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Hypermobility, ACL reconstruction & Shoulder instability:

A clinical, mechanical and histological analysis

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Abstract

Joint movements are essential for the function of human body during the activities of daily living and sports. The movement of human joints varies from normal to those which have an increased range of joint movement (gymnasts) to those with extreme disabling laxity in patients with a connective tissue disorder (Ehlers Danlos Syndrome).

“Hypermobility" is most commonly used to describe excessive movement. Hypermobility was assessed by using the current criteria of the Beighton score for signs and the Brighton criteria for symptoms of hypermobility in a group of orthopaedic patients attending the specialist knee and shoulder injury clinics.

The Beighton score was found to be higher in patients attending for primary ACL reconstruction (mean 2.9, $p = 0.002$) and revision ACL reconstruction (mean 4, $p < 0.001$) when compared with the control group. Hypermobility was a risk factor for the failure of ACL reconstruction (30% vs 0%). The mean Beighton score was higher in both the primary shoulder dislocation group (mean difference 1.8, $p=0.001$) and the recurrent shoulder dislocation group (mean difference 1.4, $p=0.004$). Bone defects were studied on the CT scan following shoulder dislocations. There was no correlation between hypermobility and the bone defects. The bone defect was a risk factor for recurrent shoulder instability (48% vs 16%).

A material testing system was used to assess the tissue laxity of discarded hamstring tendon and shoulder capsule obtained during stabilisation procedures. The mean gradient of slope for both tendon and capsule graphs was 23.8 (range 3.08-52.63). The tissue laxity was compared to the Beighton score, however no correlation was detected between the Beighton score and the gradient of the tissue laxity.

An electronic goniometer was used to measure the angle of the MCP joint of the little finger, whilst a force plate system simultaneously measured the force required to hyperextend the MCP joint. The little finger MCP joints of each hand were assessed in this manner in a group of patients undergoing primary ACL reconstruction or open shoulder stabilization. The mean force required to produce the 40 degrees angle at the little finger MCP joint was 0.04 kg with a range from 0-0.11 kg. There was a positive correlation between the gradient of tissue laxity and the force required to produce 40 degrees angle at the little finger of the dominant hand.

The expression of Collagen V and Small leucine rich proteoglycans (Decorin and Biglycan) was studied in the skin, hamstring tendon and shoulder capsule of the patients described above attending with shoulder or knee instability. These patients had different levels of hypermobility (as assessed by the Beighton score) and symptoms of hypermobility (as assessed by the Brighton criteria to diagnose Benign Joint Hypermobility Syndrome). The weaker tendon group was found to have a lower mean Beighton score, while the weaker skin group had a higher mean Beighton score.

Collagen V expression was higher in the skin dermal papillae of the weaker group.
The Beighton Scores were higher in patients with ACL and shoulder injuries. Hypermobility was a risk factor for the failure of ACL reconstruction. There was no correlation between hypermobility and the bone defects on the CT scan following shoulder dislocation. Bone defects were a risk factor for recurrence. There was no correlation between the Beighton Score and the tissue laxity. There was a correlation between the tissue laxity and the clinical assessment of laxity at the little finger MCPJ by using a force- goniometer system. There was a correlation between the collagen V expression in the dermal papillae of the skin and the Beighton score.

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Publications:

Original articles:

1. "Revision ACL Surgery and generalized ligament laxity: is there a relation? (Original Article) in press for publication by the "Knee". MA Akhtar, R Battacharya, J F Keating
2. Generalized Joint Hypermobility and traumatic anterior shoulder instability (Original article) submitted to the "Bone and Joint Journal" (BJJ) M A Akhtar, P J Jenkins, C M Robinson
3. Generalized Joint Hypermobility and CT scan assessment following Traumatic anterior shoulder dislocations (Original article) submitted to "Journal of Arthroscopy and Joint Surgery" M A Akhtar, P J Jenkins, C M Robinson
4. "Hypermobility and Collagen V expression" (Original Article) currently working on for submission to "The International Journal of Biochemistry and Cell Biology" M Adeel Akhtar, C Michael Robinson, J F Keating, A H Simpson, D M Salter.
5. "Hypermobility and Small Leucine Rich Proteoglycans (SLRP's) expression" (Original Article) currently working on for submission to "The International Journal of Biochemistry and Cell Biology" M Adeel Akhtar, C Michael Robinson, J F Keating, A H Simpson, D M Salter.
6. "Beighton score does not correlate with tissue laxity- A novel assessment method (Original Article) currently working on for submission to BMJ M Adeel Akhtar, C Michael Robinson, J F Keating, A Y Muir, A H Simpson

Abstracts:

1. Generalized Ligament Laxity in 1st time Shoulder Dislocation JBJS (Br) (Abstract) Vol 93-B, Issue SUPP_III, 307 2011 M.A. Akhtar, C.M. Robinson
2. Generalised ligament laxity in patients undergoing revision ACL reconstruction (Abstract) JBJS (Br) Vol 93-B, Issue SUPP_II, 175-176, 2011. M.A. Akhtar, T. White, J. Keating
3. Generalised ligament laxity in patients undergoing revision shoulder stabilisation (Abstract) JBJS (Br) Vol 93-B, Issue SUPP_II, 175-176, 2011 M.A. Akhtar, C.M. Robinson
4. "Benign Joint Hypermobility and sporting injuries" published in "British Journal of Sports Medicine" 2009;43:e2 (Abstract) Muhammad Adeel Akhtar, C. Michael Robinson
5. "Meniscal Injuries and Hyperlaxity (Abstract) Br J Sports Med 2010; 44:i1 doi:10.1136/bjsm.2010.078972.3 Muhammad Adeel Akhtar, J.F. Keating.
6. "Generalized Ligament Laxity and shoulder dislocations after sports injuries (Abstract) Br J Sports Med 2010; 44:i3 doi:10.1136/bjsm.2010.078972. Muhammad Adeel Akhtar, C.M. Robinson
7. "CT scan evaluation for recurrent shoulder dislocations after sports injuries (Abstract) Br J Sports Med 2010;44:i1 doi:10.1136/bjsm.2010.078972.2 Muhammad Adeel Akhtar, C.M. Robinson
8. "Small leucine rich proteoglycans (SLRP's) expression in skin, tendons and capsules of athletic population after shoulder dislocation and ACL injuries (Abstract) Br J Sports Med 2011;45:e1 doi:10.1136/bjsm.2010.081554.34 M A Akhtar, T G Ingman, C M Robinson, J F Keating, A Y Muir, H Simpson, D Salter

9. "Collagen V expression in skin, tendons and capsules of athletic population after shoulder dislocation and ACL injuries (Abstract) Br J Sports Med 2011;45:e1 doi:10.1136/bjsm.2010.081554.35 M A Akhtar, T G Ingman, C M Robinson, J F Keating, A Y Muir, H Simpson, D Salter
10. "Revision ACL reconstruction – Graft choices and Causes of failure (Abstract) Br J Sports Med 2011;45:A15-A16 doi:10.1136/bjsports-2011-090606.49 Muhammad Adeel Akhtar, R Bhattacharya, N Ohly, J F Keating
11. "Knee skin strength and hyperlaxity in athletes undergoing ACL reconstruction (Abstract) Br J Sports Med 2011; 45:A17-A18 doi:10.1136/bjsports-2011-090606.56 M Adeel Akhtar, J F Keating, A Y Muir, H Simpson.
12. "Role of CT scan in predicting recurrence following primary traumatic shoulder dislocation Br J Sports Med 2013;47:10 e3 doi:10.1136/bjsports-2013-092558.8, Muhammad Adeel Akhtar, Paul Jenkins, Fiona Ashton, Ian Beggs, Michael Robinson Christopher
13. "Hypermobility- a risk factor for recurrent shoulder dislocations Br J Sports Med 2013;47:10 e3 doi:10.1136/bjsports-2013-092558.11, Muhammad Adeel Akhtar, Paul Jenkins, Fiona Ashton, Michael Robinson Christopher
14. "Beighton score does not correlate with tissue laxity-A novel assessment method (Abstract) accepted for publication in JBJS (Br) M A Akhtar, C M Robinson, J F Keating, A Y Muir, H Simpson
15. "Revision ACL Surgery and generalized ligament laxity: is there a relation? accepted for publication in JBJS (Br) R Battacharya, M A Akhtar, J F Keating.
16. "Recurrent shoulder dislocations and hyperlaxity, J Bone Joint Surg Br 2012 vol. 94-B no. SUPP XXI 85, Akhtar M.A. Robinson C.M.
17. "Hypermobility- a risk factor for failure following ACL reconstruction" Br J Sports Med 2013;47:e4 doi:10.1136/bjsports-2013-093073.29 M A Akhtar, F Ashton, J F Keating
18. "Collagen V - biomarker of tissue strength in athletes" accepted for publication in the proceedings of the "Bone and Joint Journal". M.A. Akhtar, C.M. Robinson, J.F. Keating, T.G. Ingman, D. Salter, A.Y. Muir, H. Simpson.
19. "Collagen V, Small Leucine Rich Proteoglycans (SLRP) and surgical scar: is there a correlation?" M A Akhtar, C M Robinson, J F Keating, D M Salter, H Simpson, accepted for publication in British Journal of Sports Medicine (online edition).
20. "Biological Failure following ACL Reconstruction" M Adeel Akhtar, John F Keating, accepted for publication in British Journal of Sports Medicine (online edition).

Presentations

Oral Presentations - International Meetings

1. "Generalized Ligament Laxity in Patients undergoing Revision ACL Reconstruction" Podium presentation to 11 EFFORT Congress in Madrid 2-5 June 2010. Akhtar M. A.*, White, T, Keating J. F.
2. "Generalized Ligament Laxity in Patients undergoing Revision Shoulder Stabilizations" Podium presentation to 11 EFFORT Congress in Madrid 2-5 June 2010. Akhtar, M. A.*, Robinson, C. M.
3. "Recurrent Shoulder Dislocations and Hyperlaxity" Podium presentation to COMOC meeting in Glasgow 12-17 Sep 2010. Akhtar, M. A.*, Robinson, C. M.
4. Meniscal Injuries and Hyperlaxity Podium presentation at the International Sports Science and Sports Medicine conference Newcastle Upon Tyne 19-21 August 2010. Muhammad Adeel Akhtar, J.F. Keating.
5. CT scan evaluation for recurrent shoulder dislocations after sports injuries Podium presentation at the International Sports Science and Sports Medicine conference Newcastle upon Tyne 19-21 August 2010. Muhammad Adeel Akhtar, Christopher Michael Robinson
6. Small leucine rich proteoglycans (SLRP's) expression in skin, tendons and capsules of athletic population after shoulder dislocation and ACL injuries, Podium presentation at 2nd Congress of European College of Sports and Exercise Physicians 9-11 Sep 2010, London. M A Akhtar, T G Ingman, C M Robinson, J F Keating, A Y Muir, H Simpson, D Salter
7. Collagen V expression in skin, tendons and capsules of athletic population after shoulder dislocation and ACL injuries, Podium presentation at 2nd Congress of European College of Sports and Exercise Physicians 9-11 Sep 2010, London. M A Akhtar, T G Ingman, C M Robinson, J F Keating, A Y Muir, H Simpson, D Salter
8. "Benign Joint Hypermobility and sporting injuries" Podium presentation at the International Sports Science and Sports Medicine conference 2009 Newcastle Upon Tyne 20-22 August 2009. Muhammad Adeel Akhtar, Christopher Michael Robinson
9. "Timing of Return to Play after ACL Reconstruction", podium presentation at the "1st E.C.O.S.E.P CONGRESS" 13-14 December 2008, Thessaloniki, Greece. M.A. Akhtar, N. Allen, J.F. Keating.
10. "Correlation of electronic goniometer assessment of fingers with Beighton score for generalized ligament laxity in athletic population" podium presentation at 6thinternational Congress of European Federation of Sports Traumatologists Belgium 25-27 Nov 2010. M A Akhtar, C Robinson, J Keating, A Muir, H Simpson
11. CT scan and Hyperlaxity in patients with primary shoulder dislocation after sports injuries accepted for podium presentation to 12 EFFORT Congress, June 2011, Denmark. M A Akhtar, C M Robinson
12. Role of CT scan in predicting recurrence following primary traumatic shoulder dislocation, Podium presentation to the 3rd Congress of European College of Sports and Exercise Physicians 24-27 April, Frankfurt, Germany. M A Akhtar, P J Jenkins, F Ashton, I Beggs, C M Robinson

13. Hypermobility- a risk factor for recurrent shoulder dislocations, Podium presentation to the 3rd Congress of European College of Sports and Exercise Physicians 24-27 April, Frankfurt, Germany. M A Akhtar, P J Jenkins, F Ashton, C M Robinson
14. Revision ACL reconstruction – Graft choices and Causes of failure, Podium presentation to International Sports Science and Sports Medicine conference Newcastle Upon Tyne August 2011. M A Akhtar, R Bhattacharya, N Ohly, J F Keating

Oral Presentations – National Meetings

1. Generalized Ligament Laxity in 1st time Shoulder Dislocation Podium presentation to BOA Annual Meeting on 15-18 September 2009, Manchester M.A. Akhtar, C.M. Robinson
2. Revision ACL Surgery and generalized ligament laxity: is there a relation? Podium presentation at BASK 2011, Cardiff. R Battacharya, M A Akhtar, J F Keating.
3. Beighton score does not correlate with tissue laxity-A novel assessment method podium presentation at SCOT meeting 2011.MA Akhtar, CM Robinson, JF Keating, AY Muir, H Simpson
4. Collagen v - Biomarker of tissue strength in athletes, accepted for podium presentation at the SCOT meeting, June 2012, Dunblane. M.A. Akhtar, C.M. Robinson, J.F. Keating, T.G. Ingman, D. Salter, A.Y. Muir, H. Simpson

Poster Presentations - International Meetings

1. "Generalized Ligament Laxity in Patients undergoing Revision ACL Reconstruction" poster presentation to COMOC meeting in Glasgow 12-17 Sep 2010. Akhtar, M. A.* T O White, J F Keating.
2. Generalized Ligament Laxity in Patients undergoing Revision Shoulder Stabilizations" poster presentation to COMOC meeting in Glasgow 12-17 Sep 2010. Akhtar, M. A.* C M Robinson
3. "ACL injuries and Hyperlaxity", poster presentation to 11 EFFORT Congress in Madrid 2-5 June 2010. Akhtar M. A.*, White, T. O., Keating J. F.
4. "Recurrent Shoulder Dislocations and Hyperlaxity" poster presentation to 11 EFFORT Congress in Madrid 2-5 June 2010. Akhtar, M. A.*, Robinson, C. M.
5. "Hypermobility and sporting injuries" poster presentation to 11 EFFORT Congress, Madrid 2-5 June 2010. Akhtar, M. A.*, Robinson, C. M., Keating, J. F., Simpson, H.
6. Poster Presentation: "Improving Continuity of Care in Patients with Shoulder Stabilization presented at the "International Forum on Quality and Safety in Health Care" 17- 20 March, 2009 Berlin, Germany. M.A. Akhtar, N. Dempster, C.M. Robinson
7. Generalized Ligament Laxity and shoulder dislocations after sports injuries poster presentation at the International Sports Science and Sports Medicine conference Newcastle upon Tyne 19-21 August 2010. Muhammad Adeel Akhtar, Christopher Michael Robinson
8. Collagen v expression in skin of athletic population undergoing open shoulder stabilization or ACL reconstruction, poster presentation to 6th International Congress

of European Federation of Sports Traumatologists in Belgium 25-27 Nov 2010. M A Akhtar, T G Ingman, C M Robinson, J F Keating, A Y Muir, H Simpson, D Salter

9. Small leucine rich proteoglycans (SLRP's) expression in skin of athletic population undergoing open shoulder stabilization or ACL reconstruction poster presentation to 6th international Congress of European Federation of Sports Traumatologists in Belgium 25-27 Nov 2010. M A Akhtar, T G Ingman, C M Robinson, J F Keating, A Y Muir, H Simpson, D Salter
10. Shoulder Skin Strength and Hyperlaxity in athletes undergoing Open stabilization poster presentation to 6th international Congress of European Federation of Sports Traumatologists in Belgium 25-27 Nov 2010. M A Akhtar, C M Robinson, A Y Muir, H Simpson.
11. Shoulder capsule Strength and Hyperlaxity in athletes undergoing Open stabilization poster presentation to 6th international Congress of European Federation of Sports Traumatologists in Belgium 25-27 Nov 2010. M A Akhtar, C M Robinson, A Y Muir, H Simpson.
12. CT scan and Hyperlaxity in patients with recurrent shoulder dislocation after sports injuries poster presentation to 12 EFFORT Congress, June 2011, Denmark. M A Akhtar, C M Robinson
13. Knee skin strength and hyperlaxity in athletes undergoing ACL reconstruction poster presentation to International Sports Science and Sports Medicine conference Newcastle, August 2011. M.A Akhtar, J F Keating, A Y Muir, H Simpson.
14. Collagen V expression in capsule of athletic population undergoing open shoulder stabilization poster presentation to the annual conference of European College of Sports Science, Liverpool. July 2011. MA Akhtar, CM Robinson, JF Keating, TG Ingman, D Salter, AY Muir, H Simpson.
15. Small leucine rich proteoglycans (SLRP's) expression in shoulder capsules of athletic population undergoing shoulder stabilization poster presentation to the annual conference of European College of Sports Science, Liverpool. July 2011. MA Akhtar, CM Robinson, JF Keating, TG Ingman, D Salter, AY Muir, H Simpson.
16. "Hypermobility- a risk factor for failure following ACL reconstruction" M A Akhtar, F Ashton, J F Keating poster presentation at the International Sports Science and Sports Medicine conference Newcastle Upon Tyne 21-23 August 2013.
17. "COLLAGEN V - Biomarker of tissue strength in athletes" poster presentation to EFORT London 2014. M.A. Akhtar, C.M. Robinson, J.F. Keating, T.G. Ingman, D. Salter, A.Y. Muir, H. Simpson.
18. Collagen V, Small Leucine Rich Proteoglycans (SLRP) and surgical scar: is there a correlation? M A Akhtar, C M Robinson, J F Keating, D M Salter, H Simpson Poster presentation at the International Sports Science and Sports medicine conference 8-10th Sep 2015.
19. Biological Failure following ACL Reconstruction M Adeel Akhtar, John F Keating accepted for poster presentation at the International Sports Science and Sports Medicine conference 8-10th Sep 2015.

Poster Presentations – National Meetings

1. Comparison of Beighton score with electronic goniometer for generalized ligament laxity in athletic population, poster presentation to British Association of Sports and Exercise Medicine 24-27 Nov 2010, London. M A Akhtar, C M Robinson, J F

Keating, A Y Muir, H Simpson

2. Does ligament laxity affect surgical scar or athlete's satisfaction following open shoulder stabilization? Poster presentation to British Association of Sports and Exercise Medicine 24-27 Nov 2010, London. M A Akhtar, F Akhtar, C M Robinson
3. CT Scan Evaluation after 1st time Shoulder dislocation poster presentation to BOA Annual Meeting on 15-18 September 2009, Manchester M.A. Akhtar, I. Beggs, C.M. Robinson
4. Hyperlaxity, Capsule strength, Collagen V and Small leucine rich proteoglycans expression: is there a link? Poster presentation at the annual conference of British Orthopaedic Research Society, June 2011, Cambridge. MA Akhtar, CM Robinson, JF Keating, TG Ingman, D Salter, AY Muir, H Simpson.
5. Hyperlaxity, Tissue strength, Collagen V and Small leucine rich proteoglycans expression: is there a link? Poster presentation at the annual conference of British Orthopaedic Research Society, June 2011, Cambridge. MA Akhtar, CM Robinson, JF Keating, TG Ingman, D Salter, AY Muir, H Simpson.
6. Benign Joint Hypermobility in Southeast of Scotland" poster presentation at RCGP Annual Conference 5-7 November, Glasgow, Muhammad Adeel Akhtar, C. Mike Robinson, J.F. Keating.

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Hypermobility

Joint movements are essential for the function of the human body during the activities of daily living and sports. The movement of human joints varies from normal to those which have an increased range of joint movement to those with extreme disabling laxity in patients with a connective tissue disorder. A number of terms are used to describe the abnormal joint movement, these include “Hypermobility", "hyperlaxity" and "hyperextensibility". These have been used interchangeably by many authors without a clear definition. The word "hypermobility" is most commonly used to describe excessive movement in the normal plane of movement, most frequently hyperextension, and "laxity" is used to describe excessive movement in an abnormal plane of movement e.g. inferior subluxation of the shoulder giving an inferior sulcus sign. (Tofts, Elliott, Munns, Pacey, & Sillence, 2009a)

Hypermobility is defined as an excessive range of joint motion, taking into consideration the age, gender and ethnic origin in otherwise healthy subjects. (Beighton, Grahame, & Bird, 2012a) (R Grahame, 1999). The maximal range of movement that a joint is capable of is determined by the tightness or otherwise of the restraining ligaments (R Grahame, 1999). Hypermobility is caused by increased length and elasticity of the normal joint restraints, allowing a greater degree of translation of the articular surfaces (S. M. Johnson & Robinson, 2010). Joint hypermobility (JH) is seen either as a localized condition in a single joint or a more generalized one (Juul-Kristensen, Røgind, Jensen, & Remvig, 2007). The definition of ‘**generalised joint hypermobility’** (GJH) still remains arbitrary, and rationally should reflect both the number of joints involved and the extent to which they move. Hypermobility may represent one extreme of a Gaussian distribution of joint laxity throughout the population. (Beighton, Grahame, & Bird, 2012b). Hypermobility does not necessarily give rise to symptoms but when it does, Hypermobility Syndrome is deemed to be present (Beighton et al., 2012a).

Prevalence:

Hypermobility is more prevalent in younger people, females and in those of Asian or African origin. (Beighton et al., 2012a) (L Remvig, Jensen, & Ward, 2007a) (H. A. Bird, 2011) (Juul-Kristensen et al., 2007) (Tofts et al., 2009a) (Smits-Engelsman, Klerks, & Kirby, 2011) (Simpson, 2006) (Hirsch, Hirsch, John, & Bock, 2007) (Quatman, Ford, Myer, Paterno, & Hewett, 2008). The prevalence of GJH in published reports varies from 5% to 43% in adults (Verity Pacey, Nicholson, Adams, Munn, & Munns, 2010) (R Grahame, 1999) (A J Hakim & Grahame, 2003) (Seçkin et al., 2005) (Juul-Kristensen et al., 2007) (Rahman & Holman, 2010) (Simpson, 2006) and 2% to 55% in children (Verity Pacey et al., 2010) (Tofts et al., 2009a) (Smits-Engelsman et al., 2011). Generalized joint hypermobility is said to be more prevalent among girls than boys with sex ratios of approximately 3:1 to 2:1, females/males (Smits-Engelsman et al., 2011). GJH may be present in 10-30% males and 20-40% females in adolescent and young adulthood. (Rahman & Holman, 2010) (Seçkin et al., 2005)

Hypermobility is also a common feature of other connective tissue diseases like Marfan syndrome, Ehlers-Danlos syndrome, and osteogenesis imperfecta. (R Grahame, 1999) (Verity Pacey et al., 2010) (F Malfait, Hakim, De Paepe, & Grahame, 2006) (A. Hakim & Grahame, 2004)(Simpson, 2006) (S. M. Johnson & Robinson, 2010) and there is debate in the literature as to whether isolated joint hypermobility represents the end of the normal spectrum of joint range of movement or whether it represents a polygenic group at the mild end of the spectrum of Heritable Disorders of Connective Tissue (HDCTs) (Tofts et al., 2009a).

Subtypes:

Hypermobility can be divided into physiological, acquired, joint hypermobility syndrome, connective tissue disorder associated hypermobility.

Physiological Hypermobility:

Generalized joint hypermobility increases in females after puberty (Quatman et al., 2008). Peripheral and pelvic joint laxity increases during pregnancy and the hormone relaxin has been implicated in the pathogenesis of pelvic girdle relaxation (Calguneri, Bird, & Wright, 1982) (A. Hakim & Grahame, 2004).

Acquired hypermobility:

Greater agility provided by joint laxity may favour performing artists, where the prevalence of hypermobility is significantly higher than that of the general population. Hypermobility acts as a positive selection factor for entry into ballet for both girls and boys and polyarticular hypermobility is seen in as many as 90% of individuals in this group. Hypermobile violinists, flautists and pianists with lax finger and/or wrist joints suffer less pain than their less flexible peers. Here, too, laxity may act as a positive selection factor. (A. Hakim & Grahame, 2004) (Beighton et al., 2012a). Some authors believe that "Favourable genes'' could determine the level of success in a specific sport in the young athlete and therefore a longer career. (Jansson, Saartok, Werner, & Renström, 2005)

Joint range can also be increased into the hypermobile range by the sheer hard work of training. Ballet dancers who are not inherently lax jointed need to acquire hypermobility in certain joints to perform their art. (Klemp, Stevens, & Isaacs, 1984) (Klemp & Learmonth,

1984) Generalised joint hypermobility may follow in the wake of irreversible changes that occur in connective tissues in certain acquired diseases including acromegaly, hyperparathyroidism, chronic alcoholism, and rheumatic fever (R Grahame, 1999). In patients with acquired shoulder joint laxity, repeated minor injuries (so-called micro trauma), or repetitive use during training and competition, stretch the normal capsule-ligamentous restraints (S. M. Johnson & Robinson, 2010)

Joint Hypermobility Syndrome:

Hypermobility is not a diagnosis but when hypermobility becomes symptomatic, the "hypermobility syndrome" is said to exist. The term 'hypermobility syndrome' was first used in 1967 to denote the occurrence of musculoskeletal symptoms in the presence of joint laxity and not attributable to other rheumatic diseases. Because of its favourable prognosis by comparison with other more serious HDCTs, the term benign joint hypermobility syndrome is also used. (Beighton et al., 2012a) (R Grahame, 1999) (A. Hakim & Grahame, 2004).

Aetiology of Hypermobility:

In a British Medical Journal article Bird et al. described that Collagen is ubiquitous and when lax in the joint capsule it may also be lax at other sites in the body. The structure of collagen and the shape of the bony articulating surfaces both contribute to the range of joint movement along with the neuromuscular tone (Knight & H A Bird, 2011). In a systematic review and meta-analysis Smith et al. found that people with Benign Joint Hypermobility Syndrome (BJHS) demonstrated poorer lower limb joint position sense (T. O. Smith et al., 2013).

Ehlers–Danlos syndrome (EDS)

Ehlers–Danlos syndrome (EDS) is a prototypic connective tissue disorder with characteristic skin and joint involvement. The first clinical description detailing this disorder dates back to 1892 by Dr Tschernogobow, a Russian dermatologist, and subsequently, Drs. Ehlers and Danlos, Danish and French dermatologists, respectively, expanded on the systemic nature of this condition. Traditionally, EDS has been subdivided into **11 distinct variants (types I–XI)**, based on clinical observations, mode of inheritance, and/or molecular characterization. A consensus conference held in 1997 (Villefranche), however, proposed a revised nosology, which recognizes **six distinct subtypes**. (Uitto & Ringpfeil, 2004)

EDS type	Clinical finding	Inheritance	Gene defects
Classical (I/II)	Skin and joint hypermobility, atrophic scars, easy bruising	Autosomal dominant	COL5A1, COL5A2
Hypermobility (III)	Joint hypermobility, pain, dislocations	Autosomal dominant	Unknown
Vascular (IV)	Thin skin, arterial or uterine rupture, bruising, small joint hyperextensibility	Autosomal dominant	COL3A1
Kyphoscoliosis (VI)	Hypotonia, joint laxity, congenital scoliosis, ocular fragility	Autosomal recessive	Lysyl-hydroxylase
Arthrochalasia (VIIa,b)	Severe joint hypermobility, skin mild, scoliosis, bruising	Autosomal dominant	COL1A1, COL1A2
Dermatosparaxsis (VIIc)	Severe skin fragility, cutis laxa, bruising	Autosomal recessive	Procollagen *N*-peptidase

Table 1-The Villefranche classification of EDS *(Adapted from* (J. Mao & Bristow, 2001)

The **molecular basis** of the major forms of EDS is now well established, and the clinical manifestations are based primarily on mutations in the genes encoding collagen polypeptide subunits or enzymes that modify the primary collagen translation products. Each collagen molecule is composed of three α-chain subunits, which can be identical in **homotrimers**, or consist of two or three different kinds of polypeptides in **heterotrimers**. Thus, there are over **40 different genes** encoding the distinct α-chains that are synthesized as precursor polypeptides, pro-α-chains. These polypeptides are hydroxylated and glycosylated in reactions catalyzed by specific enzymes, three of the pro-α-chains then fold into the characteristic triple-helical conformation, and the collagen molecules are secreted into the extracellular milieu where they undergo proteolytic processing, fibre assembly, and formation of stabilizing inter and intra molecular crosslinks. In different forms of EDS, specific mutations have been identified in type I, III, and V collagen polypeptides, as well as in two enzymes that modify the collagen molecules. These molecular defects explain the connective tissue weakness and ultrastructural abnormalities in collagen fibrils. The collagen fibrils show considerable variability in their diameter by transmission electron microscopy, and although individual fibrils can be unusually large with an irregular contour, the density of collagen fibrils is often reduced. Thus, EDS has been considered as a disease of collagen. (Uitto & Ringpfeil, 2004)

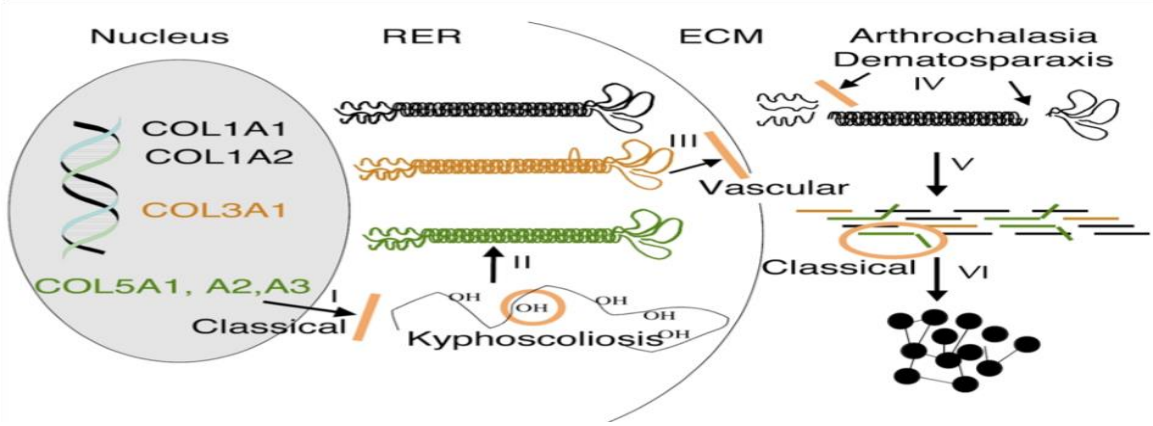


Figure 1- The biosynthetic pathway for the fibrillar collagens expressed in skin*, identifying steps that are affected in different forms of EDS. (I) Collagen gene transcription is highly regulated, but haploinsufficiency for COL5A1 is uncompensated. This accounts for 30–50% of classical EDS cases. (II) Many proline and lysine residues in the translated procollagen chains are hydroxylated by lysyl- and proline hydroxylases. Lysyl-hydroxylase deficiency causes the kyphoscoliosis form of EDS. (III) Procollagen α-chains are assembled into trimers within the rough endoplasmic reticulum (RER). Mutations in COL3A1 can cause the vascular form of EDS. (IV) In the ECM, the NH_2- and COOH-terminal propeptides are cleaved by specific peptidases. Mutations in COL1A1 and COL1A2 can cause arthrochalasia, and dermatosparaxis. (V) Collagen molecules self-assemble into heterotypic fibrils. Mutations in COL5A1 and COL5A2 can cause some cases of classical EDS. (VI) Collagen fibrils are deposited in tissue-specific arrangements in close association with many fibril-associated proteins and proteoglycans.* ***(Adapted from*** (J. Mao & Bristow, 2001)

EDS Classical Type (**EDS type I/II):**

The classical variety of EDS is usually inherited in an **autosomal dominant** fashion. The skin is soft and velvety in texture and can be extended several centimetres away from attachment sites in the classical forms. In the majority of individuals with classical forms of EDS, the underlying mutations have been reported in the type V collagen genes, COL5A1 and COL5A2. The most striking alterations were in the large dermal collagen fibrils, composed largely of type I collagen. (Byers, 2013)(Byers & Murray, 2013). The COL5A1 gene encodes the α1 chain of type V collagen (α1(V) chain), a minor fibrillar collagen. Although present in much smaller amounts than the other fibrillar collagens, type V collagen plays a critical role in the regulation of collagen fibril assembly and lateral growth **(fibrillogenesis)**. Type V collagen isoforms are heterotrimers made up of various

combinations of the α1(V), α2(V), and α3(V) chains. The major isoform contains two α1(V) and one α2(V) chains, which are encoded by the COL5A1 and COL5A2 genes, respectively. (Malcolm Collins & Posthumus, 2011)

EDS hypermobility type (EDS type III):

Several authors (Rodney Grahame, 1999) (Petersen & Douglas, 2013) (Tinkle et al., 2009) (Tofts, Elliott, Munns, Pacey, & Sillence, 2009b) (Castori, 2013) have stated that benign joint hypermobility syndrome is identical to Ehlers-Danlos syndrome type III. Rather than being monogenic, benign joint hypermobility syndrome is more likely to have multiple causes involving many extracellular matrix proteins. (Rodney Grahame, 1999) (Petersen & Douglas, 2013) The hypermobile type of EDS, is an **autosomal dominant** disorder with variable expression in which the major clinical features are large and small joint hypermobility. The skin changes are mild (Byers & Murray, 2013) when compared with EDS Type I/II and may be mildly hyper-extensible. Subluxations and dislocations are common; they may occur spontaneously or with minimal trauma and can be acutely painful. The diagnosis of EDS, hypermobility type is based entirely on clinical evaluation and family history. In most individuals with EDS, hypermobility type, the causative gene is unknown and unmapped. Haploinsufficiency of TNXB and heterozygosity for missense mutations in TNXB, the gene encoding tenascin X, have been associated with EDS, hypermobility type in a small subset of affected individuals. (Fransiska Malfait, Wenstrup, & De Paepe, 2010) (Byers, 2013)

Benign joint hypermobility syndrome (BJHS), is a **heritable disorder of connective tissue** that comprises symptomatic hypermobility predisposing to arthralgia, soft tissue injury, and joint instability. It is indistinguishable from the hypermobility type of Ehlers-

Danlos syndrome. Complications may include autonomic dysfunction, proprioceptive impairment, premature osteoarthritis, intestinal dysmotility, and laxity in other tissues causing hernias or uterine or rectal prolapse. It is diagnosed by means of the 1998 Brighton Revised Criteria for the Benign Joint Hypermobility Syndrome. (Ross & Grahame, 2011)(Tofts et al., 2009a)(Simpson, 2006)

Joint Hypermobility syndrome is believed to be far less common than asymptomatic hypermobility. BJHS is related to age, sex and ethnicity, being higher in younger people, in females and in non- whites. In recent studies 5-8% of females had features of JHS as compared with 0.6% of men. Clinical surveys conducted in places as far apart as London and Santiago indicate that the prevalence of BJHS far outstrips that of inflammatory and other arthritides in community rheumatology clinics, accounting for up to 45% of routine general rheumatology referrals. (A. Hakim & Grahame, 2004) (Simpson, 2006). (Rodney Grahame, 2008) (Beighton et al., 2012a).

Molecular Basis of Hypermobility

Extracellular Matrix: (ECM)

The connective tissue's primary function is to bind together and support various body structures and to fill the spaces between them. It mainly consists of **extracellular matrix** (ECM) within which the cells are sparsely distributed. The ECM is primarily made of fibrous proteins like type I collagen that accounts for 25% of the total protein mass of our bodies. ECM also contains a multitude of other glycoproteins, proteoglycans and the carbohydrate polymer hyaluronic acid (Chiquet-Ehrismann & Tucker, 2004). The extracellular matrix (ECM) was originally thought to serve only as a structural support for tissues, however there is growing evidence now that ECM molecules have functions other than structural roles and act as a central regulator of cell and tissue behaviour (Karsdal et al., 2013). ECM molecules interact with receptors on the surface of cells that then transmit signals across the cell membrane to molecules in the cytoplasm; these signals initiate a cascade of events through the cytoskeleton into the nucleus, resulting in the expression of specific genes, whose products, in turn, affect the ECM in various ways. Cell-ECM interactions can regulate cell adhesion, migration, growth, differentiation, and programmed cell death; modulate cytokine and growth factor activities; and activate intracellular signalling. (Petreaca & Martins-Green, 2013).

There are two main types of ECM. The first is the **basement membrane (BM**), which interacts directly with the epithelium and endothelium. It is composed primarily of type IV collagen, laminins, entactin/nidogen, and heparan sulfate proteoglycans (e.g., perlecan). The second type is the **interstitial matrix**, which makes up the bulk of the ECM in the body. The interstitial matrix consists of many types of collagens, including types I and III, together with fibronectin. The interstitial matrix additionally consists of tenascin and

proteoglycans that provide tissue hydration, enable binding of growth factors and cytokines to the tissue, and cross-link the matrix to enhance its integrity. (Karsdal et al., 2013)

Collagen is one of the most abundant proteins in the body. It has several functional roles but its mechanical function remains the most prominent. (Abou Neel et al., 2013)

Collagens:

The collagens are a heterogeneous superfamily of 28 glycoproteins located in the extracellular matrix (ECM) of almost all tissues. They maintain the structural integrity of tissues and regulate a variety of biological processes. The versatility of collagen as a building material is essentially due to its complex hierarchical structure. Collagens are **classified** by function and domain homology into:

i) fibril forming collagen;
ii) fibril-associated collagens with interrupted triple helices (FACITs);
iii) network-forming collagens;
iv) transmembrane collagens;
v) endostatin-producing collagens;
vi) anchoring fibrils;
vii) beaded-filament-forming collagens.

Collagens I, II, III, V, XI, XXIV and XXVII are members of the fibril-forming class of collagens. Types I, II, and III collagens are referred to as the major fibrillar collagens. The fibrillar types V and XI collagens are referred to as the minor fibrillar collagens and co-assemble with collagens I, II and III. The most abundant collagens (types I, II, and III) form elongated fibrils in fibrous connective tissues. Type II collagen is the basic building block of fibrils in cartilaginous tissue, whereas type I collagen fibril is found in non-cartilaginous tissues, such as tendon, ligaments, and the connective tissue components of the skeletal muscle. (Malcolm Collins & Posthumus, 2011) (S. M. Smith & Birk, 2012) (Abou Neel et al., 2013)

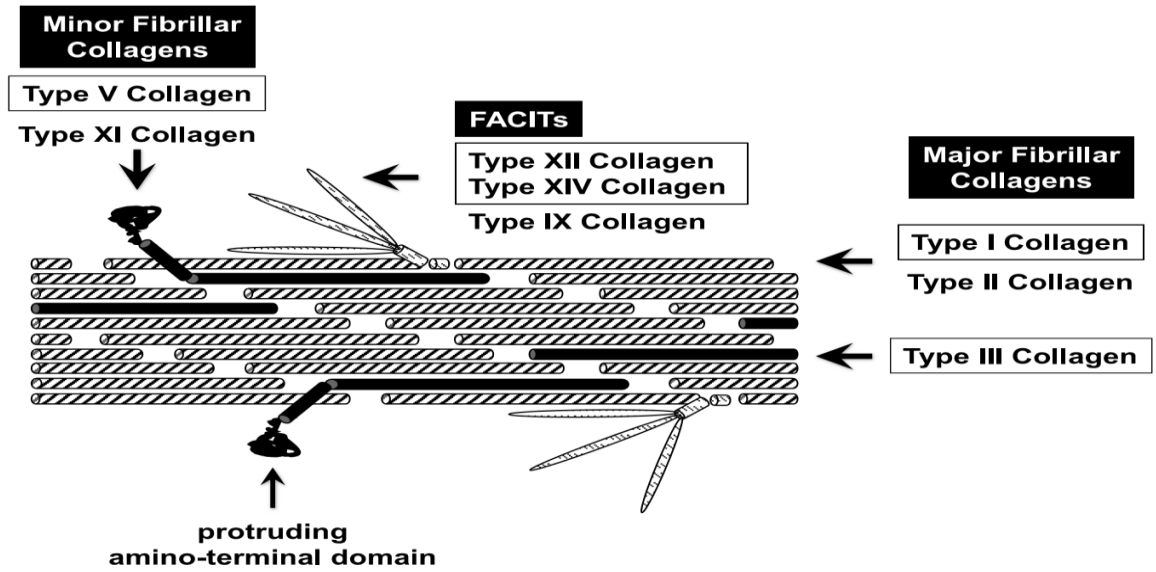


Figure 2- A schematic diagram of the collagen fibril. *The major fibrillar type I collagen molecule (hatched cylinders) is the major macromolecular component of the fibril in noncartilaginous tissues. Type II collagen (hatched cylinders), on the other hand, is the major macromolecular component of the fibril in cartilage. Noncartilaginous fibrils also contain type III collagen (solid cylinders), which also is classified as a major fibrillar collagen. Both types V and XI collagens are minor fibrillar collagens, which are imbedded in the fibrils of noncartilaginous tissues and cartilage, respectively. The amino-propeptide domains of these molecules protrude from the surface of the fibril. The major isoform of type V collagen is a heterotrimer consisting of two α 1(V) and one α 2(V) chains, which are encoded for by the COL5A1 and COL5A2 genes, respectively. Types XII and XIV collagen are associated with the surface of the noncartilaginous fibril and belong to the subfamily of FACITs. Type IX collagen is the FACIT found in cartilage. The proteins are not drawn necessarily to scale.* ***Adapted from*** *(Malcolm Collins & Posthumus, 2011)*

Fibrillar collagens are made up of combinations of 10 polypeptide chains, denoted by α1(I), α2(I), α1(II), α1(III), α1(V), α1(V), α3(V), α1(XI), α2(XI) and α3(XI). All these chains have been considered as strings of five or six amino acid triplets of **glycine–proline–hydroxyproline** (Gly–X–Y). In all fibrillar collagens the α-chain consists of an uninterrupted sequence of about 300 Gly–X–Y triplets, flanked by much shorter terminal domains of different structure. Depending on the tissue and collagen type, triple helices can be either homo-or heterotrimers. **Collagen type I** is usually a heterotrimer consisting of two α1(I) chains and one α2(I) chain. The triple helix is a long, rod-like structure, 1.5 nm in diameter and >300 nm long, flanked by non-helical domains (N- and C-propeptides) called telopeptides. The presence of C-propeptide is thought to be essential for triple helix formation but prevents collagen from forming intracellular fibrils during synthesis and transport, whilst the N-terminal is thought to be involved in controlling the primary fibril diameter. Intra- and intermolecular crosslinks bring stability to the collagen molecules, contributing to the characteristically high tensile strength and minimal extensibility of collagen. (Karsdal et al., 2013) (Lu et al., 2011) (Fransiska Malfait et al., 2010) (Tsipouras & Ramirezt, 1987) (Abou Neel et al., 2013) (Linsenmayer et al., 1993)

Type I is a structural collagen and plays a major role in providing tensile strength to tissues while type V collagen is a low-abundance fibrillar collagen which plays a crucial role in the assembly and regulation of the diameter of fibers. (*Disorders of Collagen*, n.d.)

Type I collagen:

Type I collagen accounts for 25% of the dry protein in mammals; constituting more than 90% (by weight) of the organic matrix of bone, and is also the major collagen component of tendons, skin, ligaments, cornea and many other interstitial tissues. Type I collagen is

composed of the heterotrimer α1α1α2(I) and is the most abundant type of collagen that is ubiquitously expressed. (Abou Neel et al., 2013)

Type V collagen:

Type V collagen is essential for life. Type V collagen is expressed in tissues containing type I collagen, but is a quantitatively minor component. The most common structure of type V collagen is **α1α1α2** (V), although homotrimers of three α1(V) chains and heterotrimers of the α1α2α3(V) isoforms have also been detected. It intercalates with the type I collagen molecules to form heterotypic fibrils in non-cartilaginous connective tissues, where it modulates fibrillogenesis. Expression from both copies of the COL5A1 gene is required for normal fibrillogenesis. The N-terminal domain contains a high level of tyrosine sulfated residues that contribute to the strong interactions that type V collagen has with triple-helical domains of other collagen types. This enhances the stability of fibrils (Karsdal et al., 2013). Type V collagen has been implicated in the early fibril initiation, correct assembly of collagen fibrils and to regulate their size and organization. The reduced type V collagen content is associated with a 50% reduction in fibril number and dermal collagen content. (Wenstrup et al., 2004) (Birk, 2001) (J. Mao & Bristow, 2001) (Malcolm Collins & Posthumus, 2011)

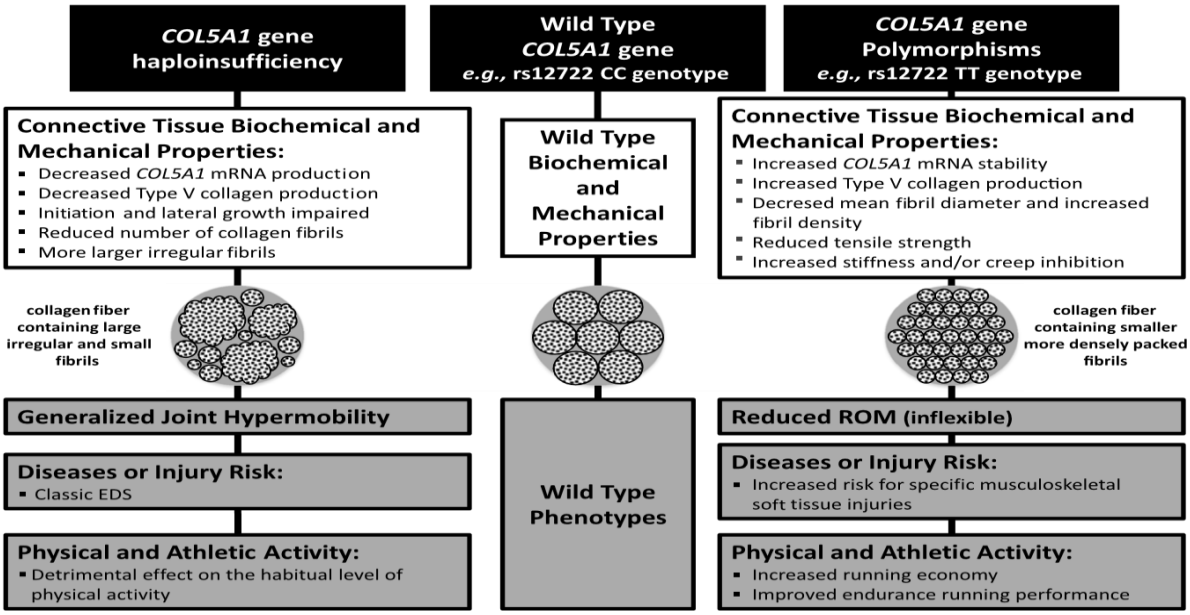


Figure 3. A schematic summary of the relationship between (i) COL5A1 genotype (black boxes), (ii) connective tissue biochemical and mechanical properties (white boxes), (iii) flexibility, (iv) disease or injury risk, and (v) physical activity. *The left panel illustrates the effects of disease-causing COL5A1 mutations on decreased type V collagen production, abnormal fibrillogenesis, and generalized joint hypermobility. These mutations cause Ehlers-Danlos syndrome (EDS), which has been shown to have a detrimental effect on the habitual level of physical activity within these patients. A mixture of large and small irregular fibrils in EDS is shown. The scenarios illustrated in the middle and right panels described the normal inter individual biological variation. The middle panel represents the wild-type COL5A1 gene and phenotypes. It is proposed that larger regularly shaped fibrils are produced from the wild-type gene, which is stronger and more compliant. These fibrils are associated with increased joint range of motion (ROM) decreased risk for specific musculoskeletal soft tissue injuries, and slower endurance running performance. The right panel illustrates the effect of functional common polymorphisms within the COL5A1 gene on increased type V collagen production. Smaller regularly shaped weaker fibrils are produced during fibrillogenesis. These fibrils, which are proposed to have an increased stiffness and/or creep inhibition, are associated with reduced joint ROM, increased risk for specific musculoskeletal soft tissue injuries, and faster endurance running performance.* ***Adapted from*** *(Malcolm Collins & Posthumus, 2011)*

Collagen Fibrillogenesis:

There is limited information on how collagen fibrillogenesis is initiated and regulated. Kadler et al described a working hypothesis of collagen fibrillogenesis in which fibronectin and integrins (the **organizers**) determine the site of fibril assembly, collagens V and XI (the **nucleators**) initiate collagen fibrillogenesis and Small leucine-rich proteoglycans (decorin, biglycan, fibromodulin, and lumican) influence the rate of assembly, size, and structure of collagen fibrils. (Kadler, Hill, & Canty-Laird, 2008) Collagen fibrillogenesis is a multiple step process that is tightly regulated by the interaction of various molecules. The initial step involves heterotypic collagen I/V nucleation at the cell surface, then SLRPs bind to the protofibril surface, regulating the linear growth and lateral growth of protofibrils to mature collagen fibrils. Deficiency of SLRPs leads to dysfunctional linear and lateral fusion, with alterations in fibril structure and function. (S. Chen & Birk, 2013)

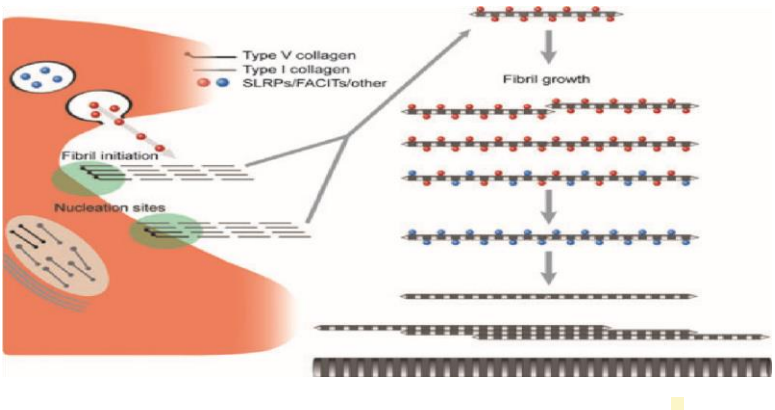


Figure 4. Model illustrating the involvement of SLRPs in the regulation of linear and lateral fibril growth. *Collagen fibrillogenesis is a multiple step process that is tightly regulated by the interaction of various molecules. The initial step involves heterotypic collagen I/V nucleation at the cell surface, then SLRPs bind to the protofibril surface, regulating the linear growth and lateral growth of protofibrils to mature collagen fibrils. Deficiency of SLRPs leads to dysfunctional linear and lateral fusion, with alterations in fibril structure and function.* ***Adapted from*** *(S. Chen & Birk, 2013)*

Functional roles of Collagen:

Collagen has several functional roles within the body although its **mechanical** function remains the most prominent. It is involved in a wide range of tissue functions, including scaffolding, morphogenesis and repair. Collagen transmits the force between the bone and neighbouring muscles as well as storing the excess **energy** in tendons or ligaments. This role of collagen is essential, as without this interconnection between bone and muscles by collagen, it would be impossible for the skeleton to move. Collagen is also present in a variety of mineralised tissues such as teeth or bones and confers a degree of **toughness** to these hard tissues and provide them with fracture resistance. Collagen plays a key role in cartilage, skin, blood vessel and muscles by providing flexibility so that each individual tissue type can fulfil its desired function. Collagen is also present in the cornea and confers specific **optical** properties in addition to its established mechanical stability due to its high degree of alignment and ordering. (Abou Neel et al., 2013)

Proteoglycans:

Collagens in the extracellular space interact with proteoglycans which are ECM macromolecules formed by a protein core with one or more glycosaminoglycans (GAGs) bound covalently. Due to the negative charge and structural conformation of GAGs, proteoglycans can interact with a large variety of macromolecules. The **small leucine-rich proteoglycan (SLRP)** family is formed by proteoglycans that bind specifically to other ECM constituents and contribute to the structural framework of connective tissues. SLRPs are small molecules, with core proteins of 40 kDa, and possess characteristic 6–10 leucine residues at conserved locations between the flanking cystein-rich disulfide-bonded domains at the N- and C-terminus that participate in protein–protein interactions with collagens, matrix glycoproteins, and cell membrane components. Based on several parameters, including gene organization and amino acid homologies, SLRPs are divided into five classes: **class I** includes decorin, biglycan, and asporin; **class II** includes fibromodulin, lumican, keratocan, proline arginine-rich end leucine-rich repeat protein (PRELP), and osteoadherin; **class III** includes epiphycan, mimecan, and opticin; **class IV** includes chondroadherin and nyctalopin; and **class V** includes podocan. Decorin, fibromodulin, asporin, lumican, PRELP, and chondroadherin can interact with collagen and influence collagen fibril formation and interaction. Decorin, biglycan, and lumican have many modulation roles in different biological processes. These functions highlight the important effect of ECM components in the cellular phenotype by influencing cell communication through, for instance; **signal transduction**, **cytokine modulation**, **adhesion**, and **migration**. (Karsdal et al., 2013) (Byers & Murray, 2013) (Birk, 2001) (S. Chen & Birk, 2013)

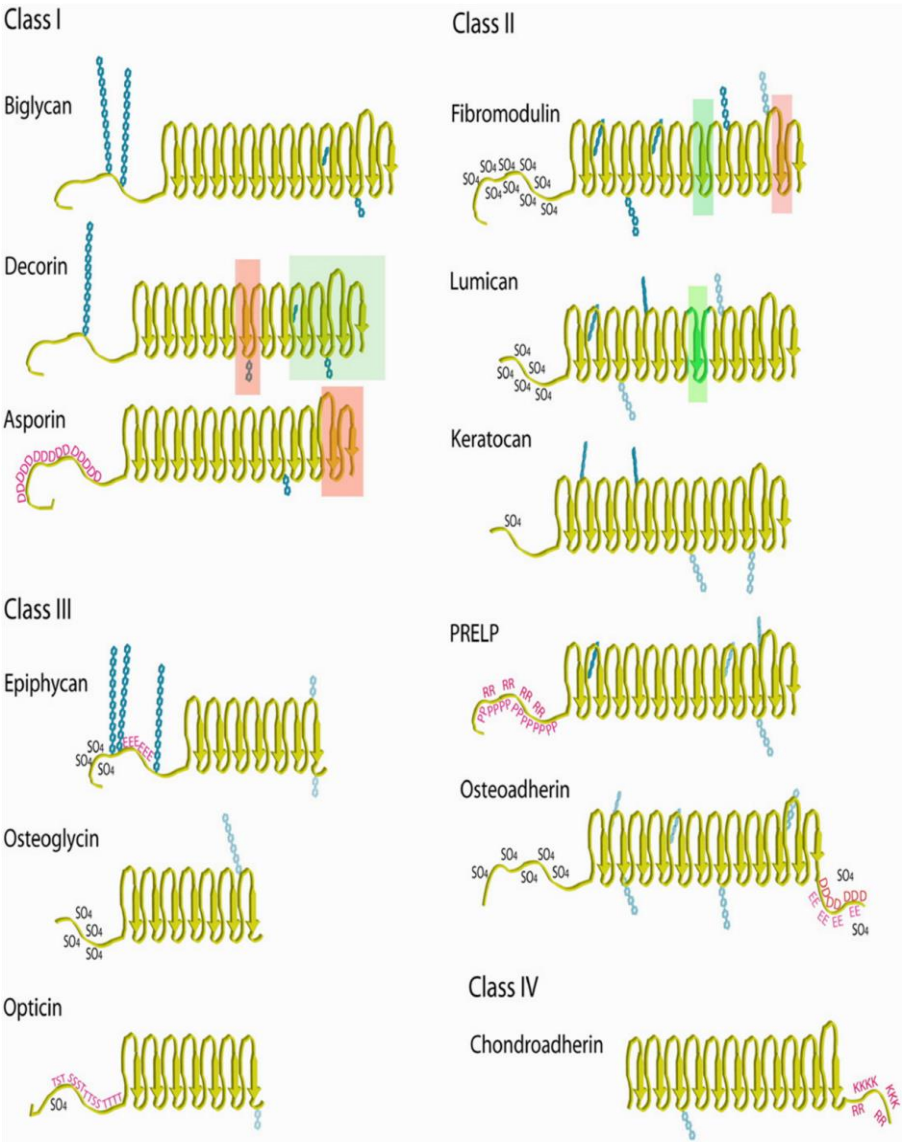


Figure 5- Scheme of the SLRP family with identified collagen-binding regions marked with red rectangles (high affinity) and green rectangles (low affinity). *Protein modifications are denoted as follows: glycosylations (blue chains) that are confirmed experimentally (solid chains); or potential glycosylations implied from consensus protein sequence (faded chains); possible tyrosine sulfations are marked by SO_4. Red letters indicate characteristic amino acids present in specific SLRPs.* ***(Adapted from*** (Kalamajski & Oldberg, 2010)

Functional roles of SLRPs:

During development, SLRPs regulate **cell migration**, **differentiation** and **proliferation** via cytokines and cell receptors. In later stages, they regulate matrix assembly through regulation of linear and lateral fibril growth by binding to the collagen fibril surface. They are indispensable constructional components of the matrix in mature tissues, interacting with other extracellular matrix components such as fibril associated collagens with interrupted triple helices (FACIT) and collagen VI. They also modulate the function of cytokines in the extracellular matrix. In pathological conditions, such as inflammation and the injury response to wounding, SLRPs facilitate tissue repair and regeneration. (S. Chen & Birk, 2013)

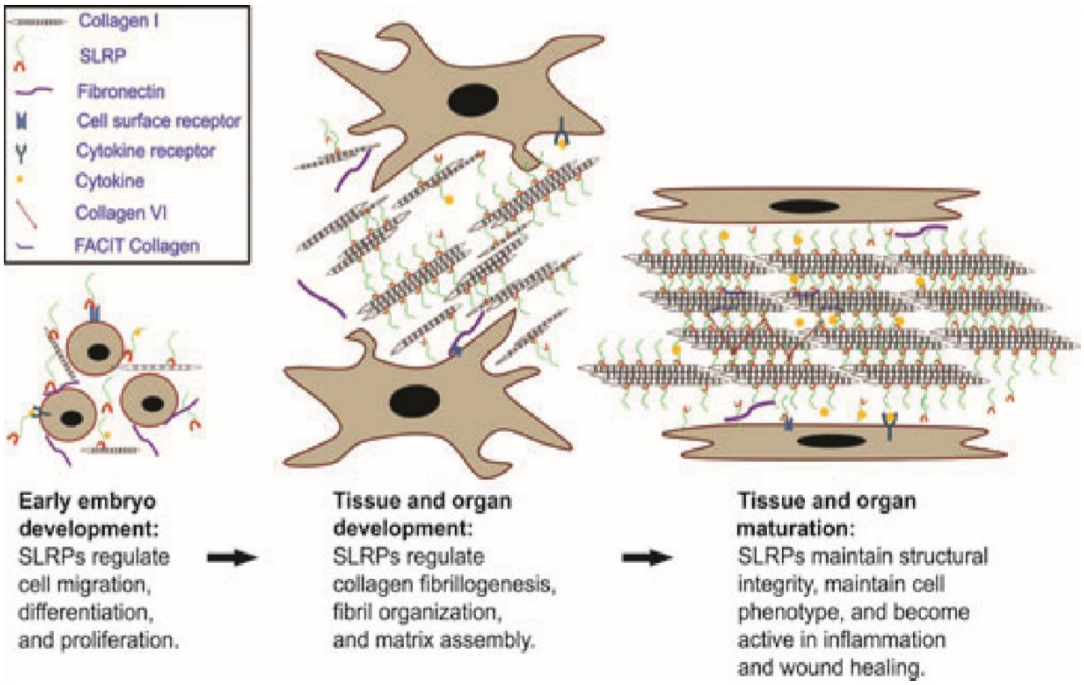


Figure 6. Roles of SLRPs in extracellular matrix assembly during development and maturation. *Dynamic interactions involving SLRPs, cells and their extracellular matrix result in the diversity and modulation of tissue-specific function. During development, SLRPs regulate cell migration, differentiation and proliferation via cytokines and cell receptors. In later stages, they regulate matrix assembly through regulation of linear and lateral fibril growth by binding to the collagen fibril surface. They are indispensable constructional components of the matrix in mature tissues, interacting with other extracellular matrix components such as FACIT collagens and collagen VI. They also modulate the function of cytokines in the extracellular matrix. In pathological conditions, such as inflammation and the injury response to wounding, SLRPs facilitate tissue repair and regeneration. FACIT, fibril associated collagens with interrupted triple helices.* ***Adapted from*** *(S. Chen & Birk, 2013)*

One major function of the SLRPs is the assembly of a specialized collagen matrix. The special requirements on the fibrils, and the different stages of fibril formation, require different SLRPs. **The SLRPs work in concert to assemble the collagen fibrils into a functioning ECM.** The level at which the SLRPs exactly act is not known, but one possibility is that when they bind to collagen it prevents an uncontrolled assembly of collagens into fibres by sterical hindrance. Alternatively the SLRPs may allow the collagen molecules that have more than one collagen-binding site (and can therefore bind more than one collagen monomer) to gather together into multimers. They could also bridge fibrillar and FACIT collagens. Finally, the SLRPs could regulate the cross-linking between two collagens by concealing some of the potential cross-linking lysine residues present on each collagen monomer.

