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Upper Limb Muscle:Bone Asymmetries and Bone Adaptation in Elite Youth Tennis Players

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Running Title: Upper Limb Asymmetry in Elite Tennis Players.

Abstract:

Introduction: The study of tennis players allows the non-racquet arm to act as an internal control for the exercising racquet arm. In addition, study of the upper limbs removes the influence of gravitational loading allowing examination of the influence of muscular force on bone adaptation.

Methods: To examine the role of muscular action on bone, strength parameters of the radius, ulna (both at 4% and 60% distal-proximal ulnar length) and humerus (at 35% distal-proximal humerus length) as well as muscle size in both arms of 50 elite junior tennis players (mean age 13.5 ± 1.9 y) were measured with pQCT.

Results: Strong relationships were found between muscle size and bone size in both arms (all correlations $P < 0.001$, $R^2 = 0.73-0.86$) However, muscle:bone ratio was significantly lower ($P < 0.001$) in the upper arm on the racquet side (compared to the contralateral arm). In addition, material eccentricity analysis revealed that bone strength in bending and torsion increased more than strength in compression as the moment arms for these actions (bone length and width respectively) increased (in all cases $P > 0.001$, $R^2 = 0.06-0.7$) with relationships being stronger in torsion than in bending. Large side differences were found in bone strength parameters and muscle size in all investigated sites, with differences in distal radius total BMC ($+37 \pm 21\%$) and humerus cortical CSA ($+40 \pm 12\%$) being most pronounced (both $P < 0.001$).

Conclusion: These results support a strong influence of muscular action on bone adaptation – however, inter-arm muscle:bone asymmetries suggest factors other than local muscle size determine bone strength. The results also suggest that torsional loads provide the greatest stress experienced by the bone during a tennis stroke.

Key Words: pQCT; Exercise; BMD; Physical Activity.

Introduction:

Paragraph Number 1 Bone strength (as determined by its density, mineral mass and micro and macro-structure and geometry) is an important contributant to fracture risk. Genetic and nutritional factors certainly play a part in determining bone strength, as does mechanical loading. The Mechanostat theory(13) suggests that bone adaptation is a homeostatic control system whose aim is to regulate the peak habitual strain experienced in bone, by means of continual modeling and remodelling. In the case of low or reduced levels of loading (such as in bed rest, space flight or spinal cord injury), bone strength decreases at rates of up to 2.5%/month (12, 19, 25). Conversely, regular exercise (particularly with a weight-bearing or high-impact element) leads to increases in bone strength parameters (21).

Paragraph Number 2 Whereas it was previously assumed that passive loading as a result of body weight bearing was the major contributing load causing bone strains and hence adaptation, it was proposed less than 15 years ago that muscle forces are a significant (and in some cases the primary) cause of bone strain and subsequent adaptation (32). Proponents of this hypothesis cite the short levers that muscles work against requiring the internally-generated forces imposed on bone to be up to 10 times that of external reaction forces. In addition, the strong relationship between muscle size (as a surrogate of force production capabilities) and bone strength (23, 34) is also proffered as evidence of this link.

Paragraph Number 3 As much as spinal cord injury patients offer a model of long-term low habitual loading, athletes conversely offer an opportunity to study the effects of years or even decades (in the case of master athletes) of high-level loading. Indeed, athletes in a number of sports have been shown to have higher bone strength parameters than less active peers, with

the effects more pronounced in sports requiring high impacts or rates of limb acceleration or deceleration (and hence higher peak muscle forces) (1, 11, 22). Associations of participation in these sports with higher bone strength have also been shown in junior (6) and veteran (39) sportspeople.

Paragraph Number 4 However, it is not clear whether these group differences are attributable to the effects of exercise, or whether an element of self-selection affects the results – critics of these studies cite possible genetic, nutritional and environmental factors which could also contribute. Therefore, some recent studies have looked at bone asymmetries in sports where one arm or leg is loaded more than the other such as tennis (2, 5, 7, 14), baseball (38), cricket (35), gymnastics (18, 40) or jumping events (18) – allowing the less active limb to act as an internal control. Such studies have found significant and (in the case of tennis players) very large differences in volumetric bone density, size and geometry between the dominant and non-dominant limb whereas in controls these differences were not evident or much reduced (0.5-5.2% side difference in bone size or bone mass dependent on site (14, 38)).

Paragraph Number 5 Study of asymmetries in the upper limbs also allows experimenters to control for gravitational loading as the upper limbs are not weight-bearing. This offers an opportunity to study the effects of muscular action on bone adaptation, as it is certainly the primary (if not the only) cause of strain on these bones. Despite these benefits, the role of muscle in the development of these asymmetries has not been thoroughly explored – whilst bone and muscle asymmetries in tennis players have been studied separately (5, 7, 10), only one (that the authors are aware of) has looked at muscle:bone relationships in the two arms (7). In this study, it was concluded that whilst muscle contributed to bone side differences it only played a minor role. However, this study combined MRI assessment of average bone

size throughout a 30% bone length diaphyseal section and DXA assessment of BMC in this section. Therefore, it cannot be deduced from that study whether the differences observed were related to variation in density and distribution, and how strongly bone strength was affected. Moreover, the participants were sub-elite players only, generally playing tennis less than 10 hours/wk and so their training volume and hence exercise stimulus to the bone would be less than that of an elite cohort.

