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ACL-Injured Subjects Have Smaller ACLs Than Matched Controls: An MRI Study

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Abstract

Background—Very few studies examining the predisposing anatomical factors leading to anterior cruciate ligament (ACL) injuries have examined the ACL itself, and none of these directly examined the difference in ACL properties between injured and matched control subjects.

Hypothesis—ACL total volume of people who have experienced a non-contact ACL injury is smaller than that of matched controls.

Methods—Contours of the ACL were manually identified in sagittal MR images and volumes were calculated for 27 contralateral, healthy knees of individuals after non-contact ACL injury and for 27 control subjects matched for gender, height, age, and weight. Validation of this method was performed on 5 porcine knees. Stepwise multiple regression was used to determine the difference in ACL volume between injured and control subjects while considering gender, height, weight, and age as potential covariates.

Results—Contralateral ACL volume for injured subjects was significantly smaller than non-injured subjects (p=0.0208) by 231 mm^3 after adjusting for weight, which was also a significant contributor to ACL volume (p<0.0001). At the average body mass of 72.7kg, subjects with a non-contact ACL injury had an average contralateral ACL volume of 1921 mm^3, while the corresponding control group had an average volume of 2151 mm^3. Gender, height, and age were not significant when weight was included in the regression model.

Conclusions—This study shows that there are anthropometric differences between the knees of subjects with a non-contact ACL injury and those without an ACL injury, suggesting that ACL volume may play a direct role in non-contact ACL injury.

Introduction

The relative risk of a non-contact anterior cruciate ligament (ACL) injury has been shown to be two to eight times higher in female intercollegiate athletes than their male counterparts.[3] Hormonal,[9, 22, 26, 35] intrinsic anatomical,[2, 6, 7, 16] biomechanical,[2, 9, 19, 31] and neuromuscular[3, 11, 20, 25, 28, 33] factors have all been investigated as causes for this disparity. Up to 70% of ACL injuries are noncontact in nature,[32] suggesting that early identification of those at elevated risk may assist in injury prevention. ACL injuries can be devastating to

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people of all ages, especially younger athletes. These types of injuries can be difficult to repair and even after reconstruction can have various degrees of persistent instability. A prolonged alteration of the kinematics of the knee may lead to major problems such as cartilage degeneration and osteoarthritis.[14, 27, 32]

Identifying structural and material properties of the ACL has been an intense area of research. Studies have determined that ACL volume and cross-sectional area are correlated to gender, height, age, and weight.[2, 7] Other cadaver studies have also observed that female ACLs have lower material properties[6] and a smaller fibril area fraction[16] than male ACLs, independent of height. Previous research has also shown females to have smaller ACLs than men through *in vivo* imaging.[12, 13] However, no direct link between ACL size and injury has been investigated, as previous comparative studies on ACL morphology have all focused on gender comparisons. These previous studies have all inferred importance of ACL morphology for injury based on gender comparisons alone, although a gender difference in ACL morphology may instead be appropriate given other gender differences in height, weight, or strength. A narrower ACL *in vivo* could identify a predisposition for non-contact ACL injury for males as well as females. Given previous data that the material properties of individual fascicles of the ACL are similar to other ligaments within an individual[4] and that average fascicle diameter is conserved across the sexes,[16] a narrower ACL may just contain fewer fascicles, leading to inferior strength. Cross-sectional area and volume are both appropriate measures of a narrow ACL, but volume may potentially be a more robust measurement *in vivo* than a single point measurement of cross-sectional area because of MRI resolution limitations, most notably slice thickness.

The purpose of this study was to test the hypothesis that a smaller ACL correlates with ACL injury, controlling for gender, height, age, and weight, by comparing the ACL volumes of the contralateral knees of individuals who previously experienced a non-contact ACL injury to control subjects matched for gender, height, age, and weight.

Subject Population

54 subjects (34 male) participated in this study after providing IRB-approved informed consent. All subjects were recruited through community advertising, and the only exclusion criteria for either group were a history of prior lower extremity soft-tissue injury requiring surgical repair/reconstruction (except for ACL rupture); meniscectomy of more than 25% of the meniscus; or fracture requiring internal fixation. They were divided equally into 2 groups. The injured group consisted of the healthy, contralateral knees of individuals who had experienced a self-reported, noncontact ACL injury. The control group consisted of knees of uninjured subjects matched for gender, height, age, and weight to the injured group. Height differences were not to exceed 2 inches in any matched pair, and the average heights for the control and injured subjects were within 0.30 inches (68.90 in vs. 68.61 in). There was no significant difference between the two groups for height ($p=0.095$), age ($p=0.154$), or weight ($p=0.128$) using a paired two tailed Student t-test ($\alpha = 0.05$) (Table 1).

