This is to certify that the

thesis entitled

MECHANICAL AND BIOLOGICAL RESPONSES OF THE CANINE PATELLAR TENDON AFTER REMOVAL OF ITS CENTRAL VERSUS MEDIAL ONE-THIRD FOR LIGAMENT RECONSTRUCTION

presented by

Lucinda Howlett Linder

has been accepted towards fulfillment
of the requirements for

M.S. degree in M.S.M

Roger C. Haut, Professor

Roger C Haut
Major professor

Date 3/17/1992

MECHANICAL AND BIOLOGICAL RESPONSES OF THE CANINE PATELLAR TENDON AFTER REMOVAL OF ITS CENTRAL VERSUS MEDIAL ONE-THIRD FOR LIGAMENT RECONSTRUCTION

By

Lucinda Howlett Linder

A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree

MASTER OF SCIENCE

Department of Material Science and Mechanics

1992

ABSTRACT

MECHANICAL AND BIOLOGICAL RESPONSES OF THE CANINE PATELLAR TENDON AFTER REMOVAL OF ITS CENTRAL VERSUS MEDIAL ONE-THIRD FOR LIGAMENT RECONSTRUCTION

By

Lucinda Howlett Linder

The use of a portion of the patellar tendon as an autograft for surgical reconstruction of a damaged anterior cruciate ligament is common. In the present research, biomechanical and histological properties of the dog patellar tendon were investigated after removal of the medial one-third. The results were compared to an earlier study on the dog patellar tendon after removal of the central one-third. The biomechanical and histological properties of the tendon with the medial defect were closer to their controls than the tendon with a central defect at all time points post-surgery. In an attempt to explain the mechanisms of this response, we studied the spatial variations in mechanical properties across the width of the tendon. While this was not a factor, we found that the host tendon after the central procedure had relatively more tissue removed during the surgery, possibly causing the tendon to experience higher stresses during the healing response. Further work is needed to better understand the mechanisms of healing in the patellar tendon especially with immediate post-surgical exercise.

ACKNOWLEDGMENTS

I would like to express my appreciation to : Roger C. Haut, Ph.D, for his guidance and advice, and Dan Linder for his encouragement and support throughout this project.

I would also like to thank the following people for their significant contributions to this research: Shawn Kosnik for the biochemical analyses, Jane Walsh for the histological analyses and Scott Hannum for his assistance in the tissue preparation and mechanical tests.

TABLE OF CONTENTS

Page

LIST OF TABLES..... vi

LIST OF FIGURES..... vii

LIST OF SYMBOLS.....viii

Chapter

I. INTRODUCTION..... 1

II. SURVEY OF LITERATURE..... 5

III. PART I. MEDIAL PROCEDURE..... 19

MATERIALS AND METHODS..... 19
Specimens..... 19
Mechanical Test Preparation..... 21
Pre Conditioning..... 27
Tensile Tests..... 28
Histology..... 29
Statistics..... 30

RESULTS AND DISCUSSION..... 31
Tissue Dimensions..... 31
Histological Observations..... 34
Tensile Failure Mechanisms..... 35
Structural Failure Properties..... 39
Tensile Response Data..... 41

ANALYSIS OF CENTRAL VERSUS MEDIAL PROCEDURE. 43
Animals..... 43
Tissue Dimensions..... 43
Tensile Failure Mechanisms..... 46
Structural Failure Properties..... 48
Tensile Response Data..... 50
Histological Analysis..... 54

IV. PART II. SPATIAL VARIATIONS..... 55

MATERIALS AND METHODS..... 56
Specimens..... 56
Mechanical Test Preparation..... 56
Mechanical Test..... 59
Microstructural Model..... 61
Biochemistry..... 63
Statistics..... 65

RESULTS..... 65
Tissue Dimensions..... 65
Physiological Properties..... 67
Microstructural Model..... 69
Modes of Failure..... 71
Structural Failure Properties..... 71
Tensile Response Data..... 73
Biochemistry..... 75

V. CONCLUSIONS..... 77

VI. DISCUSSION OF CONCLUSIONS AND SUMMARY..... 78

VII. REFERENCES..... 83

LIST OF TABLES

		Page
Table 1.	Dimensions of the Patellar Tendon in Control and Operated Preparations.....	33
Table 2.	Tensile Failure Properties of Control and Operated Preparations.....	40
Table 3.	Tensile Response Parameter for Control and Operated Preparations.....	42
Table 4.	Dimensions of the Patellar Tendon Thirds.....	66
Table 5.	Energy Absorbed in the Physiological Range.....	68
Table 6.	Crimp Geometry.....	70
Table 7.	Structural Failure Properties.....	72
Table 8.	Tensile Response Data.....	74
Table 9.	Biochemical Properties.....	76

LIST OF FIGURES

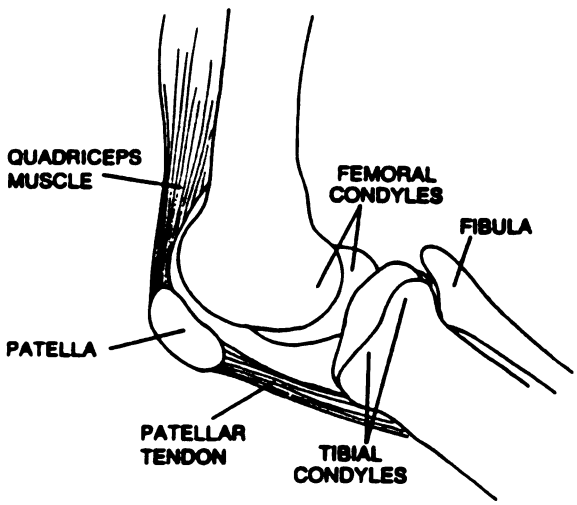
		Page
Figure 1.	Knee Anatomy (taken from Nordin, 33).....	2
Figure 2.	Mechanism of ACL injury (taken from Kennedy, 28).....	3
Figure 3.	Force-deformation curve of human PT and ACL (taken from Noyes, 37).....	7
Figure 4.	Collagenous tissue response curve (taken form Noyes, 36).....	8
Figure 5.	Tendon and ligament fiber alignment (taken from Nordin, 33).....	10
Figure 6.	Loaded versus unloaded tendon fibers (taken from Nordin, 33).....	10
Figure 7.	Mechanics of central defect versus medial defect.....	15
Figure 8.	Homeostatic responses of biological tissues (taken from Woo, 50).....	16
Figure 9.	Tendon dimension measurements.....	22
Figure 10.	Potted tibia and patella.....	24
Figure 11.	Mounted PPT complex.....	26
Figure 12.	Typical force-deformation curve (taken from Burks et al, 4).....	29
Figure 13.	Control versus operated tendon at six months.....	35
Figure 14.	Failure by avulsion.....	37
Figure 15.	Failure in the midsubstance.....	38
Figure 16.	CSA: central versus medial.....	45
Figure 17.	Mechanisms of failure: central versus medial.....	47
Figure 18.	Failure Loads: central versus medial.....	49
Figure 19.	Stiffness: central versus medial.....	51
Figure 20.	Modulus: central versus medial.....	53
Figure 21.	Cutting the patella.....	57
Figure 22.	Dividing the tendon into thirds.....	58
Figure 23.	Test fixture with saline bath.....	60
Figure 24.	Tendon crimp distribution.....	62
Figure 25.	Experimental data and model fit.....	69
Figure 26.	Stress-strain plot: central, medial and lateral thirds.....	73

I. INTRODUCTION

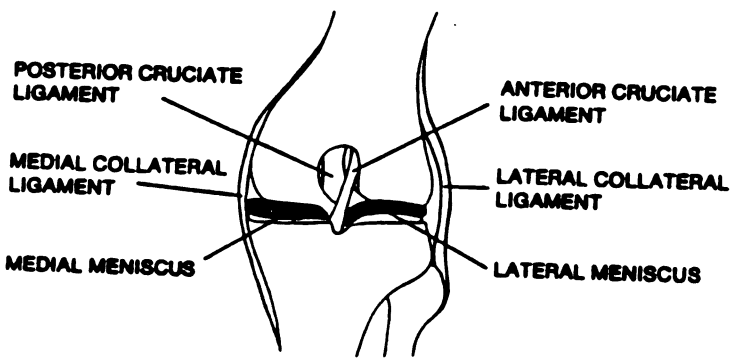
The knee is one of the most complex and frequently injured joints in the body. It allows rotation about two axes. It restrains rotation about a third axis and controls translation in all three planes. The knee bears loads that frequently exceed the body's weight by two to three times, and its location between the body's two longest lever arms makes it especially prone to injury. Because it lacks any great amount of bony stability, it depends upon a complex arrangement of ligaments, menisci, and musculotendinous units to maintain normal alignment and stability (Figure 1).

Of all the ligaments in the knee, the anterior cruciate ligament (ACL) is one of the most frequently ruptured (37). The ACL attaches to the posterior femoral condyle and to the anterior tibial plateau (Figure 1). The primary functions of the ACL continue to be debated, however, the general purpose of all ligaments is to act as a guide and restraint within the joint. There are many mechanisms of injury causing tears to the ACL. The injuries are usually caused by a deceleration and cutting action of the knee joint (15). Several common mechanisms of ACL injury are external or internal rotation (as in downhill skiing accidents) (28) (Figure 2) or a direct force to the posterior surface of the tibia (as in a clipping

injury in football). If the injury goes unrepaired, the knee becomes progressively unstable and develops meniscal tears and degenerative arthritis (18,38). Because the ACL is a key stabilizer of the knee joint (15) and an unrepaired ACL produces undesirable long term results, the ruptured ACL is commonly repaired using one of many surgical procedures practiced today.



Lateral View



Anterior View Without Patella

Figure 1. Knee Anatomy (taken from Nordin, 33).

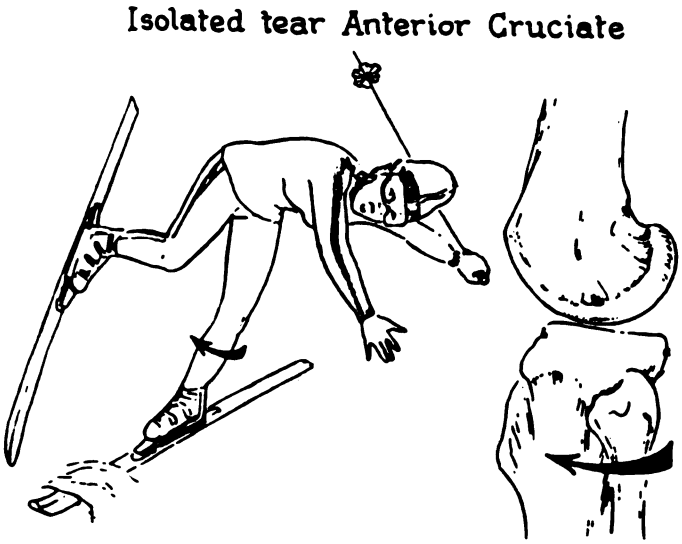


Figure 2. Mechanism of ACL Injury (taken from Kennedy, 28).

The approaches used to treat the ACL-deficient knee include primary repair (suturing the ACL), extra-articular lateral repair, ligament augmentation, and ligament replacement using autografts, allografts, xenografts and synthetic materials. Each of these techniques has pros and cons, nevertheless the technique most often used by surgeons today is to repair the ruptured ACL with an autograft, a tissue taken from another site in the same patient (24). Although this approach has been used for the past 75 years (21) questions persist about which tissue to use and what effects removal of the normal graft tissue might have on the knee and limb function.

There are many different collagenous tissues which have been used as an ACL autograft. The only one which has the strength and stiffness equal to or greater than the ACL is the bone - one-third patellar tendon - bone complex. The patellar tendon is also favorable because of the bony ends for bone to bone healing, as apposed to tendon to bone healing for the other tissues (39). For these two reasons, surgeons use the patellar tendon complex frequently as their autograft of choice for ACL reconstruction.

A question arises as to which portion of the patellar tendon is the best choice for use as an autograft. Factors to be considered when making this decision are the performance of the tissue as an autograft, the fate of the remaining tendon, and the consequences of the surgery on the function and strength of the limb, post-operatively.

This thesis is divided into two parts (Part I and II). First, the dog patellar tendon is examined after removal of the medial one-third. Second, an attempt is made to explain differences between removal of the central and medial thirds by considering spatial variations across the width of the tendon.

II. SURVEY OF LITERATURE

All types of collagenous tissues have been transferred in and around the knee for intra-articular and extra-articular ligament reconstruction. Until the 1970's, however, the mechanical properties of such grafts had not been well documented. Butler (7,8) and Noyes (39) compared subfailure and failure mechanical properties of nine commonly used autografts from young donors, since patients in this age range typically receive reconstructive procedures. Also, age and disuse-related factors are known to cause significant reductions in properties of the ACL (9,35). These investigators failed the tissues at high strain rates and induced soft tissue failures (35), commonly seen in patients; and compared the results to earlier mechanical property data for ACL-bone units from young donors (35). In this way, tissues with the best initial properties were identified. The tissue structures examined included the bone-patellar tendon (medial and central portions, each 14 mm wide), semitendinosus and gracilis tendons, quadriceps tendon-patellar retinaculum-patellar tendon (medial, central and lateral thirds), fascia lata, and distal iliotibial band. Each tissue was loaded to failure in tension at 100% of its initial length per second. The bone-patellar tendon-bone specimens developed the highest

maximum force (about 160% the strength of the anterior cruciate ligament). These tendons were the only tissues having strength greater than that of the ACL. Semitendinosus and gracilis tendons developed 70% and 49% of ACL strength, respectively. Fascia lata (16mm wide) and the iliotibial band (18mm wide) followed (36 and 38 percent, respectively). Patellar retinacular tissue was the weakest, bearing only 14-21 percent of the average maximum load for the ACL (39). The central and medial patellar tendons also generated three times the stiffness and failure energy of the ACL. Noyes (39) indicated that the width of the patellar tendon examined was comparable to that used in surgery. On a mechanical basis, other tissues could also be acceptable ACL grafts. Semitendinosus and gracilis tendon would be suitable if these structures could be adequately fixed to bone, and if the forces on them could be kept below 50% of the maximum in vivo force in the ACL. If fascia or iliotibial band were used, it would have to be almost full width to match the initial properties of the ACL. This extreme width could compromise important lateral knee restraints. The authors recommended the surgeon not use the retinacular tissues (39). Figure 3 shows the force-deformation curve of the medial third patellar tendon and the human ACL (39). There have been several studies done to determine the level of forces the ACL withstands during daily activities. Morrison (32) estimated the force levels in the ACL to be 169N for level walking, only

27N for ascending stairs, and as much as 445N for descending a ramp. Noyes and Grood (40) calculated values of 200-400N for younger humans and 80-160N for older humans. Chen and Black (12) estimated the tensile forces in the ACL during normal function to range from 67N for ascending stairs to as much as 630N for jogging.