It is possible that the SLRPs control the collagen fibril assembly through multiple or even all of these mechanisms. (Kalamajski & Oldberg, 2010)

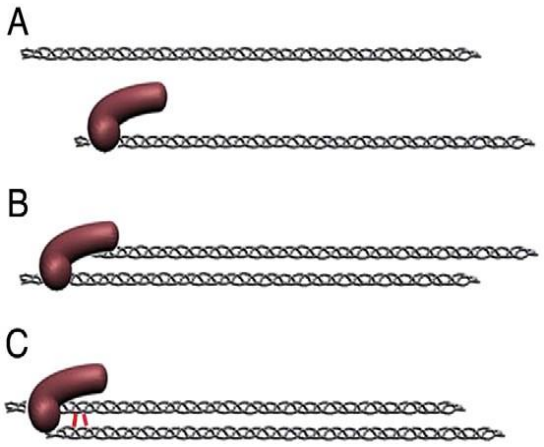


Figure 7 - Possible SLRPs regulation of collagen fibrillogenesis. *A. Steric hindrance of fibril assembly by SLRP binding to collagen. B. SLRPs with more than one collagen-binding site may connect together two assembling collagens. C. SLRP can bind near a crosslinking site and determine which cross-links (red lines) will form between the collagens.* ***(Adapted from*** (Kalamajski & Oldberg, 2010)

Decorin and biglycan may fulfill different functions in extracellular matrix assembly. Decorin and biglycan protein core demonstrated a **reciprocal expression pattern**. Biglycan compensates for, and potentially provides the regulatory roles normally associated with decorin. Both decorin and biglycan compete for collagen binding, suggesting the use of identical or adjacent binding sites on the fibril**.** (Guiyun Zhang et al., 2006)

Decorin:

Decorin (NM_133507) is an ubiquitous small extracellular proteoglycan. It is a composite molecule w100 kDa in size with a protein core and attached GAGs. It was cloned from a human embryonic fibroblast line and named PG40 for its protein core, w40 kDa. It has been known as PG-S2, bone proteoglycans-II, small leucine-rich protein-1B, dermatan sulphate proteoglycan-II, but decorin (DCN) was adopted based on its association with collagen fibrils, i.e., it "decorated" fibrils. Human decorin has 359 amino acids. Decorin has a high affinity binding site for collagen at LRRs 4-6 and a low affinity site at the C-terminus. Molecular modelling suggested that decorin may interact with multiple collagen molecules simultaneously (Guiyun Zhang et al., 2009) . Decorin is involved in the regulation of collagen fibrillogenesis. (S. Chen & Birk, 2011)

Decorin is one important member of the family of small leucine-rich proteoglycans (SLRPs), the major small proteoglycans in connective tissues. It is widely distributed in connective tissues in the body, such as **Skin, tendon, ligament, cartilage, kidney, muscle, predentin** as well as **bone**. Decorin is the most prominent proteoglycan in tendon, ligament and cartilage. It contains one GAG chain, often dermatan sulfate, which can adopt complex secondary structures and form specific interactions with matrix molecules. Its main function is to regulate **collagen fibrillogenesis** and to maintain tissue integrity by its binding with

fibronectin and thrombospondin. Decorin is an important antifibrotic agent: it influences fibrogenesis in different organs by inhibiting TGF-b; it regulates ECM synthesis and turnover, and it is involved in regulation of cell death, adhesion, and migration. (Karsdal et al., 2013) The interactions of decorins and collagen results in linkage of collagen fibrils which is important for the normal structure and function of these tissues. Previous works have shown reduced mechanical properties with irregular collagen fibrils in patellar tendons in decorin-null mice. (Liu, Yeh, Lewis, & Luo, 2005)

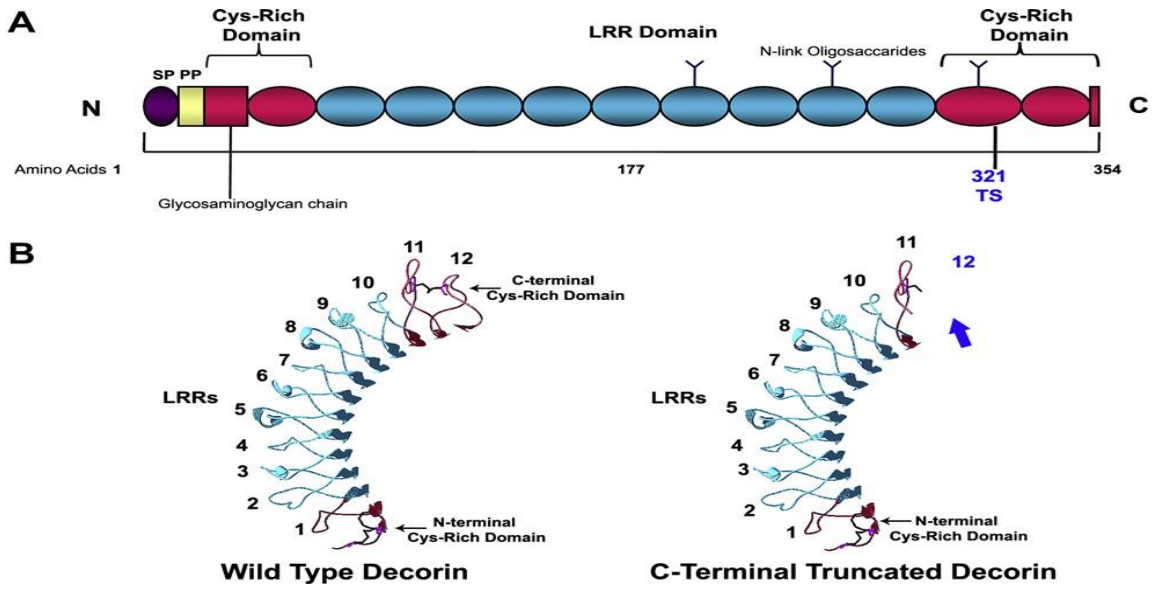


Figure 8- Structural features of decorin. *(A) Domain structure of the decorin protein core. From N- to C-terminus: signal peptide (SP); propeptide (PP); the glycosaminoglycan attachment site at a serine residue in the N-terminal Cys-rich domain; central LRR repeats; C-terminal Cys-rich domain. There are 3 N-linked oligosaccharide attachment sites in the LRR domains. (B) Swiss model of a normal mouse decorin and a truncated decorin lacking the C-terminal 33 amino acids. Blue arrow indicates the region of C-terminal truncation involving a deletion of LRR12 and part of LRR11, i.e., "ear repeat".* ***(Adapted from*** (S. Chen & Birk, 2011)

Biglycan:

Biglycan, a Class I SLRP, consists of a **LRR protein** core, which is covalently bound to two negatively charged polysaccharides: chondroitin sulfate (CS) and/or dermatan sulfate (DS) GAG chains. The protein core of Biglycan takes on a horse-shoe shaped structure, the GAGs attract calcium and phosphate ions and are responsible in part for nucleation of apatite nodules in bone matrices. Biglycan maintains the ECM structure of skeletal bone by covalently interacting with collagen fibrils. (Chiu et al., 2012) Biglycan is found in many connective tissues such as **skin, bones, cartilage, tendon**, **teeth, muscle** and **blood vessels**. Within the **hyaline cartilage** tissue, biglycan is localized mainly pericellularly. Together with decorin, biglycan is a key regulator of the lateral assembly of collagen fibres, and it interacts primarily with type VI collagen. Biglycan is thought to have a role also in **fibrogenesis** and in assembly of **elastin fibers**. Moreover, this proteoglycan is able to bind to the membrane-bound proteoglycan, dystroglycan, and to a wide variety of proteins. It has been reported that Biglycan is localized in bone and dentin matrices and is predominantly composed of CS-GAG, while softer tissues such as skin and ligament are composed of DS-GAG. (Waddington et al., 2003) Biglycan is involved in cell signal transduction during cell growth and differentiation and in regulating cytokine activity through its capacity to bind transforming growth factor (TGF) b and tumour necrosis factor (TNF) a. Biglycan is required for the maintenance of the integrity of the musculoskeletal system (Karsdal et al., 2013)(Young & Fallon, 2012)**.** In the absence of biglycan, there is decreased bone formation due to defects in the maturation of osteogenic precursors that form bone. (X.-D. Chen, Fisher, Robey, & Young, 2004)

Tenascins:

Tenascins are a family of four **ECM glycoproteins** in vertebrates, which are present in many different connective tissues. Tenascins contribute to matrix structure and influence the behaviour of the cells in contact with the ECM. (Chiquet-Ehrismann & Tucker, 2004) **Tenascin-X** deficiency causes a clinically distinct, recessive form of the Ehlers–Danlos syndrome. This finding indicates that factors other than the collagens or collagen-processing enzymes can cause the syndrome and suggests a central role for tenascin-X in maintaining the integrity of collagenous matrix. (Schalkwijk et al., 2001) (Hsia & Schwarzbauer, 2005)(Manon C Zweers et al., 2004). Tenascin-X regulates both the **structure** and **stability of elastin fibres** and organizes collagen fibrils in the extracellular matrix (ECM), impacting the rigidity or elasticity of virtually every cell in the body. (Petersen & Douglas, 2013) (M C Zweers, Kucharekova, & Schalkwijk, 2005) Tenascin-X is a large **extracellular matrix protein**, which is abundantly expressed during foetal development and in the adult. (Burch, Bedolli, McDonough, Rosenthal, & Bristow, 1995) Genetically determined deficiency of TNX is associated with the fragmentation of the elastic fibres and reduction of collagen. These findings suggest an important role of Tenascin-X in maintaining homeostasis of the extracellular matrix. (J. R. Mao et al., 2002) Hypermobility-type EDS is autosomal dominantly inherited and is the most frequently occurring type of EDS. (Wilcox, 2003) The phenotype of completely Tenascin-X deficient patients differs from the haploinsufficient patients, as skin hyperextensibility and easy bruising are characteristics of completely deficient patients, but are not found in Tenascin-X haploinsufficient patients. Elastin fibre abnormalities in hypermobility type EDS are specific for Tenascin-X haploinsufficient individuals and confirm an important role for Tenascin-X in regulating elastic fibre integrity. (Mc, Wb, Th, Bristow, & Schalkwijk, 2005).

Diagnosis of Benign Joint Hypermobility Syndrome

Joint hypermobility is a common feature of the heritable disorders of connective tissue (HDCTs) which include the Ehlers–Danlos syndrome (EDS), Marfan syndrome (MFS), and osteogenesis imperfecta (OI). Benign joint hypermobility syndrome (BJHS) is considered to be a HDCT. The HDCTs manifest as chronic musculoskeletal pain, fatigue, soft-tissue and visceral injury, cardiovascular pathology, skin abnormalities, and neurogenic dysfunction. Apart from a few specific genetic and histological investigations of connective tissue, there are no particular laboratory tests that either identify or separate these conditions. The diagnosis is made on clinical grounds, often employing investigations such as echocardiography to delineate cardiovascular involvement and slit-lamp examination to establish ocular involvement. (A. Hakim & Grahame, 2004).

The 1998 revised Brighton criteria can be used to diagnose joint hypermobility syndrome. It is important for clinicians to appreciate that a single hypermobile joint may be symptomatic and that is sufficient for the basic definition of the hypermobility syndrome to be satisfied. (Grahame R, Bird HA, & Child A, 2000). Another view that hypermobility becomes clinically significant (i.e. gives rise to symptoms) only if the Beighton score is >4/9 is erroneous (A. Hakim & Grahame, 2004).

Brighton Criteria:

The joint hypermobility syndrome is diagnosed in the presence of two major criteria, or one major and two minor criteria, or four minor criteria. Two minor criteria will suffice where there is an unequivocally affected first-degree relative.

Major criteria

• A Beighton score of 4/9 or greater (either currently or historically)

• Arthralgia for longer than 3 months in 4 or more joints

Minor criteria

• A Beighton score of 1, 2 or 3/9 (0, 1, 2 or 3 if aged 50+)

• Arthralgia (>3 months) in one to three joints or back pain (>3 months), spondylosis, spondylolysis/spondylolisthesis

• Dislocation/subluxation in more than one joint, or in one joint on more than one occasion

• Soft tissue rheumatism >3 lesions (e.g. epicondylitis, tenosynovitis, bursitis)

• Marfanoid habitus (tall, slim, span/height ratio >1.03, upper: lower segment ratio <0.89, arachnodactyly [positive Steinberg/wrist signs])

• Abnormal skin: striae, hyperextensibility, thin skin, papyraceous scarring

• Eye signs: drooping eyelids or myopia or antimongoloid slant

• Varicose veins or hernia or uterine/rectal prolapse

The reproducibility of diagnosing Generalized Joint Hypermobility and Benign Joint Hypermobility Syndrome was found to be high and additionally, the Beighton tests for Generalized Joint Hypermobility (either currently or historically) and the Brighton criteria for Benign Joint Hypermobility Syndrome showed a good reproducibility. (Juul-Kristensen et al., 2007) (L Remvig, Jensen, & Ward, 2007b) (L Remvig et al., 2007a)

Beighton Score:

The conventional method of identifying hypermobility, irrespective of cause, is to apply the Beighton nine-point scoring system established in 1973. This requires the performance of number of manoeuvres. (A. Hakim & Grahame, 2004). Several differing cut off points to indicate the presence of Generalized Joint Hypermobility have been used in the literature for the same tests (Verity Pacey et al., 2010) which has resulted in calls for revision of criteria for joint hypermobility. (Lars Remvig et al., 2011)(Lars Remvig & Juul-Kristensen, 2011)

Currently, standardized criterion of $\geq$ 4 of 9 as recommended by the British Society of Rheumatology had been most commonly used in the literature to indicate Generalized Joint Hypermobility (Verity Pacey et al., 2010) (Lars Remvig & Juul-Kristensen, 2011) (Reider, 2012).

History of Simple Scoring Systems for Hypermobility:

The first scoring system to diagnose hypermobility was devised by **Carter and Wilkinson** in conjunction with their work on congenital dislocation of the hip. They defined generalised joint laxity as being present when three of the following tests were positive, provided both upper and lower limbs were involved:

1. Passive apposition of the thumb to the flexor aspect of the forearm

2. Passive hyperextension of the fingers so that they lie parallel with the extensor aspect of the forearm

3. Ability to hyperextend the elbow more than 10°

4. Ability to hyperextend the knee more than 10°

5. An excess range of passive dorsiflexion of the ankle and eversion of the foot

Beighton et al. described in his latest book that the system of Carter and Wilkinson was revised by **Beighton and Horan** for the measurement of joint laxity in persons with the Ehlers–Danlos syndrome (EDS). Passive dorsiflexion of the little finger beyond 90°, with the forearm flat on the table, was substituted for passive hyperextension of the fingers, as the latter test had proved too severe; the range of ankle movement was replaced by measurement of forward flexion of the trunk. Patients were given a score between 0 and 5. **Grahame and Jenkins** modified this system to include passive dorsiflexion of the ankle beyond 15°. This was partly an adaptation to the particular subjects under study, half of whom were ballet dancers. Subsequently, **Beighton et al.** amended the 1969 system for use in an epidemiological survey of bone and joint disorders in an indigenous rural South African community. They employed the same tests, but gave one point for each side of the body for the paired tests. The range of scoring was thus between 0 and 9, with high scores denoting greater joint laxity. The manoeuvres used in this scoring system are listed below.

1. Passive dorsiflexion of the little fingers beyond 90° (one point for each hand) – two points

2. Passive apposition of the thumbs to the flexor aspects of the forearm (one point for each thumb) – two points

3. Hyperextension of the elbows beyond 10° (one point for each elbow) – two points

4. Hyperextension of the knee beyond 10° (one point for each knee) – two points

5. Forward flexion of the trunk with knees fully extended so that the palms of the hands rest flat on the floor – one point (Beighton et al., 2012b)

Beighton score validity:

A comparison has been made between the Carter and Wilkinson scoring system, as modified by Beighton et al., the Leeds finger hyperextensometer and a 'global index' constructed by using goniometry to assess the range of movement at almost all the joints in the body. This comparison follows the guidelines suggested by the American Academy of Orthopaedic Surgeons and sums the measured arcs of movement. Individuals were selected from different sporting groups thought to reflect more generalised hyperlaxity than that seen in the normal population. Beighton et al.'s modification of the Carter and Wilkinson system correlated well with the global index, endorsing the value of a simple scoring system that could be applied to large populations (Beighton et al., 2012b). The Beighton tests for GJH (either currently or historically) were found to be a valid measure and also showed a good reproducibility in other studies. (Juul-Kristensen et al., 2007) (L Remvig et al., 2007b) (L Remvig et al., 2007a) (Hirsch et al., 2007) (Beighton et al., 2012b).

Establishing joint hypermobility as a causative factor of symptoms in children can be difficult whose joints in any case display an unusually large range of movement compared to adults. Recently Beighton score was found to be a valid measure for generalized joint hypermobility in children, on the basis of the detailed analysis of the ranges of motion of all major joints. However it was recommended that 7/9 be the cut off for the Beighton score in white children between 6 and 12 years of age (Smits-Engelsman et al., 2011).

Strengths of Beighton Score:

This method has found favour for the following reasons:

1. Scoring systems using hyperextension of the middle rather than the little finger exclude too many persons.

2. Scoring systems using ankle movements, although perhaps appropriate for dancers, are unlikely to show much variation between individuals in a normal population.

3. Scoring systems that include trunk and hip movement (composite joint movement) are more likely to reflect generalised articular laxity. (Beighton et al., 2012b)

Weaknesses of Beighton Score:

Beighton scale is an imprecise instrument with which to recognize a hereditary connective tissue disease (Rodney Grahame, 2008). It has a number of shortcomings.

1. It gives no indication of the **severity** of the hypermobility. It merely indicates how widely it is distributed throughout the body.

2. There is also a risk that in **pauciarticular** involvement the hypermobility could pass unnoticed. Other areas that it has been suggested would be worth looking at include the proximal and distal inter-phalangeal joints, shoulders, cervical spine, hips, patellae, ankles, hind and forefeet, metacarpophalangeal joints and temporomandibular joint (TMJ) (R Grahame, 1999) (A. Hakim & Grahame, 2004) (A J Hakim & Grahame, 2003).

3. The Beighton tests have only been described by five photos and a legend. Several different **cut off points** for Generalized Joint Hypermobility have been used, e.g. a Beighton score of >6 positive tests, >5 positive tests and >4 positive tests.

4. In none of the two basic test descriptions for the Beighton tests is it stated precisely whether the tests should be performed **actively or passively**, and this may be a potential source of discrepancy in the interpretation of results from different studies. (Juul-Kristensen et al., 2007)

Other Scoring Systems:

An alternative scoring system was developed based upon work by JP Contompasis, which is more complex than the modification by Beighton et al. of the Carter and Wilkinson scale. A multiple-point scoring system based on six manoeuvres, five of which replicate Beighton, its scores span from the normal to the hypermobile range with a maximum total of 72. Initial studies had suggested that it was highly correlated with Beighton's score ($r = 0.92$; $p = 0.0001$) in original work by the editors, and it had been claimed that it was particularly useful in the assessment of ligamentous laxity in children. The greater experience produced problems in measurement, particularly in the use of foot flexibility tests, the major feature on which it differed from the Beighton score. Since the **Contompasis score** takes significantly longer and, in spite of the theoretical greater sensitivity, conveys little more information, the score is now only occasionally used. (Beighton et al., 2012b) Another score described in literature is the **Hospital Del Mar score,** which ranges from 0 to 10 and is derived by assigning one point for each of the following:

1. Passive hyperextension of the metacarpophalangeal joint of the little finger 90 or more.

2. Passive apposition of the thumb to the flexor aspect of the forearm at less than 21 mm.

3. Passive elbow hyperextension of 10 or more.

4. Passive shoulder external rotation of 85 or more.

5. Passive hip abduction of 85 or more.

6. Hyperextension of the first metatarsophalangeal joint beyond 90.

7. Patellar hypermobility, defined as excessive passive displacement medially and laterally as assessed by 3 or more quadrants of displacement.

8. Excessive range of passive ankle dorsiflexion and eversion of the foot with the knee flexed to 90.

9. Passive knee hyperflexion, defined as knee makes contact with the buttock.

10. Appearance of ecchymosis after hardly noticed, minimal traumatism (historical point).

A score of 4/10 or higher for men and 5/10 or higher for women suggests the presence of generalized ligamentous laxity. (Chahal, Leiter, McKee, & Whelan, 2010)

A **simple five-part self-report questionnaire** for joint hypermobility has been developed which can be used in screening individuals with diffuse or localized musculoskeletal symptoms in whom no clear-cut degenerative or inflammatory disease can be found. (A J Hakim & Grahame, 2003).

Mechanical Device for Hypermobility assessment: (Hyperextensometer)

A Mechanical device has been used for the accurate quantification of joint laxity. The hyperextensometer is a simple piece of apparatus comprising a carrier for the index finger which is mounted on a shaft supported in rolling element bearings which are themselves mounted in a housing and this complete assembly is fastened to a baseplate. The hyperextensometer is capable of applying preset torque varying between 2.0 and 7.0 kg cm (0.19 and 0.68 Nm). The amplitude of rotation of the index finger at the moment of slip is indicated by a pointer fixed to the operating shaft and in close proximity to a protractor which is fixed to the bearing housing.

Operation of the apparatus is simple. The patient is made comfortable with the forearm and hand supported on the baseplate, and the axis of the MCP joint of the index finger is aligned to the axis of the shaft by purely visual means. Pads of foam rubber are used where necessary to bring the hand to the correct height relative to the baseplate. The finger is then strapped to the carrier, the indicating pointer is zeroed, and the knurled knob of the clutch rotated manually until the present level of torque is reached when the clutch slips. The amplitude of rotation is then read off at leisure.

The authors have studied the correlation of the hyperextensometer both with this scoring system and a more complex 'global score of joint laxity' devised by themselves and calculated by summating the range of movement, measured by goniometer, of all the joints of the body. In a population of fifty-four individuals, some selected to demonstrate extreme joint laxity, both correlations were highly significant, $r = 0.67$, $p < 0.001$ for Carter and Wilkinson and $r = 0.61$, $p < 0.001$ for the global index. (Jobbins, Bird, & Wright, 1979)

Bird et al. compared the finger hyperextensometer, as the most accurate non-invasive measuring device, with Beighton's 1973 adaption of the Carter and Wilkinson scoring

system, as the most commonly used system in ninety-six individuals. For each individual the range of movement of each joint was measured by goniometry following the scheme of the American Academy of Orthopaedic Surgeons (1965). Correlations were then calculated between each joint measured, the hyperextensometer and the component parts of the Carter and Wilkinson system. Finally, both systems were correlated with a 'Global Index of joint laxity' calculated by summing the measured arcs of movement of most joints in the body for each individual. The finger hyperextensometer correlated best with finger extension (measured by goniometer). Correlations between scoring systems were all highly significant. However, a system measuring several attributes (Carter and Wilkinson) correlated better with a global index than a system measuring movement at a single joint. (H. a Bird, Brodie, & Wright, 1979). Thus hyperextensometer appeared to convey more applied information in an accurate fashion, emphasising that the range of movement at a single joint can correlate with overall joint laxity. (Calguneri et al., 1982) (Jobbins et al., 1979)

Effects of Hypermobility on Musculoskeletal system:

Most hypermobile subjects have few or no problems and enjoy a symptom free life. Others seem to have problems at certain times of their life and not at others. It is not always possible to pinpoint the determining factors, although a change in lifestyle, particularly unaccustomed physical exercise, is the usual precipitating factor (R Grahame, 1999). Normal ‘tight’ ligaments protect joints both by limiting the range of movement and by imposing stability (Beighton et al., 2012a) although sometimes the tight hamstrings predisposes them to injury (Watsford et al., 2010) and knee joint stiffness can result in back pain (Hamill, Moses, & Seay, 2009).

The lax joint is deprived of such safeguards and is, therefore, more vulnerable to the effects of injury from trauma and overuse. Soft tissues too are less resilient, so that ligament and muscle tears and tendon−osseous attachment lesions such as epicondylitis and plantar fasciitis may occur with increased frequency. The spectrum of clinical manifestations in the hypermobility syndrome is wide. Common features include arthralgia and myalgia, soft tissue lesions, chondromalacia patellae, acute articular and periarticular traumatic lesions, chronic polyarthritis or monoarticular arthritis, dislocation of joints, temporo-mandibular joint dysfunction, premature osteoarthritis, spinal complications, bone fragility and chronic pain. (Beighton et al., 2012a) (H. A. Bird, 2011) (Wolf, Cameron, & Owens, 2011) (R Grahame, 1999)

Risk of Injuries in Hypermobile individuals:

Generalized joint hypermobility may appear to be an advantage in some sports such as gymnastics, but it can be potentially dangerous in other sports like netball (R. Smith, Damodaran, Swaminathan, Campbell, & Barnsley, 2005) rugby (Stewart & Burden, 2004) football (Matt D Konopinski, Jones, & Johnson, 2012) and Ballet dancers (Scheper et al., 2013).

The loss of stability due to ligamentous laxity may result in recurrent dislocation after comparatively minor trauma. Dislocations, subluxations, and sprains are commonly reported in individuals with GJH and it is assumed that the risk of such injuries is magnified during activities that are more physically challenging. A systematic review with meta-analysis found that the risk of ankle injury while participating in sporting activities is not altered by the presence of GJH, yet individuals with GJH do have an increased risk of knee injury during sporting activities, particularly during contact sporting activities. (Verity

Pacey et al., 2010) Conflicting evidence between hypermobility and joint injuries has led to varying recommendations for individuals with GJH on the risks incurred by sports participation with some advising participation in noncontact activities only, such as swimming, pilates, and tai chi; Others suggest that hypermobile participants can undertake sporting activities such as netball with the use of strapping and supports in order to limit injury while others recommends full involvement in sporting activities for pain-free hypermobile individuals (Vaishya & Hasija, 2009)(Myer, Ford, Paterno, Nick, & Hewett, 2008) (Ramesh, Von Arx, Azzopardi, & Schranz, 2005) (H. A. Bird, 2011).

ACL Injuries, Reconstruction and Failure of reconstruction

Structure and Function of ACL:

The anterior cruciate ligament (ACL) runs from the posteromedial aspect of the intercondylar notch on the lateral femoral condyle to a triangular space on the tibia between the medial intercondylar eminence and the anterior horns of the meniscus. Between the femur and tibia, the ACL averages a length of 31 to 38 mm with a mid-substance width of 10 to 12 mm. The ACL has an anteromedial and a posterolateral bundle. The ACL plays an important role in knee stability. The native ACL serves as the primary restraint to prevent anterior translation of the tibia relative to the femur and acts as a secondary restraint to tibial rotation and varus/valgus stress in the presence of collateral ligament injury. There has been debate in literature about its proprioceptive role. (Beard, Dodd, Trundle, & Simpson, 1994) ACL reconstruction is indicated to prevent knee laxity and functional instability during activities of daily living and athletic activity. Reconstruction also serves to decrease the risk of meniscal injury and the eventual development of degenerative joint disease. (Claes, Hermie, Verdonk, Bellemans, & Verdonk, 2013) (Mehran, Skendzel, Lesniak, & Bedi, 2013) (Mehran et al., 2013)(Beasley et al., 2005) (Frank, Jackson, Douglas, & Beach, 1997)

Prevalence of ACL injuries:

The integrity of the anterior cruciate ligament (ACL) is important to athletes who participate in running, cutting, and jumping sports; it is particularly prone to rupture in these types of sport especially during sudden stops or when landing from a jump. Certain sports such as football, basketball, and soccer are considered to be among the higher risk sports for ACL injuries. At least 100,000 ACL injuries occur each year in young athletes in United States of America and they are becoming more common as participation in athletics increases. Approximately $1 billion are spent annually on reconstructive surgery alone. Acute ACL

rupture is a devastating injury that can significantly affect patients' activity levels and quality of life. Anterior cruciate ligament ruptures lead to moderate-to-severe disability in 77% of those who suffer the injury. While some athletes can continue to participate in their sport after an ACL injury, many require reconstruction. Anterior cruciate ligament (ACL) reconstruction is one of the most common orthopaedic procedures performed, with the goal of restoring anterior-posterior and rotational stability to the knee. It is generally a successful procedure that restores stability of the knee and allows patients to return to athletic activities. Complete ACL tears can lead to chronic knee problems, such as knee instability, meniscus and chondral surface damage, and osteoarthritis. (Beasley et al., 2005) (R. K. Flynn, 2005) (Yu, Ph, Kirkendall, & Garrett, 2002) (Shimokochi & Shultz, 2008) (J. L. Chen et al., 2013).

Mechanism of ACL injuries:

Most ACL injuries are noncontact in nature. Anterior cruciate ligament injuries often happen when an individual attempts to decelerate the body from a jump or forward running while the knee is in a shallow flexion angle. Over 70% of noncontact ACL injuries in basketball, volleyball, and gymnastics occurred in stop–jump tasks. At the time of injury, combined motions such as knee valgus and knee internal-external rotation are often noted. The final pathway of a non-contact ACL rupture could be hyperextension of the knee due to an eccentric contraction of the quadriceps from a 'position of no return' (Yu et al., 2002) (Ramesh et al., 2005)(Shimokochi & Shultz, 2008)

ACL injuries in Females:

Female athletes have a 2 to 8-fold higher incidence of noncontact ACL injuries than male athletes in basketball, soccer, volleyball, handball, rugby, and track and field. Risk factors explaining the increased incidence of anterior cruciate ligament (ACL) injury in female athletes are commonly classified as **anatomic, neuromuscular**, and **hormonal**. Among the reported anatomic risk factors for the increased risk of ACL rupture are: differences in ACL size in female, who have smaller ACLs, which are considered to be more prone to injury than larger ligaments subjected to similar loads. (Yu et al., 2002) (Quatman et al., 2008).

In an epidemiological study of the collegiate athletes over 13 years Agel et al. reported that the rates for all anterior cruciate ligament injuries for women were statistically significantly higher ($P<.01$) than the rates for all anterior cruciate ligament injuries for men, regardless of the sport. (Agel, Arendt, & Bershadsky, 2005).

Hashemi et al. analysed eighteen tissue samples by transmission electron microscopy from the distal, proximal, and middle sections of the anteromedial and posterolateral bundles of ACL from Six male and six female patients and reported that the female ACLs have lower number of fibrils per unit area but larger fibril diameters ($p<0.05$) when compared to males. (Hashemi, Chandrashekar, Mansouri, Slauterbeck, & Hardy, 2008)

Chandrashekar et al. tested 20 cadaveric femur-ACL tibia complex to failure in a tensile testing machine and reported that female ACL was found to have lower mechanical properties (8.3% lower strain at failure; 14.3% lower stress at failure, 9.43% lower strain energy density at failure, and 22.49% lower modulus of elasticity) when considering age, ACL, and body anthropometric measurements as covariates. Authors concluded female

ACL has lower mechanical properties when compared to males and this might play a major role in the higher incidence of ACL injury in female athletes. (Chandrashekar, Mansouri, Slauterbeck, & Hashemi, 2006)

Generalized joint hypermobility increases in female during puberty. This potentially leads to **decreased static stability** in female athletes. Female athletes also demonstrate measurable neuromuscular deficits. Hewett et al. demonstrated that compared to males, females display greater maximum lower extremity valgus angles and greater total medial motion of the knees during a jump task following the onset of puberty. These decreases in dynamic knee stability are coupled with decreased knee flexor torques in female athletes after puberty, compared to males. (Hewett, Myer, & Ford, 2004) In addition, while males show a neuromuscular spurt during puberty, demonstrated by increased vertical jump height and increased ability to attenuate landing forces, females do not experience a similar increase in neuromuscular performance. (Quatman, Ford, Myer, & Hewett, 2006) Poor dynamic knee control and the absence of a neuromuscular spurt, coupled with increased generalized joint laxity in maturing females, may be linked to the increased dynamic coronal plane knee motions and loads, which may increase ACL injury risk following the onset of puberty. (Hewett et al., 2004). These factors may be related to increase in knee and ACL injury incidence in female athletic populations after puberty (Quatman et al., 2008).

Risk Factors for ACL injuries:

Several internal and external factors have been proposed as potential contributors to the increased incidence of noncontact ACL ruptures, especially for women. These potential risk factors were categorized as environmental, anatomic, hormonal, and biomechanical.

Environmental factors include the type of playing surface and the type of shoes. ACL injuries appear to occur most frequently when the playing surfaces are dry. Higher level of friction between the shoe and the playing surface are associated with better performance but a higher risk of injury.

Anatomic factors include lower extremity alignment (increased femoral anteversion, increased Q angle, excessive tibial torsion and excessive foot pronation), knee joint laxity, muscle strength, femoral notch and ACL size.

Hormonal risk factors include the effect of oestrogen on the mechanical properties of the ACL.

Biomechanical factors include the increased joint loads associated with lower extremity motion patterns due to altered neuromuscular control.

In addition, an athlete's level of **conditioning**, strength, coordination, and skill may also play a role in noncontact ACL injuries. Rupture of an ACL in this way is characterised by an absence of collision, but an awkward, single-leg landing or stopping, or rapid changes in direction, especially lateral movements (Yu et al., 2002) (Ramesh et al., 2005)

Generalized joint hypermobility in ACL injuries and prevention strategies:

It has been suggested (Loudon, Goist, & Loudon, 1998) that patients with hyperextension of the knee may have a poor proprioception feedback loop and that the poor proprioceptive feedback seen in both hyperextension and increased joint laxity can reduce the ability to initiate protective reflexes. (Hall, Ferrell, Sturrock, Hamblen, & Baxendale, 1995) In a case controlled study Ramesh et al. assessed hyperextension of the knee and joint laxity in 169

consecutive patients who underwent an anterior cruciate ligament reconstruction and concluded that anterior cruciate ligament injury is more common in those with joint laxity and particularly so for those with hyperextension of the knee. (Ramesh et al., 2005) In a prospective cohort study of 110 patients undergoing ACL reconstruction Vaishya et al. found that more than 92.7 % of the patients, with ACL injuries, have significant joint laxity when compared with the control group 16.4%. (Vaishya & Hasija, 2009). Neuromuscular interventions have been reported in a study (Kraemer, Duncan, & Volek, 1998) help to compensate for decreased passive joint stability by increasing dynamic joint stability. The resistance training increased muscle strength and improved joint stability. (Quatman et al., 2008) Therefore, increased joint awareness and improved active joint motion restraints may provide joint protection and should be considered for athletes with ligament laxity to reduce the risk of ACL injuries (S.-J. Kim, Kim, Lee, & Oh, 2008) (Myer et al., 2008) (Mandelbaum et al., 2005).

Genetic Component of ACL injuries:

In a case–control retrospective questionnaire-based study, Flynn et al. investigated the familial predisposition to tears of ACL in a total of 348 affected and 384 control subjects and reported that a greater proportion of subjects with an ACL tear had a relative with an ACL tear suggesting a genetic contribution to tears of the ACL. (R Kevin Flynn et al., 2005)

There is a spectrum of connective tissue disorders with a genetic component. At one end are the classical Mendelian disorders, wherein the genetic factors are the major determinants of the severity and prognosis of the disorders like osteogenesis imperfecta (OI), Ehlers–Danlos syndrome (EDS) and Marfan's syndrome. At the other end are the complex, multifactorial conditions, wherein the development of the condition is determined by the complex

interactions of multiple gene products (i.e., proteins) and the environment. The identification of the genetic components of the underlying Mendelian disorders is usually achieved using linkage analysis or direct candidate gene sequencing. This search is complicated by the likelihood that a number of genes are involved, each having a small contribution, and by the gene–environment interactions.

Type V collagen is found in tendons and other connective tissues where it regulates the assembly (fibrillogenesis) of collagen fibres. The COL5A1 gene encodes for a component of type V collagen, and mutations in COL5A1 have been associated with both EDS and benign joint hypermobility syndrome. Individuals with an ACL tear are twice as likely to have a relative with an ACL tear and more than twice as likely to have a first-degree relative with an ACL tear, suggesting that there may be a genetic contribution to tears of the ACL. (September, Schwellnus, & Collins, 2007) (R. K. Flynn, 2005) (Dourte, Kuntz, & Soslowsky, 2008)

Treatment of ACL injuries:

Conservative management:

Management of ACL injury is one of the most controversial topics in sports medicine because there is a differential response to the injury. The majority of patients cannot return to high level athletic activities after ACL injury because of continued episodes of knee giving way, but a small percentage make a full, asymptomatic return to all pre injury activities. Successful return to pre injury activities with non-operative management after ACL rupture depends on the development of dynamic knee stability, which is accomplished

via neuromuscular adaptations in the absence of ligamentous support. (Hurd, Axe, & Snyder-Mackler, 2008)(Frank et al., 1997) (Beard et al., 1994)

Surgical management– ACL reconstruction:

Surgical management of the anterior cruciate ligament deficient knee has evolved from primary repair to extra capsular augmentation to anterior cruciate ligament reconstruction using biologic tissue grafts (Fu, Bennett, Ma, Menetrey, & Lattermann, 2000). Reconstruction is now widely accepted as the treatment of choice for the patient with a functionally unstable anterior cruciate ligament-deficient knee. By current estimates, more than 100,000 anterior cruciate ligament reconstructions are performed annually in the United States with long-term rates of good and excellent results, in terms of functional stability, relief of symptoms, and return to pre injury level of activity between 75 and 90 percent. When ACL re-ruptures and objective clinical failures (defined by one of the following: an overall IKDC objective score of C or D, IKDC grade C or D and pivot shift (i.e., >2+ or pivot shift), IKDC grade C or D Lachman examination or KT arthrometer (MEDmetric, San Diego, CA) measurement (i.e., >5mm), and / or identified grossly abnormal stability examination without IKDC scoring available) are collectively considered, the cumulative failure rate of primary ACL reconstruction at more than 10 years' follow-up is nearly double that reported in the literature 11.9% (Crawford, Waterman, & Lubowitz, 2013).

Graft choices for ACL reconstruction:

The surgeon controls the initial structural properties of a ligament reconstruction by the type and size of graft selected. (Noyes, Butler, Grood, Zernicke, & Hefzy, 1984). Three

additional factors should be considered when a graft is chosen on the basis of its strength. **First**, it is known that, for at least the first one to two months after implantation, the main factor affecting the structural strength of either patellar ligament or hamstring grafts is not the graft itself but rather the point of fixation of the graft to the bone (Rodeo, S. A.; Arnoczky, S. P.; Torzilli, P. A.; Hidaka, C.; and Warren, 1993). **Second**, a number of studies (Butler, 1989) (McFarland, E. G.; Morrey, B. F.; An, K. N.; and Wood, 1986) agree that the tendon tissue loses a considerable amount of its initial strength during the early healing period. **Third**, the effects of the initial strength, size, surface area, and origin of the graft on its potential for weakening during healing should be taken into account (Frank et al., 1997).

Autografts are selected by most surgeons as the appropriate substitute after rupture of the ACL, but allografts continue to use in some cases (Bartlett, Clatworthy, & Nguyen, 2001). Two types of autografts are used most often: an autogenous bone-tendon-bone graft involving the central one-third of the patellar tendon or an autogenous graft involving the equally tensioned quadruple hamstring tendons to approach the strength of a normal anterior cruciate ligament at the time of the operation (Hamner, Brown, Steiner, Hecker, & Hayes, 1999).

The aim of ACL reconstructive surgery is to obtain an ideal outcome for the patient for the rest of their life, not for the short period of their career (Pinczewski, Roe, & Salmon, 2009). The ideal graft for ACL reconstruction should have biomechanical properties similar to those of the native ACL, allow for stable fixation, rapidly incorporate into host tissue, and have a low rate of morbidity. As the ideal graft choice is patient specific, the surgeon must have a thorough understanding of the risks and benefits of each graft choice to determine,

which graft option best fits the patient's demands and goals and the surgeon's preferences and technical capabilities (Mehran et al., 2013).

The bone-patellar tendon-bone graft is recommended because of the graft's load to failure, stiffness, quality of fixation and durability while the hamstring tendon graft is recommended as it requires a smaller incision and has been reported to result in less anterior knee pain (Li et al., 2011) (Kartus, Movin, & Karlsson, 2001) and a thicker tendinous portion within the knee joint and the bone tunnels (Fu et al., 2000) (Hamner et al., 1999). Recent data on the use of allograft tissue suggests that an equal result to that obtained with these two autologous techniques can be expected with allograft techniques. Good-to-excellent results can be expected with any of these techniques provided the surgeon has the appropriate expertise, appropriate fixation devices have been selected and diligent rehabilitation is carried out. (Beasley et al., 2005)

Kim et al. retrospectively studied eighty-three patients who had undergone anterior cruciate ligament reconstruction. Of the thirty-one patients who had generalized joint laxity, twenty were managed with an autologous bone-patellar tendon-bone graft and eleven were managed with a four-bundle hamstring graft. Of the fifty-two patients who had normal joint laxity, thirty-three were managed with a bone-patellar tendon-bone graft and nineteen were managed with a hamstring graft. In both male and female patients, who have excessive joint laxity, the two-year outcomes of anterior cruciate ligament reconstruction with bone-patellar tendon-bone grafts were better than those with four-bundle hamstring grafts in terms of both side-to-side anterior laxity and clinical results. The proposed reason for this increased laxity in females who had hamstring grafts is a combination of physiologic laxity, the small diameter of the hamstring tendons and delayed incorporation of the hamstring tendons into

the tunnels as compared with the more rapid incorporation of bone plugs in the tunnels (S.-J. Kim et al., 2008).

Allografts:

Tendon allografts play an important role in tendon and ligament reconstruction, particularly where there is a shortage of suitable available local tissue. The use of allogenic tissue for primary ACL reconstruction is gaining popularity. (McGuire & Hendricks, 2009) (Indelli, Dillingham, Fanton, & Schurman, 2004) (Kleipool, Zijl, & Willems, 1998). Commonly used allograft sources include: BPTB, hamstrings tendons, anterior tibialis tendon, posterior tibialis tendon, and Achilles tendon with bone block. In the United Kingdom, tendo-achilleis and patellar tendon allografts are most commonly used for ACL reconstruction. The advantages of allografts include lack of donor site morbidity, high tensile strength, decreased surgical time, smaller surgical incisions and a low risk of arthrofibrosis. The disadvantages include their limited availability, high cost, susceptibility to rejection due to immuno-incompatibility and potential risk for disease transmission. All tendon grafts, whether autogenous or allogenic, undergo a similar process of integration with graft necrosis, revascularisation, cell repopulation and remodelling. Tendon allografts are rarely indicated in primary reconstruction of the ACL unless there are particular concerns regarding the morbidity associated with graft harvest. Despite this, a number of recent studies have demonstrated comparable clinical and radiological results when using allograft tissue in primary reconstruction of the ACL, whether cryopreserved or fresh-frozen, when compared to autografts. (Robertson, Nutton, & Keating, 2006) (Beasley et al., 2005) (Kustos, Bálint, Than, & Bárdos, 2004) (Bartlett et al., 2001)

Causes of failure of ACL reconstruction:

Major improvements in anterior cruciate ligament (ACL) reconstructive surgery have been made in the past decades and it is now widely accepted as the treatment of choice for individuals with functional instability due to an ACL-deficient knee. Nonetheless, 0.7–10% of patients develop graft failure with recurrent instability and may then be candidates for revision ACL reconstruction (Ménétrey, Duthon, Laumonier, & Fritschy, 2008). There are many factors that can lead to graft failure and possible revision surgery. The University of Pittsburgh group classified the mechanisms of ACL graft failure as related to (a) surgical technique; (b) graft incorporation; and (c) trauma. (Ménétrey et al., 2008), (Harner, Giffin, Dunteman, Annunziata, & Friedman, 2000), (George, Dunn, & Spindler, 2006a) (Sahu, 2009)

University of Pittsburgh classification for mechanisms of ACL graft failure:

A. Surgical technique

1. Technical errors (Tunnel location, Graft impingement, Graft tension, Graft fixation)

2. Mechanical/biomechanical factors Graft strength (size, hamstring versus BPTB, irradiation) Synthetic graft

3. Secondary stabilizers combined ligament involvement Meniscal/articular cartilage loss

B. Failure of graft incorporation

1. Avascularity 2. Immunology 3. Stress shielding

C. Trauma

1. Traumatic re-injury 2. Aggressive rehabilitation

Outcomes following revision ACL reconstruction are not as predictable as with primary ACL reconstruction and it should be considered as a salvage surgery. Allografts have been used in ACL reconstruction and ACL revision surgery and long term follow-up shows comparable results to autograft tissue for ACL revision. (A. H. Smith, Bach, & Bush-joseph, 2005).

Biological failure:

Biological failure should be suspected in patients presenting with recurrent instability without a history of trauma or an identifiable technical error (Harner et al., 2000). Biological failure can also be defined as a failure of graft incorporation and 'ligamentization', which results in an atonic, disorganized, and non-viable graft. It is a complex pathological entity and any factor affecting graft revascularization, cellular repopulation, or matrix remodelling can lead to biological failure. Graft incorporation is influenced by many factors, primarily technical and biomechanical (Ménétrey et al., 2008). The graft undergoes a process of necrosis, followed by re-vascularization, cellular repopulation, collagen deposition, and finally matrix remodelling (George et al., 2006a). This ligamentization process is influenced by the graft source, the host response, and the biomechanical loads during rehabilitation. The rate of incorporation depend on both the type of graft material and the method of fixation. (Harner et al., 2000)

Shoulder instability

Diagnosis:

The glenohumeral joint balances mobility and stability (Matsen, Chebli, & Lippitt, 2006). The maintenance of the balance between stability and mobility of the glenohumeral joint requires the synchronous function of static and dynamic stabilizers. The static stabilizers include the capsular ligaments, the glenoid labrum, negative intra-articular pressure, and articular cartilage surface contact forces, while dynamic stabilizers include the rotator cuff and long head of biceps. Shoulder instability is not the same as joint laxity. Laxity is a necessary attribute of the capsule and ligaments of the shoulder and allows for the normal large range of motion of this joint. Instability, however, is abnormal symptomatic motion of the humeral head relative to the glenoid during active shoulder motion. Shoulder instability develops when the normal stabilizing mechanisms are disrupted. (Flatow & Warner, 1998) (Cordasco, 2000) (S. M. Johnson & Robinson, 2010).

The shoulder is the most commonly dislocated joint in the body, with traumatic anterior dislocation accounting for the vast majority (98%) of these injuries. Traumatic shoulder dislocations are associated with persistent deficits of shoulder function and a high risk of recurrent instability in young adults. Patients with traumatic shoulder instability experience recurrent dislocations or subluxations due to a structural weakness produced by the injury to the capsulolabral complex. They also develop secondary osseous lesions of the glenoid and the humeral head, which further compromise stability. Structural damage can occur in both the osseous architecture and the soft tissues surrounding the shoulder joint. In studies involving arthroscopic examination, a Bankart lesion was demonstrated in 87% to 100% of shoulders of patients with a first-time dislocation and a Hill-Sachs defect was seen in 90% to 100%. Important risk factors for recurrence were age and male sex. Early surgical stabilization should be considered for young athletes involved in collision sports after a

first-time shoulder dislocation. In the absence of osseous deficiencies, the results of arthroscopic repair for the treatment of traumatic anterior shoulder instability appear to be similar to the results of open repair. (Taylor & Krasinski, 2009) (C. M. Robinson, Jenkins, White, Ker, & Will, 2008)

Rehabilitation:

Rehabilitation aims to enhance the dynamic muscular and proprioceptive restraints to shoulder instability. Proprioceptive deficits have been shown for patients with traumatic anterior shoulder instability. The high incidence of recurrent shoulder dislocation in the adolescent population may be explained, in part, by the collagen profile of encapsulating shoulder tissues. Collagen is the major protein of ligaments and tendons. The changing ratio of collagen types I (stiff) and III (elastic) with age results in reduced risk of recurrent shoulder dislocations with ageing.(Hayes, Callanan, Walton, Paxinos, & Murrell, 2002)

Risk Factors for Recurrent Shoulder Dislocation:

A number of patient-related and injury related risk factors have been identified to contribute to the risk of recurrence following arthroscopic Bankart repair and capsular shift. Patient related risk factors include younger age at the time of surgery, (Balg & Boileau, 2007) (Porcellini, Campi, Pegreffi, Castagna, & Paladini, 2009) male sex (Porcellini et al., 2009), bilateral shoulder instability (O'Driscoll & Evans, 1991), joint hyperlaxity (S. M. Johnson & Robinson, 2010), participation in collision sports (Rhee, Ha, & Cho, 2006), and an early return to contact sports (S. H. Kim et al., 2003). Injury associated risk factors include glenoid erosion or deficiency, the size of the posterior humeral head defect (Hill-Sachs lesion), and whether the lesion is engaging the anterior aspect of the glenoid (Burkhart &

De Beer, 2000). Ahmed at el. identified that the percentage of glenoid bone loss and an engaging Hill-Sachs lesion were independently predictive of recurrence following arthroscopic Bankart repair and capsular shift. (Ahmed, Ashton, & Robinson, 2012)

Hypermobility and the risk of Shoulder injuries:

The incidence of primary anterior shoulder dislocation in the general population has been reported to occur 8.2 to 23.9 per 100,000 person-years. The most common sequelae arising from a first-time shoulder dislocation in younger patients is recurrent instability, with an overall mean rate of 67% (range, 17%-96%). The relation between ligamentous laxity or hypermobility and the overall occurrence of injury has not been examined in controlled trials, and the research that has been carried out has produced conflicting results. (Stewart & Burden, 2004) Several authors have speculated on a relationship between generalized joint hypermobility and glenohumeral joint instability. Cameron et al observed a relationship between measures of generalized joint hypermobility and a history of glenohumeral joint instability, regardless of the influence of sex and race. Participants with a Beighton Scale score of 2 or greater were nearly 2.5 times more likely to have experienced an episode of glenohumeral joint instability than were participants with lower scores. (Cameron et al., 2010). Chahal et al observed that generalized ligamentous laxity was more common in individuals with a primary traumatic anterior shoulder dislocation and may therefore be a significant risk factor for this injury. (Chahal et al., 2010)

It remains unclear why subjects with hypermobile joints have an increased susceptibility to injury. There is no doubt that there is an increased maximal stretch angle in the hypermobile muscle tendon unit with an enhanced tolerance to passive tension, but this has not been shown to have a direct link with muscular complications. (R. Smith et al., 2005) Individuals

who do not have any signs of generalized ligament laxity had significantly better joint-position and kinesthetic sense than individuals with generalized ligamentous laxity (Blasier, Carpenter, & Huston, 1994). These findings suggest that prophylactic rehabilitation programs may be able to decrease the risk of primary traumatic shoulder dislocations in individuals with generalized ligamentous laxity by improving joint-specific proprioceptive capabilities. (Chahal et al., 2010)

Generalized ligamentous laxity has been proposed as a significant risk factor for failure after arthroscopic anterior shoulder stabilization although there was no significant difference in patient-rated outcome in normal versus ligamentously lax patients undergoing arthroscopic anterior shoulder stabilization. (Koyonos et al., 2013)

Bony defects after shoulder dislocations and their assessment:

Anterior shoulder dislocation leads to bone loss on the anterior aspect of the glenoid and compression fracture of the posterosuperior aspect of the humeral head (Hill-Sachs deformity). Glenoid bone loss decreases the glenohumeral contact area. A reduced glenohumeral contact area may increase joint instability and the likelihood of further dislocation. (Griffith et al., 2008)

The prevalence of glenoid bone loss ranges from 41% after a first-time dislocation to 86% with recurrent dislocation. Postoperative recurrence can occur in up to 10% of cases. When bone loss is sufficiently severe, it will contribute to failure of a Bankart repair. Before surgery, quantification of glenoid bone loss would help in selecting the preferred operative procedure (arthroscopic Bankart repair versus bone block transfer) and may be beneficial in predicting the outcome of these procedures. Flattening of the anterior glenoid curvature is

shown in most patients with anterior dislocation and increases exponentially with increasing number of dislocations. CT scan of the shoulder should be performed after primary dislocation to apply the correct treatment early and avoid potential further dislocations. (Griffith, Antonio, Tong, & Ming, 2003)(Auffarth et al., 2013) It has been reported in the literature that an inverted-pear glenoid represents a loss of 25% of the diameter of the inferior glenoid. (Itoi, Yamamoto, Kurokawa, & Sano, 2013) The Latarjet procedure is commonly performed when a glenoid bony defect exists that is greater than 25 % of the glenoid width or when the risk of recurrent instability is higher (i.e., collision-sport athletes). (Itoi et al., 2013) (Pansard et al., 2013)

Hypothesis, Aims and Clinical Relevance

Hypothesis

There is a correlation between the clinical, mechanical and histological properties of tissues.

Research Question

Is there a correlation between the clinical, mechanical and histological properties of tissues?

Specific Aims

- Development of a Force-plate Goniometer system to assess the generalized joint hypermobility and compare the results of clinical testing with Beighton score with mechanical properties and Immunohistological markers (Collagen V, SLRP's) of tissue specimens (skin, tendon, capsule) obtained during surgery.
- To explore the possible link between anterior cruciate ligament injuries, shoulder dislocations, failure of ACL reconstruction, recurrent shoulder instability and generalized joint hypermobility.
- To study the role of CT scan in predicting shoulder instability.

Null Hypothesis:

There is no correlation between the clinical, mechanical and histological properties of tissues.

Clinical Relevance:

The results of this study will guide the orthopaedic surgeon in their treatment of ACL injuries and shoulder instability in the presence of generalized joint hypermobility. The findings will be of particular relevance, to advise sports persons about the risk of injuries and prevention strategies in the presence of generalized joint hypermobility. The results will help to develop a more reliable method to assess generalized joint hypermobility.

Clinical Studies

ACL Studies:

After obtaining informed consent to take part in the study, a consecutive series of patients with ACL injuries attending a specialist knee injury clinic at the Royal Infirmary of Edinburgh between June 2008 and July 2013 were studied. The control group was made up of an age and sex-matched cohort of patients with meniscal pathology or clavicle fractures. The control group was recruited from the fracture clinics within the same hospital. The cases and controls had no injuries to other joints, which might have confounded results. All patients approached to take part in the study were explicitly asked for their consent after discussion of the risks and benefits of the study. They also had the opportunity to discuss options further with a Consultant Orthopaedic Surgeon at the Royal Infirmary of Edinburgh, who agreed to act as an independent advisor during the study. If they did not wish to enter the study, the standard treatment was offered without any modification.

Design:

- Prospective observational study

Inclusion criteria:

- Age 18-40
- Patients who reside locally and present to the knee injuries clinic with confirmed anterior cruciate ligament rupture or failure of primary ACL reconstruction.
- Control group: Patients, who reside locally, and present to the fracture clinic with knee injuries (but with intact ACLs) or clavicle fractures.

Exclusion criteria:

- Temporary residents who received primary treatment in Edinburgh Royal Infirmary's emergency department but were unable to return for any further follow-up assessments.
- Patients with significant medical co morbidities.
- Patients presenting with anterior knee pain due to patellofemoral problems.
- Patients with confirmed diagnosis of severe connective tissue diseases for example Marfans syndrome, Systemic Lupus Erythematosis (SLE), Rheumatoid arthritis (RA).
- Patients who were unable to speak English.
- Patients with Learning disabilities.
- Patients with multiple ligament knee injuries

Clinical assessment:

A detailed history was taken from the patients attending the knee injury clinic about the mechanism of injury, duration of symptoms and their level of activity. The knee injuries were assessed by complete knee examination involving the anterior and posterior draw test for the cruciate ligaments and Mcmurray's test for meniscal injury.

Radiological assessment:

Plain film evaluation included AP and lateral views of the knee. If the clinical examination was not conclusive then further radiological investigations in the form

of an MRI scan was organized to confirm the ACL tear or failure of ACL Graft before any surgical intervention was performed.

Surgical Technique:

Patients undergoing primary ACL reconstruction had an arthroscopically assisted single incision procedure utilising a quadruple hamstring tendon autograft. Fixation was achieved using an endobutton (Smith and Nephew-UK) on the femoral side and Intrafix screw (PEEK) (Dupey-UK**)** on the tibial side. Revision ACL reconstruction was performed as a single-stage arthroscopically assisted procedure using a middle third patellar tendon autograft. Graft fixation was achieved using Softsilk or RCI interference screws (Smith and Nephew-UK**)** on both sides.

Causes of Failure of ACL Graft:

The revision ACL patients were evaluated to identify any technical errors at the time of the primary procedure or any traumatic injury that could have contributed to primary graft failure. Prior to the revision surgery, preoperative radiographs and MRI scans were obtained and examined for tunnel placement. The tunnel position was directly visualised at the time of revision surgery and incorrect placement documented. It was also noted whether the graft was intact but lax (non-functional) or whether it was ruptured at the time of the revision surgery.

Test population:

The test population consisted of (1) patients undergoing ACL reconstruction, (2) revision ACL reconstruction and (3) patients who had sustained an ACL tear, but had opted for non-operative management.

The test population comprised:

139 patients with ACL tears undergoing primary reconstruction.

44 consecutive revision ACL reconstruction cases, of which 28 patients were tertiary referral cases. All cases were operated on by a single surgeon at the same institution.

A subgroup of 28 patients undergoing primary ACL reconstruction with quadruple hamstring autograft were followed up at 4 years to assess the integrity of ACL graft.

Control group:

The control group comprised a cohort of 70 age and sex matched control subjects without any knee ligament injuries. 34 patients had meniscal injuries and 36 patients had clavicle fractures.

Shoulder instability:

A consecutive series of patients attending a specialist shoulder clinic with primary traumatic anterior shoulder dislocation or with chronic instability following failed conservative or operative treatment were prospectively studied between August 2008 and August 2009 at the Royal Infirmary of Edinburgh. The control group was made up of an age and sex-matched cohort of patients with clavicle fractures or meniscal pathology. The control group was recruited from the fracture clinics within the same hospital. The cases and controls had no injuries to other joints, which might have confounded the results. All patients approached to take part in the study were explicitly asked for their consent after discussion of the risks and benefits of the study. They also had the opportunity to discuss options further with a Consultant Orthopaedic Surgeon at the Royal Infirmary of Edinburgh, who agreed to act as an independent advisor during the study. If they did not wish to enter the study, the standard treatment was offered without any modification.