Paragraph Number 6 The authors believe that a study on muscle:bone side differences in elite tennis players will provide useful information on the role of muscular action in bone adaptation. pQCT also allows analysis of bone's resistance to compressive, bending and torsional strains (26), which may give some evidence as to the primary loading modality straining the bone during tennis. An elite tennis-playing population will likely have larger side differences in muscle and bone than athletes studied previous as training volume affects the magnitude of inter-arm asymmetries in tennis (29). It is hypothesised that muscle size will be shown to be the primary determinant of bone stiffness and strength in the upper limbs, even when potentially confounding allometric factors (*e.g.* bone length) are controlled for.

Methods:

Participants

Paragraph Number 7 Fifty junior tennis players (30 male, 20 female – mean age 13.5 ± 1.9 yrs) competing at the British Junior Tennis Championships in Bournemouth, England in 2011 and Nottingham, England in 2012 were recruited for this study. Participants were included when they reported to be in good health and with no leg or arm fractures within the preceding

24 months. The study conformed to Declaration of Helsinki guidelines and was approved by Manchester Metropolitan University's Ethics Committee prior to the start of the study.

Written informed written consent was obtained from all subjects prior to their participation.

Paragraph Number 8 Information on training history in tennis and other sports was collected from each participant during a structured interview with the main author. They were then asked whether they regularly played any other sports and if so for how long – particular note was taken if the sport involved favouring one arm over the other (such as in golf, javelin throw or cricket). Participants were also asked at what age they started to play tennis regularly and how many hours they played per week as well as details of any other tennis-related training (fitness work, strength and conditioning sessions, *etc.*). Participants were asked for their preferred racquet hand, and whether they played single or double-handed backhand and forehand. Finally, they were asked if they had suffered an injury that forced them to stop playing within the last two years or whether for any other reason (lack of interest, schoolwork pressures, *etc.*) they had ceased playing regularly. In either of these cases, further details of the cause for absence, timing and length of absence were recorded. For females, date of menarche (if applicable) was also recorded.

Bone measurements

Paragraph Number 9 Scans were taken with a Stratec XCT-3000 (6 participants) or XCT-2000 (remainder of cohort) pQCT scanner (Stratec Medizintechnik GmbH, Pforzheim, Germany). In all cases, all scans for a particular participant were taken with the same scanner, and scanners were cross-calibrated with the European forearm phantom. Voxel size of 0.5mm was used, with scan speed of 50mm.s⁻¹ Scans were taken at two sites of the left

and right forearm, corresponding to 4% and 60% distal-proximal ulnar length (measured between the olecranon and the ulnar styloid process). Muscle cross-sectional area (CSA) measurements were also examined at the 60% site to examine the muscle-bone relationship. Where evidence of an open growth plate was seen in the scout view, the first scan was initiated at the most distal point of the growth plate rather than the end of the bone; the same measurements were then taken from this as a reference point. Scans of the left and right humerus at 35% proximal-distal humerus length (measured between the olecranon and lateral border of the acromium) as well as muscle CSA at this site were examined. Measurements were then exported using the Automated Analysis Tools in Version 6.00 of the software supplied with the machine. A peeling threshold of $650 \text{ mg}\cdot\text{cm}^{-3}$ was set for diaphyseal sections of bone, with a threshold of $180 \text{ mg}\cdot\text{cm}^{-3}$ set for the epiphyseal 4% slice. Only the inner 45% of bone was selected for analysis of trabecular bone in the epiphysis, using contour mode 1 and peeling mode 1 included in the machine software.

Paragraph Number 10 Given the lack of a standard nomenclature for pQCT results, the suggestions for reporting high-resolution CT results (<http://www.asbmr.org/StandardizationofBoneStructureandDensityNomenclature.aspx>) and those of a recent publication (25) have been followed. The parameters examined in the 4% epiphyseal slice were total bone area ($A_{r.tot}$, mm^2), total bone mineral content ($vBMC.tot$, $\text{mg}\cdot\text{mm}^{-1}$) and trabecular bone mineral density ($vBMD.tb$, $\text{mg}\cdot\text{cm}^{-3}$). In diaphyseal sites, $A_{r.tot}$, $vBMC.tot$, cortical area ($A_{r.ct}$, mm^2) and cortical density ($vBMD.ct$, $\text{mg}\cdot\text{cm}^{-3}$) were examined, with adjustments made to the cortical density values (due to partial volume effect) by equations established in an earlier publication (28). In addition, at the diaphyseal sites moments of inertia indicating bone's stiffness in bending perpendicular to the line of elbow flexion/extension, in line with elbow flexion/extension, and torsion respectively (I_x , I_y and I_p),

cortical thickness, ($Ct.Th_{der}$, mm), periosteal circumference (PsC, mm) and endosteal circumference (EcC, mm) derived from a circular ring model were measured. In addition, gross muscle cross-sectional area (MuscA, mm^2 , as a surrogate for maximal force production) in the 60% slice was obtained using a threshold of $35 \text{ mg}\cdot\text{cm}^{-3}$. In the upper arm, the flexor and extensor muscles were measured separately – indentations marking the separation of these muscles were located (points A and B on Figure 1), and lines drawn from these points to the centre of the bone's mass (point C on Figure 1, whose position is given in the loop output from the XCT software). These lines were then used to separate the flexors and extensors.