MRI-based Volume Calculation

MR images (GE Signa 1.5T, sagittal 3D-SPGR, voxel size 0.55mm×0.55mm×1.5mm) were taken of a single, randomly selected knee for the control subjects and of the healthy knee of the injured group. Under the guidance of an experienced orthopaedic surgeon, the entire ACL of each subject including femoral and tibial insertions was segmented from the MR images using standard software (Medical Image Processing, Analysis, and Visualization, v2.7.45) (Figure 1).[30] Each MR sequence was segmented on two separate days by a single observer. Sequences were segmented in random order and the observer was blinded to injury status to avoid an observer bias. The volume of each ACL was calculated by MIPAV from the manually drawn contours by summing up the area of each contour multiplied by the slice thickness. The volumes calculated on separate days were averaged. A similar procedure using MR images to measure muscle size was shown to produce only a 0.8% error.[34]

Validation of MRI-based Volume Calculation

Five porcine knees were used to validate the MRI-based volume measurement performed for this study. Fresh porcine stifle joints were obtained from a local butcher. These knees were imaged using a similar sequence to the human MRIs (Philips 3.0T, sagittal 3D-WATSf, voxel size 0.29mm×0.29mm×1.5mm). Immediately after the scan was completed, an experienced orthopaedic surgeon (DF) dissected each ACL as close to the bony insertions as possible. True volume of the dissected ACLs was assessed by volume displacement in 0.9% saline solution. MRI-based volume was estimated on two independent days for each porcine ACL by the same individual who performed the MRI-based volume calculation of the human ACLs (EZ).

The agreement between the two repeated MRI measures showed a high degree of agreement with intra-class correlation of 0.82. Regression analysis between true volume and MRI-based volume showed a correlation of 0.98, a slope of 0.75, and a constant offset of 555 mm^3 (Figure 2). The regression line was not significantly different from y =×(slope p=0.066, intercept p=0.0685). The range of porcine ACL volumes was 1850–2636 mm^3, which is similar to the range previously observed in fresh-frozen human ACLs[7] and comparable to the middle of our sample ACLs.

In addition, an inter-rater reliability test was performed to compare the results of the rater from this study (EZ) with a second rater. The intra-class correlation coefficient (ICC) for these two raters was 0.70, indicating moderate agreement. Subsequent analysis of variance showed an average systematic difference between the two raters of 173 mm^3

Statistical Analysis

Paired t-tests, simple and multiple regression analysis techniques were used. Stepwise multiple regression was used to determine the difference in ACL volume between injured and control subjects while considering gender, height, weight, and age as potential covariates. Variables were removed from the model one-by-one until only statistically significant variables remained. Interaction effects were also investigated. Paired t-tests were also performed separately for ACL volume on male and female groups, due to the previous observation of lower ACL material properties and smaller fibril area fraction in female ACLs.[16] All reported p-values are based on two-tailed tests, and $p < 0.05$ was considered significant. SAS JMP, Version 7 (SAS Institute, Cary, NC) was used for analyses.

Results

Stepwise regression showed that weight was a significant covariate ($p<0.0001$), while gender, height, and age were not significant when volume was adjusted for weight. The average contralateral ACL volume for the injured group was 231 mm^3 less than the ACL volume for a control subject at the same weight ($p = 0.0208$) (Figure 3). The 95% confidence interval of the average difference was (36.7, 424.6). Of the 27 injured subjects, 16 had smaller ACL volumes than their matched controls while only 9 had lower weights. At the average body mass for the entire population of 72.7 kg, the injured group had an average contralateral ACL volume of 1921 mm^3, while the control group had an average volume of 2151 mm^3.

Separate analyses of males and females showed a statistically significant difference in ACL volume for females (186 mm^3, p = 0.0116) but not for males (149.1 mm^3, p = 0.2149) (Table 2). In females, the average difference in body mass between injured and controls was 5.6 kg, whereas in males the average difference in body mass was only 1.6 kg.

