Longitudinal bone, muscle and adipose tissue changes in physically active subjects – sex differences during adolescence and maturity

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Introduction

Skeletal fracture risk in older adults is driven by low peak bone mass attainment in adolescence and young adulthood, and a decline in bone strength with age1. Emphasis has therefore been placed on promoting acquisition of bone mass, size and strength during the ‘window of opportunity’ over the peripubertal period, through physical activity or exercise2. There is also good evidence that bone strength adapts to long-term physical activity levels in mature adults, and throughout life3. Women are known to display lower bone mass and strength than men at young4,5 and older age6, and to display a greater osteoporotic fracture risk7. However, to what extent this is due to differences in bone, muscle and adipose tissue composition and/or lower levels of physical activity, has not been elucidated6.

The adaptation of bone to mechanical stimuli is strongly

Abstract

Objectives: To explore changes in bone, muscle and adipose tissue composition in athletes with high physical activity levels at different stages of life. Methods: Thigh MRIs were acquired at baseline and 2-year follow-up for 20 young (16±1 years) and 20 mature (46±5 years) athletes (10 males, 10 females, respectively). Longitudinal changes in cross-sectional areas (CSAs) of femoral bone, quadriceps muscle, and thigh subcutaneous (SCF) and intermuscular (IMF) adipose tissue were evaluated. Results: Adolescent males displayed significant muscle (+5.0%, 95%CI: 0.8, 9.2) and bone growth (+2.9%, 95%CI: 1.3, 4.5), whereas adolescent females did not (muscle: +0.8%, 95%CI: -2.2, 3.8; bone: +1.9%, 95%CI: -2.1, 5.6). Adolescent and mature females showed significant SCF increases (+11.0%, 95%CI: 0.9, 21.1 and +6.0%, 95%CI: 0.6, 11.4, respectively), whereas adolescent and mature males did not (+7.2%, 95%CI: -0.8, 22.5 and +1.5%, 95%CI: -9.7, 11.8, respectively). Muscle and bone changes were highly correlated in adolescent males (r=0.66), mature males (r=0.75) and mature females (r=0.68) but not in adolescent females (r=-0.11). Conclusions: The results suggest sex-specific patterns of age-related change in bone, muscle and adipose tissue, and tight coupling of bone and muscle growth. Sex-specific bone-muscle-adipose tissue relationships may have implications for understanding sex differences in fracture risk.

Keywords: Knee, Adolescence, Muscle, Adiposity, Bonemized Mice, Bone Metabolic Marker
determined by muscle forces. Similar to bone, muscle growth (e.g., an increase in cross-sectional area [CSA]) in adolescence has been reported to differ between men and women, potentially reflecting distinct hormonal profiles or different responses to a sedentary lifestyle. The bone-muscle relationship may therefore differ between sexes. More recently, adiposity has been identified as an important contributor to bone development in adolescent males and females as well as to the maintenance of bone density. The relationship between adiposity and bone is not only driven by mechanical force through increased body weight, but also through endocrine mechanisms, by secretion of hormones from adipocytes. One of these adipokines, the cytokine-like hormone leptin, appears to be a particularly powerful stimulator of bone formation, reabsorption and muscle development. Serum leptin levels appear to decrease through endocrine mechanisms, by secretion of hormones from adipocytes. One of these adipokines, the cytokine-like hormone leptin, appears to be a particularly powerful stimulator of bone formation, reabsorption and muscle development. Serum leptin levels have been reported to be robust predictors of bone mineral density, particularly in women, likely reflecting the typically greater subcutaneous adiposity levels in females. These sex-differences in adiposity and serum leptin levels may also drive sex-specific relationships between bone and adipose tissue. Compared to subcutaneous fat (SCF), however, intermuscular fat (IMF) has thus far received little attention: despite being less abundant, intermuscular fat appears to impact limb function through its intimate relationship with muscle, and to be a potential contributor to bone health. Serum leptin levels appear to decrease with age, independent of BMI, potentially altering its impact on muscle and bone morphology; however, no studies thus far have simultaneously assessed the relationships between bone, muscle and adipose tissue longitudinally, in males and females, and both during adolescence and maturity.