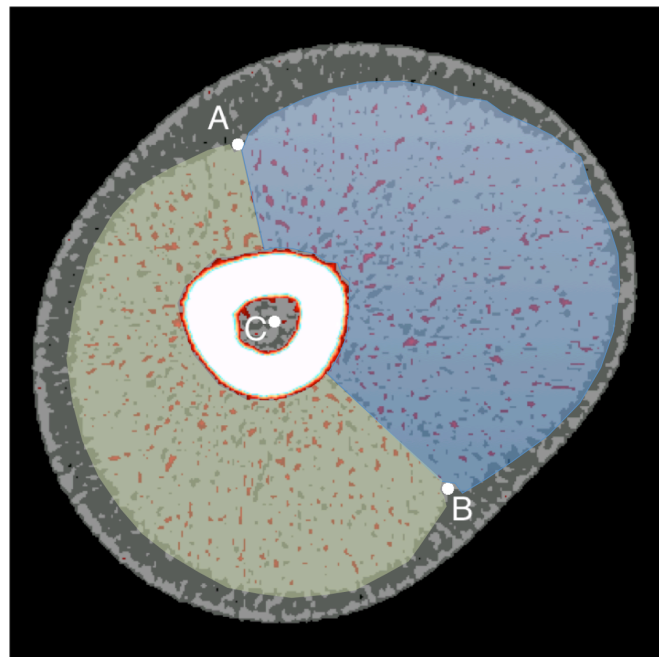


Figure 1. pQCT image of 35% humerus site showing points used to separate flexor and extensor muscles. A & B – indentations indicating separation of flexors and extensors, C – centre of bone mass. Light blue area represents area designated as flexors, light green area represents extensors.

Finally, material eccentricity (ME) was also examined. ME is the ratio between R_x , R_y and R_p (or moments of resistance in x-axis, y-axis and polar plane respectively, representing bone's strength in x-axis bending, y-axis bending and torsion) and $Ar.ct$ (which indicates

bone's strength in compression). ME thereby gives a indication of bone's relative strength in bending/torsion compared with compression (26). A greater bone length and bone circumference means a greater moment arm for bending and torsional moments respectively. Therefore the relationships of ME with bone length and bone circumference were examined to establish any link between these moments and bone geometry. *In-vivo* precision of the laboratory's pQCT measurements has been reported elsewhere (26) – precision is < 0.5% for vBMC.tot, Ar.tot and Ar.ct, 1.15% for MuscA and up to 5.1% for derived values.

Force platform data

Paragraph Number 11 A press-up was performed on a force platform (Leonardo, Novotec, Pforzheim, Germany) - participants were asked to assume a straight-armed press-up position with one hand on each force plate equidistant from the centre of the platform. They then were instructed to bend the arms and push-up as powerfully as possible with the aim of leaving the ground. Total peak power and force were recorded as well as left and right arm peak force and power. Grip strength was also measured using a Jamar+ hand grip dynamometer (Sammons Preston Inc., Bollingbrook, IL, USA). Participants completed three measures in each hand with the arm down by the side but not touching the hip and the highest force value on each side was recorded.

Statistical Analysis

Paragraph Number 12 Data were examined using PASW Statistics 18.0 (SPSS Inc, Chicago, Ill.). To test for significant differences between the dominant and non-dominant arm and effects of sex, repeated measures ANOVA with bone or muscles parameters in racquet and

non-racquet arm as within-subject factor and sex as a between-subject factor was used. Linear regression analysis was used to examine the relationship between MuscA (independent variable) and bone cross-sectional area and maximal force/velocity/power/height values (dependent values) in both the dominant and non-dominant arm. ANCOVA analysis of material eccentricity (polar or x/y-axis SSI divided by bone area, serving as a measure of how bone is adapted to resist torsion and x/y-axis bending relative to compression) was also carried out using univariate ANCOVA with material eccentricity as dependent variable, side (racquet/non-racquet arm) as fixed factor and bone length as covariate. A custom model was used, examining main effect of bone length, main effect of side and interaction between bone length and side. In the case of a side difference or bone length*side interaction, parameter estimates were used to assess differences in intercepts and gradients. Differences were considered significant at $P < 0.05$. Data are shown as mean \pm SD.

Results:

Cohort characteristics

Paragraph Number 13 Cohort characteristics and training habits are displayed in Table 1. There were no significant sex differences in participant age, training hours, tennis starting age, height or mass.

Athletic history

Paragraph Number 14 Eighteen players regularly participated in other sports on a weekly basis – these sports included swimming, association football, running, netball, basketball and ballet. Six players also regularly played another sport which favoured one arm - hockey, table tennis, badminton and cricket. In all cases the player played this additional sport with the same favoured arm as in tennis, and in no case did they train more than 2 hours per week in the secondary sport.

Parameter	Group mean (s.d.)	
	Male	Female
Age (years)	13.5 (1.9)	13.5 (2.0)
Tennis start age (years)	6.5 (2.3)	7.4 (2.4)
Tennis training hours/week	10.8 (3.7)	11.3 (3.5)
Height (m)	1.64 (0.15)	1.61 (0.10)
Body mass (kg)	52.8 (13.6)	50.5 (10.4)

Table 1. Cohort characteristics and training habits.