Discussion

The results of this study show that patients who have suffered a non-contact ACL injury have a significantly smaller ACL volume in their contralateral knee than matched controls. This result is consistent with a smaller ACL being weaker, and therefore at greater risk of injury.

This study provides the strongest evidence to date of a link between ACL size and injury risk, because it directly compares injured subjects to matched controls. Another study comparing male vs. female ACL volume,[7] found a larger difference due to gender than what we have observed between injured and control subjects. While this previously reported result did suggest ACL volume as a potential risk factor for injury, it did so only on the basis of the known gender disparity in ACL injury rates. An ACL rupture occurs when a complex interaction of both intrinsic and extrinsic factors combine to result in biomechanical loading that exceeds the ACL's failure strength.[18, 21, 23] Therefore, an isolated gender difference in a single factor such as ACL volume may not have any relevance to ACL injury, because it may be compensated for by a gender difference in another factor. In contrast, this study compared injured subjects to matched controls to directly show a relationship between ACL volume and injuries.

In this population, weight was a very strong predictor of ACL volume, whereas gender was not a significant predictor. The non-contact injury group tended to have lower weight when compared to the non-injured group, and women tended to have lower weight when compared to men. However, after adjusting for weight, the difference between men and women disappeared. Age and height were not significant factors in ACL volume. When adjusted for weight, the difference between injured and controls is enhanced, and based on the regression model one would expect that for two subjects with the same weight (irrespective of gender), the injured subject would have 231mm^3 smaller contralateral ACL volume than the control subject. This value represents about 11% of the average ACL volume observed in the data and an Effect Size of 0.52, which represents a moderate effect.[10]

Due to the previously reported results of female ACLs having lower material properties and a small fibril area fraction than male ACLs,[16] separate statistical analyses were performed that showed a statistical significance in female ACL volume but not male ACL volume (Table 2). However, weight remains the most dominant factor affecting the ACL volume. As shown in Table 2, a larger difference in body mass between injured and controls was observed in the female population than in the male population, which may explain the lack of an observed statistical difference between ACL volume in our male population.

Pre-injury screening has the potential to identify at-risk individuals. Individuals identified as having smaller ACL volumes could potentially counteract this pre-disposing factor by neuromuscular training designed to reduce the biomechanical loads on the knee.[5, 17, 29] Strengthening the surrounding muscles and training them to act as agonists for the ACL can potentially reduce ACL loading, thereby reducing the risk of injury. The response of ACL tissue to repetitive loading remains unknown, but it may be possible to prescribe a training program for at-risk individuals that will result in hypertrophy of the ACL, especially in a skeletally immature population. A molecular approach may even promote ACL health as growth factors have been shown to stimulate proliferation of ACL cells.[1]

Other studies have observed relationships between extrinsic factors such as strength and neuromuscular control and ACL injury risk, [3, 9, 11, 19] as well as a potential to reduce injury risk through training that alters these extrinsic factors.[20, 25] Pre-injury screening that includes both intrinsic and extrinsic factors may hold the best possibility of success of identifying individuals for preventative training. A review of the literature shows relatively few studies that comprehensively examine intrinsic and extrinsic factors together, making it impossible to compare the relative importance of these factors that have been identified in isolation. It is only by performing such a comprehensive investigation that the appropriate decisions can be made as to which screening tests are appropriate and cost-effective at preventing injury.

One major limitation of this technique was the use of manual segmentation of the ACL from MRI. The ACL can often be difficult to distinguish from surrounding tissue, possibly leading to errors in the subsequently calculated volume. The image produced from the sagittal view was much clearer than the axial or frontal views, but is still slightly ambiguous near the attachments. This error can be minimized by decreasing the slice thickness and improving the resolution in subsequent experiments. As the intra-class correlation coefficient of 0.70 shows, with this method one can only expect moderate agreement between raters. Moreover, a systematic bias between one rater and another can exist, making it impossible to compare the ACL volumes of subjects measured by different raters. This limitation prevents the use of the method as described as a screening tool, but further improvements such as using a training set to correct for individual rater bias may eliminate this limitation. This limitation supports our use of a single rater for all data used to test our primary hypothesis to eliminate any bias.