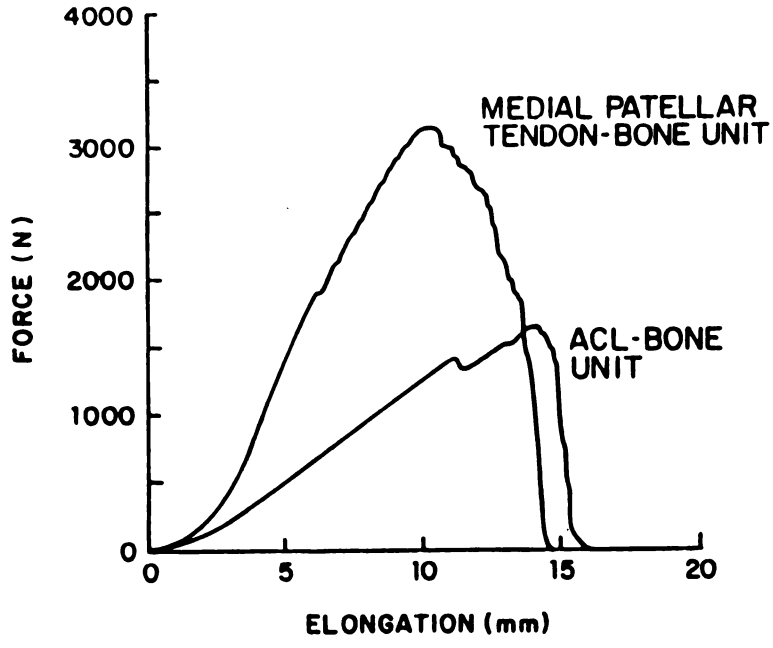


Figure 3. Force Deformation Curve of Human PT and ACL (taken from Noyes, 37).

Figure 4 is a typical representation of collagenous tissue tensile response curves. The curve typically exhibits four regions (36). The initial concave part of the curve, Region I, is called the nonlinear toe region where uncrimping and progressive recruitment of collagen fibers occurs. It is within this range of loading that most physiological activities and clinical diagnosis take place. In Region II, the nearly linear region, collagen fibers are further elongated until first significant failure occurs at the linear load point. In Region III, a serial failure process and maximum loading occurs. This is characterized by a series of small, sudden force drops and fiber separation until maximum force when catastrophic failure begins. Finally in Region IV, the post-failure region, the tissue loses its load-carrying ability, although the fibers may still appear to be in continuity.

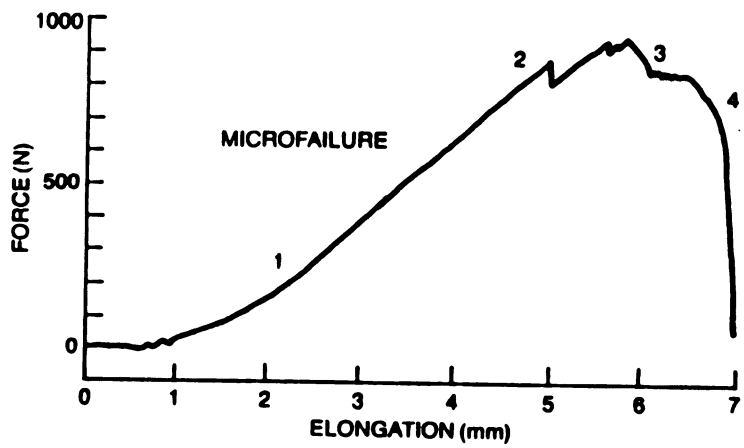


Figure 4. Collagenous Tissue Response Curve (taken from Noyes, 36).

The shift in the tensile response between the patellar tendon and the ACL (Figure 3) is caused by differences in the alignment of the constituent collagen fibers and content of these fibers between the patellar tendon and the ACL (33). Tendons have nearly a parallel alignment, which makes tendon well suited for withstanding high tensile loads. The fibers in ligaments have a less consistent structural orientation (Figure 5). In tensile loading of ligaments, where the fibers are not as aligned, only the fibers oriented in the direction of the principal load straighten out completely at first and sustain maximum loads. Also, ligaments contain less collagen than do tendons. The main components of tendons and ligaments are collagen fibers and elastic fibers, which together constitute approximately 90% of the tissues. Tendons consist almost entirely of collagen, while the ACL consists of approximately 90% collagen (a high percentage of collagen for a ligament). These fibers of the collagenous tissues have a different configuration when loaded and unloaded. When the fibers are relaxed, they have a wavy or crimped configuration (Figure 6). When loads are imposed, low loads are sustained until all fibers oriented in the direction of loading straighten out. At this point the straightened fibers sustain loads in the physiological range.

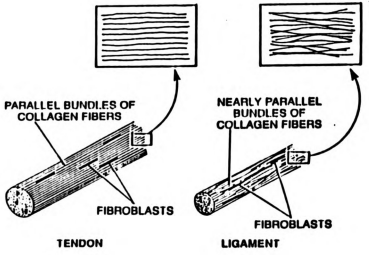


Figure 5. Tendon and Ligament Fiber Alignment (taken from Nordin, 33).

Figure 6. Loaded versus unloaded tendon fibers (taken from Nordin, 33).

Because of its superior mechanical properties and healing characteristics, the use of the patellar tendon as a substitute for a torn anterior cruciate ligament has grown in popularity in recent years. Campbell (11) first described its use for anterior cruciate reconstruction in 1936. In 1963, Jones (25) outlined the use of the central one-third of the patellar tendon in an anterior cruciate reconstruction procedure that closely parallels that which is used by many surgeons today. Since that description, the use of the medial third or central third of the patellar tendon has been reported in numerous studies.

Complications from the use of patellar tendon grafts have also been reported. Bonamo (3) reported two cases of rupture in the remaining patellar tendon at 3 1/2 months and 8 months, after use of the central third for ACL reconstruction. McCarroll (31) reported a transverse patellar fracture 6 months after ACL reconstruction with a patellar tendon (PT) graft. Hughston (22) reported medial or lateral subluxation and dislocation as potential complications of this procedure. More recently, Sachs (43) compared results from a group of patients who had ACL reconstruction with semitendinosus tendon to that using a PT graft. The PT group had a significant reduction in quadriceps muscle strength at 1 year. Tibone and Antich (45) recently published a 2 year follow-up study on patients with a central one third patellar tendon reconstruction and found similar decreases in thigh

circumference and quadriceps muscle strength, without deficits in the hamstrings as compared to the normal side. Obrien (41) also reported on patients with a central one-third patellar tendon ACL reconstruction and found a high incidence of patellar pain two years post-op. Pre and post-op radiographic analysis of the length of the host patellar tendon indicated that in 75% of cases the host patellar tendon had changed length. Fifty-five percent of these had shortened, and 20% had lengthened. The incidence of patellar pain increased as the amount of shortening or lengthening increased.

Most experimental studies of patellar tendon reconstruction for ACL deficits have focused solely on the fate of the intra-articular graft. Only Cabaud (10) and Burks et al (4) examined the remaining patellar tendon after harvesting one third for ACL reconstruction. In Cabaud's study, the ACL was reconstructed with the medial one-third patellar tendon in eleven dogs. Biomechanical tests were conducted on the remaining patellar tendon. The dogs were immobilized for six weeks post-op, prior to remobilization with unrestricted activity in a run. Patellar tendons from six dogs were examined at 4 months and showed only a slight decrease in maximum load compared to contralateral controls. In five dogs tested at 8 months the operated patella-patellar tendon-tibia (PPT) preparation carried more load than the contralateral control side. Histologic evaluation of the operated tendons showed microscopically normal orientation of

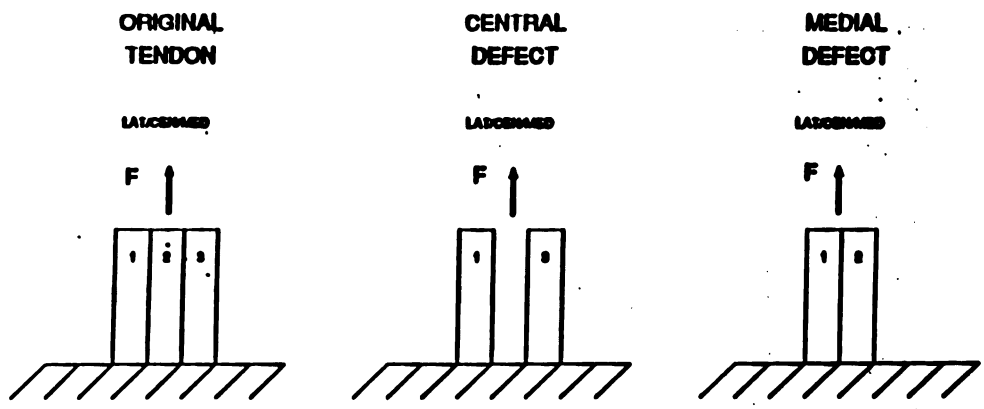
collagen. The tendons showed no abnormalities on gross inspection, except for a thickening of the undersurface where the fat pad had been removed.

Burks et al studied the host patellar tendon after removal of its central one-third in twenty-five adult mongrel dogs. The dogs were allowed free use of their limbs post-operatively. Unlike Cabaud, Burks et al documented a significant increase in cross-sectional area at 3 months, and further increase in size at 6 months when compared to contralateral controls. The failure load was 70% of controls at 3 months and 60% of controls at 6 months. The structural stiffness and tensile modulus of the operated tendon within the physiologic range were dramatically reduced to 70% and 33% of controls at 6 months, respectively. Upon gross inspection, it was found that in all tendons the defect left from graft removal was completely filled in with scar tissue. The histologic sections revealed extensive scar formation on all sides of the remaining two thirds of the patellar tendon, as well as poor alignment of collagen. The scar had not only filled in the defect but had engulfed much of the original tendon, leaving few thickened and clouded original patellar tendon fibers, especially at six months. Histologically at three months the central area of the operated tendon was hypercellular. Collagen fibers were disorganized with small aggregates of adipose tissue throughout the area. Nuclei were short and round. Demarcation between normal and disrupted

tendon was evident. At six months the collagen was still very disorganized, but the hypercellularity was markedly decreased. Nuclei were longer but flatter in shape than at three months post-op. The demarcation between normal and disrupted tendon was less pronounced. The control tendons at three and six months appeared essentially normal, but at three months some areas exhibited an increase in cellularity. The study of Burks et al indicated that the canine patellar tendon had not fully recovered 6 months after removal of its central one-third.

The contrasting results from these two studies is under investigation here. One current explanation may be that there is a variation in the tensile stiffness (or modulus) across the thirds of the tendon. In the human patellar tendon, the tensile modulii of the central and lateral thirds are approximately two times higher than that of the medial one-third (13). Removing the central third (as in Burk's study) may have resulted in excess stresses in the remaining tendon compared to removal of the more compliant medial third (Figure 7). The increased stresses in the remaining tendon after removal of the central versus medial third may result in micro-damages of the remaining collagen fibers. This may then act as a stimulant for fibroblasts to synthesize relatively more new collagen fibers (47) after removal of the central one-third.

On the other hand, if removal of the central versus medial thirds results in more normal stresses in the host tissue, collagen synthesis may be enhanced.



ASSUME LENGTH AND AREA OF EACH THIRD IS CONSTANT AND $E_1=E_2=2E_3$

$F=F_1+F_2+F_3$	$F=F_1+F_3$	$F=F_1+F_2$
$A/L\delta=F_1/E_1=F_2/E_2=F_3/E_3$	$A/L\delta=F_1/E_1=F_3/E_3$	$A/L\delta=F_1/E_1=F_2/E_2$
$F_1=2/5\ F$ $F_2=2/5\ F$ $F_3=1/5\ F$	$F_1=2/3\ F$ $F_3=1/3\ F$	$F_1=1/2\ F$ $F_2=1/2\ F$

THE LOADS IN THE TWO REMAINING THIRDS OF TENDON AFTER REMOVAL OF THE CENTRAL THIRD INCREASE 67% OVER THE ORIGINAL LOADS. THE LOADS IN THE TWO REMAINING THIRDS OF THE TENDON AFTER REMOVAL OF THE MEDIAL THIRD INCREASE 25% OVER ORIGINAL LOADS.

Figure 7. Mechanics of central defect versus medial defect.

The phenomenon of increased stress on collagen causing increased synthesis has been shown in several recent studies (14,17,47). For a normal, uninjured ligament Woo (51) found that there exists a general relationship between increased or decreased stress and strain levels and soft tissue homeostasis. An immobilized limb has a rapid reduction in the mechanical properties, as well as slight atrophy. On the other hand, exercise or increased levels of stress and motion increase the mass and strength of the tissue. Figure 8 shows a curve representing the stress and motion dependent homeostatic responses of biological tissues from Woo (51).

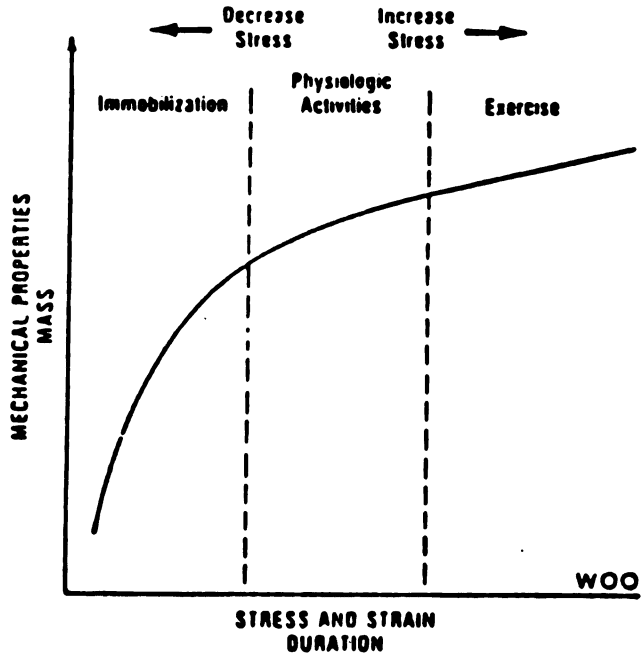


Figure 8. Homeostatic responses of biological tissues (taken from Woo, 50).