Muscle differences

Paragraph Number 15 Muscles in the racquet forearm and upper arm were 9-20% larger than those in the non-racquet arm (Table 2; all $P < 0.001$). All but six players were able to perform the power press-up and higher press-up maximal force ($4.9 \pm 6.9\%$, $P < 0.01$), maximal power ($13.0 \pm 11.4\%$), and grip strength ($20.0 \pm 15.7\%$; both $P < 0.001$) were recorded in the racquet arm. Maximal press-up force and power were positively correlated with MuscA in both upper and lower arms – with correlation coefficients of press-up maximal force ($R^2 = 0.76$ racquet arm, 0.68 non-racquet arm) being higher than those of press-up maximal power ($R^2 = 0.61$ racquet arm, 0.47 non-racquet arm) and maximal handgrip force ($R^2 = 0.49$ racquet arm, 0.56 non-racquet arm).

<Table 2>

Table 2. Results of pQCT scans of dominant and non-dominant arms in males and females. – data are presented as mean(s.d.). ^aSee *Methods section* for abbreviations. ^bSide – significant result indicates significant side difference in favour of racquet arm. Asterisks denote significant side difference or significant effect on magnitude of side difference of age or sex; * - $P < 0.05$, ** - $P < 0.01$, *** - $P < 0.001$.

Bone differences

Paragraph Number 16 In all cases bone strength parameters (except radius and humerus vBMD.ct, and humerus and ulna EcC) had significantly higher values in the racquet than in the non-racquet arm (Table 2). At the 4% forearm slice, vBMC.tot differences ($37.2 \pm 21.4\%$ radius, $23.5 \pm 32.4\%$ ulna) were due to greater Ar.tot and vBMD.tb in the racquet arm (all $P < 0.001$, except ulnar Ar.tot $P < 0.01$). In contrast, vBMC.tot differences at the 60% forearm slice and 35% upper arm slice were a result of 17-40% greater Ar.ct (all $P < 0.001$) and not vBMD.ct differences. Compared to age-matched reference data (24) radius Ar.tot was $19.7 \pm 17.0\%$ and MuscA $17.1 \pm 17.8\%$ greater than average in the racquet arm (both $P < 0.01$) - non-racquet arm values were not significantly different. In both arms vBMD.tb was $5.2 \pm 3.0\%$ greater in this cohort than in the reference population ($P < 0.001$).

Bone geometry:

Paragraph Number 17 At diaphyseal locations, Ct.Th_{der} and PsC were all greater in the racquet arm (all $P < 0.001$). However, EcC side differences differed greatly in the three bones – racquet arm radius EcC was $11.2 \pm 10.3\%$ greater, humerus EcC $7.1 \pm 5.1\%$ smaller (both $P < 0.001$) and

ulna EcC no different compared to the non-racquet arm.. As a result of these size and shape differences, I_x , I_y and I_p were 26-59% greater in all three bones – all $P < 0.001$.

Paragraph Number 18 Diaphyseal radius and humerus (but not ulna) in both arms were stiffer in bending in the y-axis (along the line of elbow flexion/extension) than in the x-axis (all $P < 0.001$). Material eccentricity (ME) was also examined (Figure 2). There was a significant effect of bone length on x-axis and y-axis ME and of PsC on polar ME in both arms in radius, ulna and humerus (all $P < 0.001$) – except x-axis ME in non-dominant humerus. Coefficients of determination of these regressions were much higher for polar ME and PsC ($R^2 = 0.4-0.7$), compared to x or y-axis ME and bone length ($R^2 = 0.06-0.4$). This shows that (with the exception of the non-dominant humerus) longer bones and bones with a larger PsC are more than proportionally stronger in response to bending and torsional strains (compared to compressive strain) respectively as opposed to a shorter bone or one with a smaller circumference.