Given that changes in bone, muscle and adipose tissue are all linked to physical activity, the relationship between bone, muscle, intermuscular fat, and subcutaneous fat likely is determined by physical activity level. Studies that have evaluated bone-muscle-adipose tissue relationships thus far, have done so isolated to either adolescents or adults in today’s predominantly sedentary populations, in which the level of physical activity can be assumed to be much less than it genuinely was in human species development. The typical sedentary lifestyle and unparalleled consumption of energy-rich processed food may thus impact findings on sex differences in the bone-muscle-adipose tissue relationship. For this reason, the current study was undertaken to evaluate the relationship between bone, muscle and adipose tissues in athletes with high levels (and similar types) of physical activity at different stages of life, in order to reveal sex differences in a non-sedentary lifestyle.

Specifically, we studied the following questions:
1) What are the differences in longitudinal change in (quadriceps) muscle, (cortical) bone and IMF/SCF CSAs between physically active male and female adolescent or mature volleyball athletes, taking into consideration the compositional differences at baseline?
2) What associations exist between the longitudinal changes of bone, muscle and adipose tissue, respectively, in each sex in physically active persons during adolescence and adulthood?

We hypothesized that a significant increase in muscle and bone CSA would occur in adolescent athletes, more so in males than females, and that no significant change would occur in these tissues in mature athletes. We also hypothesized that coupling of muscle and bone would occur in adolescent athletes, but not adipose tissue due to high activity levels minimizing adipose tissue increases.

Materials and methods

Participants

We studied 40 competitive volleyball players (20 elite adolescent athletes and 20 former elite mature athletes). The adolescent athletes (10 male and 10 female) were recruited from the Olympiastützpunkt (OSP) Berlin, were aged 16±1 years at baseline and trained twice per day for approximately 2 hours (training identical for male and female athletes, with some minor individual variation based on health and personal status) (Table 1). Training consisted of strength, endurance, individual volleyball skills, and team playmaking strategies. At baseline, the adolescent men and women were in the process of closing their tibial and femoral epiphyseal plates, whereas almost all plates were closed at follow-up. All adolescent women were post-menarcheal and none were taking oral contraceptives. The mature athletes (10 male and 10 female), also former members of the OSP, were still currently playing volleyball at least twice per week and were 30 years older than the adolescent group (46±5 years) (Table 1). Demographic characteristics of the 39 participants (one male athlete had to be excluded due to missing follow-up MRI) including injury history (self-reported), height and weight were recorded, and body mass index (BMI) calculated (kg.m⁻²). The study complied with the Helsinki Declaration of “Ethical Principles for Medical Research Involving Human Subjects”, was approved by the local ethics committee, and all participants (and/or their parents) signed informed consent prior to participation.

MR imaging acquisition

Unilateral MR images of the thigh of the dominant limb (preferred take-off leg) were acquired at baseline and 2-year follow-up using a 1.5T system (Avanto, Siemens Medical Systems, Erlangen, Germany) and a custom body coil. Participants lay supine with their dominant knee close to full extension. Axial thigh MR images, extending from the femoral neck proximally to the quadriceps tendon distally, were acquired using an axial T1-weighted spin echo sequence (8 mm slice thickness; between 0.82 and 0.85 mm in-plane resolution, repetition time between 641 and 915 ms, echo time 11ms). Follow-up images were acquired 2 years later (24±1 months; range 21–27) using the same scanner, with all acquisition parameters being kept identical to the baseline acquisition.

Image segmentation and analysis

Thigh muscle (quadriceps), bone (total femoral and cortical bone), and adipose tissue (SCF and IMF) CSAs were calculated from a single axial MR image that was located 30% from

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proximal to distal, since muscle CSAs at this location have been shown to correlate best with muscle volumes\textsuperscript{28}. The baseline and follow-up image data sets for each participant were processed in pairs, but with blinding to the acquisition time-point (i.e., baseline or follow-up). A random time-point was processed first; the other time-point was then processed using the first data set as a reference.