For a tendon or ligament healing after a reconstruction procedure, the tissue goes through three phases (48). The first is an inflammatory phase which takes place during the first three to four days of healing. During this period the tissue defect is filled with clotted blood, tissue debris and fluid. Local edema and an increase in the number of macrophages (debris eaters) occur. Towards the end of this phase fibroblastic cells begin to migrate into the wound area. These fibroblasts secrete collagen and ground substance components. The second phase is called "fibroplasia" and is characterized by a rapid production of granulation tissue. The granulation tissue consists of endothelial cells, fibroblasts and macrophages. During the fifth to seventh day of healing, collagen synthesis peaks. This increased rate of collagen turnover is related to defect bridging and debris removal (1). The collagen fibrils are laid down in a random pattern and, in the beginning, possess little mechanical strength. Gradually, a more aligned pattern emerges and strength is gained. This third and final phase of healing is termed the "maturation phase". This phase is extended over a period of years. The turnover of collagen is still higher than normal and the mechanical strength of the tissue continues to increase. There is not an increase in the collagen content, however, there is a shift in the type of collagen which is synthesized, which may explain the increase in strength of the tissue. The remodeling process takes place

partly as a response to mechanical stress, and therefore the results of these three phases depend on whether or not the tissue is immobilized. These factors largely determine post-surgical protocols for rehabilitation. Immobilization causes reduced strength of the bone-ligament-bone complex largely due to resorption of bone at the ligament insertion sites (30,36) with increased joint stiffness (50) and decreased maximum failure loads (1,34). It has also been suggested that collagen is laid down in a disorganized manner in the absence of a stress field (49). For these reasons, remobilization of the limb following surgery is the current practice. However, strenuous, forced exercise has been shown to be detrimental to the mechanical properties of the healing tissue (5,26).

III. PART I - MEDIAL PROCEDURE

The objective of Part I of this project was to repeat the surgical procedure of Cabaud (10) (remove the medial one-third of the dog patellar tendon) using the same surgical, testing and data analysis procedures as Burks et al (4). In Cabaud's paper, his primary focus was on the graft itself and not on the host patellar tendon. As a result, the biomechanics performed on the host tendon were limited.

MATERIALS AND METHODS

Specimens

This study contained 17 adult mongrel dogs with an average weight of 27.7 ± 5.6 kg. The dogs were divided into three groups. The first group (time zero dogs) consisted of five dogs for immediate sacrifice and testing of the patellar tendon after removal of its medial one-third. The contralateral limb served as a control. Six dogs were originally planned for group one, but one specimen failed in the growth plate during mechanical tests and was removed from the study. The three month and six month groups consisted of seven dogs. One dog from each chronic group was used for

histological evaluation of the tendon, while the remaining six were used for mechanical tests.

Anesthesia was induced with intravenous Biotal (thiamylal sodium) at a dose of 17 milligrams per kilogram at the University of Utah Medical Center. The dogs were maintained on Halothane at 2% for the duration of the procedure. They were placed supine, the left or right hind limb selected arbitrarily for the surgical procedure, and a standard surgical preparation was performed. A longitudinal incision was made directly over the patellar tendon. The length and width of the patellar tendon was observed. The medial one-third of the patellar tendon was incised sharply and extended 1 cm into the patella and tibia. The tendon defect was left open. The overlying fascia was closed with an absorbable suture and the skin with 3-0 nylon. Post-operative analgesia consisted of acetaminophen, 10 mg per kilogram as needed. The dogs were allowed immediate use of the knee, and unrestricted activity in a run.

All dogs were sacrificed using a commercial euthanasia solution (T-61). After sacrifice, the animals had the patella, patellar tendon and tibia dissected as a composite from the operative and nonoperative sides. The specimens were wrapped in phosphate buffered saline soaked gauze and shipped overnight to Michigan State University in refrigerated packaging for mechanical tests. The tissues were not frozen because studies have shown that healing wounds contain more

fragile tissue components and their mechanical properties are more susceptible to the adverse effects of freezing (42,46,52). All experiments were performed within 30 hours of sacrifice. All soft tissue, except for the patellar tendon was removed from the specimens immediately prior to mechanical tests.

Mechanical Test Preparation

The patellar tendon measurements were made by a single examiner using vernier calipers. Width and thickness of the tendon were measured at three points along its length and averaged (Figure 9). The initial length was measured from the inferior pole of the patella to the superior-posterior attachment of the tendon on the tibia. The tendon was wrapped in physiological saline (0.9% NaCl) soaked gauze during preparations for mechanical tests.

Figure 9. Tendon dimension measurements.

The tibia was cut at the distal end approximately six inches from the knee. A 1.5 inch diameter, 6 inch long polyvinyl chloride (PVC) tube was placed vertically on a paper mat under a ventilation hood. A room curing fiberglass resin (NAPA brand Fiberglass Reinforced Plastic Filler 6371) was mixed with compatible hardener and poured into the PVC tube. Alcohol was used to remove all fatty residue from the tibia to

ensure bonding with the epoxy. The tibia was then pushed into the resin such that the tibial insertion of the patellar tendon was just above the epoxy (Figure 10). Care was taken to ensure that the tendon did not come in contact with the resin. The potted configuration was then allowed to harden at room temperature. The tendon remained wrapped in moistened gauze throughout this procedure (Figure 10). The time between potting and mechanical tests varied from 1 to 4 hours. The tendons which had a long interim time (> 2 hrs.) between potting and testing were put on ice to minimize tissue degradation. Just prior to mechanical tests the patella was potted. A special stainless steel box-like grip (1.5 x 1.5 x 1.25 inches) was used to hold the patella. A slot cut in the bottom of the box allowed the tendon to pass through without restricting its motion. The front lid of the box was removed to pot the patella. The inside was sprayed with a silicone based lubricant to allow easy removal of the potted patella after the tensile test. The same fiberglass resin/hardener mix, described previously, was used to secure the patella. The box was nearly filled with the fiberglass. The patella was pressed into the resin up to the anterior (tendon-covered) surface and covered by the front lid of the box. The apex (inferior pole) of the patella was allowed to rest against the bottom of the box. The tendon was passed through the slot described earlier, the front was secured, and the resin was allowed to harden for ten to fifteen minutes.

Figure 10. Potted tibia.

A standard servo-hydraulic tensile testing machine (Instron model 1331) was used to mechanically extend the preparation. A specially designed fixture was built to allow exact alignment of the tendon along the load axis (Figure 11). The fixture was composed of three horizontal 1/4 inch stainless steel plates and a vertically mounted rotating disc of the same material (29). The bottom plate was secured to the base of the Instron machine. Screw drives were mounted between each set of plates and permitted 2 dimensional motions of the fixture. This x-y table configuration enabled the operator to precisely align the tendon along the axis of the Instron actuator. The rotating disc, welded to the top plate, simultaneously served two important functions. Its primary function was to provide a secure mounting mechanism for the potted tibia. Its secondary function was to allow for angle changes between the tibia and the load axis. An inclusive angle of 110 degrees was used to simulate a physiological loading configuration, and orient the patellar tendon in tension without a tearing or cutting action at the tibial tuberosity (Figure 11).

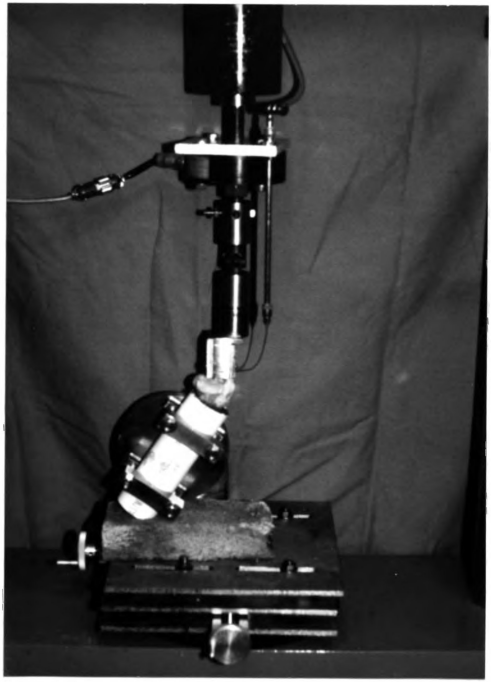


Figure 11. Mounted PPT complex.

The upper part of the loading mechanism consisted of a 2000 lb. load cell (Instron model 1010-AF) mounted in line with the actuator. Below the load cell a universal joint was used to correct minor offsets in the alignment of the tendon. The box containing the potted patella was connected to the lowest portion of the universal joint.

Physiological saline was heated to 37° C by a heat exchanger within a tank placed above the crosshead of the Instron. Gravity fed saline was passed through a tube to a drip mechanism which allowed the operator to control the flow of saline over the tendon. The drip tube was positioned at the top of the tendon, where it first exited the box grip, which permitted continuous moistening of the entire length of tendon during the mechanical tests.

Pre Conditioning

Each PPT complex was cyclically loaded from 90 N to 180 N at one Hertz for 20 cycles prior to the failure experiments. A standard linearly variable displacement transducer (accurate to ± .016 mm) within the Instron actuator measured the grip-to-grip deformations. A Nicolet oscilloscope and compatible disk drive continuously stored the load and deformation data at a rate of 100 samples per second.

Tensile Tests

Immediately after pre-conditioning, the complex was loaded to failure at a nominal rate of 100% strain per second. The actual rate of deformation varied for each test depending on the initial length of the tendon. Continuous load and deformation data were collected and stored in the same manner as the cyclic tests at a rate of 1000 samples per second. The mechanism of failure in the PPT complex was visually determined and documented.

Force versus deformation plots were generated. Figure 12 illustrates a typical force-deformation curve. The structural parameters documented in each experiment were the stiffness in the linear range of loading just prior to failure (tangent stiffness), maximum load and energy to maximum load. The tensile modulus was calculated as the product of stiffness times the ratio of initial length to cross-sectional area.

The load-deformation data were loaded into a computer program which calculated the area under the curve using the trapezoidal technique. The failure stain energy was defined as the area under the load-deformation curve.

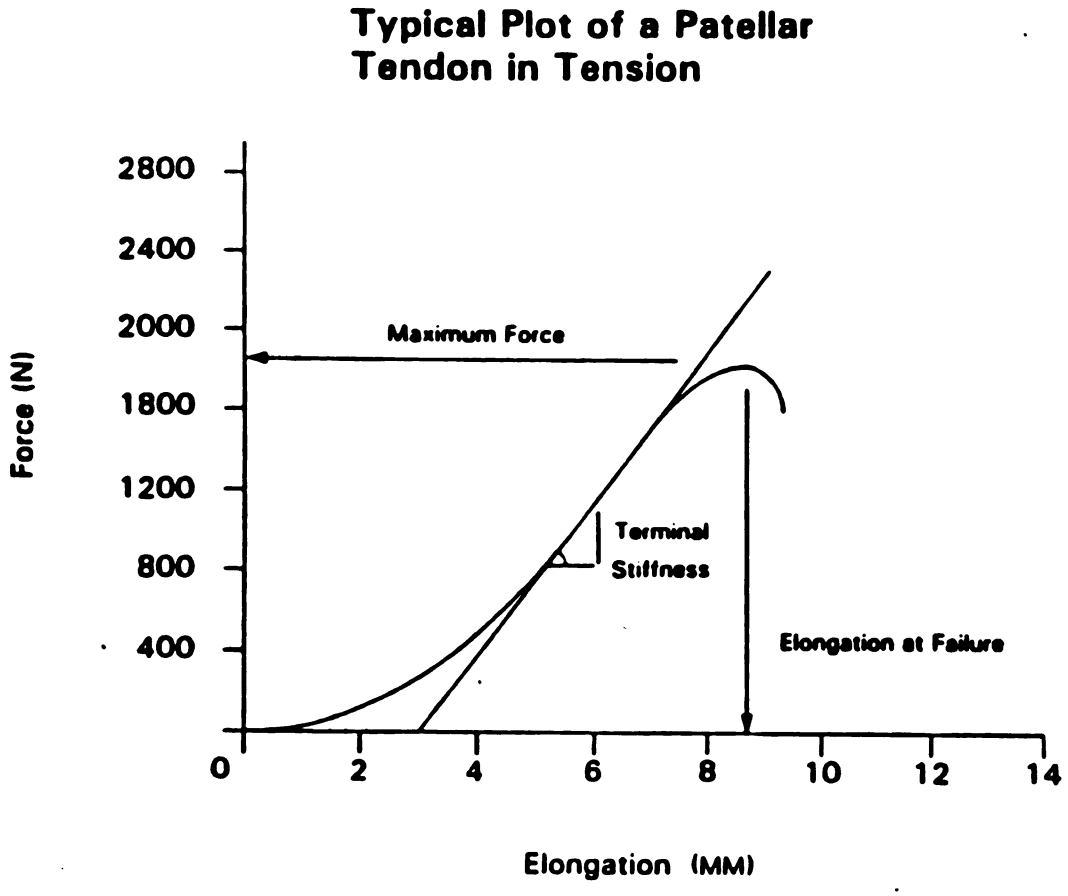


Figure 12. Typical force-deformation curve (taken from Burks et al, 4).

Histology

Two dogs were used for histology. Each had the medial one-third of the patellar tendon removed with the contralateral side serving as control. One of the animals was sacrificed three months post-operatively and the second was sacrificed six months post-operatively. Once harvested, the tendons were fixed in 10% neutral buffered formalin. After fixation the tendons were processed for routine histology, embedded in paraffin, sectioned at 6 um and stained with hematoxylin and eosin.

Statistics

All statistical calculations used for this study were performed using Systat, (Systat, Inc.). All results are presented as the mean ± standard deviation. To analyze the medial procedure, one way ANOVAS (analysis of variance) were done for each parameter (length, stiffness etc.). When a significant difference was detected ($p<.05$) a contrast post-hoc test was used to separate time effects. The control and test data were examined for statistical differences ($P<.05$) using the paired t-test.

To analyze the medial data versus the original Burks study (4), a two way ANOVA was used to detect significant differences for the interaction term and the main effects. The main effects for this study were time (0,3 and 6 months) and procedure (central versus medial). The interaction term determines if the two procedures vary the same with time. If the interaction term for a parameter was significant ($P<.05$) then a one way ANOVA and a contrast post-hoc test was performed for each procedure separately. This was done to determine which times varied for the procedure. If there was not a significant interaction term, then both procedures varied with time in the same manner. It there was not a significant interaction term, but there was a significant main effects term then a contrast post-hoc test was only necessary if the significant main effect was time. This was true because there were only two procedures, therefore a

significant main effect would automatically indicate that the central procedure was significantly different than the medial procedure. If this was the case, an independent t-test was used at each time to locate significant differences.

RESULTS AND DISCUSSION

The dogs usually favored the operative limb for approximately 2 to 3 days after surgery. In the time zero dogs, the patellar tendon was easily defined with exact borders. In the three and six months groups, there was a visible difference in the amount of scar tissue in a number of specimens. Three months after surgery two of the animals had extensive scar tissue. The ratio of operated to control cross-sectional areas in these two dogs was statistically different than the remaining four. At six months of age, one dog exhibited extensive scar tissue. There was no documented difference in the behavior pattern, surgical methods, etc. for the dogs that exhibited extensive scar tissue. Although the operated patellar tendons from other dogs at three and six months did not appear normal, the degree of scarring was fairly limited, as reflected in the tissue dimensions.

Tissue Dimensions

Patellar tendons from the control limb of all groups averaged 32.5 ± 6.5 mm (n = 17) in length (Table 1). At time zero, no difference in initial length of the patellar tendon

was noted. The operated tendon, on the average, was 11% shorter than contralateral controls three months after surgery. While, on the average, operated tendons tended to be 6% shorter six months after surgery, these differences were not statistically significant.