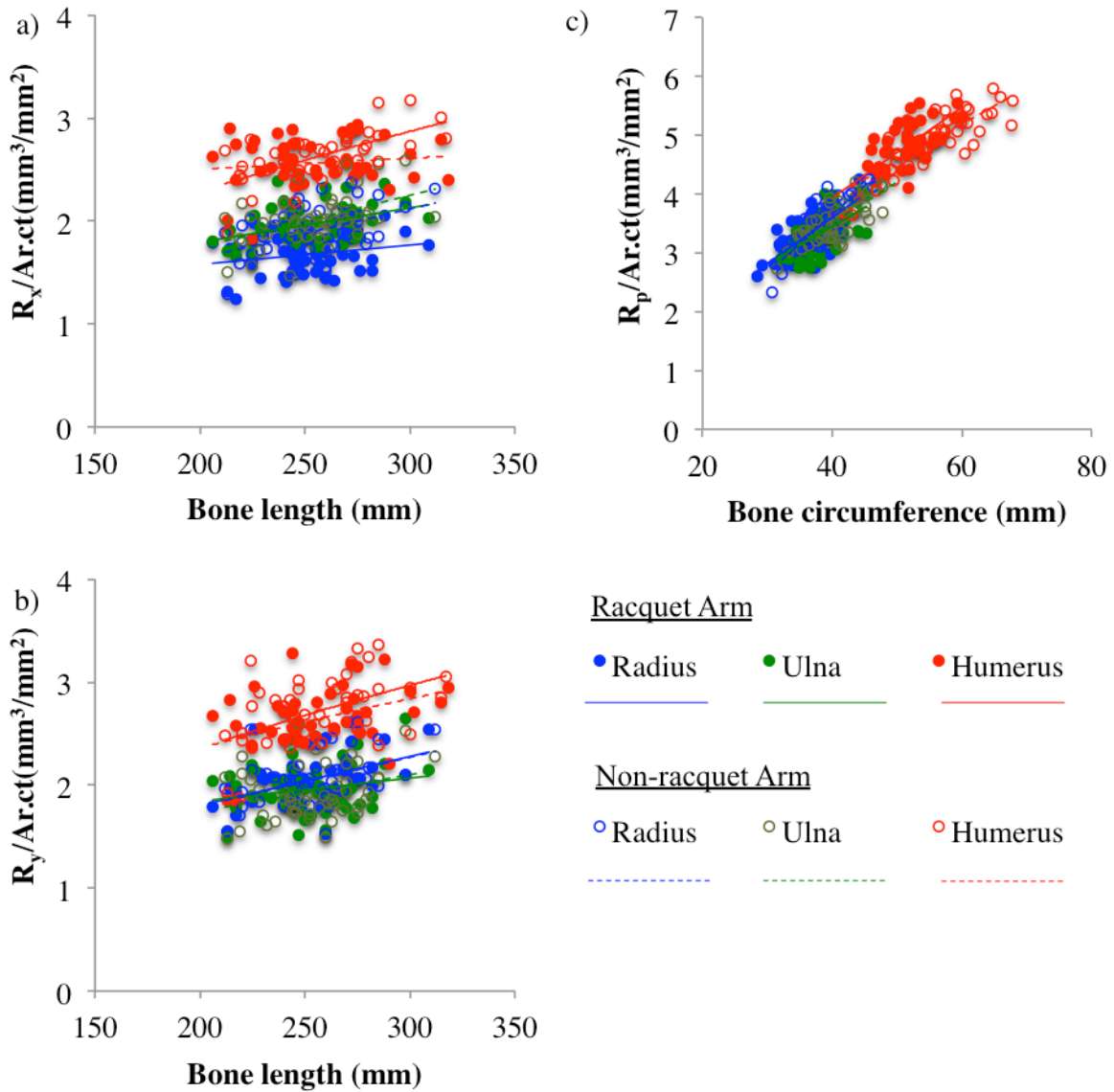


Figure 2. Material eccentricity in radius ulna at 60% distal-proximal ulna length and humerus at 35% distal-proximal length of both arms in a) anteroposterior bending and b) mediolateral bending with respect to bone length and c) torsion with respect to bone's outer circumference. All regressions $P < 0.001$, $R^2 = 0.06-0.7$, with exception of anteroposterior bending in non-dominant humerus ($P > 0.05$).

Effects of sex

Paragraph Number 19 Side differences in distal radius vBMC.tot, Ar.tot and vBMD.tb, proximal radius Ar.tot, PsC, I_x , I_y and I_p , proximal ulna I_x and humerus Ar.ct, Ct.Th_{der}, PsC, I_x

and I_y were more pronounced in men than women ($P < 0.05$, except distal radius vBMC.tot and humerus Ar.ct - $P < 0.01$ and distal radius Ar.tot - $P < 0.001$).

Effects of menarche

Ten female players had not reached menarche by the time of testing, whilst ten was postmenarcheal – the mean time since menarche for postmenarcheal players was 1.83 ± 1.14 y. Premenarcheal and postmenarcheal players had similar training volumes (11.7 ± 1.9 h.wk⁻¹ and 11.0 ± 4.3 h.wk⁻¹ respectively), and had been playing for a similar time (5.9 ± 0.8 y and 6.5 ± 3.0 y), although premenarcheal players were younger (12.4 ± 1.7 y compared to 14.5 ± 1.8 y). However, when age was controlled for (by inclusion as a covariate in ANOVA) there were no significant differences in muscle, bone or force parameters (or magnitude of side difference) between the two groups.

Muscle-bone relationship

Paragraph Number 20 There were strong correlations between MuscA and Ar.ct in both forearms and upper arms ($R^2 = 0.79-0.86$, all $P < 0.001$), with the correlation coefficients in the dominant arm being higher in all cases (Figure 3). Similarly, there were strong correlations between humerus Ar.ct and maximal press-up force (dominant arm $R^2 = 0.86$, non-dominant arm $R^2 = 0.72$) and power (dominant arm $R^2 = 0.71$, non-dominant arm $R^2 = 0.57$) and maximal hand grip force and racquet and ulna Ar.ct ($R^2 = 0.51-0.60$) - all $P < 0.001$. These strong relationships remained (albeit with lower coefficients) even when bone length was accounted for (partial correlation). Muscle-bone ratio was higher in the racquet

arm in the proximal ulna ($4.0 \pm 9.9\%$, $P < 0.05$) and radius ($6.1 \pm 7.8\%$, $P < 0.001$) and lower in humerus ($20.8 \pm 7.5\%$, $P < 0.001$).