The evaluation of muscle, bone and adipose tissue CSA has been described in detail previously\textsuperscript{29,30}. Briefly, custom software was used to firstly manually segment muscle (quadriceps), bone (total and cortical) and adipose tissue (SCF and IMF) CSAs (cm\textsuperscript{2}). Secondly, the outer circumference of the thigh was identified by an edge-detection algorithm (Open CV, http://opencv.org/); a second algorithm was then used to delineate a ‘sling’ enclosing the previously segmented muscle tissue, separating the SCF from other thigh tissue. Femoral bone CSA was determined by the same edge detection and a shape identification algorithm from Open CV. The tissue between the outer femoral bone edge and inner SCF circumference was separated into individual muscle tissue (manual segmentation), and IMF was identified from other inter-muscular tissue by applying a user-controlled Open CV signal intensity threshold (Figure 1). The intra-observer test-retest preci-

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Table 1. Demographic data of the study participants*.

|                        | Adolescent Females | Adolescent Males | Mature Females | Mature Males |
|------------------------|--------------------|------------------|---------------|--------------|
| Participants, number   | 10                 | 10               | 10            | 9            |
| Age baseline, years    | 16±1               | 16±1             | 47±6          | 46±3         |
| Height baseline, cm    | 182±4              | 194±5            | 176±5         | 191±5        |
| Weight baseline, kg    | 70±9               | 84±5             | 71±6          | 95±14        |
| Body mass index baseline, kg/m\textsuperscript{2} | 20.9±2.0 | 22.3±0.9 | 22.7±1.9 | 26.1±2.9 |
| Follow-up duration, years | 2.1±0.1     | 2.0±0.0          | 2.0±0.0       | 2.0±0.1     |
| Height change, cm      | 0.5±1.0            | 1.4±1.3          | -0.1±0.3      | 0.1±0.3      |
| Weight change, kg      | 1.4±3.3            | 6.5±2.9          | 0.0±2.6       | 2.4±4.5      |

Index knee injury history, number^

- Osgood-Schlatter disease 0 2 0 0
- Patellar tendinopathy 0 2 1 0
- ACL tear/surgery 1 0 0 0
- Undiagnosed knee pain 2 0 2 1
- Meniscus surgery 0 0 2 3
- Other knee surgery 0 0 1 1

* numbers are mean ± standard deviation unless indicated otherwise.

BMI, body mass index; ACL, anterior cruciate ligament.

^ Other reported medical issues: Adolescent males: back pain=3 (2 in combination with patellar tendinopathy), scoliosis=1, kidney issues=1, meniscectomy in contralateral knee=1; Adolescent females: ACL/ meniscus tear in contralateral knee=1, back pain=2 (1 in combination with knee pain, and 1 in combination with patellar and Achilles tendinopathy), muscle pain=2, shoulder pain=1, bilateral tibiae pain=1; Mature males: back pain=6 (1 in combination with lateral meniscectomy, and 1 in combination with other previous knee surgery); Mature females: history of Achilles rupture=1 (in combination with patellar tendinopathy), hip arthritis=3 (1 with endoscopic surgery), back pain=1 (in combination with knee pain), meniscectomy in contralateral knee=1.
All statistical analyses were completed using Pearson correlation coefficients. Correlation coefficients <0.40 were considered weak, those between 0.40 and 0.80 moderate, those between 0.80 and 0.90 strong, and >0.90 very strong. One adolescent female, two adult females and one adult male had to be excluded from SCF analyses, because of incomplete depiction of SCF layer in the image.

**Statistical analysis**

Descriptive statistics were used to describe demographic and baseline data based on sex and age group. In addition to the raw data, muscle and fat CSAs were normalized to femoral bone CSA to account for differences in body size. For each tissue variable, the absolute change between baseline and follow-up, and the relative (percent) change [(follow-up – baseline) / baseline x 100] was calculated. Differences in the longitudinal changes between men and women were evaluated using an unpaired t-test. The relationship between the longitudinal changes in muscle, fat and bone were evaluated using Pearson correlation coefficients. Correlation coefficients <0.40 were considered weak, those between 0.40 and 0.59 moderate, those between 0.60 and 0.79 strong, and >0.80 very strong. All statistical analyses were completed using SPSS statistical software V22.0. *P* values <0.05 were considered significant.

**Results**

**Baseline descriptive findings**

Baseline values of bone, muscle, and adipose tissue CSAs are presented in Table 2. These relationships are descriptive and have not been tested for statistical significance as they did not pertain to the primary study questions. Men displayed approximately one third greater quadriceps muscle CSAs than women, in both adolescent and mature participants. However, as men also had approximately one quarter greater cortical and total bone CSA, the ratio of quadriceps per total bone CSA was only slightly greater in men than women (Table 2). Women had more SCF than men in both adolescents (+77%) and mature participants (+39%), and this difference was even greater when adipose tissue CSAs were normalized to total bone size (+128% / +79%, respectively). Adolescent and mature women also had more IMF than men, particularly when normalized to total bone CSA (+45% / +6%, respectively), albeit the differences were considerably smaller than for SCF (Table 2).