Design:

- Prospective observational study

Inclusion criteria:

1. Age 18-40
2. Study group: Patients, who resided locally, and presented to the shoulder injury clinic with dislocation of the shoulder.
3. Control group: Patients, who resided locally, and presented to the fracture clinic with knee injuries or clavicle fractures.

Exclusion criteria:

1. Patients with confirmed diagnosis of severe connective tissue diseases for example Marfans syndrome, Systemic Lupus Erythematosis (SLE), Rheumatoid arthritis (RA).
2. Patients with significant medical co morbidities.
3. Patients with Learning difficulties or unable to give consent.
4. Patients with multidirectional or atraumatic shoulder instability
5. Temporary residents who received primary treatment in the emergency department but were unable to return for any further follow-up assessments.
6. Patients unable to understand English.
7. Patients with fracture dislocation of the shoulder.

Clinical assessment:

Patients were initially evaluated with a complete history and physical examination.

The shoulder history included the mechanism of first time dislocation, number of dislocations, any previous surgical procedures performed, level, and frequency of symptoms including pain, instability, and level of function.

Physical examination of the shoulder focused on inspection for previous scars, a thorough determination of active and passive range of motion, evaluation of the integrity and strength of the rotator cuff, and a detailed examination for glenohumeral laxity in the anterior, posterior, and inferior directions.

Radiological assessment:

A comprehensive plain film evaluation was carried out, including AP and modified axial views of the involved shoulder. If these were not conclusive, then further radiological investigations in the form of an Ultrasound scan, CT scan or MRI scan were organized to define the bony architecture of the glenoid and humeral head, especially the details of any Hill–Sachs lesions. For the patients that had CT scans, a 3-dimensional reconstruction of the CT scan was arranged to assess the size and location of the defect and an estimation of the amount of the articulating arc of the humeral head that was involved.

Surgical Technique:

Each patient underwent diagnostic arthroscopy for the purpose of quantifying bone loss and identifying concomitant pathology (e.g., SLAP lesions) that would need to be addressed arthroscopically before open surgery. Diagnostic arthroscopy was performed under general anaesthesia with the patient in the beach chair position. The posterior viewing portal was created inferomedial to the posterolateral corner of the acromion, and the anterosuperior instrumentation portal was created lateral to the coracoid process, through the rotator interval. A complete evaluation of the capsular and osseous lesions was performed and the presence of a Bankart lesion or osseous glenoid rim erosion/avulsion were recorded. The labral detachment was completed, if necessary, with a Bankart rasp and electrocautery. The anterior aspect of the glenoid neck was then decorticated with use of a motorized shaver and burr to create a cancellous bed to encourage soft-tissue healing. Three to five holes were drilled from the eleven o'clock to the five o'clock position (of the right glenoid), depending on the size of the detachment of the capsulolabral complex. The drill-holes were placed at the margin of the articular surface to allow recreation of the

glenoid concavity. With use of the single anterior portal, a suture passer (Linvatec, Largo, Florida) was used to deliver a PDS suture (polydioxanone; Mitek, Johnson and Johnson, Berkshire, United Kingdom) through the detached capsulolabral complex. A Panalok absorbable anchor (Mitek, Johnson and Johnson) was placed onto the limb of the suture on the glenoid side, and this was then inserted in the most superior drill-hole. The arthroscopic core suture was then tied on the capsulolabral side, to keep the knot away from the articular surface. The same manoeuvre was then performed to pass the other anchors and sutures, proceeding in a superior-to-inferior direction. (C. M. Robinson et al., 2008). In the presence of large bone defects of the glenoid or the humeral head an open Bankart repair (with use of Biocryl rapide suture anchors) combined with an inferior capsular shift (with use of multiple number-2 Ethibond sutures) was performed.

Causes of Recurrent Shoulder instability:

Patients attending with recurrent shoulder instability were assessed clinically to assess if further traumatic injury has resulted in recurrent instability and radiologically with the help of plain radiography, MRI or 3-D CT scan to assess for large bony defects of the glenoid or humeral head. These patients were also assessed for signs and symptoms of hypermobility by using the Beighton scale and Brighton criteria. The presence of bony defects were noted during the arthroscopic assessment of the shoulder before open stabilization for recurrent instability. The shape of the glenoid was assessed to see if it approximated an inverted pear.

Test population:

The test population comprised:

44 patients with traumatic primary anterior shoulder dislocation.

59 consecutive recurrent anterior shoulder instability patients. All cases were operated on by a single surgeon at the same institution.

39 patients following first time traumatic anterior shoulder dislocation had a CT scan of the shoulder to identify structural defects of the glenoid and humeral head in the form of Glenoid bone defect, glenoid flattening and Hill sach's defect.

A subgroup of 38 patients with primary traumatic anterior shoulder dislocations were followed up at 4 years to assess for recurrent shoulder instability and effect of hypermobility on recurrence.

Control group:

The control group comprised a cohort of 54 age and sex matched control subjects without shoulder instability. The cases and controls had no injuries to other joints, which might have confounded the results.

Statistical Analysis:

Sample size:

We calculated that a total of 228 patients were required to be recruited. This was based on a standardized difference of 2.24 in the Beighton score. We will be able to

detect medium treatment effects at alpha = 0.05 and beta =0.2 giving a power of 80% to detect differences in joint hypermobility in our observational cohort and the control group. This would give 38 patients in each group; Meniscal Injury (control group), ACL injury, revision ACL reconstruction, clavicle fracture (control group), primary shoulder dislocation and recurrent shoulder dislocations.

Statistical analysis:

The Beighton scores for the different groups of knee patients (primary ACL reconstruction and revision ACL reconstruction) and shoulder patients (primary shoulder dislocations and recurrent shoulder dislocations) were compared against the control group (meniscal injuries or clavicle fractures). Statistical analysis was performed using SPSS for Windows (version 11.5; SPSS Inc., Chicago, Illinois). The chi-squared test was used to compare the number of cases with generalised joint hypermobility in the various groups using Beighton scores of 4 or more. Statistical significance was set at a p value of < 0.05. The Kruskal-Wallis test was used to assess if the components of the Beighton score were different in the knee and shoulder groups.

Joint Hypermobility Assessment:

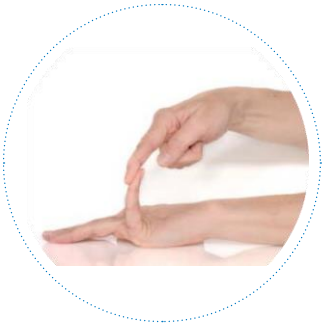
Beighton and Brighton Scores:

All patients attending specialist knee clinic for ACL or meniscal injuries or failure of ACL reconstruction or the specialist shoulder clinic for shoulder dislocation or fracture clinic with clavicle fracture were assessed for generalized joint

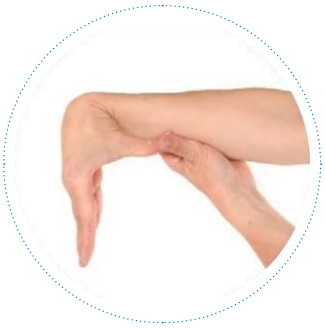
hypermobility by using the Beighton and Brighton scores. Patients were also asked about any other joints problems, arthralgias, easy bruising, or other signs of connective tissue diseases. The Beighton score (Beighton & Horan, 1969) was used to quantify the degree of joint hypermobility. The score is based on the presence or absence of physical signs associated with generalised joint hypermobility. The lowest score is 0 if there are no signs of laxity and the highest score of 9 is given for presence of all the signs. In accordance with recommendations of use of the score, subjects with a score of 4 or more were classified as having generalised joint hypermobility (Verity Pacey et al., 2010) (Lars Remvig & Juul-Kristensen, 2011) (Reider, 2012). Strict criteria were used to diagnose generalized joint hypermobility by using a score of 6 or more as previously suggested (Ramesh et al., 2005).

The affected Limb was not assessed as part of the clinical examination. An ‘‘injury allowance point’’ was used, whereby participants who tested positive for only one side of a bilateral test, but had a history of a significant injury to the contralateral joint, were presumed to be lax before that injury and were awarded an injury allowance point (Stewart & Burden, 2004)

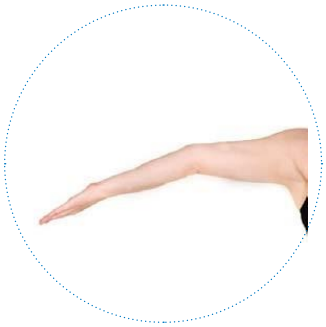
Beighton Score



1. Passive dorsiflexion



2. Passive apposition



3. Hypertension - elbow

4. Knee Hyperextension

5. Forward Flexion

	Movements	Scores	Max score
1	Passive dorsiflexion of little finger beyond 90 degrees	1 point each side	2
2	Passive apposition of thumb to flexor aspect of the forearm	1 point each side	2
3	Hyperextension of elbow beyond 10 degrees	1 point each side	2
4	Hyperextension of knee beyond 10 degrees	1 point each side	2
5	Forward flexion of the trunk with knees straight so that the palms of the hand rest easily on the floor	1 point	1
		POSSIBLE TOTAL	**9**

Figure 9 – Beighton Score

Results

Prevalence of Generalized joint hypermobility in groups of orthopaedic patients:

The total number of patients studied was 370 between April 2008 and June 2013. The mean age was 28 years with a range from 14-58 years. There were 293 males (79%) and 77 females (21%). The control group comprised of 41 patients (11%) with meniscal injuries and 43 (12%) with clavicle fracture. The ACL group comprised of 139 patients with 142 (37%) primary ACL reconstructions (3 patients had bilateral ACL reconstruction) and 44 (12%) with revision ACL reconstruction. The shoulder Group comprised of 44 (12%) patients with first time traumatic anterior shoulder dislocation, 38 (10%) with recurrent anterior shoulder dislocations and 21 (6%) with revision shoulder stabilization.

Control Group	**ACL Reconstruction Group**	**Shoulder Dislocation Group**
Meniscal Injuries = 41(11%)	Primary = 139 (37%)	First Time Dislocation = 44 (12%)
Clavicle fractures =43(12%)	Revision = 44 (12%)	Recurrent Dislocations = 38 (10%)
		Revision Shoulder Stabilization = 21(6%)

Table 2 – Different groups of orthopaedic patients

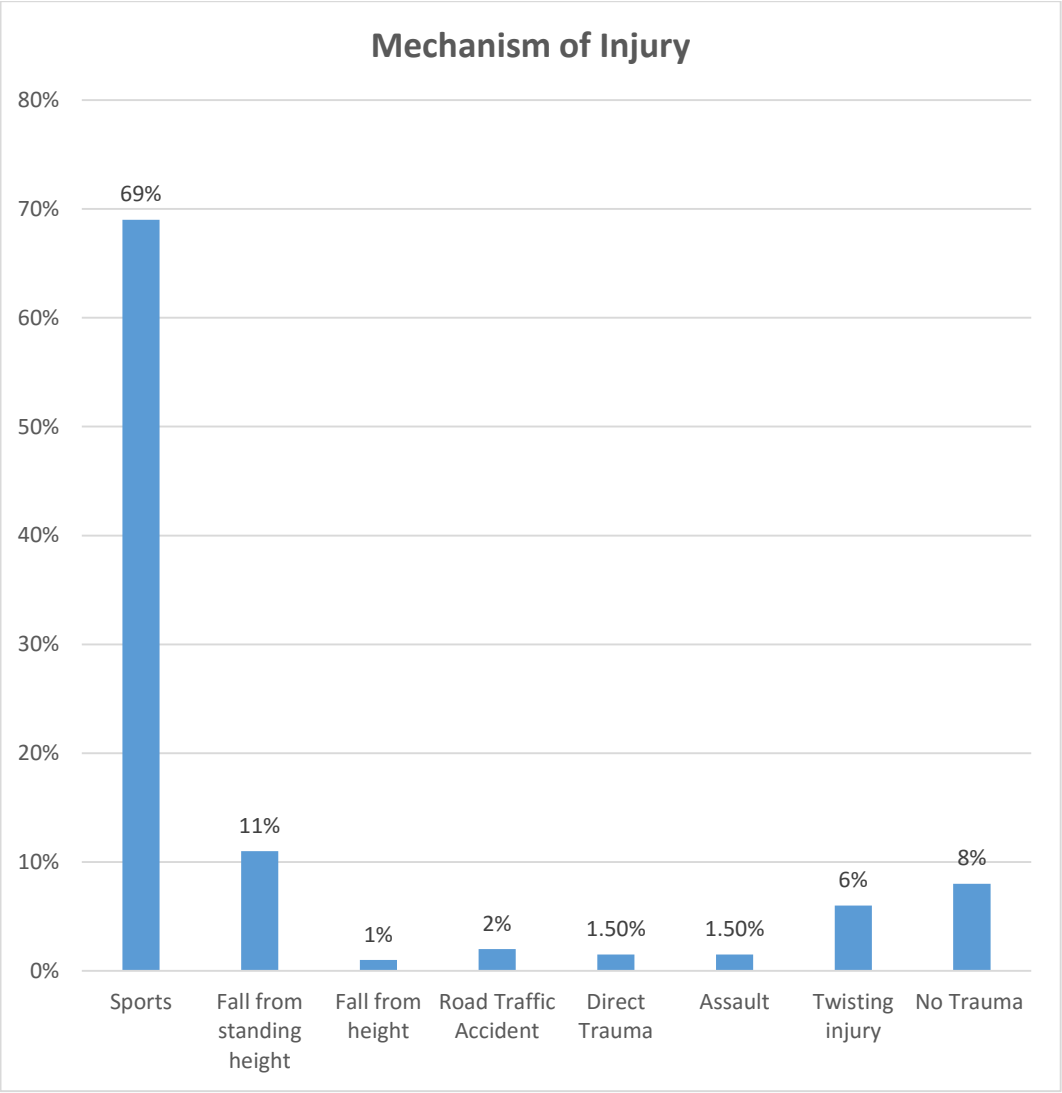


Chart 1 – Mechanism of Injury

The mechanism of injury was sports in 69%, fall from a standing height in 11%, fall from height in 1%, Road traffic accidents (RTA) in 2%, no trauma in 8%, direct trauma in 1.5%, assault in 1.5% and twisting injury in 6%.

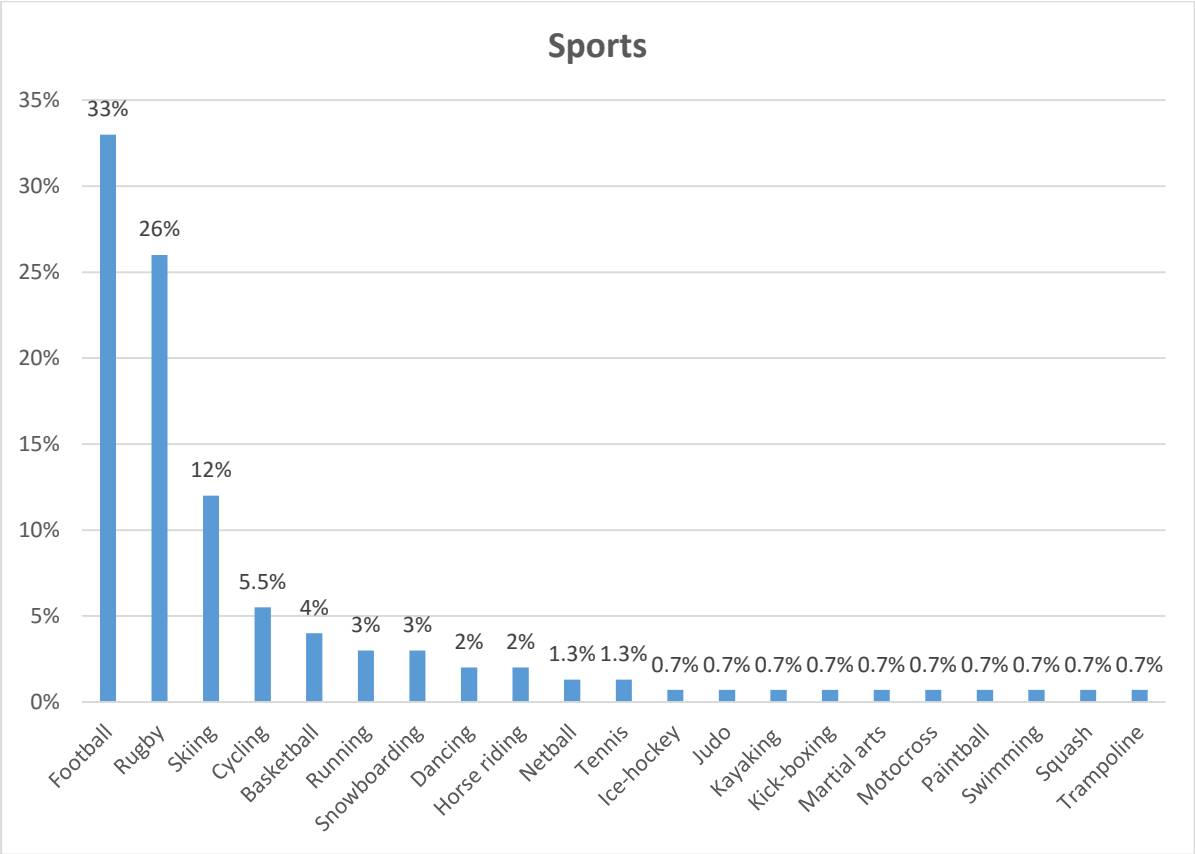


Chart 2- Sports Played

The most common sport being played when the injury occurred was football in 33%, followed by rugby in 26%, skiing in 12%, cycling in 5.5%, basketball in 4%, running in 3%, snowboarding in 3%, dancing in 2%, horse riding in 2%, netball in 1.3%, tennis in 1.3%, ice hockey in 0.7%, judo in 0.7%, kayaking in 0.7%, kickboxing in 0.7%, martial arts in 0.7%, motocross in 0.7%, paintball in 0.7%, swimming in 0.7%, squash in 0.7% and trampoline in 0.7%.

The involved limb was Right in 53%, left in 42% and Bilateral in 5%.

The mean Beighton score was 2.9 (range 0-9) and median was 2. 39% patients had a Beighton score of 4 or above and 19 % had a Beighton score of 6 or above.

a- Control Group: (Meniscal Injury / Clavicle Fracture)

The mean age was 34 years with a range from 15-58 years. There were 69 males (82%) and 15 females (18%).

The mechanism of injury was sports in 59%, fall from a standing height in 11%, no trauma in 16% and twisting injury in 14%. The involved limb was Right in 57%, left in 40% and Bilateral in 3%.

The mean Beighton score for the control group patients was 1.7 (range 0-8) and median was 1. 20% patients had a Beighton score of 4 or above and 3.5% had a Beighton score of 6 or above.

b- ACL Reconstruction Group:

The mean age was 28 years with a range from 15-57 years. There were 134 males (72%) and 52 females (28%).

The mechanism of injury was sports in 80%, twisting injury in 9%, fall from height in 2%, no trauma in 4%, RTA in 3% and direct trauma in 1%. The involved limb was Right in 55%, left in 44% and Bilateral in 1%.

The most common sport played was football in 40%, followed by rugby in 17%, skiing in 18%, cycling in 4%, basketball in 5.6%, running 1.4%, Snowboarding 1.4%, dancing 2.8%, Horse riding 2.8%, netball 2.8%, squash 1.4%, trampoline 1.4% and paintball 1.4%.

The mean Beighton score for the ACL reconstruction group patients was 3 (range 0-9) and median was 3. 41% patients had a Beighton score of 4 or above and 25% had a Beighton score of 6 or above.

c- Shoulder Dislocation Group:

The mean age was 27 years with a range from 14-58 years. There were 92 males (89%) and 11 females (11%).

The mechanism of injury was sports in 62%, fall from a standing height in 22%, no trauma in 8%, RTA in 1%, direct trauma in 2% and assault in 3.5%. The involved limb was Right in 48%, left in 42% and Bilateral in 10%.

The most common sport played was rugby in 44%, followed by football in 28%, snowboarding in 5.6%, skiing in 3.7%, cycling in 3.7%, tennis in 3.7%, basketball in 1.8%, dancing 1.8%, ice hockey 1.8%, kayaking 1.8%, martial arts 1.8% and swimming 1.8%.

The mean Beighton score for the Shoulder dislocation group patients was 3.4 (range 0-9) and median was 3. 49.5% patients had a Beighton score of 4 or above and 22% had a Beighton score of 6 or above.

d- Components of Beighton score in shoulder and knee injury groups:

The Kruskal-Wallis test showed that in the patients with knee as compared to shoulder injuries the thumb and little finger components of the Beighton score were different between groups with a P value of 0.02 for little finger and 0.04 for the

thumb while in patients with shoulder girdle injuries the knee component of Beighton score was different with a p value of 0.001. These results suggest different aetiologies in shoulder and knee injuries.

	Control Group	ACL Reconstruction Group	Shoulder Dislocation Group
Mean Age (years)	34 (15-58)	28 (15-57)	27 (14-58)
Sex			
Male	69 (82%)	134 (72%)	92 (89%)
Female	15 (18%)	52 (28%)	11 (11%)
Mechanism of Injury			
Sports	59%	80%	62%
Fall from standing height	11%	0	22%
Fall from height	0	2%	0
RTA	0	3%	1%
No Trauma	16%	4%	8%
Direct Trauma	0	1%	2%
Assault	0	0	3.5%
Twisting Injury	14%	9%	0
Beighton Score			
Mean	1.7 (0-8)	3 (0-9)	3.4 (0-9)
Median	1	3	3
Hypermobility			
BS=/>4	20%	41%	49.5%
BS=/>6	3.5%	25%	22%
BJHS	9%	13%	24%
Family History of laxity	10%	13.5%	19%
Side involved			
Right	57%	55%	48%
Left	40%	44%	42%
Bilateral	3%	1%	10%
Common Sports played			
Football	NA	40%	28%
Rugby	NA	17%	44%
Skiing	NA	18%	3.7%
Cycling	NA	4%	3.7%

Table 3- Patient Demographics, Mechanisms of Injury, Beighton scores and sports played

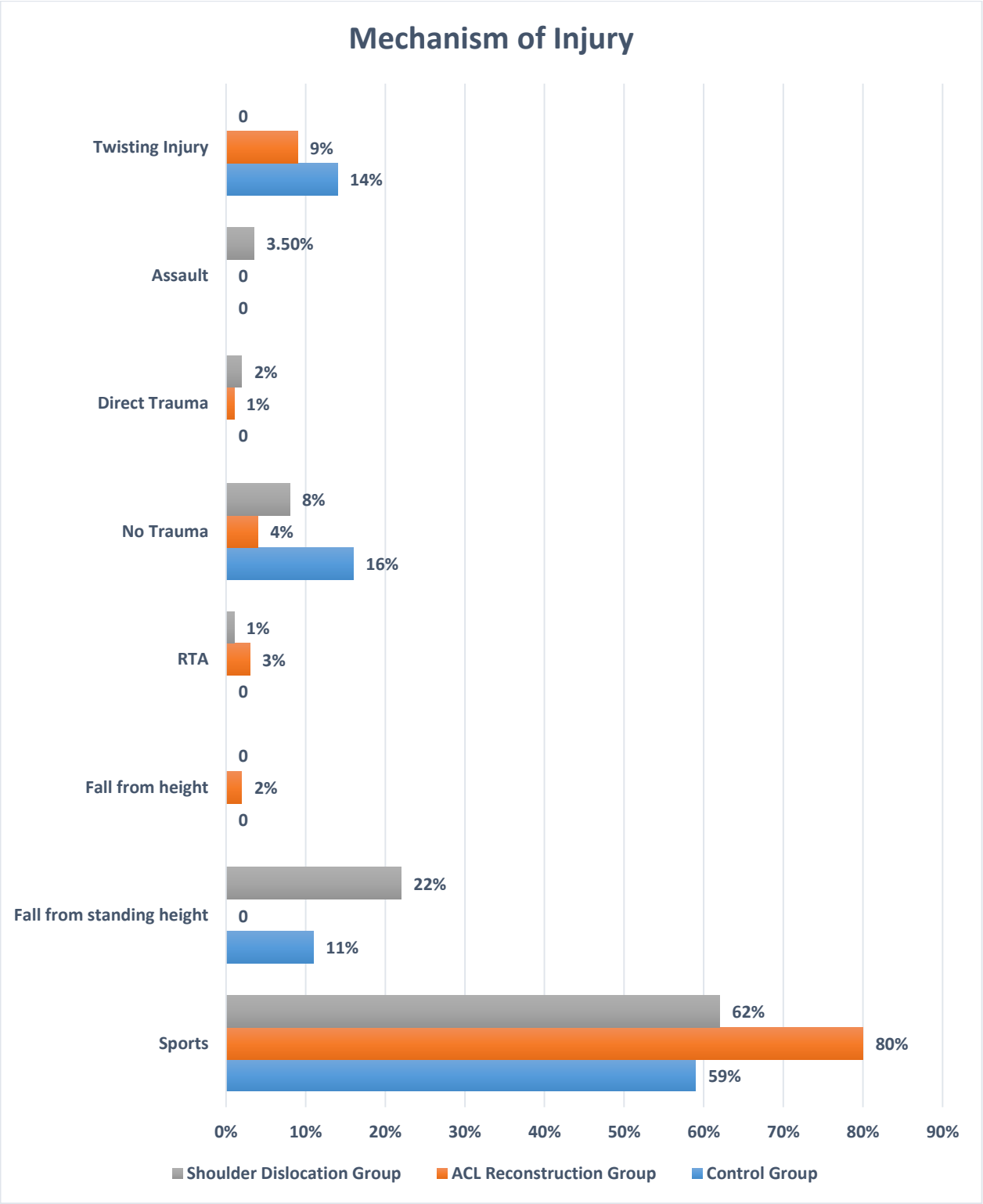


Chart 3- Mechanism of Injury in Different Groups of Orthopaedic patients

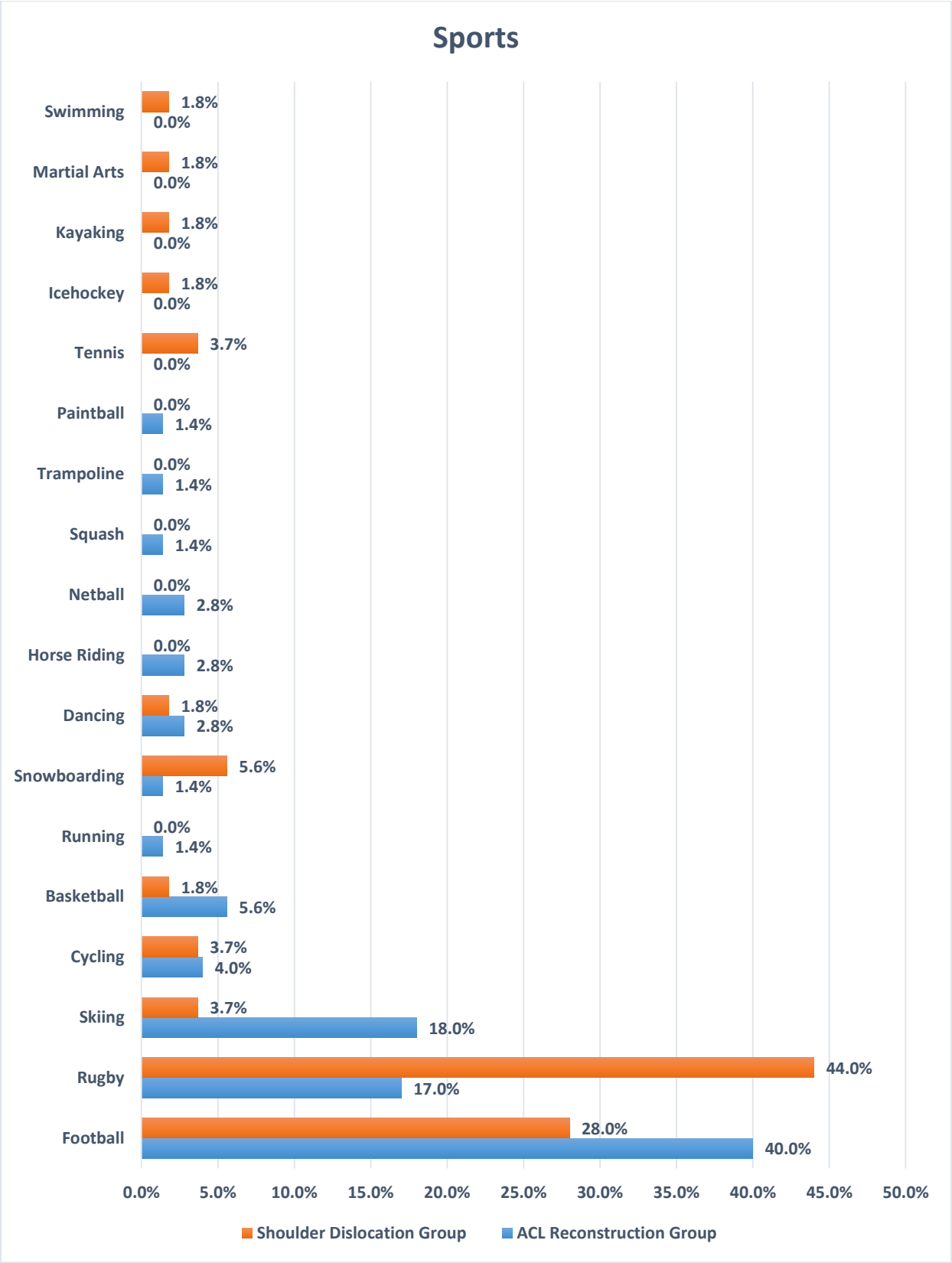


Chart 4- Sports Played in Shoulder and Knee groups

Prevalence of Benign Joint Hypermobility Syndrome (BJHS) in groups of orthopaedic patients:

There were 16% patients diagnosed with BJHS according to the Brighton score. The mean age of this group was 29 years (range 17-58). There were 33 male patients (72%) and 13 were female (28%). The mechanism of injury was sports in 67%, no trauma in 12%, fall from standing height in 12%, twisting injury in 6% and assault in 3%.

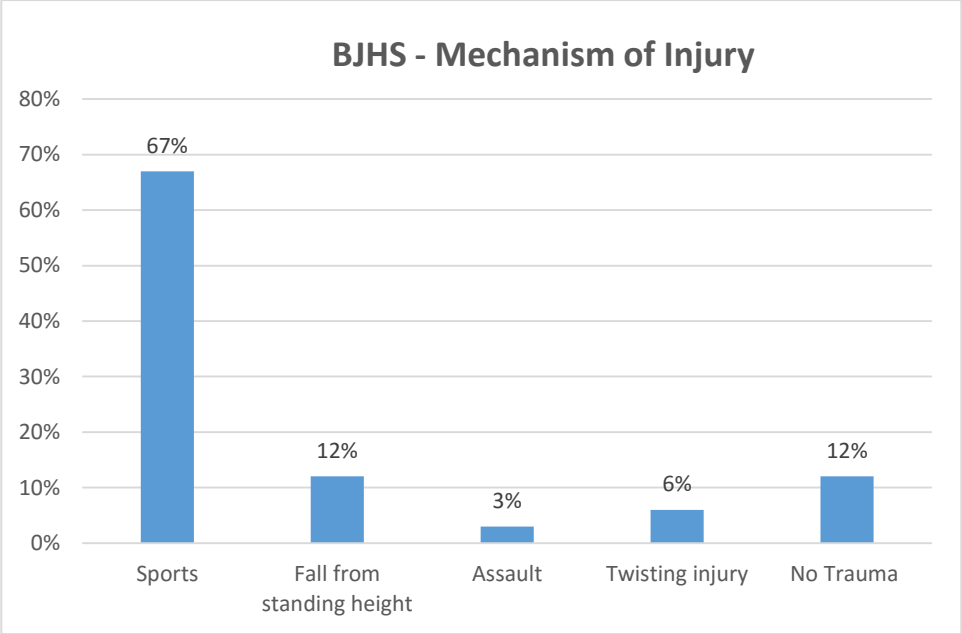


Chart 5- BJHS – Mechanism of Injury

- There were 62% patients involved in contact sports and most common sport played was football in 27% followed by rugby in 23%, skiing in 13.6%, basketball in 9%, dancing in 9%, cycling 4.5%, horse riding 4.5%, snowboarding 4.5%, ice hockey 4.5%.
- 74% patients had a Beighton score of 4 or above and 39% had a Beighton score of 6 or above.
- 45% patients had a flexible first degree relative.

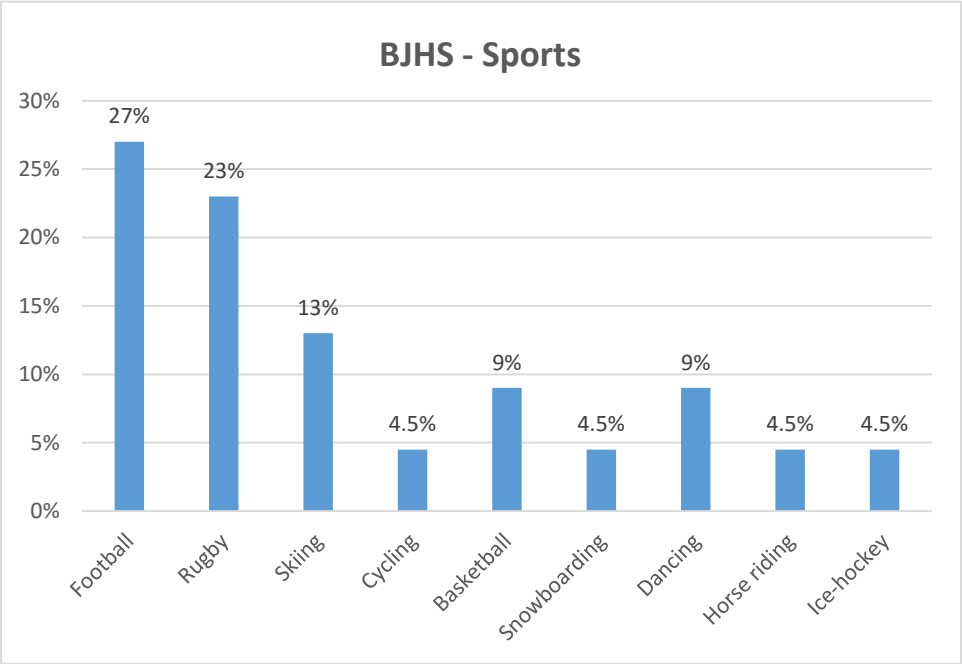


Chart 6- BJHS patient's participation in Sports

- In the control group 9% patients fulfilled the criteria for BJHS and 10% had a flexible first degree relative.
- 13% patients in the ACL reconstruction group fulfilled the criteria for BJHS and 13.5% had a flexible first degree relative.
- 24% patients in the shoulder dislocation group fulfilled the criteria for BJHS and 19% had a flexible first degree relative.

	Control Group	**ACL Reconstruction Group**	**Shoulder Dislocation Group**
BJHS	9%	13%	24%
Family History of Laxity	10%	13.5%	19%

Table 4- BJHS in different groups

There were 8.7% patients with meniscal injuries, 19.5% had primary ACL reconstruction, 11% had revision ACL reconstruction, 6.5% had clavicle fracture, 13% had first time shoulder dislocation, 17.3% had recurrent shoulder dislocations and 24% had revision shoulder stabilization.

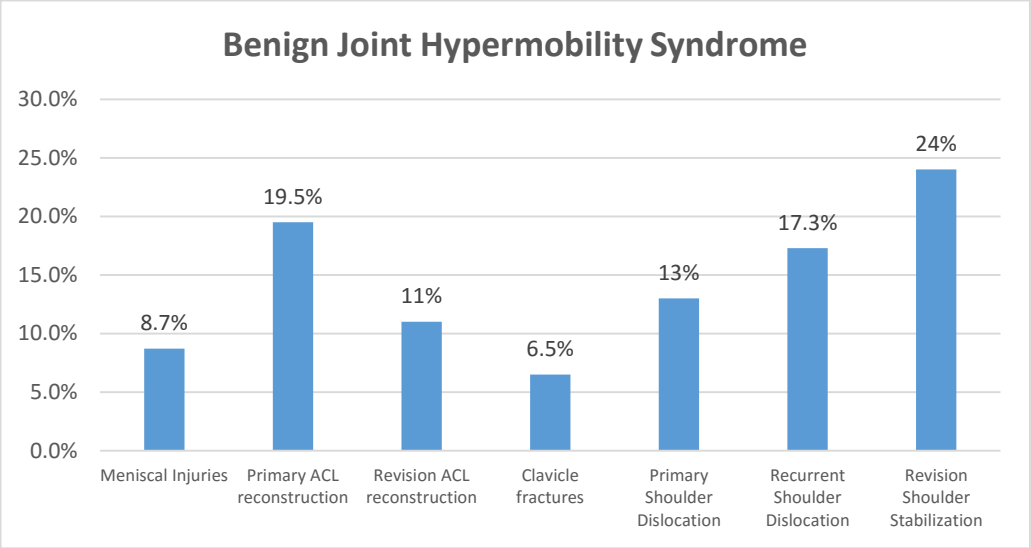


Chart 7- BJHS in different groups of Orthopaedic Patients

Meniscal Injuries and Generalised joint hypermobility:

The average Beighton score for the 41 patients with meniscal injuries was 1.5 with a range from 0-8. 4 patients (12%) in this group had a Beighton score of 4 or more as compared to control group of 43 clavicle fracture that had 8 patients (18%) There was no statistically significant difference between the 2 groups with a P value of 0.24. 9% patients fulfilled the Brighton criteria for BJHS. The most common cause of meniscal injury was sports related injuries in 46% patients. The most common sport was football in 38% patients. The most common procedure performed was partial medial menisectomy in 72% patients.

ACL reconstruction and Generalised joint hypermobility

The primary ACL reconstruction group had a mean age of 28 years (range 15 – 57 years) and a mean Beighton score of 2.9 (median 2). There were 100 males and 39 females. The mean duration from injury to surgery was 17 months.

The revision ACL group had a mean age of 28 years (range 16 – 51 years) and a mean Beighton score of 4 (median 4). There were 29 males and 15 females. The mean time to revision surgery following the primary procedure was 68 months (range 7 months to – 312 months). The choice of graft used for primary ACL reconstruction was variable and included quadruple hamstring tendon, bone patella tendon and fascia lata.

In 21 cases (48%) of revision surgery, there was an identifiable cause of primary graft failure. This included 1 case where concomitant MCL laxity was not addressed at the primary procedure resulting in persistent symptomatic instability. In the other 20 cases there was a significant further traumatic injury to the knee that was associated with rupture of the original graft. In the remaining 23 cases no clear reason for graft failure could be identified, and these were classified as "biological failures". In this group 17 patients were noted to have an intact ACL graft which was lax at the time of revision surgery. The Biologic failure group had a mean age of 28 years (range 16 – 50 years) and a mean Beighton score of 4.6 (median 4). There were 14 males and 9 females.

The control group had a mean age of 33 years (range 15 – 58 years) and a mean Beighton score of 1.4 (median 1). There were 57 males and 13 females.

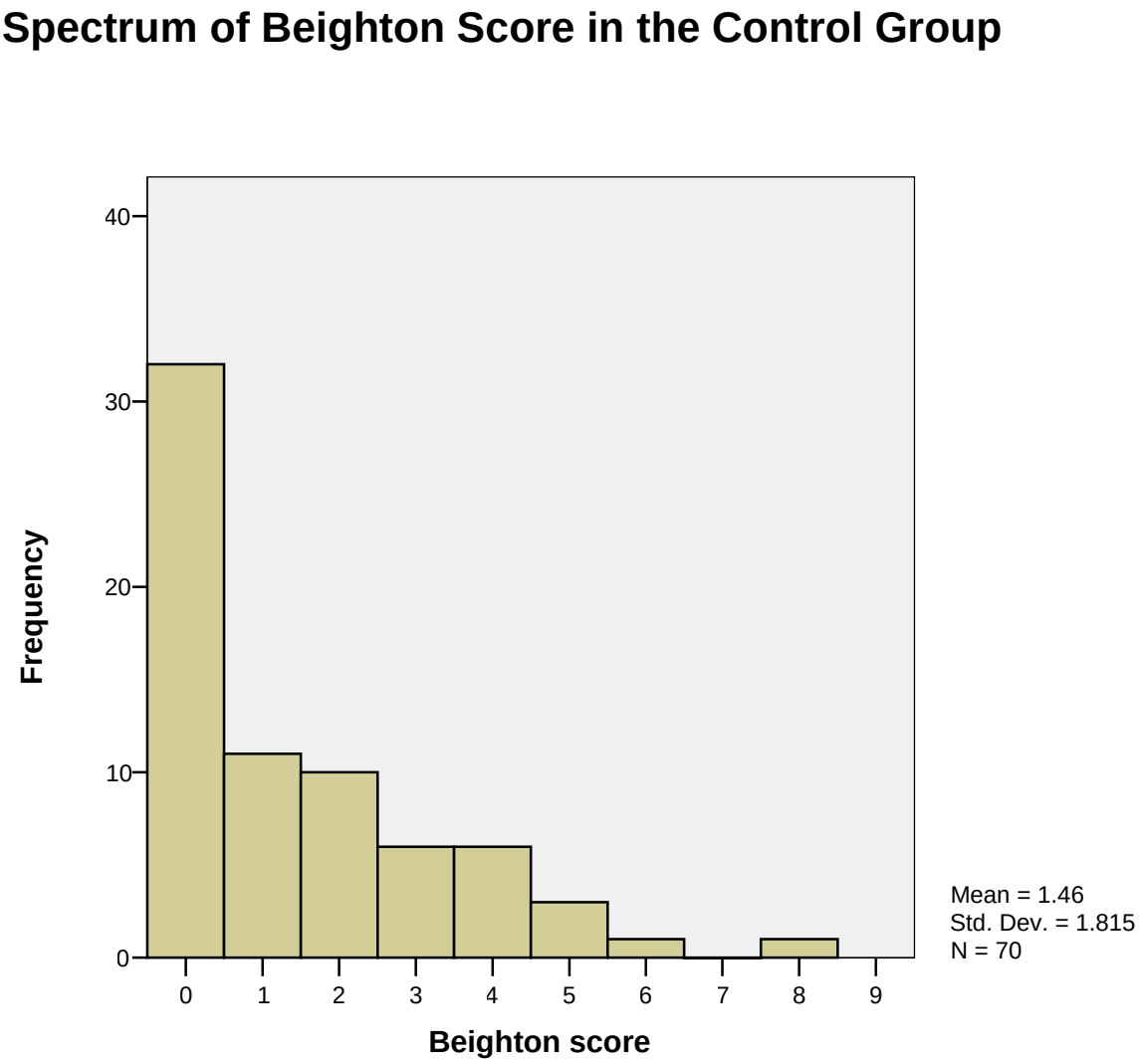


Chart 8- Control Group Beighton Score

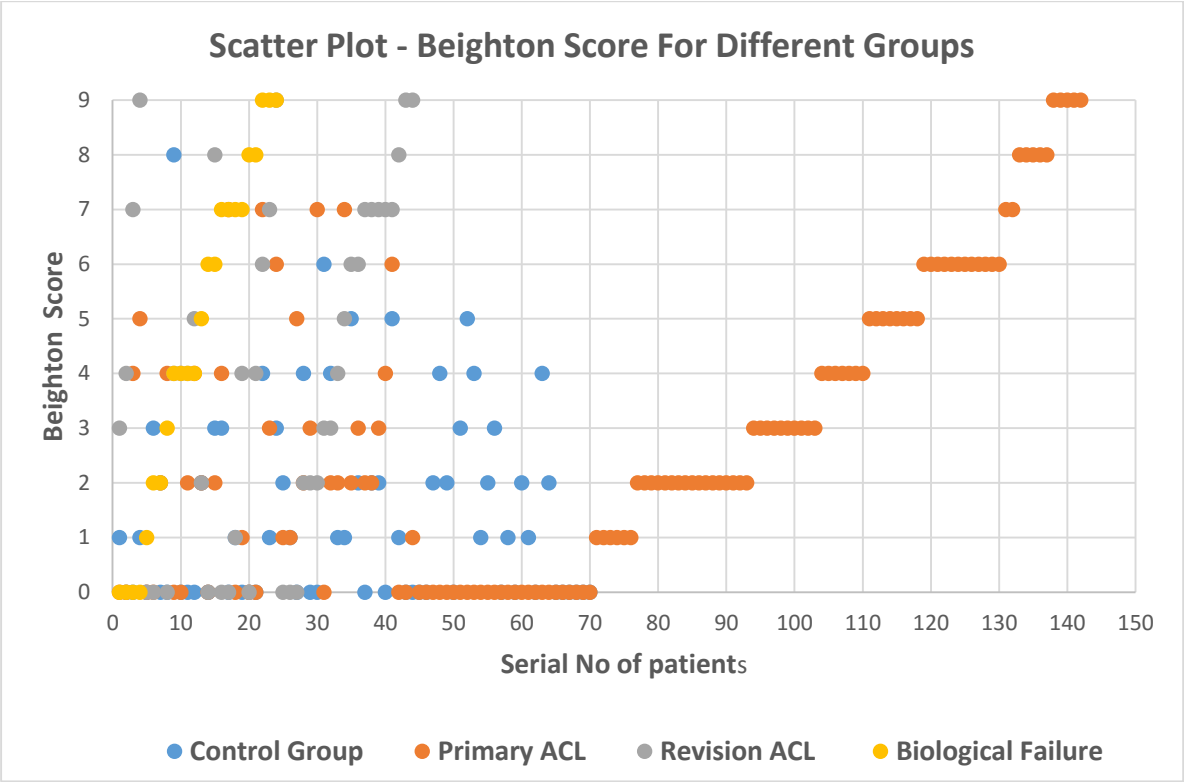


Chart 9- Beighton Score for different groups of Orthopaedic patients

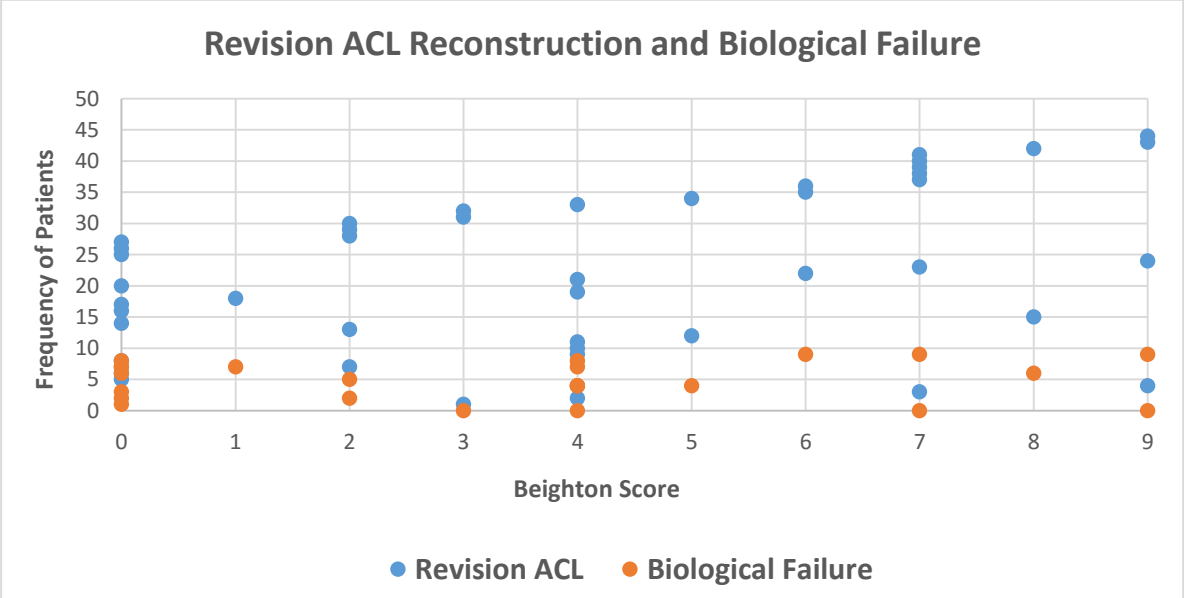


Chart 10- Beighton Score in patients with failure of ACL reconstruction & Biological Failure

The primary ACL surgery group was associated with an increased incidence of generalised joint hypermobility with higher Beighton scores compared to the control group. The mean Beighton score was 2.9 (median 2) and this difference was statistically significant ($p = 0.002$). Similarly the revision surgery group was also associated with increased generalised joint hypermobility compared to the control group ($p < 0.001$). The mean Beighton score in the revision ACL group was 4 (median 4).

Spectrum of Beighton Scores in patients Undergoing Primary ACL Reconstruction

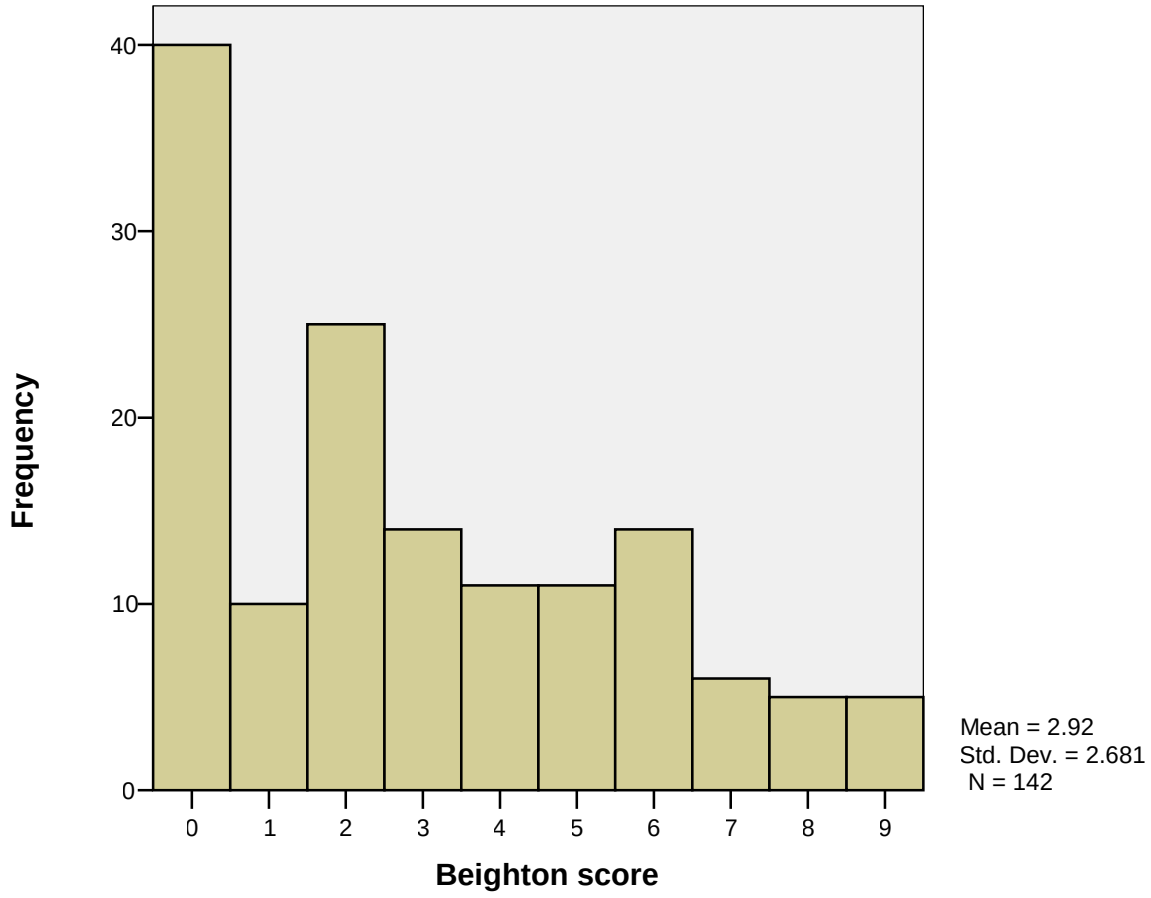


Chart 11- Beighton Score and Primary ACL Reconstruction

The revision ACL surgery group was also associated with increased generalised joint hypermobility when compared to the primary ACL surgery group and this difference was statistically significant p = 0.019. There was a subgroup within the revision cohort, who had a failure of the original surgery due to biological failure of the primary graft. The incidence of generalised joint hypermobility in this group as defined by the Beighton score was also significantly higher than the primary surgery group (4.4 vs 2.9 p = 0.010).

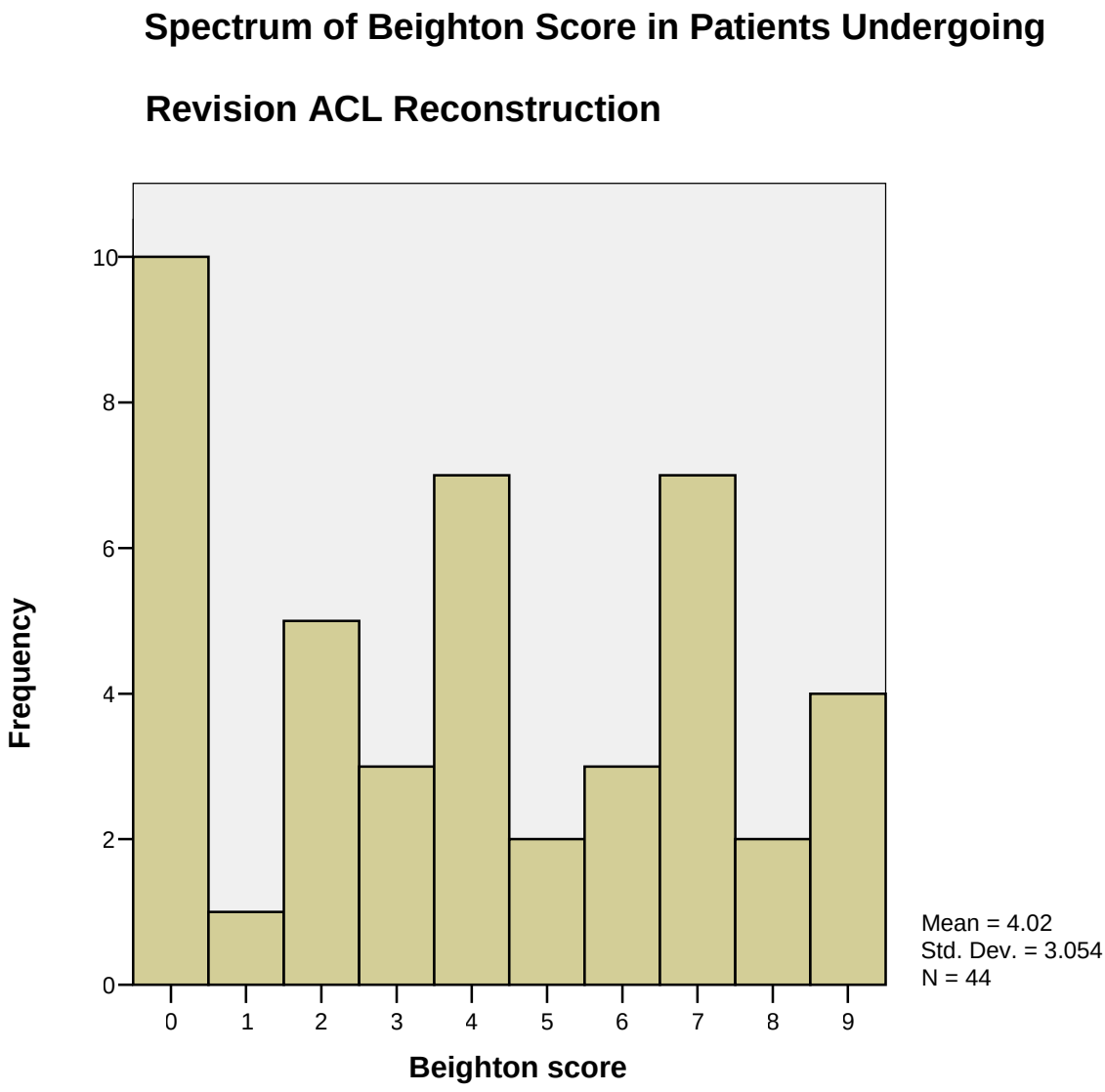


Chart 12- Beighton Score and Revision ACL Reconstruction

When Generalized joint hypermobility was considered using a strict criteria of Beighton score 6, the proportion with Generalized joint hypermobility was greater in the primary ACL reconstruction group compared with the control group (p = 0.001). It was also higher in the revision ACL reconstruction group as compared to the control group (p<0.001). There was a significant difference between the revision and primary ACL reconstruction groups (p=0.043). There was also a difference between Biological failure group and primary ACL reconstruction Group (p= 0.006).

