THE ROLE OF CONNECTIVE TISSUE IN THE FEMALE PELVIC FLOOR

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Abstract

The Role of Connective tissue in the Female Pelvic Floor

Methods:

Biomechanical studies were performed on biopsies of the rectus sheath, round and uterosacral ligaments as well as anterior and posterior vaginal walls of women with or without genital prolapse.

In the second study, 96 healthy women in their fifth decade, were compared with 94 similar women with genital prolapse surgery Their clinical connective tissue markers were assessed by standardised methods and their first childbirth data were collected from hospital notes. The progress of the first labour was analysed by multiple logistic regression in the 2 groups of women.

In the third study, 665 primiparous women who had their first baby during the previous 7 to 12 months, had their clinical connective tissue markers assessed as in the second study. Data from their first labour were then collected from the hospital notes and compared to the connective tissue markers by standard multiple regression analysis.

Results:

In-vitro biomechanical data showed that the round ligament properties were different in women with and without genital prolapse. They were also different amongst women under and over 40 years of age *without* genital prolapse. In women *without* genital prolapse, the elasticity of the rectus sheath and the round ligament differed from each other.

In women *with* genital prolapse, the anterior vaginal wall and the uterosacral ligament were different from each other.

In the second study, women with uterocervical prolapse had a shorter active first stage of labour than women without prolapse. When women with any type of genital prolapse were compared to women without prolapse, there was no difference in the duration of labour. The study is likely not to have had adequate power to detect a difference in the second stage of labour. More women in the prolapse group had knee hyperextension but there was no difference in the mobility of the upper limb joints.

In the third study, primiparous women with increased joint mobility had a shorter second stage of labour.

Connective tissue properties play an important role in pelvic floor function.

List of Abbreviations

LA nerve –levator ani nerve

PNTML –Nerve Term Motor Latency

EMG studies – electromagnetic studies

BMI- body mass index as a function of the individual's height and weight (Kg/m2)

MRI- Magnetic resonance imaging

BJHS – Benign joint hypermobility syndrome

POP-Q score – (Pelvic Organ Prolapse) Quantification system.

gh - genital hiatus

pb - perineal body

tvl – total vaginal length

NHS – National Health Service

FH – family history

FTP- failure to progress in labour

FD – fetal distress in labour

IOL- induction of labour

Emerg cs – emergency caesarean section

Instr D - Instrumental delivery

SD - standard deviation

CI – confidence interval

OR – odds ratio

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DECLARATION

The work contained in this thesis is my own. None of the data is a part of any other thesis. The studies presented here were approved by the ethics committees of the individual hospitals. All patients gave informed consent prior to their involvement in the study.

Margaret R Spiteri

I dedicate this thesis to my father Francis, and to the memory of my mother Carmen. I would have never reached this far without their unfailing love and total dedication. 'I can never thank you enough.....'

CHAPTER I

INTRODUCTION

Background

In the West, during the second half of the 20th century, there has been a change in society's attitudes to women's health. Women have always regarded femininity as an important property of visual image. However, nowadays women give considerable status to the integrity of their bodies as manifesting their gender. More open discussion has resulted in a greater realisation of the significance of pelvic floor dysfunction in the life quality of women, especially in older women. This attitude, together with a shift in the priorities secondary to an increase in life expectancy, influenced policies for both governments and health professions. Thus, it has been estimated that in the United States, over the next 30 years, growth in demand for services to care for female pelvic floor disorders will increase at twice the rate of growth of the same population [1]. For pelvic floor disorders mature age groups generate 10 times the number of consultations per 1000 woman years as do their younger counterparts.

The pelvic floor must allow for sexual function and childbirth, as well as control of storage and evacuation of urine and faeces. Thus the term pelvic floor dysfunction may implicate varied conditions, including urinary incontinence, faecal incontinence, pelvic organ prolapse, disordered sensation, emptying abnormalities of the lower urinary tract, defaecatory dysfunction, sexual dysfunction, and several chronic pain syndromes. It could also include dyspareunia and obstructed labour. However, most commonly it refers to genital prolapse and impaired control of urine and faeces. These conditions share some similar risk factors and have generated the most epidemiological data, opinion, and dogma.

1.1 Research and genital prolapse

Research is a process that produces knowledge. It may fall into one of two broad categories. Firstly it can help understanding of disease by describing and determining causes of health problems. Most work on genital prolapse is of this type. It serves to provide a base-line from which hypotheses, on preventing and managing disease, may be formulated and tested.

The second category of research is used to test interventions for preventing disease or improving its treatment. As yet we know of no measures to prevent genital prolapse. The management of pelvic floor weakness includes physiotherapy at the very initial stages, although at the time of presentation many women are beyond help by this means. A pessary that supports prolapsed vaginal walls, cervix or uterus is another possibility. The other alternative is surgery with repair and reconstruction of the pelvic floor. There have been many operations described in the literature for the correction of pelvic floor dysfunction. Typically these have come in and out of fashion as early data has been shown to be over-optimistic. Because of the lack of standardisation in the reporting of preoperative, operative outcome data and the absence of long-term follow-up, it is difficult to estimate the rate of recurrence. However for prolapse alone, it is reported to range from 11.6% to 95.7% [2-5]. Most studies report observational data rather than controlled randomised trials. There is also a tendency to report anatomical outcomes with few reporting on function in respect of continence, bowel habit and sexual function. An adequate anatomical outcome following surgery, does not mean functional success. In fact there may be deterioration or the onset of new functional pelvic floor problems[6]. Repair of prolapse with natural and synthetic mesh has only recently been introduced and long term results are not yet available[7-9].

1.2 From Association to Causation

The causal factors in the pathogenesis of pelvic floor disorders are far from clear. When observing an association, one must consider several problems in the extrapolation to cause or effect [10].

Major Criteria

1. Temporal relationship of 'exposure' to causal factors and a disorder is important in understanding the development of the disorder. To date, there are no prospective longitudinal studies reported in the literature that report on the natural history of prolapse.

2. Biologic plausibility - Often epidemiologic observations precede biologic knowledge. The limited biological knowledge related to genital prolapse and the scarcity of data of childbirth as the main aetiological factor in genital prolapse will be explored.

3. Consistency with other knowledge – recent studies have investigated the long-term pelvic dysfunction following childbirth. Caesarean delivery was not associated with a significant reduction in long term pelvic floor morbidity compared with spontaneous vaginal delivery [11]. Thus the role of pregnancy rather than vaginal delivery in pelvic floor damage warrants further investigations.

4. Consideration of alternative explanations. There are many published studies in the literature that correlate parity and mode of delivery with prolapse[2;12]. The causal implications of this have not been developed critically. For instance, none of these studies investigated the possibility of connective tissue properties acting as confounding variables.

Other considerations

1. Strength of the association – the stronger the association the more likely it is that the relation is causal.

2. Replication of the findings – It is important that the studies of one group be repeated by others. Whilst this is a well established scientific principle, a mistaken belief in the pre-eminence of originality often discourages this type of work.

3. Dose/Response relationship - its absence in no way negates a causal relationship. Nevertheless many publications seem to focus excessively on quantifying aetiological suspects.

4. Specificity is the weakest of all these guidelines and its absence in no way negates a causal relationship. Specificity supposes that a certain exposure is associated with only one disease outcome. The possibility of multiple effects from a single factor is not surprising considering the complexity of pregnancy and childbirth.

5. Cessation of exposure - if a factor is a cause of a disease, we would expect the risk of disease in most situations to decline when exposure to the factor is reduced or eliminated. However, in genital prolapse, changes in the dynamics of the pelvic floor that occur early on in the reproductive life, might predispose a patient to further damage when other factors repeatedly increase the strain on the pelvic floor.

Any conclusion that an observed association is causal is greatly strengthened when different types of evidence from multiple sources support such reasoning. Thus, it is not so much a count of the number of guidelines present that is relevant to causal inference but rather an assessment of the total pattern of evidence observed that may be expressions of one or more guidelines.

1.3 Prevention is better than cure

Natural childbirth is believed to be the source of the main damage of the pelvic floor although this has not been established by rigorous experiment [12-14]. Thus, 31% of 'well informed' obstetricians in London in the mid-1990's, said they would opt for a planned caesarean section rather than vaginal delivery even if there were no specific risk factors present. The main reason given (88%) was fear of perineal damage [15]. In the developed world, there has been a sustained increase in caesarean section rates associated with much public interest and debate on the justification of bypassing vaginal delivery completely [16]. Repeated caesarean sections might well result long-term morbidity form denervation of the bladder neck but further research is required. We do not know where to place the fulcrum between maternal fulfillment derived from avoiding intervention and protecting the pelvic floor from future dysfunction. The majority of women who bear children do not have problems with the pelvic floor whilst nulliparous women are not exempt from developing pelvic floor pathology [12;17]. It is therefore important that before this practice becomes more widespread, we establish the true risks of developing pelvic floor disorders in relation to childbirth.

Understanding both the specific predisposing factors that place an individual woman at risk and the precise events that initiate injury is important for primary prevention. Defining the relative importance of the various factors that promote further damage and lead to decompensation is essential for secondary prevention.

Ordinal classifications are a useful means to organise thoughts about the progression from a "perfect" pelvic floor that is anatomically, neurologically, and functionally

normal; to a less-than-perfect but well-compensated pelvic floor in an asymptomatic patient; to the functionally decompensated floor in the patient with end-stage disease with urinary incontinence, anal incontinence, or pelvic organ prolapse in whom most treatment efforts are concentrated [18]. However, it should be remembered that we are examining a biological system made up of continua that are ill-matched to ordinal classes.

The first pregnancy and labour are probably the first major insult to the pelvic floor in women. Their outcome has immediate as well as long-term implications on women's health. Failure to progress in labour is a major indication for emergency caesarean sections in nulliparous women. Puerperal anxiety as well as maternal and fetal morbidity are higher following emergency caesarean section than elective caesarean or vaginal delivery [19]. In its turn, the outcome of the first labour has major effect on subsequent deliveries, and maternal request for repeat caesarean section is a main indication for elective caesarean sections in multiparous women [20;21]. On the other hand, labour can result in difficult instrumental delivery, which might result in immediate and long-term physical and psychological trauma to the mother as well as increased morbidity for the baby. On the other side of the spectrum, there are women who have quick, seemingly uncomplicated labours. Despite current technological innovations we are still unable to predict the immediate outcome of labour in women, and even more so predict the long-term effects.

The objective of this thesis was to investigate the relationship of connective tissue and the first labour as well as the association of each of them to genital prolapse in older women. I will start by reviewing the literature in order to explain the reasons why these studies were performed and why the methods used were chosen.

CHAPTER II

REVIEW OF THE LITERATURE

2.1 The genital tract : structure and function

2.1.1 Introduction

Anatomy is a living science and individual to each person but the principles of applied anatomy and associated physiology are essential for the understanding of female pelvic floor pathology. The pelvic floor is very dynamic. Discrepancies are found in the descriptions in anatomical textbooks of the structure of the levator ani, perineal body and of the sphincter muscles of the anus. This is because although cadaveric dissections have supplied us with valuable information, fixation, and even anaesthesia, may alter the relationship between muscular components of the pelvic floor [22;23].

Modern imaging (endosonography and magnetic resonance) is helping to elucidate the changes that occur after delivery, hysterectomy or with genitourinary prolapse [24;25]. The combination of function and morphology allows for an innovative view of the pelvic floor, and thus adds to our understanding of the various interactions of the structures and the basic principles that underlie support of the pelvic organs.

2.1.2 The structure of the pelvic floor

The pelvis lies at the bottom of the abdominal-pelvic cavity and the *pelvic floor* closes the canal within the bony pelvis. The floor forms a supportive layer that prevents the abdominal and pelvic organs from falling through the opening within the bony pelvis. In addition to the passive elastic support of the endopelvic connective tissues, the active basal tone of the levator ani muscles keeps the urogenital hiatus closed and the upper

vagina and pelvic viscera supported over the levator plate[26-28]. The pelvic floor muscles are the only striated muscles in the human body that are under tonic contraction, even when at rest [29]. These muscles reflexly contract in response to coughing or other activities that increase intra-abdominal pressure. This reflex decreases the tension placed upon the pelvic connective tissues during periods of increased intra-abdominal pressure. There is a persistent disagreement as to the relative importance of pelvic floor muscles and fascia in relation to pelvic floor disorders. It is likely, that the contribution of each varies in different women.

The *pelvic wall* is the site of attachment of pelvic floor structures. It comprises the pubic bones, ischial spines, sacrum and coccyx to which pelvic floor structures attach directly or indirectly by fascia. It also comprises the sacrospinous triangular-shaped ligament and the internal obturator muscle whose tendon inserts into the greater trochanter of the femur. A tendinous ridge of the obturator fascia, the arcus tendineus levator ani forms the pelvic sidewall attachment of the levator ani. Histologically, this ridge resembles the tendons and ligaments of the peripheral musculoskeletal system with well-organised fibrous collagen. The fascia of the pelvic wall is a strong membrane covering the surface of the internal obturator and piriformis muscle posteriorly with firm attachment to the periosteum [30;31].

2.1.3 The support of the uterus and vagina – the pelvic fascia

The *pelvic fascia* is defined as the fascial tissue which covers the lower and upper surfaces of the levator ani muscles, together with the medial surfaces of the two obturator internus muscles. Between the pelvic fascia and the peritoneum above, all the loose tissues are best referred to as the *endopelvic fascia*. In addition, the vagina,

cervix, rectum, bladder and the urethra have their own fascial layer. Furthermore, each of these is attached to adjacent organs and the pelvic side walls by pelvic fascia which can be recognised during surgery [32]. Tears of the endopelvic fascia might be the initiating event for various types of prolapse since it normally suspends the pelvic organs to the pelvic wall and forms a continuous sheet-like mesentery made of adventitial connective tissue. The part which attaches to the uterus is called the parametrium and it is continuous with the paracolpium which attaches to the vagina. DeLancey reported cadaveric studies to delineate the discrete supporting structures of each third of the vagina in women with an intact uterus as well as following hysterectomy [22;26;33-35]. Thus Level I or uppermost vaginal support is provided by vertical fibers of the parametrium and paracolpium attached laterally to the pelvic walls by the broad ligament. At the anterior side of the parametrium, the *round ligament* might provide accessory support in maintaining anteversion of the uterus in most women. It consists of unstriated muscle fibres arranged in a central thick core of longitudinal fibres separated by a connective tissue of collagen fibres with very little elastic tissue from an outer thin layer of muscle fibres running obliquely or longitudinally.

In the middle third of the vagina (level II) the paracolpium attaches the vagina laterally to fascia of the greater sciatic foramen and posteriorly to the presacral fascia by the cardinal and uterosacral ligaments respectively. The anteromedial part of the vagina is supported by the pubocervical fascia which is embedded by smooth muscle fibres and is attached to the arcus tendineus fascia pelvis. These attachments form a supportive hammock underneath the urethra. The rectovaginal fascia supports the posterior part of the vagina. It is fibromuscular with abundant vascular supply. It is attached to the uterosacral and cardinal ligaments and fuses with the superior fascia of the pelvic

diaphragm and the perineal body. The vagina also has lateral support from the medial part of the levator ani, perineal membrane as well as the perineal body (Level III). Defects in the support provided by the mid-level (Level II/III) vaginal supports (pubocervical and rectovaginal fasciae) result in cystocele and rectocele in the anterior and posterior compartments of the vagina. Loss of the upper suspensory fibers (Level I) of the paracolpium and parametrium is responsible for the development of middle or superior defects that result in vault, cervical and uterine prolapse. These defects usually occur in varying combinations and this is responsible for the diversity of clinical problems encountered within the overall spectrum of pelvic organ prolapse [35].

The *levator ani muscles* consist of two portions: the pubovisceral muscle and the ilio-coccygeus muscle. The pubovisceral muscle is a thick U-shaped muscle whose ends arise from the pubic bones on either side of the midline and pass behind the rectum, forming a sling-like arrangement. This portion includes both the pubococcygeus and puborectalis portions of the levator ani. Laterally, the iliococcygeus arises from a fibrous band on the pelvic wall (arcus tendineus levator ani) and forms a relatively horizontal sheet that spans the opening within the pelvis and forms a shelf on which the organs may rest. Because of its firm attachment to the lateral vaginal wall, it acts functionally as a vaginal sphincter without actually encircling that organ. The puborectalis and external anal sphincter maintain a constant muscular tone that is directly proportional to the volume of the rectal content and relaxes at the time of defecation [7]. The opening within the levator ani muscle, through which the urethra and vagina pass and through which prolapse occurs, is called the urogenital hiatus of the levator ani. The hiatus is bounded anteriorly by the pubic bones, laterally by the levator ani muscles, and posteriorly by the perineal body and external anal sphincter.

2.1.4 *Nerve supply of the pelvic floor*

Innervation of the pelvic floor muscles is believed to be derived from ventral roots of the second, third and fourth sacral nerves via the pudendal nerve [36]. In 1986, Snooks and co-workers reported neurophysiological evidence that the human puborectalis muscle was innervated by direct branches of the sacral plexus that enter the muscle from its cephalad side while the external anal sphincter muscle seemed to be innervated by branches of the pudendal nerves. The striated urinary sphincter musculature also received a dual innervation. The periurethral component was innervated by perineal branches of the pudendal nerves and the intramural portion by a different pathway, probably consisting of supralevator branches derived from the pelvic splanchnic nerves [37]. Recent work on human cadavers suggests that the levator ani innervation is through the 'levator ani nerve'(LA nerve), that originates from the sacral nerve roots S3 to S5 and travels on the superior surface of the pelvic floor. Further work by the same group is in progress with the squirrel monkey as an animal model [38;39].

The role of pudendal nerve terminal motor latency (or PNTML) in the study of denervation injury leading to pelvic floor dysfunction was introduced by Snooks and Swash in 1984 for patients with idiopathic faecal incontinence. Since then, this technique has been used as a marker for pudendal neuropathy in patients with stress urinary incontinence [40], genital prolapse [41], failed anal sphincter repair [42], constipation [43] and after vaginal surgery [44] or following childbirth. Most studies found increased PNTML and interpreted this as evidence for pelvic floor muscle injury. However, it is now known that motor latency is a poor reflection of axonal loss and on its own, is not to be used to assess the integrity of the pelvic floor musculature. [45] The most appropriate test to evaluate the function of striated muscle and the nervous system that

supplies them would be EMG(electromagnetic) studies. They allow pelvic floor muscle assessment during specific activities such as coughing and can define the site, type and the degree of pathology. They are not useful for the evaluation of smooth muscle such as the internal anal sphincter which is better assessed using endoanal ultrasonography. Keeping this in mind, I will summarise the current available research.

Two groups have performed most of the neurophysiological studies of the pelvic floor in relation to childbirth and pelvic floor dysfunction. Allen and co-workers reported that nulliparous women with a second stage of labor lasting more than 83 minutes were at increased risk for denervation injury as measured by EMG, PNTML and perineometry [13]. Active maternal pushing lasting more than 57 minutes was associated with a greater risk of denervation injury. Following this study, it has become common practice to allow the fetus to descend passively and restrict active pushing during the second stage. Although the association between prolonged second stage of labour and nerve damage is plausible, the specific time/pressure relationship has not yet been established. Although the majority of women showed immediate evidence of nerve damage, forceps delivery, perineal tears and other risk factors believed to result in prolapse did not affect the degree of nerve damage seen after childbirth. Postnatally, there was evidence of re-innervation in the pelvic floor muscles in 80% of 75 nulliparous women who delivered vaginally. There was no difference in PNTML of the pudendal nerve a few days and two months post-partum.