Another limitation is the use of the contralateral ACL to represent the volume of the ruptured ACL, which cannot be measured after the injury has occurred. However, a recent side-to-side comparison of ACL volume in healthy uninjured subjects using the same methodology described here has shown that no significant difference exists between healthy ACLs within the same individual.[24] This result suggests that the contralateral ACL is an appropriate surrogate for the ruptured ACL for volume measurements.

The ACL volumes calculated for this study fall within the range of previously reported values. Previously published ACL volumes from cadaveric knees, measured using 3-D scanning software, were 2722±706 mm^3 for males and 1996±530 mm^3 for females.[7] The 3-D scanning method is prone to over-estimation of ACL volume, because it is unable to account for concavities in the ACL[15]. Charlton used a segmentation approach similar to our ACL volume measurement,[8] but estimated considerably smaller volumes (male = 839 mm^3, female = 652 mm^3). This under-estimation is most likely due to the fact that they used a 2.5mm slice thickness and a low-field MR scanner (0.2T). In our experience, it was often difficult to identify the full extent of the insertion at both femoral and tibial attachment points, which could lead to underestimating the contour in each slice. This difficulty would only be exacerbated with larger slice thickness and lower resolution. It should also be noted that the ACL volumes reported in this study are the MRI-based measurements, rather than “corrected” volumes achieved by applying the linear equation found during our validation of the porcine knees. Since the slope and intercept were not significantly different from the line y =×and the porcine knees were scanned with a different scanner, we felt that it was most appropriate to report the unaltered MRI-based volume measurements. Moreover, given the strong linearity of the validation data, one would expect that any comparisons between subjects would be unaffected by a linear transformation of the data using the calculated slope and intercept.

Another limitation in this study was the use of ACL volume rather than cross-sectional area. Given the fact that stress in a tissue is equal to force divided by cross-sectional area, the minimum cross-sectional area would be the ideal measurement to make. However, the resolution limitations of MRI made an accurate minimum cross-sectional area measurement impossible from this data set. With 1.5mm sagittal slices, the estimated cross-sectional area along the length of the ligament is extremely noisy and jagged, with the artifacts from slice thickness obscuring the actual cross-sectional area. Moreover, a validation of the cross-sectional area would have required equipment not at our disposal including a rapid freezing method for the porcine knees and an appropriate saw for making thin slices perpendicular to the long axis of the ligament. However, if one accepts the assumption that the ACL has a fairly typical shape and aspect ratio (cross-sectional area/length), the volume should be a reasonable surrogate measure for cross-sectional area.

This study was retrospective and relied on self-reported mechanisms of ACL injury. There also was no available data on the activity levels of this group of subjects, which may affect the tensile properties of the ACL.[36] Intuitively, it seems reasonable that competitive athletes should have stiffer and stronger ligaments than recreational athletes or non-athletes due to the repetitive loading that these ligaments have experienced, but to the authors' knowledge no data has been presented in the literature to support this claim. This study assumes that the control subjects have similar histories of athletic activity and competition level as the injured subjects and that their risk of ACL injury due to other factors beyond ACL volume was similar to the injured subjects. However, it is possible that the control subjects may also suffer an ACL rupture in the future. This potential confounding variable was controlled for as much as possible by age-matching the two groups, but difference in activity level affecting both prior likelihood and future likelihood of an ACL rupture remains a limitation.

Conclusion

This study is the first to explore a link between ACL volume and injury. Considering this is a relatively new investigation, additional testing is needed to substantiate our observed difference. ACL volume may be a risk factor for non-contact ACL rupture, in conjunction with the many other potential risk factors that have previously been identified. A future comprehensive study that attempts to create screening metrics based on as many of these factors as possible, including ACL volume, may prove to be the most effective in identifying at-risk individuals.

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Figure 1.
Manually drawn sagittal plane ACL volume contour.