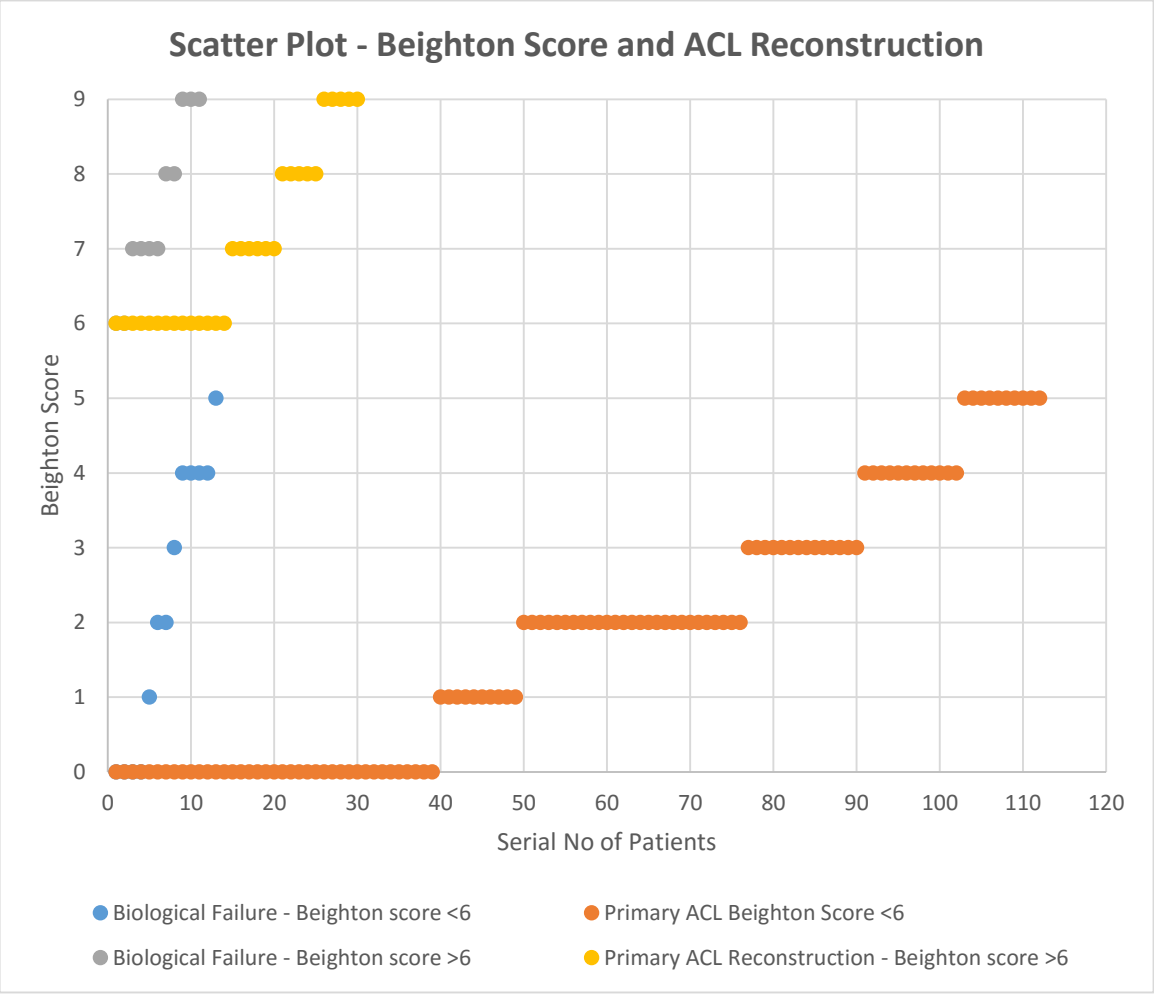


Chart 13- Beighton Score and ACL reconstruction Scatter Plot

	Control Group	**Primary ACL Group**	**Revision ACL Group**	**Biological Failure Group**
Total No of patients	70	139	44	23
Mean Age	33 (15-58)	28 (15-57)	28 (16-51)	28 (16-50)
Male	57 (81%)	100 (73%)	29 (66%)	14 (61%)
Female	13 (19%)	39 (27%)	15 (34%)	9 (39%)
Mean Beighton Score (Standard Deviation)	1.4 (1.8)	2.9 (2.6)	4 (3)	4.6 (3.2)
Median Beighton Score	1	2	4	4
Generalized Ligament Laxity BS > 4	11 (16%)	52 (37%)	25 (57%)	15 (65%)
Generalized Ligament Laxity BS > 6	2 (3%)	30 (21%)	16 (36%)	11 (48%)

Table 5- Demographics and Beighton score of patients undergoing ACL reconstruction

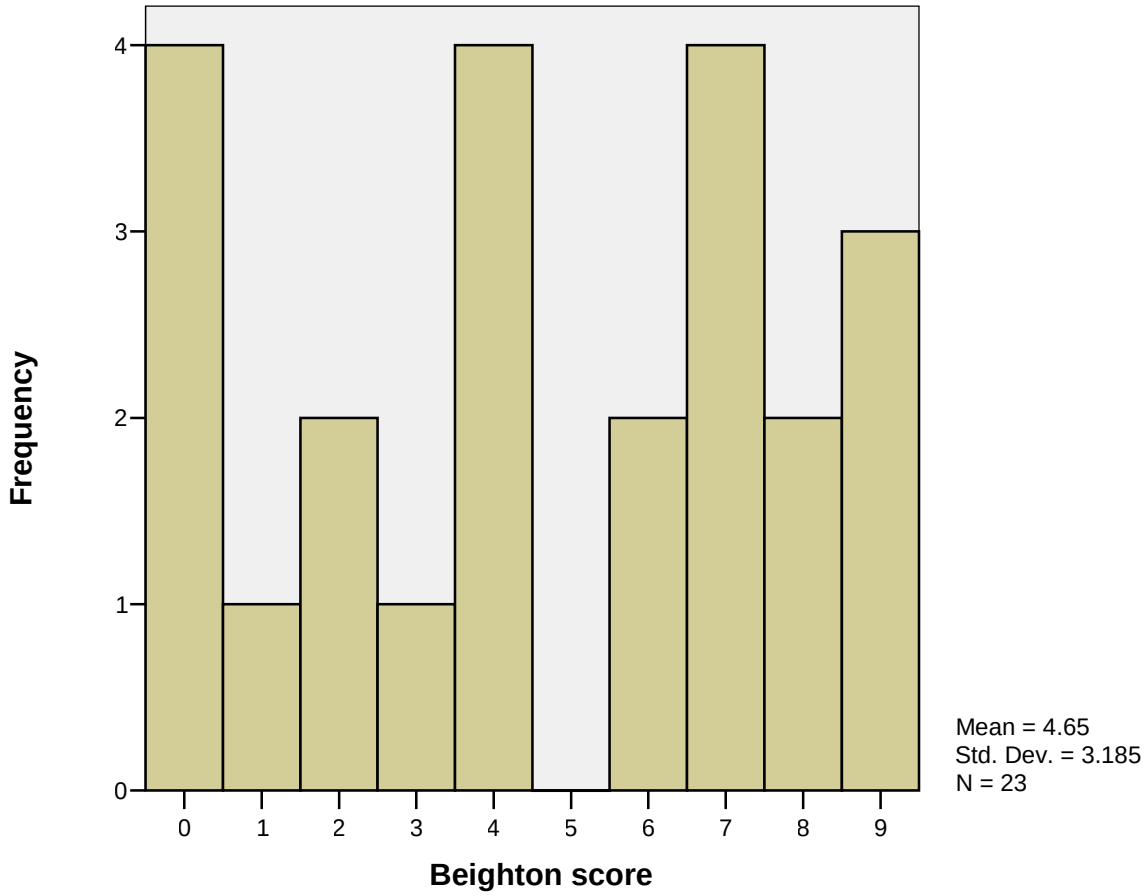


Chart 14- Beighton Score and Biological Failure

Hypermobility- a risk factor for failure following ACL reconstruction:

The primary ACL reconstruction follow-up group at 4 years had a mean age of 27 years (15-54 years). There were 19 males and 9 females. 24 patients (86%) were involved in contact sports of which football was the most common sport played (9 patients (37%)). The mean Beighton score for these patients was 2.8 (range 0-9). 10

patients in this group had a Beighton score of 4 or more (Group A) and 5 patients had a Beighton Score of 6 or more. 18 patients (64%) had Beighton score less than 4 (Group B).

The mean age for Group A (10 patients) was 26 years (range 14-40). There were 4 male and 6 females. 9 patients were involved in contact sports. 3 patients (30%) had failure of ACL reconstruction. The mean Beighton score for these patients was 5.9 (range 4-9). 2 patients had a Beighton Score of 7 and 1 with Beighton Score 5. 3 patients in this group had Benign Joint Hypermobility Syndrome.

The mean age for Group B (18 patients) was 28 years (range 15-54). There were 15 males and 3 females. 15 patients were involved in contact sports. The mean Beighton score was 1 (range 0-3). No patient had failure of ACL reconstruction.

	Group A (BS 4 or >)	**Group B (BS<4)**
Total No of Patients	10	18
Mean Age	26 (14-40)	18 (15-54)
Sex		
Male	4	15
Female	6	3
Contact Sports		
Yes	9	15
No	1	3
Failure of ACL reconstruction	3 (30%)	0
Mean Beighton score	5.9 (4-9)	1 (0-3)
BJHS	3 (30%)	0

Table 6- Demographics, Beighton score and failure of ACL reconstruction

Revision ACL reconstruction: Causes of failure and Graft Choices

The mean age of 98 patients undergoing revision ACL reconstruction between 1996 and 2010 was 26 years (range 13-50). There were 70 males (71%) and 28 females (29%). The primary surgery was performed in 71% cases in the teaching hospital locally and 29% were tertiary referrals from other hospitals in the region. In 42% cases the senior surgeon performed the primary ACL reconstruction and 58% patients were referred from the practice of other orthopaedic consultants. The primary surgery was performed between 1982 and 2008.

The graft used for primary ACL reconstruction was quadruple hamstring in 47%, patella tendon in 28%, double hamstring in 11%, synthetic graft in 8%, allograft (patella tendon) in 2.7%, fascia lata and iliotibial band in 1.4% cases each. 10 Patients (10%) had associated surgery performed with ACL reconstruction; 8 had partial medial menisectomy and 1 had subtotal lateral menisectomy and 1 had medial meniscal repair and lateral menisectomy.

73% patients were involved in sports. The mean time from primary surgery to failure of ACL reconstruction was 41 months (range 2-232). The cause of failure was trauma in 46% cases, Technical in 7%, Trauma and additional cause (technical) in 5%, infection in 2% and biological in 40% where no identifiable cause of failure was found. During revision surgery 42% cases had ACL graft completely ruptured, 8% had partial disruption of the ACL graft and 50% had a redundant ACL graft which was in continuity but avascular, lax and non-functional.

The choice of graft for revision ACL reconstruction surgery performed by the senior author was patella tendon graft in 58% of cases, allograft in 23%, (Achilles in 7% and patella tendon in 16%) quadruple hamstring in 15%, quadriceps tendon in 2% and double hamstring and synthetic graft in 1%. 25% patients had associated surgery performed with revision ACL reconstruction. 48% had partial medial menisectomy, 16% had partial lateral menisectomy and 4% each had lateral meniscal repair, medial meniscal repair, partial medial and lateral menisectomy, medial collateral ligament repair, mosaicplasty, partial medial menisectomy and mosaicplasty, partial medial menisectomy and medial collateral ligament repair, posterolateral corner repair and posterior cruciate ligament reconstruction and posterolateral corner repair.

The groups were divided into redundant ACL (Group A – 36 patients) if the ACL Graft was in continuity but lax and non-functional and ruptured ACL (Group B – 36 patients) if the graft was not in continuity on arthroscopic examination during the revision surgery.

The mean age for group A was 25.7 years (range 13-50). 69% were male and 31% female. The grafts used for primary ACL reconstruction were Quadruple hamstring tendon 55%, patella tendon in 24%, double hamstring in 7%, allograft in 7%, iliotibial band in 3.5% and synthetic graft in 3.5%. The mean time from primary surgery to graft failure was 41 months (2-232). The causes of failure of primary ACL reconstruction was trauma in 43%, technical failure in 3%, trauma and technical failure in 11%, and biological failure in 43%. 35% patients were involved in sports. The choice of revision ACL graft was patella tendon in 61%, allograft in 22% and quadruple hamstring in 17%. 4 patients had associated surgery during the

primary ACL reconstruction and 9 patients had associated surgery during the revision ACL reconstruction.

The mean age for group B was 25.9 years (range 15-47). 80% were male and 20% female. The grafts used for primary ACL reconstruction were Quadruple hamstring tendon 53%, patella tendon in 25%, double hamstring in 12.5% and synthetic graft in 9.5%. The mean time from primary surgery to graft failure was 38 months (2-120). The causes of failure of primary ACL reconstruction was trauma in 58%, technical failure in 11%, trauma and technical failure in 3%, infection in 6% and biological failure in 22%. 35% patients were involved in sports. The choice of revision ACL graft was patella tendon in 56%, allograft in 28%, double hamstring and synthetic graft in 3% and quadruple hamstring in 14%. 3 patients had associated surgery during the primary ACL reconstruction and 9 patients had associated surgery during the revision ACL reconstruction.

The mean age and gender difference were similar in both groups. The duration of time from primary ACL reconstruction to failure was 41 months in group A and 38 months in group B ($p = 0.69$). The biological failure was higher in the redundant ACL group 43% vs 22% ($p= 0.04$). The mean Beighton score was higher in redundant ACL Group 4.2 vs 1.8 but this difference was not statistically significant ($P= 0.07$).

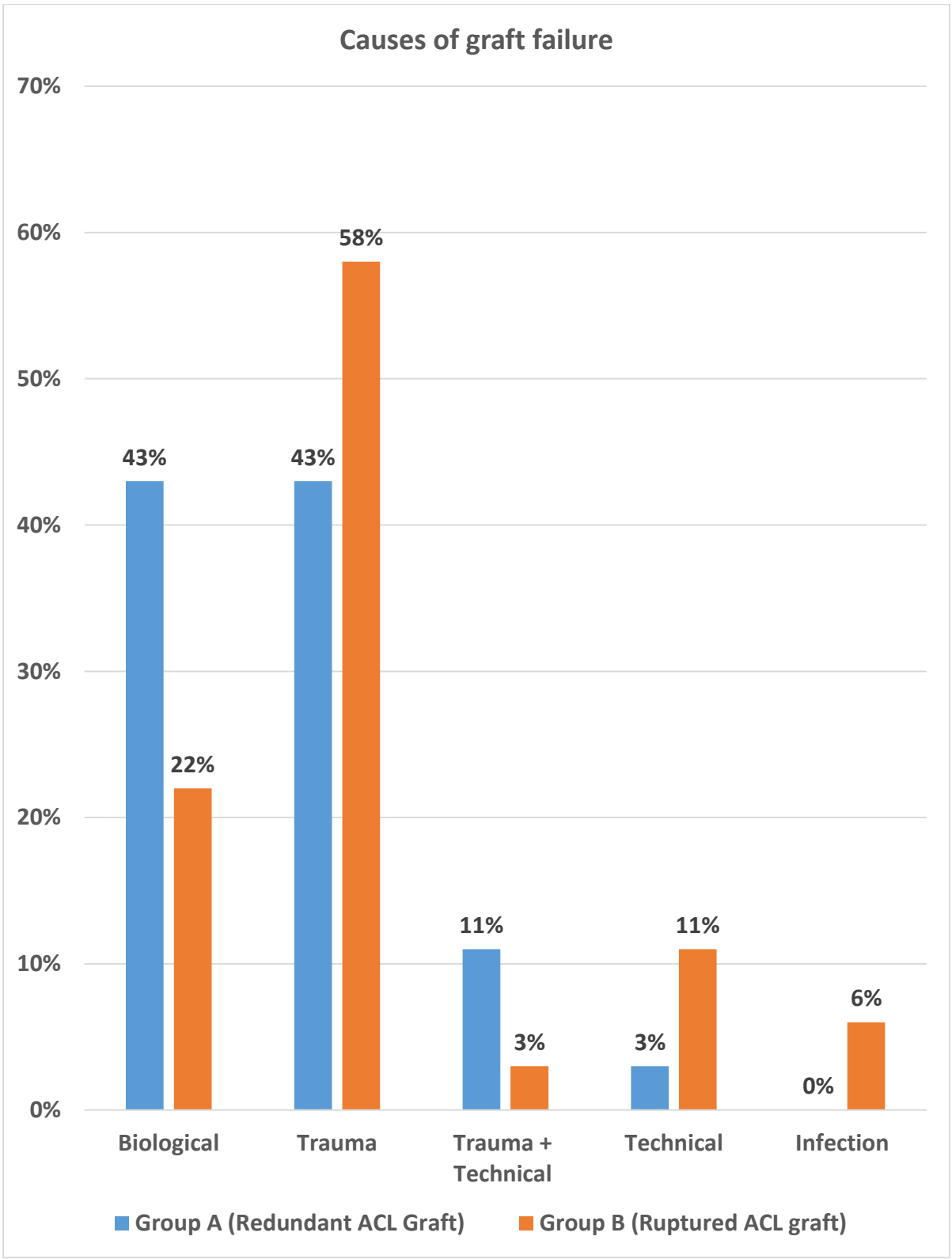


Chart 15- Causes of ACL Graft Failure

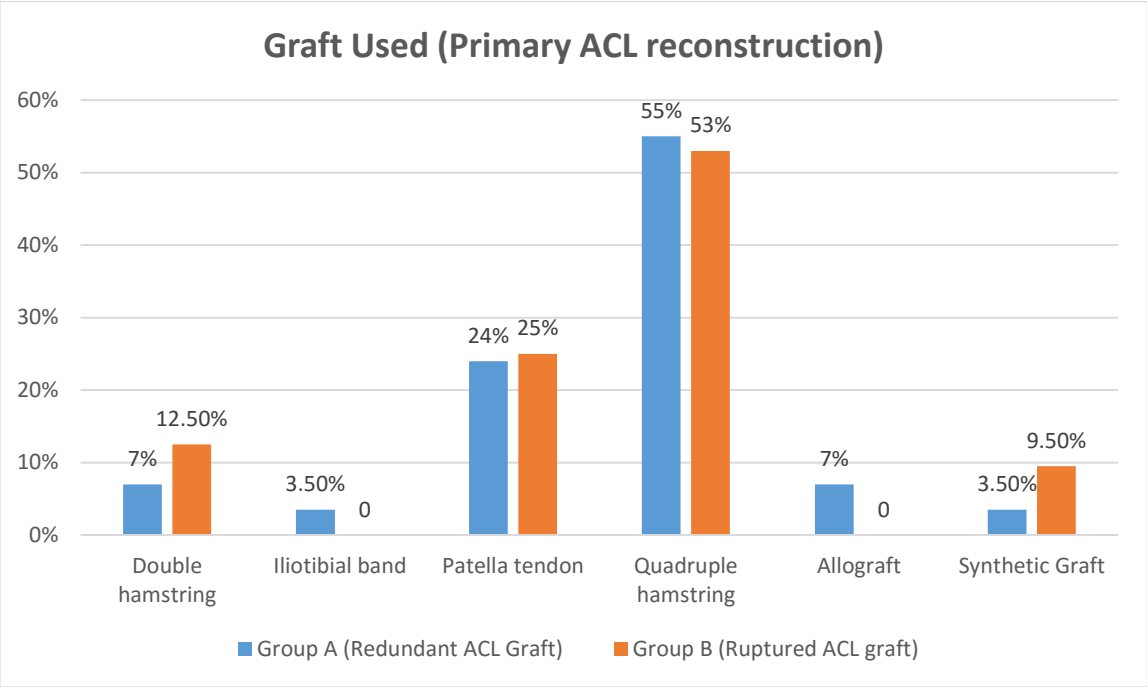


Chart 16- Grafts Used in Primary ACL reconstruction

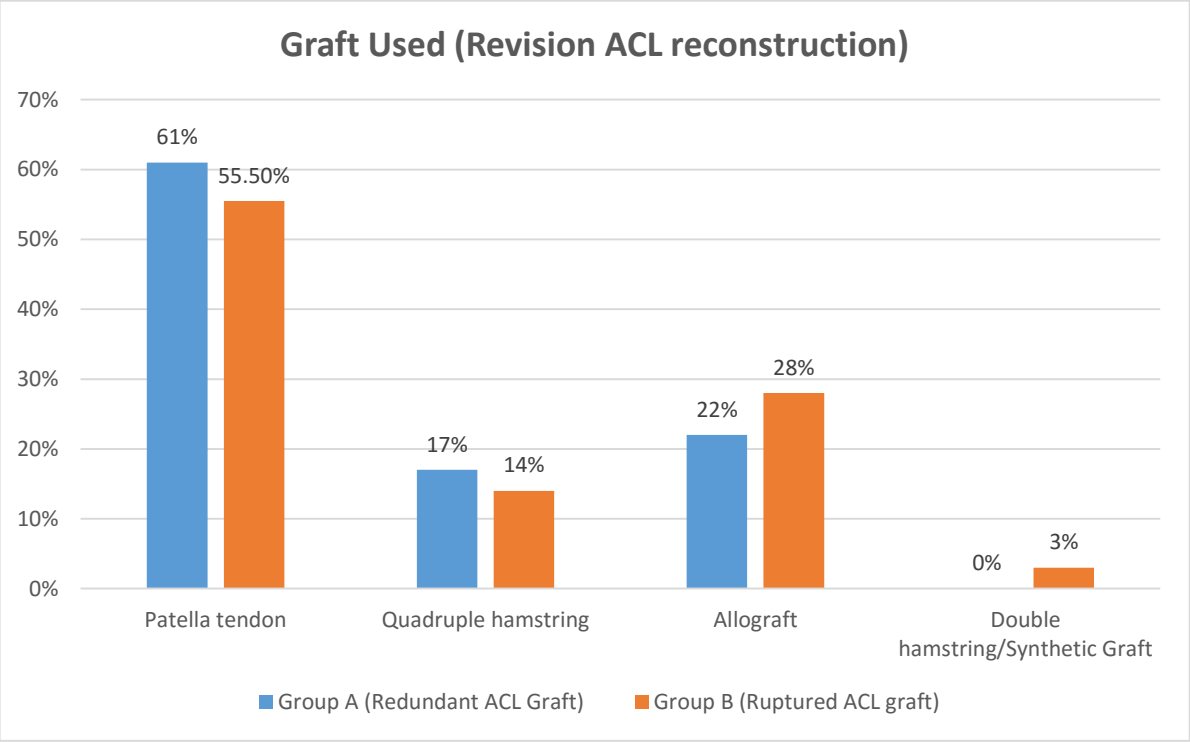


Chart 17- Grafts Used in Revision ACL Reconstruction

	Group A (Redundant ACL Graft)	**Group B (Ruptured ACL graft)**
Total No of Patients	36	36
Mean Age (years)	25.7 (13-50)	25.9 (15-47)
Sex		
Male	69%	80%
Female	31%	20%
Graft Used (Primary ACL reconstruction)		
Double hamstring	7%	12.5%
Iliotibial band	3.5%	0
Patella tendon	24%	25%
Quadruple hamstring	55%	53%
Allograft	7%	0
Synthetic Graft	3.5%	9.5%
Mean time for revision ACL reconstruction (months)	44.8 (5-232)	37.7 (2-120)
Causes of graft failure		
Biological	43%	22%
Trauma	43%	58%
Trauma + Technical	11%	3%
Technical	3%	11%
Infection	0%	6%
Sports	35%	35%
Graft Used (Revision ACL reconstruction)		
Patella tendon	61%	55.50%
Quadruple hamstring	17%	14%
Allograft	22%	28%
Double hamstring/Synthetic Graft	0%	3%
Associated surgery		
Associated surgery- Primary ACL reconstruction	11%	8%
Associated surgery-Revision ACL reconstruction	25%	25%

Table 7- Demographics, Grafts used for primary ACL reconstruction, Causes of Failure, Grafts used for revision ACL reconstruction in Redundant and Ruptured ACL Groups

Shoulder instability and Generalized joint hypermobility:

The primary shoulder dislocation group had a mean age of 25.9 years (SD- 9.5) and a mean Beighton score of 3.6 (median 3). There were 40 males and 4 females. The recurrent shoulder dislocation group had a mean age of 25.6 years (SD-6.4) and a mean Beighton score of 3.3 (median 4). There were 52 males and 7 females. The control group had a mean age of 26.8 years (standard deviation- 7.4). There were 46 males and 8 females. The mean Beighton score was 1.8 (median 1).

	Control Group (n=54)	**Primary Dislocation Group (n=44)**	**Recurrent Dislocation Group (n=59)**	**P Value**
Male Gender (n, %)	46 (85%)	40 (91%)	52(88%)	0.687
Age (years, SD)	26.8 (7.4)	25.9 (9.5)	25.6 (6.4)	0.859
Beighton Score (mean, 95% CI)	1.85 (1.33 to 2.37)	3.66 (2.82 to 4.5)	3.29 (2.8 to 3.9)	<0.001
Beighton Score (median, IQR)	1 (0 to 3)	3 (2 to 5.75)	4 (1 to 5)	
Beighton >=2 (n, %)	26 (48%)	34 (77%)	43 (73%)	0.003
Beighton >=4 (n, %)	11 (18%)	21 (34%)	30 (48%)	0.002
BJHS Criteria Present (n, %)	4 (7.4%)	6 (13.6%)	19 (32.2%)	0.002

Table 8- Demographics and Beighton's score in patients with Shoulder dislocations

The mean Beighton score was higher in both the primary dislocation group (mean difference 1.8, p=0.001) and the recurrent dislocation group (mean difference 1.4, p=0.004) compared with the control group. There was no difference between the primary and recurrent dislocation group (mean difference 0.371, p=1.00).

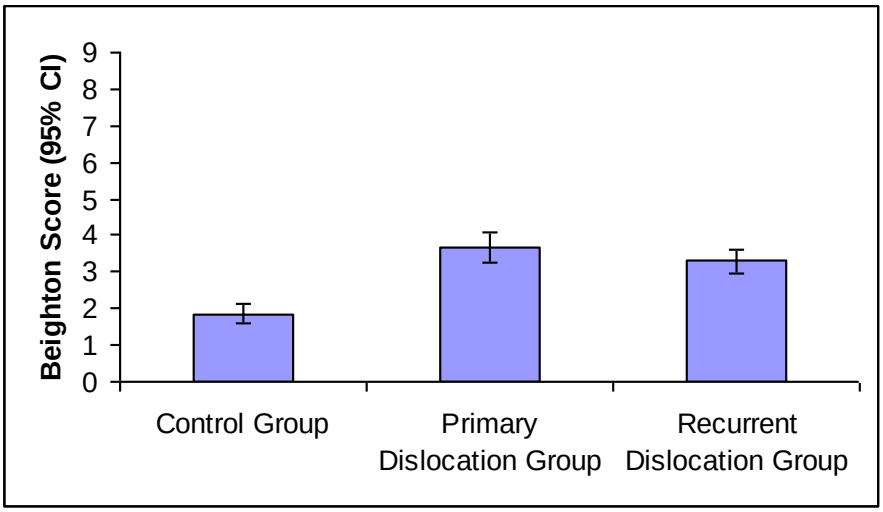


Chart 18- Beighton score and Shoulder Dislocations

The proportion of patients with the Beighton score ≥4 was greater in the dislocation group compared with the control group (OR 3.5, 95% CI 1.5 to 8.8, p=0.005). It was also higher in the recurrent group compared with the controls (OR 4.0, 95% CI 1.7 to 9.5, p<0.001). There was no difference between the primary and recurrent dislocation groups (OR 1.1, 95% CI 0.5 to 2.5, p=0.759).

Cameron et al. reported that the participants with a Beighton score of 2 or greater were nearly 2.5 times more likely to have experienced an episode of glenohumeral joint instability. (Cameron et al., 2010) When Beighton score ≥2 was used to determine the difference in laxity there were similar differences in the distribution of patients with GJH between the control group, primary dislocation (OR 3.6, 95%

CI 1.5 to 9.1, p=0.004), and recurrent dislocation groups (2.9, 95% CI 1.3 to 6.4, p=0.008). There was again no difference between primary and recurrent dislocation groups (0.624).

There was no difference in the incidence of Benign joint hypermobility syndrome (BJHS) between primary dislocation and control groups (OR 2.0, 95% CI 0.5 to 8.4, p=0.337), but there was a difference between recurrent dislocation and control groups (OR 5.9, 95% CI 1.9 to 21.5, p=0.001). The BJHS does exhibit a difference in proportions between recurrent and primary dislocation groups (OR 3.0, 95% CI 1.1 to 8.9, p=0.031).

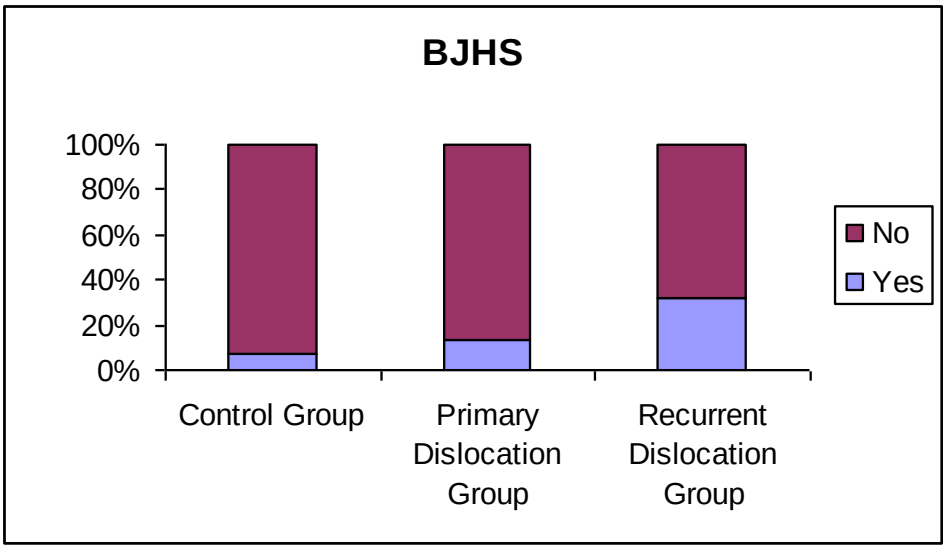


Chart 19- BJHS and Shoulder Dislocations

The most common sport played by the primary group was rugby in 14 patients followed by football in 9 patients. The most common sport played in recurrent group was football in 14 patients followed by rugby in 12 patients. The number of subluxations in primary group varies from 0-2 and in recurrent group varies from 0-100. The number of dislocations ranges from 2-20 in recurrent group. 25%

patients in primary dislocation group and 13% patients in recurrent dislocation group also had a family history of generalized joint hypermobility.

Category of Mechanism	Primary Shoulder Dislocation	Recurrent Shoulder Dislocation
Sports	25	30
Direct trauma	1	10
Fall from standing height	11	10
RTA	2	2
Assault	2	2
Fall from height	1	0
Not known	2	5
Total	44	59

Table 9- Mechanism of Injury in Shoulder Dislocations

Hypermobility- a risk factor for recurrent shoulder dislocations

The mean age of 38 patients with traumatic primary anterior shoulder dislocations followed up at 4 years was 25 years (range 15-55). There were 35 males and 3 females. 22 patients (58%) were involved in contact sports and most common sport played was rugby in 12 patients (55%). The mean Beighton score for these patients was 3.6 (range 0-9). GJH was assessed according to loose criteria of BS $\geq$ 4 and strict criteria of BS $\geq$ 6.

18 patients in this group had a Beighton score of 4 or above (loose criteria) (Group A). 20 patients had a Beighton score less than 4 (Group B).

10 patients (26%) in this group had a Beighton score of 6 or more (Strict Criteria) (Group C). 28 patients (74%) had Beighton score less than 6 (Group D).

	Group A (BS 4 or >)	**Group B (BS<4)**	**Group C (BS 6 or >)**	**Group D (BS<6)**
Total No of Patients	18	20	10	28
Mean Age	25 (15-55)	26 (15-43)	26 (15-55)	25 (15-44)
Sex				
Male	16	19	9	26
Female	2	1	1	2
Contact Sports	10	12	5	11
Recurrent Dislocation	9 (50%)	8 (40%)	6 (60%)	11 (39%)
Mean Beighton score	6 (4-9)	1.3 (0-3)	7.4 (6-9)	2 (0-5)
BJHS	4 (22%)	0	3 (30%)	1 (3%)
Family History of Laxity	7 (39%)	2 (10%)	6 (60%)	3 (11%)

Table 10- Recurrent shoulder dislocations in patients with BS <4 / >4 and BS <6 / >6

There was no significant difference when groups were compared using the loose criteria of Beighton score of 4 or above 50% vs 40% (p= 0.5) or strict criteria of Beighton score of 6 or above 60% vs 39% (p= 0.2) to assess the risk of recurrent shoulder dislocations.

CT scan Evaluation after Primary Shoulder Dislocation:

Prospective data was collected for 20 patients with a mean age of 27 years and a range of 15-56 years. 8 patients (40%) were involved in contact sports. Left shoulder was involved in 11 patients (55%). The CT scan showed glenoid fracture (bony Bankart lesion) in 7 patients (35%). Only 2 (28%) of these patients had evidence of a glenoid fracture using conventional radiographs. 5 patients (25%) showed evidence of glenoid flattening on the CT scan. Hill-Sachs lesion was present in 17 Patients (85%). The range of the defect was 8-29 mm. 1 patient had recurrence following a bony Bankart lesion and 2 patients developed adhesive capsulitis.

CT scan Evaluation for Recurrent Shoulder Dislocations after sports injuries:

Prospective data was collected for 37 patients with a mean age of 25 years and a range of 14-40 years. 24 patients (71%) were involved in contact sports. The most common sport was football in 12 (50%) patients followed by rugby in 9 (37%) patients. Left shoulder was involved in 19 patients (51%). The CT scan showed glenoid fracture (bony Bankart lesion) in 11 patients (32%), Glenoid flattening in 14 (41%) patients and Hill Sachs defect in 29 (85%). The range of the defect was 11-45 mm. 4 patients had glenoid flattening but no glenoid fracture. The number of

dislocations range from 0-17 with a mean of 3 and the number of subluxations range from 0-35 with a mean of 4.5.

	Primary Shoulder Dislocation	**Recurrent Shoulder Dislocation**
Total No of Patients	20	37
Mean Age	27 (15-56)	25 (14-40)
Contact Sports	8 (40%)	24 (71%)
CT Scan Findings		
Bony Bankart Lesion	7 (35%)	11 (32%)
Glenoid Flattening	5 (25%)	14 (41%)
Hill- Sachs Lesion	17 (85%)	29 (85%)
Size of Hill-sachs Defect	8-29mm	11-45mm
No of Dislocations	1	3 (0-17)
No of Subluxations	0	4.5 (0-35)

Table 11- CT scan findings in patients with Primary and Recurrent shoulder dislocations

Role of CT scan in predicting recurrence following primary traumatic shoulder dislocation:

The mean age of 39 patients with first time traumatic anterior shoulder dislocation who were followed up for 4 years was 26 years (range 15-56). There were 36 males and 3 females. CT scan showed glenoid bone defect in 10 patients (26%), glenoid flattening in 7 patients (18%) and Hill sach's defect in 33 patients (85%). Patients were divided into groups according to the number of bone defects on CT scan (No bone defect- 0, glenoid bone defect- 1, glenoid flattening- 1 and Hill sach's defect- 1).

Group A (no bone defect) had 6 patients (15%), Group B (Bone defect) had 33 patients (85%); 22 patients (56%) had 1 bone defect (Group C), 5 patients (13%) had 2 bone defects (Group D) and 6 patients (15%) had 3 bone defects (Group E). Group A (no bone defect) had 1 recurrent shoulder dislocation (16%) and Group B (Bone defect) had 16 recurrent shoulder dislocations (48%). Group C (1 bone defect) had 11 recurrent shoulder dislocations (50%), Group D (2 bone defects) had 2 recurrent shoulder dislocations (40%) and Group E (3 bone defects) had 3 recurrent shoulder dislocations (50%).

	Group A (no bone defect)	**Group B** (Bone defect)	**Group C** (1 bone defect)	**Group D** (2 bone defects)	**Group E** (3 bone defects)
Total No of Patients	6 (15%)	33 (85%)	22 (56%)	5 (13%)	6 (15%)
Recurrent Dislocation	1 (16%)	16 (48%)	11 (50%)	2 (40%)	3 (50%)

Table 12- Recurrent Shoulder Dislocations and number of Bone defects on CT

CT scan and Hypermobility in patients with Primary shoulder dislocation:

The mean age of 34 patients after first time shoulder dislocation who had the CT scan was 26 years with a range from 15-56 years. There were 31 males and 3 females. The most common cause of shoulder dislocation was sports related injuries in 16 patients (62%). The most common sport was rugby in 9 patients (56%).The right shoulder was involved in 24 patients (73%) and left in 9 patients (27%). 33 patients were right handed dominant. All patients had anterior shoulder dislocation. The average Beighton score for patients was 3 with a range from 0-9. 13 patients (38%) in this group had a Beighton score of 4 or more indicating joint hypermobility.

CT scan showed bony Bankart lesion in 8 patients (23%), glenoid flattening in 5 patients (15%) and Hill sach's defect in 29 patients (88%). There was no correlation between the Beighton score and the CT scan findings.

CT scan and Hypermobility in patients with Recurrent shoulder dislocations:

The mean age of 37 patients was 25 years with a range from 14-40 years. 24 patients (71%) were involved in contact sports. The most common sport was football in 12 (50%) patients followed by rugby in 9 (37%) patients. Left shoulder was involved in 19 patients (51%), right in 12 patients (32%) and 6 patients (16%) had bilateral shoulder dislocations.

CT scan showed glenoid fracture (bony Bankart lesion) in 11 patients (32%), Glenoid flattening in 14 (41%) patients and Hill-Sachs defect in 29 (85%).The

range of the defect was 11-45 mm. 4 patients had glenoid flattening but no glenoid fracture. The number of dislocations range from 0-17 with a mean of 3 and the number of subluxations range from 0-35 with a mean of 4.5

The average Beighton score for patients was 2.8 with a range from 0-7. 15 patients (40%) in this group had a Beighton score of 4 or more. There was no correlation between the Beighton Score and the CT scan findings of glenoid flattening, bony Bankart lesion and hill-sach's defects on statistical testing. (T tests, and Mann Whitney U tests on the Beighton and Brighton score and Chi squared tests on the tabulation of the CT findings with the diagnosis of BJHS.)

	Primary Shoulder Dislocation	**Recurrent Shoulder Dislocation**
Total No of Patients	34	37
Mean Age	26 (15-56)	25 (14-40)
Sex		
Male	31	36
Female	3	1
Sport injuries	8 (40%)	24 (71%)
Rugby	9 (56%)	9 (37%)
Football	2 (8%)	12 (50%)
Side involved		
Right	24 (73%)	12 (32%)
Left	9 (27%)	19 (51%)
Bilateral	0	6 (16%)
Hand Dominance		
Right	33	33
Left	1	4
Mean Beighton Score	3 (0-9)	2.8 (0-7)
BS 4 or >	13 (38%)	15 (40%)
CT Scan Findings		
Bony Bankart Lesion	8 (23%)	11 (32%)
Glenoid Flattening	5 (15%)	14 (41%)
Hill- Sachs Lesion	29 (88%)	29 (85%)
Size of Hill-sachs Defect	2.4-29mm	11-45mm
No of Dislocations	1	3 (0-17)
No of Subluxations	0	4.5 (0-35)

Table 13- Demographics, Sports played and CT scan Findings in Primary and Recurrent Shoulder Dislocations

Electrogoniometry Studies

A selective cohort of patients had their joint hypermobility assessed at the metacarpophalangeal joints of the index and little finger by using an electronic goniometer (E-link system- Biometrics Ltd).

This was correlated with Beighton score and Brighton criteria used for clinical assessment of joint hypermobility.

A- Goniometer Studies:

A cohort of 22 patients attending the specialist shoulder, knee and fracture clinics were recruited and their joint hypermobility assessed at the metacarpophalangeal joints of the index and little finger by using an electronic goniometer- force plate system (E-link system- Biometrics Ltd). These readings were correlated with the Beighton score.

Test Setup:

During the goniometry assessment, the patient sat on a chair facing the researcher with their forearm placed on a table and their fingers freely mobile at the end on the table. The hands were dried of any sweat and the angle sensor was attached to the hand using double sided adhesive tape at the ulnar border of little finger and hypothenar eminence. (Figure 10) The angle of sensors was calibrated to 0^{o} and the load of the load cell was also calibrated to 0 gram before using the data log. The load cell of the pinchmeter was placed under the distal phalanx. The data log was placed in the recording mode and using the non-sensor area of the load cell, the finger was extended to record the load required to produce the maximum angle at

the MCPJ of the index and little fingers of both hands and then returned to 0^o (Figure 9). The finger was extended (Dorsiflexed) 10 times for the first few patients and subsequently the measurements were repeated 3 times for each finger and both the angle and load sensors were zeroed between the recordings. The maximum tolerated angle by the patients without causing discomfort was recorded along with the load to produce this angle. The data was transferred to the analysis software. If no load was recorded due to the slippage of the load cell (30%) the reading was not used in the analysis. Similarly if the electronic goniometer was dislodged due to excessive sweating then that reading was also not used in the analysis.

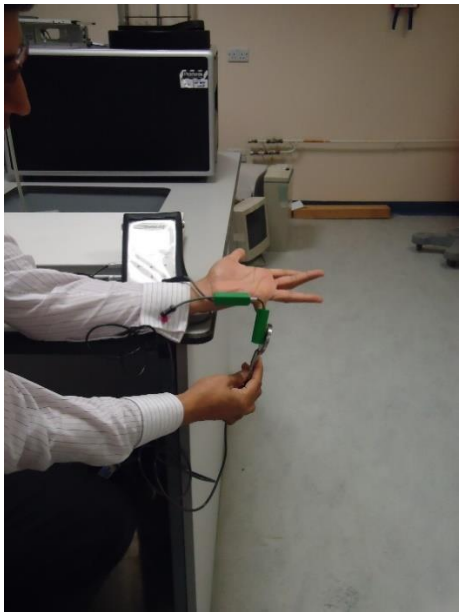


Figure 10: Electronic goniometer – load plate system (testing)

Data was collected for 86 fingers in 22 patients. There were 16 male (73%) and 6 female (27%) patients. 13 patients attended the knee injury clinic, 11 (50%) with ACL ruptures and 2 (9%) with meniscal injuries. Nine (9) patients (41%) attended the shoulder injury clinic with shoulder instability. All patients were right hand

dominant. Data was analysed for 20 right index fingers in 13 patients, 11 left index fingers in 7 patients, 14 right little fingers in 11 patients and 11 left little fingers in 10 patients. The load needed to produce 40 degrees of hyperextension at the metacarpophalangeal joint of the dominant right hand little finger was also recorded. This amount of hyperextension (i.e 40 degrees) was chosen as all patients could obtain this angle without discomfort.

Electronic Goniometer- Load plate system (Biometrics)

Twin Axis Goniometer: SG Series

The 'SG' series twin axis goniometers can simultaneously measure angles in up to two planes of movement. The goniometer has two separate output connectors, one measures flexion/extension, and the other radial/ulnar deviation. (Biometrics, n.d.-a)

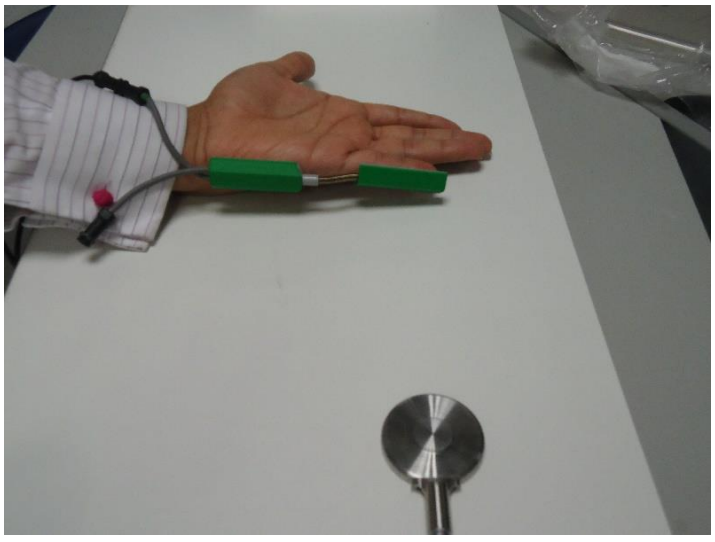


Figure 11: Angle sensor attached to the hand

Precision Pinchmeter P100

This unique electronic pinchmeter serial number M01849 which is a part of the E-LINK system (Biometrics, n.d.-b) has a low profile design that enabled the accurate quantification of the applied load, especially close to the end range. The range of the pinchmeter was 0-22 kg.

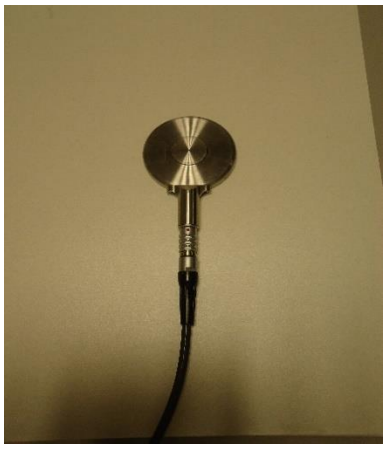


Figure 12: Isometric pinchmeter

DataLOG:

DataLOG is a general purpose, fully portable, programmable Data Acquisition Unit which was used to collect both analog and digital data from a wide range of sensors including Biometrics' Goniometers, Torsiometers, active EMG sensors, Accelerometers, Pinchmeters, Hand Dynamometers, and Contact Switches. For 'set up' the DataLOG was connected to the host PC via a cable, including simple adjustments per channel for gain, power supply, sampling rate and datum settings. When Biometrics' sensors were connected these parameters were automatically selected via a default button. During recording the data was stored on a 512 MB

MMC Flash Memory Card allowing the subject to move freely. After data collection, the MMC Flash Card was removed from the DataLOG and connected to a proprietary MMC card USB read/writer for importing the data into the Biometrics Display & Analysis Software. During recording real time feedback was obtained from the Graphics Display during the pilot phase of the study. The DataLOG accommodated a wide range of both single ended voltage inputs and differential voltage inputs. The standard unit came with 8 analog inputs, 4 general purpose digital inputs, and one dedicated to synchronizing start/stop with other hardware systems.(Biometrics, n.d.-a) A real-time clock enabled every recording to be marked with an accurate start date and time along with its duration

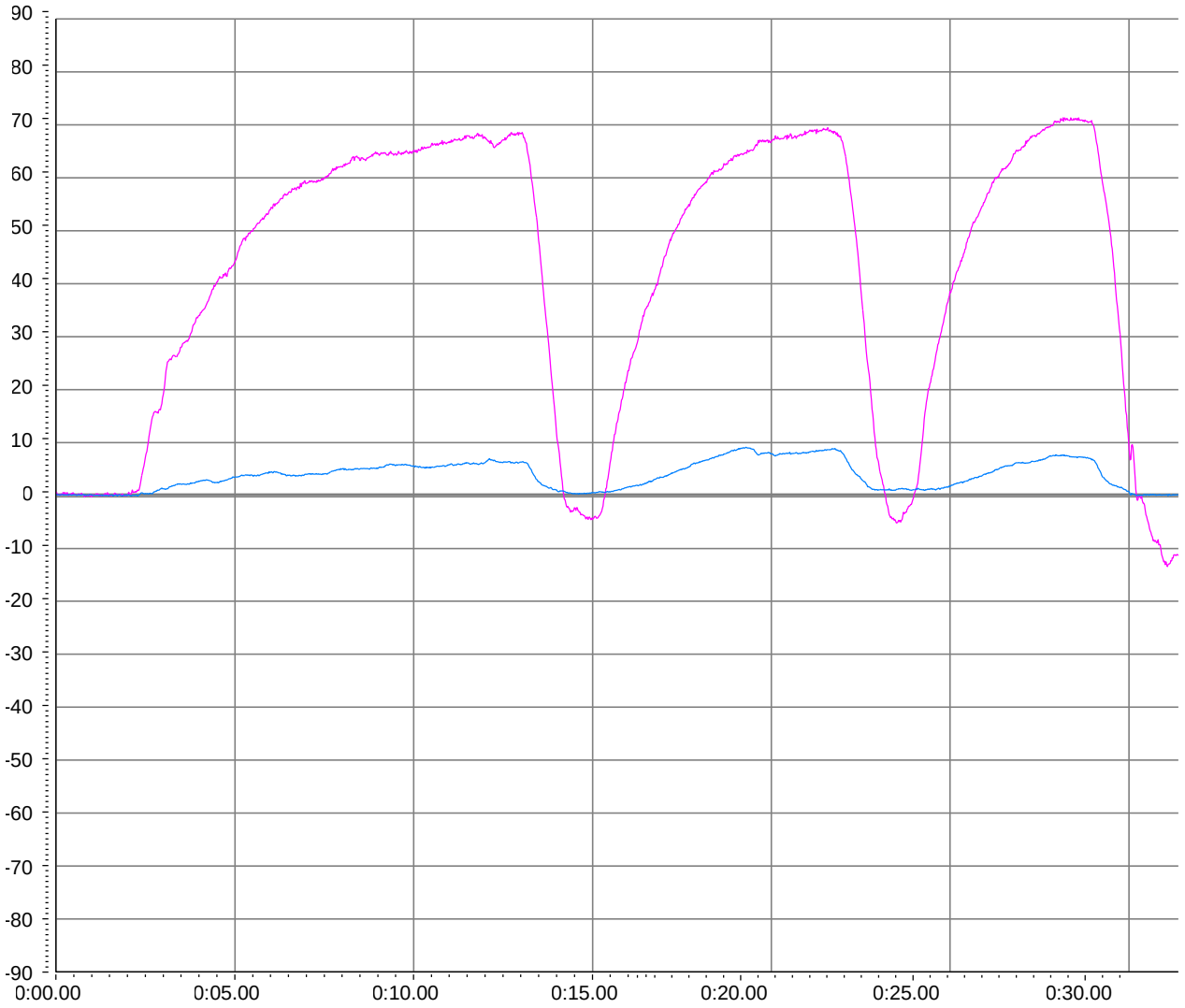
Figure 13: Electronic goniometer - Load plate system

Analysis:

Data was transferred from the DataLOG to computer software and then exported to excel. Graphs showing the load and angle were reproduced. The maximum angle measured was correlated against the Beighton score which gives a point on each

side for >90 of little finger extension. The maximum load to produce the maximum angle was recorded. Different loads were recorded for different angles produced at the fingers. The patient's pain was a major contributory factor in the load and angle measurement. It was noted that most patients were able to produce 40 degrees at the right hand little finger with little discomfort so this angle was selected and the load required to produce this angle was recorded.

Y axis- Pink graph - angle in Degrees Blue graph - load applied in kilogram

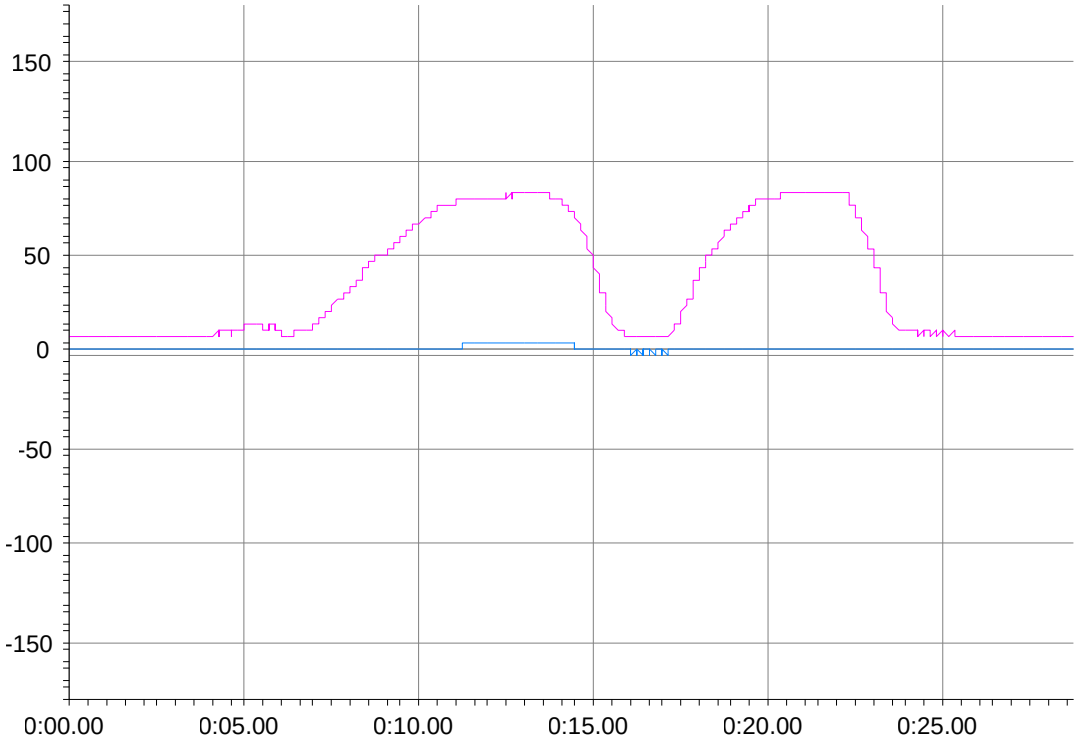


X-axis (Time - seconds)

K-5 Right index Finger - Beighton Score 0

Chart 20 (A) - Example of Graphs produced with the Biometrics Software

Y-axis- Pink graph - angle in Degrees Blue graph - load applied in kilogram



X-axis (Time – seconds)
K 16- Left index- Beighton Score 9
Chart 20 (B) - Example of Graphs produced with the Biometrics Software

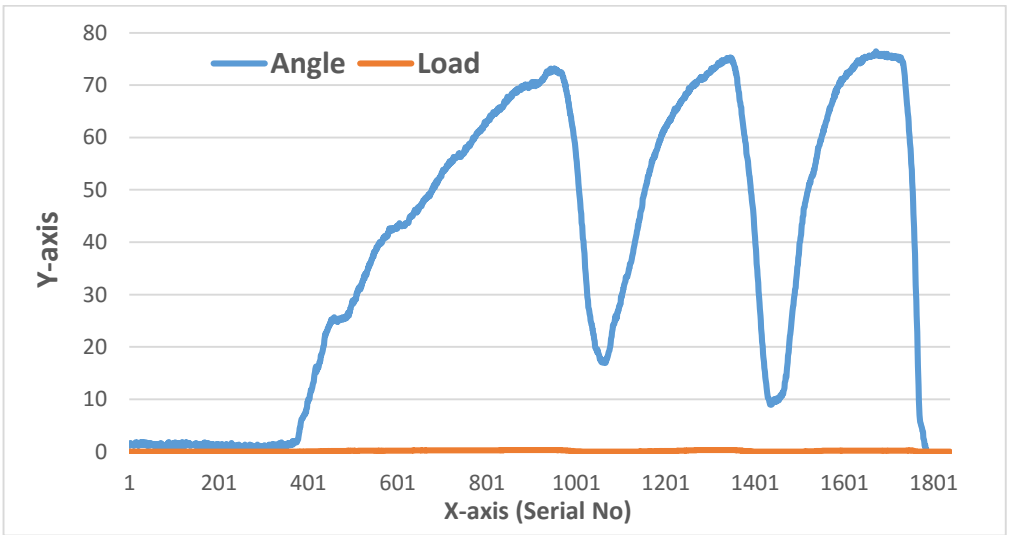


Chart 21- Load and angle graph produced with Microsoft Excel
K-2 Left Little finger- Beighton Score 0 (Microsoft Excel Graph)

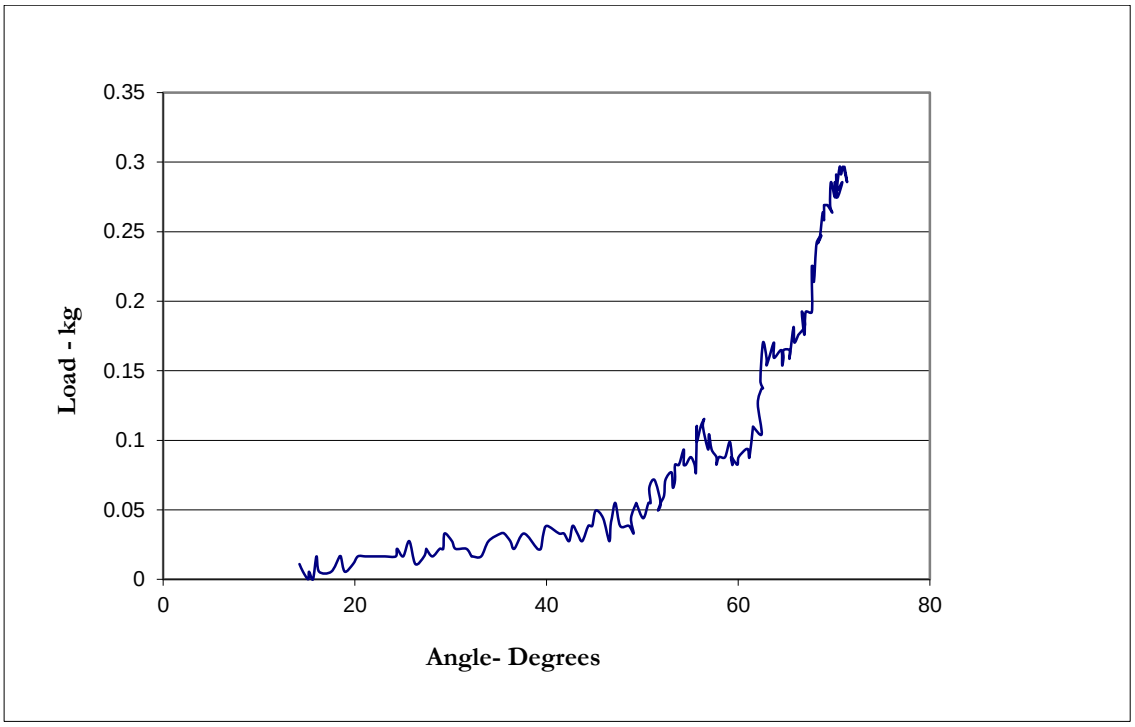


Chart 22- Load and angle graph (K-5 Right index Finger - Beighton Score 0)

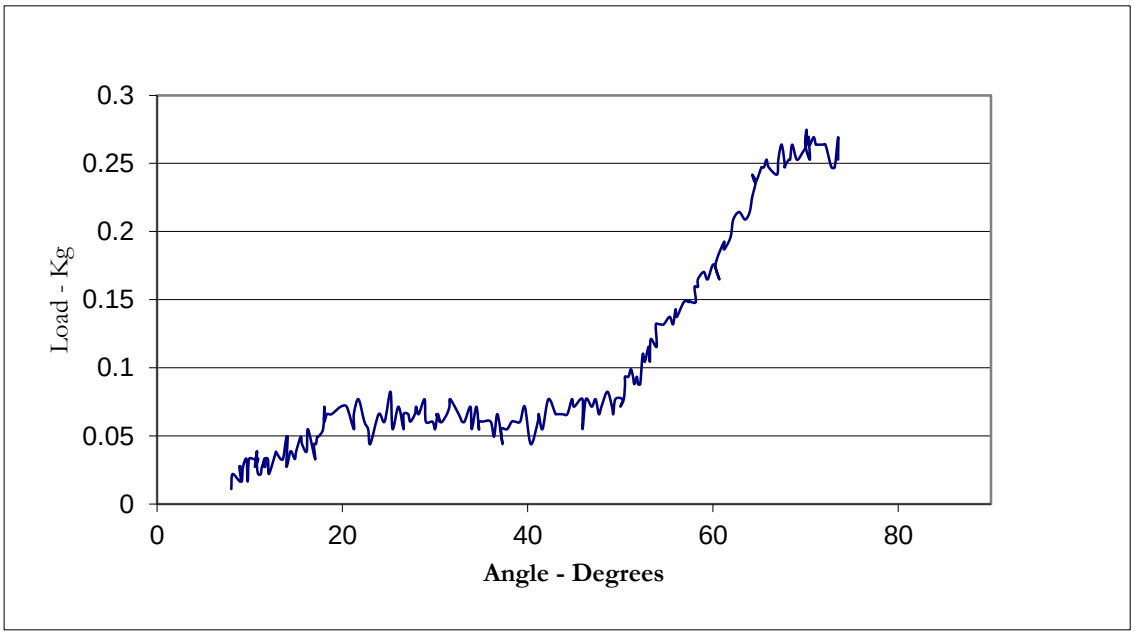


Chart 23- Load and angle graph (K 16- Left index Finger- Beighton Score 9)

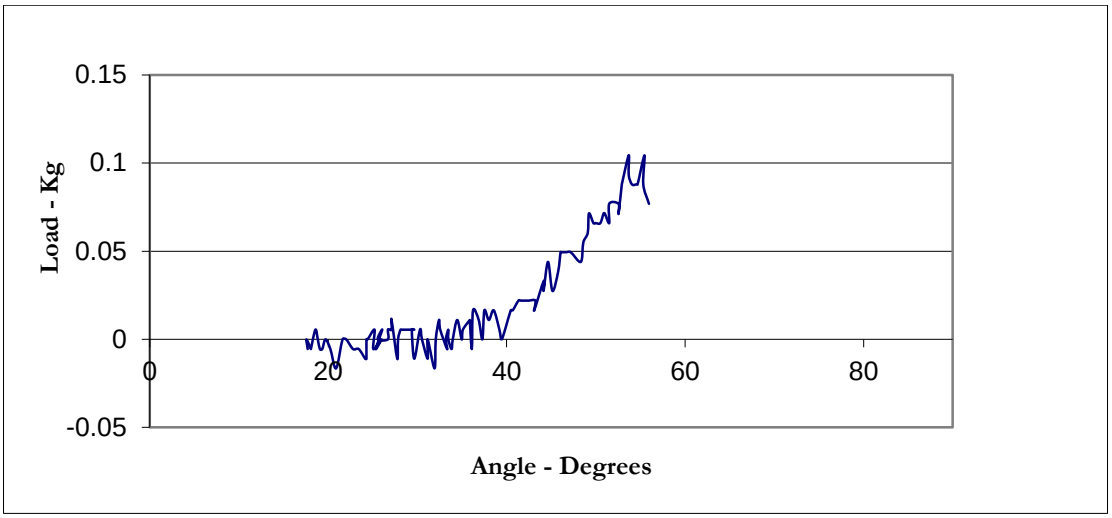


Chart 24- Load and angle graph (K-2 Left Little finger- Beighton Score 0)

Comparison of Beighton score with electronic goniometer for generalized joint hypermobility in athletic population

There were 22 patients in the study, 16 male (73%) and 6 female (27%). 13 attended the knee injury clinic, 11 (50%) with ACL and 2 (9%) with meniscal injuries. 9 patients (41%) attended the shoulder injury clinic with shoulder instability. 20 (94%) of these were right hand dominant.