Male and female adolescent participants displayed 17% / 15% greater quadriceps muscle CSA than their older counterparts after normalizing to bone size, respectively. Adolescent athletes had less SCF and IMF, and similar bone size compared with mature athletes (Table 2).

**Longitudinal change in bone, muscle and adipose tissue in adolescent and mature athletes**

Both cortical (+2.9%; 95% CI: 1.0, 4.8%) and total femoral bone CSA (+2.9%; 95% CI: 1.3, 4.5%) increased significantly in adolescent men between age 16 and 18 years, but no significant change was observed in young women (+1.9%; 95% CI: -2.1, 5.6%, and +0.8%; 95% CI: -1.8, 3.4%, respectively) (Table 3; Figure 2). The quadriceps CSA increased by 5.0% (95% CI: 0.8, 9.2%) in adolescent men and only by 0.8% (95% CI: -2.2, 3.9%) in adolescent women. Longitudinal change in muscle and fat normalized to total bone size are presented in Table 3.

The SCF CSA increased by 11.0% (95% CI: 0.9, 12.1%) in the young women between age 16 and 18 years, and by 7.2% (95% CI: 8.0, 22.5%) in young men; the longitudinal increase in IMF in young women was also statistically significant when normalized to total bone area (Table 3; Figure 2). No significant longitudinal increase in IMF CSA was observed in either sex, with or without normalization to femoral bone CSA. When compared directly with each other, differences in longitudinal changes of the above tissues between adolescent men and women did not reach statistical significance.

Mature athletes did not display any significant tissue

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**Table 2.** Adolescent and mature volleyball athletes: baseline values of muscle, adipose and bone tissue cross-sectional areas (mean ± standard deviation cm²) in females and males.

|                         | Adolescent       | Mature          |
|-------------------------|------------------|-----------------|
|                         | Female (n=10)    | Male (n=10)     |
| Femoral cortical bone   | 4.8 ± 0.6        | 5.9 ± 0.5       |
| Total femoral bone      | 6.1 ± 0.9        | 7.6 ± 1.0       |
| Quadriceps muscle       | 67.7 ± 9.9       | 87.2 ± 7.5      |
| Subcutaneous fat         | 91.2 ± 22.5      | 51.5 ± 15.5     |
| Intermuscular fat        | 10.0 ± 2.5       | 8.5 ± 2.1       |
| Quadriceps/Total bone   | 11.2 ± 1.4       | 11.7 ± 1.2      |
| Subcutaneous fat/Total   | 15.3 ± 3.1       | 6.7 ± 1.9       |
| Intermuscular fat/Total  | 1.6 ± 0.3        | 1.1 ± 0.1       |

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CSA changes over two years, except mature women continued to have a significant 6% (95% CI: 0.6, 11.4%) increase in SCF (Table 4; Figure 2). Mature women tended to demonstrate a small reduction in quadriceps CSA (-1.7% [95% CI: -6.5, 3.2%]), despite maintenance of high levels of physical activity, while mature men still displayed a small (statistically non-significant) increase (1.2% [95% CI: -2.9, 5.4]). Similarly, small non-significant increases

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**Table 3.** Adolescent volleyball athletes: longitudinal change in muscle, adipose and bone tissue cross-sectional areas (cm²) over 2 years in females and males.

|                      | Female (n=10) | Male (n =10) | p-value* |
|----------------------|--------------|--------------|----------|
|                      | Mean         | 95% CI       | Mean     | 95% CI       |          |
| Femoral cortical bone| 0.09         | -0.10, 0.27  | 0.17     | 0.06, 0.28   | 0.403    |
| Total femoral bone   | 0.05         | -0.11, 0.21  | 0.22     | 0.10, 0.34   | 0.082    |
| Quadriceps muscle    | 0.57         | -1.49, 2.62  | 4.37     | 0.71, 8.03   | 0.056    |
| Subcutaneous fat     | 10.04        | 0.84, 19.24  | 3.72     | -4.12, 11.56 | 0.246    |
| Intermuscular fat    | 0.35         | -1.01, 1.72  | 0.85     | -0.08, 1.78  | 0.508    |
| Quadriceps/Total bone| 0.00         | -0.52, 0.52  | 0.24     | -0.23, 0.70  | 0.455    |
| SCF/Total bone       | 1.41         | 0.03, 2.80   | 0.32     | -0.86, 1.51  | 0.186    |
| IMF/Total bone       | 0.04         | -0.19, 0.26  | 0.09     | -0.05, 0.23  | 0.641    |

CI, confidence interval; SCF, subcutaneous fat; IMF, intermuscular fat.