The average cross-sectional area of the patellar tendon on the control side was 33.6 ± 8.7 mm^2. The average thickness was 2.7 ± 0.5, mm and the average width was 12.5 ± 1.4 mm. The cross-sectional area, thickness and width of the control side tendons did not change following surgery. The cross-sectional area (CSA) of the patellar tendon was decreased 73%, reflecting a decreased width after removal of the medial third. Three months after surgery the cross-sectional area averaged 275% of controls. In the two animals with extensive scarring the CSA averaged 424 ± 20% of controls, while in the remaining four the CSA was 227 ± 100% of controls. The large increase in CSA for these two particular animals was not specifically due to any increase in the thickness or width parameter. Six months after surgery the CSA of the patellar tendon was, on average, 288% of controls. In the one exhibiting extensive scarring, the CSA was 487% of the contralateral control, compared to 249 ± 73% of controls for the remaining five in this group.

TABLE 1.
Dimensions of the Patellar Tendon
in Control and Operated Preparations

Time Post-op.	Length (mm)	Width (mm)	Area (mm^2)
0 Months			
Control Limb			
90-181	33.0	13.8	38.3
90-184	36.2	13.8	36.1
90-192	34.7	11.0	26.3
90-193	28.4	13.0	32.3
90-299	34.7	9.6	24.7
Mean ± SD	33.4 ± 3.0	12.2 ± 1.9	31.7 ± 5.9
Test Limb			
90-181	33.1	8.6	24.2
90-184	35.1	8.3	21.6
90-192	34.7	10.3	27.0
90-193	28.2	7.2	18.6
90-299	33.8	8.7	22.1
Mean ± SD	33.0 ± 2.8	8.6 ± 1.1	22.6 ± 2.9
3 Months			
Control Limb			
90-196	31.6	14.4	48.0
90-200	35.6	14.5	54.6
90-218	36.9	12.5	34.9
90-207	32.3	11.5	30.6
90-223	34.9	12.6	35.5
90-228	36.5	13.0	40.0
Mean ± SD	34.6 ± 13.1	13.1 ± 1.2	40.6 ± 9.1
Test Limb			
90-196	31.3	14.2	64.7
90-200	33.4	14.2	80.6
90-218	31.0	16.9	153.5
90-207	28.5	15.5	96.5
90-223	27.9	13.6	110.4
90-228	32.5	19.1	163.3
Mean ± SD	30.8 ± 2.2	15.6 ± 2.1	111.5 ± 39.5
6 Months			
Control Limb			
90-154	35.0	14.1	39.9
90-165	31.6	12.5	26.0
90-157	34.4	12.7	26.0
90-158	31.4	12.2	29.2
90-168	31.8	10.5	33.9
90-209	33.3	11.3	24.6
Mean ± SD	32.9 ± 1.5	12.2 ± 1.2	28.3 ± 6.0
Test Limb			
90-154	29.6	15.4	105.9
90-165	30.8	17.4	126.7
90-157	32.7	12.8	45.0
90-158	28.9	16.3	99.6
90-168	30.3	13.8	69.0
90-209	33.0	12.2	43.6
Mean ± SD	30.9+/-1.7	14.6 ± 2.1	81.6 ± 34.3

Histologic Observations

At the time of harvest, operated tendons used for histology appeared to have a nearly normal thickness and little scar. The scar tissue completely filled the defect and had engulfed some of the original tendon (Figure 13). Histologically at three months post-op, the medial portion of tendon was hypercellular and collagen fibers were disorganized. The cell nuclei were somewhat rounded. The area of demarcation between normal and disrupted tendon was distinct. At six months post-op, the collagen had begun to organize into defined bundles. Hypercellularity was still evident. The cell nuclei were longer and less rounded in shape than at three months post-op. Control tendons at three and six months were normal in appearance.

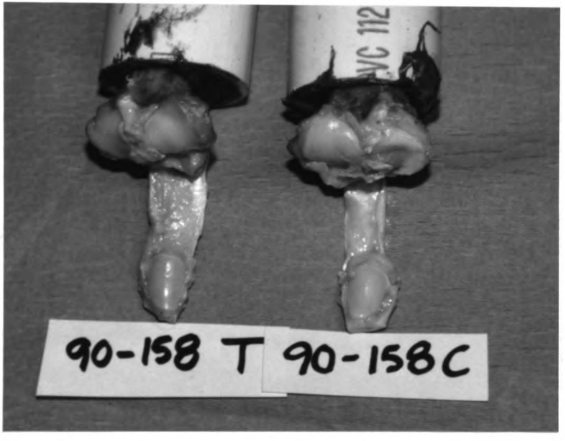


Figure 13. Control versus operated tendon at six months.

Tensile Failure Mechanisms

All specimens were stretched sufficiently to cause a readily observable failure of the patella-patellar tendon-tibia preparation. In the time zero specimens all the control preparations failed by avulsion of the patellar tendon at the patella (Figure 14). After removal of the medial third,

avulsion fractures were evident in three of five cases. In the remaining two specimens from this group, failure was in the substance (Figure 15), and it appeared to originate from the insertion points without bone avulsion.

In six out of the six cases at three months and five out of six cases at six months, control preparations failed by avulsion of bone from the distal patella. On the operated sides, avulsion fractures occurred in five out of six cases, for three and six months after surgery (Table 2).

Figure 14. Failure by avulsion.

Figure 15. Failure in the midsubstance.

Structural Failure Properties

Since the preparation did not consistently fail in the substance of the patellar tendon, it is more appropriate to discuss structural versus material properties (Table 2). The load required to fail control preparations was not significantly altered following surgery in any group. After removal of the medial third of the tendon, the load required to fail the preparation decreased 29%, on the average. At three months post-surgery the operated tendons failed at a load 83% of controls, and at six months the failure load was 79% of contralateral controls. The failure loads were statistically lower than controls on the operated preparation at three and six months. The energy required to fail control preparations was 13.7 ± 4.5 Joules (n=17). This parameter did not change on the control side after surgery on the contralateral limb. After removal of the medial third the energy required to fail the tendon was decreased by 26%, but this was not statistically significant. The energy to cause failure was decreased 15% and 17% at three and six months post-op, but at neither time was the change statistically significant.

TABLE 2.
Tensile Failure Properties
of Control and Operated Preparations

Time Post-op.	Failure Force (kN)	Failure Energy (J)	Mechanism of Failure
0 Months			
Control Limb			
90-181	2.77	18.19	Avulsion @ patella
90-184	1.80	6.93	Avulsion @ patella
90-192	2.57	11.69	Avulsion @ patella
90-193	1.80	7.48	Avulsion @ patella
90-299	2.85	15.36	Avulsion @ patella
Mean ± SD	2.4 ± 0.5	11.9 ± 4.9	
Test Limb			
90-181	1.46	8.51	Midsubstance
90-184	1.97	11.43	Avulsion @ patella
90-192	1.81	10.15	Midsubstance
90-193	0.86	2.78	Avulsion @ patella
90-299	2.38	11.29	Avulsion @ patella
Mean ± SD	1.7 ± 0.6	8.8 ± 3.6	
3 Months			
Control Limb			
90-196	2.25	9.83	Avulsion @ patella
90-200	3.24	22.19	Avulsion @ patella
90-218	3.62	20.59	Avulsion @ patella
90-207	2.52	16.87	Avulsion @ patella
90-223	2.59	11.47	Avulsion @ patella
90-228	3.19	16.71	Avulsion @ patella
Mean ± SD	2.9 ± 0.5	16.3 ± 4.9	
Test Limb			
90-196	2.96	16.93	Avulsion @ patella
90-200	2.48	21.02	Midsubstance
90-218	2.45	11.73	Avulsion @ tibia
90-207	2.26	11.89	Avulsion @ patella
90-223	2.14	8.85	Avulsion @ patella
90-228	2.25	12.40	Avulsion @ patella
Mean ± SD	2.4 ± 0.3	13.8 ± 4.4	
6 Months			
Control Limb			
90-154	3.35	10.6	Avulsion @ patella
90-165	2.30	10.52	Avulsion @ patella
90-157	3.63	27.25	Avulsion @ patella
90-158	2.96	15.01	Midsubstance
90-168	2.44	11.23	Avulsion @ patella
90-209	2.76	14.36	Avulsion @ patella
Mean ± SD	2.9 ± 0.5	14.3 ± 3.0	
Test Limb			
90-154	2.59	14.78	Avulsion @ patella
90-165	1.37	4.65	Avulsion @ patella
90-157	2.60	12.58	Avulsion @ patella
90-158	2.71	11.68	Avulsion @ patella
90-168	2.42	9.46	Avulsion @ patella
90-209	1.87	7.83	Avulsion @ patella
Mean ± SD	2.3 ± 0.5	11.8 ± 2.8	

Tensile Response Data

The tensile response curves for all preparations qualitatively appeared similar, exhibiting an initial nonlinear region, a range of linear response, and a short range of nonlinearity immediately prior to an abrupt unloading and gross structural failure of the preparation. This is a typical collagenous tissue response, as discussed in the survey of literature. The tensile response was significantly different from controls only at time zero for deformations above approximately 1.0 mm. This was reflected in the values of tangent stiffness, which were decreased 29% at time zero from contralateral controls (Table 3). At three and six months post-op the tangent stiffness of the operated patellar tendon preparations was on the average 85% and 91%, respectively, of controls. These parameters were not statistically less than contralateral control preparations. When these data were normalized for initial length and cross-sectional area to produce stress-strain data, the tensile responses of operated tendons were shown to be significantly lower than control and time zero responses (Table 3). The tensile modulus of control specimens was 340 ± 100 MPa (n=17). The tangent modulus computed for time zero, operated specimens was not different than controls. At three and six months post-op, the modulus of operated tendons was statistically less, being 31% and 28% of controls, respectively.

TABLE 3.
Tensile Response Parameters
for Control and Operated Preparations

Time Post-op.	Tensile Stiffness (kN/m)	Tensile Modulus (MPa)
0 Months		
Control Limb		
90-181	260	224
90-184	260	260
90-192	350	450
90-193	250	220
90-299	300	485
Mean ± SD	280 ± 40	330 ± 129
Test Limb		
90-181	170	233
90-184	200	325
90-192	230	304
90-193	140	212
90-299	280	428
Mean ± SD	200 ± 50	300 ± 86
3 Months		
Control Limb		
90-196	318	210
90-200	313	204
90-218	375	395
90-207	377	399
90-223	320	315
90-228	340	219
Mean ± SD	340 ± 30	290 ± 92
Test Limb		
90-196	345	167
90-200	280	116
90-218	266	54
90-207	250	74
90-223	298	75
90-228	303	60
Mean ± SD	290 ± 30	91 ± 43
6 Months		
Control Limb		
90-154	400	350
90-165	305	370
90-157	320	422
90-158	320	344
90-168	345	460
90-209	345	470
Mean ± SD	340± 30	403 ± 56
Test Limb		
90-154	282	79
90-165	260	63
90-157	304	220
90-158	330	96
90-168	365	176
90-209	310	230
Mean ± SD	310 ± 40	111 ± 77

ANALYSIS OF CENTRAL VERSUS MEDIAL PROCEDURES

A primary motivation for this study was to examine differences between removal of a medial third versus removal of a central third of the canine patellar tendon. A comparative study is conducted here using earlier experimental data from Burks (4). One and two way ANOVAS with corresponding post-hoc contrasts tests were used throughout the following analysis.

Animals

An ANOVA was performed on body weight from the medial and central studies. A significant difference showed up between the studies. Post-hoc test revealed that this effect was due to small animal body weights, on the average, in the time zero group of the medial study. No differences existed, however, between the two studies at three and six months post-op.

Tissue Dimensions

Analysis of control side tendons indicated that no significant differences in length existed between the two studies. In both studies, however, the difference between operated and control lengths of the patellar tendon was significantly longer at time zero than either three or six months post-op. These surgical procedures caused contracture of the host patellar tendon.

The mean cross-sectional area of control tendons in the medial study was 32% higher than for the central study. Post-hoc tests revealed that a significantly larger CSA was recorded for control side tendons in the three month time point in the medial study. There was a significant interaction term for CSA between the two studies. Therefore, separate one way ANOVAS were performed for each procedure. An examination of differences between control and operated tendons for each animal in the two studies indicated that while the main effect was the same across the two procedures, the time sequence varied between the studies. With the medial procedure the difference in CSA between control and test tendons was statistically less at time zero than at three or six months post-op. No change was recorded between three and six months. In contrast, for the central procedure, there was no significant change between zero and three months, while the difference between the control and operated tendons was significantly increased between three and six months (Figure 16).

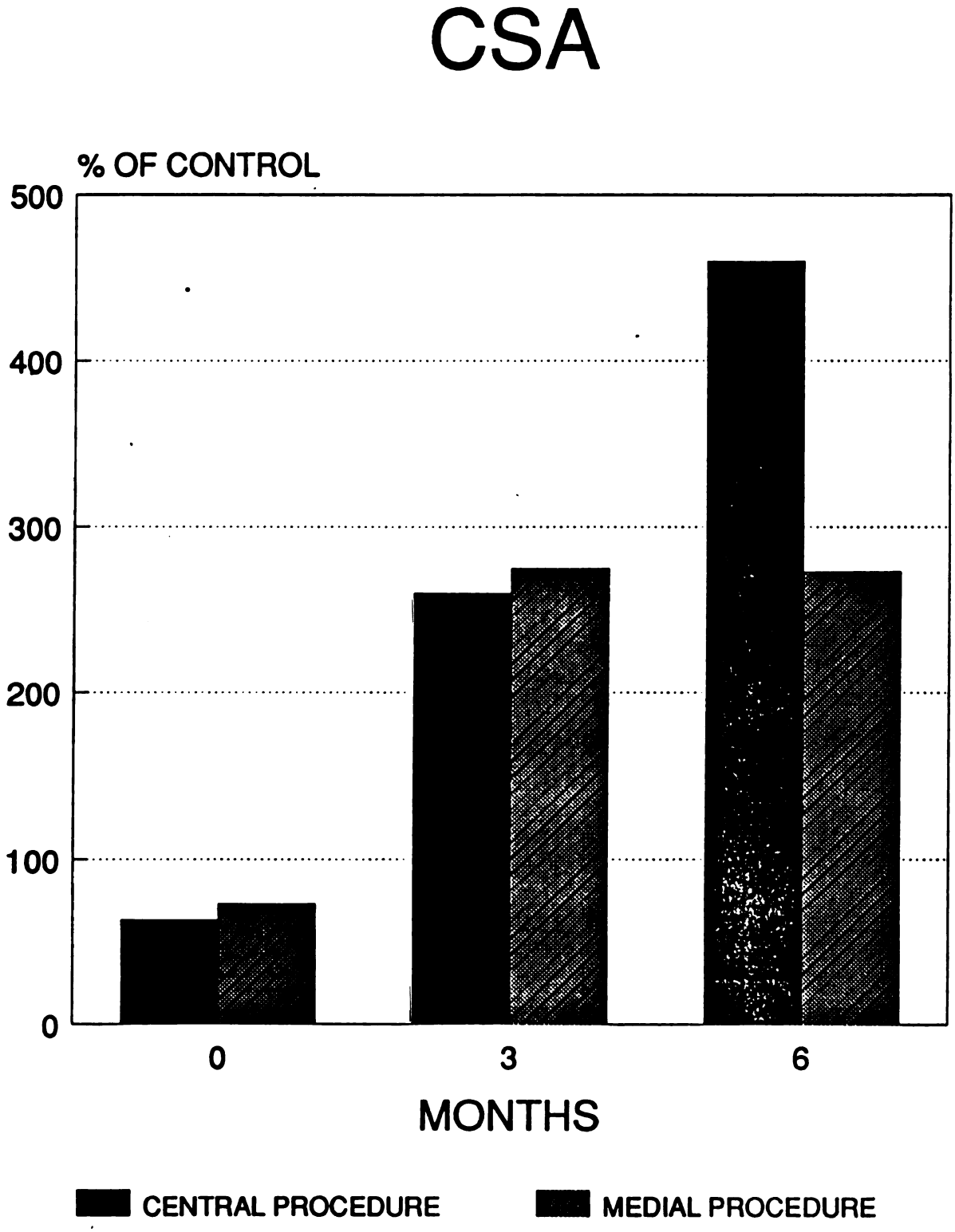


Figure 16. CSA: central versus medial.