The same group studied anal sphincters in 102 women who delivered vaginally and compared them to 34 nulliparous control subjects. When they followed up women 2 months after delivery, there was manometric and neurophysiological evidence of weakness because of partial denervation of the pelvic floor striated sphincter

musculature, with pudendal neuropathy, which was more marked in those women with incontinence. In this study, multiparity, forceps delivery, increased duration of the second stage of labour, third degree perineal tear and high birth weight were important factors leading to pelvic floor damage. The same researchers followed up 14 of the 24 (58 per cent) multiparous women in this study, 5 years later. Five of the 14 had developed clinical symptoms of stress incontinence; two of them had had a further uncomplicated vaginal delivery during this time. There was manometric and neurophysiological evidence of weakness because of partial denervation of the pelvic floor striated sphincter musculature, with pudendal neuropathy, which was again more marked in those women with incontinence [46].

Smith and colleagues reported that women with stress urinary incontinence, with pelvic organ prolapse or both had significant partial denervation of the pelvic floor as detected by single-fibre electromyography as well as histochemical studies of the pubococcygeus [47]. However, women with prolapse but no stress incontinence had normal conduction time in the innervation of the external striated urethral sphincter. In women with stress incontinence, PNTML were prolonged. The same group performed histological and histochemical analysis of biopsy samples of pubococcygeus muscle obtained from asymptomatic women and from women with stress incontinence of urine, with or without genitourinary prolapse. They found partial denervation of the pelvic floor in patients with urinary stress incontinence with or without genital tract prolapse [41].

Dimpfl et al reported that aging and vaginal childbirth lead to histomorphological changes of the pelvic floor muscle that were consistent with changes of myogenic origin [48]. Evidence of grouped fibre atrophy, small angulated fibres, or type grouping consistent with neurogenic damage could not be demonstrated in pelvic floor muscles of the female cadavers of reproductive age on whom these studies were conducted.

The levator ani have a higher proportion of slow fibers (66%) than found in other human female muscle (48%) and this explains their constant state of tonicity. Histopathological assessment has been used to assess indirectly the denervation and reinnervation of the levator ani muscles in women with incontinence and/or prolapse. In one such study, levator ani muscle biopsies were neither denervated nor reinnervated [49]. In the first study, a birth weight greater than 3,400gm was associated with a greater risk of neuropathy [13]. It is likely that the size of the fetus in proportion to the size of the pelvis is more important than birth weight alone.

In a cross-sectional study, the histomorphology of the pelvic floor muscles of 45 premenopausal unfixed and fresh female cadavers were studied [48]. The circumference of type I fibers was significantly larger in nulliparous women younger than 40 years compared to nulliparae older than 40 years. Compared to nulliparae, vaginal delivery led to a significant difference regarding the presence of centrally located nuclei, fibrosis, and variation in fiber diameter. No comparison was made with women who underwent elective caesarean section. In women with a history of vaginal delivery, no further increase in these characteristics could be detected with increasing age. Comparing the three different biopsy sites, all three changes were more pronounced in the ventral part. Increasing age as well as vaginal childbirth lead to histomorphological changes of the pelvic floor muscle that are consistent with changes of myogenic origin with no evidence of neurogenic damage.

2.2 Connective tissue

Abnormal connective tissue may be the key factor that leads to pelvic support disorders. These may be due to an intrinsic abnormality of collagen synthesis such as abnormal collagen or an imbalance between collagen types as well as an imbalance between synthesis and degradation or remodeling. Collagen is the basic structural element for soft and hard tissue in animals. It has various structural forms in different tissues and organs and is the main load carrying element in body organs attributing to most of their strength. Change of fibre configuration with straightening of the fibres attributes to 1% of the stretching. It is believed that most of the stretching is attributed to the basic alignment of the collagen molecules, the fifth segment in which the amino acids are more or less randomly placed [50]. Change in collagen metabolism result in an increased concentration of collagen and larger collagen fibrils. These alterations should rsesult in a more rigid form of extracellular matrix, suggesting a connective tissue with impaired mechanical function [51;52].

Elastin is the most linearly elastic biosolid material known. The human gene responsible for elastin synthesis is turned off at puberty and there is little change in the elastin content of an individual's connective tissue throughout life after this age. It has an almost linear loading curve. There is very little difference between the loading and the unloading curves showing very little energy dissipation.

In the human cervix, muscle forms only 10% whereas in the uterus it forms 30% to 40%; the rest is connective tissue. The surrounding ground substance consists mainly of mucopolysaccharides or glycosaminoglycans and tissue fluid. Dense connective tissue contains a small amount of ground substance whereas loose connective tissue contains a lot more. Fibroblasts are the main cells that produce the fibres and are embedded within the ground substance.

Enzymatic digestion of the noncollagen components can greatly change the mechanical properties of the tissue. There is a 'buckling' function of ground substance on collagen fibres and any change in content of the former will change the strength and elasticity properties of the tissue. Hydration of tissue greatly influences the tissue's mechanical properties. Other components of connective tissue include reticulin and the microfibrin network, which may provide a scaffold on which larger fibers are built and fibronectin which is thought somehow to modify collagen fiber formation.

Three papers and several conference abstracts have been published on collagen or other biochemical properties of the pelvic floor and its relationship to prolapse [9,10,12-14]. The methods have tended to report comparisons between vaginal epithelial tissue or endopelvic fascia from women found to have prolapse and arbitrary controls. Whether these histological and biomechanical changes are the cause or the effect remains unknown.

The focus of these studies has been piece-meal and a more systematic approach is desirable.

2.2.1 Histology and other scientific studies

Histologically the endopelvic fascia consists of moderately dense connective tissue that varies considerably in different areas of the pelvis. Smooth muscle similar to the deep aspects of the vaginal wall was reported to be present in the anterior and posterior fascia in one study [53]. Similarly Keane et al reported that the collagen in vaginal epithelial tissue closely resembles that in endopelvic fascia [54]. Makinen *et al* in a study of perivaginal fascia, demonstrated a decrease in the number of fibroblasts and an alteration in the orientation of collagen fibrils in women with prolapse compared with

normal women [55]. El Kholi and Mina reported reduced and fragmented elastic tissue in cystocoeles [56]. Yamamoto et al cultured biopsies for karyotyping by G banding from cardinal ligaments of women undergoing hysterectomies for prolapse and compared their results with controls undergoing hysterectomy for other reasons. There was a significantly less elastin production by prolapse fibroblasts and TGF-β1 increased elastin production by 4.6 fold in prolapse subjects as compared to 2.5 fold in the controls. There was an elevated elastin production in a dose-dependent manner but the maximum stimulation of elastin synthesis was significantly less in prolapse cases. The number of pregnancies and the prevalence of other predisposing factors in both groups were not discussed. These changes can therefore be secondary to differences other than prolapse. Using Northern blot analysis the levels of elastin gene expression were significantly down-regulated compared with that in the controls. The same authors reported similar findings with the growth curves of fibroblasts in patients with prolapse [57-59].

Sayer et al found that women with bladder-neck prolapse had an alteration of the collagen component of the pubo-cervical fascia in the absence of any evidence of denervation to either smooth or striated muscle [60]. Their findings were based on an increase in autofluorescence which they attributed to pyridoline crosslinking of collagen. They did not determine pyridoline directly and thus "advanced glycation products", which also fluoresce, could have been mistaken as pyridoline. Norton et al demonstrated that women with genitourinary prolapse had a high proportion of weaker type III rather than type I collagen, compared to women without [61]. Jackson et al found reduced total collagen content and decreased collagen solubility in premenopausal women with genitourinary prolapse [62]. Both intermediate intermolecular cross-links and advanced glycation cross-links were increased in prolapse tissue as compared with

controls. Collagen turnover, as indicated by matrix metalloproteinase activity, was up to four times higher in prolapse tissue. Collagen-type ratios, mature cross-link pyridinoline and total elastin content were similar in both prolapse and control tissues. They concluded that prolapse tissue had increased collagenolytic activity. The same group found that women with stress incontinence had less collagen, decreased ratio of collagen I to collagen III as well as less mature collagen compared to controls. However Norton et al found no such difference in women with urinary stress incontinence [61].

2.2.2 Biomechanical properties

Studying connective tissue is not complete unless one considers it as a whole. Biomechanical tests analyse the whole tissue rather than one or more of its components separately.

In engineering, the aim of understanding the mechanical properties of a material is to try to improve performance and maybe design better material structures. In mechanical testing, a material is elastic when it returns completely to its original shape after the force or load is removed, without the application of any compressive force. When there is residual deformation in the specimen, there is a component of plasticity in the properties of the material. In most instances, when a sustained load is applied to a biological specimen some elongation occurs with time, the relationship being influenced by viscosity (the power of resisting a change in the arrangement of the molecules) in the property of the material. Most biological specimens exhibit a mixture of elastic and plastic properties. In contrasts to elastic material, grease or putty show no tendency to recover their shape after being deformed and are said to be highly plastic. In reality, if stressed more than a certain limit, any structural material will fail to return completely to its original shape.

In 1678 Robert Hooke was the first to discover that for small strains, stress and strain are linearly proportional to one another [63]. Thus Load (F) / Area (A) = Stress σ and is the applied force per cross-sectional area. The response to stress is related to the strength of the material. Strain ε is the Displacement/Length or ΔL/ L and is the deformation of the material in relation to stress. The *modulus of elasticity* or Young's modulus of a material, E, is the material property that relates stress to strain. E is used to describe how stiff a material is in tension and compression and is independent of the size and shape of the specimen. Stress values that are critical in defining material behaviour are referred to as strength values. *Yield strength* is the stress at which plastic deformation begins. The ultimate tensile strength (UTS) is the maximum stress a material can undergo before failure or breakage of the material is imminent.

A large specimen can sustain a large force whilst a smaller specimen can take only a small force. Thus, the size of the test specimen is important to estimate the strength of the material under study. Hence we refer to force per unit cross-sectional area (a). In the International System of Units (SI units), unit of force (F) is the newton (N) and that of length is the meter (m). The unit of stress is newton per square meter (N/m^2) or pascal (Pascal). 1 MPa = 1 N/mm^2. A force of 1N can accelerate a mass 1 kg to 1 m/sec^2.

Hooke's law states that stress σ, is linearly proportional to strain ε until the material yields. The Hookean Elastic Solid is one that obeys Hooke's law. In reality, most living tissue does not obey this law. Of note is the fact that in the physiological state, many tissues are not unstressed as is evident when a broken tendon or blood vessel shrinks away when cut.

Strong structures fail either because elastic deflections become too large in them, or because plastic flow occurs leading to large plastic deflections or even worse to

fracture. Thus an understanding of the nature of plastic strength in connective tissue of the pelvic floor is considered important to explain the failure that results in genital prolapse. Plastic flow is very heterogenous, at virtually every level from atoms to large engineering structures. At each level, the heterogeneity manifests itself in different ways, all of which tend to be complicated.

2.2.3 Biomechanical properties of female connective tissue

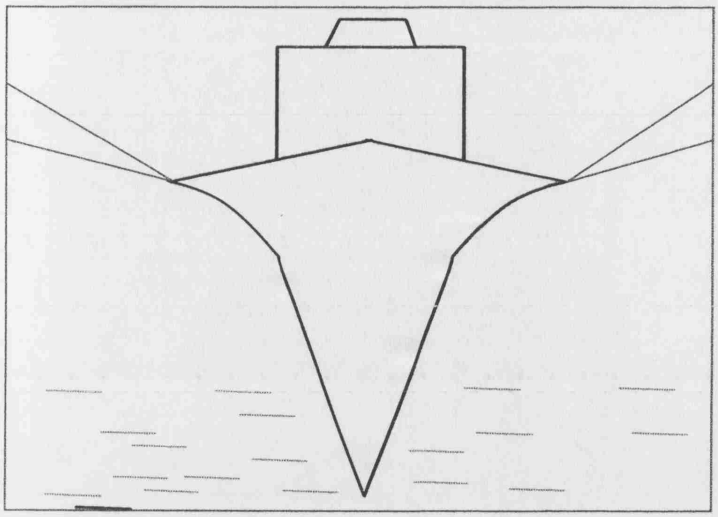
Landon and Smith investigated the effect of pregnancy on the rectus sheath [64]. A strip of fascia was obtained at the time of elective caesarean section and patients were followed up postnatally. Nine patients developed stress incontinence. When compared with continent pregnant subjects the ultimate tensile strength was reduced. The percentage strain at tissue failure and energy required for tissue failure were reduced but not significantly. The stress required for 10% tissue extension and Young's modulus were reduced whereas tissue thickness was increased in pregnant women but more so in those developing postnatal stress incontinence. These changes might indicate that in these women tissue may be more easily damaged and an insult such as vaginal childbirth can result in irreversible damage.

Most biomechanical experimental models are designed for testing ligaments and bone. Models for testing skin are often non-physiological as they involve testing the tissue to failure with the effect of strain rate not clearly defined and the elastic modulus obtained at high stress levels. For functional purposes, Goh et al reported that tissue properties at lower stress levels may be more meaningful and therefore used a different method [65;66]. They used frozen anterior vaginal wall tissue strips and measured small stiffness changes in tissue over a wide range of mechanical loading. The postmenopausal group had more severe prolapse (Baden stage 2-4, mean 2.8) than the premenopausal (range

2-3, mean 2.2) and five of the 10 postmenopausal women were on hormone replacement therapy. Premenopausal and postmenopausal women had equal elongation or long-term tissue deformation although postmenopausal tissue showed a higher elastic modulus. They interpreted this lack of difference as meaning intrinsically weak tissue in premenopausal women [66]. A control group of women without genital prolapse in their study would have helped to ensure this conclusion is justified.

2.3 Aetiology of genital prolapse

Pelvic floor dysfunction is likely to be multifactorial and this explains why it has been so difficult to identify women at risk. Although much has been written about the aetiology of pelvic floor disorders, we lack the scientific data to assess which theories are correct. It is theorised that when the levator ani muscle loses tone secondary to direct or neurological injury as well as intrinsic smooth muscle dysfunction, it drops from a horizontal to a semivertical position, predisposing to prolapse. Without adequate levator ani support, the visceral attachments of the pelvic contents are placed under tension and are thought to stretch and fail.



- levator ani muscle (pelvic diaphragm) and endopelvic fascia
- thickened endopelvic fascia forming the supporting ligaments of the uterus

Fig 2.1 Analogy of a boat in a dry dock

Fig 2.1 depicts an analogy that introduces the concept of pelvic floor disorders as a boat in a dry dock. The pelvic organs (the boat) are supported by water (levator muscles, including their fascia and ligamentous attachments to the pelvic side wall) and stabilized by its moorings (pelvic ligaments) [67]. If the water is drained, all of the weight of the boat must be supported by the moorings. If the moorings are cut or stretched, any changes in the level of the water will be evident immediately because the boat will drop in level.

Two main hypotheses exist to explain the aetiology of pelvic floor prolapse [67].

i. Anti-fascialist theory - Pelvic floor relaxation is secondary to muscle denervation or direct trauma of the pelvic supports that occurs following chronic straining or during vaginal childbirth. As the small nerves are torn away from their muscle fibers, the muscles' ability to contract is diminished and normal function is lost. Deterioration of

levator tone may result in damage to the endopelvic connective tissue and subsequent pelvic organ prolapse [47].

Site-specific defects - during childbirth, stretching or tearing can occur in the endopelvic fascia surrounding the vaginal wall resulting in herniation of pelvic organs. Nichols and Randall proposed an attenuation of the vaginal wall without loss of fascial attachment – *distension* cystocoele or rectocoele [68]. Anterior and posterior wall defects resulting from loss of attachment of the lateral vaginal wall to the pelvic side walls are described as *displacement* (paravaginal) cystocele or rectocoele.

ii. The fascialists theory – genital prolapse is secondary to inherently weak, abnormal connective tissue. Even the strongest muscle cannot exert any force without its connective tissue attachment to fixed bony structures. Failure of these tissues to provide support may be a failure of the collagen and other connective tissue that comprise the ligaments and fascial covering of the muscle fibres. Detachment of the pelvic viscera from their connective tissue attachments and inherent weaknesses in the pelvic connective tissues will result in pelvic organ prolapse [61;69].

Overall, it is likely that both suspension by the endopelvic fascia and ligaments as well as closure by the levator ani muscles interact to provide normal support.

2.3.1 Different mechanics of The Three Compartments of the pelvic floor

The mechanical axis of the pelvic floor changes following surgery. This, together with intrinsic weakness of the tissues, can result in prolapse in any of the other compartments. This is most commonly seen following Burch colposuspension for correction of stress urinary incontinence [70;71].

Similarly, after prolapse surgery, stress urinary incontinence can become apparent [72].

Amongst Swedish asymptomatic women, anterior or/and posterior vaginal wall

prolapse, were the most common forms of prolapse [73]. The findings were similar in a study of inner city Black women population [74].

Stohbehn et al reported on the characteristics and type of genital prolapse amongst young and older women [75]. Table 2.1 illustrates their results.

Table 2.1 Results of types of genital prolapse in the study by Stohbehn et al

Primary leading site	No. of young women(n=27	No. of older women(n=164)
Cystocoele/ urethrocoele	5(18.5%)	41(25.0%)
Uterine prolapse	16(59.3%)	37(22.6%)
Vault eversion	2(7.4%)	44(26.8%)
Enterocoele	0	29(17.7%)
Rectocoele	4 (14.8%)	13(7.9%)

Younger patients had prolapse at one site only, more often than older patients. These results show that middle compartment prolapse was more common in young women. Women thus might present in different ways and with different symptoms as age increases. In another study, magnetic resonance imaging showed multiple sites prolapse in chronically constipated patients, suggesting global pelvic floor weakness [76]. In contrast, in another study, the weakness was frequently posterior in faecal incontinent patients. Similarly, there was a difference in joint mobility between women with and without genital prolapse but no such difference was observed with stress incontinence [77]. Such studies can highlight the fact that the underlying intrinsic tissue properties in women with weakness in the different compartments might well be diverse.

Symptoms experienced by the patient do not necessarily correlate with compartment-specific defects. Thus an inverse correlation was observed between increasing severity of pelvic organ prolapse and urinary incontinence whereas digital manipulation and incomplete evacuation of the rectum as well as abstinence from sexual activity and sexual impairment was associated with worsening posterior compartment pelvic organ prolapse [78].

2.4 Epidemiology

2.4.1 Prevalence of Pelvic organ prolapse

Pelvic floor prolapse often does not become symptomatic until the descending segment is through the introitus and therefore it is frequently not recognized until end-stage disease. There is little specific data on the prevalence, incidence, natural history, or remission of genital prolapse in a community-dwelling population not seeking care for the condition. This points to a significant gap in our knowledge of a very common condition. Only a minority of patients with some degree of genital prolapse have, until very recently, volunteered their symptoms because of embarrassment and reluctance, under any circumstance, to admit to such problems. This forms a barrier to good prevalence data. There are no published data on remission. Clinically, pelvic organ prolapse does not seem to regress, although some improvement may be seen with the chronic use of a pessary. Some women progress rapidly from mild to advanced stages of pelvic organ prolapse, whereas others seem to remain stable for many years [18].

In 1995 a study from the USA reported a woman's lifetime risk for prolapse or incontinence surgery by the age of 80 years to be 11.1% [2]. The studied population was 149,554 women in the region whose inpatient and outpatient charts were examined. In another study of 487 women attending for cervical smear tests in Scandinavia, the

percentage of nulliparous women was 45.7%. Forty four percent of multiparous women had some degree of prolapse, including an absent urethrovesical crease, whereas the corresponding prevalence amongst nulliparous women was 5.8%. One out of 6 women reported to have prolapse, actually had absent urethro-vesical crease with no other pelvic floor defect. Amongst these women, 2% had prolapse that reached the intoitus. Two women had prolapse surgery and they were both over 50 years of age. The 487 women in this study constituted 76% of all eligible women and all were examined in the lithotomy position by any of 3 midwives who had a one-day training session to learn the ICS POP-Q examination (section 2.7.1)[73]. This study was not truly representative of the general population and any women who had undergone total hysterectomy for any reason, including uterine prolapse, would have automatically been omitted from the study, because they would no longer have required cervical smear tests.

The Oxford Family Planning Association study, followed up more than 17,000 British women aged 25 to 39 years from enrollment between 1968 and 1974 for as many as 26 years until 1994. The annual incidence of hospital admission with prolapse before age 60 was 2 per 1000 person-years [4].