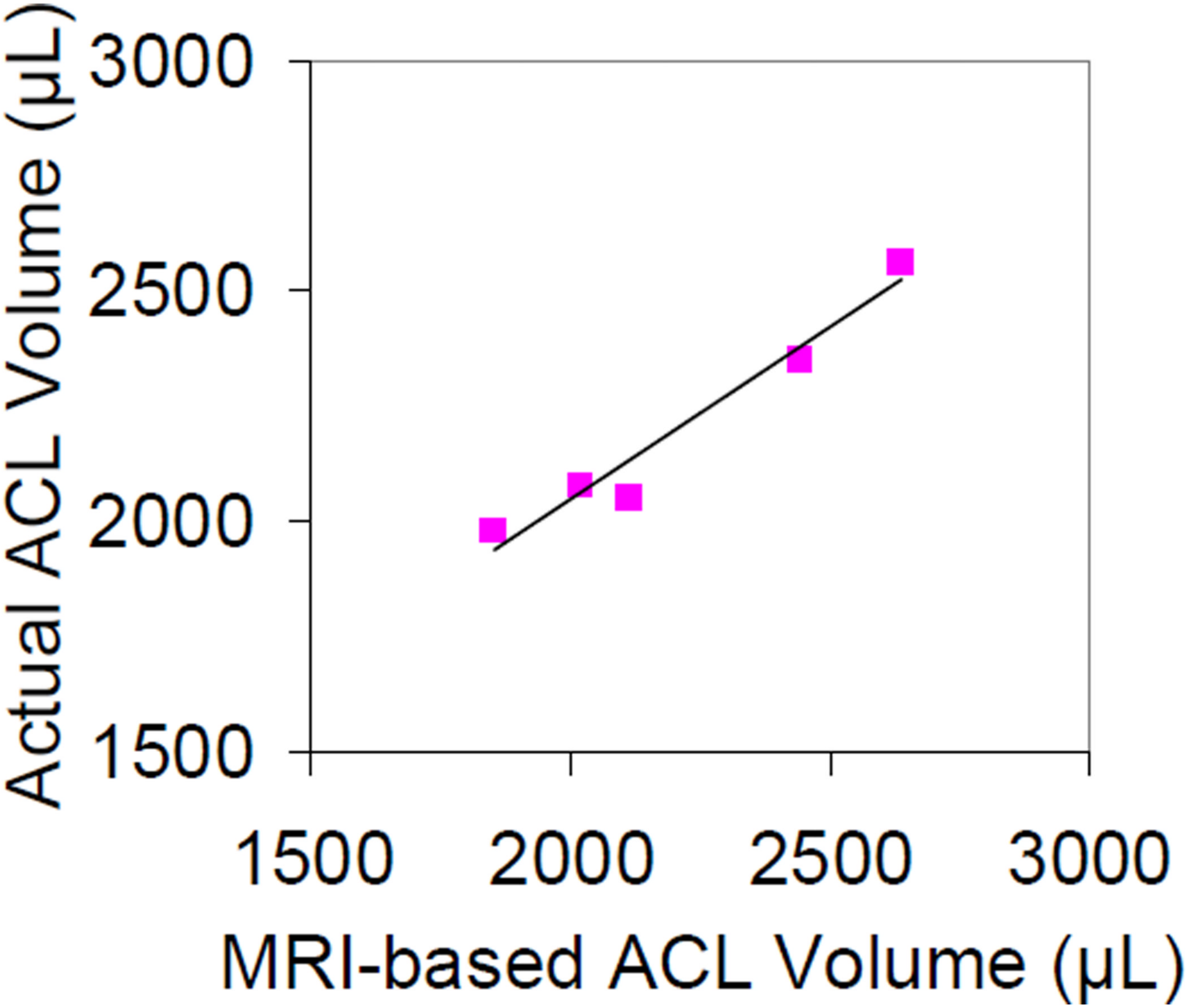

Figure 2.

Results of porcine validation of MRI-based volume measurement against actual ACL volume measured by water displacement. The two measurements were very well correlated ($r=0.98$, $p<0.05$).