Tensile Failure Mechanisms

The mechanism of failure for control preparations was by avulsion of the bone at the patella in both studies. The exceptions were three substance ruptures in the central study at three months post-op and one substance rupture at six months in the medial study. For the operated side the mechanism of failure, after removal of the central one-third of the PT, was substance rupture at time zero, while three out of five cases failed by avulsion at the patella in the medial study. This could be a result of more tissue being removed during the central procedure versus the medial procedure. The lesser amount of tissue remaining could cause the remaining tissue to be weaker than the insertion which would result in substance type failures. At three and six months post-op in the central study, the mechanism of failure was avulsion of bone, while in the medial study one out of six cases failed in the substance at three and six months post-op (Figure 17).

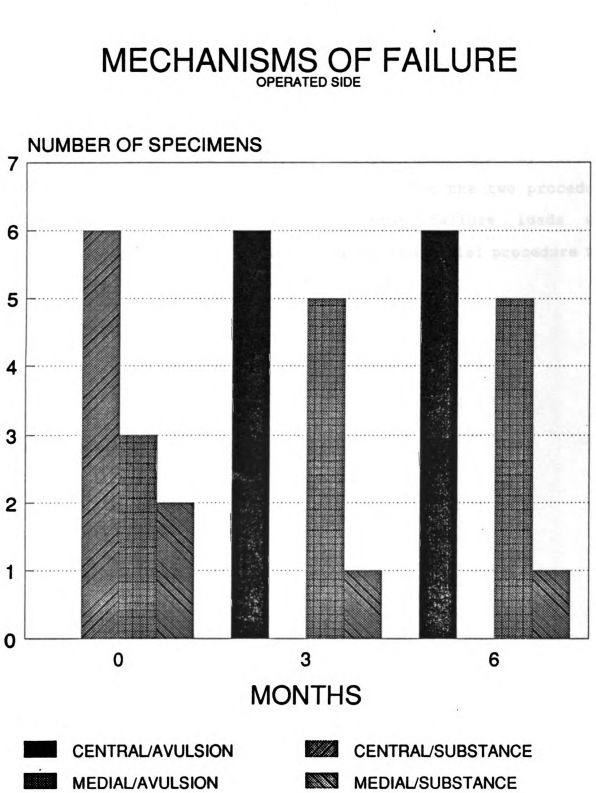


Figure 17. Mechanisms of failure: central versus medial.

Structural Failure Properties

No significant differences were measured in the loads required to cause failure of the patellar tendon preparation in controls between the two procedures. No changes were evident with time in either study. When the differences between control and operated tendons for the two procedures were examined, it was found that failure loads were statistically closer to controls for the medial procedure than for the central procedure (Figure 18).

FAILURE LOADS

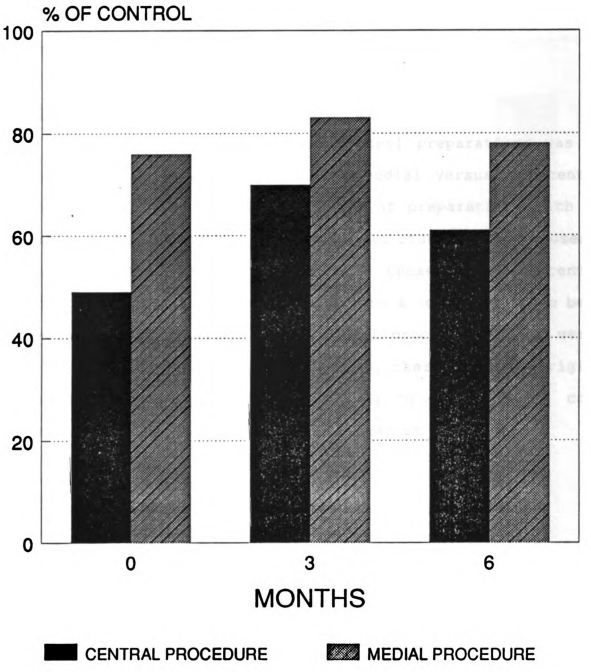


Figure 18. Failure loads: central versus medial.

The energy to fail control patellar tendon preparations was not different between studies and did not vary with time post-op. Similar results were obtained when the differences between control and operated preparations for the two studies were examined.

Tensile Response Data

The tangent stiffness of control preparations was 12% higher, on the average, for the medial versus the central procedure. The tangent stiffness of preparations with the medial procedure were, on the average, statistically closer to their contralateral controls than those with the central procedure (Figure 19). This could be a result of there being more aligned collagen in the medial procedure tendon versus the central procedure tendon. Also, there was more original tendon remaining after the medial procedure which could contribute to the increased stiffness of the tendon.

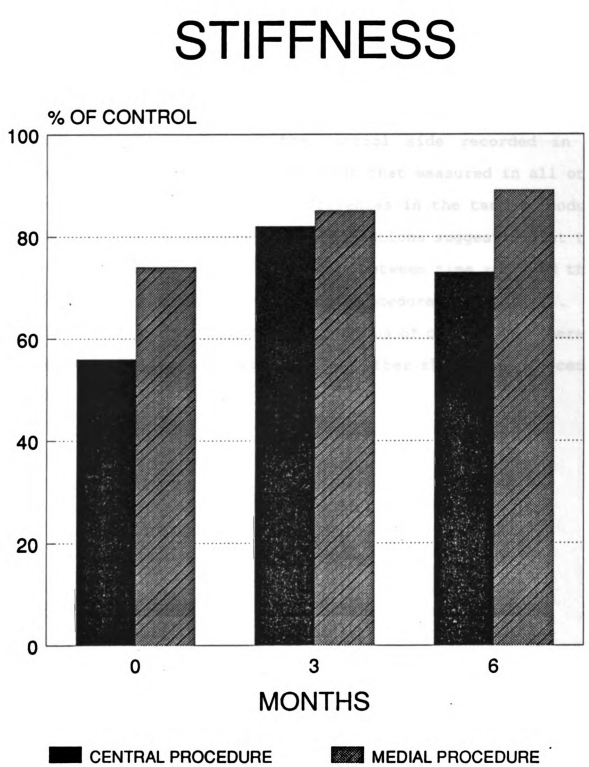


Figure 19. Stiffness: central versus medial.

The tangent modulus of control patellar tendons in the medial study was greater than that in the central study. Post-hoc tests revealed that this main effect was primarily due to a significant difference for the three month post-op animals. The tangent modulus of the control side recorded in the central study was much larger than that measured in all other controls. An analysis of differences in the tangent modulus between control and operated preparations suggested that this parameter decreased significantly between time zero and three and six months post-op with both procedures (Figure 20). The difference between the tangent modulus of control and operated tendons was statistically smaller after the medial procedure than after the central procedure.

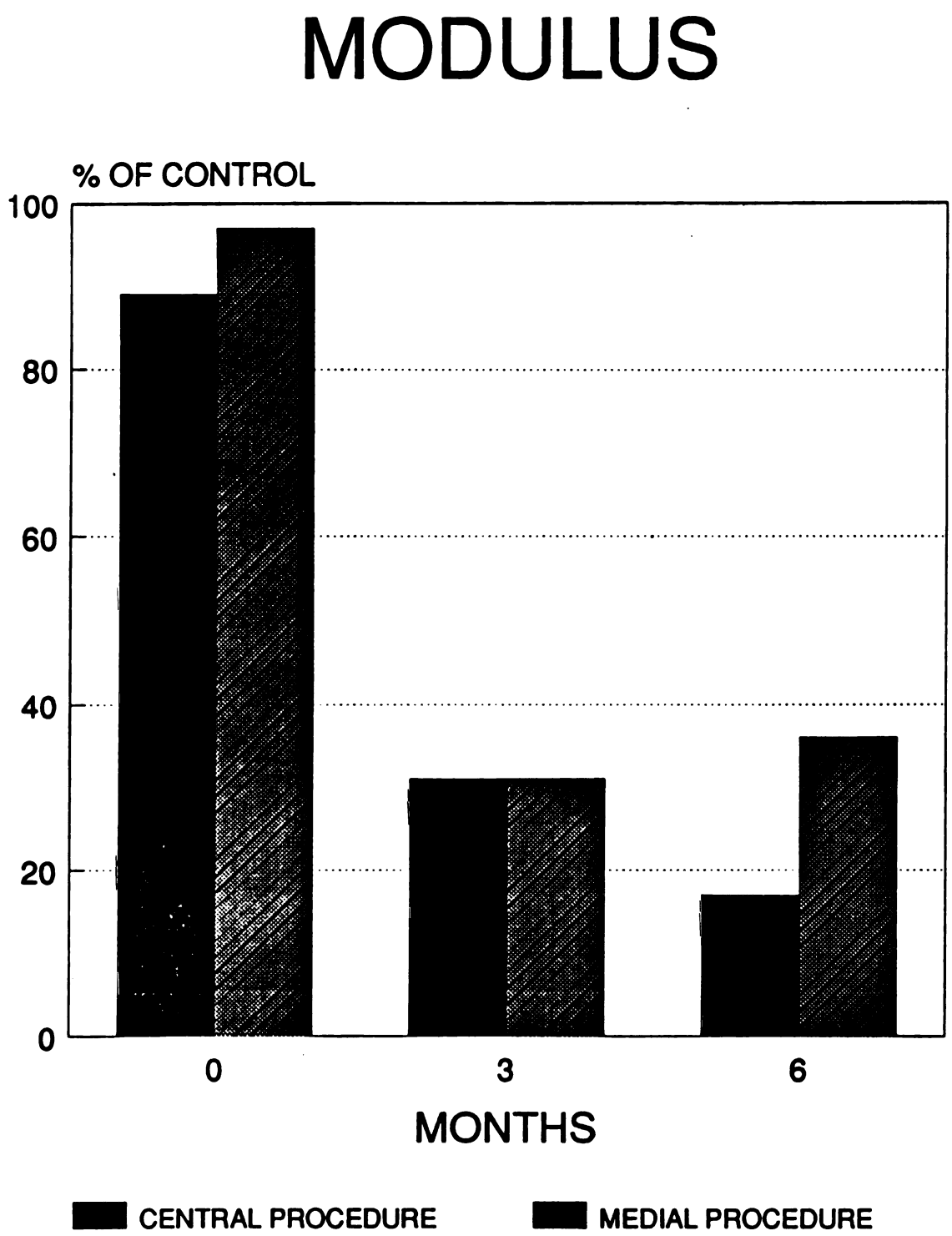


Figure 20. Modulus: central versus medial.

Histological Analysis

A comparative analysis was conducted on one control and operated tendon from the medial and central study at three and six months post-op. The histological analysis of tissues in the central study (4) was described in the survey of literature.

The patellar tendon from the two experimental studies differed markedly on gross examination at the time of harvest. The tendons from the central group were generally thicker and exhibited more scar tissue than the medial study group. While the relative degree of hypercellularity between groups was similar, the collagen fibers from the medial group were less disorganized and by six months had begun to form distinct bundles. While healing was incomplete at six months post-op, indications were that removal of the medial third is less disruptive overall to the patellar tendon than removal of a central third.

IV. PART II - SPATIAL VARIATIONS

The purpose of Part II of this project was to determine if there are any differences in the structural, material or biochemical properties between the lateral, central and medial thirds of the dog patellar tendon. The results from Part I indicate that there are statistical differences in the maximum load to failure, stiffness and modulus between the central and medial procedures. From a mechanical point of view, a variation in the properties across the width of the tendon could be the cause for this difference. For instance, if the central third was the stiffest and strongest third, then removal of the central portion would cause a greater amount of damage to the remaining tendon than if a weaker and more compliant portion were removed. Spatial variations in the human patellar tendon (9,38) have been shown to exist in previous studies, as discussed earlier in the survey of literature.

MATERIALS AND METHODS

Specimens

For this portion of the thesis, all tissues were obtained from the Michigan State University Veterinary Clinical Center. Dogs of both sexes were used from varied breeds and weights. Their average weight was 27.7 ± 4.19 kgs and ranged from 20.4 to 31.3 kgs. Eight dogs were used. The patellar tendon from each hind limb was removed along with the patella and approximately six inches of the tibia. All animals were used in a previous study, and had been caged for three months with daily exercise. All animals were disease free, in good health and with no history of knee surgery or previous injury.

Mechanical Test Preparation

After the specimens were harvested, the tissues were frozen at 0° Centigrade between 4 days and 5 weeks prior to testing. On the evening before each test, a specimen was removed from the freezer for thawing at room temperature. The following morning, the specimen was cleaned of all soft tissue except for the patellar tendon, as described earlier for the medial procedure. The patella was cut into thirds using a bone saw. In this process, even pressure was applied onto the patella while it was held in vise grips for support (Figure 21). Two cuts were made through the patella to divide it into thirds. The cuts with the bone saw were made through

approximately 80% of the thickness of the patella. The remainder of the cut was made with a knife after the patella was removed from the vise grips.

Figure 21. Cutting the patella.

Once the patella was split, the tendon was cut into thirds. The tendon was cut using a scalpel blade under a stereo microscope with a magnification of 10x (Figure 22). Care was taken to cut between the collagen fibers to avoid excessive damage. A single person prepared all the specimens for mechanical tests. The preparations were potted in the same manner as described previously for the medial procedure.

Figure 22. Dividing the tendon into thirds.