Data was collected for 86 fingers in 22 patients. These fingers were divided into 2 groups. Group-1 with Beighton score=0 and Group-2 with Beighton score >0. There were 20 fingers in Group-1 and 66 fingers in Group-2. The mean angle for group-1 was 61 with a range from (18-87) and for group-2 was 85.5 with a range from (61-128). None of the little fingers in Group-1 scored >90 which is in accordance with the clinical examination of Beighton score. 6 little fingers in group-2 were given points on clinical examination but these score <90 on electronic goniometer.

	Group 1 (Beighton score = 0)	Group 2 (Beighton score >0)
Total fingers	20	66
Mean Angle	61 (18-87)	85.5 (61-128)

Table 14- Beighton score and Finger angle measurements

Correlation of electronic goniometer assessment of fingers with Beighton score for generalized joint hypermobility in athletic population:

Data was collected for 20 right index fingers in 13 patients, 11 left index fingers in 7 patients, 14 right little fingers in 11 patients and 11 left little fingers in 10 patients. All patients were right hand dominant. The mean Beighton score was 4 for index and 3.4 for little fingers with a range from 0-9. The average load needed to produce 40 degrees angle was 0.10 kg at right index finger and 0.09 kg at left index finger as compared to 0.02 kg for right little finger and 0.01 kg for left little finger . The average load needed to produce 50 degrees angle was 0.15 kg at right index finger and 0.12 kg at the left index finger as compared to 0.02 kg for right little finger and 0.01 for left little finger. There was a negative correlation between the Beighton score and the load needed to produce 40 and 50 degrees angle for left index finger. (i.e. the load to produce 50 degrees of extension was more than the load needed to produce 40 deg.)

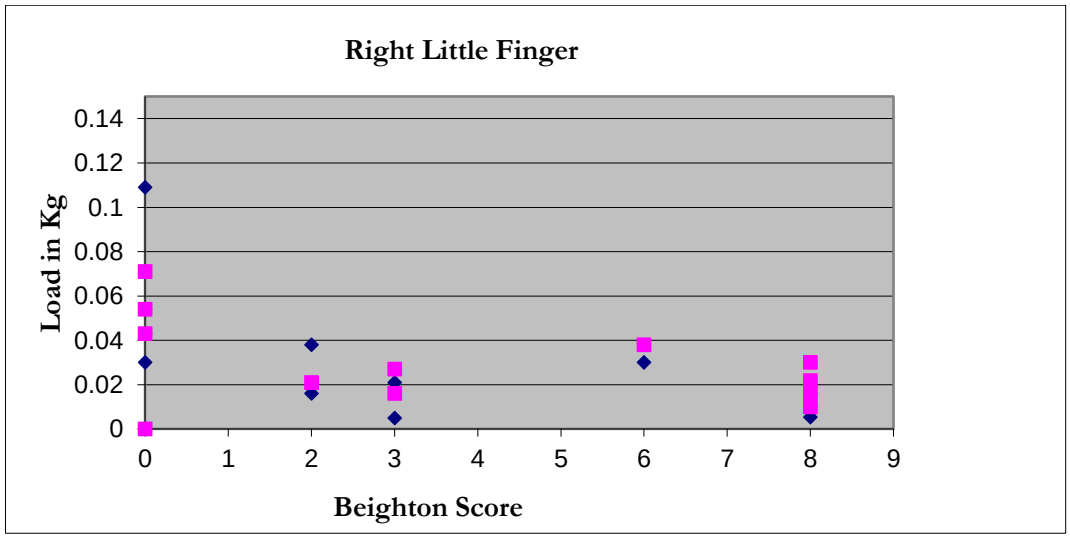


Chart 25- Load required to produce 40 (blue dots) and 50 (pink dots) degrees angle at the MCPJ of the Right Little Finger

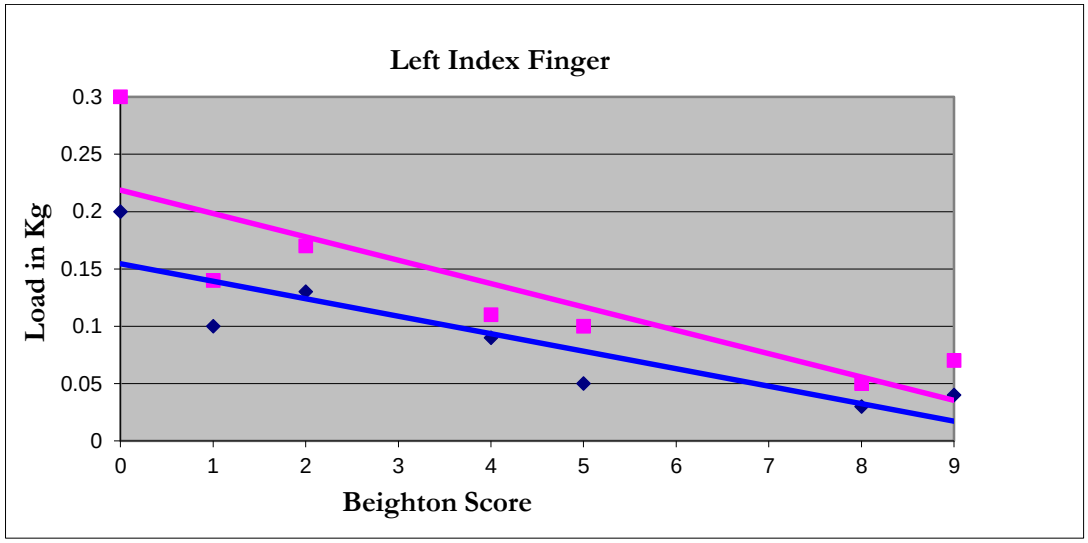


Chart 26- Load required to produce 40 (blue dots) and 50 (pink dots) degrees angle at the MCPJ of the Left index Finger

Mechanical Studies

The mechanical strength of the samples was assessed using a professional material testing machine (Zwicke-Rolle-model Z005)(Zwicke-Rolle, n.d.-a)(Zwicke-Rolle, n.d.-b) (Figure 2). This is a free standing machine which can apply a maximum force (Fmax) of 10 kN. The test speed can vary from 0.0005-2000 mm/min. The measured values were determined independently of the test setup.

Figure 14- Zwicke-Rolle tensile testing machine

Tissue specimens: (Skin, Capsule, Tendon)

Tissue specimens were obtained from patients undergoing ACL Reconstruction or Shoulder stabilization after ethics approval and informed consent.

Skin:

An ellipse of skin was obtained from patients undergoing either open shoulder stabilization or arthroscopic ACL reconstruction from the skin incision used to harvest the quadruple hamstring tendons. Twenty three (23) specimens of shoulder skin from 12 patients with a mean age of 26 years (range 17-31) were studied. All these patients were male and the average number of dislocations was 5.2 and subluxations was 6. Twenty (20) specimens of knee skin from 13 patients with a mean age of 29 years (range 20-43) were studied. There were 10 male and 3 female patients.

Shoulder Capsule:

The shoulder capsule was obtained from patients undergoing open shoulder stabilization if it was found to be lax after recurrent dislocations and needed to be removed for appropriate tensioning of the shoulder capsule during the Open Bankart repair. Fifteen (15) specimens of the shoulder capsule from 10 patients with a mean age of 26 years (range 17-31) were studied. All these patients were male and the average number of dislocations was 5.2 and subluxation was 6.

Hamstring Tendon:

Hamstring tendons were obtained following the preparation of the Quadruple hamstring tendon graft for ACL reconstruction. Eleven (11) hamstring tendons from 10 patients with a mean age of 26 years (range 16-43) were studied. There were 8 male and 2 female patients.

It was not possible to obtain the tissue specimens from all the recruited patients as some patients attending for open shoulder stabilization did not have any redundant capsule and patients attending for ACL reconstruction did not have an appropriate length of the hamstring tendons. It was noted that at least 3cm length of the tissue specimens was required for testing in the material testing systems.

Specimen Storage:

The Specimens were stored in a saline soaked swab and placed in the universal container and given a study ID number. The specimens were transferred from operating theatre to the mechanical testing lab in the University of Edinburgh and stored in a fridge (Temp 4 Degree centigrade)

Specimen Preparation:

The mean time delay from harvesting of the tissue specimen to mechanical testing was 4 days (range 0-10 Days). This was due to the availability of the professional testing system during the week days. During the mechanical testing of the tissue specimens, these were removed from saline soaked swabs and placed on a damp paper towel. The tissue samples were prepared by cutting into uniform rectangle pieces to allow for accurate measurement. The length and width was measured using a steel ruler and the thickness was measured using an electronic vernier calliper (Figure 14, 15). An average of 3 readings was taken for each dimension. The tissue samples were tested at room temperature (which was noted to be 24 degrees and centrally controlled). The samples were kept moist with saline during mechanical testing.

Figure 15- Electronic vernier callipers

Figure 16- Electronic vernier callipers- Measuring the Thickness

Specimen Testing:

In order to be correctly mounted into the tensile testing machine each sample was held with superglue between two small wooden sticks at each end. This was in order to keep the tissue sample planar thus stopping local deformation of the tissue which could influence the integrity of the collagen structures (Soden & Kershaw, 1974). The sticks were clamped in custom made small steel rectangle clamps (Figure - 16) with a decreasing angle on the inferior edge. Thus, as load was applied, clamping became firmer. Care was taken to ensure that the tissues were prepared and loaded in the machine in the correct orientation (as in vivo) to provide more accurate results. Fibre orientation was important since it has been shown that fibre organisation and orientation are the main constituents that dictate tensile strength (Wenger, Bozec, Horton, & Mesquida, 2007). The machine was set up with a 2.5 cm gap between each clamp. The clamps were tightened with the samples relaxed. Care was taken to secure tissue within the clamps to prevent slippage and also to ensure that the wooden sticks stayed as horizontal and parallel as possible. Incorporation of the free specimen in the clamps would cause a compressive force to be transmitted through the tissue, affecting the validity of the tensile test result. Similarly, if the tissue was not secured properly within the wooden sticks the tissue would ‘slip’ causing a false reading. Testing involved the uniaxial loading of tissues at a constant rate of 0.5mm per second until failure.

Figure 17- Tissue Specimen mounted in clamps

Data collection:

Data were captured including the force applied to the tissue specimens and displacement of the tissue under applied force. The yield was noted for tissue specimens after successful test. If the specimen slipped out of the clamps at the start of the test due to being loose in the clamps then one further attempt was made to hold the specimen between the wooden sticks and clamps as described earlier. If the specimen slipped during the test then the test was abandoned for that tissue specimen. Care was taken to keep the tissue moist during the test by application of saline. If the clamp was too tight then the tissue specimen failed at the stress riser near the clamp, so these results were discarded. The force-displacement graphs were produced by excel after exporting the data from the Zwick software (testXpert®).

Examples of Graphs produced with the Excel

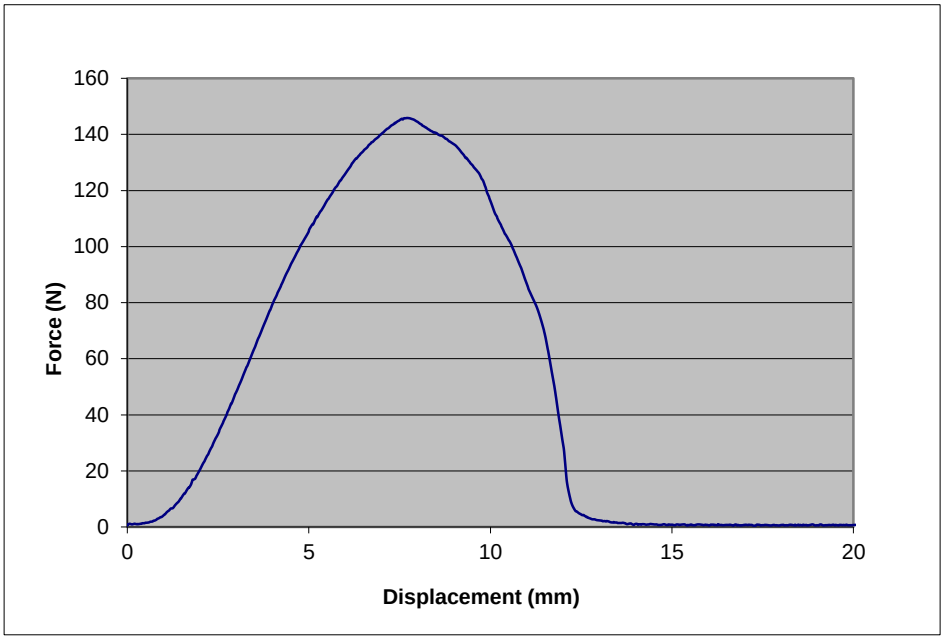


Chart 27- Force Displacement Graph (S6 – Skin: Beighton Score – 2)

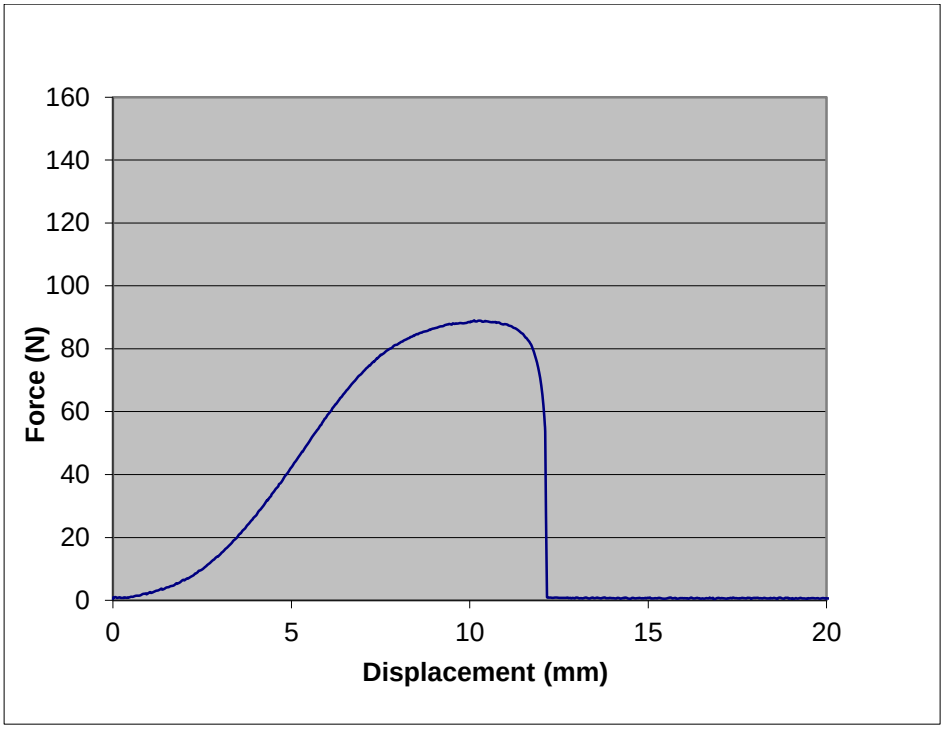


Chart 28- Force Displacement Graph (K12 – Skin: Beighton Score – 1)

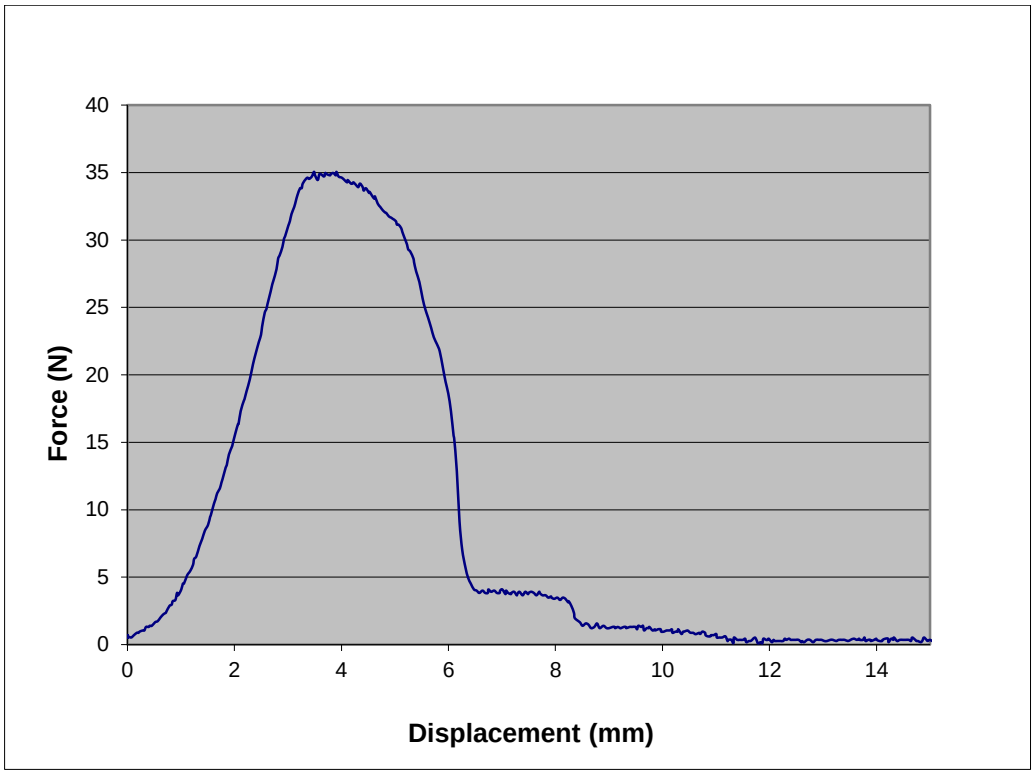


Chart 29- Force Displacement Graph (S11 – Capsule: Beighton Score – 0)

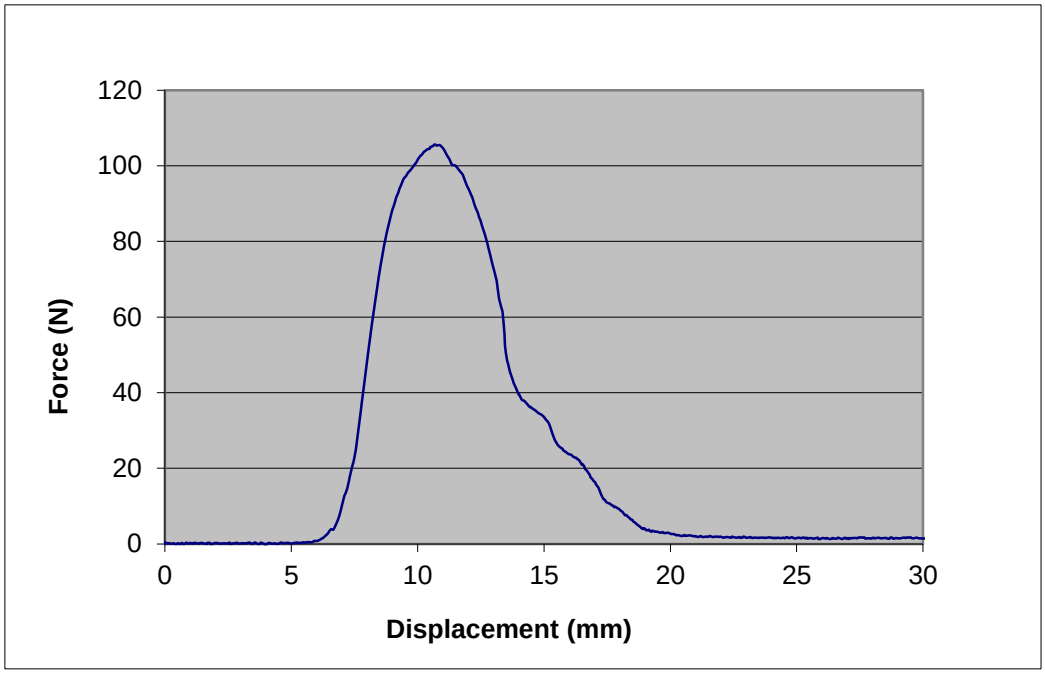


Chart 30- Force Displacement Graph (K2 – Tendon: Beighton Score – 0)

Data analysis:

The data from the testXpert software were exported to Microsoft excel and Force Displacement graphs were reproduced. The gradient of the force-displacement graphs was calculated using Microsoft excel as described by few examples below.

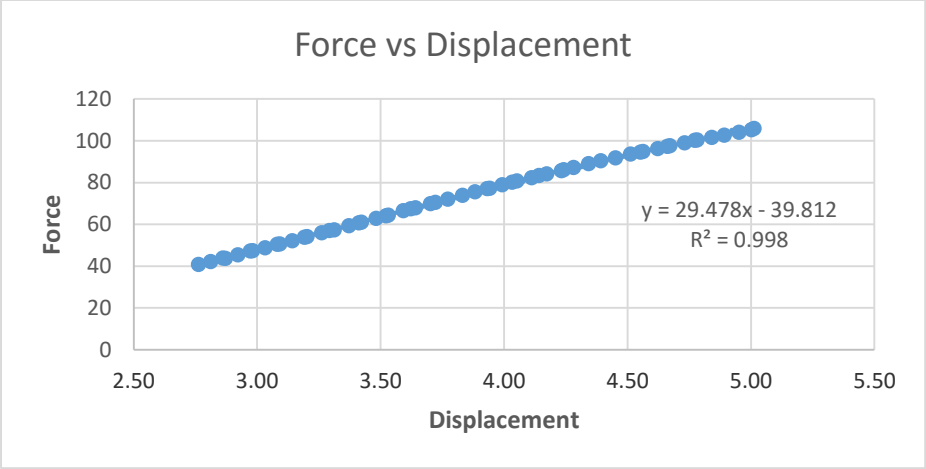


Chart 31- Gradient of the force-displacement (S6- Skin- Slope Graph)

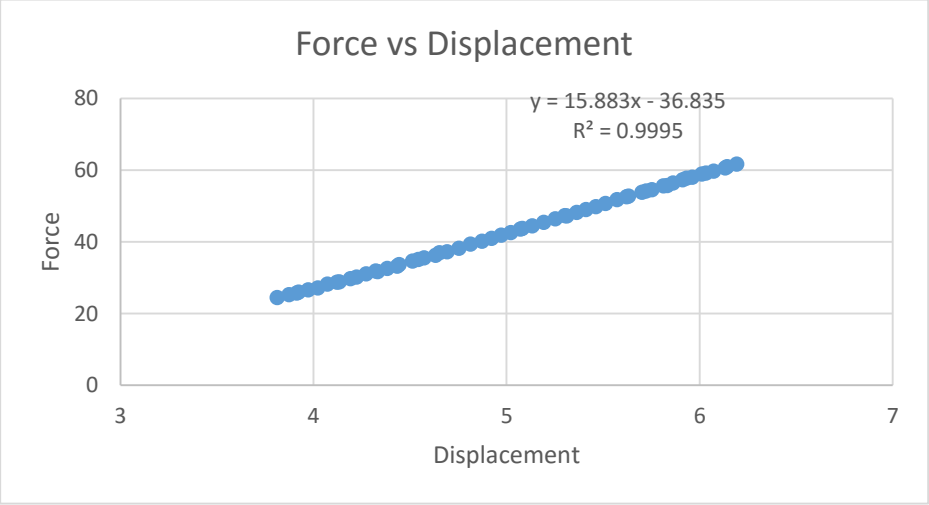


Chart 32- Gradient of the force-displacement (K12- Skin- Slope Graph)

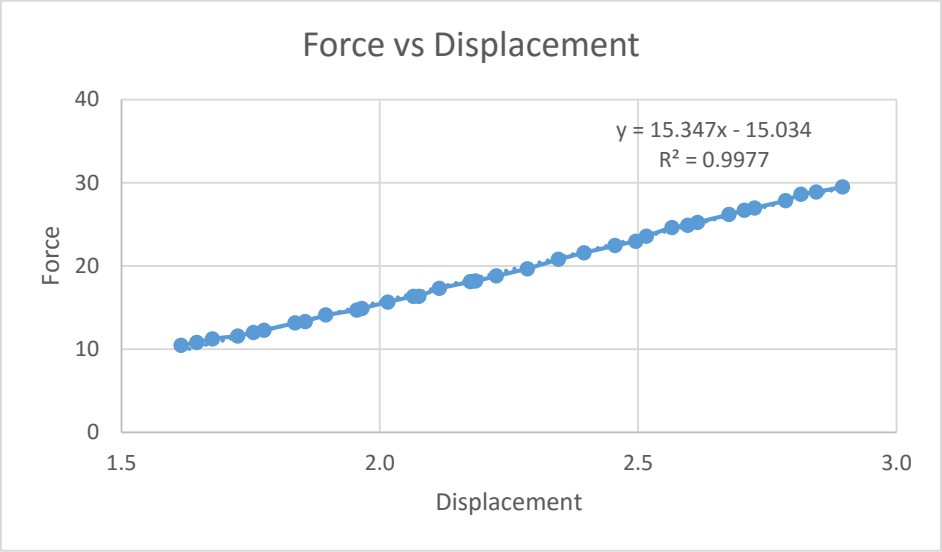


Chart 33- Gradient of the force-displacement (S-11- Capsule - Slope Graph)

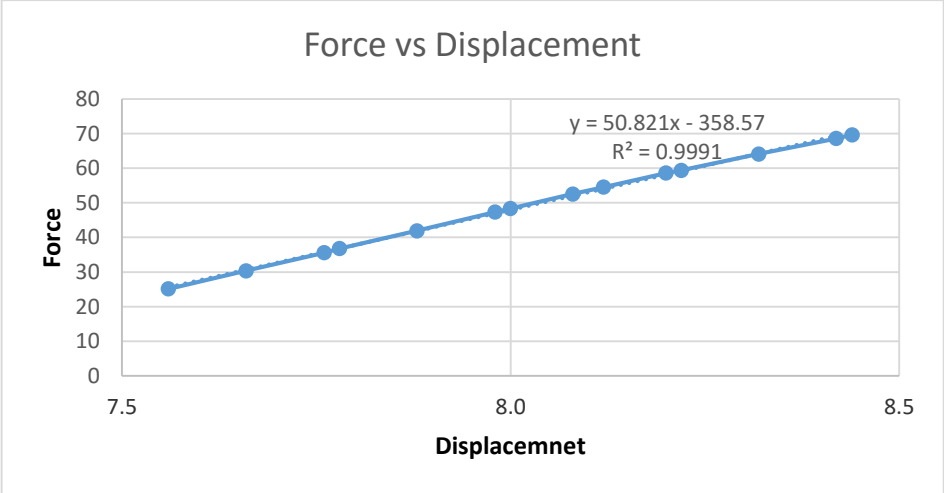


Chart 34- Gradient of the force-displacement (K-2 Tendon - Slope Graph)

Knee skin strength and Joint Hypermobility in patients undergoing ACL reconstruction:

The mean age of 20 specimens of knee skin from 13 patients undergoing primary ACL reconstruction was 29 years with a range from 20-43. There were 10 male and 3 female patients. The average Beighton score was 2.9 with a range from 0-9. 5 patients had a Beighton score of 4 or more indicating generalized joint hypermobility. The average force required to reach yield for knee skin was 56 N with a range from 12-107 N. The average force to yield for patients with Beighton score of 4 or above was 47 N (18-107 N) as compared to 61 N (15-89) in patients with Beighton score less than 4. The average force to yield for patients with Beighton score of 6 or above was 37 N (18-50) as compared to 61 N (12-107 N) in patients with Beighton score less than 6.

Shoulder Skin Strength and Joint Hypermobility in patients undergoing Open shoulder stabilization:

The mean age of 23 specimens of shoulder skin from 12 patients was 26 years with a range from 17-31. The average number of dislocations was 5.2 and subluxation was 6. All patients were male. The average Beighton score was 2.3 with a range from 0-4. 3 patients had Beighton score of 4 or more. The average force to yield for shoulder skin was 83N with a range from 27-171N. The average force to yield for patients with Beighton score of 4 or above was 64.5 N (27-113 N) as compared to 89.5 N (29-171) in patients with Beighton score less than 4.

Hamstring Tendon Strength and Joint Hypermobility in patients undergoing ACL reconstruction:

The mean age of 11 hamstring tendons from 10 patients undergoing ACL reconstruction was 26 years with a range from 16-43. Most common sport resulting in ACL injuries was football and skiing in 3 patients each followed by rugby and cycling in 2 patients each. There were 8 male and 2 female patients. The average Beighton score for these patients was 2 with a range from 0-9. 3 patients had a Beighton score of 4 or more, which (using current criteria) indicated generalized joint hypermobility. The mean thickness of the hamstring tendons tested was 0.022 cm. The average yield for individual hamstring tendon was 90N with a range from 39-105N. There was a positive correlation with the Beighton score.

Shoulder capsule Strength and Joint Hypermobility in athletes undergoing Open shoulder stabilization

The mean age of 15 specimens of the shoulder capsule from 10 patients was 26 years with a range from 17-31. Most common sport resulting in shoulder dislocations was rugby in 5 patients. The average number of dislocations was 5.2 and subluxations was 6. All patients were male. The average Beighton score for these patients was 1.9 with a range from 0-4. Two patients had Beighton score of 4 or more, which (using current criteria) indicated generalized joint hypermobility. The mean thickness of the shoulder capsule was 0.028 cm. The average force to yield for shoulder capsule was 44 N with a range from 17-78 N. The average force to yield for patients with Beighton score of 4 or more was 29 N as compared to 47 N in patients with Beighton score less than 4.

	Knee Skin	**Shoulder Skin**	**Hamstring Tendon**	**Shoulder Capsule**
Total No of Specimens	20	23	11	15
Total No of patients	13	12	10	10
Mean age	29 (20-43)	26 (17-31)	26 (16-43)	26 (17-31)
Sex				
Male	10	12	8	10
Female	3	0	2	0
Hypermobility				
Mean Beighton Score (BS)	2.9 (0-9)	2.3 (0-4)	2 (0-9)	1.9 (0-4)
BS 4 or >	5 (38%)	3 (25%)	3 (30%)	2 (20%)
BS 6 or >	3 (23%)	0	3 (30%)	0
Mean Force for yield				
All tissue specimens	56 N (12-107)	83 N (27-171)	90 N (39-105)	44 N (17-78)
Tissue Specimen- BS > 4	47 N (18-107)	64.5 N (27-113)	96 N (88-103)	29 N (26-32)
Tissue Specimen- BS < 4	61 N (15-89)	89.5 N (29-171)	88 N (39-105)	47 N (17-78)
Student T test	p=0.30	p=0.15	p=0.38	p=0.01
Tissue Specimen- BS > 6	37 N (18-50)	NA	96 N (88-103)	NA
Tissue Specimen- BS < 6	61 N (12-107)	NA	88 N (39-105)	NA
Student T test	p=0.03		p=0.38	

Table 15- Mechanical testing of different tissue specimens

Comparison of Beighton score with Patient satisfaction and Surgical Scar

Introduction:

Skin hyperextensibility defined as the ability of the skin to be stretched beyond normal limits is associated with Joint Hypermobility Syndrome (JHS). Wound healing defects are also common in JHS and may present as atrophic, non papyraceous scars due to delayed wound repair combined with skin fragility. Occasionally, defective wound healing occur after surgery (Castori, 2012).

Wound healing follows a tightly regulated sequence of events after injury.

1. Inflammation 2. Granulation tissue formation 3. Proliferation

4. Reepithelization 5. Remodelling. (Ghatak et al., 2015)

Methods:

Prospective data was collected for 34 patients attending shoulder or knee injury clinic following open shoulder stabilization or ACL reconstruction between April and July 2009 including demographic details, surgical scar grading on digital photographs, level of satisfaction on a visual analogue scale (VAS) (0 to 10) with 10 being most satisfied. Generalized joint hypermobility was assessed by using the Beighton score. Joint hypermobility was scored on a 0-9 scale. The digital pictures were taken of all the scars in the clinic after informed consent by using the same settings on the digital camera. 2 reviewers looked at all the pictures and decided a reference picture for a grade 1, a grade 2 and a grade 3 scar. These pictures were then used as a reference and the rest of the surgical scars on the digital pictures were scored on a 1-3 scale. (1=good cosmetic scar, 3=poor cosmetic scar).

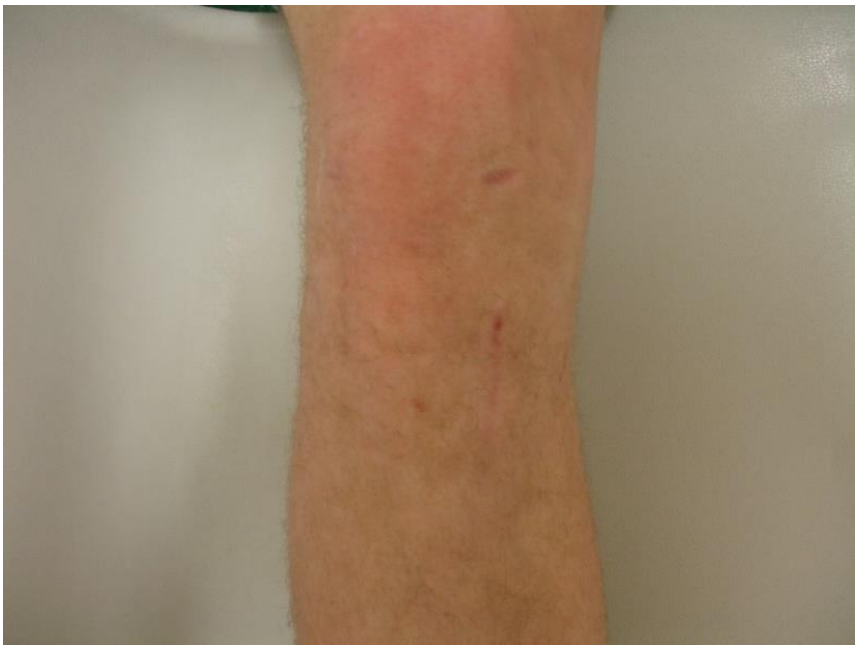


Figure 18- Picture Score -1 BS-0, VAS- 7

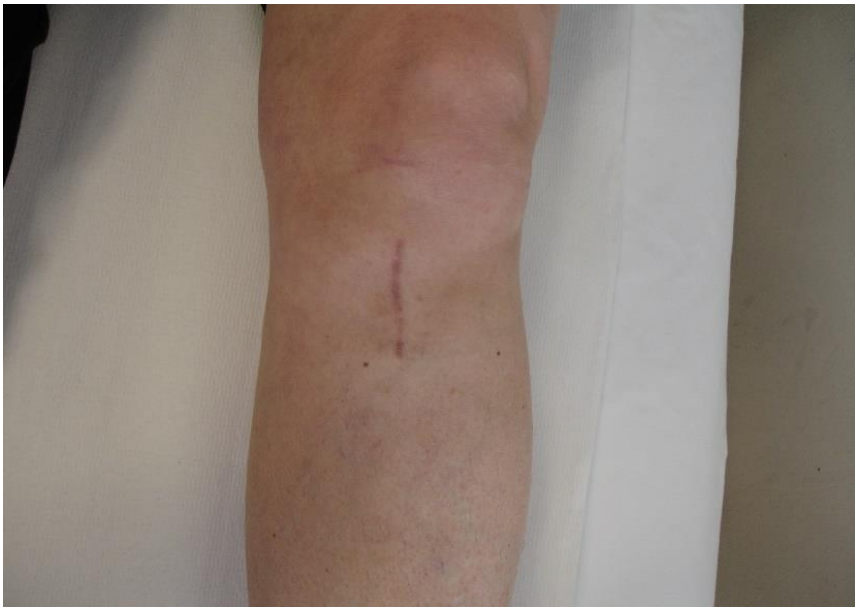


Figure 19- Picture Score -2 BS-7, VAS-8

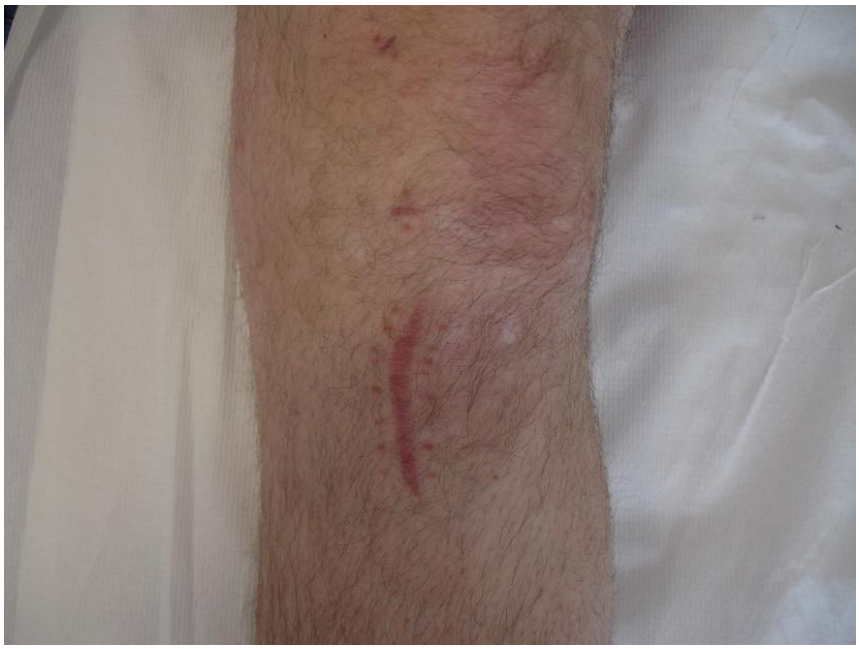


Figure 20 - Picture Score- 3 BS-1, VAS-10

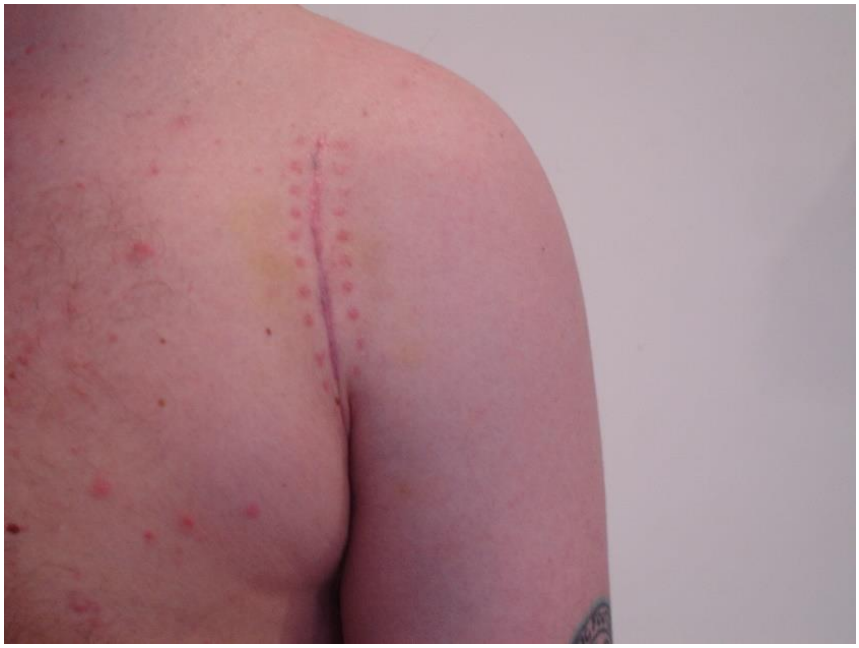


Figure 21- Picture Score- 1 BS- 0, VAS- 7

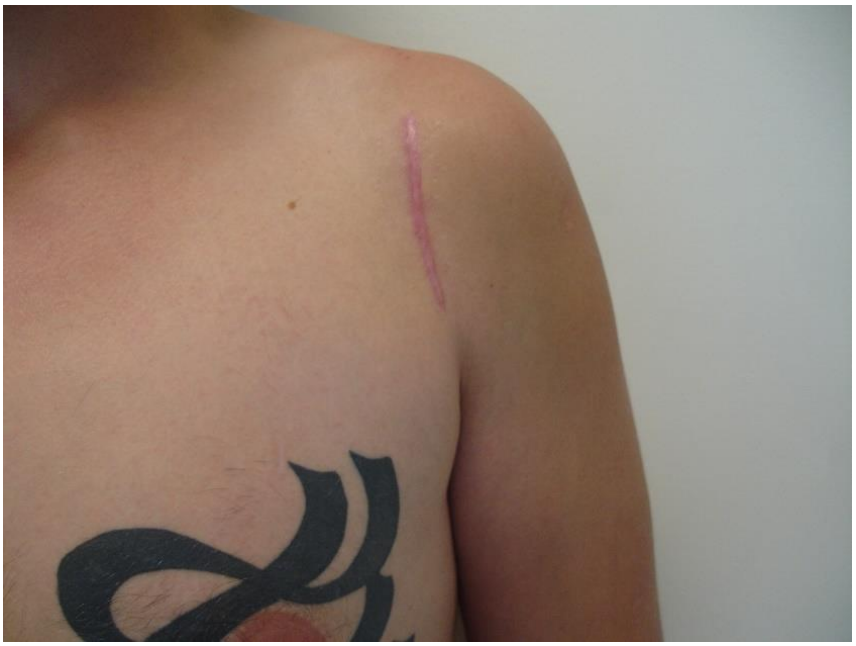


Figure 22- Picture score-2 BS-5, VAS- 10

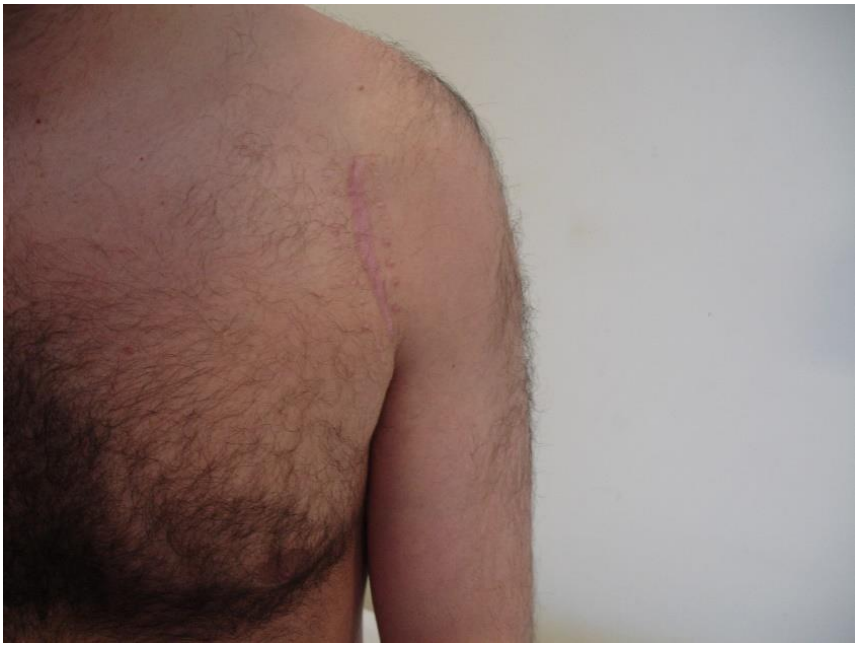


Figure 23- Picture Score-3 BS-4, VAS- 3

Results:

Does joint hypermobility affect surgical scar or patient's satisfaction following open shoulder stabilization or anterior cruciate ligament reconstruction?

21 patients attending shoulder or knee injury clinic following open shoulder stabilization or ACL reconstruction were studied. There were 18 male and 3 female patients. The mean age was 26 years (range 17-43). 16 patients had shoulder stabilization and 5 had Primary ACL reconstruction. The average scar time (time from surgery to the last clinic follow up) was 34 weeks (range 3-68 Weeks). Staples were used for wound closure in all patients. There were no wound complications. The mean overall satisfaction on visual analogue scale (VAS) was 6 (range 0-10). The mean picture score was 1.9, median was 2 and mode was 1 (range 1-3). The mean Beighton score for the whole group was 3 (range 0-7). Patients were divided into group A with Beighton score <4 and group B with Beighton score 4 or >.

Group A had 10 patients and all were male. 8 patients had shoulder stabilization and 2 had ACL reconstruction. The mean scar time was 9.7 weeks (range 5-22). The mean satisfaction score on VAS was 5.7 (range 0-10). The mean picture score was 1.8 (range 1-3). 4 patients each had score 1 and 2 and 2 patients had picture score 3. The mean Beighton score was 1.5 (range 0-3)

Group B had 11 patients, 8 were male and 3 female. 8 patients had shoulder stabilization and 3 had ACL reconstruction. The mean scar time was 34 weeks (range 3-68). The mean satisfaction score on VAS was 6 (range 4-10). The mean

picture score was 2 (range 1-3). 4 patients had a picture score 1, 2 had a picture score of 2 and 5 patients had a picture score of 3. The mean Beighton score was 4.8 (range 4-7)

There was no difference in the satisfaction with the scar on VAS scale in the lax or the stiff group 6 vs 5.7 although 5 patients in the lax group and 2 patients in the stiff group had poor cosmetic scars. The mean scar time was longer in patients with higher Beighton score group 34 w vs 9.7 w.

	Group A (Beighton score < 4)	**Group B (Beighton score 4 or >)**
Total No of Patients	10	11
Surgery		
Shoulder Stabilization	8	8
ACL reconstruction	2	3
Mean Scar age	9.7 weeks (5-22)	34 weeks (3-68)
Mean Satisfaction (VAS)	5.7 (0-10)	6 (4-10)
Mean Picture score	1.8 (1-3)	2 (1-3)
Mean Beighton Score	1.5 (0-3)	4.8 (4-7)
Poor cosmetic Scar (Picture Score=3)	2	5

Table 16- Scar satisfaction

Beighton Score and Tissue Laxity

Tissue specimens (capsule and tendon) were obtained from 24 patients and tested on the material testing system; 17 attending for ACL reconstruction (Group A) and 7 for open shoulder stabilization (Group B). 20 were male and 4 females. Mean age was 26.5 years (range 16-43). Most common mechanism of injury was contact sports in 12 patients. Mean Beighton score for the whole group was 3.2 (range 0-9) and median was 2.5. Eight (8) patients had a Beighton score > 4 indicating generalized joint hypermobility. 3 patients fulfilled the Brighton criteria for BJHS indicating that they also have symptoms of generalized joint hypermobility along with the signs as measured by the Beighton score.

The mean gradient of slope of graphs for both tendon and capsule was 23.8 N/m (range 3.08 - 52.63). The mean load required to produce 40 degrees angle at the little finger MCP joint was 0.04 kg with a range from 0-0.11 kg.

The mean age for group A (ACL reconstruction) was 27 years (range 16-53). There were 13 male and 4 female patients. 8 patients were involved in contact sports. The mean Beighton score was 3.7(range 0-9). 1 patients had BJHS. The mean gradient of slope of graphs for tendon tissue was 28.4 N/m (range 4.5 -52.6). The mean load required to produce the 40 degrees angle at the little finger MCP joint was 0.04 kg with a range from 0.01 – 0.11 kg.

The mean age for group B (Shoulder stabilization) was 25 years (range 17-30). All patients were male. 4 patients were involved in contact sports. The mean Beighton score was 2 (range 0-4). 2 patients had BJHS. The mean gradient of slope of graphs for capsule tissue was 12.5 N/m (range 3 -22). The mean load required to produce

the 40 degrees angle at the little finger MCP joint was 0.01 kg with a range from 0-0.04 kg.

	Combined Groups	**ACL Reconstruction (Group A)**	**Shoulder Stabilization (Group B)**
No of Tissue Specimen	24	17	7
Mean Age	26.5 (16-43)	27 (16-53)	25 (17-30)
Sex			
Male	20 (83%)	13 (76%)	7 (100%)
Female	4 (17%)	4 (24%)	0
Contact Sports	12 (50%)	8 (47%)	4 (57%)
Hypermobility			
Mean Beighton Score	3.2 (0-9)	3.7 (0-9)	2 (0-4)
Median Beighton Score	2.5	3	2
Beighton Score > 4	8 (33%)	7 (41%)	1 (14%)
Beighton Score > 6	5 (21%)	5 (29%)	0
BJHS	3 (12%)	1 (6%)	2 (28%)
Mean Slope Gradient (N/m)	23.8 (3.08 - 52.63)	28.43 (4.56 - 52.63)	12.56 (3.08 – 21.98)
Mean load to produce 40 degrees angle at MCPJ (Kg)	0.037 (0-0.109)	0.042 (0.007 - 0.109)	0.014 (0 - 0.038)

Table 17- Hypermobility, Mechanical testing and Load goniometer

There was no correlation between the Beighton score and the gradient of the laxity of the tissues measured by the material testing system (Chart 35)

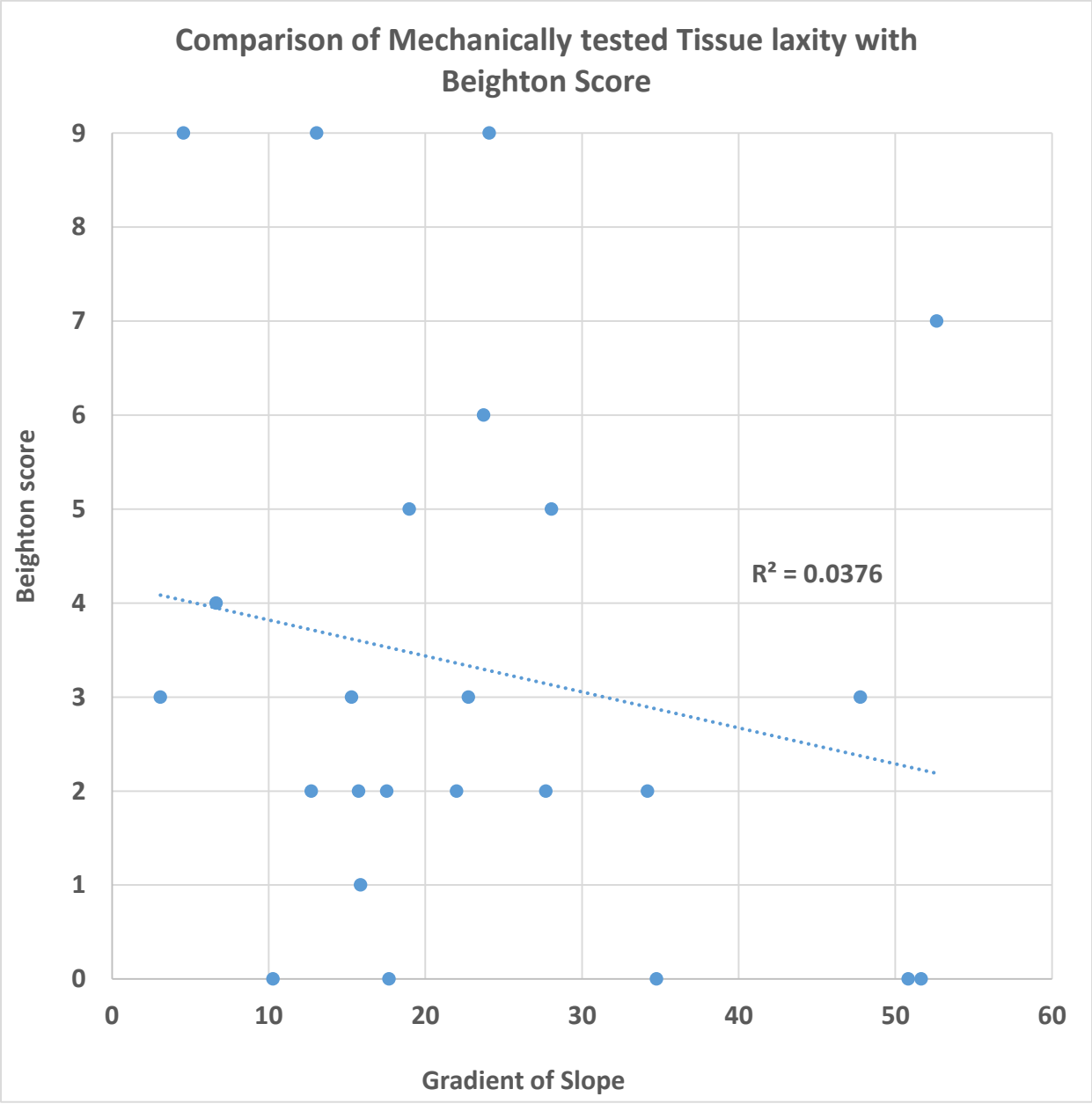


Chart 35- Comparison of mechanically tested Tissue laxity with Beighton Score

There was a positive correlation between the gradient of the force-displacement graph and the load required to produce 40 degrees angle at the little finger of the dominant hand (Chart 36)

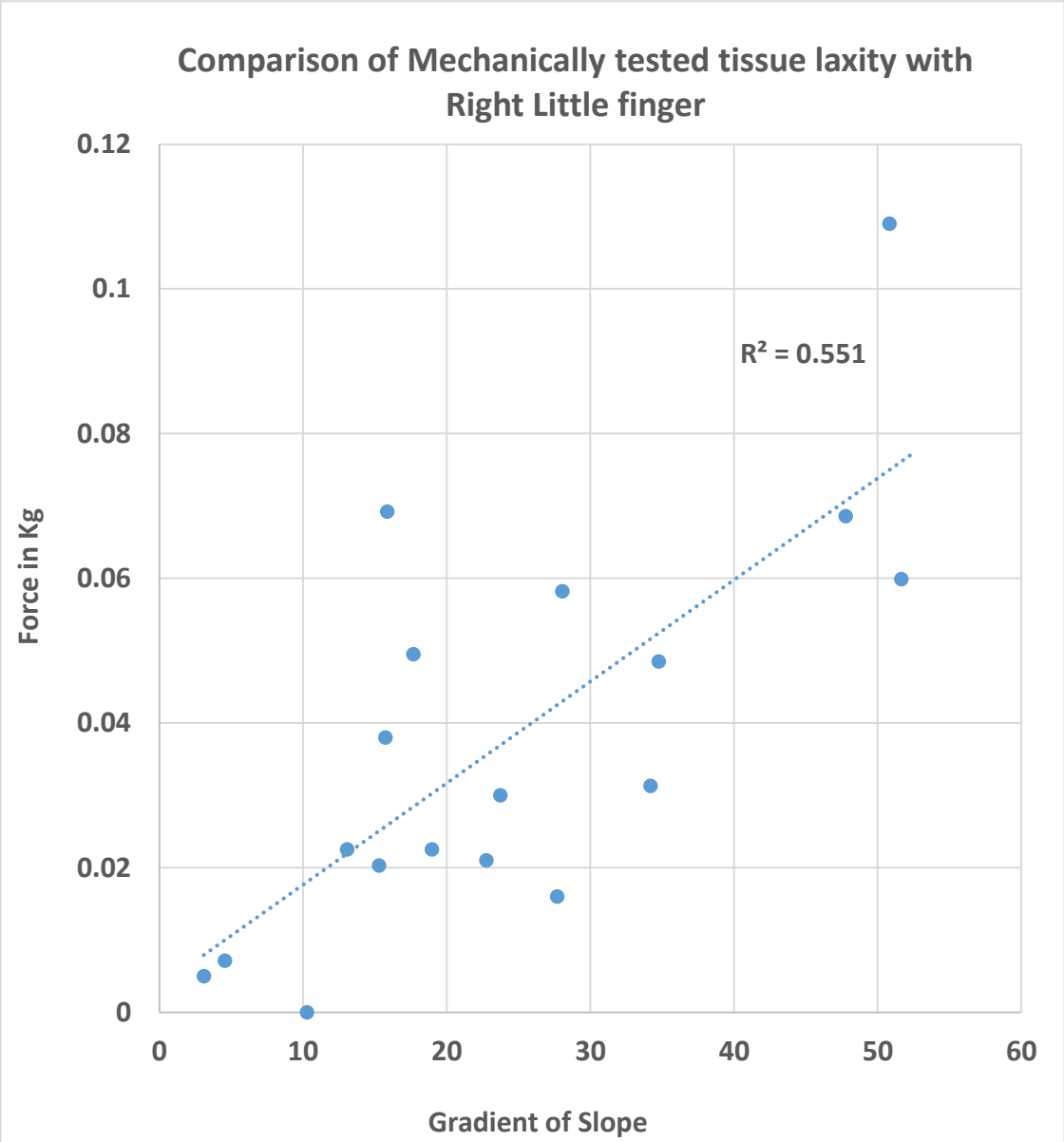


Chart 36- Comparison of mechanically tested Tissue laxity with Right Little Finger

Hamstring Tendon Graphs:

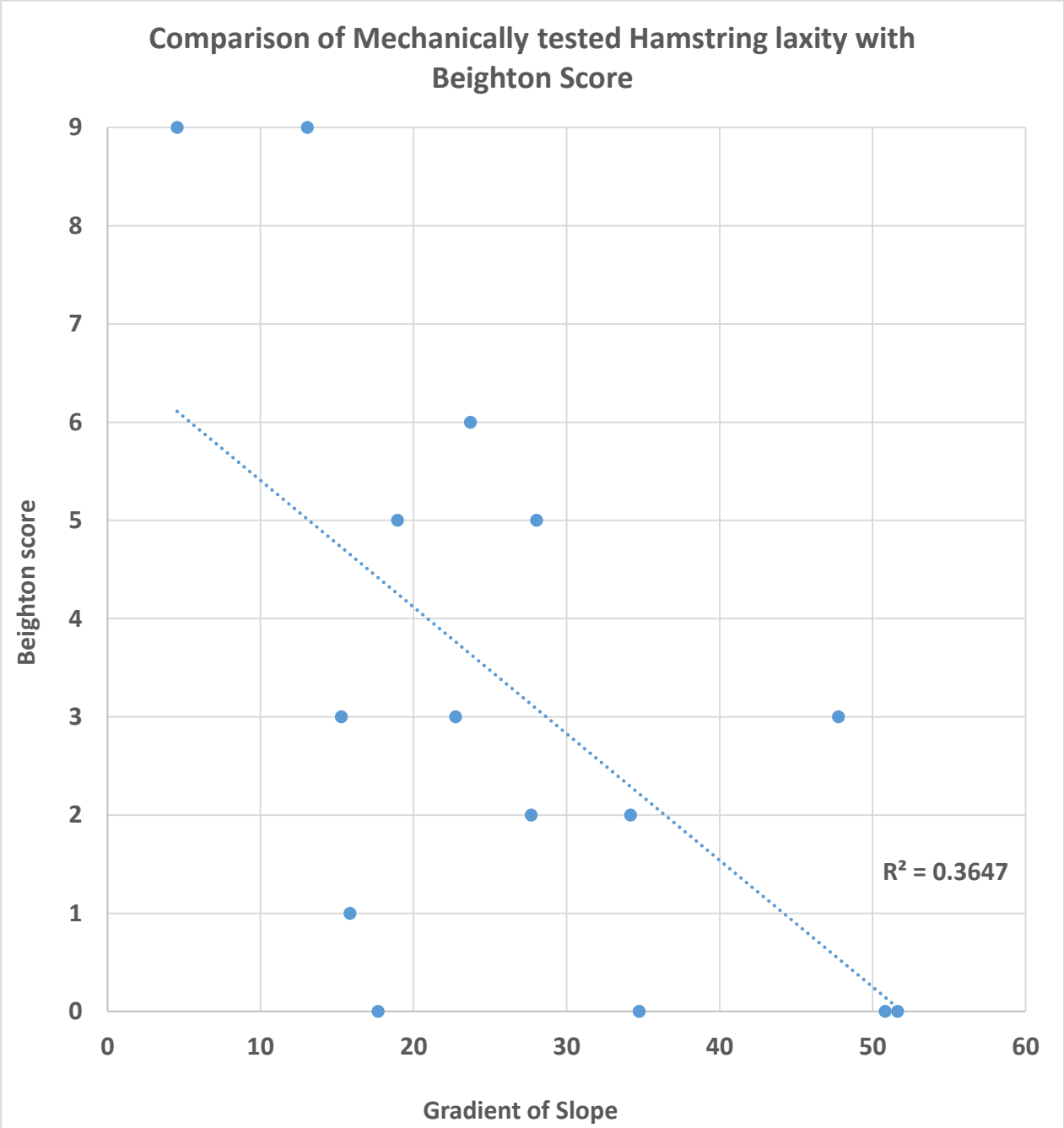


Chart 37- Comparison of mechanically tested Hamstring laxity with Beighton Score

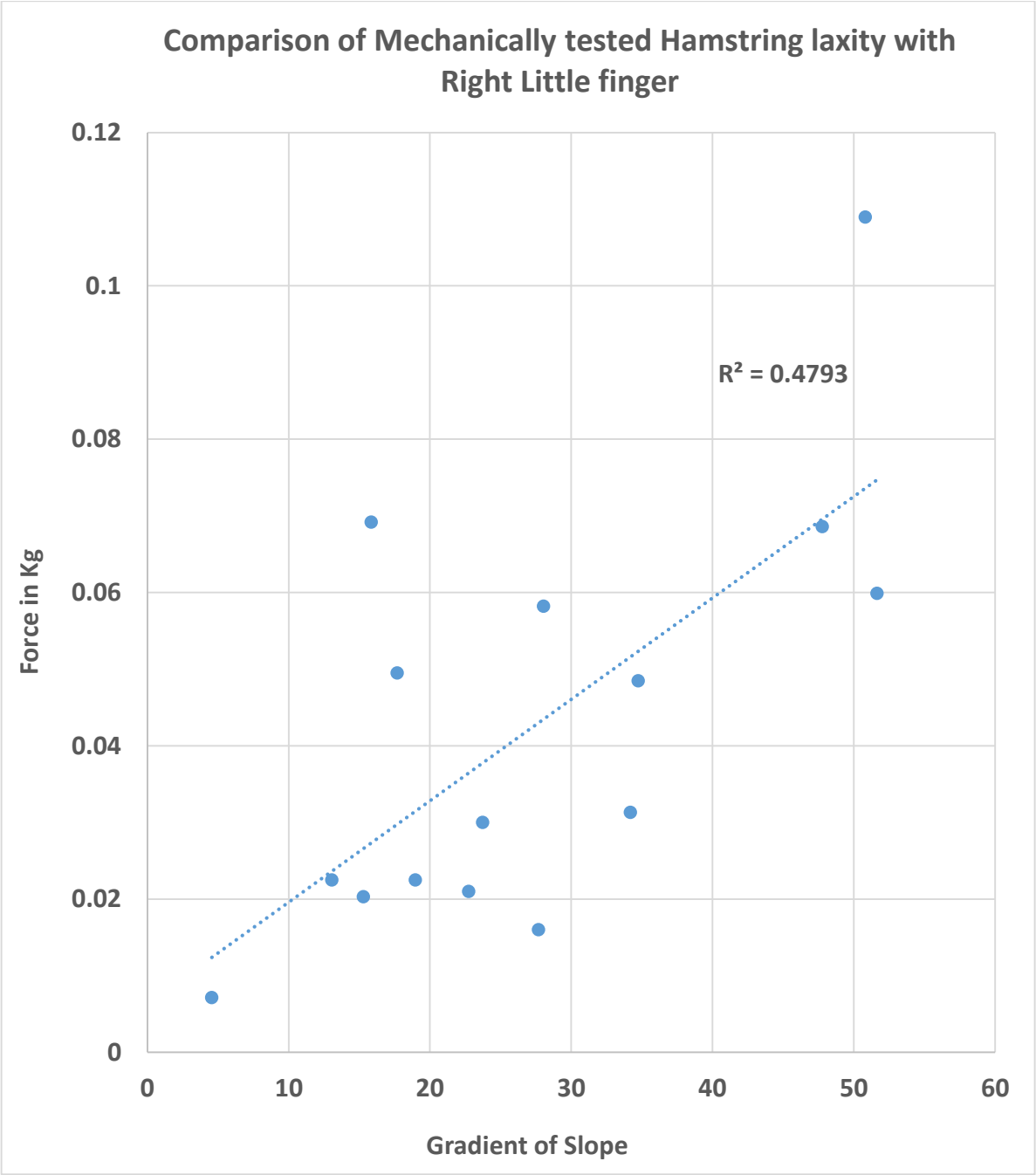


Chart 38- Comparison of mechanically tested Hamstring laxity with Right Little Finger

Shoulder Capsule graphs:

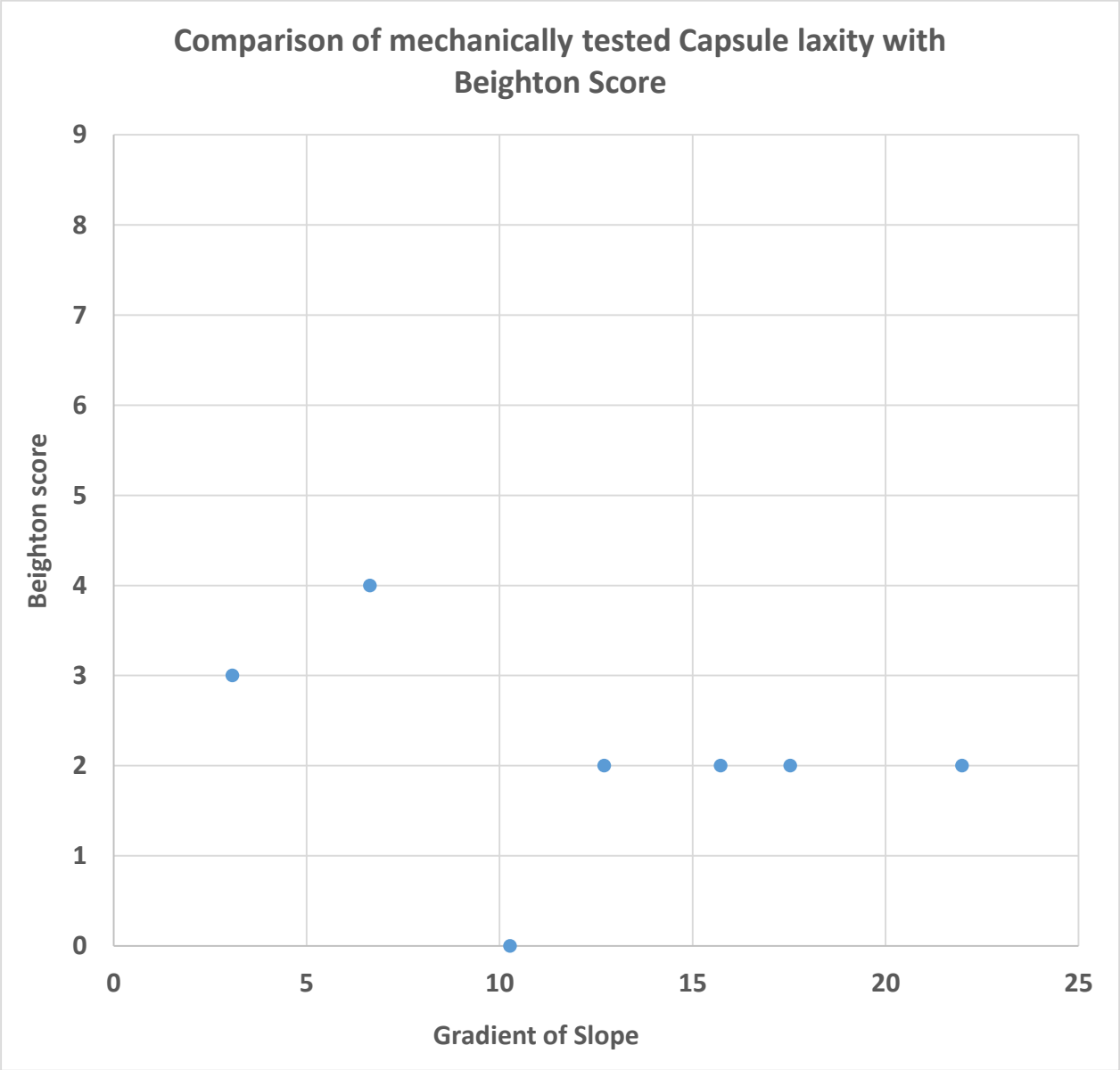


Chart 39- Comparison of mechanically tested Capsule laxity with Beighton Score

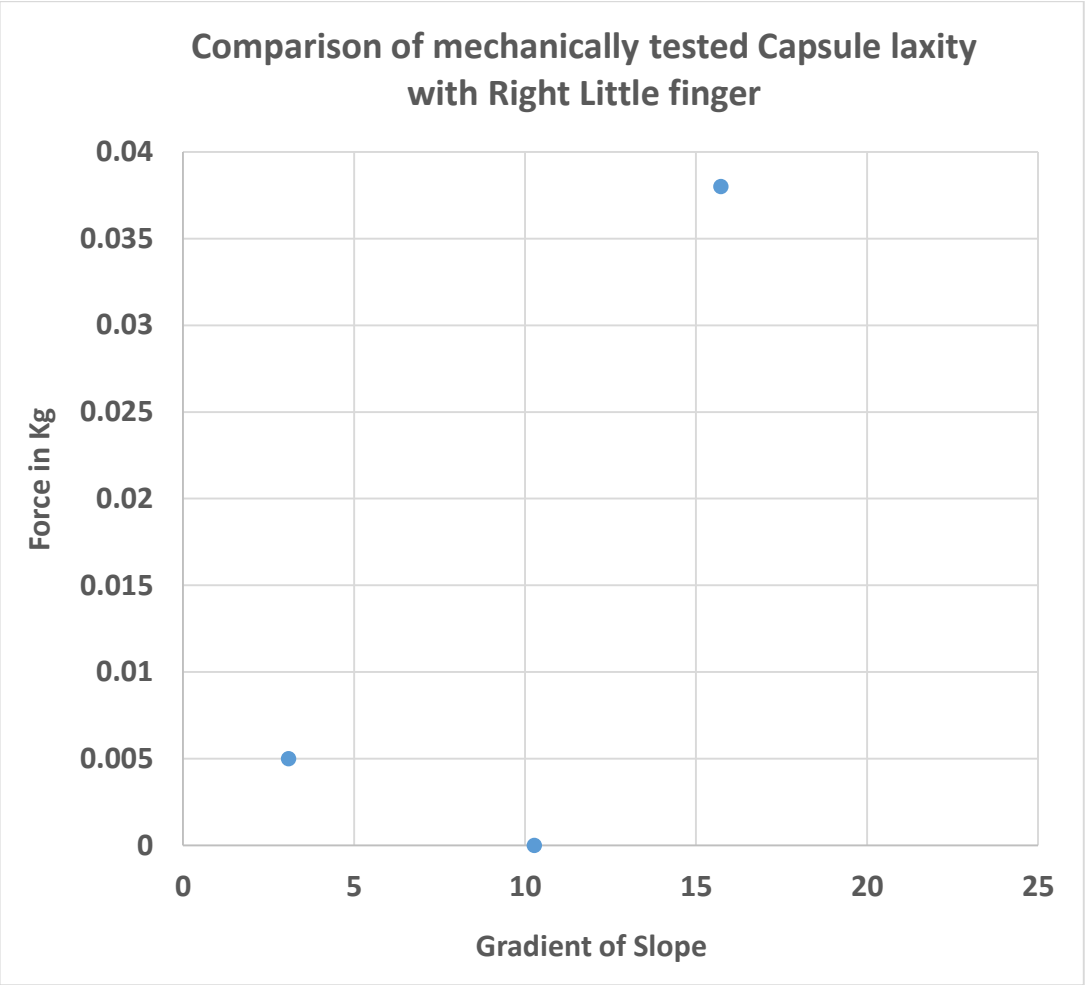


Chart 40- Comparison of mechanically tested Capsule laxity with Right Little Finger

Young's Modulus - Hamstring Tendons:

The Young's modulus of elasticity was calculated for the hamstring tendons of 9 patients undergoing primary ACL reconstruction. The mean age for 8 male and 1 female patient was 22.5 year (range 16-33). The mean Beighton score for the group was 3.1 (0-9). 3 patients had Beighton score 4 or more indicating GJH. No patient had BJHS. The mean load required to produce 40 degrees angle at the little finger MCPJ was 0.04 kg (range 0.02 - 0.06). The mean gradient of slope on mechanical testing of hamstring tendon was 26.94 N/m (range 13.06 - 51.64). The mean force to yield was 79.16 N (range 36.23 - 135.15). The mean stress was 683.92 N/m^2 (148.63 - 1126.31) and the mean strain was 0.94 (0.188 – 1.480). The mean young's modulus was 808.49 N/m^2 (range 358 - 1705). These patients were followed up for an average of 21 weeks (range 7-39 weeks). At the last FU all but 1 patient had full range of motion and stable graft so were discharged to physiotherapy. One patient had cyclops lesion resulting in stiffness.

Patient No	Beighton Score (SD-2.9)	Force to yield (N) (SD-34.6)	Stress (N/m^2) (SD-340)	Strain (SD-0.50)	Young's Modulus (N/m^2) (SD-350)	Load to produce 40 degree at little finger MCPJ(kg) (SD-0.02)	Mechanical testing gradient (Force vs Displacement) (Nm) (SD-14.57)
1	0	36.2	148.63	0.19	788.18	0.049	17.68
2	0	135.15	1126.31	1.32	853.18	0.059	51.64
3	1	52.62	487.22	1.36	358.19	0.069	15.85
4	2	101	1010	1.48	682.13	0.031	34.18
5	3	115.90	881.40	1.37	641.63	0.068	47.77
6	3	55.71	211.03	0.33	640.27	0.020	15.29
7	5	57.07	864.84	0.95	902.89	0.022	18.96
8	5	102.14	680.97	0.39	1705.87	0.058	28.04
9	9	56.61	744.90	1.05	704.10	0.022	13.06
Mean	3.1	79.16	683.92	0.94	808.49	0.04	26.94

Table 18- Hamstring Tendon; Hypermobility, Yield, Young's modulus, Gradient of Force Displacement and Load to produce 40 degrees MCPJ hyperextension at Right Little Finger

Hypermobility, Collagen V and Small Leucine rich Proteoglycans (SLRPs) expression- Biomarkers of tissue strength:

Demographics:

Group A (Skin) consisted of 25 patients; 21 male and 4 females and the mean age was 27 years (range 17-43 Y). 22 patients sustained their injuries playing sport and most common sport played was football (9 patients) followed by rugby (5 patients) and skiing (4 patients). The mean Beighton score for the whole group was 2.8 (range 0-9). Eight (8) patients in this group had Beighton score 4 or more indicating generalized joint hypermobility. The patients were divided into weak (A-weak) and strong (A-strong) groups according to the tensile strength on the material testing machine. The mean Beighton score for Group A(weak) was 3.4 and Group A(strong) was 1.9.

Group B (Hamstring Tendon) consisted of 9 patients; 7 male and 2 females. The mean age for this group was 27 years (range- 29-43). 8 patients were involved in sporting activities and the most common sport was skiing in 3 patients. The Mean Beighton score for the whole group was 2.3 and 3 patients had BS 4 or more indicating generalized joint hypermobility. The mean Beighton score for group B (weak) was 1.4 and group B(strong) was 3.2.

Group C (shoulder capsule) consisted of 10 patients and all were male. The mean age was 26 years (range 17-31). 8 patients were involved in sports and most common sport played was rugby in 5 patients. The mean Beighton score for the group was 2.1 (range 0-4). 2 patients had Beighton score 4 or more indicating

generalized joint hypermobility. The mean Beighton score for group C(weak) was 1.9 and group C(strong) was 2.

	Group A- Skin	**Group B- Tendon**	**Group C- Capsule**
Total No of Patients	25	9	10
Male	21	7	10
Female	4	2	0
Mean Age	27 (17-43)	27 (29-43)	26 (17-31)
Sports played	22	8	8
Mean Beighton Score	2.8	2.3	2.1
Beighton Score ≥ 4	8	3	2
Mean Beighton Score (weak group)	3.4	1.4	1.9
Mean Beighton Score (strong group)	1.9	3.2	2

Table 19- Demographics and Beighton score for skin, tendon and capsule specimens

Tissue Strength:

The mean force required for yield in 43 skin specimens was 70N (12-171), 10 hamstring tendons was 95N (86-105) and 15 shoulder capsules was 45N (17-78N). Data was analysed for weak (w) group below the mean force and strong group (s)

above the mean force. Group A (w) weak group with yield < 70N had 21 tissue specimens and Group A (s) strong group with yield >70N had 22 specimens. The mean force required for yield for group A (weak) was 41N (12-67) and group A (strong) was 98N (70-171). Group B (w) weak group with yield < 95N had 5 tissue specimens and Group B (s) strong group with yield >95N also had 5 specimens. The mean force required for yield for group B (w) weak group was 88N (86-93) and group B (s) strong group was 102N (98-105). Group C (w) weak group with yield <45N had 8 specimens and Group C (strong) with yield >45N strong group had 7 specimens. The mean force for group C (weak) was 31N (17-41) and group C (strong) was 59N (45-78).

	Group A-Skin	**Group B-Tendon**	**Group C-Capsule**
Total No of specimens	43	10	15
Force required for yield (N)	70 (12-171)	95 (86-105)	45 (17-78)
Weak Group specimen No	21	5	8
Force required for yield (N)	41 (12-67)	88 (86-93)	31 (17-41)
Strong Group specimen No	22	5	7
Force required for yield (N)	98 (70-171)	102 (98-105)	59 (45-78)

Table 20- Mechanical Strength for skin, tendon and capsule specimens

Immunohistochemistry Studies

Methods:

Histology specimens:

After ethics approval and informed consent 40 patients were studied, 25 undergoing primary ACL reconstruction and 15 undergoing shoulder stabilization. The mean age of patients was 26 years. 34 patients (85%) were male and 6 female (15%). Skin specimens were obtained from patients undergoing open shoulder stabilization or ACL reconstruction. Hamstring tendon was taken after the graft preparation for ACL reconstruction, using the semitendinosis and gracillis from the muskulotendinous junction end. Shoulder capsule was taken from patients undergoing open shoulder stabilization.

Slide Preparation:

For every tissue specimen, one part of the specimen was snap freeze with liquid nitrogen and the other part was fixed in formalin for processing and embedding in paraffin wax in the Department of Pathology. 5 micron sections were cut, floated and dried onto glass slides. Sections were either stained by haematoxylin and eosin or following appropriate antigen retrieval used for immunohistochemistry according to the laboratory protocols (Dako, 2001)(Nairn & Helbert, 2006).

Grading of the Immunohistological Staining:

Lens x 4 magnification was mostly used. The x 10 lens was used for detailed analysis of the staining of skin, tendon and capsule. Grading of the staining for each slide was done on a 0-4 scale (0 = no staining, 1 = mild staining <50 % of the tissue, 2 = Mild staining >50 % of the tissue, 3 = strong staining < 50 % of the tissue and 4 = strong staining > 50 % of the tissue).

Representative control slides for skin, tendon and capsule for Collagen V, Biglycan and Decorin were selected. The control slides were then used as a reference before immunohistochemical grading of each slide. An additional two independent observers scored the immunohistochemical staining on a random selection of slides of skin, tendon and capsule for collagen V, Biglycan and Decorin using the same control slides for reference and according to the agreed standards mentioned above using the similar magnifications. The inter-observer values were calculated and the kappa ratio was 0.8. After a gap of 6 months all these slides were reviewed again and by using the same control slides and according to the agreed protocols; grading of the stain was done to calculate the intra-observer value and the kappa ratio was 0.78. Images of the control slides with Haematoxylin and Eosin, Collagen V, Biglycan, Decorin and negative stain were taken. Student T test was used to compare the mean expression of collagen V, Biglycan and Decorin in different groups of skin, tendon and capsule and a P value of <0.05 was set as significant.

A- Localization of Collagen V:

Localization of Collagen V was studied by immunohistochemical staining of formalin fixed paraffin embedded sections (FFPE). Collagen V antibody (Abcam, 2009a) was used at a 1:500 dilution without an antigen retrieval step.

Collagen V- Histology slides protocol:

Collagen V expression was studied at the following locations in different tissue specimens.

A- <u>Skin:</u>

- Dermal papilla (Basement Membrane)
- Dermis (Extracellular matrix)
- Appendages (Blood vessels, sweat glands, sebaceous glands, arrector pilli)

Lens x10 was used for the grading of the stain.

Slide no: 2015Z/09 was used as a **control slide**.

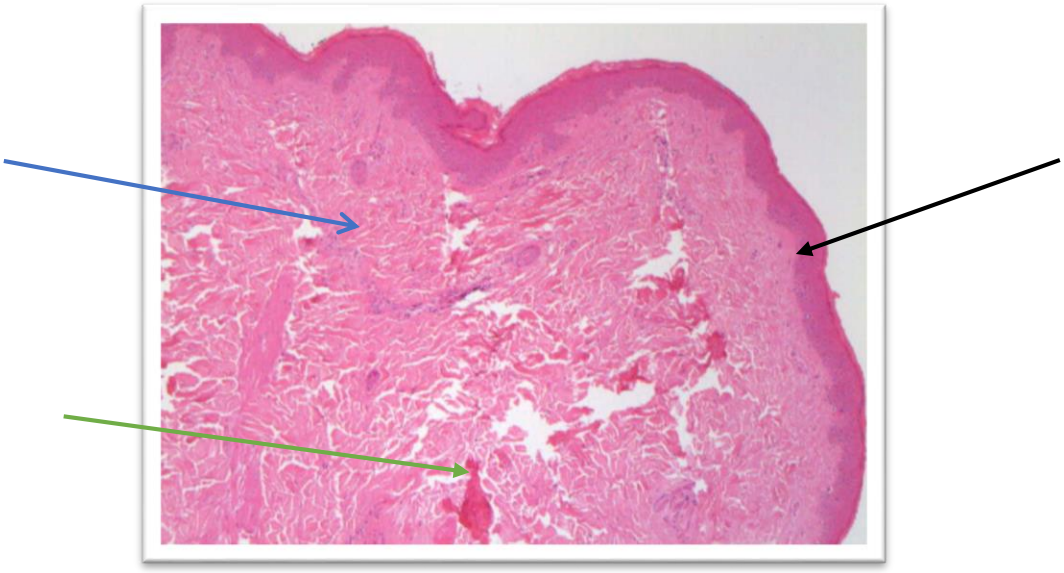


Figure 24- Skin specimen stained with haematoxylin and Eosin

Skin epidermis stained negative but dermal papilla (Black arrow), papillary and reticular dermis (Blue arrow), blood vessels, sweat glands, sebaceous glands and arrector pilli (Green arrow) stained positive to varying degrees for collagen V.

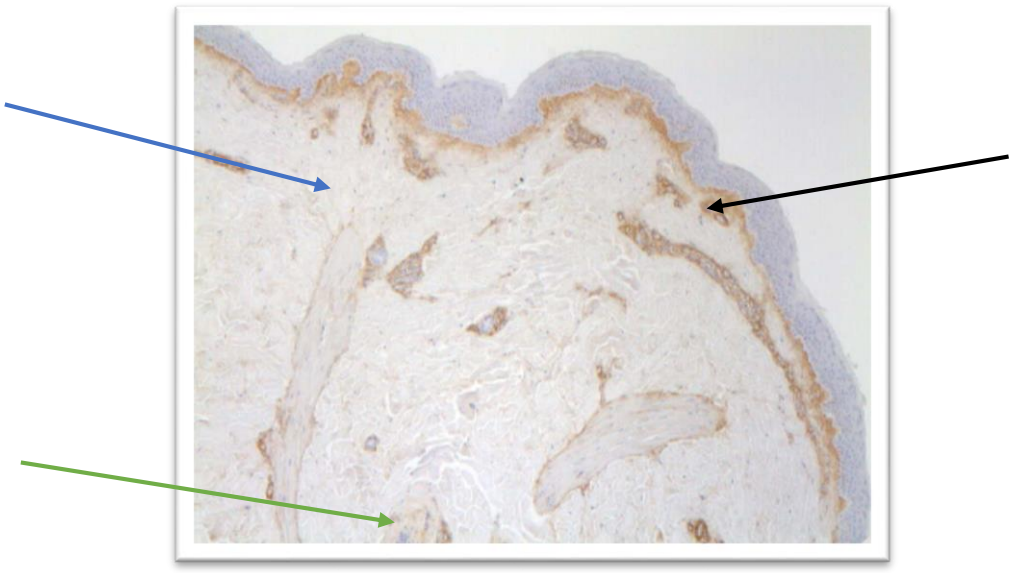


Figure 25- Skin specimen showing collagen V staining in brown

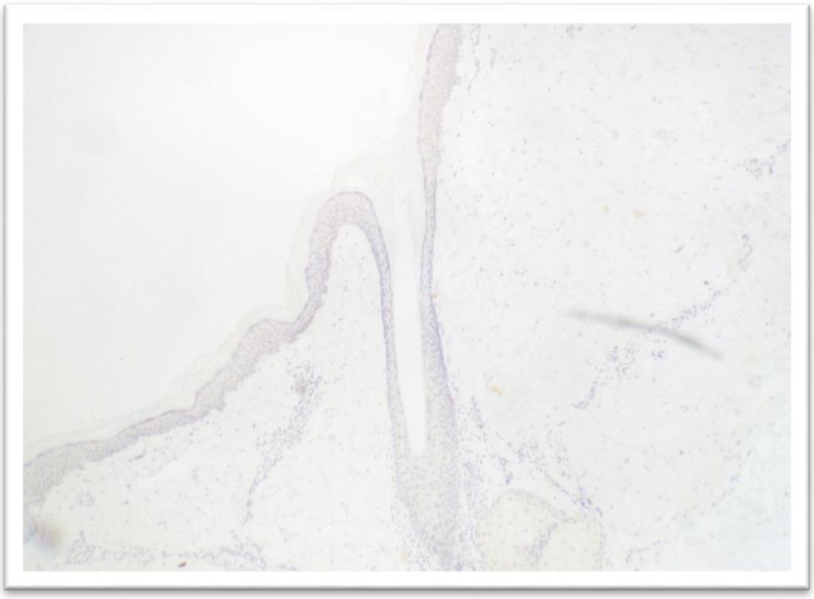


Figure 26- Skin specimen without collagen V staining (Negative Control)

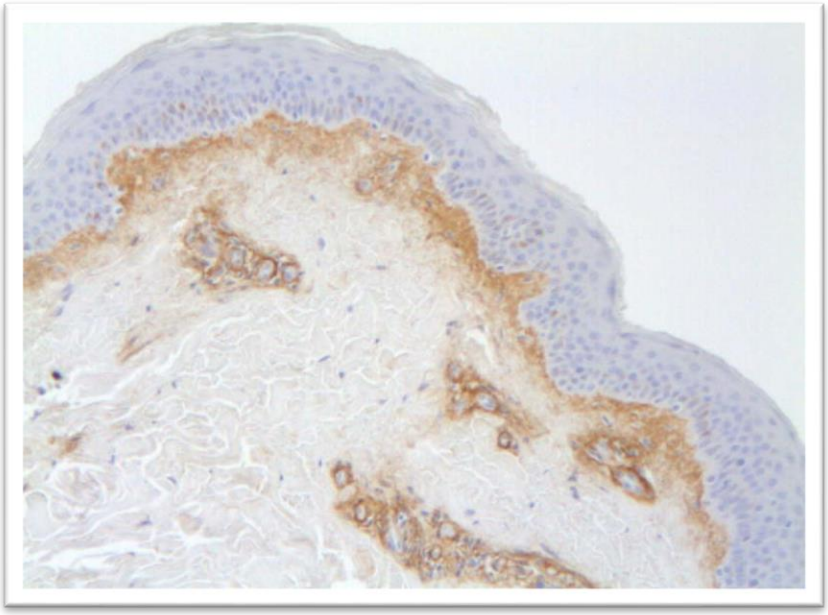


Figure 27- Skin Dermal Papillae showing collagen V staining in brown

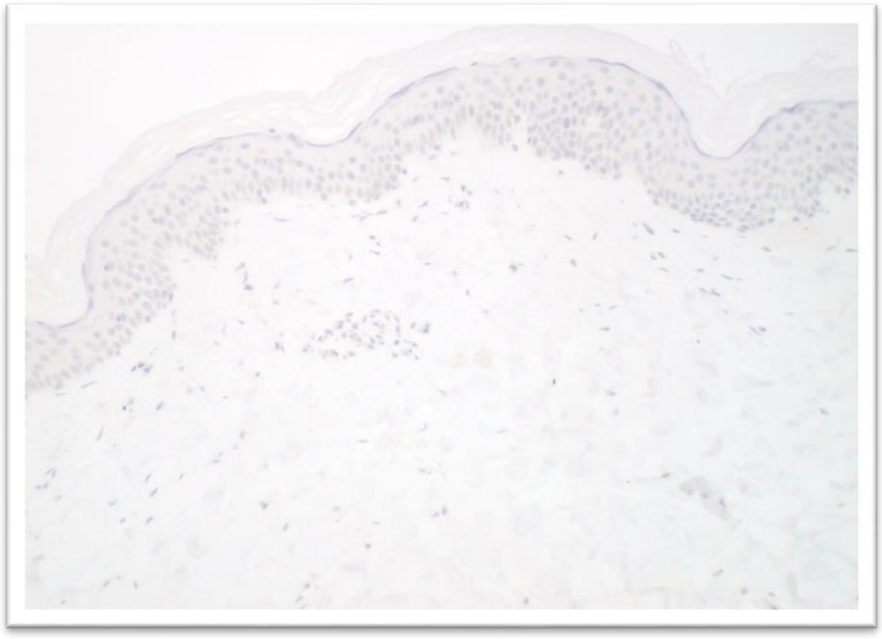


Figure 28- Skin Dermal Papillae without collagen V staining (Negative Control)

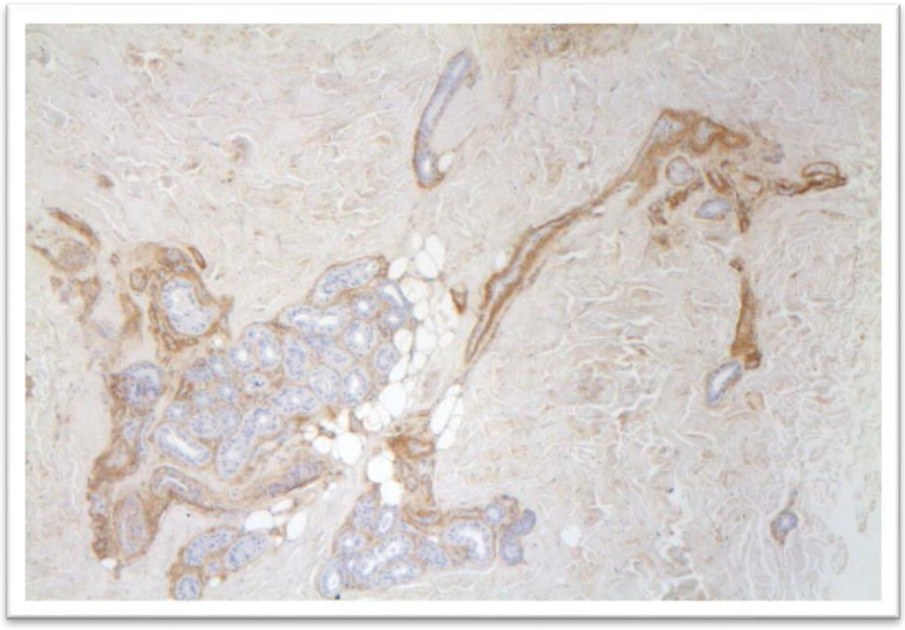


Figure 29- Skin appendages showing collagen V staining in brown

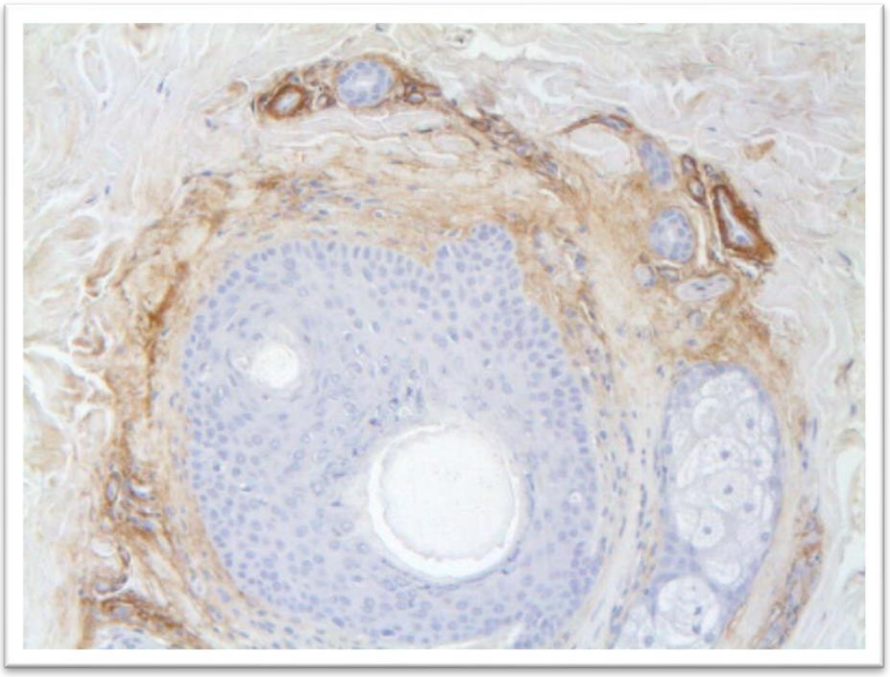


Figure 30- Skin appendages showing collagen V staining in brown

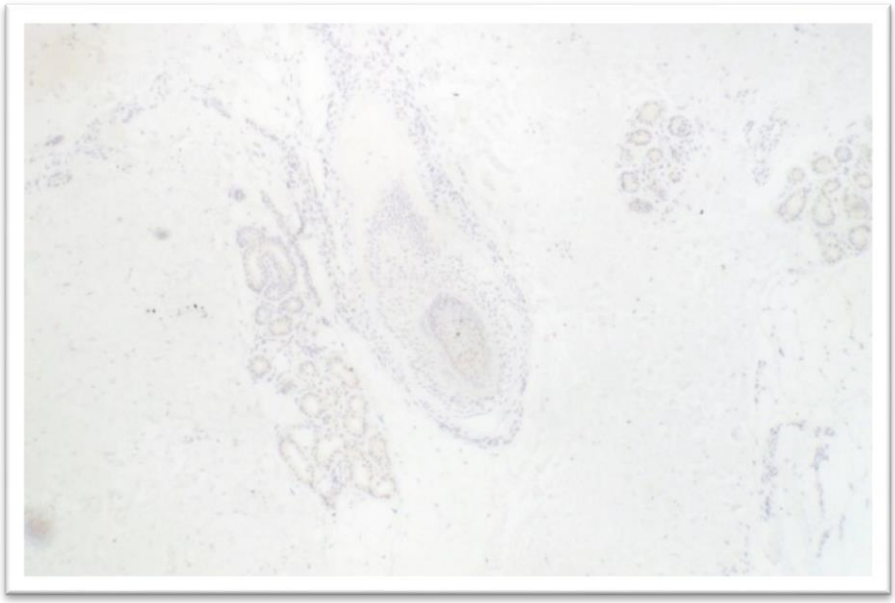


Figure 31- Skin appendages without collagen V staining (Negative Control)

B- <u>Tendon:</u>

- Tendon sheath (not visible in all cases)
- Extracellular Matrix (ECM-collagen fibers)
- Blood vessels
- Interfascicular tissue (not visible in all cases)

Lens x4 was mostly used although lens x10 was used for detailed analysis

Slide no: 2014G/09 was used as a **control slide**.

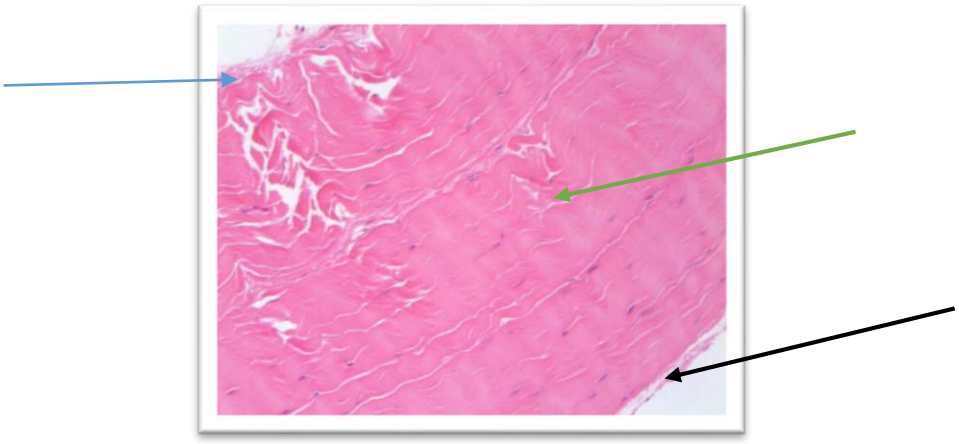


Figure 32- Tendon specimen with haematoxylin and eosin staining

Tendon sheath (Black arrow), collagen fibres (Green arrow), blood vessels and interfascicular connective tissue (Blue arrow) stained positive to varying degrees, but skeletal muscle stained negative.

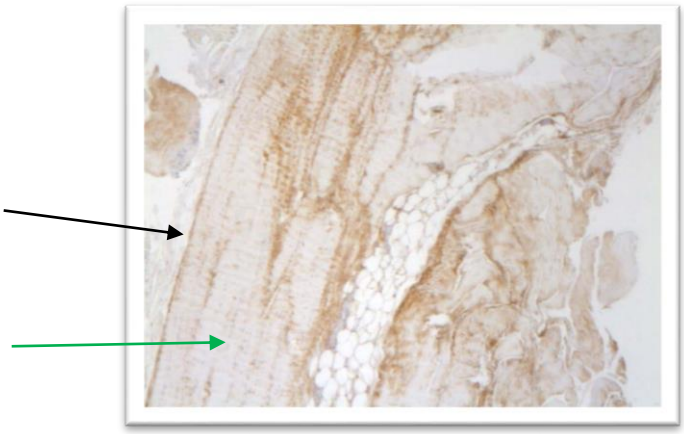


Figure 33- Tendon showing Collagen V staining in brown

C- <u>Capsule:</u>

- Synovial surface (SS)
- Blood vessels (BV)
- Extracellular Matrix (ECM-collagen fibers)

Lens x4 was mostly used although lens x10 was used for detailed analysis.

Slide no: 6558/09 was used as a **control slide**.

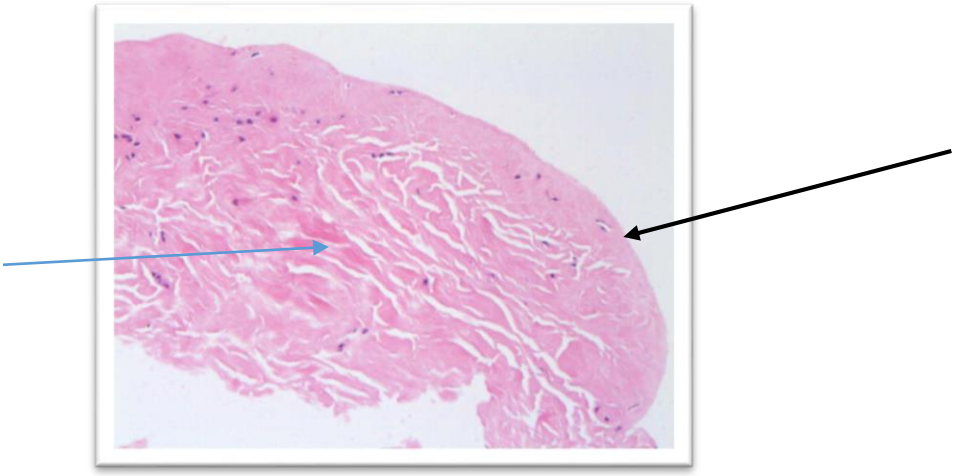


Figure 34- Shoulder Capsule specimen with Haematoxylin and eosin staining

Synovial surface of the capsule (Black arrow), blood vessels and extracellular matrix (Blue arrow) stained positive to varying degrees.

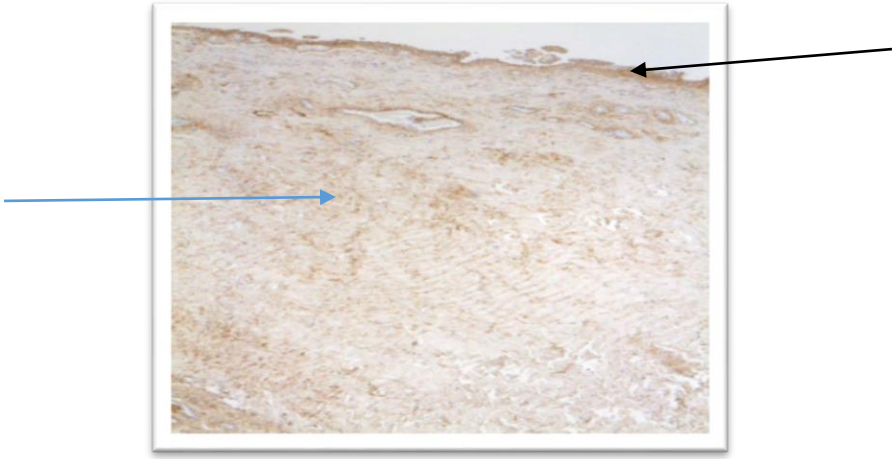


Figure 35- Shoulder capsule showing Collagen V staining in brown

B-Localization of Biglycan:

Localization of Biglycan was studied by immunohistochemical staining of paraffin embedded sections. Biglycan antibody (Abcam, 2009b) was used. Pre-treatment was performed with microwave and EDTA. A dilution of 1:100 was selected with antigen retrieval after comparing the results with dilutions of 1:50 and 1:200.

Biglycan - Histology slides protocol

Biglycan expression was studied at the following locations in different tissue specimens.

A- <u>Skin:</u>

- Skin epidermis (ED)
- Appendages (Blood vessels, sweat glands, sebaceous glands, arrector pilli)
- Superficial Dermis (Extra cellular matrix)
- Deep Dermis (Extra cellular matrix)

Lens x10 was used under full light of microscope for the grading of the stain.

Slide no: 26981X/08 and 7649T/09 were used as **control slides**.

Skin epidermis (Black arrow), blood vessels, sweat glands, sebaceous glands, hair follicle (Green arrow) and superficial dermis (Blue arrow) and Deep Dermis (Orange arrow) stained positive for biglycan to varying degrees.

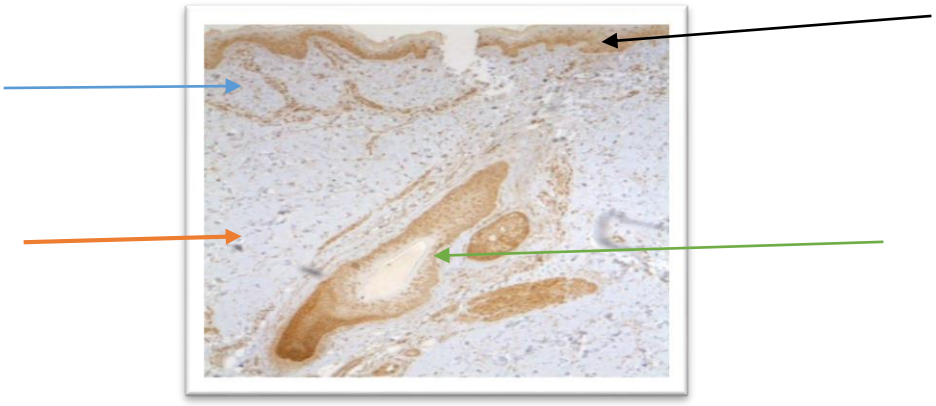


Figure 36- Skin showing Biglycan staining in brown

B- <u>Tendon:</u>

- Extracellular Matrix (ECM- collagen fibers)
- Blood vessels (BV)
- Skeletal muscle (non-specific staining)

Lens x4 was mostly used although lens x10 was used for detailed analysis

Slide no: 2014 G/09 was used as a **control slide**.

Tendon sheath (Black arrow) stained negative but collagen fibers (Blue arrow), blood vessels and skeletal muscles stained positive to varying degrees.

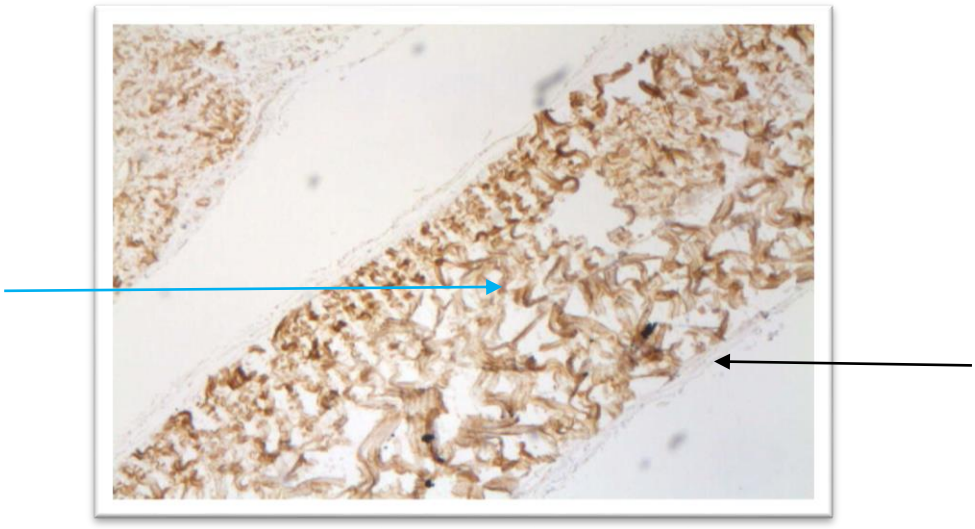


Figure 37- Tendon showing Biglycan staining in brown

C- <u>Capsule:</u>

- Synovial surface (SS)
- Extracellular Matrix (ECM – collagen fibers)
- Blood vessels (BV)

Lens x4 was mostly used although lens x10 was used for detailed analysis

Slide no: 27018R/08 was used as a **control slide**.

Synovial surface of the capsule, blood vessels and ECM stained positive to varying degrees.

Figure 38- Shoulder capsule showing Biglycan staining in brown

C-Localization of Decorin:

Localization of Decorin was studied by immunohistochemical staining of paraffin embedded sections. Decorin antibody (SANTA CRUZ BIOTECHNOLOGY, 2009a) was used. Pre-treatment was done with microwave and citrate. Decorin was used in 1:100 dilution with antigen retrieval after comparing with dilutions of 1:50 and 1:200.

Decorin - Histology slides protocol

Decorin expression was studied at the following locations in different tissue specimens.

A- Skin:

1- Skin dermis

Lens x 4 was used under full light of microscope for the grading of the stain.

Slide no: 7416D/09 was used as a **control slide**.

Skin epidermis, blood vessels, sweat glands, sebaceous glands and arrector pilli stained negative but dermis stained positive to varying degrees for decorin

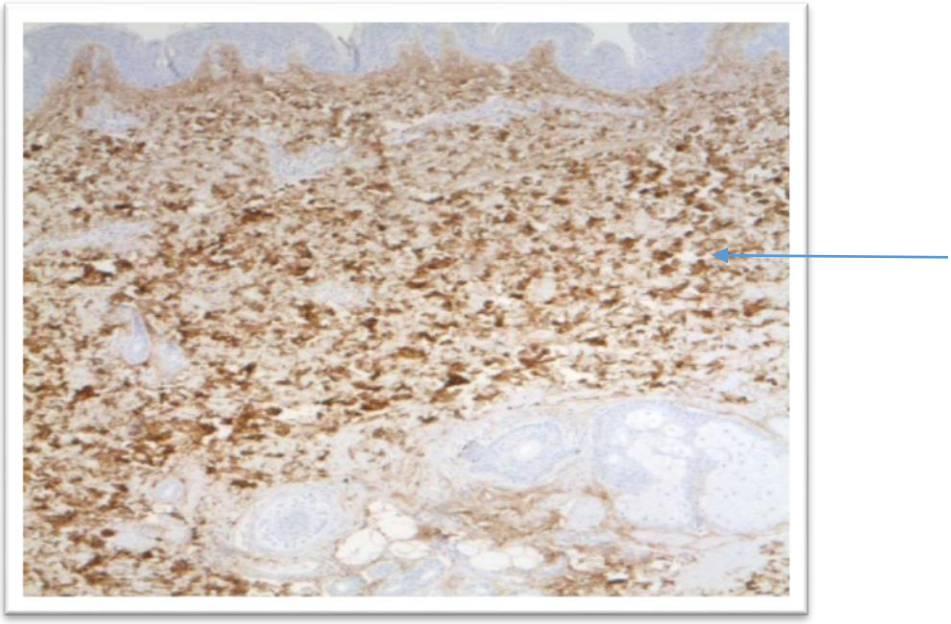


Figure 39- Skin showing Decorin staining in brown

B- Tendon:

1- Extracellular Matrix (ECM - collagen fibers)

2- Tendon sheath in some cases

Lens x4 was mostly used for the grading of the stain.

Slide no: 5240J/09 was used as a **control slide**.

Tendon sheath and collagen fibers in ECM stained positive to varying degrees but skeletal muscle did not stain for decorin.

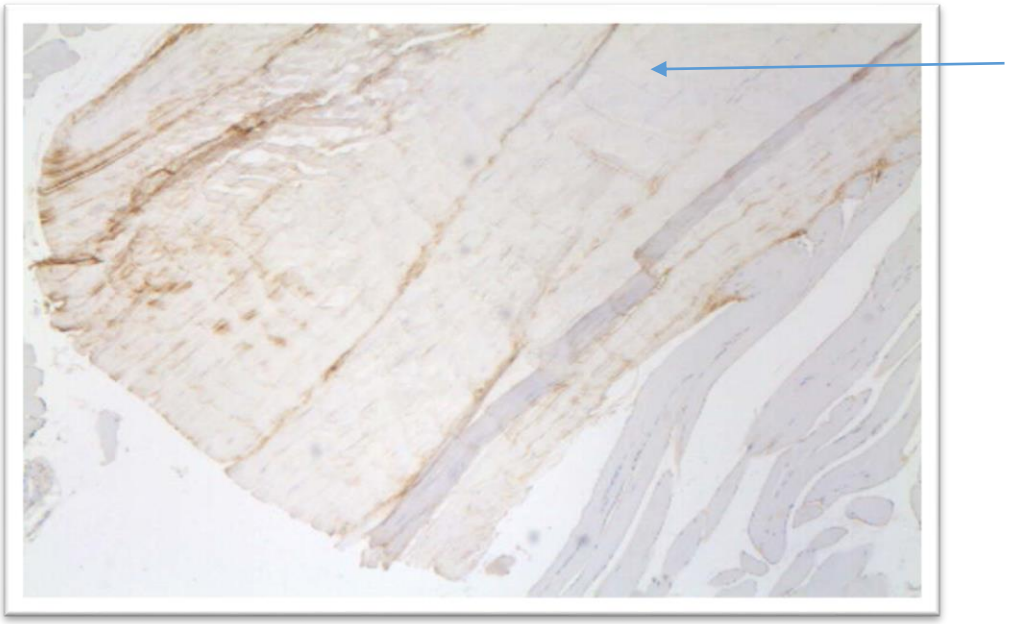


Figure 40- Tendon showing Decorin staining in brown

C- Capsule:

1- ECM (collagen fibers)

Lens x4 was mostly used under full light of microscope for the grading of the stain.

Slide no: 2288B/09 was used as a control slide.

Synovial surface of the capsule, blood vessels and skeletal muscles stained negative but ECM was positive to varying degrees.

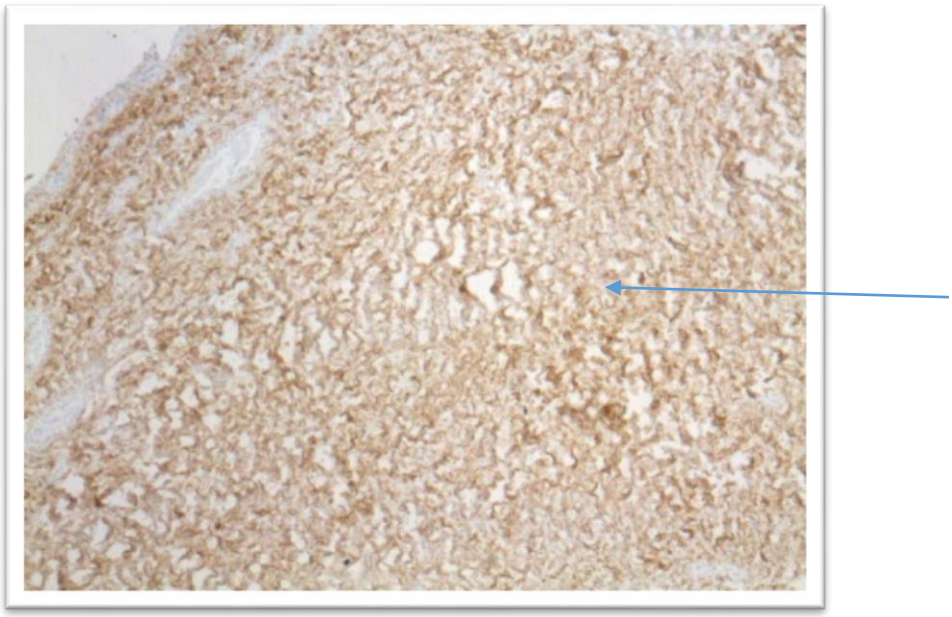


Figure 41- Shoulder capsule showing diffuse Decorin staining (brown) throughout the ECM

D- Localization of Fibromodulin:

Localization of Fibromodulin was studied by immunohistochemical staining of paraffin embedded sections. Fibromodulin antibody (SANTA CRUZ BIOTECHNOLOGY, 2009b) was used. Different methods were used for testing but unfortunately they all failed. We tried direct antigen antibody reaction without pre-treatment to varying concentrations ranging from 1:50, 1:100, 1:200. We also tried treatment with heat, trypsin, heat and citrate buffer, heat and EDTA and citrate. We also used freeze dried specimen of the skin but could not get a positive antigen-antibody reaction. After discussion with manufacturers of the antibody and acting on their advice still there was no staining of the tissue specimens with Fibromodulin. So no testing was possible for fibromodulin on the collected specimens.

E- Localization of Tenascin X:

Localization of Tenascin X was studied by immunohistochemical staining of paraffin embedded sections. Tenascin X antibody (SANTA CRUZ BIOTECHNOLOGY, 2004) was used. No pre-treatment was done for Tenascin X and 1:100 dilution was used for antigen antibody reaction with antigen retrieval after comparing with dilutions of 1:50 and 1:200. Tenascin X was only tested in skin specimens and did not show a positive antigen – antibody reaction in the dermis although basement membrane stained positive in all specimens to varying degrees. After review of the slides it was not possible to differentiate the grade of staining of different slides.

Results:

Collagen V Expression:

Skin epidermis stained negative but dermal papilla, papillary and reticular dermis, blood vessels, sweat glands, sebaceous glands and arrector pilli stained positive to varying degrees for collagen V. Tendon sheath, collagen fibres, blood vessels and interfascicular connective tissue of the tendon stained positive to varying degrees, but skeletal muscle was negative. Synovial surface of the capsule, blood vessels and extracellular matrix stained positive to varying degrees. The mean grading of collagen V expression in skin dermal papilla was 2.4, appendages 2.2 and extracellular matrix (ECM) 1.8 in A(w) and 1.3,1.8 and 1.7 respectively in A(s), tendon sheath (TS) was 2.2 and blood vessels (BV) was 2.8 in both groups (Bw/Bs) and ECM was 2.4 in B(w) and 1.4 in B(s), capsule's synovial surface was 2.6, blood vessels (BV) 1.6 and ECM 1.9 in C(w) and 4, 3.1 and 2.6 respectively in C(s).

	Skin Dermal Papilla	**Skin Appendages**	**Skin extracellular matrix**
Collagen V Grading- (Weak Group)	2.4	2.2	1.8
Collagen V Grading- (Strong Group)	1.3	1.8	1.7
Student T test	**P=0.001**		

Table 21 - Collagen V expression in Skin

	Tendon Sheath	Tendon Blood Vessels	Tendon extracellular matrix
Collagen V Grading-(Weak Group)	2.2	2.8	2.4
Collagen V Grading-(Strong Group)	2.2	2.8	1.4
Student T test			p=0.05

Table 22- Collagen V expression in Tendon

	Capsule Synovial Surface	Capsule Blood vessels	Capsule extracellular matrix
Collagen V Grading-Weak Group	2.6	1.6	1.9
Collagen V Grading-Strong Group	4	3.1	2.6
Student T test	p=0.09	p=0.07	p=0.22

Table 23- Collagen V Expression in Capsule

Student T test was used to compare the mean expression of collagen V in different groups and a P value of <0.05 was significant. Collagen V expression was higher in the skin dermal papillae of weaker group and this difference was statistically significant P=0.001.

Grading of the stain for each slide was done on a 0-4 scale (0 = no staining, 1 = mild staining <50 % of the tissue, 2 = Mild staining >50 % of the tissue, 3 = strong staining < 50 % of the tissue and 4 = strong staining > 50 % of the tissue).

Small Leucine rich Proteoglycans (SLRPs) expression:

Decorin Expression:

The mean grading of Decorin expression in skin was 3.2 in A (w) and 3.1 A (s), tendon was 3.4 in B (w) and 3.8 in B (s) and shoulder capsule was 2.7 in C (w) and 3.3 in C (s). This difference was not statistically significant.

	Skin Dermis (extracellular matrix)	**Tendon Sheath**	**Capsule (extracellular matrix)**
Decorin Grading (weak group)	3.2	3.4	2.7
Decorin grading (strong group)	3.1	3.8	3.3

Table 24- Decorin Expression in Skin, Tendon and Capsule

Biglycan expression:

The mean grading of Biglycan expression in Skin epidermis was 1.5, appendages 2.2, ECM in superficial dermis 1.5 and deep dermis 0.75 in A (w) and 1.75, 2.1, 1.5 and 0.5 respectively in A (s), Tendon ECM was 1.75 in B (w) and 3.2 in B (s), Capsule synovial surface was 2, BV 2 and ECM 2.9 in C (w) and 2, 2.5 and 4 respectively in C (s). There was no statistically significant difference in those groups.

	Skin Epidermis	Skin appendages	Skin superficial Dermis	Skin Deep Dermis
Biglycan Grading (weak group)	1.5	2.2	1.5	0.75
Biglycan Grading (strong group)	1.75	2.1	1.5	0.5

Table 25- Biglycan Expression in Skin

	Tendon extracellular matrix	Tendon Blood vessels	Tendon skeletal muscle
Biglycan Grading (weak group)	1.75	1	1
Biglycan Grading (strong group)	3.2	2.6	2
Student T test	p=0.18		

Table 26- Biglycan Expression in Tendon

	Capsule Synovial surface	Capsule extracellular matrix	Capsule blood vessels
Biglycan Grading (weak group)	2	2.9	2
Biglycan Grading (strong group)	2	4	2.5
Student T test		p=0.39	

Table 27- Biglycan Expression in Capsule

The weaker tendon group was found to have a lower mean Beighton score, while the weaker skin group had a higher mean Beighton score. Decorin expression in capsule ECM (P= 0.02) was statistically significant when compared against the Beighton score indicating GJH. Collagen V expression was higher in the skin dermal papillae of weaker group. These markers play an important role in the strength of tissues and should be assessed in professional athletes to determine the risk of injury.