* unpaired t-test

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**Figure 2.** Percent (%) total femoral bone, quadriceps muscle and subcutaneous fat cross-sectional area change in adolescent and mature volleyball athletes: bar graph showing mean change and 95% confidence interval in females vs. males.
also occurred in cortical and total bone CSA in mature athletes (approximately 1% in mature men and women). Consistent to observations made in adolescence, no statistically significant longitudinal differences were observed between mature men and women.

Correlations between longitudinal change in bone, muscle and adipose tissue

Correlations between longitudinal change in muscle, adipose and bone tissue CSA are presented in Table 5. Although longitudinal changes in cortical bone were small, an increase in quadriceps muscle CSA was significantly correlated with an increase in cortical bone CSA in adolescent men ($r=0.66$) as well as in mature men ($r=0.75$) and women ($r=0.68$) ($p<0.04$), but not adolescent women ($r=-0.11$, $p=0.755$) (Figure 3). Similar relationships were observed with total bone CSA, however, the associations failed to reach statistical significance (Table 5). Changes in IMF and SCF were strongly correlated ($r=0.5$) but only reached statistical significance in adolescent females and mature males. Most other longitudinal bone-muscle-adipose tissue changes were weakly and non-significantly correlated ($r<0.40$) (Table 5).

Table 4. Mature volleyball athletes: longitudinal change in muscle, adipose and bone tissue cross-sectional areas (cm$^2$) over 2 years in females and males.

|                         | Female (n=10) | Male (n=9) | p-value |
|-------------------------|---------------|------------|---------|
|                         | Mean 95% CI   | Mean 95% CI|         |
| Femoral cortical bone   | 0.01 -0.14, 0.16 | 0.06 -0.13, 0.25 | 0.625   |
| Total femoral bone      | 0.06 -0.06, 0.18 | 0.09 -0.06, 0.24 | 0.756   |
| Quadriceps muscle       | -1.01 -3.89, 1.88 | 0.97 -2.37, 4.31 | 0.318   |
| Subcutaneous fat         | 6.75 0.72, 12.78 | 1.23 -7.01, 9.46 | 0.221   |
| Intermuscular fat        | -0.19 -1.01, 0.64 | 0.67 -0.47, 1.80 | 0.176   |
| Quadriceps/Total bone   | -0.26 -0.63, 0.11 | 0.02 -0.30, 0.33 | 0.218   |
| SCF/Total bone          | 0.91 -0.10, 1.91 | 0.13 -0.91, 1.18 | 0.230   |
| IMF/Total bone          | -0.06 -0.17, 0.06 | 0.07 -0.07, 0.20 | 0.134   |

CI, confidence interval; SCF, subcutaneous fat; IMF, intermuscular fat.

* unpaired t-test

Figure 3. Scatter plot of longitudinal change in quadriceps muscle and femoral cortical bone cross-sectional areas in female and male adolescent (A) and mature (B) volleyball athletes.

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Table 5. Pearson correlation coefficients and p-values for longitudinal change in muscle, adipose and bone tissue cross-sectional areas over 2 years.

|                     | Quadriceps muscle | Subcutaneous fat | Intermuscular fat |
|---------------------|-------------------|------------------|-------------------|
|                     | Adolescent Female | Mature Female    | Adolescent Female | Mature Female    | Adolescent Female | Mature Female    | Adolescent Female | Mature Female    |
| Subcutaneous fat    | -0.67             | 0.03             | 0.30             | 0.07             | -                 | -                 | -                 | -                 |
|                     | 0.050             | 0.937            | 0.547            | 0.867            | -                 | -                 | -                 | -                 |
| Intermuscular fat   | -0.48             | 0.03             | 0.24             | -0.11            | 0.76              | 0.50              | 0.59              | 0.76              |
|                     | 0.160             | 0.943            | 0.502            | 0.778            | 0.017             | 0.139             | 0.124             | 0.030             |
| Femoral cortical bone | -0.11           | 0.66             | 0.68             | 0.75             | 0.44              | -0.30             | 0.27              | -0.01             |
|                     | 0.755             | 0.038            | 0.032            | 0.019            | 0.241             | 0.399             | 0.519             | 0.979             |
| Total femoral bone  | -0.35             | 0.62             | 0.63             | 0.67             | 0.55              | -0.40             | 0.12              | 0.21              |
|                     | 0.318             | 0.075            | 0.051            | 0.051            | 0.128             | 0.283             | 0.784             | 0.615             |

Pearson’s r with p-value in italics. Bold indicates p <0.05.