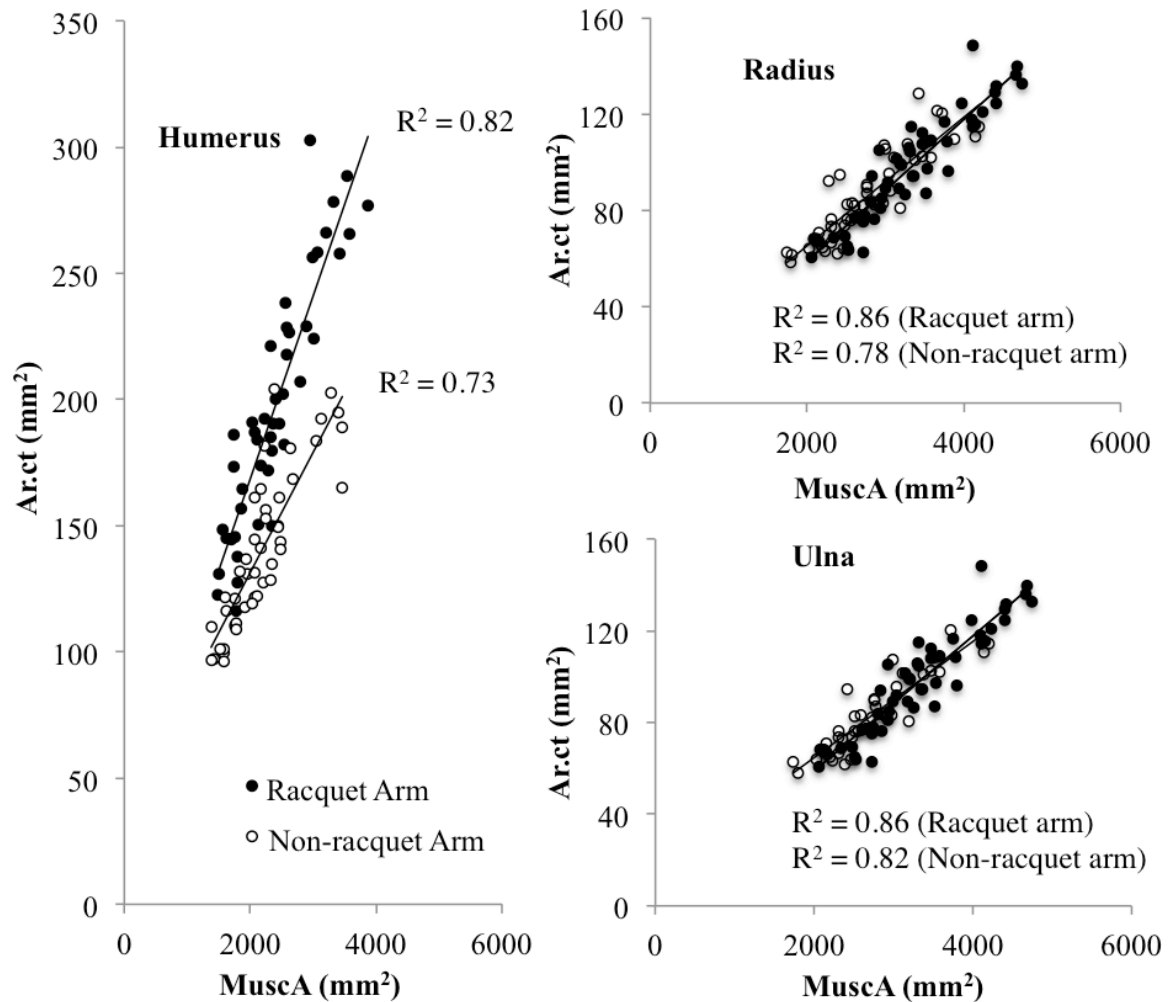


Figure 3. Linear regressions showing relationship between muscle CSA (MuscA) and cortical bone CSA (Ar.ct) at mid-shaft radius, ulna and humerus sites in racquet and non-racquet arms.

Discussion:

Paragraph Number 21 The main observations of this study are the close correlations of muscle size and bone size, in addition to a significantly lower muscle:bone ratio in the upper arm on the racquet side compared to the other arm. Also, strong relationships between bone

strength in bending and torsion and bone length and width (the moment arms for these actions respectively) were found. The results also showed large side differences in bone strength indicators, muscle size and force/power output in favour of the racquet arm.

Muscle differences

Paragraph Number 22 In line with previous observations it is shown here that regular participation in tennis during youth is associated with large, site-specific differences in bone strength and muscle size between the racquet and non-racquet arm (2, 5, 7, 14). The results qualitatively conform with the literature in that muscle size was found to be larger on the racquet side (7), and that further to this the increased upper arm muscle size is due primarily to an increase in triceps brachii rather than the elbow flexors in youth players (30) although not in adults . This suggests that the triceps have a larger role in tennis playing than the flexors and as such their action may more markedly stress the bone. Perhaps then, looking at gross muscle cross-sectional area in a limb segment as a surrogate for the force experienced by the bone is too simplistic. This could be more apparent in the upper limbs where muscles play very different roles as flexors, extensors, rotators, *etc.* In addition to muscle side differences, this study also demonstrates functional superiority on the racquet side in grip strength – again in agreement with previous studies (9) - but also in a powerful push-up whereby even in this bilateral activity the racquet arm contributes significantly more force and power to the movement.

Bone differences

Paragraph Number 23 The bone side differences in this study are comparable to previous similar studies on a qualitative level. Side differences were site-specific with differences in diaphyseal sites being almost entirely due to cross-sectional area differences, whereas greater

bone mass in the distal radius and ulna of the racquet arm was a result of a combination of cross-sectional area and bone density differences.