Benign Joint Hypermobility Syndrome (BJHS), Tissue Strength, Collagen V and Small Leucine Rich Proteoglycans (SLRPs) Expression in athletes undergoing shoulder stabilization or ACL reconstruction

	Group A- Skin	**Group B- Tendon**	**Group C- Capsule**
Total No of patients	30	9	10
Sex			
Male	26	7	10
Female	4	2	0
Mean Age	27 (17-43)	27.7 (19-43)	26 (17-31)
Sports played	21	8	8
Mean Beighton score	2.6 (0-9)	2.5 (0-9)	2.1 (0-4)
Mean Force for yield	63 N (12-144)	94.5 N (86-105)	39.7 N (17-66)
Family History of laxity	6	3	2
BJHS			
Yes	4	1	3
No	26	8	7

Table 28- Demographics of Skin, Tendon and Capsule Groups- BJHS

Benign Joint Hypermobility Syndrome (BJHS), Skin Strength, Collagen V and Small Leucine Rich Proteoglycans (SLRPs) Expression in athletes undergoing shoulder stabilization or ACL reconstruction

Data was collected for 30 patients undergoing either open shoulder stabilization or primary ACL reconstruction.

4 patients had BJHS (Group A). 26 patients did not have BJHS (Group B). The mean age for group A was 31 years. 3 were male and 1 female. 3 patients in Group A had shoulder stabilization and 1 had primary ACL reconstruction. 2 patients played football. The mean force required for yield was 71N (31-94). The mean Beighton score was 2.4 (range 1-4). 1 patient had Beighton score more than 4, which (using current criteria) indicated generalized joint hypermobility. 4 patients

had a family history of hypermobility. The mean grading of collagen V expression in the skin dermal papilla (basement membrane) was 2, appendages was 2 and skin superficial and deep dermis extracellular matrix was 2.4. The mean grading of Decorin expression in the ECM was 3.75 and Biglycan expression was 2 in the epidermis and skin appendages, 3 in the superficial dermis ECM and 1 in the deep dermis ECM.

The mean age for group B was 26 years. 23 were male and 3 females. 10 patients in Group B had shoulder stabilization and 16 patients had primary ACL reconstruction. 10 patients played football, 6 played rugby and 4 skiing. The mean force required for yield was 63N (12-144). The mean Beighton score was 2.7 (range 0-9). 7 patient had Beighton score more than 4 and 3 patients had Beighton score more than 6 indicating hypermobility. 3 patients had family history of hypermobility. The mean grading of collagen V expression in the skin dermal papilla (basement membrane) was 1.9, appendages was 2 and skin superficial and deep dermis (extracellular matrix) was 1.7. The mean grading of Decorin expression in the ECM was 3.4 and Biglycan expression was 1.9 in the epidermis, 2.3 in the skin appendages, 1.6 in the superficial dermis and 0.86 in the deep dermis ECM.

The expression of Collagen V, SLRP's, skin strength and BJHS were studied in patients undergoing shoulder stabilization or primary ACL reconstruction. The force required for yield for skin specimens was higher in patients with BJHS. The mean grading of Biglycan expression in the ECM of superficial dermis was higher in patients with BJHS. There was no difference in the expression of Collagen V in both groups.

	Group A (BJHS)	Group B (No BJHS)
Mean Age	31	26
Sex		
Male	3	23
Female	1	3
Mean force required for yield (N)	71 (31-94)	63 (12-144)
Mean Beighton score	2.4 (1-4)	2.7 (0-9)
Beighton score 4 or >	1	7
Beighton score 6 or >	0	3
Family History of Laxity	4	3
Collagen V expression		
Skin Dermal papillae (basement membrane)	2	1.9
Skin superficial Dermis (extracellular matrix)	2.4	1.7
Skin Deep Dermis (extracellular matrix)	2.4	1.7
Skin Appendages	2	2
Decorin Expression		
Skin Dermis (extracellular matrix)	3.7	3.4
Biglycan Expression		
Skin Epidermis	2	1.9
Skin superficial Dermis (extracellular matrix)	3	1.6
Skin Deep Dermis (extracellular matrix)	1	0.86
Skin Appendages	2	2.3

Table 29- Benign Joint Hypermobility Syndrome (BJHS), Skin Strength, Collagen V and Small Leucine Rich Proteoglycans (SLRPs) Expression

Benign Joint Hypermobility Syndrome (BJHS): Tendon Strength, Collagen V and Small Leucine Rich Proteoglycans (SLRPs) Expression

Data was collected for 9 patients undergoing arthroscopic ACL reconstruction for instability.

1 patient had BJHS (Group A) and 8 patients did not have BJHS (Group B). The age of patient in group A was 22 years and he was a male. The force required for yield was 88N. The Beighton score was 6 indicating generalized joint hypermobility. There was no family history of hypermobility. The grading of collagen V expression in tendon sheath was 4, in the tendon extracellular matrix (ECM) was 2, Tendon blood vessels and interfascicular tissue was 3 each. The grading of Decorin expression was 4. The grading of Biglycan expression in tendon ECM was 1 and skeletal muscle was 2.

The mean age for group B was 28.5 years (range 19-43). 7 patients were male and 2 were female. The mean force required for yield was 95N (range 86-105). The mean Beighton score was 2 (range 0-9). 2 patients had Beighton score more than 6 indicating hypermobility. 3 patients had family history of hypermobility. The mean grading of collagen V expression in tendon sheath was 2.1 (1-4), tendon extracellular matrix (ECM) was 2 (1-3), Tendon blood vessels was 3 (1-4) and tendon's interfascicular tissue was 2.8 (1-4). The mean grading of Decorin expression was 3.5 (3-4). The mean grading of Biglycan expression in tendon ECM was 2.7 (0-4), tendon blood vessels was 2 (0-4) and skeletal muscle was 1.3 (0-2).

The expression of Collagen V, SLRP's, tendon strength and BJHS was studied in a small cohort of patients undergoing ACL reconstruction. The force required for yield for hamstring tendon was lower in patients with BJHS. The grading of Biglycan expression in extracellular matrix was lower in patients with BJHS. The grading of Collagen V tendon sheath was higher in patients was BJHS. There was no difference in the expression of Decorin in both groups

	Group A (BJHS)	**Group B (No BJHS)**
Mean Age	22	28.5 (19-43)
Sex		
Male	1	7
Female	0	2
Mean force required for yield (N)	88	95 (86-105)
Mean Beighton score	6	2 (0-9)
Beighton score 4 or >	1	2
Beighton score 6 or >	1	2
Family History of Laxity	0	3
Collagen V expression		
Tendon sheath	4	2.1
Tendon extracellular matrix	2	2
Tendon Blood vessels	3	3
Tendon interfascicular tissue	3	2.8
Decorin Expression		
Tendon extracellular matrix	4	3.5
Biglycan Expression		
Tendon extracellular matrix	1	2.7
Tendon Blood vessels	_	2
Tendon skeletal muscles	2	1.3

Table 30- Benign Joint Hypermobility Syndrome (BJHS): Tendon Strength, Collagen V and Small Leucine Rich Proteoglycans (SLRPs) Expression

Benign Joint Hypermobility Syndrome (BJHS): Capsule Strength, Collagen V and Small Leucine Rich Proteoglycans (SLRPs) Expression

Data was collected for 10 patients undergoing open shoulder stabilization for recurrent instability.

3 patients had BJHS (Group A) and 7 patients did not have BJHS (Group B). The mean age for group A was 28 years and all were male. The mean force required for yield was 32 N (17-55). The mean Beighton score was 2.3 (range 1-4). 1 patient had Beighton score more than 4 indicating generalized joint hypermobility. 2 patients had family history of hypermobility. The mean grading of collagen V expression in synovial surface of capsule was 2, blood vessels was 2.3 and extracellular matrix was 1.3. The mean grading of Decorin expression in capsule ECM was 3. The mean grading of Biglycan expression in capsule blood vessels was 3 and extracellular matrix was 2.

The mean age for group B was 25 years and all were male. The mean force required for yield was 43 N (32-66). The mean Beighton score was 2 (range 0-4). 1 patient had Beighton score more than 4. No patient had family history of hypermobility.

The mean grading of collagen V expression in synovial surface of capsule was 3.3, blood vessels was 1.8 and extracellular matrix was 2.3. The mean grading of Decorin expression in capsule ECM was 3. The mean grading of Biglycan expression in capsule synovial surface was 2, blood vessels was 2 and extracellular matrix was 1.

The expression of Collagen V, SLRPs, capsule strength and BJHS was studied in a small cohort of patients undergoing shoulder stabilization for recurrent instability. The force required for yield for shoulder capsule was lower in patients with BJHS. The mean grading of Collagen V expression was lower and Biglycan expression was higher in the extracellular matrix in patients with BJHS. There was no difference in the expression of Decorin in both groups.

	Group A (BJHS)	**Group B (No BJHS)**
Mean Age	28	25
Sex		
Male	3	7
Female	0	0
Mean force required for yield	32 N (17-55)	43 N (32-66)
Mean Beighton score	2.3 (1-4)	2 (0-4)
Beighton score 4 or >	1	1
Beighton score 6 or >	0	0
Family History of Laxity	2	0
Collagen V expression		
Capsule Synovial Surface	2	3.3
Capsule extracellular matrix	1.3	2.3
Capsule blood vessels	2.3	1.8
Decorin Expression		
Capsule extracellular matrix	3	3
Biglycan Expression		
Capsule Synovial Surface	_	2
Capsule extracellular matrix	2	1
Capsule blood vessels	3	2

Table 31- Benign Joint Hypermobility Syndrome (BJHS): Capsule Strength, Collagen V and Small Leucine Rich Proteoglycans (SLRPs) Expression

Collagen V, Small Leucine Rich Proteoglycans and Surgical scar: is there a correlation?

Proteoglycans (PGs) play an important role in modulating the structure and regulating the functions of the skin. Wound healing depends on the level of PGs which if not adequate leads to abnormal scars (Prathiba & Gupta, 2000). Altered expression of decorin and a decrease in the collagen-to-decorin ratio can affect the mechanical properties of human skin (Lochner et al., 2007).

13 patients were included in the study to assess the relationship between appearances of the healed surgical scar following open shoulder stabilization or primary ACL reconstruction wounds closed with staples without any complications and the expression of Collagen V, Decorin and Biglycan in skin. There were 11 male and 2 female patients. The mean age was 26 years (range 17-43). 9 patients had open shoulder stabilization and 4 patients had primary ACL reconstruction. The mean scar age was 10 weeks (range 3-22 weeks). The mean satisfaction score on VAS scale was 5.7 (range 0-10). The mean picture score was 1.7 (range 1-3). The mean Beighton score was 2.6 (range 0-7).

The mean decorin expression in the skin superficial and deep dermis was 3.4 (range 2-4). The mean expression of collagen V for skin dermal papilla (basement membrane) was 2 (range 1-4), skin appendages was 1.7 (range 1-3), skin superficial dermis was 1.7 (range 1-4) and deep dermis was also 1.7 (range 1-4).

The mean expression for Biglycan in skin epidermis was 2.1 (range 1-4), skin appendages was 2.6 (range 1-4), skin superficial dermis was 2 (range 1-4) and skin deep dermis was 1 (range 0-2).

There were 3 patients with poor cosmetic scars with a Picture score of 3 (Group A). The mean Beighton score was 2.3. The mean scar satisfaction score was 6.3 (range 0-10). The mean scar age was 13.7 week (range 10-20 w). All patients were male and the mean age was 19.7 years (range 17-22).

The mean decorin expression in skin was 3.6 for both superficial and deep dermis. The mean Collagen V expression was 1.3 for skin dermal papillae, skin appendages, superficial and deep dermis. The mean Biglycan expression was 2 for skin epidermis, 3 for skin appendages, 2 for superficial skin dermis and 0.6 for skin deep dermis.

There were 7 patients with good cosmetic scars with a picture score of 1 (Group B). The mean Beighton score was 3 (range 0-7). The mean scar satisfaction score was 5.8 (range 4-8). The mean scar age was 9 week (range 3-22 w). There were 5 male and 2 female patients. The mean age was 28 (range 23-43).

The mean decorin expression in skin was 3.3 for both superficial and deep dermis. The mean collagen V expression was 2.4 for skin dermal papillae, 2.1 for Skin appendages, 1.7 for superficial and deep skin dermis. The mean Biglycan expression was 2.3 for skin epidermis, 2.5 for skin appendages, 2.1 for superficial skin dermis and 1.16 for skin deep dermis.

There was no difference in the laxity between the 2 groups BS 2.3 vs 3. Collagen V expression was higher in skin dermal papillae 2.4 vs 1.3 ($p=0.02$) and skin appendages 2.1 vs 1.3 ($p=0.05$) of the good cosmetic scar. The Biglycan expression

in skin deep dermis was higher 1.16 vs 0.6 for the good cosmetic scar group but this difference was not statistically significant (p=0.1).

	Group A (poor cosmetic Scars)	**Group B (good cosmetic scars)**
Total No of Patients	3	7
Mean age	19.7 (17-22)	28 (23-43)
Sex		
Male	3	5
Female	0	2
Mean Beighton score	2.3	3
Mean Decorin Expression in ECM	3.6	3.3
Collagen V Expression		
Skin Dermal papillae (basement membrane)	1.3	2.4
Skin superficial Dermis (extracellular matrix)	1.3	1.7
Skin Deep Dermis (extracellular matrix)	1.3	1.7
Skin Appendages	1.3	2.1
Biglycan Expression		
Skin Epidermis	2	2.3
Skin superficial Dermis (extracellular matrix)	2	2.1
Skin Deep Dermis (extracellular matrix)	0.6	1.16
Skin Appendages	3	2.5
Mean Scar Satisfaction (VAS 0-10)	6.3 (0-10)	5.8 (4-8)
Mean Scar Age	13.7 weeks (10-20)	9 weeks (3-22)

Table 32- Collagen V, Small Leucine Rich Proteoglycans and Surgical scar

This study suggests that the presence of Collagen V and biglycan is important for the development of surgical scars.

Discussion

Hypermobility:

Hypermobility is defined as an "excessive range of joint motion" and is a direct consequence of ligamentous laxity. The definition of 'generalised joint hypermobility' (GJH) is still not clear but should include the number of joints involved and the extent of their movement. (Beighton et al., 2012b). Beighton score is commonly used to assess hypermobility in clinical and epidemiological studies. (Beighton & Horan, 1969) (Beighton P, Solomon L, 1973). It incorporates nine genetically determined sites one of which, forward flexion, can also be acquired through training (Klemp & Chalton, 1989). Beighton score uses an arbitrary score of four or more as a cut off for hypermobility (R Grahame, 2000).

The prevalence of GJH in published reports varies from 5% to 43% in adults (Verity Pacey et al., 2010) (R Grahame, 1999) (A J Hakim & Grahame, 2003) (Seçkin et al., 2005) (Juul-Kristensen et al., 2007) (Rahman & Holman, 2010) (Simpson, 2006). 20% patients in our control group with Meniscal Injury or Clavicle Fracture had a Beighton score of 4 or above indicating generalized joint hypermobility whereas 41% patients in the ACL reconstruction group and 49.5% patients in the Shoulder dislocation group had generalized joint hypermobility.

The Kruskal-Wallis test showed that in the patients with knee as compared to shoulder injuries the thumb and little finger components of the Beighton score were different between groups with a P value of 0.02 for little finger and 0.04 for the thumb while in patients with shoulder girdle injuries the knee component of Beighton score was different with a p value of 0.001. These results suggest different aetiologies in shoulder and knee injuries.

Hypermobility is not always symptomatic but when it is, it may represent Hypermobility Syndrome which is classified by means of the Brighton Criteria for the Benign Joint Hypermobility Syndrome (BJHS) (Simpson, 2006). 9% patients in our control group with Meniscal Injury or Clavicle Fracture, 13% patients in the ACL reconstruction group and 24% patients in the shoulder dislocation group fulfilled the Brighton Criteria for BJHS.

Risk of Injuries:

Tissues such as tendon, ligament, bone, cartilage and skin, which rely on the considerable tensile strength of their collagen component for their physical integrity, are at greater risk of mechanical failure in hypermobile individuals taking part in sporting activities (Beighton et al., 2012b).

Hypermobility is common in athletic population and is associated with increased risk of injuries like ACL rupture and shoulder dislocations in Rugby, Football, Netball and basketball players (Brodie, Bird, & Wright, 1982)(Acasuso Díaz, Collantes Estévez, & Sánchez Guijo, 1993)(Decoster, Bernier, Lindsay, & Vailas, 1999)(R. Smith et al., 2005)(Stewart & Burden, 2004)(Nicholas JA, 1970a)(Keller, Noyes, & Buncher, 1988)(Matt D Konopinski et al., 2012)(Verity Pacey et al., 2010)(Reider, 2012)(Myer et al., 2008)(Ramesh et al., 2005)(Gray et al., 1985)(Uhorchak, J Scoville, C, Williams, G, Arciero, R, Pierre, P. Taylor, 2003)(Cameron et al., 2010)(S.-J. Kim et al., 2008).

67% patients with BJHS in the study were involved in sports and the most common sport played was football in 27% followed by rugby in 23% and skiing in 13.6%. 45% patients had a flexible first degree relative.

ACL injuries:

Anterior cruciate ligament injury is a very common sports injury. Generalised ligamentous laxity has been associated with an increased incidence of ACL injuries (Acasuso Díaz et al., 1993)(Nicholas JA, 1970b)(Ramesh et al., 2005)(Verity Pacey et al., 2010)(Vaishya & Hasija, 2009)(Myer et al., 2008)(Matt D Konopinski et al., 2012)(V Pacey, Ll, Rd, & Donaldson, 2012)(R. Smith et al., 2005).

The primary ACL surgery group was associated with an increased incidence of generalised joint hypermobility compared to the control group with higher Beighton scores. The mean Beighton score was 2.9 (median 2) and this difference was statistically significant ($p = 0.002$). When GJH was considered using a strict criteria of Beighton score ≥ 6, the proportion with GJH was still greater in the primary ACL reconstruction group compared with the control group ($p = 0.001$). These findings support the view that generalised joint hypermobility is associated with an increased risk of ACL injury since there was a significantly higher Beighton score in the primary ACL group compared to the control group.

Shoulder Instability:

Shoulder dislocations are common amongst athletic population and the incidence continues to increase (Headey, Brooks, & Kemp, 2007)(Owens, Dawson, Burks, & Cameron, 2009)(Zacchilli & Owens, 2010). It is important to identify the risk

factors for primary and recurrent shoulder dislocations in order to maximise future stability and return to sporting activities. There had been little evidence from literature about the incidence of GJH in primary and recurrent shoulder dislocations. In 1960 Carter and Sweetman presented a series of cases of recurrent dislocations of the shoulder that were associated with familial joint laxity (Carter & Sweetnam, 1960). Cameron et al. reported that participants with a Beighton Scale score of 2 or greater were nearly 2.5 times more likely to have experienced an episode of glenohumeral joint instability than were participants with lower scores when sex and race were held constant (Cameron et al., 2010). Chahal et al. reported in a retrospective case-control study of 57 consecutive individuals that generalized joint hypermobility was more common in patients who had sustained a primary shoulder dislocation (Chahal et al., 2010).

In this study the, mean Beighton score was higher indicating GJH in the primary dislocation group (mean difference 1.8, p=0.001) when compared with the control group. When GJH was considered with the Beighton score $\geq$4, the proportion of patients with GJH was greater in the shoulder dislocation group compared with the control group (OR 3.5, 95% CI 1.5 to 8.8, p=0.005). When Beighton score $\geq$2 was used to determine generalized joint hypermobility as reported by Cameron (Cameron et al., 2010) there were similar differences in the distribution of patients with GJH between the control group and primary shoulder dislocation (OR 3.6, 95% CI 1.5 to 9.1, p=0.004).

The findings of the present study support the view that GJH is associated with an increased risk of primary shoulder dislocation since there was a significantly higher

Beighton score in the primary dislocation group compared to the control group representing the general population.

There was no difference in the incidence of Benign joint hypermobility syndrome (BJHS) between primary shoulder dislocation and control groups (OR 2.0, 95% CI 0.5 to 8.4, p=0.337). This finding suggests that patients with first time traumatic shoulder dislocation were not symptomatic with the hypermobility before they sustain this injury. Brighton criteria for BJHS assess the symptoms of hypermobility in addition to signs of hypermobility which are assessed with clinical examination of Beighton score.

ACL reconstruction and Generalised Joint Hypermobility

The success of modern techniques of arthroscopically assisted ACL reconstruction has been associated with a steady increase in the annual incidence of ACL reconstruction surgery (Bollen & Scott, 1996)(Lyman et al., 2009). Satisfactory results following ACL reconstruction have been reported in 75% to 95% of patients (Getelman & Friedman, 1999)(Vorlat & Verdonk R, 1999)(Bourke, H E ; Gordon, D J ; Salmon, L J ; Waller, A ; Linklater, J ; Pinczewski, 2012). Failure of primary ACL reconstruction surgery can occur due to various factors (Jaureguito & Paulos, 1996)(Vergis & Gillquist, 1995) and outcomes after revision surgery have been reported to be worse than primary procedures (George, Dunn, & Spindler, 2006b)(Ferretti, Conteduca, Monaco, De Carli, & D'Arrigo, 2007). In order to minimise the risk of a poor outcome from revision surgery it is important to identify the causes of failure of the primary procedure to achieve successful outcome after revision surgery.

Surgical reconstruction is now widely accepted as the treatment of choice for individuals with functional instability due to an ACL-deficient knee. Nonetheless, 0.7–10% of patients develop graft failure with recurrent instability and may then be candidates for revision ACL reconstruction.(Ménétrey et al., 2008)

Failure of primary ACL reconstruction can be due to several factors. The well-documented reasons include surgical technical errors with improper tunnel placement, impingement from inadequate notchplasty, inadequate graft tensioning or fixation, unaddressed concomitant combined ligament injury patterns, loss of motion or traumatic re-injuries (Getelman & Friedman, 1999) (Ménétrey et al., 2008)(George et al., 2006a). Occasionally, there have been cases of lack of graft incorporation without any history of trauma or identifiable technical errors. The term “biological failure” has been used to denote this mode of failure in the absence of other identifiable causes. (Ménétrey et al., 2008).

However there has been no study in the literature looking at the relationship between generalised joint hypermobility and the incidence of revision ACL reconstruction. If GJH is associated with an increased risk of ACL tears, it may also be associated with an increased risk of graft failure after primary ACL reconstruction. If this is true, it was hypothesised that individuals who undergo revision ACL surgery are more likely to have generalised joint hypermobility compared to normal population or patients undergoing primary ACL reconstruction. The aim of this study was to investigate the relationship of generalised joint hypermobility and requirement for revision ACL reconstruction.

Generalised joint hypermobility is well recognised to be associated with an increased incidence of ligamentous injuries during sporting activity (Acasuso Díaz et al., 1993)(Nicholas JA, 1970b)(Hopper, Hopper, & Elliott, 1995)(Decoster et al., 1999)(Stewart & Burden, 2004)(R. Smith et al., 2005)(Verity Pacey et al., 2010)(M. D. Konopinski, Jones, & Johnson, 2012)(Donaldson, 2012)(Smith R, Damodaran AK, Swaminathan S, Campbell R, 2005). Although the surgical treatment of ACL injuries has been the subject of intensive clinical and laboratory based research in the orthopaedic sports medicine literature (Uhorchak, J Scoville, C, Williams, G, Arciero, R, Pierre, P. Taylor, 2003) there has been less attention in the literature given to on the specific association between ACL injuries and ligamentous laxity (Ramesh et al., 2005) (Uhorchak, J Scoville, C, Williams, G, Arciero, R, Pierre, P. Taylor, 2003) (S.-J. Kim et al., 2008)(Vaishya & Hasija, 2009)(Myer et al., 2008). Moreover, no previous study has considered the relationship between joint hypermobility and failure of primary ACL surgery.

The revision ACL surgery group was associated with increased generalised joint hypermobility when compared to the primary ACL surgery group and this difference was statistically significant $p = 0.019$. There was a subgroup within the revision cohort, who had a failure of the original surgery due to biological failure of the primary graft. The incidence of generalised joint hypermobility in this group as defined by the Beighton score was also significantly higher than the primary surgery group (4.4 vs 2.9 $p = 0.010$).

When GJH was considered using a strict criteria of Beighton score $\geq$6, the proportion with GJH was greater in the primary ACL reconstruction group

compared with the control group (p= 0.001). It was also higher in the revision ACL reconstruction group as compared to the control group (p<0.001). There was a significant difference between the revision and primary ACL reconstruction groups (p=0.043). There was also a difference between Biological failure group and primary ACL reconstruction Group (p= 0.006).

The primary ACL reconstruction group had a mean Beighton score of 2.9 while the revision ACL group had a mean Beighton score of 4. In 48% of revision surgery cases, there was an identifiable cause of primary graft failure. This included 1 case where concomitant MCL laxity was not addressed at the primary procedure resulting in persistent symptomatic instability. In the other 20 cases there was a significant further traumatic injury to the knee that was associated with rupture of the original graft. In the remaining 52% cases no clear reason for graft failure could be identified, and these were classified as “biological failures”. In this group 17 patients were noted to have an intact ACL graft which was lax at the time of revision surgery. The Biologic failure group had a mean age of 28 years (range 16 – 50 years) and a mean Beighton score of 4.6 (median 4). There were 14 males and 9 females.

Generalised joint hypermobility has been related to abnormalities in extracellular matrix composition of which collagen is an important constituent. It would be reasonable to assume that for the same reason, tendon material from the same individuals may also have an inferior mechanical structure, including the hamstrings and patellar tendons. As these are the commonest autografts used in ACL reconstruction, there is therefore a possibility that in hypermobile individuals the use of these autografts for ACL reconstruction may lead to time dependent

stretching of these grafts and eventual failure due to graft insufficiency. This mechanism may well be a contributory cause of the so-called “biological failure” of the primary ACL graft, a concept whose causes remain ill defined (Ménétrey et al., 2008).

The findings of the present study support the view that generalised joint hypermobility is associated with an increased risk of ACL injury since there was a significantly higher Beighton score in the primary ACL group compared to the control group. There was also a difference between primary and revision ACL cases with increased laxity in the revision cases. When comparing the primary surgery group with overall revision surgery group a statistically significant difference in laxity was found. The subgroup without obvious cause for graft failure i.e. the “biological failures”, were also studied and there was a statistically significant higher incidence of generalised ligamentous laxity when compared to the primary ACL surgery group. As most of these cases had autografts used for the primary procedure, the cause of failure may well be attributable to the laxity of the ligament autografts, causing insufficiency under load and ultimately graft failure. This is of course difficult to confirm unless pathological testing of the failed graft is undertaken and this would be a useful subject for further research.

Limitations:

It was acknowledged that there are some inherent flaws in a study of this nature. Beighton’s modification (Beighton & Horan, 1969)(Beighton P, Solomon L, 1973) of the Carter and Wilkinson scoring system (Carter C, 1964) is very popular for measuring generalised joint hypermobility (Nicholas JA, 1970b) (Alan J Hakim,

Keer, & Grahame, 2010) (Lars Remvig, Jensen, & Ward, 2007)(L Remvig et al., 2007b)(Juul-Kristensen et al., 2007)(Ross & Grahame, 2011)(Simpson, 2006)(A. Hakim & Grahame, 2004)(Baum & Larsson, 2000)(Smits-Engelsman et al., 2011)(Alan J Hakim et al., 2010). However it has an inherent problem in that there is no accurate and specific cut off point to denote increased laxity, even in Beighton's own papers (Beighton & Horan, 1969)(Beighton P, Solomon L, 1973). A cut off score of 4 or more has been considered as being indicative of generalised joint hypermobility in the literature (Alan J Hakim et al., 2010) (Lars Remvig et al., 2007)(L Remvig et al., 2007b)(Juul-Kristensen et al., 2007)(Ross & Grahame, 2011)(Simpson, 2006)(A. Hakim & Grahame, 2004)(Baum & Larsson, 2000)(Smits-Engelsman et al., 2011)(Alan J Hakim et al., 2010), although some authors have suggested the use of 2 as a cut off to diagnose generalized joint hypermobility using the Beighton score (Cameron et al., 2010) while others had used 6 as a cut off to diagnosing generalized joint hypermobility (Ramesh et al., 2005). There is a need for a consensus on the methods by which to measure joint mobility and the definition of norms for hypermobility that reflect age, gender and ethnic-dependent variation (Lars Remvig et al., 2011).

Clinical Significance:

The findings of the study suggest a relationship between generalised joint hypermobility and ACL injury and the risk of failure after surgical reconstruction. The increased Beighton scores in the revision ACL group is consistent with this observation. Based on the results of this study it was felt that in the presence of generalised joint hypermobility an autogenous graft may not be the best mode of reconstruction for either primary or revision ACL reconstruction. An allograft

tendon may be an alternative choice in these patients. Further prospective studies comparing allograft and autograft failure rates in patients undergoing primary and revision ACL surgery in the presence of generalized joint hypermobility are required to confirm these observations.

Hypermobility- a risk factor for failure following ACL reconstruction

There were 28 patients followed up for 4 years after primary ACL reconstruction to study the failure of primary ACL autograft and it was found that hypermobile patients had a higher incidence of failure of ACL reconstruction (30% vs 0%) ($p=0.01$). The mean Beighton score for these patients who had failure of ACL reconstruction was 5.9. 3 patients (30%) fulfilled the Brighton criteria for BJHS. 2 patients had a Beighton score of 7 and 1 with Beighton score 5.

Prevention of ACL Injuries:

Neurophysiological defects may occur in the joint hypermobility syndrome. Ferrell et al. studied the proprioception at the proximal interphalangeal and knee joints of 12 patients with hypermobility and found this to be significantly impaired in hypermobile patients when compared to age- and sex-matched controls. (Ferrell, 1994). Hall et al. studied the proprioceptive acuity at the knee joint using an accurate determination of the onset and direction of knee joint displacement at constant angular velocity and showed that 10 female hypermobile patients showed poorer proprioceptive feedback when compared to age- and sex-matched controls. (Hall et al., 1995). These studies form the basis of the rationale for using proprioceptive enhancement as a form of treatment. (A. Hakim & Grahame, 2004)

It has been suggested in the literature that patients with hyperextension of the knee may have a poor proprioception feedback loop. The poor proprioceptive feedback seen in both hyperextension and increased joint laxity can affect both limbs and reduce the ability to initiate protective reflexes (Ramesh et al., 2005). The findings of the current study agree with other authors (Ramesh et al., 2005) (Vaishya & Hasija, 2009) (Myer et al., 2008) that patients with GJH participating in sports should be identified and offered a targeted programme to improve landing technique and proprioception to prevent ACL tears.

Graft Incorporation:

The process of successful incorporation of both autografts and allografts includes graft necrosis, revascularization, cellular repopulation, collagen deposition, and matrix-remodelling. The ligamentization process is influenced by the graft source, the host response, and the biomechanical loads affecting the graft during rehabilitation. Inadequate graft vascularity caused by graft over tensioning, postoperative immobilization, infection, and immunologic reactions may delay or prevent graft incorporation. Surgical factors such as roof impingement and excessive graft tensioning may also play a role in decreased vascularity and delayed graft incorporation. Furthermore, the rate of incorporation has been shown to depend on both the type of graft material and the method of fixation (Harner et al., 2000)(George et al., 2006a).

Biological failure:

Biological failure should be suspected in patients presenting with recurrent instability following ligament reconstruction without a history of trauma or an identifiable technical error. (Harner et al., 2000). Failure of graft incorporation and ligamentization as described by Amiel et al. (Amiel, Kleiner, Roux, Harwood, & Akeson, 1986) is commonly referred to as ‘‘biological failure’’ of the ACL graft. Biological failure can also be defined as a failure in the completion of the ligamentization process, leading to an atonic, disorganized, and non-viable graft.

Biological ACL graft failure is a complex pathological entity. Any factors affecting graft revascularization, cellular repopulation, or matrix remodelling can lead to biological failure. Graft incorporation is influenced by many factors, primarily technical and biomechanical, and cannot always be appreciated objectively. Biological failure of an ACL graft’’ should be considered more as an exclusion diagnosis rather than a real pathological entity. The surgeon is directly responsible for the mechanical aspects, and the patient, is responsible for providing the appropriate bio-chemical environment. (Ménétrey et al., 2008)

The redundant ACL group was compared with the ruptured ACL group and it was found that the biological failure was common in the redundant ACL group than the ruptured ACL group 43% vs 22% ($p= 0.04$). There was no difference in the primary ACL Graft used in the redundant or ruptured ACL groups; quadruple hamstring 55% vs 53% and Patellar tendon 24% vs 25%. The duration of time from primary ACL reconstruction to failure was 41 months in group A and 38 months in group B ($p = 0.69$). Unfortunately the Beighton score was not available for all the

patients in the study to assess the role of GJH on graft incorporation and difference between different graft types (Quadruple hamstring vs Patella tendon vs allograft) which can result in biological failure. The mean Beighton score for available patients was higher in redundant ACL Group 4.2 vs 1.8 but this difference was not statistically significant (P= 0.07). The mean age and gender difference were similar in both groups. The findings of the study suggest that biological failure was higher in the redundant ACL grafts.

Future graft options:

1- Synthetic ACL Graft:

The ideal artificial ligaments should demonstrates biocompatibility (chemical stability, degree of polymerization, absence of soluble additives, scarce water adsorption, presence of pores for fibroblasts ingrowth) and mechanical characteristics (traction resistance, stiffness, elongation, torsion and abrasion resistance) as similar as possible to those of the natural ligament. The ideal substitute graft has not been found as yet despite much effort and many experimental studies (Legnani, Ventura, Terzaghi, Borgo, & Albisetti, 2010).

2- ACL repair:

In the absence of an ideal substitute ACL graft, research has been aimed to develop a tissue-engineered ACL repair technique that addresses the shortcomings of existing strategies. The ACL has a latent repair capacity that becomes active when a suitable scaffold is placed between the ruptured ends of the ligament. A type I collagen hydrogel laden with adenoviral vectors is able to transduce cells as they

migrate into the scaffold and enhance the synthesis of repair tissue. (Steinert et al., 2008)

3- Tissue Engineered ACL Graft:

Fan et al. investigated the ACL regeneration in a pig model which closely mimics the human knee joint. Their regenerated ligament exhibited abundant ECM and proliferating fibroblast-like cells at 24 weeks postoperatively. The tensile strength could meet the mechanical requirements for daily activities. It implies that silk scaffold has great potentials in clinical applications. (Fan, Liu, Toh, & Goh, 2009).

4- Application of thermal energy:

The application of thermal energy to collagen results in modification of its microscopic structure. The potential indication for ACL thermal shrinkage is laxity in association with continuity of either a native ACL or an ACL graft. The results of shrinkage of a lax, intact native ACL seem promising, but the complication of catastrophic, spontaneous ACL rupture has been described. In cases of shrinkage of lax, intact ACL grafts, clinical outcome studies report contradictory results (Lubowitz, 2005).

5- Living related donor allograft:

Tállay et al. presented the case of the use of a living related donor allograft for the ACL reconstruction in a skeletally immature patient who presents with the need for revision ACL reconstruction (Tállay, Lim, & Morris, 2008).

Shoulder Instability: Risk Factors

The stability of the shoulder joint depends on static (capsular ligaments, the glenoid labrum, negative intra-articular pressure and articular cartilage surface contact forces) and dynamic stabilizers (rotator cuff, deltoid, and long head of the biceps) (Flatow & Warner, 1998)(Matsen et al., 2006) (S. M. Johnson & Robinson, 2010).

1- Generalized Joint Hypermobility:

The association between GJH and increased incidence of athletic injuries has been extensively debated (Headey et al., 2007) (R. Smith et al., 2005)(Myer et al., 2008)(Ramesh et al., 2005)(Stewart & Burden, 2004) (Lintner, Levy, & Kenter, 1996)(Zemek & Magee, 1996)(Bigliani, Codd, & Connor, 1997)(McFarland & Campbell G, 1996) and many authors have demonstrated a relationship between generalized joint hypermobility and glenohumeral joint laxity in asymptomatic healthy athletes (Flatow & Warner, 1998)(Lintner et al., 1996)(Bahk, Keyurapan, Tasaki, Sauers, & McFarland, 2007)(Jia, Ji, Petersen, Freehill, & McFarland, 2009)(Borsa, Sauers, & Herling, 2000). There is recent evidence of association of generalized joint hypermobility with history of glenohumeral joint instability (Cameron et al., 2010)

Although treatment of shoulder instability is one of the most popular topics in the orthopaedic sports medicine literature and generalized joint hypermobility is thought to be associated with multidirectional instability (Flatow & Warner, 1998)(Matsen et al., 2006) (S. M. Johnson & Robinson, 2010)(Flatow & Warner, 1998), there has been less focus in the literature on the more specific association between traumatic anterior shoulder instability and joint hypermobility (Cameron

et al., 2010) and even more so on the relationship between joint hypermobility and the risk of recurrent shoulder dislocations.

It would be reasonable to assume that increased ligamentous laxity noted overtly in normal individuals would apply to all capsular tissue in the body, including the shoulder. Patients with generalized joint hypermobility might be at an increased risk of shoulder dislocation in dangerous shoulder positions due to capsular laxity.

Generalized joint hypermobility has been proposed as a significant risk factor for failure after arthroscopic anterior shoulder stabilization. There is no significant difference in patient-rated outcome in normal versus ligamentously lax patients undergoing arthroscopic anterior shoulder stabilization (Koyonos et al., 2013).

In this study the mean Beighton score was higher indicating GJH in both the primary dislocation group (mean difference 1.8, p=0.001) and the recurrent dislocation group (mean difference 1.4, p=0.004) compared with the control group. There was no difference between the primary and recurrent shoulder dislocation group (mean difference 0.371, p=1.00).

When GJH was considered with the Beighton score $\geq$4, the proportion with GJH was greater in the dislocation group compared with the control group (OR 3.5, 95% CI 1.5 to 8.8, p=0.005). It was also higher in the recurrent group compared with the controls (OR 4.0, 95% CI 1.7 to 9.5, p<0.001). There was no difference between the primary and recurrent dislocation groups (OR 1.1, 95% CI 0.5 to 2.5, p=0.759).

When Beighton score $\geq$2 was used to determine GJH as recently suggested by Cameron et al. (Cameron et al., 2010) there were similar differences in the

distribution of patients with GJH between the control group, primary dislocation (OR 3.6, 95% CI 1.5 to 9.1, p=0.004), and recurrent dislocation groups (2.9, 95% CI 1.3 to 6.4, p=0.008). There was again no difference between primary and recurrent dislocation groups.

There was no difference in the incidence of Benign joint hypermobility syndrome (BJHS) between primary dislocation and control groups (OR 2.0, 95% CI 0.5 to 8.4, p=0.337), but there was a difference between recurrent dislocation and control groups (OR 5.9, 95% CI 1.9 to 21.5, p=0.001). The BJHS does exhibit a difference in proportions between recurrent and primary dislocation groups (OR 3.0, 95% CI 1.1 to 8.9, p=0.031).

The findings of the present study support the view that GJH is associated with an increased risk of primary shoulder dislocation since there was a significantly higher Beighton score in the primary dislocation group compared to the control group representing the general population. There was also a difference in GJH between the “recurrent instability” group and the controls.

Although the incidence of generalized ligament laxity was higher in both primary and recurrent shoulder dislocation group it is not possible to identify it as a causal independent factor in this cross-sectional study. The presence of associated soft tissue and bony defects may affect the development of chronic instability. The findings of this study suggest an association between GJH and the shoulder instability.

Hypermobility- a risk factor for recurrent shoulder dislocations:

There were 38 patients with traumatic primary anterior shoulder dislocations who were followed up for 4 years to assess for recurrent shoulder instability. There was no significant difference when groups were compared using the loose criteria of Beighton score $\geq$ 4 (50% vs 40%) or strict criteria of Beighton score $\geq$6 (60% vs 39%) to assess the risk of recurrent shoulder dislocations. 30% patients had associated symptoms of Generalized joint hypermobility (GJH) and fulfilled the Brighton criteria for BJHS and 60% had a family history of laxity. The results of this study suggest that GJH was not an independent risk of recurrence following primary traumatic shoulder dislocation. However patients should be counselled about the potential risk of recurrence in association with bone defects and appropriate rehabilitation with emphasis on proprioception should be offered.

Prevention of Shoulder Dislocations:

Blasier et al. demonstrated that individuals who were clinically ‘‘tight’’ had significantly better joint-position sense and kinesthetic sense than individuals who demonstrated at least 3 signs of generalized joint hypermobility. Individuals with generalized joint hypermobility presumably have looser capsular structures and hence have perception of shoulder rotation that is less sensitive. These findings suggest that prophylactic rehabilitation programs may also be able to decrease the risk of primary traumatic shoulder dislocations in individuals with generalized joint hypermobility by improving joint-specific proprioceptive capabilities. (Blasier et al., 1994)

2- Bone Defects:

Anterior shoulder dislocation leads to bone loss on the anterior aspect of the glenoid and compression fracture of the posterosuperior aspect of the humeral head (Hill-Sachs deformity). Glenoid bone loss decreases the glenohumeral contact area. A reduced glenohumeral contact area may increase joint instability and the likelihood of further dislocation. Overall, approximately 70% of patients with first-time dislocations can expect dislocation again within 2 years, age-related. Preoperative quantification of glenoid bone loss would facilitate decision making as to the type of operative procedure is required (arthroscopic Bankart repair versus bone block transfer) and may be beneficial in predicting the outcome of these procedures (Griffith et al., 2008).

The Latarjet procedure is commonly performed when a glenoid bony defect exists that is greater than 25 % of the glenoid width or when the risk of recurrent instability is higher (i.e., collision-sport athletes). Hill–Sachs lesions need to be assessed as well. For the purpose of assessing the bipolar lesions, the glenoid track concept is useful. A Hill– Sachs lesion that is located more medially than the medial margin of the glenoid track is defined as an engaging Hill– Sachs lesion (Itoi et al., 2013). Anterior bone loss of the glenoid superior to 25% can lead to a high risk of failure of the Bankart arthroscopic procedure. This bone loss decreases the glenoid's arc length and reduces its concavity, so for such patients, bony augmentation procedures are recommended (Pansard et al., 2013).

CT assessment of Bony defects:

Griffith et al. noted two distinct forms of glenoid bone loss in recurrent anterior dislocation; anterior or anteroinferior glenoid rim fracture and anterior glenoid flattening. The routine use of reformatted CT images enface to the glenoid fossa is recommended to assess glenoid bone loss as an exponential relationship was found between the degree of anterior flattening (as evidenced by the length of the anterior straight line) and the number of dislocations sustained. A defect resulting in a glenoid width of less than 21% of glenoid length was considered to induce instability. Flattening of the anterior glenoid curvature is shown in most patients with anterior dislocation and increases exponentially with increasing number of dislocations. (Griffith et al., 2003)

Compared with arthroscopy, the sensitivity and specificity of CT in detecting glenoid bone loss are 93% and 79%. A high correlation ($r = 0.79$) also exists between CT and arthroscopy regarding severity of bone loss. Glenoid bone loss is almost as common as Hill-Sachs deformity in anterior shoulder dislocation. Glenoid bone loss is probably multifactorial in origin. Dislocation frequency cannot accurately predict the degree of bone loss, which helps to justify the use of CT. (Griffith et al., 2008)

CT scan Evaluation after Primary Shoulder Dislocation:

The CT scan assessment following primary traumatic shoulder dislocations showed glenoid fracture (bony Bankart lesion) in 35% patients and only 28% of these patients had evidence of a glenoid fracture using conventional radiographs. 25%

patients showed evidence of glenoid flattening on the CT scan and the Hill-Sachs lesion was present in 85%. The range of Hill-Sachs defect size was 8-29 mm. The prevalence of glenoid bone loss is reported to be 41% after a first-time shoulder dislocation. Postoperative recurrence can occur in up to 10% of cases. Thus, misdiagnosis of bony glenoid rim lesions has been assumed a major cause for failure. Radiographs seem inferior to CT scans for assessing osseous lesions especially at the glenoid rim. An osseous glenoid rim lesion after a traumatic shoulder dislocation left untreated can cause the onset of recurrent shoulder instability. It had been reported that with plain radiographs alone, even osseous fragments that would need refixation are likely to be overlooked. Therefore, a CT scan of the shoulder should be performed after a first-time traumatic shoulder dislocation so that the appropriate treatment can be applied at the correct time. (Auffarth et al., 2013)

Role of CT scan in predicting recurrence following primary traumatic shoulder dislocation:

The structural defects of glenoid and humeral head on the CT scan following primary shoulder dislocations and the risk of recurrence in the presence of bone defects was studied. Hill sach's defect was the most common finding on the CT scan (85%) followed by glenoid bone defect in 26%, glenoid flattening in 18%. Patients with bony defects had a higher rate of recurrent shoulder dislocations (48% vs 16%) although this difference was not statistically significant (p=0.14). The use of CT scan following primary shoulder dislocations was recommended to identify structural defects of humeral head and glenoid to predict the risk of recurrence and decide the appropriate treatment available.

CT scan Evaluation for Recurrent Shoulder Dislocations:

Bony lesions of the glenoid and of the humeral head are known to be the risk factors of recurrent instability after surgery. The glenoid defect is related to the number of dislocations and the age of initial dislocation (Itoi et al., 2013). The prevalence of glenoid bone loss following recurrent shoulder dislocation is reported to be 86% (Auffarth et al., 2013).

The CT scan findings of glenoid and humeral head following recurrent shoulder dislocations were studied. The most common finding was a Hill Sachs defect in 85%, followed by Glenoid flattening in 41% and glenoid fracture (bony Bankart lesion) in 32%. The range of Hill-Sachs defect size was 11-45 mm. 4 patients had glenoid flattening but no glenoid fracture. The prevalence of Hill Sachs defect was same as after primary dislocation 85% but glenoid flattening was more after recurrence rather than primary dislocation 41% vs 18%. This finding may be due to the compressive fracture of the glenoid during recurrent episodes of dislocation as suggested by (Griffith et al., 2003). These findings should be carefully assessed on the CT scan and compared with the opposite shoulder.

Clinical significance of CT scan:

There had been suggestion in the literature about the role of bone defects (Bony Bankart lesion, Glenoid flattening, large hill Sachs defect,) in predicting recurrent shoulder dislocations (Griffith et al., 2008). Itoi et al reported that the engaging hill sach's lesion that is located more medially than the medial margin of the glenoid track could be another important risk factor for symptomatic shoulder instability.

(Itoi et al., 2013). The numbers of bone defects on the CT scan rather than their size or position was studied and it was found that the presence and number of bone defects was higher in patients with recurrent shoulder dislocations. Although a large Bony Bankart lesion or a large Hill Sachs defects in itself can cause recurrent shoulder instability but a number of small defects can contribute to the shoulder instability as suggested in the follow up study to predict recurrent shoulder instability.

CT scan and generalized joint hypermobility in patients with primary shoulder dislocation:

The generalized joint hypermobility and structural defects of glenoid and humeral head on CT scan following primary shoulder dislocations were studied in 34 patients. There was no correlation between the generalized joint hypermobility and CT scan findings of glenoid flattening, bony Bankart lesion and hill sach's defects on statistical testing. It was recommended to identify the risk factors for shoulder dislocation by the use of CT scan for structural defects of humeral head and glenoid and clinical examination to assess generalized joint hypermobility.

CT scan and generalized joint hypermobility in patients with Recurrent shoulder dislocations:

The generalized joint hypermobility and structural defects of glenoid and humeral head on CT scan following recurrent shoulder dislocations were studied in 37 patients. There was no correlation between hyperlaxity and the CT scan findings of glenoid flattening, bony Bankart lesion and hill-sach's defects on statistical testing.

It was recommended to identify the risk factors for recurrent shoulder dislocation by the use of CT scan for structural defects of humeral head and glenoid and clinical examination to assess GJH. It was suggested that in the presence of GJH and bone defects on the CT scan an open procedure to address the soft tissue and bone defects might be better than an arthroscopic stabilization.

Assessment measures of Joint Hypermobility:

Different methods have been used for the precise measurement of movements of a hinge joint e.g. Loebl hydrogoniometer (Loebl WY, 1967), MIE clinical goniometer and Myrin goniometer (Beighton et al., 2012b). Some additional devices, like an electromagnetic movement sensor for the shoulder (G. R. Johnson, Fyfe, & Heward, 1991) and a plurimeter at the hip (Croft, Nahit, Macfarlane, & Silman, 1996) has been devised, validated and used for more sophisticated measurement of the range of movement at those joints.

Surface goniometry frequently proves to be inadequate when correlated with movements measured radiologically due to distension and unpredictable movement of the skin and other soft tissues as compared to underlying bones (Beighton et al., 2012b). A comprehensive account of techniques for measuring joint movement throughout the body is described in a booklet by the American Academy of Orthopaedic Surgeons (American Academy of Orthopaedic Surgeons., 1965). Wright also studied the available methods of measurement of movement at each major joint in the body to define the normal range of movement at each joint in males and females and provided estimations of inter-observer and intra-observer variations (Seedhom BB, 1981).

Measurement at the metacarpophalangeal joint (MCPJ) has been extensively studied for the assessment of laxity. MCP joint is easily accessible and is a component part of the conventional scoring systems for the assessment of laxity (Beighton & Horan, 1969). Different methods have been used for the assessment of laxity at the MCPJ. Harris and Joseph developed a radiological technique for measuring the range of extension at the MCP joint (Harris H, 1949) and Loebl devised a mechanism for abducting the fingers to investigate movement at the MCP joints (Loebl, 1972).

A finger arthrograph has been used to quantify the resistance encountered when the index finger is moved in sinusoidal fashion at a constant speed through a pre-selected angle of displacement and is of value in measuring stiffness (Jobbins, Bird, & Wright, 1981). This concept has also been revisited and a microprocessor-controlled arthrography has been devised which used movement of the MCP joint in a lateral rather than a flexion/extension plane. (Howe, Thompson, & Wright, 1985).

Grahame and Jenkins described a simple spring device that applied a predetermined force (2 lb (0.91 kg)) to the fifth MCP joint which mimicked the passive range of movement as measured in the clinical scoring system to the nearest 30° (Grahame R, 1972). The Leeds finger hyperextensometer allows greater precision in quantification of the range of movement and has good inter-observer and intra-observer reliability. It applies a torque of 2.6 kgcm−1 and can be used in epidemiological surveys as it is light, portable, and inexpensive (Jobbins et al., 1979). Most recently, an electronic gravity goniometer has been developed for

determining the passive range of movement of the MCP joints by the use of preset fixed torques (Wagner & Drescher, 1984).

There has been little validation of Beighton score and concerns have been raised about the cut off point for hyperlaxity (L Remvig et al., 2007b) (H. a Bird et al., 1979). In the current work, an electronic goniometer was used to assess the angle at the MCPJ of the fingers.

Electronic Goniometer:

Comparison of Beighton score with electronic goniometer for generalized joint hypermobility:

The hyperextension of MCPJ of 86 little and index fingers by electronic goniometer in 22 patients was studied by dividing those into 2 groups; Stiff Group (Group-1 with BS=0) and lax Group (Group-2 with BS >0). The BS >0 was selected for the lax group in this study as hyperextension of little fingers MCPJ will give 2 points on the Beighton score. There was a strong correlation between clinical examination and electronic goniometer findings for both index and little fingers in the stiff group. None of the little fingers in stiff group scored >90 which is in accordance with the clinical examination of BS. 30% of the little fingers were not in agreement with electronic goniometer findings in the lax group. 6 little fingers in the lax group were given points on clinical examination for Beighton score but these score <90 on electronic goniometer. Electronic goniometer is an easy and reliable method of assessing patients for GJH and should be used as an adjunct to the clinical examination. This study helped to improve the clinical

examination of little finger MCPJ hyperextension by taking into account the patients and assessor factors for Beighton score measurements.

Correlation of Electronic goniometer assessment of fingers with the Beighton score for generalized joint hypermobility:

The correlation between the GJH measured by clinical assessment for Beighton score and the load required to produce 40 and 50 degrees angle at the MCPJ of the little and index finger was studied. The average load needed to produce 40 degrees angle was 0.10 kg at right index finger and 0.09 kg at left index finger as compared to 0.02 kg for right little finger and 0.01 kg for left little finger . The average load needed to produce 50 degrees angle was 0.15 kg at right index finger and 0.12 kg at the left index finger as compared to 0.02 kg for right little finger and 0.01 kg for left little finger. There was a negative correlation between the Beighton score and the load needed to produce 40 and 50 degrees angle for left index finger. Index finger assessment recorded the load better than the little fingers. Index finger assessment could be a better indicator as compared to the little finger for GJH which is currently used in the Beighton score.

Initially the index finger was considered for electronic goniometer-load plate system comparison with force-displacement graphs of a sample of tissues obtained during the surgery. However, it was noticed that not all the patients were able to achieve 40 or 50 degrees angle at the index finger but all patients were able to achieve 40 degrees angle at the Right hand little finger. Although the load required to produce this angle was less than the load required to produce the same angle at the index finger. The load sensor in the load plate system was able to measure the

load from 0-22 Kg. It was decided to select 40 degrees angle at the Right hand little finger achieved by all the patients in the study so comparison can be made with the clinical and mechanical properties of the tissue specimens. Fairbank et al. used goniometry at 6 different joints in a group of 446 normal adolescents and concluded that there was a weak but significant correlation between the ranges of movement at each of the different joints measured, except for elbow hyperextension (Fairbank, Pynsent, & Phillips, 1984). Cameron et al. observed the relationship between sex and generalized joint hypermobility for each individual Beighton score item except for hyperextension of the metacarpophalangeal joint of the fifth finger bilaterally (Cameron et al., 2010), which suggest that the little finger assessment will not be different in male or females. This was another reason to choose little finger for assessment of hyperextension at the MCPJ in the current study.

The test used in this thesis was simple to perform and was able to identify the movement at MCPJ of little finger in response to applied force without causing any discomfort. It does not rely on the extreme of range of movement as does the Beighton score instead it identifies the angle produced in response to the force applied, achieved by most people. This test correlated with the laxity of the tissues as well and can supplement the Beighton score in diagnosing GJH in those cases which are between the 2 extremes of mobility BS=0 and BS=9.

Mechanical Testing of the Tissues:

Connective tissues are the structural materials of the body. They consist of networks of protein fibres embedded in a matrix of amorphous ground substances

and tissue fluids. Cells and other structures are also present, but are considered to contribute comparatively little to the mechanical properties (HARKNESS RD, 1961). The load-bearing protein fibres are of two main types; collagen fibres which are strong, stiff and comparatively inextensible, and elastic fibres which are less strong and stiff but highly extensible and exhibit rubber like elastic recovery (HARKNESS RD, 1961) (Ross R, 1971).

The mechanical properties of any particular tissue will depend on the proportions of collagen, elastin and non-fibrous material present, the arrangement and orientation of the fibres, the nature of the matrix and interactions between the components, all of which may show striking variations from one tissue to the next (Soden & Kershaw, 1974).

The most widely used test for comparing the mechanical properties of materials is the uniaxial tensile test in which a suitably shaped strip of material is gripped at both ends and stretched while the load and extension are recorded. The potential problems with this technique are encountered whilst (1) preparing the specimens, (2) measuring their cross-sectional area, (3) gripping the ends of the specimen, (4) providing a suitable test environment, (5) determining the initial settings and (6) measuring the loads and particularly the extensions applied to the specimen. Different solutions to all these problems have been described (Soden & Kershaw, 1974). Mechanical testing of the tissues can be performed either under constant load (Vladimir, Engineering, & Engineering, 1980) or constant displacement (Iatridis, Wu, Yandow, & Langevin, 2003). In the current work constant load was applied for mechanical testing of the tissues.

Tendons:

Orientation of collagen fibers can help to assess the mechanical properties of the tendons by applying load till failure and assess for displacement. Patients attending for ACL reconstruction had higher Beighton scores indicating GJH which mean that there were only a few tendon tissue from patients with extremes of mobility.

Capsule /skin:

The mechanical properties of skin and shoulder capsule were difficult to assess, which may have been due to the varied orientation of the collagen fibres and the effect of plastic deformation in the case of the shoulder capsule due to recurrent dislocations and the effect of age on skin. Patients attending for open shoulder stabilization had recurrent dislocations. All these patients were male and there were no patients with higher Beighton scores (BS > 6) indicating severe GJH. This means that it was not possible to assess the tissue strength or expression of Collagen V and SLRP's in patients with severe GJH. An alternative was to assess the patients with BS=9 but most of these patients were diagnosed with connective tissue diseases which was one of the exclusion criteria for the studies.

Knee skin strength and generalized joint hypermobility in patients undergoing ACL reconstruction:

The knee skin strength in 20 specimens from 13 patients undergoing ACL reconstruction was studied. Patients with higher Beighton scores indicating GJH had weaker knee skin. The mean force required to produce yield was 47N (SD-31) vs 61N (SD-24) with Beighton score of 4 or above and 4 or less and this difference

was not significant p=0.30. The mean force required to produce yield was 37N (SD-12) vs 61N (SD-37) with Beighton score of 6 or above and 6 or less and this difference was significant p=0.03. These observations suggest that patients with GJH may have weaker skin which might be related to the composition and organization of the collagen fibres in the ECM.

Shoulder Skin Strength and generalized joint hypermobility in patients undergoing open stabilization:

The shoulder skin strength in 23 specimens from 12 patients undergoing open shoulder stabilization was studied. Patients with higher Beighton scores suggesting GJH had weaker shoulder skin. The mean force required to produce yield was 65N (SD-32) vs 90N (SD-39) with Beighton score of 4 or above and 4 or less. This difference was not statistically significant p=0.15.

Hamstring Tendon Strength and generalized joint hypermobility in patients undergoing ACL reconstruction:

The Hamstring tendon strength in 11 specimens from 10 patients undergoing ACL reconstruction was studied. The mean force required to produce yield was 96N (SD-7.6) vs 88N (SD-21.4) with Beighton score of 4 or above and 4 or less. This difference was not statistically significant p=0.38.

Shoulder capsule strength and generalized joint hypermobility in patients undergoing open stabilization:

We studied the shoulder capsule strength in 15 specimens from 10 patients undergoing open shoulder stabilization. Patients with higher Beighton scores

indicating GJH had weaker shoulder capsule. The mean force required to produce yield was 29N (SD-4) vs 47N (SD-17) with Beighton score of 4 or above and 4 or less. This difference was statistically significant p= 0.01. These observations suggest that patients with GJH have weaker shoulder capsule which might be related to the composition and organization of the collagen fibres in the extracellular matrix. No difference in the Beighton score was found between the weak and strong capsule groups. However when the groups were studied according to the Beighton score then patients with higher Beighton score (4 or above) had weaker capsule tissue. The collagen V expression was higher in the strong capsule group.

Beighton score does not correlate with tissue laxity:

A commercial system (Biometrics) was used to measure the joint angle which uses the electronic goniometer. A load plate system was developed to record the angle produced by the applied load. An angle of 40 degrees at the MCP joint was selected to record the load needed as this angle was achieved by all the patients with the dominant hand little finger without discomfort.

There was no correlation between the Beighton score and the gradient of the laxity of the tissues measured by the material testing system (r^2 =0.03)

There was a positive correlation between the gradient of the force-displacement graph and the force required to produce 40 degrees angle at the little finger of the dominant hand (r^2 =0.55)

.

The tensile strength of the tissues such as hamstring tendon and capsule depends on the collagen component for their physical activity. These tissues can be at higher risk of injury due to different organization of extracellular matrix and collagen contents in patients with hypermobility. In the current study the gradient of force displacement graphs of these tissues did not correlate with the clinical testing of hypermobility as done by commonly used Beighton score. Instead the readings from the force displacement graphs did correlate with the tissue laxity as measured with the mechanical testing system. This novel technique is suggested as an additional assessment tool in professional athletes to identify the risk of sports related injuries in the presence of equivocal signs of hypermobility.