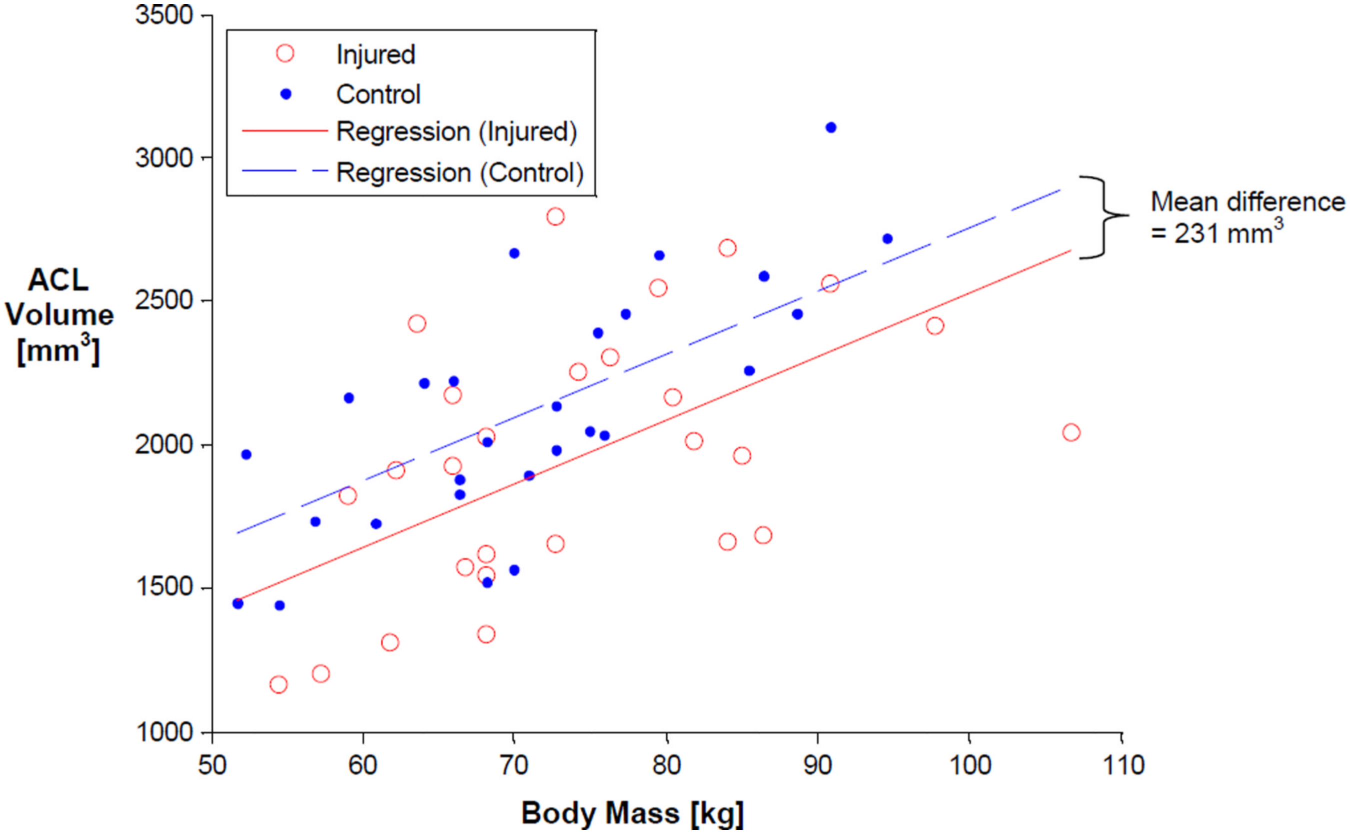

Figure 3.

ACL volumes for injured (red circles) and control (blue dots) subjects, with results of stepwise regression shown (solid and dashed lines). The non-contact injured subjects' volume was significantly smaller than non-injured subjects ($p=0.0208$) by 231 mm^3 after adjusting for weight, which was also a significant contributor to ACL volume ($p<0.0001$).

Table 1

Anthropometric Data.

	Female (n=20)		**Male (n=34)**		**Overall**		
	Injured	**Control**	**Injured**	**Control**	**Injured**	**Control**	**p-value**
Height [m]	1.67	1.68	1.79	1.79	1.74	1.75	0.095
Age [y]	30.9	35.0	34.2	33.9	33.0	34.3	0.154
Bodymass [kg]	66.3	60.8	78.8	77.2	74.2	71.1	0.128

Table 2

Gender-specific ACL volume comparison.

	Female (n=20)		**Male (n=34)**	
	Mean ACL Volume [mm^3]	**Mean Bodymass [kg]**	**Mean ACL Volume [mm^3]**	**Mean Bodymass [kg]**
Injured	1694.5	66.3	2107.5	78.8
Control	1880.3	60.8	2256.5	77.2
Difference	-185.8	+5.6	−149.1	+1.6
p-value	0.0116		0.2149	