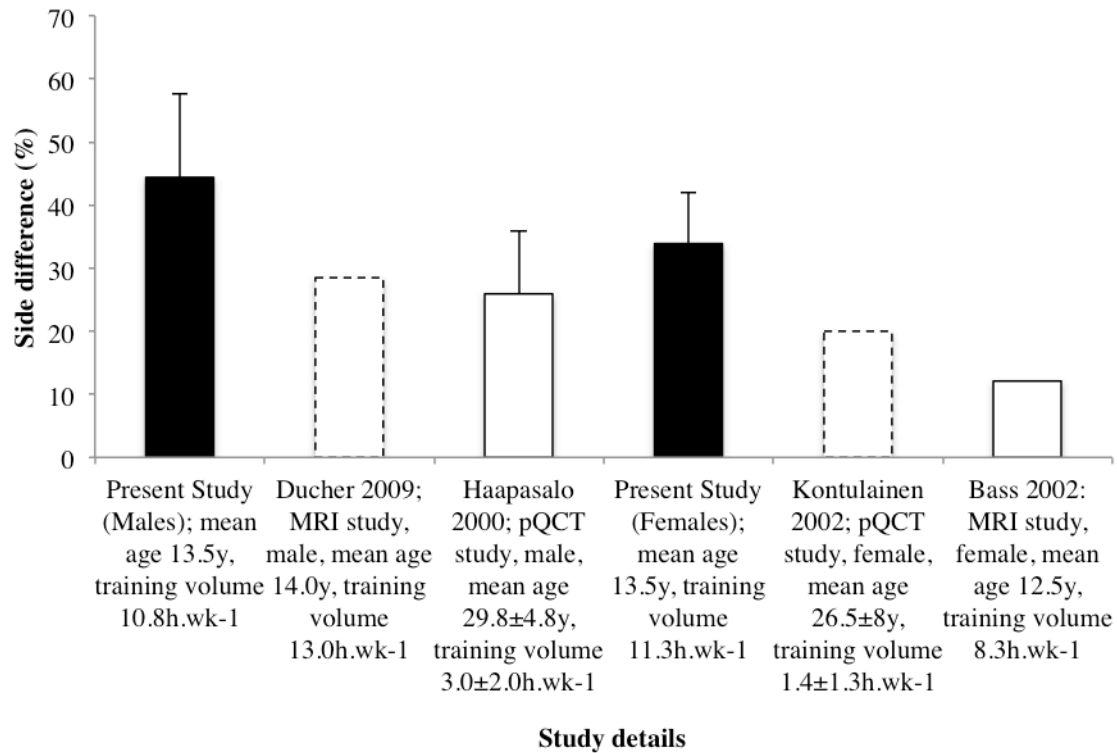


Figure 4. Mean side differences in humerus cortical area and details of cohort age, sex and training volume data in this and similar studies. Error bars indicate SD - dashed bars indicate studies where cohort dispersion value (*e.g.* SD) was unfortunately not reported.

Paragraph Number 24 On a quantitative level, the bone side differences seen in this study are larger than those in previous similar studies (2, 10, 14, 20) (Figure 4). However, compared to players in the first three of those studies, players in the present study had ~270%, ~700% and ~35% higher training volume respectively which has been shown to result in greater side differences (29). In addition, the players in this study had been playing for around 20% longer than participants in the Bass study, and a number of participants in the Haapasalo study had not started to play tennis regularly until adulthood – previous studies have seen greater side differences in those who start playing tennis before the onset of puberty (20). The two studies recording the smallest side differences in bone included solely female

participants, we have seen in this study that side differences in females were smaller than those in males. The authors believe that these factors largely explain why the side differences in this study are much greater than those previously found. Whilst the Ducher study participants were of a similar age with a similar training volume and length of time played to the cohort in this study they were not reported to be elite-level players. Perhaps the increased demands of elite-level training and competition are responsible for the differences found – however, the Ducher cohort side differences were most similar to those found in this study.

Bone geometry

Paragraph Number 25 A previous study on female youth tennis players found that humerus periosteal and endocortical radii were greater in the racquet arm (except in post-pubertal players where there was no significant difference) (2). In this study, in both sexes the racquet arm humerus had a larger periosteal and smaller endocortical radius (although it is unclear whether this was as a result of increased endosteal apposition or retention of bone mass on this surface). In comparison, in racquet arm proximal radius and ulna both periosteal and endosteal circumferences were greater (although not significantly in the case of ulna endocortical circumference). Perhaps the larger adaptation in the humerus in this group (when combined with the large skeletal changes already happening in an adolescent group) means that the bone is unable to remodel by bone drift and so only completes periosteal apposition during this period - although only a longitudinal study could clarify this.

Paragraph Number 26 The results also showed that longer bones and bones with a larger outer circumference are more than proportionally stronger in bending and torsion than compression, compared to a shorter bone or a bone with a smaller circumference. This is

likely to be due to an increase in the moment arm for bending and torsional moments that accompanies an increase in bone length or width – this is supported by the linear nature of these relationships. The relationships with bone width and strength in torsion are stronger (and the increase in torsional strength with increased bone width greater) than those of bone length and strength in bending, suggesting that torsional strains may be the dominating influence on bone adaptation as a result of regular tennis. The one anomaly is the lack of an association of bone length and resistance to bending in the non-dominant humerus, the reason for this is currently unclear.

Muscle-bone relationships

Paragraph Number 27 The most important finding of this study is that the muscle:bone relationship is quantitatively different in both upper arms; the racquet arm has a much lower muscle:bone ratio than the non-racquet arm. In previous studies, side differences in muscle and bone in the arms of tennis players were found to be similar (5, 7), although side differences in arm muscles in golfers were larger than those in bone (8). Whilst different muscle:bone relationships have been found in males and females and in groups of differing age or developmental status (particularly during puberty (7, 33) and old age(36)), in groups of similar age and sex muscle:bone relationships have followed the same regression line. This is true even in the extreme case of spinal cord injury (3). The sole exception the authors are aware of is young women with anorexia nervosa, in which case muscle:bone ratio increases as the disease progresses (4). Clearly, none of these explanations can account for the observed discrepancy in muscle:bone slope in the two arms. The observation of differing muscle:bone relationships in the two upper arms also rules out, on a profane level, that the strong muscle:bone-relationships often observed are a mere consequence of our genetic-anatomical make-up. By contrast, and if we follow the idea that muscular forces constitute

the most influential (if not the only) source of mechanical stimulation to the arm bones, and that this guides their adaptation, then it seems that muscle cross-section (as well as muscle function as per our testing) is modulated by another influence.