Beighton Score – Assessment:

Beighton score is easy to use after some training and practice in few minutes in clinic and can be used as a screening tool to assess patients with stiffness (Beighton score-0) and generalized joint hypermobility (Beighton score = 9). There will be few patients without a known diagnosis of hereditary connective tissue diseases with Beighton score of 9. Most of the general population will have a Beighton score between the upper and lower extremes. One way to determine GJH might be to use multiple groups 0-2, 2-4, 4-6, 6-9 (R. Smith et al., 2005) to determine the generalized nature of the hypermobility which the Beighton score can identify. However there can be a number of factors which can affect the Beighton score despite its good inter and intra-observer reliability (Juul-Kristensen et al., 2007). A number of patient related factors were noted which could affect their Beighton score.

A- Patient Factors:

1- Pain:

Pain threshold was a major factor in determining the angle achieved by most patients to qualify for a point on the Beighton score (Beighton & Horan, 1969) as there is no consensus whether the different joint movements to give a Beighton score should be performed actively or passively. The anecdotal evidence was that when patients were asked to copy certain joint movements for the Beighton score for example the little finger hyperextension and thumb apposition, they all apply different force which is not accounted for during the Beighton score. Another observation was that without causing any discomfort a number of patients were able to achieve 2 points for the little finger hyperextension in the Beighton score when the movements were performed passively. This can potentially change some one diagnosed with no hypermobility BS=2 to hypermobility BS=4.

2- Mood:

Another factor which could potentially affect the Beighton score was the patient's mood. It was not possible to assess the mood in clinic but a simple observation was that if the patients were happy with the overall outcome of their treatment then they were willing to apply more force to achieve a particular angle to get a point in the Beighton score. This can potentially change some one diagnosed with no hypermobility BS=2 to hypermobility BS=4.

3- Warmup:

Another factor which can potentially change the Beighton score for the patients was warmup. This was more relevant for the active movements of the little fingers, thumbs and forward bending which can be improved by repetitive movements and warmup. Previous studies have shown that the range of movement of a joint can be different before and after a warm up (Bird HA., 2004).

The patients who had their hypermobility assessed with force-goniometer system had performed a number of movements of the little finger for the warmup to counter this effect but it was not possible to do that for all the patients attending clinics due to time restraints.

B- Assessor Factors:

A number of assessor factors were also noticed which could potentially change the Beighton score.

1- Pain threshold:

Pain threshold was an important factor for the assessor as well as for the patient. This relates to the passive test for little finger hyperextension, thumb apposition, elbow and knee hyperextension.

2- Force application:

As the Beighton score does not take into account the force applied to achieve a particular angle it can vary according to different force applied by the assessor during active tests.

Suggestions:

It was recommended that patients who had a Beighton score between 2-6 on active testing of different joint movements should have it repeated passively and if there is any change in the score then they should have their laxity assessed at the little finger MCPJ by load plate-electronic goniometer system before the diagnosis of GJH.

Components of ECM:

Collagens are triple helical proteins that occur in the extracellular matrix (ECM) and at the cell–ECM interface. There are more than 30 collagens and collagen-related proteins but the most abundant are collagens I and II. (Kadler et al., 2008). Collagen V is essential for the assembly of collagen I-containing fibrils in vivo. Small leucine-rich proteoglycans decorin, biglycan, fibromodulin, and lumican have been shown to influence the rate of assembly, size, and structure of collagen fibrils formed in vitro. (Kadler et al., 2008).

Collagen V:

Collagen fibrils are composed of a quantitatively major and a minor fibril collagen. In non-cartilaginous tissues, type I collagen accounts for the majority of the collagen mass, and collagen type V is a minor component. Type V collagen has been implicated in the regulation of fibril diameter and is also required for collagen fibril nucleation. The reduced type V collagen content is associated with a 50% reduction in fibril number and dermal collagen content. The complete dependence of fibril formation on type V collagen is indicative of its critical role in the

regulation of fibrillogenesis (Sun et al., 2011) (Wenstrup et al., 2011) (Wenstrup et al., 2004).

Collagen type V is widely distributed in tissues and helps regulate the diameter of fibrils of the collagen type I. Mutations in the COL5A1 and the COL5A2 gene, encoding the alpha 1 and the alpha 2-chain of type V collagen respectively, are identified in approximately 50% of patients with a clinical diagnosis of classic EDS. In approximately one third of patients, the disease is caused by a mutation leading to a non-functional COL5A1 allele, and resulting in haploinsufficiency of type V collagen. In a smaller proportion of patients, a structural mutation in COL5A1 or COL5A2, resulting in the production of a functionally defective type V collagen protein, is responsible for the phenotype (Fransiska Malfait & Paepe, 2005)(Uitto & Ringpfeil, 2004)(J. Mao & Bristow, 2001)(Imamura, Scott, & Greenspan, 2000) (Wenstrup et al., 2006) (Symoens et al., 2009) (Fransiska Malfait et al., 2010) (Symoens et al., 2009).

Collagen V is a quantitatively minor component (1 to 3%) of the tissues such as dermis, tendon, ligament and bone. However, collagen V has a key role in the regulation of initial fibril assembly. A critical density of type V collagen would favour the initiation of new fibrils rather than the continued growth of existing fibrils. The collagen fibril diameter was inversely proportional to type V/type I collagen ratios, i.e. the higher concentration of type V, the smaller the fibril diameter. A greater fibril diameter generates greater tensile strength and the relative smaller fibril diameter provides greater elastic properties. So for the tendon to function properly it seems that there is need for both small and large diameter fibrils (Lu et al., 2011). During all stages of tendon development there is a constant

small, but detectable amount of type V collagen (Birk & Mayne, 1997). Collagens V regulate early steps in fibrillogenesis during tendon development (Wenstrup et al., 2011)(Fichard, Kleman, & Ruggiero, 1995).

Diameter of collagen fibrils in soft tissues has a positive correlation with collagen mechanical strength (Lu et al., 2011). The larger fibril radius is a primary determinant of higher tendon stiffness and strength and Fibril-fibril interactions may also improve tendon strength (Rigozzi, Müller, & Snedeker, 2010).

Collagen V Expression:

Skin:

Skin epidermis stained negative but dermal papilla, papillary and reticular dermis, blood vessels, sweat glands, sebaceous glands and arrector pilli stained positive to varying degrees for collagen V. The mean grading of collagen V expression in skin dermal papilla was 2.4, appendages 2.2 and extracellular matrix (ECM) 1.8 in weak skin group and 1.3, 1.8 and 1.7 respectively in strong skin group. The mean Beighton score for the weak skin group was 3.4 and strong skin group was 1.9.

This study highlights that collagen V expression was higher in the skin dermal papillae of weaker skin group which shows the signs of GJH by higher Beighton score. This confirms the findings of (Lu et al., 2011)that the increased amount of collagen V can results in weaker ECM in the skin. Collagen V expression is also noted to be higher in patients with GJH which was also a finding in this study.

Tendon:

Tendon sheath, collagen fibers, blood vessels and interfascicular connective tissue of the tendon stained positive to varying degrees, but skeletal muscle was negative. The mean grading of collagen V expression in tendon sheath (TS) was 2.2 and blood vessels (BV) was 2.8 in both weak and strong tendon groups and ECM was 2.4 in weak tendon group and 1.4 in strong tendon group. The mean Beighton score for weak tendon group was 1.4 and strong tendon group was 3.2.

These results highlight the fact that the expression of collagen V in the extracellular matrix of the weaker tendon group was higher, which confirms the observation (Lu et al., 2011) that increased amount of collagen V can alter the mechanical properties of the tissues. The study also suggest that the weaker tendon group was stiffer which can predispose individuals to the risk of injury. Collagen V can be present in varying degrees in general population and can affect the connective tissue organization in the ECM which can affect the mechanical properties of the tissue and can predict injury patters. If these patients suffer from GJH then proprioception training might be useful to address the biofeedback and prevent certain injuries like ACL rupture or Shoulder dislocations.

Collins et al. proposed a novel hypothesis that there is an increased type V collagen production among individuals with a COL5A1 rs12722 TT genotype, resulting in structural and architectural changes within the collagen fibril. They further propose that these changes result in altered mechanical properties of musculoskeletal soft tissues, which in turn associates with increased risk of specific injuries, reduced

joint ROM (flexibility), and increased endurance running ability. (Malcolm Collins & Posthumus, 2011)

Capsule:

Synovial surface of the capsule, blood vessels and extracellular matrix stained positive to varying degrees. The mean grading of collagen V expression in capsule's synovial surface was 2.6, blood vessels (BV) 1.6 and ECM 1.9 in weak capsule group and 4, 3.1 and 2.6 respectively in strong capsule group. The Beighton score for weak capsule group was 1.9 and strong capsule group was 2.

These results show that the grading of collagen V expression was higher in strong capsule group's synovial surface, blood vessels, and ECM. This observation was different from the skin and tendon group. One explanation might be that there could have been a different healing process present in the presence of recurrent shoulder dislocation resulting in plastic deformation of the shoulder capsule. It would be interesting to study the effect of the number of shoulder dislocations on plastic deformation and correlation of collagen V expression with the plastic deformation. It was also noted that in the shoulder capsule group there was no difference in the Beighton score between the weak and strong shoulder capsule groups. This might be due the fact that all patients in shoulder capsule group were male and none had very high Beighton score (BS > 4) to suggest severe GJH.

After the statistical analysis it was found that only collagen V expression in the skin dermal papillae of the weaker skin group was significant P=0.01. This

suggests that collagen V is present in abundance in the human skin and can affect the mechanical properties of skin more than the tendon or capsule.

Weaker skin tissue had a higher expression of collagen V. These results support a model testing of collagen which showed that the network stiffness strongly decreases with increasing collagen V content, even though the network structure does not substantially change when studied by a combination of fluorescence and atomic force microscopy, turbidimetry, and rheometry of the networks of purified collagen I and V. Collagen fibers are rather stiff polymers with a Young's modulus in the range of 1-800 MPa. This co assembly is thought to provide a mechanism for regulating fibril diameter. This view is mainly based on studies of collagen I and V, which form heterotypic fibrils whose diameter decreases with increasing collagen V content. In most adult tissues, collagen fibrils contain only 2-5 % collagen V and have a broad distribution of diameters in the range of 40-200 nm. Ehlers-Danlos syndrome is associated with increased collagen V content, indicating that a correct stoichiometry of collagen I and V is critical for normal tissue function. (Piechocka, van Oosten, Breuls, & Koenderink, 2011).

Collagen V and Sports:

Musculoskeletal soft tissues injuries are common and multiple risk factors including genetic factors are implicated in the aetiology of these injuries. Sequence variants within the COL1A1 and COL5A1 genes have been shown to be associated with chronic Achilles tendinopathy, cruciate ligament ruptures and/or shoulder dislocations (Malcolm Collins & Raleigh, 2009) (Posthumus, September, Schwellnus, & Collins, 2010) (Khoschnau et al., 2008) (September et al., 2007)

(Laguette, Abrahams, Prince, & Collins, 2011) (Posthumus et al., 2009) (G. G. Mokone, Schwellnus, Noakes, & Collins, 2006).

A variant within COL5A1, which encodes a subunit of type V collagen, is associated with injury and performance phenotypes, which might suggest that these phenotypes are associated directly or indirectly with the mechanical properties of musculoskeletal soft tissue (Malcolm Collins & Posthumus, 2011). Genetic and histologic studies performed on the blood and tendon tissues in patient with COL5A1 polymorphism and spontaneous simultaneous quadriceps tendon rupture showed a statistically significant reduction in collagen type V expression and an alteration in collagen structure in the tendon (Galasso et al., 2012).

There is an interest in identifying the intrinsic risk factors including altered musculotendinous flexibility that may be associated with musculotendinous injuries. A sequence variant, namely the BstUI restriction fragment length polymorphism (RFLP), within the COL5A1 gene is independently associated with lower limb ROM and sit and reach range of movement (SR ROM) (M Collins, Mokone, September, van der Merwe, & Schwellnus, 2009) (J C Brown, Miller, Schwellnus, & Collins, 2011). Sit and reach range of motion (SR ROM) is negatively associated with running economy, suggesting that reduced SR ROM is advantageous for endurance running performance. The COL5A1 genotype was found to be significantly associated with performance in a 56 km ultra-endurance run. Authors speculate that the COL5A1 gene alters muscle-tendon stiffness (James C. Brown, Miller, Posthumus, Schwellnus, & Collins, 2011). Running economy, a key component of endurance ability, has been shown to be associated with flexibility. Increased stiffness (inflexibility) may improve running economy and

therefore endurance running ability. COL5A1 BstUI RFLP has been identified as a marker for endurance running performance (Posthumus, Schwellnus, & Collins, 2011).

Small Leucine rich Proteoglycans (SLRPs):

SLRPs and Skin:

Adult human skin is a layered organ consisting of an epidermis that is attached to a dermis by an elaborate connective tissue structure, the basement membrane (BM). The dermis is divided into two functional layers: the papillary dermis and reticular dermis. Decorin is produced at high levels by papillary fibroblasts (PF) and at low levels by reticular fibroblast (Nomura, 2006) (Sorrell & Caplan, 2004). These two layers are separated by a vascular plexus, the rete sub papillare.

Extracellular matrix (ECM) organization is a complex process that requires the coordinated efforts of many molecules. The proper development of collagen fibrils requires facilitating molecules like proteoglycans. Among others (Hildebrand A, Romaris M, Rasmussen LM, Heinegard D, Twardzik DR, Border WA, 1994)(Krumdieck R, Hook M, Rosenberg LC, 1992)(Schmidt et al., 1987), the small leucine-rich proteoglycan decorin has the ability to bind primarily collagen type I but also type III (El-Domyati et al., 2002)(Vogel, Paulsson, & Heinegård, 1984). Decorin is synthesized and secreted by fibroblasts and comprises 30–40% of all proteoglycans of the skin (Longas & Fleischmajer, 1985).

In the presence of decorin, collagen fibrils form more slowly allowing time for optimal interaction with one another and ultimately resulting in structurally ideal

fiber diameters (Reed & Iozzo, 2002) (Vogel & Trotter, 1987). Altered expression of decorin mRNA in the different dermal strata and a decrease in the collagen-to-decorin ratio inflicted by both age and ultraviolet irradiation possibly affect collagen bundle diameter and subsequently the mechanical properties of human skin. The extracellular matrix (ECM) of the human dermis is primarily comprised of collagen, elastin, proteoglycans, fibronectin, and hyaluronan (Fisher et al., 1997)(Hascall V, 1997)(Tammi R, 1998).

The reticular dermal layer of human skin is composed primarily of large-diameter collagen fibrils organized into interwoven fiber bundles, whereas the papillary dermis is characterized by smaller collagen bundles. While the proteoglycan decorin facilitates a distinct collagen bundle formation coupled with characteristic matrix assembly in the reticular dermis, formation of a less orderly but fine fibrous collagen network in the papillary dermis might be dependent on another mechanism (Stenn KS, 1983). This is suggestive of the influence of further regulators of collagen fibrillogenesis in addition to decorin. Several other proteoglycans could be possible candidates for ensuring proper fibrillogenesis. An obvious candidate would be biglycan, which displays close homology to decorin. However, biglycan does not interact with collagen fibrils under usual assay conditions and does not bind collagen in human skin. (Lochner et al., 2007)

SLRPs and Tendon Development:

Tendons are uniaxial connective tissues that transmit mechanical forces. They are composed primarily of aligned columns of fibroblasts, collagen fibrils grouped as fibers, and an inter-fibrillar matrix (Benjamin & Ralphs, 2000)(Kjaer, 2004)(G. Zhang et al., 2005). The structure and function of a mature tendon are determined by the tendon-specific assembly of the extracellular matrix. Fibrillogenesis and matrix assembly are multistep processes and each step is independently regulated during tendon development. The interactions of collagen fibrils with small leucine-rich proteoglycans have been implicated as important regulators of collagen fibrillogenesis.

The predominant SLRPs in tendon are decorin and biglycan— class I SLRPs, with one or two chondroitin sulfate glycosaminoglycan (GAG) chains, respectively. The prevalence of decorin and biglycan in tendon tissue has been well documented (P. S. Robinson, Lin, Jawad, Iozzo, & Soslowsky, 2004)(P. S. Robinson, Lin, Reynolds, et al., 2004)(P. S. Robinson et al., 2005)(Dourte et al., 2012).

Decorin and biglycan were differentially expressed during normal tendon development. Decorin and biglycan protein core demonstrated a reciprocal expression pattern. Both decorin and biglycan compete for collagen binding, suggesting the use of identical or adjacent binding sites on the fibril. (Guiyun Zhang et al., 2006)

Decorin:

Decorin serves an important role in regulating fibril development, growth, fusion, and orientation during tendon development. Fibril alignment is known to lead to enhanced mechanical properties (Lake, Miller, Elliott, & Soslowsky, 2009). Decorin also appears to assist in alignment of collagen molecules in tendon as well as facilitates sliding during mechanical deformation (Silver, Freeman, & Seehra, 2003). Since fibrils with a wide distribution of sizes pack poorly, this latter effect is likely to reduce fibril area fraction. (Dunkman et al., 2013)

Biglycan:

Biglycan has been shown to be expressed at particularly high levels during early development of tendon structure while decorin expression rises and remains relatively constant in the mature tendon (Guiyun Zhang et al., 2006)(Ansorge, Adams, Birk, & Soslowsky, 2011).

Biglycan is up regulated in decorin-deficient cornea and may replace the function of decorin. Studies (Svensson, Heinegard, & Oldberg, 1995) show that biglycan does not bind to collagen and the potential biglycan–collagen binding mechanism is not well characterized. (Kalamajski & Oldberg, 2010)

Small Leucine rich Proteoglycans (SLRP) expression:

1- Decorin Expression:

Skin:

The mean grading of Decorin expression in skin was 3.2 in weak skin group and 3.1 strong skin group.

Tendon:

The mean grading of Decorin expression in tendon was 3.4 in weak tendon group and 3.8 in strong tendon group.

Capsule:

The mean grading of Decorin expression in shoulder capsule was 2.7 in weak capsule group and 3.3 in strong capsule group.

The results showed that Decorin was present in abundance in the ECM of the skin, tendon and capsule but there was no significant difference in its expression between the weak and strong tissue specimens.

2- Biglycan expression:

Skin:

The mean grading of Biglycan expression in skin epidermis was 1.5, appendages 2.2, ECM in superficial dermis 1.5 and deep dermis 0.75 in weak skin group and 1.75, 2.1, 1.5 and 0.5 respectively in strong skin group.

Biglycan expression was present in skin epidermis, appendages and ECM but there was no significant difference in the expression between the weaker and strong skin groups.

Tendon:

The mean grading of Biglycan expression in tendon ECM was 1.75 in weak tendon group and 3.2 in strong tendon group.

Biglycan expression in the ECM of tendon was higher in the strong tendon group. This was opposite to the expression of the collagen V in the ECM of tendon which was higher in the weaker tendon groups. These results suggest that higher Biglycan expression along with Decorin Expression can help in the organization of the Collagen Fibres in the tendons which results in higher tensile strength of the tendons on mechanical testing whereas the collagen V has the opposite effect.

Capsule:

The mean grading of Biglycan expression in Capsule synovial surface was 2, BV 2 and ECM 2.9 in weak capsule group and 2, 2.5 and 4 respectively in strong capsule group.

The results show that the expression of Biglycan in the ECM of the capsule was higher in the strong capsule group which suggest that Biglycan can help in the organization of the collagen fibres in the ECM and can result in stronger capsule. However there was no statistically significant difference in the expression of Biglycan in different parts of the capsule between the strong and weak capsule groups.

Biomarkers:

1. **Skin:**

The patients with generalised joint hypermobility have ***weaker skin*** and ***higher Collagen V expression*** in the ***skin dermal papillae***.

2. **Tendon:**

The patients with generalised joint hypermobility have ***strong tendons*** and **lower *collagen V expression*** *and* ***higher Biglycan expression*** *in the* ***extracellular matrix.***

3. **Capsule:**

The patients with generalised joint hypermobility had ***weaker capsule*** and ***Decorin expression*** in ***extracellular matrix*** was statistically significant.

Clinical Significance:

These markers play an important role in the strength of tissues and potentially can affect the performance and the risk of injuries. These biomarker potentially could be used in professional athletes to determine the risk of injury and prevent it by gene therapy if necessary.

Benign Joint Hypermobility Syndrome (BJHS):

Benign Joint Hypermobility Syndrome (BJHS), Skin Strength, Collagen V and Small Leucine Rich Proteoglycans (SLRP's) Expression in athletes undergoing shoulder stabilization or ACL reconstruction

The expression of Collagen V, SLRP's and skin strength was studied in 30 patients undergoing shoulder stabilization or primary ACL reconstruction and compared those for the patients who had signs and symptoms of GJH and without those signs and symptoms. It was found that patients who had signs and symptoms of Hypermobility (BJHS) had strong skin and the Biglycan expression in the ECM of superficial dermis was higher. There was no difference in the expression of Collagen V in patients with or without the signs and symptoms of GJH.

Benign Joint Hypermobility Syndrome (BJHS): Tendon Strength, Collagen V and Small Leucine Rich Proteoglycans (SLRP's) Expression

The expression of Collagen V, SLRP's and tendon strength was studied in 9 patients undergoing primary ACL reconstruction and compared those for the patients who had signs and symptoms of GJH and without those signs and symptoms. It was found that patients who had signs and symptoms of Hypermobility (BJHS) had weaker tendons and the Biglycan expression in extracellular matrix was lower and Collagen V expression in tendon sheath was higher in patients who had signs and symptoms of GJH (BJHS). There was no difference in the expression of Decorin in patients with or without the signs and symptoms of GJH.

Benign Joint Hypermobility Syndrome (BJHS): Capsule Strength, Collagen V and Small Leucine Rich Proteoglycans (SLRP's) Expression

The expression of Collagen V, SLRP's and capsule strength was studied in 10 patients undergoing shoulder stabilization for recurrent instability and compared those for the patients who had signs and symptoms of GJH and without those signs and symptoms. It was noticed that the patients who had signs and symptoms of Hypermobility (BJHS) had weaker capsule and the collagen V and Biglycan expression in extracellular matrix was lower in patients who had signs and symptoms of GJH (BJHS). There was no difference in the expression of Decorin in patients with or without the signs and symptoms of GJH.

Surgical Scar and patient satisfaction:

Does Ligament Laxity affect surgical scar or patient's satisfaction following open shoulder stabilization?

The effect of GJH on surgical scars and patient's satisfaction was studied following open shoulder stabilization by dividing the 21 patients into stiff group with Beighton score <4 or lax group with Beighton score >4. There was no difference in the scar satisfaction on VAS scale in the lax or the stiff group 6 vs 5.7. However 5 out of 11 patients in the lax group and 2 out of 10 patients in the stiff group had poor cosmetic scars. The mean scar time was longer in the lax group 34 w vs 9.7 w. The scars in patients with GJH were cosmetically poor but did not affect their satisfaction.

Collagen V, Small Leucine Rich Proteoglycans and Surgical scar: is there a correlation?

The expression of collagen V, decorin and Biglycan was studied in the skin of 13 patients with poor cosmetic scars or good cosmetic scars following the open shoulder stabilization or primary ACL reconstruction. There was no difference in the ligamentous laxity between the 2 groups as the Beighton Score was 2.3 vs 3. Collagen V expression was higher in skin dermal papillae 2.4 vs 1.3 (p=0.02) and skin appendages 2.1 vs1.3 (p=0.05) of good cosmetic scars. This study indicates that higher skin content of Collagen V is associated with better cosmetic scars. Biglycan expression was higher in skin deep dermis 1.1 vs 0.6 (p=0.1) of good cosmetic scars.

Clinical Significance:

There can be a number of other factors which can affect the patient's satisfaction with their scar.

1- **Outcome of the surgery:** The outcome of their surgery and improvement in their symptoms could be the most important factor in their satisfaction with the overall treatment and hence the surgical scar despite the appearance of it.

2- **Presence of laxity / Stretchy scars:** The lax tissues heal differently than the stiff tissue which could be due to the composition of the extracellular matrix in the skin which could be different in patients with GJH (F Malfait et al., 2006).

3- **Effect of time on the scar satisfaction:** The surgical scar usually becomes lighter in colour with time which can affect the appearance of a scar with time and in turn can affect patient satisfaction with the scar over time. As the scar time was different in different patients and it was difficult to control this variable, this might be responsible for the different appearances of the scars and hence the scar satisfaction.

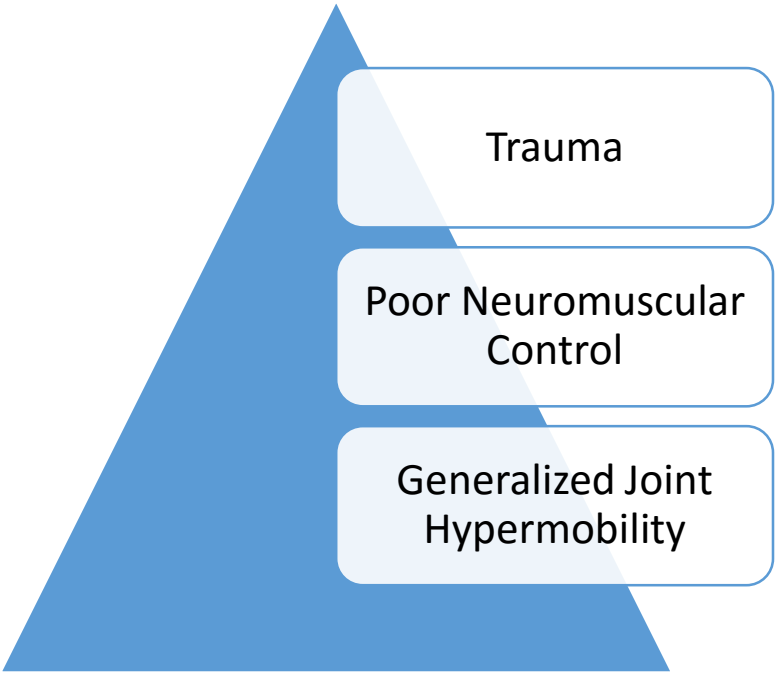


Figure 42- Musculoskeletal Injury Risk Pyramid

Limitations:

- Beighton's modification of the Carter and Wilkinson scoring system is very popular for measuring GJH. However there is no consensus on the accurate and specific cut off point denoting GJH. A cut off score of 4 has mostly been used in the literature.
- Multifactorial Nature of the Generalized joint Hypermobility- could not account for other intrinsic factors; Elastin, Relaxin, Tenascin X, and extrinsic factor; training
- Small sample size for the mechanical testing and force electronic goniometer system
- Most patients in the study were male.
- There is no inter- or intra observer reading for the Beighton scoring system.

Conclusions

The Beighton Scores were higher in patients with ACL and shoulder injuries.

Hypermobility was a risk factor for the failure of ACL reconstruction.

There was no correlation between hypermobility and the bone defects on the CT scan following shoulder dislocation.

Bone defects were a risk factor for recurrence of shoulder dislocation.

There was no correlation between the Beighton Score and the tissue laxity.

There was a correlation between the tissue laxity and the clinical assessment of laxity at the little finger MCPJ by using a force- goniometer system.

There was a correlation between the collagen V expression in the dermal papillae of the skin and the Beighton score.

Collagen V expression was higher in the skin dermal papillae of the weaker group.

Future Research and Further Work

A- Future Projects: ACL

1- Case series of ACL reconstruction with autografts / allografts in presence of GJH:

We are currently studying the role of allograft for primary ACL reconstruction in the presence of Generalized Joint Hypermobility.

2- Immunohistochemical analysis of the Failed ACL stump in the suspected Biological failure:

We are planning to study the process of ligamentization and graft incorporation by the histological analysis of the stump of failed ACL Graft retrieved during the revision surgery.

3- Mechanical testing and histological analysis of the Redundant ACL graft:

Another future project is to study the mechanical and histological properties of the redundant ACL graft found at the revision ACL reconstruction and study the role of Generalized Joint Hypermobility for the ACL graft incorporation.

4- Role of Synthetic Graft (LARS-Ligament) in ACL reconstruction in the presence of GJH:

Another interesting study will be to assess the role of newer synthetic ligaments (LARS) in the ACL reconstruction in the presence of GJH.

B- Future Studies: Shoulder Instability

Trial of **E**valuation of Surgical **T**reatment Techniques for **R**ecurrent **I**nstability of the **S**houlder **(TETRIS):**

Further work is in progress with the above study to assess the role of Open Surgery (capsular shift / Latarjet) in the presence of Bone defects on the CT scan by one of the supervisor.

C- Future Studies: Mechanical Properties of Tissues

Currently we are conducting a large series of tissue laxity assessment by comparing the mechanical properties of tissue with Force plate electronic goniometer system. Future changes (www.velamed.com) to the force plate goniometer system will be

1. Wireless measurement (Overview, 2009) (Tbd, 2011)
2. Small sensors (Usd, 2010)
3. Add to the clinical assessment tool

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Appendix

Appendix 1: Force Goniometer

Technical Details:

A switch-mode power supply was used to obtain the maximum life from a set of four AA batteries. The Graphics Display shows the remaining battery power at all times and audible and visual warnings were provided when there was less than 10 minutes of recording time left. The batteries were changed when required without losing the recordings held in memory.

The two way sensor of the electronic goniometer was attached to the DataLOG through the J1000 interconnecting lead. The polarisation marks on each of the black sockets were aligned before insertion. The silver plug of the J1000 interconnect lead was connected to channel 1 of the analogue sockets of the DataLOG. The red dots were aligned before the J1000 plug was engaged with the socket. The goniometer pre-set from within the Channel Configuration Dialogue was selected. The isometric pinchmeter was connected to channel 3 via the H1800 cable. Once again the red dots of the plug and socket were aligned and engaged. The P100 pre-set default was selected which set the channel up as follows; Channel Sensitivity - 3mVdc, Sampling rate - 6 per second, Excitation Output - 2000mV, Full scale - 22.68 Kg, Units - Kg.

Appendix 2: Mechanical Testing Machine (Dartix) Setup:

The test setup for the tensile testing of the materials was

Tensile Test- Setting:

A: Verifications:

- Test Environment Name: tensile test without Torque cell
- Type of test: Tensile
- Test area: Upper
- Upper soft end Switch: 857.675mm
- Lower soft end Switch: 857.675mm
- Current LE: 5.774mm
- Selection of Load Cells: Force Sensor- 10KN
- Upper Force Limit: 5500 N
- Lower Force Limit: -5500 N
- Selection of standard extensometers: Crosshead travel monitor (WN: 156490)

B- Pre-cycle/Cycle/Steps:

- Pre-cycles- 2
- Number of Cycles- 1
- Upper Reversal point- Standard Force – 200N
- Upper waiting time: 10 sec
- Lower reversal point- Strain- 0mm
- Lower waiting time- 15 sec
- Cycle Speed- strain controlled- 0.5mm/sec
- Other speed for load removal- position controlled- 1000mm/min

Creep Test: Setting

A: Verifications:

3- Test Environment Name: tensile test without Torque cell
4- Type of test: Tensile
5- Test area: Upper
6- Upper soft end Switch: 857.675mm
7- Lower soft end Switch: 857.675mm
8- Current LE: 5.774mm
9- Selection of Load Cells: Force Sensor- 10KN

10- Upper Force Limit: 5500 N
11- Lower Force Limit: -5500 N
12- Selection of standard extensometers: Crosshead travel monitor (WN: 156490)

B- Pre-cycle/Cycle/Steps:

- Pre-cycles- 2
- Upper Reversal point- Standard Force – Adjustable depending on yield (80% of yield) e.g 50 N.
- Upper waiting time: 60 sec
- Upper type of hold: Position controlled
- Cycle Speed- strain controlled- 1mm/sec

Cyclic Loading:

A: Verifications:

13- Test Environment Name: tensile test without Torque cell
14- Type of test: Tensile
15- Test area: Upper
16- Upper soft end Switch: 857.675mm
17- Lower soft end Switch: 857.675mm
18- Current LE: 5.774mm
19- Selection of Load Cells: Force Sensor- 10KN
20- Upper Force Limit: 5500 N
21- Lower Force Limit: -5500 N
22- Selection of standard extensometers: Crosshead travel monitor (WN: 156490)

B- Pre-cycle/Cycle/Steps:

- Pre-cycles- 2
- Number of Cycles- 10
- Upper Reversal point- adjustable (80% of the Standard Force) e.g 50 N for tendon
- Upper waiting time: 2 sec
- Upper type of hold: Force controlled
- Lower reversal point- Strain- 0.00mm
- Lower waiting time- 15 sec
- Cycle Speed- strain controlled- 1mm/sec

Appendix 3- Mechanical Testing Experiment Notes:

Specimen Type	Pt Details	Date	Timing of Test	Type of Test	Comments
Tendon Size- 4.5/4.5/4.0 cm (? 3 specimens) <0.011399>	**K-2**	21.11.08	14.50 14.54 15.11 15.14 15.18 15.27 15.32	Tensile Tensile Creep test Repeat Creep Repeat Tensile Cyclic Loading Repeat Cyclic Loading	Failed Tendon very dry ? Failed Success
Tendon Size- 5x1 cm 5x1 cm 5x1 cm Dry specimens <0.022798>	**K-3**	Same	15.44 15.46 15.58 16.04 16.15?	Tensile Tensile Creep Test Repeat Creep Cyclic Loading	 Failed near lower end 60 sec 180 sec
Tendon Size- 4.0x0.5cm <0.011399> **Tendon** Size-4x0.5cm Same Specimen **Tendon** Size- 5x0.5cm <0.011399>	**K-5**	28.11.08	14.06 14.16 14.18 ?	Tensile Creep Repeat Tensile Cyclic Loading	Failed near upper end (85N) 60 sec Failed at lower end 60 N Failed (Tensile?)
Tendon Size-<0.011399> 5x0.5cm **Tendon** Size-<0.011399> 5x0.5cm (thin sample) **Tendon** Size- 5x0.5cm <0.011399>	**K-8**	30.1.09	14.07 14.15 14.20	Tensile (P14) Creep (P15)	Failed near lower end Test Failed (behave like creep) Failed near lower end act as (45N) tensile test

Skin <0.021405> Size-2x0.5cm Same specimen Same specimen Same specimen			14.30 14.35 14.40 14.45	Cyclic Loading (P16) Tensile (P17) Cyclic Load Creep Tensile	Failed at Lower End H OK(20N) 20 N Failed at/near upper end
Tendon Size<0.011399> 4x0.5cm (Thin specimen) **Tendon** Size-<0.0045596> 4x0.2cm **Tendon** Size-<0.0068394> 2.2x0.3cm **Skin** Size<0.008562> 1.5x0.2cm (small sample)	**K-9**	Same	14.55 15.00 Sample too small to test 15.20	Tensile Creep Cyclic loading Tensile	Break near the upper end/ middle (40N) Failed near lower end (30N) Failed test (25N) Break near the upper end
Tendon Size<0.011399> 6x0.5cm **Tendon** Size-<0.0068394> 6x0.3cm **Tendon** 6x0.3cm<same> **Skin** Size- 3x0.5cm <0.021405> Same Specimen reattached	**K-11**	5.2.09	09.53 10.03 10.16 10.24 ? 	Tensile Creep Cyclic Loading Tensile Creep	Failed near upper End (70 N) Successful (50 N) (40 N) Rupture near lower end still attached ?stress riser clamp 20 N

Capsule Size<0.0143055> 2.5x0.5cm Same specimen Same specimen Same specimen **Skin** Size-<0.012843> 2x0.3cm (small sample)	**S-5**	Same	15.53 15.59 16.03 16.06 16.15?	Tensile Creep Cyc. Loading Repeat Tensile Tensile (P)	Break near upper end (55N) 40 N 40 N Break near upper end Break/Slipped near upper end (52N)
Capsule Size<0.0143055> 3x0.5cm Same specimen **Skin** Size-<0.021405> 2.5x0.5cm Same specimen	**S-6**	Same	16.22 16.29 16.36 16.41	Tensile (P) Repeat Tensile (P) Tensile (P) Tensile (P)	Tear near the lower end Failed near lower end Failed near upper end? Slipped(60 N) Failed near lower end/slipped (150N)
Capsule Size- 2.5x0.5x0.2cm (working length 2cm) <0.01> Same specimen reattached at lower end Same specimen reattached at lower end Same specimen reattached at lower end(Wet) Same specimen Same specimen Same specimen Same specimen (sample became dry with time-? Change the stiffness) **Skin** Size- <0.015>	**S-7**	Same	11.34 11.40 11.45(approx) 11.49 11.52 11.54 11.57 ?	Tensile (P) Tensile (P) Tensile Creep Creep Creep Creep Tensile	Elongated at then break near the lower end (30N) Elongated near the lower end and then break (50N) Elongated at then break near the lower end (50N) Successful At 35 N Successful At 40 N 45 N 50 N Break near upper end- 60 N

3x0.5x0.3cm <u>Same specimen</u> reattached at upper end <u>Same specimen</u> reattached at lower end and soaked with saline(slightly twisted)			12.08 12.12 12.15	 Tensile Tensile Tensile	Break near the upper end (30N) Break at lower end (100N) Break near the upper end (150N)
<u>Capsule</u> Size- 3x0.3x0.01cm (uneven specimen) Working length (1.7cm) <0.003> <u>Same specimen</u> reattached **<u>Capsule</u>** Size- 2.5x0.5x0.01cm <0.005> **<u>Skin</u>** Size- 4x0.3x0.02cm Working length (1.7cm) <0.006>	**S-10**	4.3.09	11.07 11.15? 11.23 11.33	Tensile Tensile (P) Creep Tensile	Slipped/Break near upper end (20N) Break in the middle (35N) Break inside the clamp from lower end (spec. looks small) Specimen break in the middle (30N) less skin more S/C tissue?
<u>Capsule</u> Size- <0.00375> 2.5x0.3x0.01/0.015cm **<u>Skin</u>** Size- <0.004> 2.5x0.2x0.02 cm Thin specimen <u>Same specimen</u> reattached (thickness 1 mm on the machine)	**S-9**	Same	11.45? 11.55 11.58?	Tensile Tensile (P) Tensile (P)	Break near the upper end (25N) Break at the upper end (30N) Break near the lower end

Tendon Size- 5x0.5x (0.0098/0.016/ 0.0120) <0.0063> Working length 3 cm	**K-16**	Same	13.37	Tensile (P)	Break near the upper end (100N) Fibres looked stretched at the upper end Stretched and break again
Same specimen reattached Working length 3 cm			13.44	Tensile (P)	near the upper end (120N) Sample attached but break with the cocktail stick
Same specimen reattached and became dry			13.46	Tensile (P)	Break near upper end (85N)
Tendon Size- 3x0.5x(0.0102/0.0098/ 0.01)cm <0.005> Working length (1.7cm)			13.55	Creep (P)	Break near the lower end (40N)
Skin Size- 2x0.3x(0.0134/0.0131/ 0.0112)cm <0.00377> (Last Fibre Standing)			14.??	Tensile	
Capsule Size- <0.0075> 2.5x0.3x(0.040/ 0.020/0.015) cm	**S-12**	Same	11.35	Tensile (P)	Break near the lower end 11 N
Skin Size- 3x0.5x (0.042/ 0.041/0.040)cm Working length 3 cm <0.0205>			11.43	Tensile (P)	Break/Slipped near the lower end (LFS) 65 N

Capsule 2.5x0.6x (0.045 /0.023/0.021) Working length 1.7cm <0.0178> Same specimen **Skin** Size- 4x0.3x (0.026/ 0.028/0.032)cm <0.0086>	**S-11**	Same	12.25 12.38? 12.45	Tensile Repeat Tensile (P) Tensile (P)	Break at the lower end (? Slipped) Dry specimen Break near the upper end at 35 N (LFS) Thin skin specimen Break suddenly near the lower end at 45 N

Appendix 4: Immunohistochemistry - Datasheets

Collagen V: Datasheet: (Abcam, 2009a)

Product Name: Collagen V antibody **Product type:** Primary antibodies

Description: Rabbit polyclonal to Collagen V **Isotype:** IgG

Purity: Immunogen affinity purified

Immunogen: Full length native Collagen Type V (purified) from adult human and bovine tissues.

Reacts with: Human, Mouse

Specificity: Negligible cross-reactivity with Type I, II, III, IV or VI collagens. Non-specific cross reaction of anti-collagen antibodies with other human serum proteins or non-collagen extracellular matrix proteins is negligible.

Tested applications: ELISA, ICC/IF, IHC-Fr, **IHC-P**, IP, WB

Recommended dilutions: IHC-P: 1/500

Cellular localization: Extracellular matrix

Storage buffer: Preservative: None

Constituents: 0.005% EDTA, 0.125M Sodium Borate, 0.075M Sodium

Chloride. pH 8.0

Form: Liquid **Concentration:** 1.100 mg/ml

Storage instructions: Add glycerol to a final volume of 50%, aliquot and store at -20°C. Avoid repeated freeze / thaw cycles.

Testing Sequence: Collagen V

The following sequence was followed for antigen antibody reaction.

PBS-5min

Block H2O2- 5min

PBS-5 min

Primary antibody (Collagen V) - 30 min

PBS-5min

Secondary antibody (Envision) - 30 min

PBS- 5 min

Deionised water- 5min

DAB 1- 5min

DAB 2- 5min

DAB 3- 5min

Deionised water- 5min

After staining the slides with primary and secondary antibody (Dako, 2013a)(Dako, 2013b) , these were washed with water and counterstained with haematoxylin and Eosin. Slides were dehydrated through graded alcohol, cleaned in xylene and left to dry in air before being examined under the microscope.

Biglycan – Datasheet: (Abcam, 2009b)

Product Name: Biglycan antibody **Product type:** Primary antibodies

Description: Mouse monoclonal to Biglycan **Isotype:** IgG2a

Immunogen: Recombinant full length protein, corresponding to amino acids 1-369 of Human

Biglycan

Reacts with: Human **Tested applications**: **IHC-P**, WB

Light chain type: kappa **Purity:** Protein G purified

Storage buffer: Preservative: None

PBS, pH 7.2

Form: Liquid **Concentration:** 0.500 mg/ml

Storage instructions: Shipped at 4°C. Upon delivery aliquot and store at -20°C or -80°C. Avoid repeated freeze / thaw cycles

Testing Sequence: Biglycan

The following sequence was followed for antigen antibody reaction.

PBS-5min

Block H2O2- 5min

PBS-5 min

Primary antibody (Biglycan) - 30 min

PBS-5min

Secondary antibody (Envision) - 30 min

PBS- 5 min

Deionised water- 5min

DAB 1- 5min

DAB 2- 5min

DAB 3- 5min

Deionised water- 5min

After staining the slides with primary and secondary antibody, these were washed with water and counterstained with haematoxylin and Eosin. Slides were dehydrated through graded alcohol, cleaned in xylene and left to dry in air before being examined under the microscope.

Decorin- Datasheet: (SANTA CRUZ BIOTECHNOLOGY, 2009a)

SOURCE:

Decorin (9XX) is a mouse monoclonal antibody raised against full length recombinant Decorin of human origin.

PRODUCT:

Each vial contains 100 µg IgG1 in 1.0 ml of PBS with < 0.1% sodium azide and protein stabilizer.

STORAGE:

Store at 4° C, **DO NOT FREEZE**. Stable for one year from the date of shipment.

APPLICATIONS:

Decorin (9XX) is recommended for detection of Decorin of human origin by Western Blotting (starting dilution 1:200, dilution range 1:100-1:1000), immunofluorescence and **immunohistochemistry (including paraffin-embedded sections) (starting dilution 1:50, dilution range 1:50-1:500)** and solid phase ELISA (starting dilution 1:30, dilution range 1:30-1:3000). Suitable for use as control antibody for Decorin siRNA (h): sc-40993. Molecular Weight of Decorin is 43 kDa.

RECOMMENDED SECONDARY REAGENTS:

To ensure optimal results, the following support (secondary) reagents are recommended: 1) Western Blotting: use goat anti-mouse IgG-HRP: sc-2005 (dilution range: 1:2000-1:32,000) or Cruz Marker™ compatible goat anti- mouse IgG-HRP: sc-2031 (dilution range: 1:2000-1:5000), Cruz Marker™ Molecular Weight Standards: sc-2035, TBS Blotto A Blocking Reagent: sc-2333 and Western Blotting Luminol Reagent: sc-2048. 2) Immunofluorescence: use goat anti-mouse IgG-FITC: sc-2010 (dilution range: 1:100- 1:400) or goat anti-mouse IgG-TR: sc-2781 (dilution range: 1:100-1:400) with UltraCruz™ Mounting Medium: sc-24941. 3) **Immunohistochemistry: use ImmunoCruz™: sc-2050 or ABC: sc-2017 mouse IgG Staining Systems.**

Testing Sequence: Decorin

The following sequence was followed for antigen antibody reaction.

PBS-5min

Block H_2O_2- 5min

PBS-5 min

Primary antibody (Decorin) - 30 min

PBS-5min

Secondary antibody (Envision) - 30 min

PBS- 5 min

Deionised water- 5min

DAB 1- 5min

DAB 2- 5min

DAB 3- 5min

Deionised water- 5min

After staining the slides with primary and secondary antibody, these were washed with water and counterstained with haematoxylin and Eosin. Slides were dehydrated through graded alcohol, cleaned in xylene and left to dry in air before being examined under the microscope.

Fibromodulin- Datasheet: (SANTA CRUZ BIOTECHNOLOGY, 2009b)

SOURCE:

Fibromodulin (N-14) is an affinity purified goat polyclonal antibody raised against a peptide mapping near the N-terminus of Fibromodulin of human origin.

PRODUCT:

Each vial contains 200 µg IgG in 1.0 ml of PBS with < 0.1% sodium azide and 0.1% gelatin.

Blocking peptide available for competition studies, sc-25857 P, (100 µg peptide in 0.5 ml PBS containing < 0.1% sodium azide and 0.2% BSA).

APPLICATIONS:

Fibromodulin (N-14) is recommended for detection of Fibromodulin of human origin by Western Blotting (starting dilution 1:200, dilution range 1:100- 1:1000), immunoprecipitation [1-2 µg per 100-500 µg of total protein (1 ml of cell lysate)], immunofluorescence (starting dilution 1:50, dilution range 1:50-1:500) and solid phase ELISA (starting dilution 1:30, dilution range 1:30-1:3000). Molecular Weight of Fibromodulin: 67 kDa.

RECOMMENDED SECONDARY REAGENTS:

To ensure optimal results, the following support (secondary) reagents are recommended: 1) Western Blotting: use donkey anti-goat IgG-HRP: sc-2020 (dilution range: 1:2000-1:100,000) or Cruz Marker™ compatible donkey anti-goat IgG-HRP: sc-2033 (dilution range: 1:2000-1:5000), Cruz Marker™ Molecular Weight Standards: sc-2035, TBS Blotto A Blocking Reagent: sc-2333 and Western Blotting Luminol Reagent: sc-2048. 2) Immunoprecipitation: use Protein A/G PLUS-Agarose: sc-2003 (0.5 ml agarose/2.0 ml). 3) Immunofluorescence: use donkey anti-goat IgG-FITC: sc-2024 (dilution range: 1:100-1:400) or donkey anti-goat IgG-TR: sc-2783 (dilution range: 1:100-1:400) with UltraCruz™ Mounting Medium: sc-24941.

STORAGE:

Store at 4° C, **DO NOT FREEZE**. Stable for one year from the date of shipment. Non-hazardous. No MSDS required.

Testing Sequence: Fibromodulin

The following sequence was followed for antigen antibody reaction.

PBS-5min

Block H2O2- 5min

PBS-5 min

Primary antibody (Fibromodulin) - 30 min

PBS- 5 min

PBS-5min

Secondary antibody (Rabbit anti-goat) - 30 min

PBS- 5 min

PBS- 5 min

ABC- 30 min

PBS- 5 min

Deionised water- 5min

DAB 1- 5min

DAB 2- 5min

DAB 3- 5min

Deionised water- 5min

Tenascin X: (SANTA CRUZ BIOTECHNOLOGY, 2004)

PRODUCT:

Tenascin-X (h): 293T Lysate represents a lysate of human Tenascin-X transfected 293T cells and is provided as 100 µg protein in 200 µl SDS-PAGE buffer.

APPLICATIONS:

Tenascin-X (h): 293T Lysate is suitable as a Western Blotting positive control for human reactive Tenascin-X antibodies. Recommended use: 10-20 µl per lane.

Control 293T Lysate: sc-117752 is available as a Western Blotting negative control lysate derived from non-transfected 293T cells.

Tenascin-X (H-90): sc-25717 is recommended as a positive control antibody for Western Blot analysis of enhanced human Tenascin-X expression in Tenascin-X transfected 293T cells (starting dilution 1:100, dilution range 1:100-1:1,000).

Genetic locus: TNXB (human) mapping to 6p21.3.

RECOMMENDED SECONDARY REAGENTS:

To ensure optimal results, the following support (secondary) reagents are recommended: 1) Western Blotting: use goat anti-rabbit IgG-HRP: sc-2004 (dilution range: 1:2000-1:100,000) or Cruz Marker™ compatible goat anti- rabbit IgG-HRP: sc-2030 (dilution range: 1:2000-1:5000), Cruz Marker™ Molecular Weight Standards: sc-2035, TBS Blotto A Blocking Reagent: sc-2333 and Western Blotting Luminol Reagent: sc-2048.

STORAGE:

Store at -20° C. Repeated freezing and thawing should be minimized. Sample vial should be boiled once prior to use. Non-hazardous. No MSDS required.

Testing Sequence: Tenascin X

The following sequence was followed for antigen antibody reaction.

PBS-5min

Block H2O2- 5min

PBS-5 min

Primary- 30 min

PBS-5min

Envision- 30 min

PBS- 5 min

Deionised water- 5min

DAB 1- 5min

DAB 2- 5min

DAB 3- 5min

Deionised water- 5min

Appendix 5- Immunohistological Slides Grading

Collagen V skin Expression:

Study No	Collagen V- Skin-BM (A)	Collagen V- Skin-BM (T)	prof	Collagen V-Skin-Appendages (A)	Collagen V-Skin-Appendages (T)	prof	Collagen V-Skin-Dermis=ECM (A)	Collagen V-Skin-Dermis=ECM (T)	prof
K1	1+	1+		1+	1+		1-	1-	
K2	1+			2-			1+		
K3	2+			2+			1+		
K4	1+	1+		2-	1+		0	1-	
K5	2+			2+			1+		
K6	2-			2-			1-		
K7	1+			1-			0		
K8	2-			2-			1+		
K9	2-/1+			2-/1+			1+		
K10	2+		2+	2+		2+	1+		1+
K11	2+			2-			1-		
K12	1+			1+			1+		
K13	2+	2+		2+	2+		2+	2+	
K14	0	1-		1+	2-		0/1-	0/1-	
K15	1+			1+/2-			1+		
K16	2+			1+			1+		
K17	1+			1+			1+		
K18	1+	1+		1-	1-		0	0	
K19	2+			1+			1-		
K20	2+			2+			2+		
K21	1+			2-			1+		
K22	NA			NA			NA		
K23									
K24	1-	1-	1-	1+	1+	1+	1-	1-	1-
K25	2+			2+			2+		
S1	1-			1-			1-		
S2	0	0	1+	1-		1+	1-		1-
S3	1-			1-			0/1-		
S4	1-			1+			1+		
S5	2-			1-			1-		
S6	1-			1-			1-/0		
S7	1+			1+			2+?		
S8	2-	2-		2+	2+		1+	1+	
S9	1+			1+			1+		
S10	1-			1-			1-		
S11	1+			1-			1-		
S12	1+	1+		1+	1+		1+	1+	
S13	1+			1+			1+		
S14	2+			2+			2-		
S15	2-			2+			2+		

Collagen V Tendon Expression:

Study No	Collagen V Tendon-Sheath (A)	Collagen V Tendon-Sheath (T)	Prof	Collagen V Tendon ECM=collagen (A)	Collagen V Tendon ECM=collagen (T)	Prof	Collagen V Tendon-BV (A)	Collagen V Tendon-BV (T)	Prof	Collagen V tendon-interfascicular tissue (A)	Collagen V tendon-interfascicular tissue (T)	Prof
K1	1-	1-		2-	1+		2-	2-		2-	2-	
K2	1-			1-			1-			1-		
K3	1-			1-			1-			NA		
K4	2+	2+		1+	1+		2-	2-		2-	2-	
K5	2-			1+			2-			2-		
K6	2-/1+			1-			1-			1-		
K7	2-			1+			2-			NA		
K8	2-			1-			2+			2-		
K9	2+		2+	1+		1+	2+		2+	2+		2+
K10	2-			1+			2-			2-		
K11	2+			2-			2-			2-		
K12	1+			2-			2+			2+		
K13	2-	2-		2-	2-		2+	2+		2+	2+	
K14	1-	1-	1-	1+	1+	1-	2-	2-	2-	NA	NA	
K15	1-			1-			1-			1-		
K16	2+			1+			2+			2+		
K17	2-			1-			1+			NA		
K18	1+	1+		1+	1+		1+	1+		2-	2-	
K19	2-			2-			2-			2-		
K20	1-			2-			1-			NA		
K21	1+			1+			2+			1+		
K22												
K23												
K24	2-		2-	2+		2+	2+	2+	2+	2+	2+	
K25	2+			2+			2+			2+		

Collagen V Capsule Expression:

Study No	Collagen V Capsule SS (A)	Collagen V Capsule SS (T)	prof	Collagen V Capsule BV (A)	Collagen V Capsule BV (T)	prof	Collagen V Capsule ECM (A)	Collagen V Capsule ECM (T)	prof
S1	2-			1+			1+		
S2	1+			1+			1+		
S3	1-			0			0		
S4	2+			1+			2-		
S5	2+			1+			2-		
S6	2+			2-		2+	2-		2-
S7	2+			2+			1+		
S8	2+	2+	2+	1+	1+	1+	1-	1-	1-
S9	0			1-			0		
S10	2-			1+			1+		
S11	2+			1+			2-		
S12	1-	1-		1+	1+		1+	1+	
S13	2-			1+			1+		
S14	NA			NA			NA		
S15	NA			NA			NA		

Biglycan Skin Expression:

Study No	Biglycan-Skin Epidermis (A)	Biglycan-Skin Epidermis (T)	Prof	Biglycan-Skin Appendages (A)	Biglycan-Skin Appendages (T)	Prof	Biglycan-Skin Dermis (Superficial) =ECM (A)	Biglycan-Skin Dermis (Superficial)=ECM (T)	Prof	Biglycan-Skin Dermis (Deep) =ECM (A)	Biglycan-Skin Dermis (Deep) =ECM (T)	Prof
K1	1+	1+		1+	2-		1+	1+		1-	1-	
K2	1+/2-		1+	1+		2+	2+		2+	1-		1-
K3	1+			2-			1+			1-		
K4	1+	1+	1+	2-	2-	2+	1+	1+	1+	0/1-	1-	1-
K5	1+			1+			2-			1-		
K6	1+			1+			1+			1-		
K7	1+			1+			2-			0		
K8	NA			NA			NA			NA		
K9	1+			1+			1+			1-		
K10	2+			2+			2+			1+		
K11	1+	1+		1+	2-		2+	2+		1+	1+	
K12	1+	1+		2-	2+		1-	1-		0		
K13	1+			1+			1-			1-		
K14	1+			1+			1-			0		
K15	NA			NA			NA			NA		
K16	1+/2-			1+			1+			1-		
K17	0			0			0			0		

K18	2+	2+		2+	2+		1-	1-		1-	1-	
K19	2+			2+			1+			1+		
K20	NA			NA			NA			NA		
K21	1+			1+			1+			1+		
K24	1+			2-			1+			1-		
K25	1+			2-			1+			1+		
S1	1+			2-			2-			1-/0		
S2	1+			2+			2+			NA		
S3	1+			1+			1-			0		
S4	1+			1+			1+			1-		
S5	1+			2-			1+			1-		
S6	1+			2-			1+			1-		
S7	1+			1+			1+			1-		
S8	1+			2-			1+			1+		
S9	1+			1+			1+			1-		
S10	1+			2-			1+			1-		
S11	1+			1+			1+			1-		
S12	1+			2-			1+			0/1-		
S13	1-			1+			1-			1-		
S14	2-			2-			1-			0		
S15	1+			2-			1-			1-?		

Biglycan Tendon Expression:

Study No	Biglycan Tendon ECM (A)	Biglycan Tendon ECM (T)	prof	Biglycan Tendon BV (A)	Biglycan Tendon BV (T)	prof	Biglycan Tendon SM (A)	Biglycan TendonSM (T)	prof
K1	2+	2+	2+	NA		1+	1-	1-	1-
K2	2-			NA			1+?		
K3	2+			NA			1+		
K4	1-	1-		NA			1+	1+	
K5	2+			1+			NA		
K6	2+			NA			1-		
K7	2+			1-			1-		
K8	2+			1+			NA		
K9	2+			1+		1+	NA		
K10	NA			NA			NA		
K11	2-	2-		1+	1+		1+	1+	
K12	0			0			0		
K13	1-			NA			1+		
K14	NA			NA			NA		
K15	1-			NA			NA		
K16	2+?			2+?			NA		
K17	2-			NA			NA		
K18	2+	2+		1+	1+		1+	1+	
K19	2+?			NA			1+		
K20	1+			NA			NA		
K21	1+			1+			1+		
K22									
K23									
K24	1+			2+			1+		
K25	1-			2+			1-		

Biglycan Capsule Expression:

Study No	Biglycan Capsule SS (A)	Biglycan Capsule SS (T)	prof	Biglycan Capsule ECM (A)	Biglycan Capsule ECM (T)	prof	Biglycan Capsule BV (A)	Biglycan Capsule BV (T)	prof
S1	2+			1+			1+		
S2	NA			NA			NA		
S3	NA			2-			NA		
S4	1-			1-			1+?		
S5	1+			2+			2-		
S6	1+			2+			1+		
S7	NA			2+			NA		
S8	1+	1+	1+	2+	2+	2+	1+	1+	1+
S9	NA			1+			2-		
S10	2-			2+			1+		
S11	1+			2+			1+		
S12	2+	2+		2+	2+		2+	2+	
S13	1+			1+			1+		
S14	NA			NA			NA		
S15	NA			NA			NA		

Decorin Expression:

Study No	Decorin-Skin-Adeel	Decorin-Skin-Tim	Prof Salter	Decorin tendon/capsule A	Decorin tendon/capsule T	pro f
K1	2+	2+		2+	2+	
K2	2-			2+		
K3	2+			2+		
K4	2+	2+		2+	2+	2+
K5	2-			2-		
K6	2+	2+		2-	2-	
K7	2+			2+		
K8	2+			1+/2-		
K9	2+			2+		
K10	2+			2+		
K11	2+			2+		
K12	2+			2-		
K13	1+ ? 2-	1+		1+	2-	
K14	2+	2+		2+	2+	
K15	NA			2+		2+
K16	2+			2+		
K17	2+			2-		
K18	1+	1+	1+	2-	2-	
K19	NA			2+		
K20	1+			2-		
K21	2+			2+		
K22	NA			NA		
K23	NA			NA		
K24	2-	2-	2+	2+	2+	2+
K25	2+			2+		
S1	2+			2+		
S2	2-	1+		1+	1+	1+
S3	1+			2-		
S4	2+ ?			1+		
S5	1+			2+		
S6	2-			2-		2+
S7	2+			2-/1+		
S8	2+	2+		1+	1+	1+
S9	2+			2+		
S10	2+			2-		
S11	1+			1+		
S12	2-	1+/2-		2+	2+	
S13	2+		2+	2+		
S14	2+			NA		
S15	2-			NA		