2.4.2 Risk factors for Genital prolapse

a. Pregnancy and Childbirth

Increasing parity has shown a strong association with the development of pelvic organ prolapse. The great majority of women deliver vaginally and therefore vaginal birth, resulting in damage to the pelvic floor is thought to be the main aetiological factor for development of prolapse [2;4;12;14;17;28;79].

However, many women who bear children never develop symptomatic prolapse whereas nulliparous women can develop the condition [17;80]. The role of specific events of the pregnancy, labour and delivery process in the development of genital prolapse is controversial. Most symptomatic genital prolapse occurs temporally distant from pregnancy or vaginal childbirth and therefore it has been difficult to study the relationship between the two in detail. Boyle et al examined 21 pregnant and 21 non-pregnant nulliparous women between the ages of 18 and 29 years [81]. All patients in the non-pregnant group had a POP-Q stage (see section 2.7.1) of 0 or 1, whereas 47.6% of the pregnant subjects had POPQ stage 2 ($p < 0.001$). Significant differences were noted for points Aa and Ba, Ap and Bp, as well as pb and tvl. Thus pregnancy was associated with increased POP-Q stage compared with non-pregnant control subjects. A report from Australia on pelvic floor symptoms from a five-year prospective follow-up after childbirth are expected shortly. Five years are unlikely to show any trends for genital prolapse but this is a start [82]. Although it is generally believed that genital prolapse develops secondary to prolonged complicated labours which are preventable by elective caesarean section, it has been reported that prolapse can also follow a precipitate labour which can cause over-distention and tearing [83;84]. However, precipitate labour may reflect intrinsically weak pelvic floor tissue and these women may have a genetic predisposition to prolapse. The controversy between the role of childbirth and pregnancy in the development of urine incontinence and genital prolapse has recently been given ample attention in urogynaecology. Chaliha et al screened all women who had three elective caesarean sections as selected from a database of 40,000 women delivering between 1977 and 1998 [85]. They also recruited age-matched women having three vaginal births and all women answered validated urinary and bowel symptom questionnaires. The results of this study showed that caesarean section was associated

with a lower risk of urinary incontinence, although a protective effect on development of faecal symptoms was not seen.

In a study from the West Midlands, a total of 184 primiparous women who delivered by caesarean section (104 emergency; 80 elective) and 100 other women who delivered vaginally were interviewed 8 to 12 months postpartum [86]. A comprehensive bowel function questionnaire was completed. Severe anal incontinence followed elective and prelabour emergency caesarean in some women.

Symptoms in most women with stress urinary incontinence start during pregnancy rather than in the post-natal period, indicating that pregnancy rather than childbirth damage the continence mechanism [87;88].

Recently, MacLennan et al, surveyed 4410 households and interviewed women between 15 and 97 years of age [11]. Pregnancy (> 20 weeks), regardless of the mode of delivery, greatly increased the prevalence of major pelvic floor dysfunction, defined as any type of incontinence, symptoms of prolapse or previous pelvic floor surgery. Compared with nulliparity, pelvic floor dysfunction was significantly associated with caesarean section (OR 2.5, 95% CI 1.5-4.3), spontaneous vaginal delivery (OR 3.4, 95% CI 2.4-4.9) and at least one instrumental delivery (OR 4.3, 95% CI 2.8-6.6). The difference between caesarean and instrumental delivery was significant ($p<0.03$) but was not for caesarean and spontaneous delivery.

The precise role of pregnancy and delivery towards different types of pelvic floor dysfunction remains unclear. The evidence in the literature is controversial.

It is imperative that we consider the long-term implications of our obstetric practices, as well as the immediate effects. Norton described three phases to describe the effect of childbirth [67]. Injury during delivery is followed by the immediate repair phase and the

long-term maintenance phase during which factors such as age and disease influence the healed injury and may alter the body's ability to compensate for it.
On the one-hand what may seem like a major injury at childbirth may soon heal with no long-term consequences. On the other hand, what may initially seem to be a minor injury, can become clinically important years later after age and disease have taken their toll. Thus, up to 35 % of nulliparous and 44% of multiparous women with vaginal deliveries get anal sphincter disruption but few are symptomatic [89]. Incontinence of flatus or faeces can develop in some women with seemingly intact anal sphincters [90;91].

Other epidemiological studies tried to investigate the effect of parity as well as which pregnancies cause the main damage to the pelvic floor. In the Oxford family planning study, women with two children were 8.4 times as likely as nulliparous women to have prolapse requiring hospital referral [4]. The risk was increased eleven fold in women with more than four deliveries. In a cross sectional study of perimenopausal women attending a network of menopause clinics in Italy, with a prolapse incidence of 5.5%, the odds ratio for women having three or more births was 3 as compared to nulliparous women [92]. The total number of nulliparous women was not reported. In these studies, no comparison could be made between women with vaginal deliveries and those with emergency and elective caesarean section only as their number was too small.

The mother's position in second-stage labour, method of pushing, and administration of epidural analgesia were all claimed to be protective against perineal trauma and future pelvic floor weakness but definite evidence is lacking [93]. Descent of the perineum six to eight weeks post- partum during rest or during straining was observed by several investigators [94-96]. Simultaneously there was a considerable decrease in strength of

contraction assessed with vaginal cones, intravaginal squeeze pressure measurements and with a digital muscle strength score [97-99]. Anal sphincter pressures [94] as well as other clinical anal function [100-104] were used by other researchers to assess changes in the pelvic floor following childbirth but none of these methods have been shown to be reliable.

Since the opening in the pelvic floor through which the fetal head must pass is relatively small, the head pushes the floor downward until it has dilated the opening sufficiently to pass through. It is believed that descent causes not only potential elongation damage of the levator ani muscles, but also stretches their attached nerves, raising the possibility of neuromuscular injury. Since the nerve begins at the ischial spine and runs on the muscles' inner surface, damage can occur from direct elongation. The more the floor must descend before the head passes through it, the greater the possible neural injury. In addition, further downward descent would cause more damage to connective tissue supports and attachments.

To minimize the amount of floor descent, a strategy originated in the 1920s to perform a generous mediolateral episiotomy to open the birth canal. An outlet forceps delivery was believed to reduce the descent. Similarly, an anaesthetic that paralyzed the pelvic floor muscles would further ease birth. Because of easier passage, there would be less forceful descent and less traction on the nerves going to the pelvic floor musculature. Also lessened would be traction on the pelvic connective tissue, thus diminishing chances of immediate injury. These strategies were predicated on intervening before floor descent occurred, and therefore needed to be instituted in advance of marked downward displacement. Studies comparing 1,000 women with controls indicate that they did, in fact, help limit immediate damage to the pelvic floor. [105]damage to the pelvic floor [105].

Moreover, long-term follow-up data, covering a period beginning in the late 1940s, revealed that protecting the pelvic floor by the methods cited above effectively reduced incidence of genital prolapse later in life. It would be expected that prevalence of prolapse would be unaffected during the first 20 years after instituting a program of protecting the pelvic floor since women of an age likely to develop prolapse would have been delivered with older methods. However, once the women studied reached an age when prolapse might be expected, prevalence of cystocele, rectocele, and enterocele decreased significantly. Analysis showed this result couldn't be explained simply by changes in demographics or parity [106].Women in this study were not randomised and therefore results should be interpreted with caution.

More recently, midline episiotomies have unequivocally been associated with an increased incidence of third and fourth degree tears whereas mediolateral episiotomies were shown to be protective [107;108 109].

Röckner found that patients with mediolateral episiotomy had considerable impairment of pelvic floor muscle strength compared with those with spontaneous or no laceration [110]. A similar study concluded that episiotomy does not prevent pelvic relaxation [111].

Klein and co-workers found that for every 30-minute increase in the second stage of labour the odds of having a vaginal tear decrease by a factor of 0.49 [112]. Thirteen women had such a tear amongst 459 mothers in this randomized study of liberal versus restricted use of midline episiotomy. Seventy five women sustained a third or fourth degree tear. These women significantly performed less strenuous exercise at least three times per week. However, exercise did not protect women against vaginal tears.

The correlation between birthweight, malposition such as persistent head deflexion and consequent operative vaginal delivery and prolapse is inconsistent [2;73;113;114].

Maternal age and weight at the time of delivery, forceps delivery and prolonged second-stage of labour have all been mentioned as aetiological factors but reports are conflicting [2;11;12;17;79;114-116].

b. Connective tissue Weakness

Connective tissue is the 'cement' of the body. Genitourinary prolapse is a common finding in women with Ehler-Danlos syndrome [117]. This syndrome together as well as Marfan syndrome and osteogenesis imperfecta form the heterogeneous group of rare genetically determined connective tissue or collagen diseases.

Joint hypermobility, as is most commonly estimated by the Carter and Wilkinson or Beighton score, is a feature common to all connective tissue diseases, but it is also a feature of the general population [118]. It will be described in more detail in sections 2.6 and 3.1. In conjunction with joint hypermobility, there are well recognised clinical markers of weak connective tissue as named in the Revised Brighton criteria for diagnosis of the Benign Joint Hypermobility Syndrome- BJHS [119]. These criteria are also discussed in more detail in section 2.6.

The association of joint hypermobility and genital prolapse was originally described by Al Rawi et al in Iraq [120]. In the control group (n=76), 18% of women had joint hypermobility as compared to 66% in the prolapse group. The mean parity in both groups was more than 6.0.

Norton et al further modified Beighton criteria and used only the upper limb joints for assessment of joint hypermobility, using an arbitrary lower angle to diagnose little finger's hypermobility [77]. They recruited 108/110 consecutive women from the practices of three university gynaecologists. Recruited women were predominantly white caucasian and their mean age was 53.9 years and their mean parity was 2.8

amongst cases and 3.1 amongst controls. After answering a questionnaire, each patient was examined for genital prolapse and stress incontinence in the standing straining position. Joint mobility was assessed by a blinded researcher and patients were divided into 2 groups; those with and those without joint hypermobility. Although there was no difference in stress incontinence between the 2 groups, women with joint hypermobility had a higher prevalence of cystocoele, rectocoele and vault or uterine prolapse that reached the introitus and beyond (odds ratio of 2.7 to 3.7) even after adjusting for confounding variables such as previous pelvic surgery. One should note that the above 2 studies investigated women with different ethnicity, age and parity.

c. *Chronic medical disease and lumbar lordosis*

Young patients undergoing surgery for genital prolapse have a higher than expected prevalence of congenital anomalies, as well as rheumatology and neurological diseases [75;77;121]. Olsen et al reported that women with prolapse had higher incidence of chronic lung disease and smoking history; the two being related [2]. Similarly, women with prolapse had reduced lumbar lordosis and a pelvic inlet that is orientated less vertically downwards than women without prolapse [122].

d. *Ethnicity and Family history*

There may be differences in frequency among racial groups or families, but it is unclear whether these differences are intrinsic or due to the effects of bowel habits, diet, work patterns or access to health care. Sometimes, the family history is unclear in these patients and may be related to the stigma associated with these conditions in past generations, where women simply did not report prolapse or incontinence to their

physicians or families. However, several studies showed up to 30% familial incidence of genital prolapse [17;123].

In 1977, Zacharin, reported a low prevalence of uterine prolapse and stress incontinence in Chinese women. After performing dissections on cadavers he reported differences in the pelvic supporting tissue in Chinese compared with caucasian females.[124] In 1996, Brieger and colleagues from the Chinese University of Hong Kong reported that an audit of major gynaecological surgery for benign conditions in 5 major public teaching hospitals in Hong Kong revealed that genital prolapse and/or urinary problems were not uncommon [125]. These authors concluded that there appeared to have been a significant change in the prevalence of these two conditions in Hong Kong in the previous 20 years. This apparent increase may represent a legacy of Western diet or practices, as well as the consequent increase in longevity and in the standard of living in Hong Kong Chinese. Previous under-reporting of the condition by women might play a significant role.

In a retrospective study at the university of Michigan, 176 black nulliparous women were less likely to deliver with second-degree or greater lacerations and more likely to deliver with their perineums intact than 1633 white women [126]. For Asian women of Chinese or Indonesian origin a nonsignificant increase in risk of 'severe tears' was found. Combs *et al.* also found a similar increase for Asian women compared with North European caucasian women [127]. Green & Soohoo reported a significant odds ratio of 2.9 for Chinese women compared with North European caucasians and speculated that a possible explanation for this increased risk might be the commonly observed short perineum in Chinese/Indonesian women [128]. These differences may also be

explained by ethnic differences in the tensile strength and elasticity of tissues.

Functional and morphologic differences were also detected in the urethral sphincteric and support system of black and white women [129]. Other cross sectional studies consistently found a lower prevalence of urodynamic stress incontinence in black women [74;130]. Additional comparative studies are required.

e. Age

There is much interest in the effects of aging on tissue structure and function. In skin, the conversion of proline to hydroxyproline increases with age, and this increase seems to be influenced by environmental factors including physical exercise. In cartilage, proteoglycans undergo rapid reversible deformation, resulting in fragmentation and weakness. [25,26]

Similarly, tissue atrophy in the pelvic connective tissue would occur, precipitating genital prolapse. Paradoxically, in most elderly women the vagina and supportive tissues are unusually rigid and stenotic and prevent genital prolapse.

f. Constipation

The role of constipation and pelvic floor weakness is not well known, although its presence and change in pattern following pelvic floor surgery is well documented [6;131-133].

Chronic constipation with repeated prolonged defaecatory straining efforts has been shown to contribute to progressive neuropathy and perineal descent. Thus constipation is considered to be a major inciting event for pelvic floor dysfunction [134]. Older women with urinary incontinence have been shown to be significantly more likely to have both constipation and faecal incontinence than are women without urinary incontinence,

lending further evidence to the association [135]. In one case-control study, constipation and straining at stool as a young adult before the onset of pelvic floor dysfunction were significantly more common in women who subsequently had pelvic organ prolapse (61%) or stress urinary incontinence (30%) than in women who did not have pelvic floor dysfunction(4%) [136]. Many of these women also needed to digitate to achieve rectal evacuation. Magnetic resonance imaging clearly shows the pelvic floor configuration on straining with pelvic visceral prolapse. Prolapse frequently involves multiple sites in constipated patients, which is suggestive of global pelvic floor weakness[76] . Pregnancy may lead to the onset of chronic constipation which might be an aetiological factor in its own right [136].

g. Occupational and Recreational Stresses

The incidence of prolapse is higher in housewives than in women with a career and tertiary education [17]. Similarly, it is higher in assistant nurses [137] . It seems that occupational or recreational activities that result in excessive or repetitive increases in abdominal pressure contribute to the development of pelvic floor weakness. Just as heavy lifting predisposes as individual to an inguinal hernia or lumbar discopathy, the same process can cause prolapse or herniation of the pelvic floor [138]. A high prevalence of urinary incontinence is found in women performing high impact activities such as gymnastics, track and field games [139].

h. Surgery

The mechanical axis of the pelvic floor changes with pelvic surgery, precipitating genital prolapse [4]. Bladder neck suspensions either contribute or unmask pelvic organ prolapse. Surgery for pelvic organ prolapse which unmasks urinary incontinence is

familiar to all surgeons who operate on the pelvic floor [71;140]. Other predisposing surgery includes hysterectomy, rectal surgery and extensive pelvic dissection [141]. Davis et al reported genital prolapse in 3 young female soldiers during or after rigorous airborne training. All three women had undergone laparoscopic uterine nerve ablation (LUNA) for refractory dysmenorrhea [138].

2.5 Labour and childbirth

2.5.1 Connective tissue in the pregnant female

When the uterus expands during pregnancy, the amount of collagen is increased seven to eightfold, only to be rapidly resorbed in the first few weeks after delivery [142]. In the rat cervix, the ratio of fibre to ground substance is 1:1.5 in the non- pregnant state whereas it becomes 1:5 towards term pregnancy [143]. During pregnancy, the ground substance increases at the rate of the overall growth while collagen increases more slowly and elastin and reticulin almost not at all. Post-partum, the distensibility increases but the strength decreases. If stress is calculated by excluding ground substance and including only the estimated collagen fibres cross-sectional area, the collagen framework per se is actually stronger at one day post-partum in the rat uterus than in the nulliparous animal. In the non-pregnant uterus, there are cyclical changes in water content but not in dry weight [144]. The maximum tensile strength decreases when the tissue is swollen. It is unknown whether these data can be extrapolated to the human cervix.

Peripherally, pregnant women seem to develop a generalised joint laxity. Thus, Bird et al observed the laxity of the metacarpophalangeal joint of the left index finger in 68

pregnant women (33 weeks gestation) and again postpartum (mean 15.1 weeks) using a finger hyperextensometer. This device records deflection in response to a constant preset torque [145]. Laxity was independent of age. Mean laxity during pregnancy was 5 degrees more than postpartum ($p<0.02$). They reported an increase in laxity which was significantly greater in women in their second pregnancy as compared to their first, but did not alter further in the third and fourth pregnancy.

Joint laxity in pregnancy may cause backpain in 50% of all pregnant women [146;147]. Two main types of such pain have been described [148]. The first is low lumbar back pain thought to be a result of increased laxity of the posterior longitudinal ligament. The second type is sacro-iliac pain thought to be due to decrease in tensile strength of capsule and ligaments of the sacro-iliac joint resulting in instability. The overall incidence of back pain 1 to 2 months post partum in one study was 44% [148]. Predisposing factors for new-onset post partum back pain were increased weight and shorter stature but other clinical connective tissue markers were not assessed. Chan et al reported a strong correlation between high signal MRI intensity in the cervix due to hydration and softening of the cervix and back pain which was not explained by MRI changes in the spine. They suggested soft tissue laxity as an important cause of backpain in pregnancy [149]. Unfortunately this study did not report the progress of labour in women with and without backpain or with different tissue intensity in the cervix.

At present we do not know the exact mechanism that initiates and supports labour. There have been multiple rejected hypotheses in the literature. Many chemical substances have been investigated but as yet no one substance has been proven to be useful. A positive correlation was found between duration of labour and tumour and cytoskeleton protein tissue polypeptide antigen (TPA) [150]. Similarly a negative correlation was detected between the 2,3-DPG in maternal red blood cells and the

duration of labour [151]. In one study, cervical "ripening" was attributed to a breakdown of collagen and elastin by enzymes that are present in precursor forms [152]. Given the appropriate signal, these enzymes convert the inactive collagenase and elastase, and can rapidly degrade collagen and some elastin. This breakdown results in a loss of mechanical strength of the cervix, which eventually permits passage of the fetus. The connective tissues responsible for support of the uterus and upper vagina are immediately adjacent to the collagen that breaks down in the dilating cervix. It is easy to imagine that a similar degradation occurs in the pelvic ligaments and fascia.

2.5.2 The progress of labour

There are several known factors which are associated with the duration of labour but the exact mechanism that triggers or supports labour is unknown. Childbirth is a multifactorial process and at present we cannot identify women who will have a long obstructed labour from those who will not.

In the 1960's through to the early 1980's, O'Driscoll and colleagues suggested that any nulliparous women with a rate of cervical dilatation below the average of 1cm/hr should be augmented [153]. Philpott et al demonstrated that the rate of maximum slope of cervical dilatation had a median of 1.25cm/hour amongst 200 women in South Africa and the partograph was introduced [154] . In 1975, Duignan et al reported the characteristics of normal labour in 1306 white, Asian and black women in a London hospital [155]. This was soon after the introduction of the Philpott partograph into obstetric practice at the Maternity and Dudley Road hospitals in Birmingham, UK. Cervical dilatation-time curves revealed no significant differences in the progress of normal labour in the

different racial groups. Thus active management of labour with artificial rupture of membranes and use of syntocinon would be used in about 50% of nulliparous women. As explained in section 1.1, caesarean section rates have continued to rise in Britain over the last decade. On the other hand, the Dublin National maternity hospital reported a stable caesarean section rate of 15.6% in 2002 (web page for the hospital- www.nmh.ie). Their annual delivery rate is more than 8000 births and 48% of women are nulliparous. This hospital emphasizes on antenatal education and the continuous presence of a 'personal nurse' in labour. There is evidence that support from caregivers reduces the need for analgesia in labour [156]. This in turn might affect the duration of labour and the cascade of other possible complications leading to interventions. Strict criteria for diagnosis of labour with early amniotomy, 2-hourly vaginal examinations and oxytocin augmentation if progress of labour is slow are also used in this hospital. Several studies however, showed that active management of labour using these latter measures alone, reduced the duration of the first stage of labour without affecting the rate of caesarean section [157] .