Paragraph Number 28 It is well known from basic muscle physiology that muscles require resistance to work against in order to generate force (17). One interpretation would therefore be that the way the arm muscles are used during tennis enable them to produce greater forces than the habitual usage most of us follow – which seems a reasonable assumption. Muscle activity during the tennis stroke peaks around time of ball impact (15) - this may be a direct response to the high-impact nature of the tennis stroke, whereby a large impulse is imparted to the ball over a short contact time requiring a high level of force. Athletes competing in high impact sports have greater bone strength than those in low-impact sports, and in turn controls (1, 11, 22). Alternatively, this peak in muscle activity could be a response to the large impact-induced vibrations transferred to the arm during the tennis stroke (16). Whilst the effects of vibration on bone strength in the lower limbs have been studied in a number of populations (37), the effects on upper limb bone remain unexplored. Another possibility would be that certain muscles have particular influence on the forearm bones, and that these muscles were not able to be fully identified by our pQCT approach – an interpretation that is equally attractive to us.

Paragraph Number 29 In the radius and humerus, the bone was much stronger in resisting bending along the line of action of the elbow flexor and extensors suggesting that their action is a major cause of strain within these bones in the areas scanned. However, that side differences in bending strength in this axis and the perpendicular axis were similar and that in the racquet arm the difference in bending strength between the two axes was greatly reduced

suggests that torsional stress may be the greatest stress experienced by the bone during a tennis stroke. In addition, the stronger relationships seen between polar material eccentricity and bone width also support the idea that torsion is the primary stressor of bone.

Paragraph Number 30 Whilst the muscle:bone relationships in both limbs were strong, side differences in bone CSA only had a weak correlation with side difference in muscle size (similar to the results in the only previous paper to examine muscle-bone relationships in the playing and non-playing arms of tennis players (7)). However, there is a certain measurement error in pQCT scans (around ~1%) - as there are two measurements (muscle area and cortical area) there are two sets of measurement errors within each muscle:bone relationship. Because only the smaller side difference values are taken the measurement error becomes proportionally larger. For instance, mean side difference in humerus muscle area was 9.2% and previous error measurement by one of the authors has revealed muscle area measurement error to be 1.2% (27). Therefore the standard error is 13% of the side difference value, it may follow that this is a significant reason for the weak correlation in side differences in muscle and bone. Further to this, there are a number of other factors (tendon stiffness, muscle specific tension, motor unit recruitment and rate of force development, muscle architecture, *etc.*) which may be different between the two limbs, affect the muscle forces produced (and hence strains experience by the bone) and hence correlating side differences in muscle and bone introduces the influence of these factors. Whilst side differences in the majority of these factors have not yet been explored, fibre type distribution has been shown not to differ significantly between the two arms in tennis players (31). Finally, the gross muscle cross-sections obtained by pQCT do not allow for the full separation of individual flexors, extensors, rotators, *etc.* – as we have seen in this and a previous study (30) there are differential adaptations between muscle groups in tennis players

and so (as discussed earlier) analysing the gross muscle CSA may not give the most accurate representation of the strains experienced by the bone.

Limitations

Paragraph Number 31 There are several limitations to this study – firstly, no sedentary controls were examined. However, we have seen in previous studies (14, 18, 30, 40) that in arms and legs of controls side differences in bone strength and muscle size are non-existent or minimal (a maximum of 5.2% in bone mass or area, and 5.9% in muscle size) when compared to those completing regular asymmetric exercise. In addition, comparison of the player's muscle and bone size relative to reference data (24) revealed that regular participation in tennis and even use of the non-racquet arm in double-backhand strokes was not sufficient to confer significant size increases in muscle or bone on this limb. This supports the use of the youth tennis player model as a means of obtaining control and exercise data within a single participant, and hence largely discounting any of the inter-subject factors listed in the introduction. Another potentially confounding factor is the use of pre, peri and post-pubertal children – however, in previous studies in male (10) and female (2) youth tennis player cohorts no effects of pubertal status on side differences were found. In addition, comparison of premenarcheal and postmenarcheal females revealed no effect on menarche on magnitude of side difference. That the muscle-bone relationships found are so strong despite the different maturation levels and sex of the participants adds further weight to the idea of dominating influence of muscular force on bone adaptation.

Conclusion

Paragraph Number 32 In summary, this study provides further evidence of the large

osteogenic potential of regular tennis participation, and demonstrates a strong relationship between muscle and bone size. In addition, differing muscle:bone relationships in the two upper arms suggest that other factors aside from local muscle size dictate exercise-induced adaptations in bone. There is also some evidence that the predominant stress experienced by the diaphyseal radius and humerus is torsional. Finally, that these adaptations are site-specific with differences between endocortical and periosteal adaptation as well as adaptation in the upper and lower arm – possibly due to either the dominating force at play, or else the ability of the body to adapt to a large exercise stimulus.

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Conflicts of Interest:- the authors declare that there are no conflicts of interest.

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