Mechanical Tests

The tests were run as described earlier for the medial procedure; with two exceptions. First, a saline bath was used in place of the saline drip (Figure 23). A study done by Haut (20) showed that there were significant differences for the tangent moduli, failure stresses and failure strains between tendons tested in drip and bath environments. The bath is believed to be more representative of physiological conditions. The saline was heated in a separate glass container to 37° C and pumped into a plexiglass box after the specimen was mounted for testing. When the mechanical tests were completed, the saline was drained back into the glass container to be reheated for the next test.

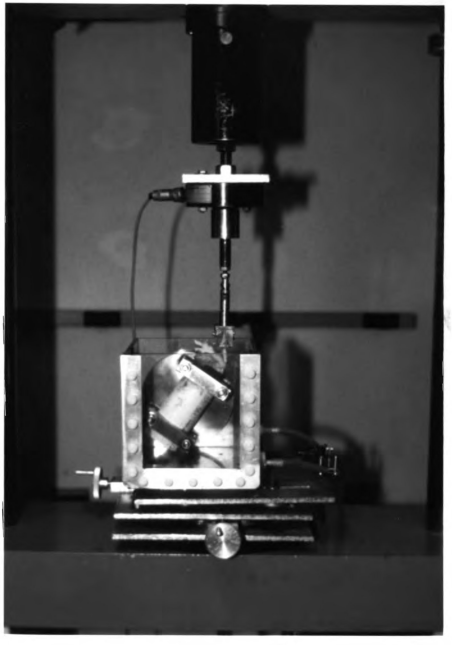


Figure 23. Testing fixture with saline bath.

The second exception was that preconditioning of the specimens consisted of a 3% strain versus 90-180 Newtons in the medial procedure. 2-3% strain is widely accepted as the amount of strain normally undergone by tendons under physiological conditions (19,27).

Microstructural Model

In an attempt to document spatial variations across the various thirds of the tendon, several approaches were taken. One approach was to use a mathematical model of its collagenous microstructure (2). A major advantage here might be that via model parameters, the tensile responses in the toe region and the early linear range can be compared. These regions of response are likely those which encompass the physiological responses of the patellar tendon. The model assumes a naturally occurring waviness (crimp) of collagen fibers in the stress-free tendon (Figure 24). As the tendon is deformed, the crimping gradually disappears, as fibers become straight. Once straightened, the fibers are able to resist deformation and generate load. It was assumed that the fibers were straightened gradually with elongation of the tendon (Gaussian distribution). Using the Marquardt method, the model was fit to stress-strain curves in order to determine model parameters μ and σ, where μ is the stretch in the tendon at which half of the fibers are straightened and σ is a dispersion of crimp angles (standard deviation).

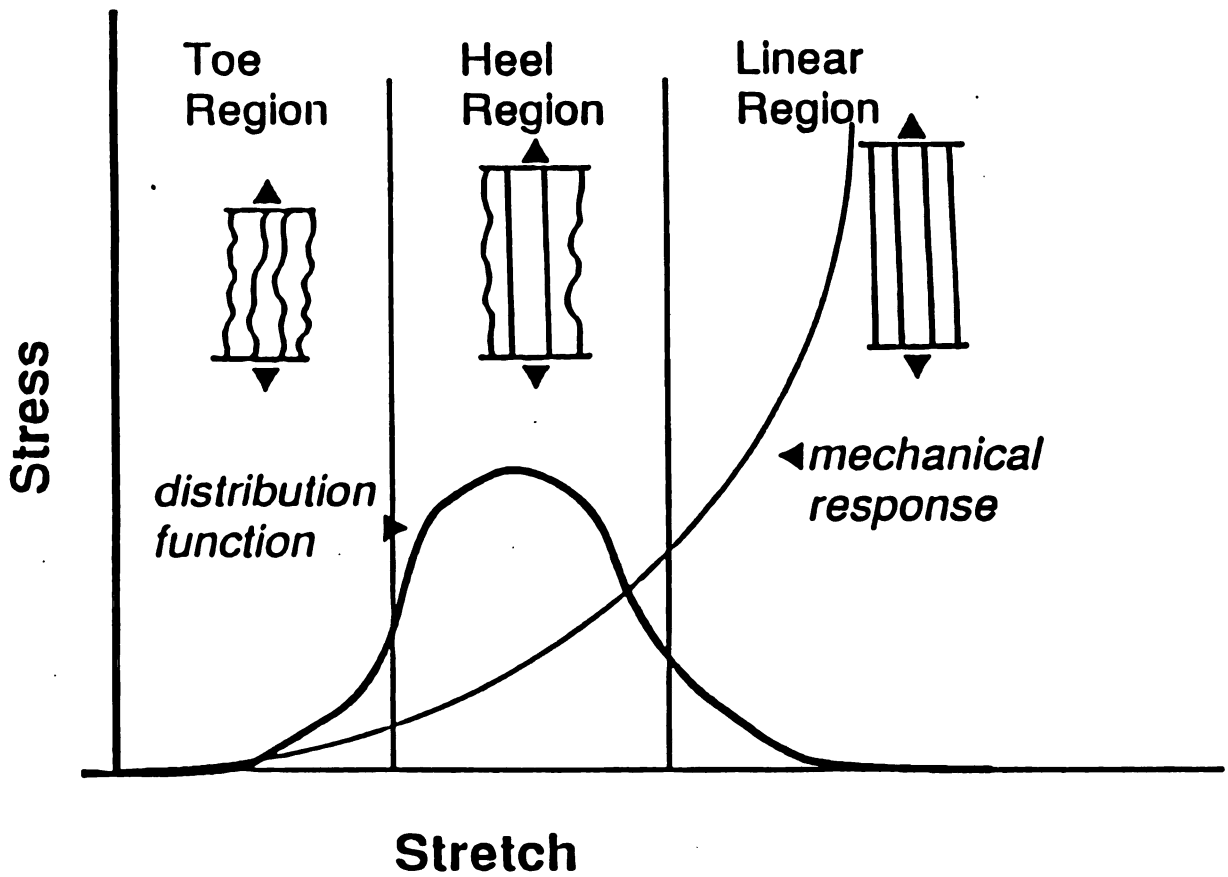


Figure 24. Tendon crimp distribution.

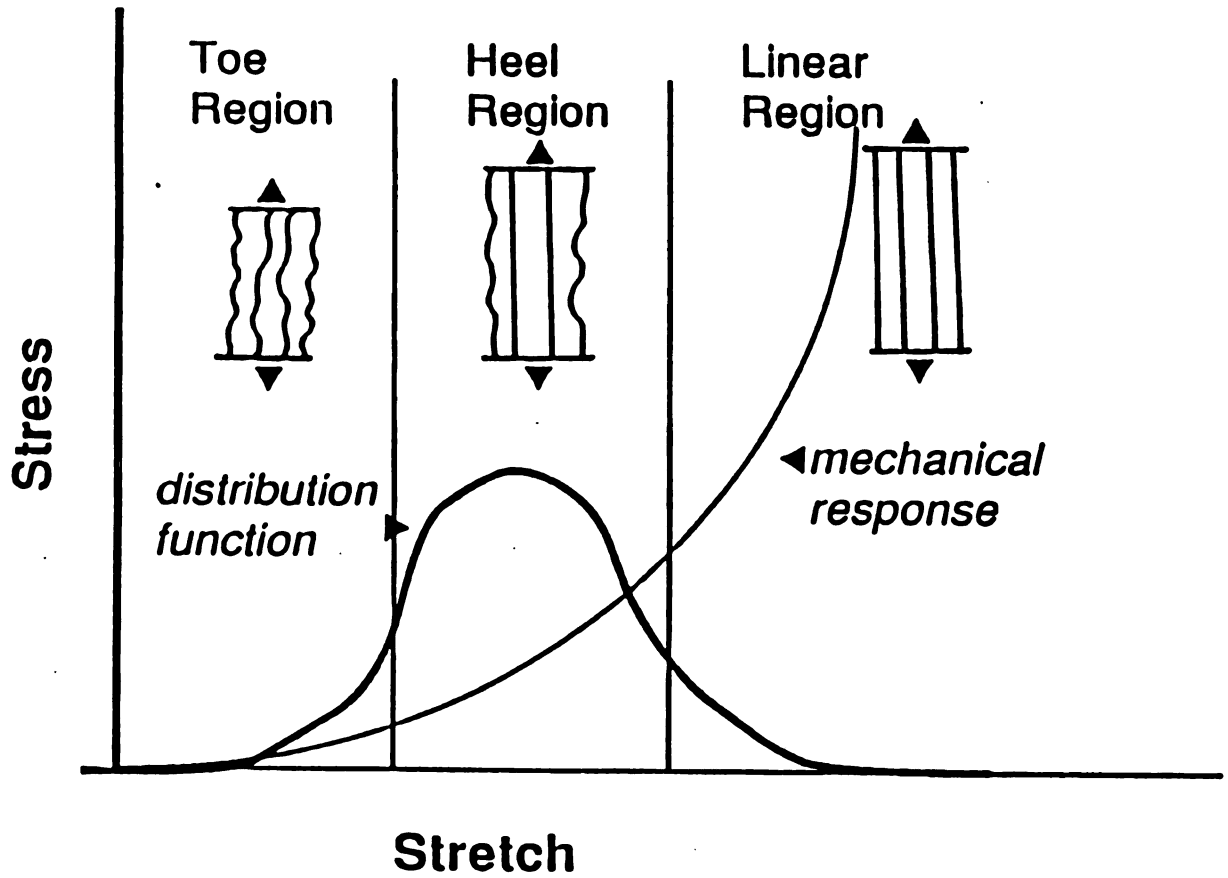


Figure 24. Tendon crimp distribution.

Biochemistry

Another approach to examine spatial variations in the tendon was to analyze the collagen content of each third. Three samples of equal length were taken from each third of the tendon following the mechanical tests. The samples were weighed and frozen. At a later date, the samples were removed from the freezer, and the total collagen content was determined in each portion.

For each assay approximately 15-20 mg. of tendon was lyophilized overnight, weighed, and placed into small test tubes. The lipids were removed by agitating the samples overnight in chloroform-methanol (2:1 V:V) at 4° C. The following day the samples were centrifuged at 2000 rpm for 1.5 hours. The supernatant was removed and discarded. The above procedure was repeated two additional times for a total of 72 hours of extraction. After removal of the supernatant on the third day, the sample was dried with nitrogen gas. The samples were then hydrolyzed and neutralized by the procedures to follow.

Hydrolysis

2 ml of 6 M HCl was added to the test tube to dissolve the precipitate. The sample was incubated for approximately 18 hours at 110° C in a sealed test tube.

Neutralization

The following day, the sample was neutralized by the following procedure: 2-3 drops of methyl-red indicator was added to the hydrolyzed sample. 2400 ul of 2.5 M NaOH was then added and vortexed. Additional NaOH was added until a pH of 7.0 was reached. The samples were then brought to 10 ml with distilled water.

Collagen Assay

Content of collagen was determined, based on hydroxyproline, according to Stegemann (44). An assay for collagen was conducted on all samples. A set of ten standards (1-10 ug/ml) was prepared from a stock of 1.0 mg hydroxyproline/100 ml. Two water samples were used for zero standards. The assay was conducted using 1 ml aliquots of the sample. Two solutions were used in the assay preparation: 3 M $HClO_4$ was added to each test tube, vortexed, and left to settle for five minutes; 1 ml. of p-dab solution was added, vortexed and incubated at 65° C for 18 minutes. Once the test tubes had cooled to room temperature, the absorbances of the standards and samples were measured on a Perkin-Elmen Lambda 2 (UV/VIS) Spectrophotometer. The water samples were prepared with reagents as above and served as reference. The absorbances of the samples were compared with the standard curve for the calculation of hydroxyproline per dry weight of tissue.

The force-deformation curves were normalized by the amount of collagen per mm of original length of tendon. This was done in an attempt to determine the modulus of an individual collagen fiber in each third of the tendon. This was done under the assumption that all collagen fibers participate equally in the tensile load-bearing function and that a constant fraction of the weight per unit length is collagen (47). The amount of collagen per mm of tissue was also normalized by the cross-sectional area to determine the density of collagen for each third of the tendon.

Statistics

For Part II of this thesis, one way ANOVAS were used to detect significant differences between thirds of the tendon. If the ANOVA indicated a significant difference for a parameter, contrast post-hoc tests were conducted.

RESULTS

Tissue Dimensions

The tissue dimensions for each third of the tendons are shown in Table 4. There were no statistical differences between the thirds.

TABLE 4

Dimensions of the Patellar Tendon (mean ± SD)

Portion of Tendon	Length (mm)	Width (mm)	CSA (mm^2)
Lateral Third			
1 left	36.7	4.4	8.4
right	36.7	2.7	5.6
2 left	37.9	5.7	11.6
right	38.8	5.6	12.4
3 left	39.3	4.3	8.8
right	38.1	3.6	7.7
4 left	35.5	3.2	6.4
right	35.3	4.6	9.1
5 left	36.0	5.1	10.8
right	38.2	4.1	8.8
6 left	42.4	3.5	10.9
right	42.6	2.4	4.6
7 left	39.2	4.5	8.6
right	41.0	3.5	7.2
8 left	34.8	4.7	9.1
right	36.1	3.5	7.1
Mean ± SD	38.0 ± 2.4	4.1 ± 1.0	8.6 ± 2.2
Central Third			
1 left	34.0	5.1	9.9
right	35.4	3.1	6.4
2 left	37.7	4.2	8.5
right	37.1	3.9	8.7
3 left	39.8	4.6	9.4
right	38.6	4.5	9.6
4 left	32.4	4.8	9.6
right	34.2	4.6	9.3
5 left	36.1	6.3	13.2
right	37.3	4.3	9.4
6 left	43.0	5.8	12.5
right	43.6	3.5	7.5
7 left	39.7	4.8	9.2
right	40.0	5.1	10.6
8 left	35.6	4.2	8.0
right	36.0	3.5	6.9
Mean ± SD	37.6 ± 3.2	4.6 ± 0.8	9.5 ± 1.7
Medial Third			
1 left	36.0	3.7	7.0
right	35.8	5.3	10.9
2 left	37.0	2.5	5.1
right	39.3	4.4	9.8
3 left	40.0	3.0	6.0
right	40.9	4.1	8.7
4 left	31.2	4.5	9.0
right	36.0	4.0	7.9
5 left	37.6	1.8	3.7
right	38.5	4.4	9.6
6 left	46.0	3.6	7.7
right	45.2	7.0	15.1
7 left	41.5	3.0	5.8
right	40.2	5.1	10.5
8 left	37.7	3.2	6.0
right	36.1	4.5	9.1
Mean ± SD	38.8 ± 3.8	4.1 ± 1.1	8.5 ± 2.6

Physiological Properties

The energy absorbed by each third of the tendon was determined in the estimated physiological range of loading. The data are shown in Table 5. There was a significant difference between the energy absorbed for the lateral and central thirds ($p<.05$), but not between the central versus medial thirds or the lateral versus medial thirds.