Several other factors contribute to the progress of labour. Mechanical factors such as occiput posterior presentation and persistent head extension also result in prolonged labour in nulliparous women [158;159].

Meta-analyses of randomised controlled trials concluded that in women using epidural analgesia, the first stage of labour is prolonged by 26 minutes and the second stage of labour is prolonged by about 15 minutes [160]. Multiple physiological factors and decreased maternal effort during the second stage of labour resulting in instrumental delivery, make it difficult to isolate the effects of epidural analgesia on uterine function and the pelvic floor. Recent studies comparing ambulation versus no ambulation during epidural analgesia have shown that ambulation does not shorten the duration of labour

[161]. In another study involving seventeen maternity units in the North West Thames Health Region, involving 25,069 women, the duration of the second stage and the use of operative intervention and instrumental deliveries were positively associated with the use of epidural analgesia [162]. Maternal age, fetal birthweight and maternal height were also independently associated with the duration of the second stage. There were small but significant differences in the characteristics of women using epidural analgesia and those using alternative methods of pain relief. Parous women using epidural analgesia behaved in a similar manner to nulliparous women without epidurals. Within the region, the epidural rate in individual units positively correlated with the overall forceps rate, the rate of caesarean section in the second stage of labour as well as the duration of the second stage. Nesheim et al found that induced labours were 1.9 hours shorter that spontaneous labours in nulliparous women. In the same study, the duration of labour was positively correlated with BMI, negatively correlated with maternal height but was not affected by maternal age [159]. Birthweight showed strong correlation with the duration of labour in 1000 term nulliparous women observed in 1988 by Turner et al [159;163].

Intrinsic properties of the pelvis might also be a factor [164]. In a recent study, Dietz et al showed that relaxation of pelvic ligaments as measured by translabial ultrasound during the third trimester in 160 nulliparous women, was significantly associated with a reduced length of the second stage of labour [165]. This was mainly due to short passive stage rather than the active stage. In this study, the distance in millimetres between the lowest position of the bladder neck, bladder, cervix and rectum after three to six Valsalva manoeuvres and the lower margin of the symphysis pubis were measured. There was a statistically significant association between the mode of delivery and pelvic

organ descent with the lowest mobility seen in women who required a caesarean section in the second stage. Upper limb joint mobility of the dominant side did not correlate with the mode of delivery. To avoid the confounding effect of cervical compliance, women who did not reach the second stage (16%), were not included in the analysis. Some of these women must have had caesarean section for failure to progress. Labour was induced in 21/160(13%) women and the rate of epidural analgesia or its potential confounding effect was not accounted for. Similarly, there was no stratification of indication for intervention deliveries and potential confounding factors such as maternal age or BMI and birthweight were not assessed.

2.6 Joint Mobility

Although joint hypermobility in its extreme form may be associated with a hereditary disorder of connective tissue, it is now widely acknowledged that normal subjects exhibit varying degrees of joint laxity and so-called hypermobile people merely represent the upper end of the Gaussain distribution for joint mobility. Measurement of joint laxity is the least invasive and most promising clinical method described in the literature to measure connective tissue properties. In 1964, Carter and Wilkinson described five criteria to measure joint laxity [166]. Beighton et al modified these criteria in 1973 [15]. These researchers and others after them, showed good intra- and inter-observer reliability [15,16,18]. One intrinsic difficulty in measuring passive movements, is that the observed movement depends on the force applied to the moving part. This is likely to depend on both the enthusiasm of the observer and the pain threshold of the subject.

For this reason, several other methods have been reported in the literature to investigate joint mobility. They include radiological assessments[167] as well as the use of specially designed devices known as joint hyperextensometers[168]. Although these methods led to more reproducible results, they fell out of fashion because they are invasive and not practical to screen a large number of patients.

The Beighton criteria are:

1. passive dorsiflexion (hyperextension) of the little fingers beyond 90° from the horizontal to lie parallel to the extensor aspect of the forearm.
2. passive opposition of the thumbs to the flexor aspect of the forearms.
3. hyperextension of the elbows beyond 10° from the horizontal.
4. hyperextension of the knees beyond 10° from the vertical plane.
5. forward flexion of the trunk with the knees straight so that the palms of the hands rested easily on the floor.

One point may be gained for each side for manoeuvres 1 to 4, so that the hypermobility score will have a maximum of 9 points if all are positive. Norton et al found that women with genital prolapse had an increased joint mobility score. In this study, they used a modified Beighton score and assessed upper limb joint mobility only [77]. An angle of 55° or more from the horizontal was required to diagnose hyperextension of the little finger.

The 9- point Beighton score is an arbitrary all-or none test that takes no account of the degree of laxity itself. The Contompasis semi-quantitative scoring system [169] is often used by rheumatologists to quantify the degree of hypermobility but to our knowledge

there has been no attempt to quantify joint laxity or rigidity by any scoring system, in the general population.

Many population–based studies in the literature, reported trends in Beighton score amongst different age groups and ethnic populations [170-172]. The range of normal joint movements decreases rapidly throughout childhood and adult life. Although no large scale comparative studies have been carried out, there is a strong clinical impression of a racial variation in joint mobility [173]. For instance, Indians show more hyperextension of the thumb than Africans, who in turn have greater hyperextension than Europeans [167].

No large studies have screened for joint laxity amongst adult women. Amongst 637 white blood donors in the United States, 10% of white American women in the sample were hypermobile [174]. In 18% of women in the control group in Al Rawi's study [120], and up to 20% of South African adult females in Beighton's study [170] had clinical joint hypermobility using the Beighton score.

Assuming an intact tendon apparatus, normal articular movement is a function both of the stretch of joint capsule and ligaments as well as of muscle tone. Most studies confirmed that joints on the dominant side are less mobile than joints on the other side [170;171 167]. The dominant side is likely to have more muscle tone than the less frequently used non-dominant side. This would be reflected in a smaller range of movement for a given force on the dominant side. In the literature, there are no prospective general population studies that reported on the rate of change of joint mobility with increasing age.

Amongst individuals who are not affected by connective tissue disease, joint hypermobility has long been associated with multi-systemic signs and symptoms which reflect the hyper-elastic characteristics of their connective tissue [175]. Consequently, the

term benign joint hypermobility syndrome or BJHS was gradually recognized by rheumatologists over the last few decades [119]. A set of validated diagnostic criteria - The Revised Brighton criteria for the diagnosis of BJHS, were eventually published in July 2000. They are:

Major criteria

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

2. Arthralgia for longer than 3 months in 4 or more joints

Minor criteria

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

2. Arthralgia (≥ 3months) in 1-3 joints, or back pain (≥ 3 months), spondylosis, spondylolysis/spondylolisthesis

3. Dislocation/subluxation in more than one joint, or more than once in one joint

4. Soft tissue rheumatism ≥ 3 lesions (e.g., epicondylitis, tenosynovitis, bursitis).

5. Marfanoid habitus (tall, slim, span/height ratio >1.03)

6. Abnormal skin: striae, hyperextensibility, thin skin, papyraceous scarring.

7. Eye signs: drooping eyelids or myopia or antimongoloid slant.

8. Varicose veins or hernia or uterine/rectal prolapse.

9. Requirement of high doses of local anaesthetic as normal doses are not effective

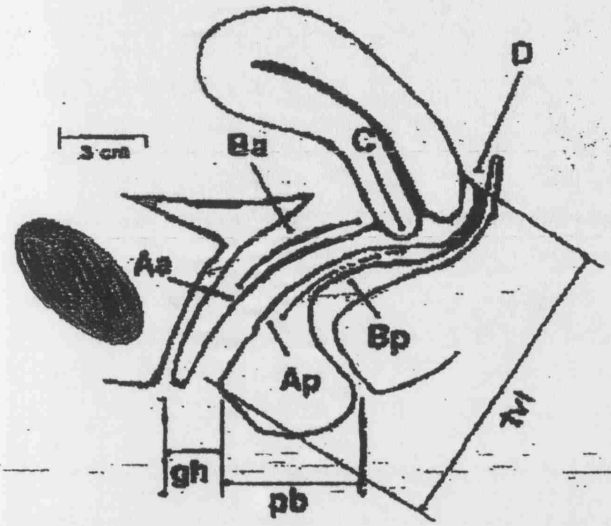
Further reference will be made to these criteria in the studies described in this thesis.

2.7 Examining pelvic organ prolapse

2.7.1 The pelvic organ prolapse quantification system (POP-Q)

Until recently, different gynaecologists have used different classifications for genital prolapse. The most popular classifications were described by Porges[176], Baden[177] and Beecham [176]. The POP-Q system, accepted by the International Continence Society, the American Urogynecologic Society, and the Society of Gynaecologic Surgeons for the description of pelvic organ prolapse is currently the most quantitative, site-specific system for describing pelvic organ prolapse [178]. It describes the topographic position of six vaginal sites (Aa, Ba, Ap, Bp, C, D) and gives information regarding perineal descent and the change in axis of the levator plate based on increases in genital hiatus(gh) and perineal body(pb) measurements. The POP-Q examination proforma used in our department is shown here.

Name Hospital number Date of birth Consultant Date	PLEASE COMPLETE DETAILS OR AFFIX STICKY LABEL



Key to diagram

Anterior wall Aa	Anterior wall Ba	Cervix or cuff C
Genital hiatus gh	Perineal body pb	Total vaginal length tvl
Posterior wall Ap	Posterior wall Bp	Posterior fornix D

Aa Fixed point on anterior vaginal wall, in midline, 3cm proximal to external urethral meatus
Ba Variable point on anterior vaginal wall - most distal/dependent point of any part of ant vaginal wall from vault to point Aa
C Distal edge of cervix or vaginal cuff if hysterectomy
D Posterior fornix/POD in women with cervix
Ap Fixed point on posterior vaginal wall as point Aa but 3cm proximal to hymen
Bp Variable point on posterior vaginal wall as point Ba
gh middle of urethral meatus to posterior midline hyme
pb from distal genital hiatus to midanal opening

The reference points are the external urethral meatus anteriorly and the hymen posteriorly. All measurements are made with the patient straining maximally.

Patient

Aa	Ba	C
gh	pb	tvl
Ap	Bp	D

Normal values

- 3 Aa	- 3 Ba	- 8 C
2 gh	3 pb	10 tvl
-3 Ap	-3 Bp	-10 D

Reference

RC Bump et al. The standardization of terminology of female pelvic organ prolapse and pelvic floor dysfunction. *Am J Obstet Gynecol* 1996;175(1):10-17

ProlapsescoringApril2000

2.7.2 Staging of Pelvic organ Prolapse based on the POPQ examination

The staging of the POP-Q classification is described as follows:

Stage 0 - No prolapse in demonstrated

Stage 1 - The criteria for stage 0 are not met but the most distal portion of the prolapse is more than 1cm above the level of the hymen (ie. its quantitation value is < -1cm).

Stage 2 - The most distal portion of the prolapse is 1cm or less proximal to or distal to the plane of the hymen (ie. its quantitation value is ≥ -1cm but ≤ +1cm)

Stage 3 - The most distal portion of the prolapse is more than 1cm below the plane of the hymen but protrudes no further than 2cm less than the total vaginal length in centimetres [ie. its quantitation value is > +1cm but < + (TVL-2)cm]

Stage 4 - Complete eversion of the total length of the lower genital tract. The distal portion of the prolapse protrudes to at least (TVL-2)cm. In most instances the leading edge of the prolapse will be the cervix or the vaginal vault if a hysterectomy had been performed.

It has been shown in several studies that the lithotomy, sitting or standing positions are better than the supine position to evaluate the full extent of genital prolapse [178-180].

2.8 Summary

1. Our understanding of the role of different support structures and nerve supply of the pelvic floor in genital prolapse is limited. Pelvic floor prolapse is likely to be multifactorial and this explains why it has been so difficult to identify women at risk.

Although much has been written about the causes of pelvic floor disorders, we lack the scientific data to assess which theories are correct.

2. Connective tissue properties might be the key factor that leads to pelvic support disorders. Added risk factors, then result in genital prolapse. Each factor may interact in a variety of ways. Collagen is the basic structural element for soft and hard tissue in animals but it is certainly not the only component of connective tissue. Extracellular matrix and the other fibres might well have an important role but their assessment in the pelvic floor has been very limited. Various histological and biomechanical changes have been found in genital prolapse but association is not causation and whether these are the cause or the effect remains unknown.

3. Increasing age, ethnicity, pelvic floor surgery, occupational and recreational stress and constipation as well as joint hypermobility and chronic medical diseases have all been associated with genital prolapse. Pregnancy, vaginal childbirth and increased birthweight of the newborn are also associated with the condition. The extent to which pregnancy, labour and vaginal delivery attribute to genital prolapse is currently unknown. Childbirth itself is a multifactorial process and at present we do not understand the exact mechanism that leads to a long obstructed labour in some women but not in others.

2.9 Objectives of this thesis

Our hypothesis is that connective tissue plays a role in the outcome and progress of labour. Events occurring during childbirth as a reflection of intrinsic connective tissue properties, then further increase the risk of prolapse in genetically predisposed women. This thesis investigates the role of connective tissue in childbirth and genital prolapse. We hypothesised that prolapse of different compartments has different underlying pathology as manifested in a different pattern of women's first labour.

Strategy and Study Plan used to investigate the objectives:

Initially, clinical and in-vitro biomechanical connective tissue properties were investigated in women with and without genital prolapse. The former was investigated mainly through the assessment of joint mobility. Several in-vitro biomechanical parameters simulating physiological activity, were investigated in women with and without prolapse, using rectus sheath to represent systemic connective tissue and the anterior/posterior vaginal wall as well as the round and uterosacral ligaments to represent the pelvic connective tissue.

In the second and third studies, the first labour in women with genital prolapse as well as in nulliparous women was investigated in some detail. Joint mobility was the main clinical connective tissue parameter assessed but several other signs and symptoms were investigated. A model equation predictive of the duration of the second stage of labour in nulliparous women was generated in the last study. In the literature, this is the first model that attempts to include connective tissue as represented by joint mobility as a predictor of the progress of labour.

CHAPTER III

METHODS

3.1 Investigation techniques

3.1.1 Measuring Connective tissue function in vivo - Joint Mobility

Joint mobility was measured as follows: I demonstrated the methods to each patient who then performed the required tasks. Standard pictures of joints were used to demonstrate what I was aiming to assess – Images 1 to 5 in fig 3.1. The measurement was performed after three attempts at the task to ensure 'warming up' of the joint.

1. Passive dorsiflexion (hyperextension) of the little fingers : the angle of the main axis of the finger with the horizontal was measured using a small joint goniometer.

2. Passive apposition of the thumbs to the flexor aspect of the forearms was performed and the distance between tip of distal phalanx and forearm was measured using a small ruler in increments of 0.5cm.

3. Hyperextension of the elbows: subjects placed their elbows against a plain cardboard on the wall and marks were made along the main axis of the humerus and forearm bones, just proximal and distal to the elbow joint. Each mark was extended to meet the other and the angle made with the horizontal was then measured using the small joint goniometer. This angle was recorded as 1 on the database if it formed an angle of 190 ° or beyond. Otherwise the joint mobility was recorded as 0. Women were asked to passively hyperextend their elbows as much as possible.

4. Hyperextension of the knees was recorded in the same way. Women were asked to push their knees as backwards as possible as they stood against a plain cardboard on the wall. The angle made by the long bones, just above and below the knee anteriorly, was drawn in pencil on the cardboard. A goniometer was then used to record the angle. This

angle was recorded as 1 on the database if it formed an angle of 190 ° or beyond. Otherwise the joint mobility was recorded as 0.

5. Forward flexion of the trunk was then performed with the knees extended and attempt was made to rest the palms of the hands on the floor. A successful attempt was recorded as 1 on the database. If unsuccessful, a zero was recorded on the database.

Women with a history of fracture, severe trauma or other disability in any single joint, which could have affected intrinsic mobility, did not have the affected joint assessed and on the data was left blank.

For the reasons explained in section 2.7, the left-sided joints were used in the analysis. Dominance was not taken into account and this could have skewed the final results.

Knee joint mobility as well as the ability to touch the palm of the hands to the ground were not assessed in the first study which compared the in-vivo and vitro connective tissue properties in genital prolapse. This follows the study by Norton et al which found a difference between upper limb joint mobility in women with and without prolapse [77].

Fig 3.1 Demonstration of joint hypermobility

3.1.2 Examination for Genital Prolapse

I used the POP-Q classification system to examine women participating in the in-vitro/in-vivo connective tissue and genital prolapse study.

Prior to examination, all women were asked to empty their bladder. In the United Kingdom, it is uncommon to have lithotomy beds or birthing chairs in gynaecology wards or out-patients clinics. Hence, after examination in the semisitting position, each subject underwent examination in the standing position. All women were asked to perform Valsalva maneuver as well as cough strongly. The most dependent findings were recorded. Women with known genital prolapse were asked to confirm the full extent of the perceived prolapse by self-digital palpation. Measurements were obtained using a wooden spatula marked at 1cm intervals and recorded at 0.5cm increments. Gh, pb and tvl (total vaginal length) were measured in the semi-sitting position as they supported their backs against pillows or against the lifted head rest of the examination couch. These points were obtained at rest when the patient was not straining. An accompanying health carer recorded the measurements instantly.

A minus sign for the measurements in centimetres reflected a point above the introitus inside the vagina. All other measurements without the minus sign were those beyond the hymenal ring.

3.1.3 Structured Interviews /connective tissue assessment

a. *The first labour and Genital Prolapse*

This study was conducted in Malta (section 3.3.2).

Each subject was interviewed and the questions in Appendix I - Interview No.1 were asked. To assess a history of heavy lifting, present and past occupations were recorded. A history of farming or caring for bed-ridden patients or relatives as well as factory work involving lifting of heavy bundles was recorded as positive. A positive smoking history was recorded if subjects had ever smoked more than 10 cigarettes daily for more than 5 years in their lifetime. These 2 questions are arbitrary and have not been validated.

If the subject passed hard stools requiring straining, three times per week or less often, it was recorded as a positive finding. This question was not validated, either.

A family history of genital prolapse, urinary stress incontinence, constipation, haemorrhoids and all the symptoms assessed by the Brighton criteria was also recorded. If two or more first or second degree relatives had the condition, the response was considered positive.

The Brighton revised criteria (section 2.6) were assessed [119]. These criteria have been validated by the rheumatologists. The relevant signs and symptoms were assessed. Rheumatologists subjectively assess skin hyperextensibility by holding a flap of skin over the dorsum of the hand and then watch it unfold back into place. In the beginning of the study, I attended several rheumatology clinics at University College London hospital to learn how to assess for this sign but assessment is entirely subjective. For this reason, this sign was not assessed in this study. The span/height ratio was not measured either.

I subjectively decided whether the patient had enough varicose veins or skin striae. No objective scoring system was used to ensure an accurate quantification.

b. Connective tissue markers in nulliparous women and their progress of labour

This study was also conducted in Malta (section 3.3.2).

The questions shown in Interview 2 - Appendix I, were asked as translated in English.

A positive smoking history was recorded if subjects had ever smoked more than 10 cigarettes daily for more than 5 years in their lifetime. This question has not been validated.

If prior to pregnancy, the subject passed hard stools requiring straining, three times per week or less, it was recorded as a positive finding. This question was not validated, either.

A family history of genital prolapse, urinary stress incontinence, constipation, haemorrhoids and all the symptoms assessed by the Brighton criteria were also recorded. If two or more first or second degree relatives had the condition, the response was considered positive.