Discussion

In this longitudinal study of highly active adolescent and mature athletes, we aim to elucidate innate bone-muscle-adipose tissue relationships in a non-sedentary lifestyle. We observed sex-specific patterns of tissue change at different stages of life. At baseline, adolescent and mature male athletes displayed a mean 23-35% greater bone and muscle CSA than female athletes, but <5% greater muscle/bone CSA ratios. Women, in contrast, displayed a mean 39-77% greater SCF CSA than men, and 79-128% greater SCF when normalized to bone CSA. Over the two-year longitudinal observation period, adolescent males displayed significant muscle and bone growth. Adolescent and mature women displayed significant increases in SCF, despite being physically active, and despite displaying minimal increases in body weight over the observation period and having approximately twice the amount of SCF observed at baseline, compared with male athletes. Longitudinal changes in muscle CSAs were highly correlated with cortical bone geometry changes in adolescent males, and in mature males and females, providing evidence of a bone-muscle unit throughout life.

A limitation of the current study is the modest sample size, which limits the power in detecting significant differences. However, the unique study design examining elite volleyball athletes limited the number of available participants; this approach to studying sex differences ensured similar levels of physical activity in men and women. Due to the small sample size, we focused our analysis on longitudinal relationships only and did not statistically test baseline sex-differences in order to minimize the number of statistical tests performed and reduce the chances of a type I error. We did not correct for conducting multiple tests on the longitudinal data in view of the exploratory nature of the analysis. Further, the same athletes were not evaluated at adolescence and middle-age, and although the mature athletes were less active than the adolescent athletes, the selection ensured that the mature athletes had gone through the same “history of physical activity” as the adolescent study participants, approximately 30 years earlier. We did not specifically evaluate the age that participants commenced volleyball training; however, from the time that the athletes entered the OSP program (16 years of age – just prior to baseline assessment for adolescent athletes), training was the same for all male and females athletes. Similarly, we did not record the level of physical activity of athletes outside of their volleyball involvement nor dietary intake, which may have influenced bone-muscle-adipose tissue relationships, particularly the increase in SCF observed in adolescent females and the generally large variability in SCF changes. Volleyball is a high-impact sport which is arguably more likely to influence tissue changes, particularly bone and muscle than activities of daily living. We did not evaluate non-athletic controls, which limited our ability to directly compare our findings in high-impact athletes to changes due to growth alone in a sedentary population. Previous data from non-athletic controls enabled us to extrapolate the influence of high-impact activity on tissue change. While bone, muscle and adipose tissue were analyzed using CSA from a single axial MR image, this image was very carefully selected from a volumetric acquisition, to represent a consistent anatomical location. Further, quadriceps muscle measurements from this location were previously shown to correlate highly with total muscle volume. Importantly, the longitudinal changes observed were larger than the measurement error reported for thigh tissue CSAs. Using a cross sectional imaging approach rather than, for instance dual energy X-ray absorptiometry (DXA), enabled separation of SCF and IMF components of adiposity, which may have distinct functional relevance.

A significant increase in muscle size occurred over 2 years.
in young male athletes only. This extends previous findings reporting that, from 16 years of age, athletic males become significantly stronger\textsuperscript{46} and continue to display greater increases in muscle strength during their final teenage years as opposed to their female counterparts\textsuperscript{8}. However, when normalized to bone size, no significant increase in muscle was observed, reflecting the tight association between bone and muscle growth. The greater muscle and bone changes from 16 years of age in males compared with females likely reflect hormonal differences that lead to a later onset of puberty in males, with abundant levels of testosterone driving bone and muscle growth\textsuperscript{37}. The approximately three times greater increase over 2 years in body height and weight in adolescent males compared to adolescent females supports this notion of continued linear growth in adolescent males during the follow-up period. Nevertheless, previous thigh CSA data comparing 16-18 year old jumping athletes to non-athletic controls shows that adolescent athletes have 10-20\% greater quadriceps CSA than controls\textsuperscript{20}, suggesting that the increases in muscle CSA we observed in adolescent athletes may also be observed independent of growth.