TABLE 5.
Energy Absorbed in the Physiological Range (mean ± SD)

Portion of Tendon	Energy (Joules)
Lateral Third	
1 left	.78
right	1.88
2 left	.79
right	1.12
3 left	.86
right	---
4 left	.99
right	.98
5 left	.83
right	1.28
6 left	1.16
right	1.74
7 left	1.00
right	1.61
8 left	.96
right	---
Mean ± SD	1.14 ± .36
Central Third	
1 left	.62
right	.93
2 left	.79
right	.88
3 left	.62
right	.67
4 left	.70
right	.55
5 left	.80
right	1.11
6 left	1.03
right	1.33
7 left	.82
right	.77
8 left	1.38
right	---
Mean ± SD	.87 ± .25
Medial Third	
1 left	.90
right	.61
2 left	---
right	.63
3 left	.62
right	.67
4 left	.93
right	.70
5 left	---
right	.65
6 left	1.71
right	.77
7 left	1.79
right	.96
8 left	1.04
right	.74
Mean ± SD	.97 ± .39

Microstructural Model

The microstructural model fit the experimental data very well (Figure 25). The parameters describing crimp geometry (u,) are shown in Table 6. The mean straightened length (u) of the fibers and the dispersion ( ) of the straightened lengths about the mean were not significantly different for the tendon thirds. This indicates that there was not a difference in the crimping of the collagen between thirds, and that the heel region of their mechanical response was located at the same stretch ratio.

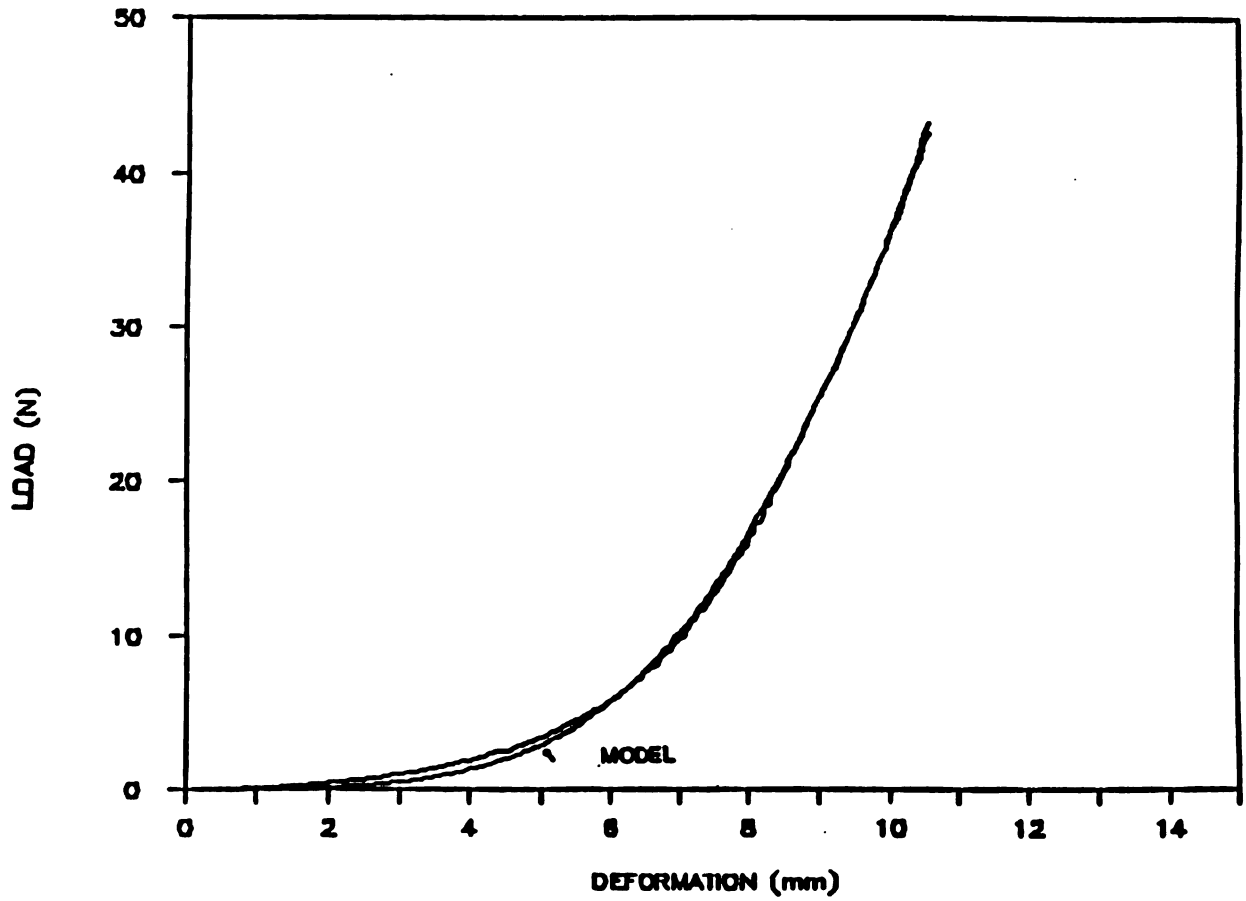


Figure 25. Experimental data and model fit.

TABLE 6.
Crimp Geometry (mean ± SD)

Portion of Tendon	μ*	σ*
	(* Dimensionless Stretch Ratios)	
Lateral Third		
1 left	1.010	.008
right	1.105	.008
2 left	1.020	.019
right	1.018	.026
3 left	1.008	.008
right	1.010	.009
4 left	1.011	.012
right	1.013	.012
5 left	1.041	.033
right	1.002	.033
6 left	1.030	.042
right	1.100	.050
7 left	1.037	.038
right	1.038	.050
8 left	1.020	.011
right	1.032	.020
Mean ± SD	1.025 ± .023	.024 ± .015
Central Third		
1 left	1.066	.005
right	1.029	.014
2 left	1.025	.017
right	1.012	.010
3 left	1.011	.012
right	1.020	.009
4 left	1.063	.021
right	1.013	.013
5 left	1.071	.050
right	1.020	.011
6 left	1.010	.025
right	1.020	.051
7 left	1.028	.021
right	1.013	.034
8 left	-----	----
right	1.013	.033
Mean ± SD	1.028 ± .021	.022 ± .014
Medial Third		
1 left	1.010	.009
right	1.013	.018
2 left	1.021	.011
right	1.007	.007
3 left	1.001	.024
right	1.010	.006
4 left	1.055	.044
right	1.014	.014
5 left	-----	----
right	1.015	.015
6 left	1.024	.014
right	1.048	.029
7 left	1.019	.015
right	1.036	.020
8 left	1.027	.040
right	1.009	.006
Mean ± SD	1.021 ± .015	.018 ± .012

Modes of Failure

The modes of failure for the different thirds varied significantly (Table 7). The lateral third failed 4 times in the midsubstance of the tendon, 8 times by avulsion of the patella and 4 times by the patellar insertion of the tendon. The central one-third failed in the midsubstance 4 times, while the remaining 12 failures were avulsions. The medial third differed from the central and lateral thirds in that it failed in the midsubstance of the tendon 15 of 16 times. The other failure was an avulsion of the patella.

Structural Failure Properties

The load to failure was determined from load deformation curves (Table 7). There was no statistical difference between the thirds.

TABLE 7.
Structural Failure Properties (mean ± SD)

Portion of Tendon	Max Force (kN)	Mechanism of Failure
Lateral Third		
1 left	7.62	Midsubstance
right	2.30	Midsubstance
2 left	8.29	Avulsion
right	4.00	Avulsion
3 left	6.50	Avulsion
right	1.49	Midsubstance
4 left	5.31	Patellar Insertion
right	3.53	Patellar Insertion
5 left	10.74	Midsubstance
right	5.17	Avulsion
6 left	8.68	Avulsion
right	4.08	Patellar Insertion
7 left	7.07	Patellar Insertion
right	4.00	Avulsion
8 left	12.66	Avulsion
right	5.60	Avulsion
Mean ± SD	6.1 ± 3.0	
Central Third		
1 left	12.87	Avulsion
right	4.30	Avulsion
2 left	5.54	Avulsion
right	4.65	Avulsion
3 left	7.52	Avulsion
right	8.71	Avulsion
4 left	9.13	Avulsion
right	7.24	Avulsion
5 left	9.76	Midsubstance
right	5.24	Avulsion
6 left	6.31	Midsubstance
right	4.98	Midsubstance
7 left	8.03	Avulsion
right	6.85	Avulsion
8 left	4.59	Midsubstance
right	----	Avulsion
Mean ± SD	7.0 ± 2.4	
Medial Third		
1 left	5.18	Midsubstance
right	9.60	Midsubstance
2 left	1.07	Midsubstance
right	8.93	Midsubstance
3 left	2.55	Midsubstance
right	7.33	Midsubstance
4 left	3.29	Midsubstance
right	7.45	Midsubstance
5 left	----	Midsubstance
right	8.92	Avulsion
6 left	6.40	Midsubstance
right	11.37	Midsubstance
7 left	2.72	Midsubstance
right	6.67	Midsubstance
8 left	5.06	Midsubstance
right	8.66	Midsubstance
Mean ± SD	6.3 ± 3.0	

Tensile Response Data

The tensile stiffness and modulus of the thirds is shown in Table 8. There was no significant difference between the thirds for the stiffness or the modulus. A stress-strain plot showing all three thirds together indicates the modulus of the central and medial third to be very comparable (Figure 26).

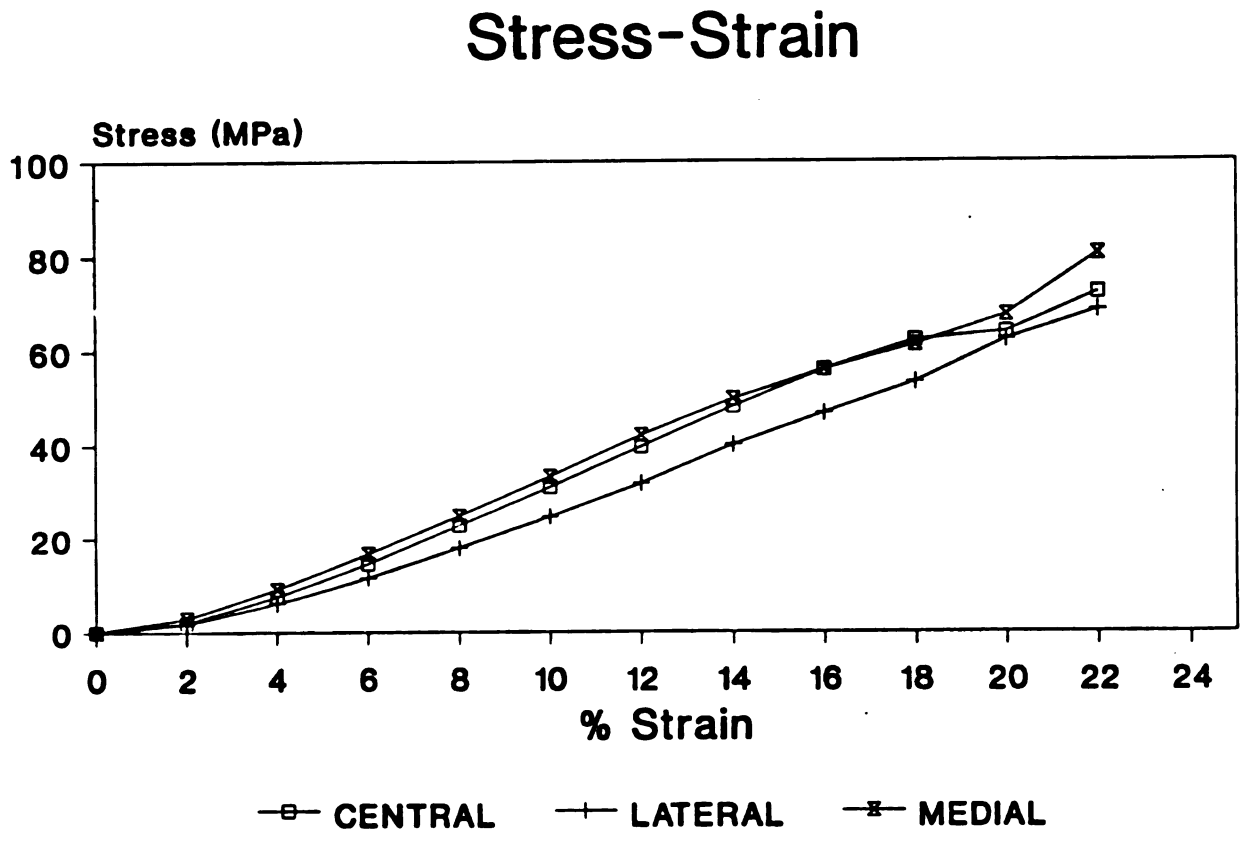


Figure 26. Stress-strain plot: central, medial and lateral thirds.

TABLE 8.
Tensile Response Data (Mean ± SD)

Portion of Tendon	Stiffness (N/mm)	Modulus (N/mm)
Lateral Third		
1 left	98	428
right	45	297
2 left	120	394
right	72	225
3 left	150	673
right	40	199
4 left	103	574
right	75	291
5 left	138	461
right	75	326
6 left	81	316
right	60	560
7 left	112	513
right	70	398
8 left	150	577
right	100	509
Mean ± SD	71 ± 30	421 ± 139
Central Third		
1 left	158	544
right	83	461
2 left	87	384
right	87	370
3 left	133	564
right	123	495
4 left	137	461
right	153	566
5 left	150	411
right	105	418
6 left	93	321
right	78	454
7 left	115	497
right	126	475
8 left	78	349
right	--	---
Mean ± SD	114 ± 29	451 ± 76
Medial Third		
1 left	83	424
right	138	454
2 left	24	174
right	127	508
3 left	50	334
right	110	520
4 left	92	319
right	110	500
5 left	---	---
right	160	643
6 left	90	539
right	140	419
7 left	112	513
right	95	365
8 left	75	469
right	160	635
Mean ± SD	100 ± 41	443 ± 124

Biochemistry

The modulus of the collagen fibers for each third of the tendon is shown in Table 9. A one way ANOVA indicated that there was no statistical difference between thirds. The concentration of collagen in each third of the tendon is also documented in the table. Once again, there was no statistical difference between thirds. Yet, it is interesting to note that as there was a tendency for the concentration of the collagen fibers to decrease from the lateral to the medial side of the tendon, the modulus of the collagen fibers tended to increase from the lateral side to the medial side.