The Brighton revised criteria (section 2.6) were assessed as discussed above in section 3.1.3 (a).

For backpain, the response was recorded as positive if the symptoms were present prior to the pregnancy. I assessed whether the patient had abundant striae or varicose veins during the interview. I then decided on a positive or negative finding.

3.1.4 Data Collection for patients' obstetric notes

Following consent, history taking and examination, the following information was collected from the patients' obstetric notes:

Maternal Age at the first delivery

Maternal Weight and Height at the first delivery

Induction of labour

Augmentation of labour with syntocinon

Length of first stage of labour

Length of active first stage of labour (4 to 10 cms)

Length of second stage of labour

Child's Birthweight/ Head circumference

Type of perineal trauma

Indication for caesarean section / instrumental delivery

Cervical dilatation at Caesarean section

3.2 The in-vitro and in-vivo Connective Tissue Study in Genital Prolapse

3.2.1 Methods - women in this study were recruited from the Whittington hospital and the Elizabeth Gareth Anderson hospital in London. The study had local ethical committees' approval- 2001/07 – The Whittington hospital and 2001/101 at The Elizabeth Gareth Anderson hospital. All women between 18 and 70 years of age, who required surgery through a lower median, paramedian or suprapubic transverse incision were asked to participate for the rectus sheath study. Amongst eligible women, 85% consented to take part in the study. Seventy patients underwent gynaecology surgery and 3 patients underwent general surgery.

The criteria which excluded women from entering this study were:

- known collagen disorders or long-term steroids use. In these two groups of patients, connective tissue elasticity is known to be altered.
- pregnant or lactating women and women less than twelve weeks postpartum were also excluded for the same reason.
- patients with expected metastatic malignant disease were excluded.
- rectus sheath and round ligament biopsies only were taken from women with localised gynaecology malignancy.
- women with previous abdominal scars at the line of incision of the current surgery did not have any rectus sheath biopsies taken.

Recruitment for elective surgery was performed either during pre-assessment clinics or on the gynaecology wards prior to their surgery.

Women undergoing abdominal hysterectomy were eligible for rectus sheath and pelvic tissue sampling. Pelvic connective tissue biopsies were taken from the round and uterosacral ligaments as well as anterior and posterior vaginal adventitia. Rectus sheath was not taken from women undergoing vaginal hysterectomy with or without repair. In

women undergoing anterior or posterior wall repair, corresponding biopsies were taken from the midline of the vagina nearest the cervix.

I explained the study to all patients and gave them written information to read. If they agreed to participate, they signed a consent form. Each patient was asked relevant demographic data together with relevant personal history. Each patient then had a Beighton joint examination as well as a vaginal examination for genital prolapse using the POP-Q scoring system.

All data were recorded immediately onto a personal computer. Under the Data Protection Act (1984), information on patients was stored using a coded system. Interpreters accompanying patients who spoke or understood little or no English were asked to interpret for me when required.

Biopsies:

Each hysterectomy or repair procedure was performed routinely. Rectus sheath biopsies were sampled during entry. For vertical abdominal incisions the rectus sheath at the lowermost part of the incision was sampled. The central part of the sheath was sampled in low suprapubic transverse incisions. At hysterectomy, the round and uterosacral ligaments on one side as well as both anterior and posterior vaginal edges in the midline, were excised by the surgeon to ensure a 1cm length of tissue for the study. For anterior and posterior wall repair, biopsies were taken from the redundant vaginal skin. Biopsies were taken as near as possible to the cervix in the midline. A blade or scissors was used to take the biopsies. Tissue desiccated with diathermy was avoided. Tissue biopsies were collected immediately and stored in a Ca- free HEPES- Tyrode's solution in a polypropylene container. They were put inside a 4°C refrigerator or

transported immediately to the laboratory at the Urology Institute in the Middlesex hospital and processed. Transport was at room temperature and took up to 30 minutes. All biopsies were processed within 8 hours of surgery.

Solutions: *Tyrode's solution:*

The superfusing solution used to irrigate tissue strips during all the experiments was modified Tyrode's solution. This solution is a standard physiological solution used to ensure that tissue is kept in the best possible conditions. Each litre of freshly made solution consisted of:

Chemicals	Concentration (mM)	
NaCl	6.896g	118.0
$NaHCO_3$	2.016g	24.0
KCl (1M stock)	4 ml	4.0
$MgCl_2.6H_2O$ (1M stock)	1 ml	1.0
$NaH_2PO_4.H_2O$(1M stock)	0.4 ml	0.4
$CaCl_2.6H_2O$(1M stock)	1.8 ml	1.8
Glucose	1.10g	6.1
Sodium pyruvate	0.55g	5.0

HEPES Tyrode's solution	Concentration (mM)
NaCl	105.4
$NaHCO_3$	22.3
HEPES	19.5
KCl (1M stock)	3.6
$MgCl_2.6H_2O$ (1M stock)	0.9
$NaH_2PO_4.H_2O$(1M stock)	0.4
Glucose	5.4
Sodium pyruvate	4.5
NaOH(1M stock) to buffer	

Fresh Tyrode's solution was made up almost daily in deionised water from a Milli-RO 10 plus system (Millipore, Croxley Green, Watford, UK). The constituents were added in solid form or from 1M stock solutions (KCl, $MgCl_2$, and NaH_2PO_4). The stock solutions had been made up in Analar grade water and stored at 4°C in a refrigerator for less than 4 weeks except $CaCl_2$ which was purchased as a 1M solution (BDH Chemicals Ltd). The compounds in the solution were Analar grade agents from BDH Chemicals Ltd. (Poole, Dorset, UK). Solids were weighed using a Sartorius balance accurate to ±1mg and liquids were measured using calibrated, fixed or variable volumetric pipettes.

Care was taken to avoid dessication of tissue at any time from tissue sampling to processing during experimentation. The tissue was handled using disposable gloves according to the departmental safety instructions and all wastes were disposed for autoclaving in yellow bags for incineration. Tissue wastes were placed in their transport plastic containers prior to disposal. All instruments were soaked in Haz –tablets (sodium dichloroisocyanurate) solution which was made by dissolving one Haz tablet in 500mls of water.

Tissue preparation

Tissue samples were placed in a dissection dish containing Tyrode's solution and secured with fine entomology pins. With the aid of a binocular dissecting light microscope (x 20 magnification: Nikon S.M.5, Projectina company, Skelmorlie, Ayreshire) the fibre orientation was ascertained, and parallel cuts made along the fibres to dissect tissue strips. This was relatively easy for rectus sheath but could be difficult with any of the other biopsies.

Fine suture silk (8/0) was then passed under the ends of the biopsies and tied sufficiently tight in a reef knot to secure the tissue without cutting into it. The free ends of thread were loosely knotted, and the anchored tissue ends were then carefully cut free. A strip, ideally 3mm in length and <1mm in diameter resulted. Avoidance of any stretching was paramount.

Tissue mounting and equipment set up

Strips of tissue were then transferred to the superfusion trough. Transfer of the tissue from the dissection dish to the trough was as swift as possible, to prevent tissue exposure to the air being in excess of a few seconds. The horizontal Perspex trough was 3mm in cross section. One end of the tissue strip was tied to a hook on an isometric force transducer (Stratham UC2, Bishops Stortford, Herts,UK). This facilitated adjustment of the resting length of the tissue strip.

Care was taken to ensure the transducer hook was only in contact with the tissue preparation and in no way touching the trough as this would prevent accurate tension recording. The other end of the tissue strip was tied to a hook projecting from a lever-arm system (see below).Throughout the experiment the tissue was completely immersed in superfusate to prevent desiccation.

The mean diameter of the strip was then estimated using the graticule of the light microscope. Three measurement were taken and the mean value was used in the analysis. There was a maximum difference of 10% in the diameter of a minority of strips. The raw data is presented in the attached CD (Strip measurements). These measurements enabled later computation of the cross sectional area.

Cross sectional area = πr^2 where π is a constant and r is the radius of the strip.

Similarly, the length of the strip was measured in mm using the graticule.

The apparatus was mounted on a heavy metal frame, standing in tubs of sand in order to minimise external vibration.

Mechanical stability of the preparation was ensured by performing experiments on a steel table top (24" x 24 "x 1 ") which was isolated from its base by anti-vibration padding (Neoprene-Teflon composite, S.K. Bearings Ltd. Cambridge UK). The perfusion bath was screwed into aluminium slots bolted to the steel table top.

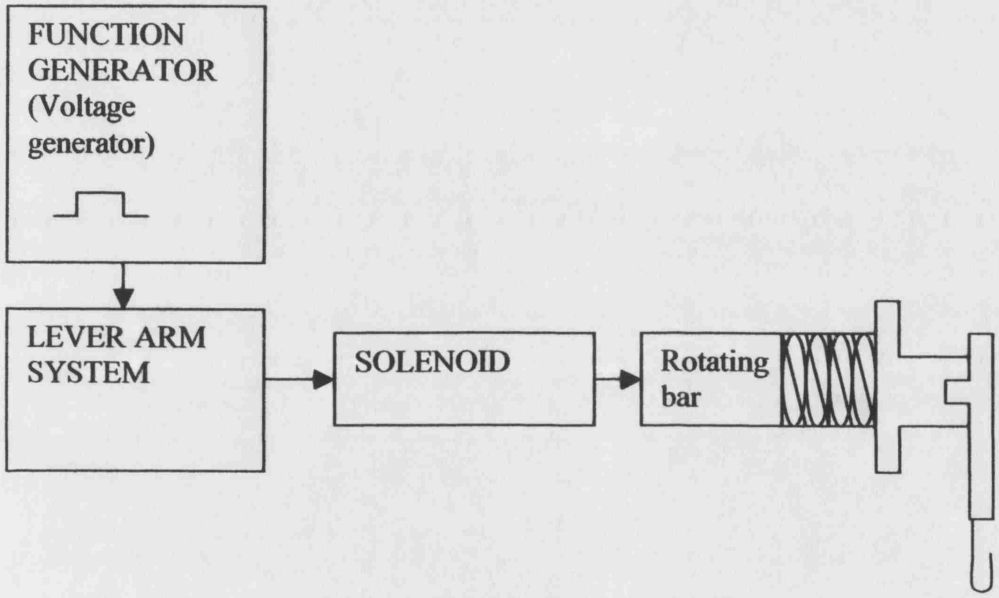
The perfusing Tyrode's solution was warmed in a water bath maintained at 37°C (Tecam E7, Tempette), supported on a shelf three feet higher than the experimental table, and equilibrated with 95% oxygen and 5% carbon dioxide, maintaining a pH of 7.33+/- 0.02. The solution was fed to the tissue preparation by gravity, in tubing enclosed within circulating water at 37°C, at a flow rate of 4 ml/min. The superfusate then drained directly to waste.

Lever-arm system

Fig 3.1 shows the Lever-arm system used to impose rapid maintained changes of strain to the tissue strips.

In principle, the lever-arm system (Cambridge- Model 6800 HP) consisted of a metal bar within a solenoid. Imposition of a voltage across the ends of the solenoid rotated the bar. Changes to voltage were delivered via a lever-arm system (Model 308B) (Cambridge Technology Inc., Cambridge MA. USA. Model 6800 HP). Lever-arm system was in turn driven by a function generator that delivered square-wave voltage signals of adjustable duration and magnitude- Fig 1. This signal was then amplified by the lever-arm system and was then transmitted to a solenoid. Across the coil in the solenoid, the square wave voltage signal reached a rotating bar attached to it. This bar had an attached hook to which the tissue biopsy strip was attached in the trough. The rotating bar moved each time the signal was generated and stretched the attached tissue

strip. A force-transducer transmitted the extension and relaxation changes in stress of the tissue strip to the attached oscilloscope. Thus the extension and relaxation stress curves were generated as seen on the oscilloscope(red curves in Fig 3.3). The lever arm system was also attached to the oscilloscope as seen in fig 3.2. Thus the square strain waves showing the generated voltage and change in length of the tissue strip were recorded (blue wave lines in fig 3.2).



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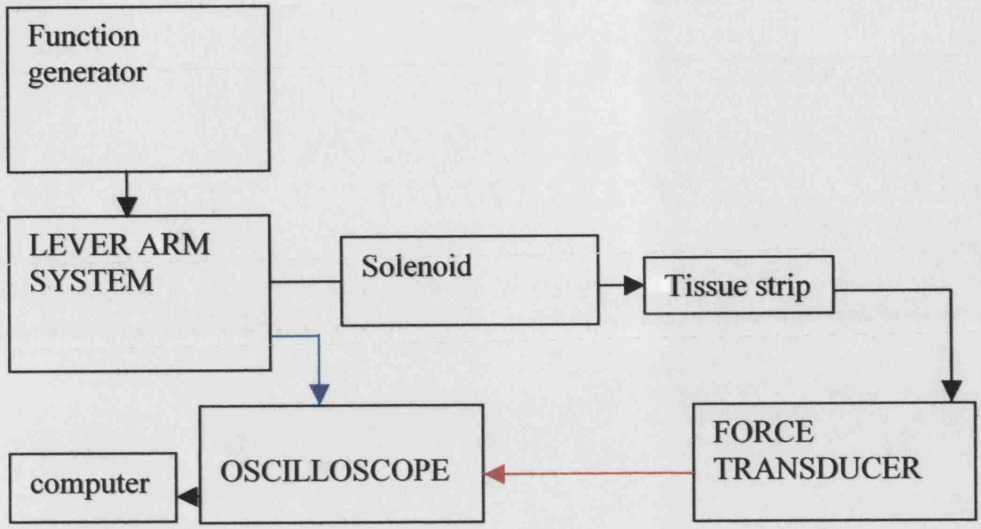


Fig 3.3 The tension- recording system

Tension Recording

The force transducer output was connected to a bridge amplifier, with variable gain (ORMED Amplifier Series;ORMED Ltd. Welwyn Garden city, Herts, UK). Strain was applied to the other end of the specimen via the lever-end system.

Isometric tension was recorded via the tension transducer which formed one variable resistance of a Wheatstone bridge $R_{SG,}$ within the bridge amplifier. The Wheatstone bridge was polarised to 5V. Changes to stress on the tension transducer were manifested as a change to R_{SG} and a change of voltage across the bridge output which was subsequently amplified with an operational amplifier in closed loop configuration.

To zero the voltage output of the bridge amplifier to correspond to a state of zero strain, the bridge was balanced by imposing a voltage on the negative input of the amplifier. The output from the bridge amplifier was subsequently low pass filtered with a corner of frequency 15Hz. The signal was displayed on a digital storage oscilloscope (Model OS4200, Gould Electronics Ltd., Ilford, Essex, U.K.). Parallel output of signals to a storage oscilloscope was helpful for checking calibration levels, verifying that all

circuits/strain gauge were intact, and for general monitoring purposes during an experiment. Strain voltage signals were then sampled directly into a computer via a 12-bit A/D converter. A block diagram of the experimental system is shown in fig 3.3. This equipment been used by 2 other research fellows at Middlesex hospital. It was purpose-built for the biomechanical study of bladder and pelvic floor tissue.

3.2.2 Experimental Protocol

For most strips, three increasingly larger changes of strain were applied to the tissue strips. Each strain was applied as a step change for 45 to 50 seconds, after which it was returned to control. The stress response to each change in strain was then recorded via the force transducer.

To allow for preconditioning, a minimum of five loading /unloading cycles of the tissue strips were performed before recording was started. The same procedure was performed for each different strain applied to the strip.

Stored data were then transferred to a personal computer and subsequently processed. Initially, data were converted to Microsoft Excel® format and then imported into Kaleidagraph® (Synergy software, Reading USA). A stress-strain curve was constructed for the different loading and unloading cycles, from which the principal steady state and time dependent parameters were obtained.

Strain values were normalised to the length of each strip to obtain proportional changes of strain imposed on the tissue preparation. Stress values were normalised, by dividing values with the cross sectional area of the preparation.

Other studies investigated Young's modulus and the maximum tensile strength and strain of tissues. For this study, it was felt more appropriate to investigate tissue

undergoing physiological stress and strain situations that mimicked the pelvic floor in real-life situations. Tissue was strained (stretched) and relaxed back to its original length. Stress and strain changes were recorded as follows:

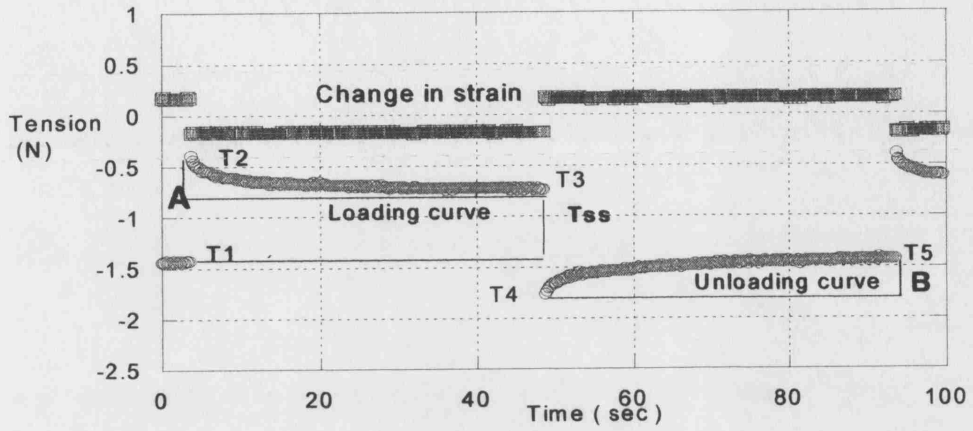


Fig 3.4 – Loading and unloading curves in response to one change of strain

Fig 3.4 shows an example of loading and unloading curves for one change in strain on a tissue strip. The blue tracing represent the change of strain and the red curves show the consequent change of stress in the tissue preparation. As displayed in this figure, a downward deflection on the strain curve represents lengthening of the tissue strip (loading).

A change of length to the preparation generates an instantaneous increase of stress followed by a partial time-dependent dissipation to a new steady-state level. Return of strain to the control level resulted in an under-shoot of stress followed by a return to the control level. The following parameters were measured:

T1 is the *resting stress* in the tissue preparation

T2 is the *instantaneous maximum change in stress on extension* of the tissue strip. The stress decreases exponentially with time as the length of the specimen remains constant.

T3 is the *steady state stress on tissue extension*

T4 is the *instantaneous undershoot in stress on return of strain to control*

T5 is the *steady state stress on return to control.*

If changes in stress were non-plastic during this cycle, T5 should be equivalent to T1.

Analysis

The stress curves exhibited both a time-dependent component and a steady state component. In other words, the sudden change in stress generated by stretching or relaxing the tissue gradually reverted back towards its resting state although the length of the tissue remained constant. Analysis of the curve was carried out to estimate the magnitude of these two components as well as the time dependence of the temporal component.

In general the analysis of stress during imposition and relaxation of strain were identical. The derivation below applies to the period of the test-cycle when the tissue strip was stretched, ie. strain was increased. The change of stress, T, as a function of time, T(t), was modeled as an instantaneous change of T(t), followed by an exponential decay to a steady state value, Tss. Equation1 describes this function.

Equation 1: $T(t)=A^* \exp(-t/\tau)+Tss$

where A is the magnitude of the time-dependent component, τ is the time constant of stress-relaxation to a steady state level, Tss -refer to fig 3.3.

In Fig 3.3, A + Tss represents the instantaneous change in stress equivalent to T2. This analysis was achieved by fitting the stress curve to equation 1, using Kaleidagraph®. The programme provides a curve-fitting routine employing a least squares analysis with the operator inputting estimated values of the desired parameters A, Tss and τ.

In the results section, values of τ for an increase of strain (as above) are quoted as τ1, and corresponding values of τ2 were obtained for the unloading curves.

Values of the magnitude of the time dependent relaxation of stress during the unloading curves are quoted as values of B, by analogy to values of A for the loading curves.

Fig 3.4 shows an example of a decay curve with stress values estimated according to equation 1 shown above. The initial estimates of the parameters were altered if necessary to optimise the fit as judged by achieving a value of correlation coefficient, τ, as near to 1.0 as possible.