Although the adolescent and mature athletes were compared by cross-sectional analysis, our results suggest that approximately 85\% of muscle mass of highly trained young athletes can be maintained in both mature women and men, approximately 30 years after adolescence, when regular high-impact activity is continued. This stands in contrast to sedentary adults of similar age, who have been reported to lose >8\% of their muscle mass per decade starting with age 40, with this loss accelerating to >15\% per decade after 75 years of age\textsuperscript{28,39}. These findings, to some extent, contrast the common notion that muscle mass and strength decline as a function of aging alone, and support data suggesting maintenance of muscle mass in master’s athletes \approx40 years of age competing in a variety of sports\textsuperscript{40}. In terms of bone, mature male and female athletes displayed 2-10\% greater total femoral and cortical femoral bone CSA than their adolescent counterparts. This small difference likely reflects ongoing bone development during late adolescence that continues slowly until approximately 50 years of age\textsuperscript{41,42}. The bone CSA increases we observed in adolescent athletes (particularly males), is likely to be greater than sedentary populations who lack the influence of high-impact exercise on bone growth – an important driver of bone accrual\textsuperscript{2}, although this was not directly tested in the current study.

Significant longitudinal SCF changes occurred in both adolescent and mature athletic women, but not in men. These results, combined with the significant increases in muscle and bone observed in adolescent males only, suggest some sex-specific patterns of change in bone, muscle and adipose tissue, despite examining men and women with very high levels of physical activity. Previous research suggests that healthy women tend to increase SCF with increasing age even in the presence of stable weight\textsuperscript{40,43}. Although we evaluated SCF in an anatomical location where women, more so than men, deposit adiposity, results of the current study reveal that increases in SCF occur even in highly active athletes, likely reflecting a crucial role that the SCF appears to play in metabolic homeostasis in women\textsuperscript{44}. Despite increases in SCF in young and mature female athletes, no increase in IMF was observed in any group. These findings are inconsistent with data from older women (>50 years of age), where 2-year change in IMF CSA was observed and related to aging\textsuperscript{33}. Maintaining physical activity may thus prevent IMF deposits, and in turn potentially prevent detrimental implications of IMF on physical function and performance\textsuperscript{17,18}. The lack of correlation of longitudinal change in adipose tissue with that in bone in adolescent males and females, and in mature men, contrasts with the relatively high correlations in longitudinal growth of muscle and bone. These findings appear to suggest that, in terms of bone apposition and growth, there is a tighter relationship between muscle and bone than between adipose tissue and bone in physically highly active subjects, with the potential exception of mature women. It is also important to note that no correlation between muscle and bone growth was observed in adolescent female athletes. That the adolescent females gained the most SCF, which trended strongly towards being correlated with less muscle growth (Table 5), may partly explain the only lack of relationship observed between muscle and bone growth (i.e., due to the mediating influence of increased SCF). Nevertheless, the coupling of muscle and bone change in mature adolescents extends previous findings of muscle forces being a strong determinant of bone structure during peak musculoskeletal growth\textsuperscript{1,22}, by providing evidence of a muscle-bone unit many years after peak growth has ceased. The weak relationship between adipose tissue and bone may be due to the relatively small amounts of body fat observed in the highly active athletes, despite a significant longitudinal SCF increase in adolescent and mature women, or may indicate that leptin and other adipokines influence trabecular bone density and mineral content more so than cortical bone mass and diaphyseal bone size.

In conclusion, adolescent and mature female athletes gained significant levels of SCF over two years despite high physical activity levels. Adolescent males continued to display significant muscle and bone growth, with approximately 85\% of adolescent male and female muscle mass being maintained in adulthood. Generally strong correlations between muscle and bone change (except in adolescent females) provide some evidence of a bone-muscle unit throughout life, however, the influence of local fat mass on bone geometry in highly active athletes appears small. These results provide insight into the innate bone-muscle-adipose tissue relationships during growth and maturation in a non-sedentary lifestyle. The findings have potential implications for a greater understanding of the role of muscle and adipose tissue in bone fracture risk.

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Ethical approval

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

Informed consent

Informed consent was obtained from all individual participants included in the study.

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