TABLE 9.
Biochemical Properties (Mean ± SD)

Portion of Tendon	Modulus (MN/ug/mm)	Concentration (ug/mm)
Lateral Third		
1 left	49	84
right	28	107
2 left	69	56
right	37	67
3 left	114	38
right	45	80
4 left	40	121
right	40	75
5 left	85	56
right	48	66
6 left	170	31
right	55	88
7 left	50	95
right	48	84
8 left	44	117
right	35	142
Mean ± SD	57 ± 36	82 ± 30
Central Third		
1 left	86	59
right	50	107
2 left	53	71
right	50	80
3 left	67	83
right	68	81
4 left	64	70
right	55	94
5 left	121	36
right	134	37
6 left	35	97
right	89	49
7 left	63	80
right	88	53
8 left	39	85
right	--	97
Mean ± SD	70 ± 29	74 ± 22
Medial Third		
1 left	63	67
right	79	64
2 left	14	122
right	58	98
3 left	245	14
right	84	64
4 left	37	79
right	70	76
5 left	--	4
right	126	52
6 left	--	11
right	116	42
7 left	65	53
right	73	50
8 left	55	86
right	63	101
Mean ± SD	82 ± 55	61 ± 33

V. CONCLUSIONS

1. Mechanical and histological results from the current medial procedure study varied significantly from Cabaud's study. Six months post surgery in the current study, the tendons had inferior structural and material properties and a large increase in cross-sectional area when compared to controls. In Cabaud's study, the tendons had strength and stiffness values equal to controls and no increase in size.

2. When compared to Burk's central study, the current medial study showed the tendons to have a decreased amount of scar tissue, statistically greater failure loads at all times and statistically greater stiffness and modulus at six months.

3. The results of the tests for spatial variations in the dog patellar tendon indicated that there were no statistical differences between any of the thirds for any of the structural, material, physiological response and biochemical parameters.

VI. DISCUSSION OF CONCLUSIONS AND SUMMARY

The primary objective of this research was to examine the remaining dog patellar tendon after removal of the medial one third and explain the mechanisms of response. The results from the medial study were compared with those from Cabaud (10) and Burks et al (4). The mechanical properties and histological results were significantly different than those from Cabaud. One factor which may, in part, help explain the difference is that in Cabaud's study, the animals were immobilized for six weeks. In the studies of Burks (4) and the present investigation the dogs were not immobilized after surgery. Perhaps early immobilization is an advantage, protecting the initially weakened patellar tendon from developing excessive scar tissue.

It has been shown that for the healing tendon, lack of joint movement (via immobilization) may result in better alignment of collagen, possibly due to an isometric field of stress created by a state of low level in vivo muscle contraction (16,23). Also, immobilization of rabbit tendons has been shown to increase the modulus of the tendon when compared to the tendon which underwent short periods of strenuous activity (ten minutes each day on a treadmill) and

subsequent periods of rest (26). This type of exercise environment was shown to be detrimental to the healing process and the mechanical properties of the tendon following this surgery. The dogs in the medial and central studies were allowed unprotected activity in cages with runs, and most likely underwent exercise patterns similar to that just described (high intensity stress followed by periods of rest).

The results from the current study on removal of the medial one-third of the patellar tendon were also different from Burks (4) after removal of the central one-third, even though the surgery, post-surgical housing and biomechanical testing procedures were the same in both studies. After the medial procedure, the mechanical properties of the operated tendon at six months were closer to the control values than those after the central procedure. Length, cross-sectional area, maximum load to failure, stiffness and modulus all exhibited this trend. Two hypotheses were created to explain these differences. The first hypothesis was that making two cuts for the central procedure versus one cut for the medial procedure may have caused more tissue damage initially with subsequent enhanced phagocytosis, etc. The second hypothesis was that there may be spatial variations across the width of the dog patellar tendon. If the central third of the tendon was the stiffest and strongest third, then in a mechanical sense, its removal would have the greater detrimental effect on the remaining tendon. The operated tendon with the central

procedure may have increased damage when compared to the tendon with the medial procedure either because of more cuts or increased stresses. This damage may then stimulate fibroblastic cells to synthesize relatively more collagen and a larger scar after the central defect was created. The basis for my second hypothesis was tested in this thesis by examining spatial variations across the width of the dog patellar tendon. The results from Part II indicated that there was no statistical difference between the thirds. However, the idea of the remaining tendon having a decreased stiffness and increased stresses may still be true. By revisiting the time zero data for both the central and the medial studies, it was found that the surgeon consistently removed more tissue during the central procedure than during the medial procedure; 37% versus 27%, respectively. In fact, the surgeries were performed by two separate surgeons. The extent of post-surgical scar may depend on how much the host tendon is compromised during surgery. The increased stresses in the tendon after removal of relatively more tissue may stimulate the healing process, as has been observed in past studies. Vailus (46), for example, examined rats in which the medial collateral ligament had been transected, repaired and immobilized for two weeks, followed by six weeks of either exercise or cage activity. He concluded that the healing process was enhanced by exercise, as evidenced by the more rapid return of tissue DNA, collagen synthesis and the return

of ultimate load to normal levels. Burroughs (5) found that in rats, the unstable knee was adversely effected by forced exercise. He concluded that the healing ligament in an unstable knee joint does not receive the same benefits from exercise that is seen in stable joints. These data suggest that increased stress benefits the healing process to a point.

FUTURE RESEARCH DIRECTIONS

While the results from this study suggest that the medial procedure may be less harmful to the host patellar tendon than the central procedure, there are several contributing factors to this conclusion. First, the amount of tissue removed during surgery may be a critical factor influencing the healing process in the tendon. In addition, the question of immobilization versus remobilization or a combination of both needs to be examined. It is possible that a short period of immobilization (per Cabaud's study) followed by a low stress form of continuous exercise may be most beneficial to the healing tendon. Future studies need to determine the best combination and form of immobilization and remobilization for the healing tendon.

On a microstructural basis, there was a tendency for the dog patellar tendon to contain less collagen, with higher modulii, on the medial side versus the lateral side of the tendon. This indicates that the fibers in the less dense

an apparent decrease in the number of collagen fibers. On the other hand, these data may reflect a more efficient response of collagen fibers and less entanglement in less dense areas. Or, if the density of fiber bundles is comparable across the width, these data indicate that larger diameter fiber bundles have relatively lower modulii. More morphometrical studies on the size and distribution of collagen fiber bundles is needed.

In summary, this study provided some new data and directions of research to more fully understand the consequences of using a portion of the patellar tendon for reconstructive surgery.

VII. REFERENCES

REFERENCES

1. Amiel D, Woo SL-Y, Harwood FL, Akeson WH. The effect of immobilization on collagen turnover in connective tissue: a biochemical-biomechanical correlation. Acta Orthop Scand 1982;53:325-332.

2. Belkoff SM, Haut RC: A microstructurally based model analysis of gamma irradiated tendon allografts. J Orthop Res 1992;

3. Bonamo JJ, Krinick RM, Sporn AA: Rupture of the patellar ligament after use of its central third for anterior cruciate reconstruction. A report of two cases. J Bone Joint Surg 1984;66A:217-224.

4. Burks RT, Haut RC, Lancaster RL: Biomechanical and histological observations of the dog patellar tendon after removal of its central one-third. Am J Sports Med 1990;18:146-153.

5. Burroughs P, Dahners L: The effect of enforced exercise on the healing of ligament injuries. Am J Sports Med 1990;18:376-378.

6. Butler DL, Grood ES, Noyes FR, et al: Biomechanics of ligaments and tendons. Exerc Sport Sci 1978;Rev 6:125-181.

7. Butler DL, Noyes Fr, Grood ES, Miller EH, Malek M: Mechanical properties of transplants for the anterior cruciate ligament. Trans Ortho Res Soc 1979;4:81.

8. Butler DL, Grood ES Noyes FR, Zernicke RF, Brackett K: Effects of structure and strain measurement technique on the material properties of young human tendons and fascia. J Biomechanics 1984;17(8):579-596.

9. Butler DL, Grood ES, Noyes FR, Sodd AN: On the interpretation of our anterior cruciate ligament data. Clin Orthop Rel Res 1985;196:26-34.

10. Cabaud HE, Feagin JA, Rodkey WG: Acute anterior cruciate ligament injury and augmented repair. Experimental studies. Am J Sports Med 1980;8:395-401.

11. Campbell WC: Reconstruction of the ligaments of the knee. Am J Surg 1939;43:473-480.

12. Chen EH and Black J: Material design analysis of the prosthetic anterior cruciate ligament. J Biomedical Mat Res 1980;14:567-586.

13. Chun KJ, Butler DL, Bukovec DB, Gibbons MJ, Stouffer DC: Spatial variations in material properties in fascicle-bone units from human patellar tendon. Trans Ortho Res Soc 1989;14:214.

14. Dahners LE, Torke MD, Gilbert JA, Lester GE: the effect of motion on collagen synthesis, DNA synthesis and fiber orientation during ligament healing. Trans Orthop Res Soc 1989;14:299.

15. Feagin JA: The syndrome of the torn anterior cruciate ligament. Orthop Clin North Am 1979;10:81-90.

16. Frank CB, MacFarlane B, Edwards P, Rangayyan R, Liu Z-Q, Walsh S, Bray R: A quantitative analysis of matrix alignment in ligament scars: A comparison of movement versus immobilization in an immature rabbit model. J Orthop Res 1991;9:219-227.

17. Frank CB, Amiel D, Woo SLY, Akeson W: Normal ligament properties and ligament healing. Clin Orthop 1985;196:15-25.

18. Fried JA, Bergfeld JA, Weiker G, et al: Anterior cruciate reconstruction using the Jones-Ellison procedure. J Bone Joint Surg 1985;67A:1029-1033.

19. Fung YC: Biomechanics:Mechanical properties of living tissues. New York, Springer Verlag,1981.p.222.

20. Haut RC, Powlison, AC: The effects of test environment and cyclic stretching on the failure properties of human patellar tendons. J Orthop Res 1990;8:532-540.

21. Hey Groves EW: Operation for the repair of the cruciate ligaments. Lancet 1917;2:674-675.

22. Hughston JC: Complications of anterior cruciate ligament surgery. Orthop Clin North Am 1985;16:237-240.

23. Inoue M, Woo SLY, Gomez MA, Amiel D, Ohland KJ, Kitabayashi LR: Effects of surgical treatment and immobilization on the healing on the medial collateral ligament: A long-term multidisciplinary study. Conn Tiss Res 1990;25:13-26.

24. Johnson RJ, Eriksson E, Haggmark T, et al: Five-to ten-year follow up evaluation after reconstruction of the anterior cruciate ligament. Clin Orthop 1984;183:122-140.

25. Jones KG: Reconstruction of the anterior cruciate ligament. A technique using the central one-third of the patellar tendon. J Bone Joint Surg 1963;45A:925-932.

26. Kamps B, Linder LH, Haut RC, Render J: Influence of forced exercise on the healing of the patellar tendon after removal of its central third; (Unpublished Data).

27. Kear M, Smith RN: A method for recording strain in sheep during locomotion. Acta Orthop Scand 1975;46:896-905.

28. Kennedy JC, Weinberg HW, Wilson AS: The anatomy and function of the anterior cruciate ligament. J Bone Joint Surg 1974;56A:223-235.

29. Lancaster RL: Biomechanical properties of the canine patellar tendon as a function of age and diabetes. M.S. Thesis, Michigan State University, 1990.

30. Laros GS, Tipton CM, Cooper RR: Influence of physical activity on ligament insertions in the knees of dogs. J Bone Joint Surg 1971;53A:275-286.

31. McCarroll JR: Fracture of the patella during a golf swing following reconstruction on the anterior cruciate ligament. A case report. Am J Sports Med 1983;11:26-27.

32. Morrison JB: Bioengineering analysis of force actions transmitted by the knee joint. Biomech Eng 1968;3:164-170.

33. Nordin M, Carlstedt CA: Biomechanics of tendons and ligaments: Basic biomechanics of the musculoskeletal system, Nordin, M (ed) Philadelphia,Lea & Febiger,1989;59-74.

34. Noyes FR, Delucas JL, Torvik PJ: Biomechanics of anterior cruciate ligament failure: An analysis of strain-rate sensitivity and mechanisms of failure in primates. J Bone Joint Surg 1974;56A:236-253.

35. Noyes FR, Grood ES: The strength of the anterior cruciate ligament. Age and species-related changes. J Bone Joint Surg 1976;58A:1074-1082.

36. Noyes FR: Functional properties of knee ligaments and alterations induced by immobilization. A correlative biomechanics and histological study in primates. Clin Orthop Rel Res 1977;123-210.

37. Noyes FR, Basset RW, Grood ES Butler DL: Arthroscopy in acute traumatic hemarthrosis of the knee. Incidence of anterior cruciate tears and other injuries. J Bone Joint Surg 1980;62A:687-695.

38. Noyes FR, Butler DL, Paulos LE Grood ES: Intraarticular cruciate reconstruction. I. Perspectives on graft strength, vascularization, and immediate motion after replacement. Clin Orthop Rel Res 1983a;172:71-77.

39. Noyes FR, Butler DL, Grood ES, Zernicke RF, Hefzy MS: Biomechanical analysis of human ligament grafts used in knee ligament repairs and reconstructions. J Bone Joint Surg 1984;66A:344-352.

40. O'Brien SJ, Warren RF, Pavlov H, Panariello R, Wickiewicz TL: Reconstruction of the chronically insufficient anterior cruciate ligament with the central third of the patellar tendon. J Bone Joint Surg 1991;73A:278-286.

41. Quirinia A, Viidik A: Freezing for postmortal storage influences the biomechanical properties of linear skin wounds. J Biomech 1991;24:819-823.

42. Sachs RA, Daniel DM, Stone ML, et al: Patellofemoral problems after anterior cruciate ligament reconstruction. Am J Sports Med 1987;17:760-765.

43. Stegemann H: Mikro-vestimmung von hydroxyproline mit chloramin-Tund p-Dimehylamino Benzaldehyd. Physiol Chem 1958;311:41-45.

44. Tibone JE, Antich TJ: A biomechanical analysis of anterior cruciate ligament reconstruction with the patellar tendon. A two year follow up. Am J Sports Med 1988;16:332-335.

46. Vailus AC, Tipton CM, Matthes RD, Gart M: Physical activity and its influence on the repair process of medial collateral ligaments. Connect Tissue Res 1981;9:25-31.

47. Viidik A: Biomechanical behavior of soft connective tissue: Progress in Biomechanics. Akkas, N (ed) Sijthoff & Noordhoff, 1979;75-108.

48. Viidik AV, Gottrup F: Mechanics of healing soft tissue wounds: Frontiers in Biomechanics, Schmid-Schonbein, G, Woo, S, Zweifach, B (ed) New York, Springer-Verlag, 263-278.

49. Woo SLY, Matthews JV, Akeson WH, et al: Connective tissue response to immobility: Correlative study of biomechanical and biochemical measurements of normal and immobilized rabbit knees. Arthritis Rheum 1975;18:257-264.

50. Woo SLY, Gomez MA, Woo YK, et al: Mechanical properties of tendons and ligaments. II. The relationships of immobilization and exercise on tissue remodeling. Biorheology 1982;19:397-408.

51. Woo SLY, Orlando CA, Camp JF, Akeson WA: Effects of postmortem storage by freezing on ligament tensile behavior. J Biomech 1987;20:557-563.