All values were entered in a Microsoft Excel® database and then imported into STATA® for statistical analysis.

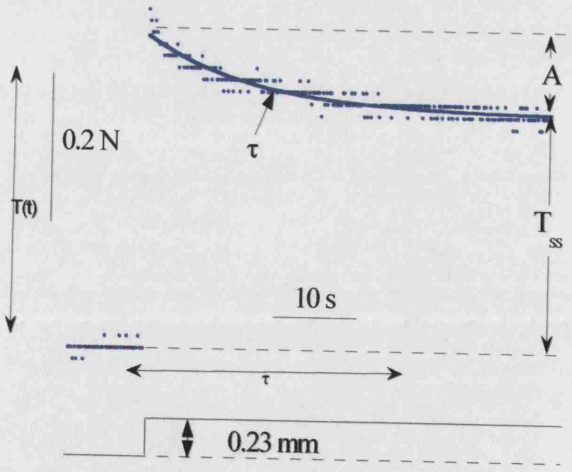


Fig 3.5 –Stress changes due to a step change of strain of 0.23mm on a strip of rectus sheath. The curve is the best-fit to the stress-relaxation component

Calibration of the force transducer

The signal from the tension recorder was regularly calibrated by suspending fixed weights from the hook of the transducer at each gain setting of the recording apparatus. The deflection was then plotted as a function of the weight m. The mass of the weight was expressed as a force, F, in Newtons. F = mg where g =9.81m/sec^2. Fig 3.6 shows an example of such a calibration. The slope of the graph was then estimated to produce a calibration factor to equate deflexion (mm) to force (N).

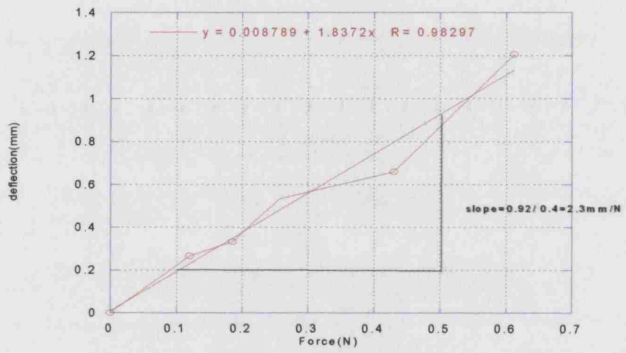


Fig 3.6 – Calibration of the force transducer; an example

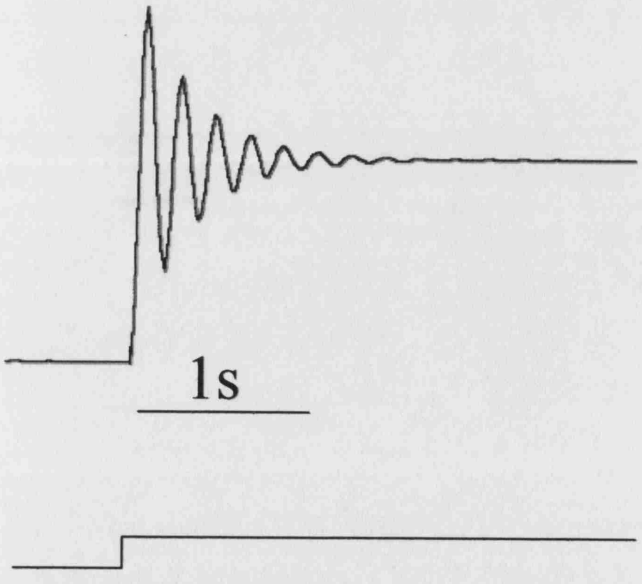


Fig 3.7 Frequency response of the experimental system

Fig 3.7 shows a recording of a force recorded by the transducer when a metal bar was substituted for the tissue sample in the experimental system. A step change of the solenoid bar caused a damped oscillation which can be attributed to the resonance within the force-transducer. The oscillations subsided completely within approximately one second. This was much more rapid than the visco-elastic relaxation rate with the tissue strip in place. Therefore the resonance within the experimental system itself did not interfere with the estimation of the visco-elastic properties of the tissue preparations.

Fig 3.8 shows an example of a graph performed to calibrate the voltage imposed on the solenoid and the distance moved by the attached rotating bar/hook.
In the example given in fig 3.8, each 3.04V imposed on the solenoid moved the attached strip by 1mm. Thus the distance between the blue lines in fig 3.4 was then multiplied by 3.04 to obtain the actual amount of stretching on the tissue strip.

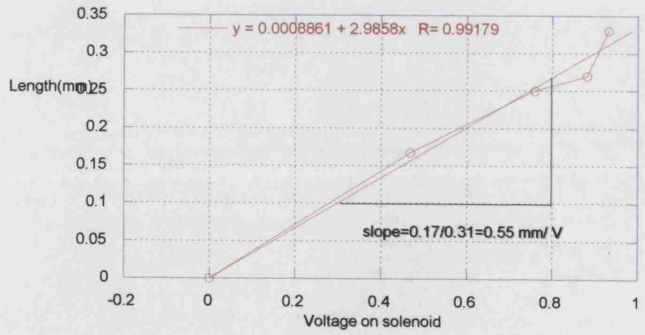


Fig 3.8 – Calibration of the solenoid; an example

3.3 Genital prolapse and the progress of the first labour

The local ethics committee approved the study- Ref No. 2002/73. The objective of this study was to compare obstetric data of the first delivery in women with or without genital prolapse surgery. From available literature, an 80% power for the study required the recruitment of 90 women in each arm, to detect a 20-minute difference in the first and second stage of labour.

The inclusion criteria to enter this study were:

- patients who underwent genital prolapse surgery between 1999 and 2001 as recorded on the gynaecology operating theatre register at St Luke's teaching hospital, Malta were eligible if they were under 50 years of age.
- the control group of women were recruited from gynaecology/smear test clinics in district health centres as well as in hospital. They were recruited if they denied any history of genital prolapse or a history of a genital bulge or protrusion.
- women must have delivered their first baby at St Luke's teaching hospital, in Malta. It was not possible to retrieve obstetric notes from private obstetric hospitals practicing in the 1970's as they have closed down and others have replaced them. Women who had their first baby in some other country, were also excluded.

Exclusion criteria were:

- women with diagnosed collagen disorders or on long-term steroids
- pregnant women or women less than twelve weeks postpartum as pregnancy is known to enhance the viscoelastic properties of connective tissue
- lactating women for the same reasons mentioned above
- patients with metastatic malignant disease

Women who had undergone prolapse surgery, were contacted by telephone and the aim of the study explained. Amongst eligible women, 90.4% (94/104) agreed to participate and were given an appointment to be seen by myself. Women in the control group were recruited whilst they were waiting to be seen in clinics as explained above. All participants were given a patient information leaflet to read, and then signed a written consent form for participation in the study as well as to permit access to their obstetric notes.

Each patient was asked a structured set of questions about their demographic data and family history as well as the Brighton criteria and bowel habit- Appendix I (A). Beighton joint examination was then performed for each participant. Obstetric data was collected during the following few weeks, from the patient's obstetric notes stored at the medical records department of the hospital. All the data were stored immediately on a personal computer and a number was assigned to each patient to maintain confidentiality.

The objectives of this study was to compare the obstetric history of the first labour in women with and without genital prolapse. All women were under or 50 years of age. The caesarean section rate amongst the former was 13.8% as compared to 7.3% in women without prolapse. This will be discussed further in section 4.5.2 in page 159. Logistic regression was used to account for confounding variables.

3.4 The relationship of connective tissue markers and the progress of labour in nulliparous women.

The population of Malta in 2001 was 393,447. The national obstetric database in Malta detected 1684 nulliparous women over 18 years of age who had their first baby in 2001. Women who had their baby in private clinics or who underwent elective caesarean sections were excluded from the study. For this cross-sectional study, 1153 eligible women who fitted the inclusion criteria discussed below, were detected. The local ethics committee in Malta approved the study- Ref No. 2002/74. It was started in July 2002. Consecutive nulliparous women who delivered their first child between January and December 2001 were eligible for this study. St Luke's teaching hospital and Gozo district general hospital are the only two obstetric national health care hospitals on the islands. In both hospitals, epidural analgesia is not available except in very special circumstances. Onset of labour is defined by regular uterine contractions associated with progressive effacement and dilatation of the cervix. Following spontaneous ruptured membranes at term, induction of labour is commenced within 24 hours with the use of syntocinon. Examinations are usually performed by the same midwife/obstetrician and the aim is for one to one midwifery care in labour. Cervical assessment during the latent first stage is performed every four hours and every two hours in the active phase. Amniotomy is performed if progress is not satisfactory. Poor progress in labour is defined as less than 1cm/hour in the active first stage of labour. Oxytocin augmentation using a standard protocol, is commenced if progress in labour is delayed and continuous monitoring is used thereafter. Fetal scalp pH analysis is not available for suspected cases of fetal distress and emergency caesarean section is performed for all such cases.

The absence of descent of the baby's head after 30 minutes of pushing or delivery not imminent after one hour of pushing in the second stage of labour defines poor progress of the second stage of labour.

Eligible women were selected from the labour ward registers for 2001 held at the Department of Obstetrics at St Luke's hospital in Malta as well as the Gozo General district hospital. The information collected from the register was:

i. Name of patient

ii. Address

iii. Telephone number if present.

Missing telephone numbers were retrieved from the PAS hospital system or from the national telephone directory website.

The exclusion criteria were:

i. Mothers less than eighteen years of age at the time of delivery

ii. Breech deliveries

iii. Stillbirth or early neonatal deaths as detected on the register and from the Special Care Baby Unit register (information collected by SCBU sister)

iv. History of drug abuse as recorded on the register

iv. Less than 37 weeks gestation

v. Elective caesarean section or emergency caesarean when labour had not already started

vi. The few patients who had epidural analgesia were excluded

Between July and the end of September 2002, eligible women visiting the well-baby clinics in the local health centres were asked to participate in the study. The paediatric national health service, offers free regular baby check-ups as well as vaccinations to

everyone. All other mothers were contacted by phone and after explaining the aims of the study, were asked to participate. Women were excluded from the study if at the time of recruitment they were:

i. Pregnant again

ii. Had their second child and were now less than three months post-partum

iii Breastfeeding

iv. On oral steroid use or had known connective tissue disorders

Women who agreed to participate, were given an appointment to be seen at one of the five local main health centres. To encourage good attendance, some interviews and examinations were conducted on the premises of local town councils. Interviews were held as early as 07.00 hours to enable women to attend on their way to work and as late as 20.00 hours including Saturdays. This involved intense concentration on the project on my side. On Sundays, I embarked on contacting patients as I considered it the best day to find women at home, if during the week I had been unsuccessful.

After reading an information leaflet, they signed a written consent form. Then they answered all the relevant questions of a standardised interview. Their joint mobility was assessed according to Beighton criteria.

The obstetric data were then obtained from the patients' notes which are kept at the medical records department. Data were entered directly on a personal computer and patients were given an identification number so as to safeguard patient confidentiality.

3.5 STATISTICS

STATA intercooled Version 7.0 was used for statistical analysis. The Null hypothesis was rejected if $p \leq 0.05$. Shapiro Francia W` test was used to check for normal distribution. Cross tabulation of categorical data were analysed with Pearson's x^2 test or Fisher's exact test. For continuous variables which were normally distributed, Pearson correlation (r) test and Student's 2-sample t test were used as appropriate.
Wherever appropriate a relative risk and 95% confidence intervals were calculated. The confidence intervals for the difference in means was estimated for normally distributed continuous data.

Non-parametric methods which are based on analysis of ranks rather than actual data were used to analyse continuous variables which did not have a normal distribution. To analyse continuous- with- categorical variables the Kruskal-Wallis test or the Mann-Whitney test (also known as the Wilcoxon two sample test) were used. The Mann-Whitney test requires all the observations to be ranked as if they were from a single sample. Then the sum of the ranks in one group is calculated and a p value found from specific tables.[181] The Kruskal-Wallis test is a more general form of the Mann-Whitney test and if the Kruskal-Wallis test is applied to just 2 groups of observations, exactly the same result is obtained as that from the Mann-Whitney test.
Spearman correlation (r_s) was used to analyse the association between two continuous variables. Correlation evaluated the extent to which two random variables were related and regression analysed the relationship between the variables.

For the in-vitro tissue study, *Wilcoxon matched-pairs signed rank sum* non-parametric test was used to compare tissue biopsies taken from different sites of the same patient. The probability (p) values are presented. (Table B9-page 531; Practical statistics for medical research; DG Altman; 1991 edition).

Regression analysis

When the outcome variable was continuous, the independent variables which showed statistical significance were then analysed in a standard regression model. The *likelihood ratio* test was then performed to assess the significance of each of the variables in standard regression. The test is based on the difference in the 'likelihood' of the model with and without the variable under consideration. To predict how well the final regression model predicted the outcome variable (duration of the second stage of labour – page 182), the proportion of the total sum of squares that were explained by the regression (*R-sq*) was estimated using STATA®. The higher R-sq, the better the predictability of the model.

When the outcome variable was binary, the statistically significant independent variables were analysed by multiple logistic regression. Goodness of fit: the Hosmer - Lemeshow test was used to assess how well the logistic model fitted the binary data in the genital prolapse/progress of labour study. The observed and expected number of successes and failures within the groups were used to form a χ^2 statistic with G-2 degrees of freedom (where G is the number of groups, taken to be 10). Significance of this statistic ($p<0.05$) indicates lack of fit in the model.

3.3.1 Test for Normality:

One simple method to calculate deviations from normality in a quantitative way is called Shapiro Francia W' test statistic. This is based on the correlation of the original data with their Normal scores. The correlation coefficient, r, is calculated first and then squared to give W' ie $W' = r^2$. The W' test statistic is then compared to the probability points given in W' tables (eg. Page 538-9; Appendix B. Practical statistics for medical research; DG Altman; 1991 edition). The Null hypothesis that the distribution of the data differs from a Normal distribution is thus tested. Small values of W' indicate non-normality with probability values of <0.05.

3.3.2 Two sample variance

Under the Null hypothesis, the ratio of the two sample variances is expected to have a sampling distribution known as the F distribution. The variance ratio is the ratio of the sample variances or the square of the ratio of the sample standard deviations. The variance ratio observed in results, is estimated by dividing the larger SD by the smaller SD. The result is then compared to values in standard F distribution tables, using n-1 degrees of freedom for each variable under test - Table B6; pg. 525 (DG Altman- Practical statistics for medical research- 1991 edition) The coefficient of variation in the tissues is then divided by the coefficient of variation in the experiments.

The standard deviation is subsequently divided by the mean value of all the means of the experiments to estimate the coefficient of variation of all the experiments.

3.6 THE NULL HYPOTHESES

i. The in-vitro and in-vivo Connective Tissue Study in Genital Prolapse

Null hypothesis: In-vitro biomechanical tissue properties and clinical in-vivo connective tissue markers are not different in women with genital prolapse as compared to women without.

The secondary hypothesis is that the biomechanical properties of the rectus sheath are not different from the properties of pelvic connective tissue.

ii. Genital prolapse and the progress of the first labour

Primary null hypothesis: no difference exists in the obstetric history of women with genital prolapse surgery and those without.

There is no difference in connective tissue clinical markers in women with genital prolapse and those without.

Secondary hypothesis: there is no difference in the duration of labour between women who require middle compartment surgery and women who had no genital prolapse surgery.

iii. Connective tissue markers in nulliparous women and their progress of labour.

Primary null hypothesis: in nulliparous women, no difference exists in the mode of delivery as well as in the duration of labour amongst women with different joint mobility and connective tissue clinical markers.

CHAPTER IV

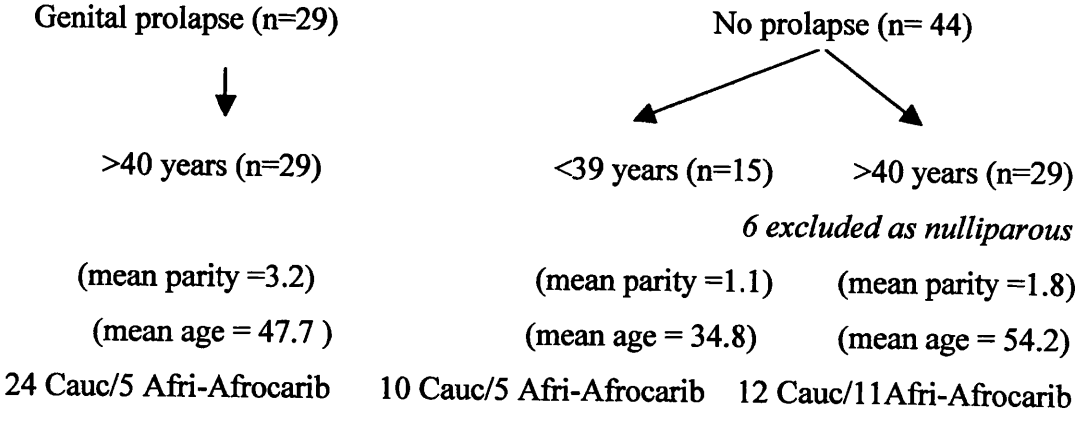
RESULTS

4.0 In vitro tissue studies and (in vivo) clinical connective tissue markers

This study was conducted to test the primary hypothesis that in-vitro biomechanical tissue properties reflect clinical in-vivo connective tissue markers and that these biomechanical properties are different in women with and without genital prolapse.

In this study, all 15 patients under 39 years of age, had POP-Q stage 0 or I (see section 2.8) and therefore were all in the control group. All but 6 in the group of 58 women over 40 years of age were parous. These 6 women were excluded from the main analysis. Depending on the type of surgery undertaken, as well as on the technical problems encountered during sampling of the biopsy, some women had biopsies from one site only and some had biopsies from different sites. Three women with grade II genital prolapse had their rectus sheath biopsied. Demographic data were distributed as shown in the flow diagram. In the group of women older than 40 years (n=58), women with genital prolapse (n=29) had a higher mean parity and were younger than women without genital prolapse (mean of difference was –7.9, CI: -11.8 to –4.01; $p<0.0001$ using the 2-sample t test). For age, the Shapiro Francia W' value was 0.952; p=0.06; showing a normal distribution.

The flow diagram below represents the demographic data of all patients in the study.



4.1 Joint mobility and genital prolapse

4.1.1 Joint mobility and genital prolapse in women over 40 years of age

The left thumb and little finger mobility for all patients in this study were normally distributed - table 4.1

Table 4.1 The Shapiro-Francia W' tests for the left thumb and little finger mobility

	n=	W'	P value	Mean	SD
Lt thumb (cms)	70	0.98	0.31	4.75	3.10
Lt little finger (degrees)	71	0.98	0.32	63.46	19.02

For the reasons explained in section 2.7, the left-sided joints were used in the analysis. Dominance was not accounted for and an assumption was made that all women were right-handed. This created an error in the results as up to 10% of the general poulation can be left-handed [182].

4.1.2 Individual compartment POP-Q score and Joint Mobility

With reference to the POP-Q genital prolapse classification discussed in section 2.8, Aa and Ba are standard landmarks on the anterior vaginal wall. Ap and Bp are standard landmarks on the posterior vaginal wall. Ba and Bp are defined as the lowest extent of the segment of the vagina between points A and the apex of the vagina anteriorly and posteriorly. Points A are located 3cm proximal to or above the urethral meatus anteriorly and the hymen posteriorly. By definition, Aa and Ap can have values between – 3cm (in a patient without prolapse) and + 3cm (in a patient with maximal prolapse) The position of the cervix and posterior fornix are represented by c and d respectively- fig 4.1.

A minus sign for the measurements in centimetres reflected a point inside the vagina,

whilst the measurements without the minus sign are those beyond the hymenal ring, signifying a high degree of genital prolapse.

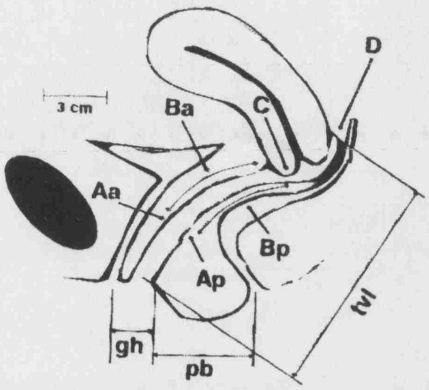


Fig 4.1 Landmarks for quantitative pelvic examination. Aa=point A anterior; Ap=point A posterior; Ba=point B anterior; Bp=point B posterior; C=cervix or vaginal cuff; D=posterior fornix; gh =genital hiatus; pb = perineal body; tvl=total vaginal length (From Bump RC, Mattiasson A, Bo K, et al: The standardisation of terminology of female pelvic floor dysfunction. Am J Obstet Gynecol 175:10-17, 1996)

4.1.3 The comparison of different POP-Q scores in parous women over 40 years of age and joint mobility

In the first instance, the findings from 29 parous women suffering from stage II or III genital prolapse (section 2.8.2) were compared with those from 23 parous women without genital prolapse. Individual joint mobility was analysed in women over 40 years of age with increasing prolapse of the anterior and posterior walls of the vagina as well as cervix(c) and posterior fornix(d) – table 4.2. There was no correlation between either points Aa or Ba on the anterior wall and Ap or Bp on the posterior wall of the vagina and any of the measured joints (Kruskal Wallis test for the left thumb and little

Table 4.2 The relationship of the *anterior vaginal wall* prolapse and joint mobility in women over 40 years of age

	Lt. elbow; χ^2 (p)	Lt. little finger; χ^2(p)	Lt. thumb; χ^2 (p)
Anterior wall prolapse	1.38 (0.71)	2.45 (0.48)	3.10 (0.38)
Posterior wall prolapse	0.55 (0.91)	2.17 (0.54)	1.74 (0.63)
Cervical prolapse	4.19 (0.24)	2.70 (0.44)	1.95 (0.44)
Prolapse of the posterior fornix	3.21 (0.52)	2.95 (0.56)	1.48 (0.83)

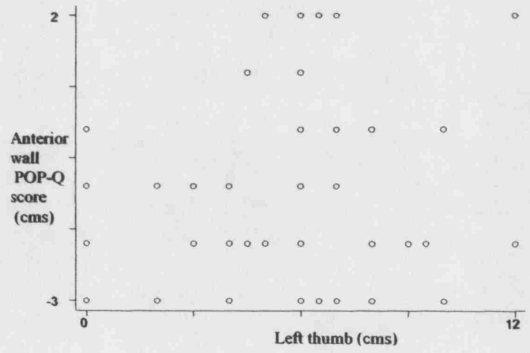


Fig 4.2 Scatter plot of the anterior vaginal wall prolapse and left thumb mobility (χ^2=3.10; p=0.38)

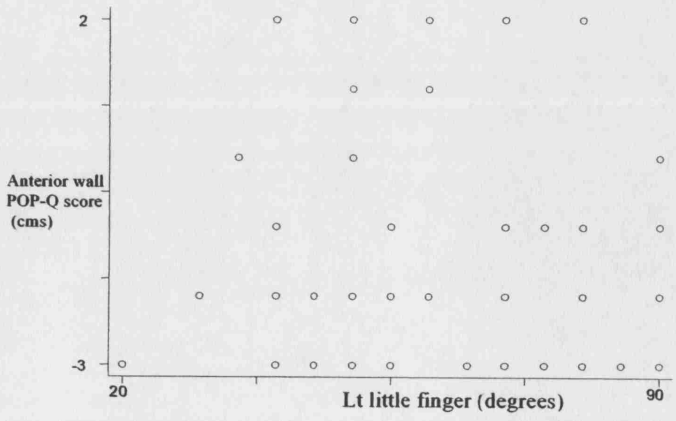


Fig 4.3 Scatter plot of the anterior vaginal wall prolapse and left little finger mobility (χ^2=2.45; =0.48)

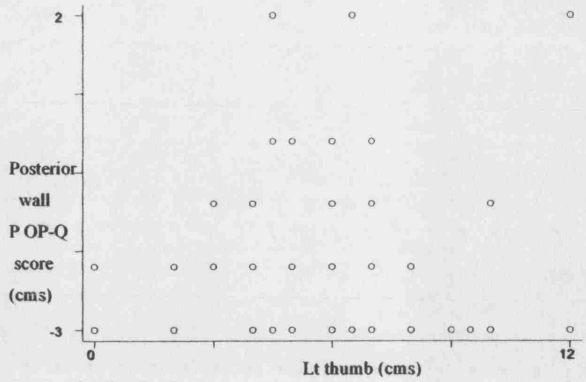


Fig 4.4 Scatter plot of the posterior vaginal wall prolapse and left thumb mobility (χ^2=1.74; p=0.63)

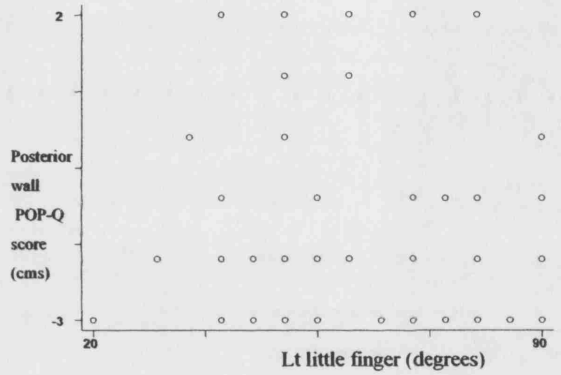


Fig 4.5 Scatter plot of the posterior vaginal wall prolapse and left little finger mobility (χ^2=2.1 p=0.54)

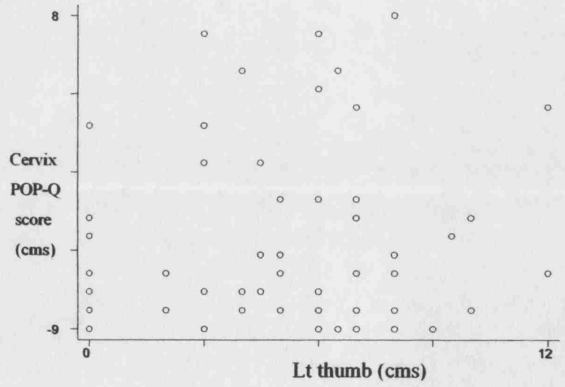


Fig 4.6 Scatter plot of the prolapse of the cervix and left thumb mobility (χ^2=5.48; p=0.96)

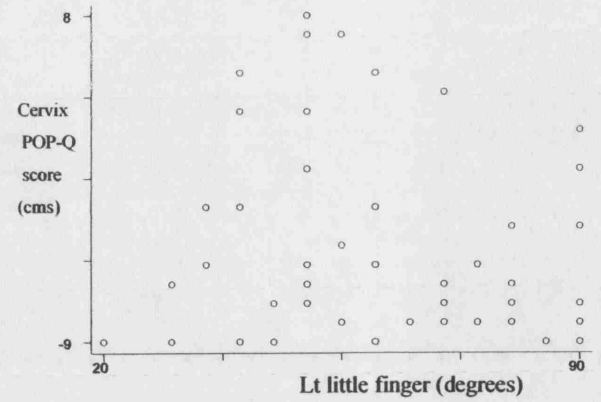


Fig 4.7 Scatter plot of prolapse of the cervix and left little finger mobility

(χ^2=15.23; p=0.29)

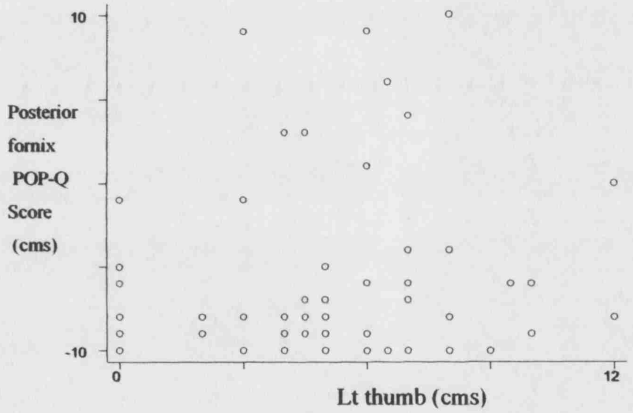


Fig 4.8 Scatter plot of prolapse of the posterior fornix and left thumb mobility

(χ^2=1.48; p=0.83)

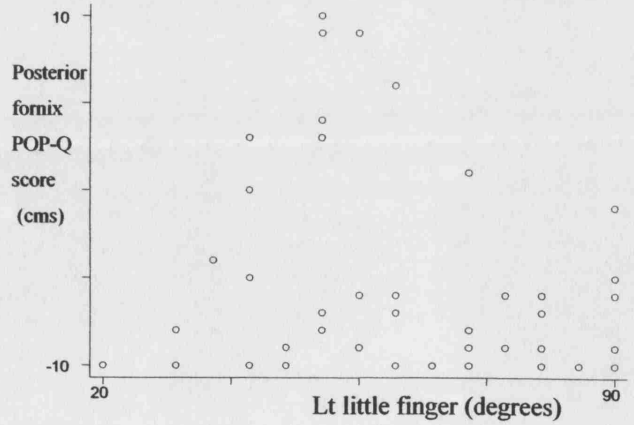


Fig 4.9 Scatter plot of prolapse of the posterior fornix and left little finger mobility

(χ^2=2.95; p=0.56)

Table 4.3 shows the prevalence of joint hypermobility as defined by Norton et al [77] amongst parous women over 40 years of age. There was no evidence of a difference in left elbow mobility between women with genital prolapse (grade II and III) and women without (grade 0 or I) (χ^2 =3.9;p=0.14). There was also no difference in left little finger mobility (p=0.55; CI -12.1 to 6.5) or left thumb mobility (p=0.58; CI –1.93 to 1.1). The chi squared (χ^2) test was used to analyse left elbow mobility and the 2-sample t test was used for the thumb and little finger mobility.

Table 4.3 Joint hypermobility amongst parous women over 40 years of age

	Lt elbow ≥ 190°	Lt thumb/forearm apposition	Lt little finger ≥ 55°
Women with gen. prolapse	5/23(21.7%)	3/24(12.5%)	17/26(65.4%)
No genital prolapse	4/26(15.4%)	3/23(12.5%)	12/23(52.2%)

In summary, clinical connective tissue properties, as assessed by upper limb joint mobility, did not correlate with increasing genital prolapse. There was also no association between the mobility score (0 to 3) as defined by Norton et al [77] and the overall POP-Q genital prolapse score (χ^2=6.39; p=0.89).

4.2 Analysis of in-vitro tissue data

Testing the Null hypothesis: In-vitro biomechanical tissue properties are different in women with and without genital prolapse. In section 3.2.1, I explained the biomechanical values that we measured on the tissue biopsy strips. The value of (A) is the magnitude of the time-dependent component of stress, $\tau 1$ is the time constant of stress relaxation to a

steady state level, Tss. Fig 4.10 is a replication of fig 3.4 in section 3.2.1. Values of the magnitude of the time-dependent relaxation of stress during the unloading curves are quoted as values of B, by analogy to values of A for the loading curves.

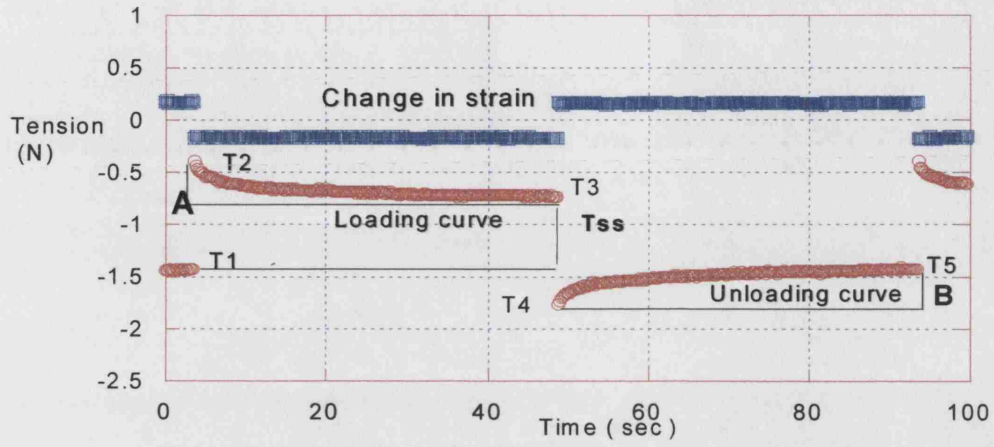


Fig 4.10 Loading and unloading curves in response to one change of strain

4.2.1 The values of T1 and T5

T1 is the resting stress before imposition of strain and T5 is the steady state stress after imposition of strain. Table 4.4 shows T1 and T5 ratios. The third column shows that using the 1-sample t test for each of the tissue biopsies, the T1/T5 ratios were not significantly different from 1.0. This means that after a sudden change of strain, the tissue's stress returned to its pre-strain value. Therefore, no plastic deformation occurred during imposition of strain. Overall the values of T1 were used to derive Tss for analysis of the results.

The values of the T1/T5 ratio for the round ligament showed the largest standard deviations- table 4.4.

Table 4.4 The values of T1/T5 ratio for different tissues

	T1/T5 ratio (Newtons)	Range T5 and T1	p value
Rectus sheath (n=55)	0.98± 0.11	-16.4 to 24.52;-16.4 to 23.9	0.18
Anterior vag. wall(n=35)	0.99± 0.03	-9.7 to 6.6; -9.5 to 6.7	0.06
Posterior vag. wall(n=52)	1.00± 0.01	-8.34 to 8.43; -8.33 to 8.44	1.00
Round ligament(n=58)	0.97± 0.20	-6.82 to 7.7; -6.81 to 7.8	0.26
Uterosacral lig.(n=20)	1.00± 0.01	-8.3 to 11.1; -8.3 to 11.08	1.00

The observed intra-group variability in tissue biomechanical properties could have been due to the experimental methods or due to true wide biological variability.

To ensure accurate measurements, three experiments were repeated on each biopsy strip. The estimated average value was then used in the analysis.

Table 4.5 shows the mean values and their standard deviations for all measured parameters of the tissue biopsy sites in the study.

Table 4.5 Summary of Tss, (A), (B), (τ1),(τ2)of all the tissue biopsy sites

	Tss ($N.mm^{-3}$) Mean ± SD	(A) in $N.mm^{-3}$ Mean ± SD	(B) in $N.mm^{-3}$ Mean ± SD	(τ1) in sec. Mean ± SD	(τ2) in sec. Mean ± SD
Rectus sh.	9.02±10.36	2.68±2.46	1.82±1.86	11.57±14.31	10.20±7.25
Anterior vag wall	4.75±5.36	1.50±1.26	1.09±0.99	12.12±6.90	15.25±15.89
Posterior vag wall	8.71±10.37	2.24±2.23	1.36±1.13	11.81±7.81	10.98±5.10
Round lig	10.11±11.02	3.86±4.83	2.67±2.46	8.76±6.04	11.18±6.21
Uterosacral ligament	11.80±14.16	2.71±2.30	2.35±2.35	14.41±12.83	12.51±9.18

4.2.2 *Differentiating between intra-experimental variability or biological tissue variability*

The round ligament biopsy results were used to estimate the intra-experimental tissue variability. For each of the 35 tissue biopsy strips of the round ligament, the standard deviation was divided by the mean value of all three experiments to estimate the coefficient of variation within each experiment. This was done for each of the 5 biomechanical tissue parameters.

Estimate of tissue variability: the raw data from all three experiments from which the mean values of the in-vivo tissue parameters of the round ligament (n=35) were obtained, were analysed. The standard deviation was divided by the mean value of all the means of the experiments to estimate the coefficient of variation in the experiments. This was done for each of the 5 biomechanical tissue parameters. (section 3.2)

Table 4.6 shows that the main variability was biological rather than intra-experimental variability.

Table 4. 6 Intra - and - inter - tissue variability

	SD/ mean of all round lig samples	SD/mean of each patient's 3 biopsies	F test	P value
Tss round lig	1.09	0.05	13624	<0.001
(A) round lig	1.25	0.04	1471	<0.001
(B) round lig	0.92	0.05	234	0.004
τ1 round lig	0.69	0.11	1860	<0.001
τ2 round lig	0.55	0.06	1636	<0.001

4.2.3 Analysis of women in different groups

Women in the study were subdivided into two sets of 2 groups:

The first set was formed by a group of parous women over 40 years of age who had genital prolapse as defined by POP-Q score 2 and 3. These women were compared with a group of parous women over 40 years of age who had no genital prolapse (POP-Q score 0 or 1). The objective of this analysis was to find differences in clinical and in-vitro characteristics between women with or without genital prolapse. The second set was formed by a group of women under 39 years of age, none of whom had genital prolapse. These women were compared with a group of women over 40 years of age with no genital prolapse. Nulliparous women over 40 years of age were included in this group. The objectives of the analysis for this set was to investigate the effect of age on the clinical and in-vitro connective tissue markers amongst women without genital prolapse. This was not part of the initial hypothesis.

4.2.4 The comparison of in-vitro tissue parameters in parous women over 40 years of age with or without genital prolapse

The demographic data of parous women over 40 years of age are shown in the flow diagram in section 4.0.

Table 4.7 shows a summary of the raw data for Tss in the different tissue biopsies in both women with and without genital prolapse. The values for women without genital prolapse are in bold face type. Fig 4.11 to fig 4.15 show comparison between data for women with and without genital prolapse. The χ^2 test and p values from the Kruskal-Wallis test are presented. Outliers are shown as open circles.

Table 4.7 Comparison of (Tss) of different tissues in women with or without genital prolapse

	n=	Median	25th centile	75th centile	**n=**	**Median**	**25th centile**	**75th centile**
Tss rectus sheath(Nmm^{-3})	3	9.15	8.20	10.73	**18**	**4.42**	**3.00**	**8.96**
Tss anterior vaginal wall (Nmm^{-3})	21	3.12	1.89	5.15	**8**	**4.17**	**2.20**	**5.22**
Tss posterior vaginal wall (Nmm^{-3})	20	6.02	2.65	14.65	**4**	**3.61**	**2.80**	**4.51**
Tss round lig. (Nmm^{-3})	11	6.45	4.22	10.71	**19**	**9.40**	**4.64**	**16.55**
Tss uterosacral lig. (Nmm^{-3})	14	4.53	2.95	11.51	**6**	**9.37**	**2.31**	**23.46**

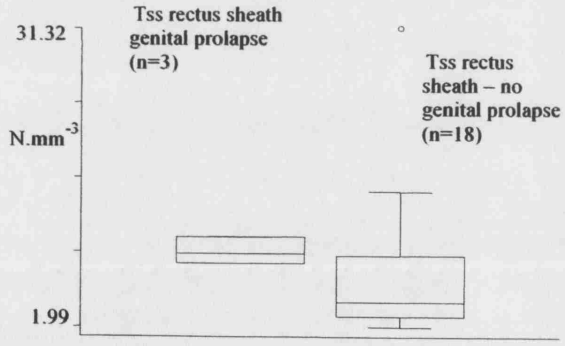


Fig 4.11 Box plot for Tss ($N.mm^{-3}$) of the rectus sheath amongst women with or without genital prolapse. (χ^2 =3.88; p=0.05)

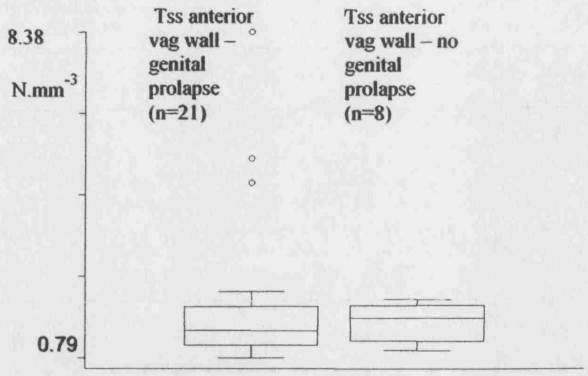


Fig 4.12 Box plot for Tss ($N.mm^{-3}$)of the anterior vaginal wall amongst women with or without genital prolapse. (χ^2 =0.34; p=0.56)

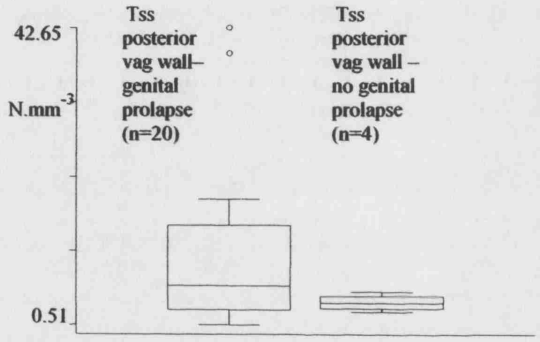


Fig 4.13 Box plot for Tss ($N.mm^{-3}$) of the posterior vaginal wall amongst women with or without genital prolapse. (χ^2 =1.18; p=0.28)

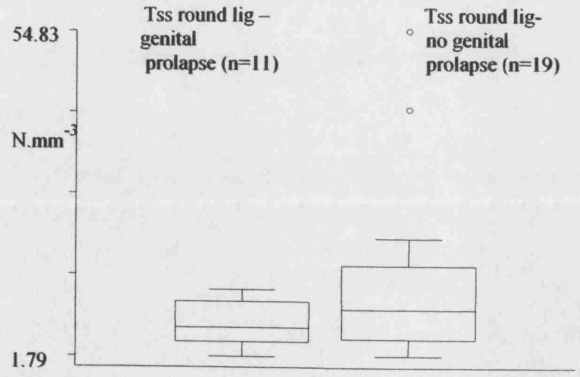


Fig 4.14 Box plot for Tss ($N.mm^{-3}$) of the round ligament amongst women with or without genital prolapse. (χ^2 =1.24; p=0.26)

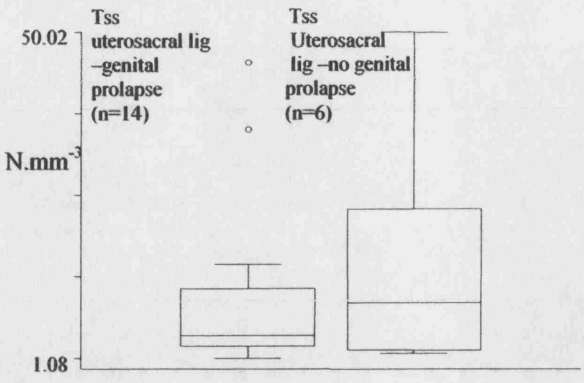


Fig 4.15 Box plot for Tss (Nmm^{-3})of the uterosacral ligament amongst women with or without genital prolapse. (χ^2 =0.03; p=0.85)

Values of (A) in different tissues

Tables 4.8 shows a summary of the raw data for values of (A) in the different tissue biopsies in both women with and without genital prolapse. The values for women without genital prolapse are in bold face type. Fig 4.16 to fig 4.20 show comparison between data for women with and without genital prolapse. The χ^2 test and p values from the Kruskall Wallis test are presented. Outliers are shown as open circles. A number of outliers are way above or below the 25th and 75th centile box plot. All theses values were checked on the raw data and deemed true values. This will be discussed further in section 5.5.3.

Table 4.8 The values of (A) in the different tissue biopsies in women with or without genital prolapse.

	n=	Median	25th centile	75th centile	n=	**Median**	**25th centile**	**75th centile**
(A)rectus sheath (Nmm^{-3})	3	2.83	1.70	3.28	**18**	**1.77**	**1.28**	**3.05**
(A) anterior vag wall (Nmm^{-3})	21	1.00	0.72	1.64	**8**	**1.24**	**0.84**	**1.71**
(A) poster. vag wall (Nmm^{-3})	20	1.36	0.62	3.43	**4**	**1.28**	**0.69**	**1.85**
(A)round lig (Nmm^{-3})	11	1.79	1.10	3.34	**19**	**3.72**	**2.16**	**5.52**
(A)uterosacral lig (Nmm^{-3})	14	1.40	0.90	2.58	**6**	**3.18**	**0.58**	**5.23**

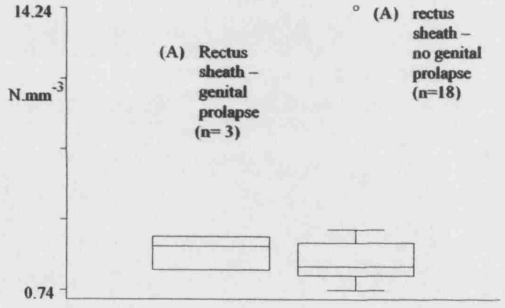


Fig 4.16 Box plot for the values of (A) in $N.mm^{-3}$ of the rectus sheath amongst women with or without genital prolapse. (χ^2 =0.65; p=0.42)

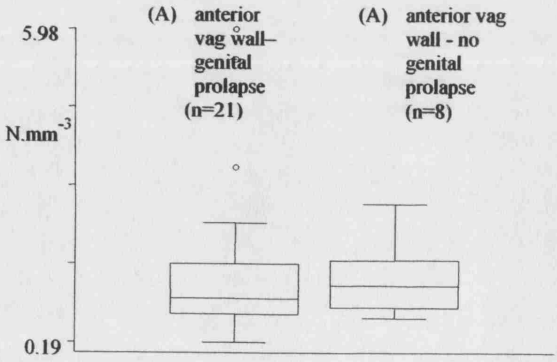


Fig 4.17 Box plot for values of (A) in $N.mm^{-3}$ of the anterior vaginal wall amongst women with or without genital prolapse. (χ^2 =0.47; p=0.49)

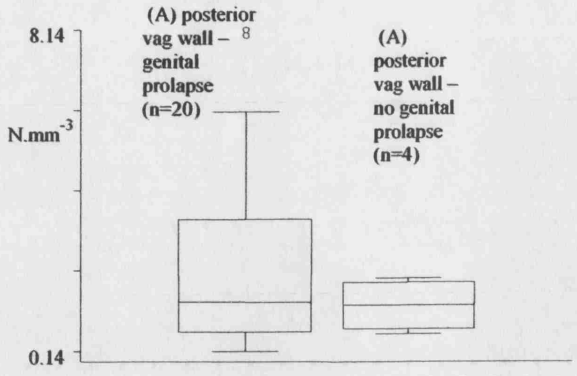


Fig 4.18 Box plot for values of (A) in $N.mm^{-3}$ of the posterior vaginal wall amongst women with or without genital prolapse. (χ^2 =0.15; p=0.70)

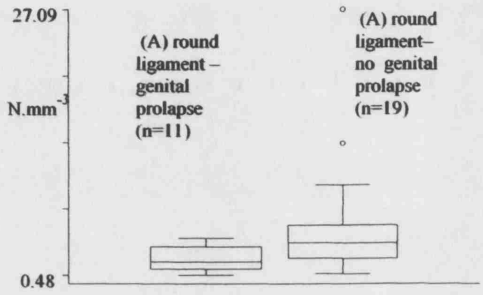


Fig 4.19 Box plot for values of (A) in $N.mm^{-3}$ of the round ligament in women with or without genital prolapse. (χ^2 =5.39; p=0.02)

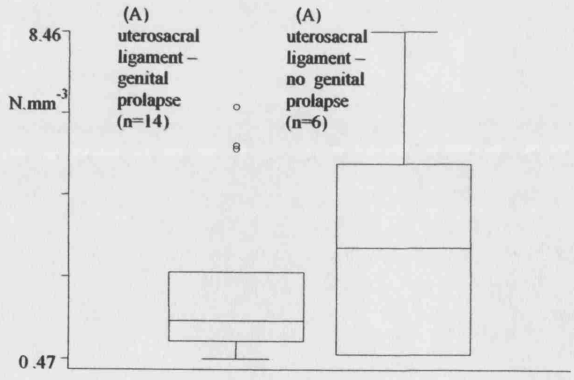


Fig 4.20 Box plot for values of (A) in $N.mm^{-3}$ of the uterosacral ligament in women with or without genital prolapse. (χ^2 =0.009; p=0.93)

Values of (B) in different tissues

Table 4.9 shows a summary of the raw data for values of (B) in the different tissue biopsies in both women with and without genital prolapse. The values for women without genital prolapse are in bold face type. Fig 4.21 to fig 4.25 show comparison between data for women with and without genital prolapse. The χ^2 test and p values from the Kruskal-Wallis test are presented. Outliers are shown as open circles.

Table 4.9 Comparison of the values of (B) in the different tissue biopsies in women with or without genital prolapse.

	n =	Median	25th centile	75th centile	**n =**	**Median**	**25th centile**	**75th centile**
(B)rectus sheath (Nmm^{-3})	3	1.87	1.01	7.50	**18**	**0.96**	**0.73**	**1.80**
(B) anterior vag wall (Nmm^{-3})	21	0.71	0.52	1.22	**8**	**0.72**	**0.59**	**1.13**
(B) posterior vag wall (Nmm^{-3})	20	1.02	0.50	1.82	**4**	**0.77**	**0.53**	**1.28**
(B) round lig (Nmm^{-3})	11	1.04	0.69	2.70	**19**	**2.84**	**1.29**	**5.11**
(B) uterosacral lig (Nmm^{-3})	14	0.79	0.61	1.57	**6**	**2.25**	**0.76**	**5.77**

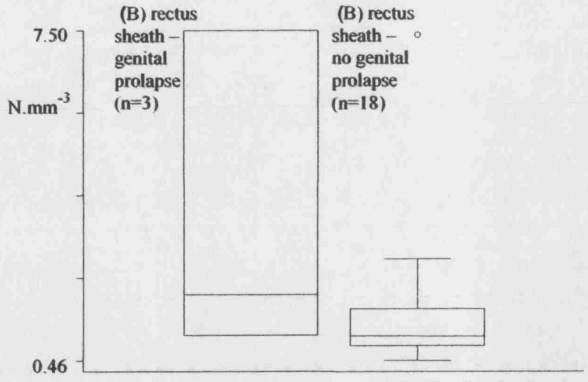


Fig 4.21 Box plot for the values of (B) in $N.mm^{-3}$ of the rectus sheath amongst women with or without genital prolapse. (χ^2 =2.90; p=0.09)

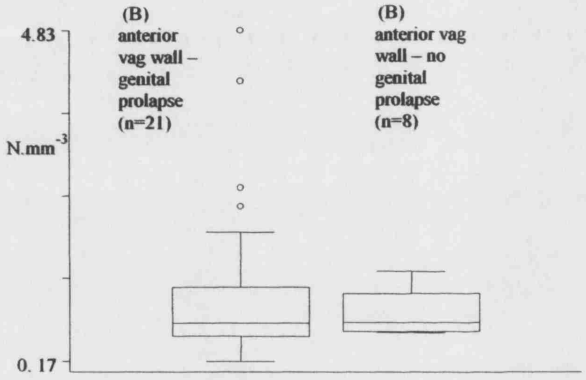


Fig 4.22 Box plot for values of (B) in $N.mm^{-3}$ of the anterior vaginal wall in women with/without genital prolapse. (χ^2 =0.24; p=0.62)

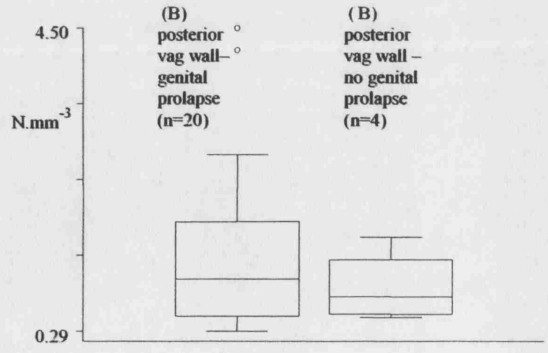


Fig 4.23 Box plot for values of (B) in $N.mm^{-3}$ of the posterior vaginal wall amongst women with or without genital prolapse. (χ^2 =0.15; p=0.70)

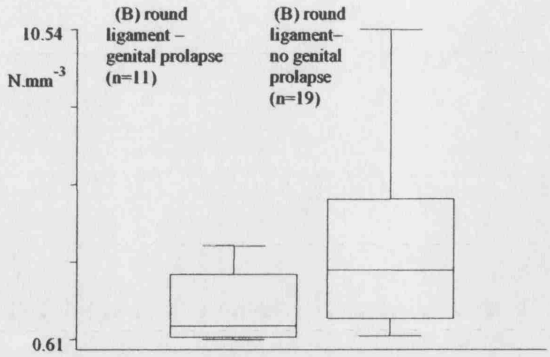


Fig 4.24 Box plot for values of (B) in $N.mm^{-3}$ of the round ligament in women with or with no genital prolapse. (χ^2 =8.28; p=0.004)

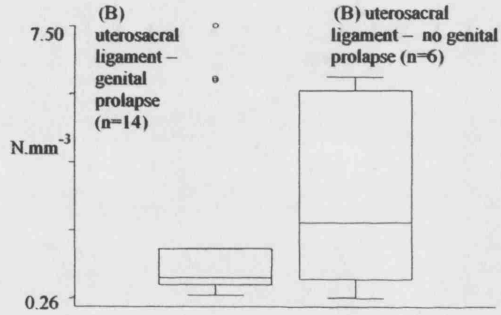


Fig 4.25 Box plot for values of (B) in $N.mm^{-3}$ of the uterosacral ligament amongst women with or without genital prolapse. (χ^2 =0.03; p=0.85)

The values for (τ1)

Table 4.10 shows a summary of the raw data for the values of (τ1) in the different tissue biopsies in women with or without genital prolapse. The values for women without genital prolapse are in bold face type. Fig 4.26 to fig 4.30 show comparison between data for women with and without genital prolapse. The χ^2 test and p values from the Kruskal-Wallis test are presented. Outliers are shown as open circles.

Table 4.10 Comparison of the values of (τ1) in the different tissue biopsies in women with or without genital prolapse.

	n =	Median	25th centile	75th centile	**n =**	**Median**	**25th centile**	**75th centile**
(τ1) rectus sheath (secs)	3	20.15	17.65	91.60	**18**	**9.47**	**6.32**	**12.22**
(τ1) anterior vag wall (secs)	21	9.80	8.08	15.10	**8**	**12.72**	**10.50**	**13.95**
(τ1) posterior vag wall (secs)	20	11.56	9.42	14.00	**4**	**8.56**	**5.70**	**11.79**
(τ1) round lig (secs)	11	6.53	5.73	12.43	**19**	**7.58**	**5.03**	**10.11**
(τ1)uterosacral lig (secs)	14	10.37	8.35	22.90	**6**	**7.10**	**5.80**	**11.89**

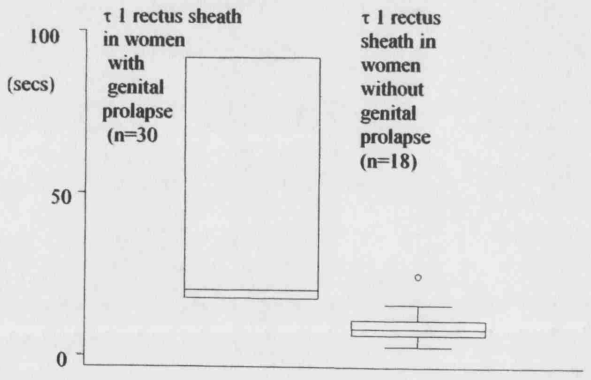


Fig 4.26 Box plot for the values of (τ1) in seconds of the rectus sheath amongst women with or without genital prolapse. (χ^2 =4.62; p=0.03)

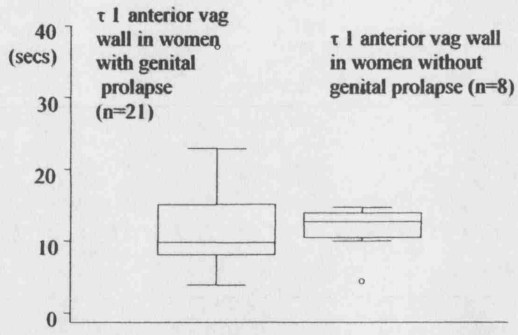


Fig 4.27 Box plot for values of (τ1) in seconds of the anterior vaginal wall amongst women with or without genital prolapse. (χ^2 =0.77; p=0.38)

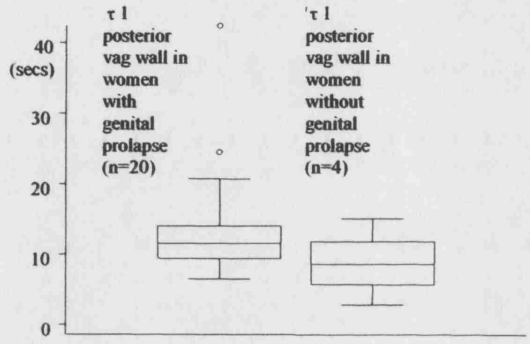


Fig 4.28 Box plot for values of (τ1) in seconds of the posterior vaginal wall amongst women with or without genital prolapse. (χ^2 =1.94; p=0.16)

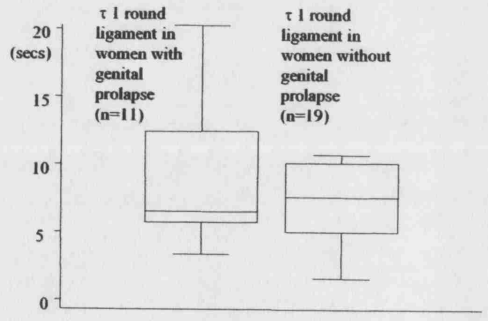


Fig 4.29 Box plot for values of (τ1) in seconds of the round ligament amongst women with or without genital prolapse. (χ^2 =0.36; p=0.55)

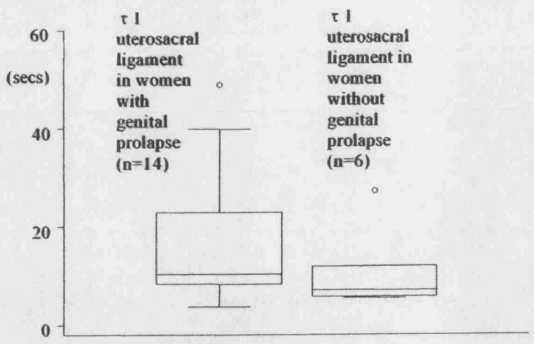


Fig 4.30 Box plot for values of (τ1) in seconds of the uterosacral ligament amongst women with or without genital prolapse. (χ^2 =2.48; p=0.11)

The values for (τ2)

Table 4.11 shows a summary of the raw data for the values of (τ2) in the different tissue biopsies in women with or without genital prolapse. The values for women without genital prolapse are in bold face type.

Fig 4.31 to fig 4.35 show comparison between data for women with and without genital prolapse. The χ^2 test and p values from the Kruskal-Wallis test are presented. Outliers are shown as open circles.

Table 4.11 (τ2)of different tissue biopsies in women with or without genital prolapse.

	n=	Median	25th centile	75th centile	**n=**	**Median**	**25th centile**	**75th centile**
(τ2) rectus sheath(secs)	3	6.75	4.75	14.46	**18**	**9.62**	**7.98**	**12.48**
(τ2) anterior vag wall(secs)	21	12.27	6.64	15.15	**8**	**14.37**	**11.40**	**17.30**
(τ2) posterior vag wall (secs)	20	10.97	8.92	15.27	**4**	**8.30**	**6.47**	**13.80**
(τ2) round lig (secs)	11	9.33	7.10	11.00	**19**	**11.17**	**7.44**	**15.30**
(τ2) uterosacral lig (secs)	14	11.03	10.25	13.30	**6**	**9.01**	**7.58**	**12.16**

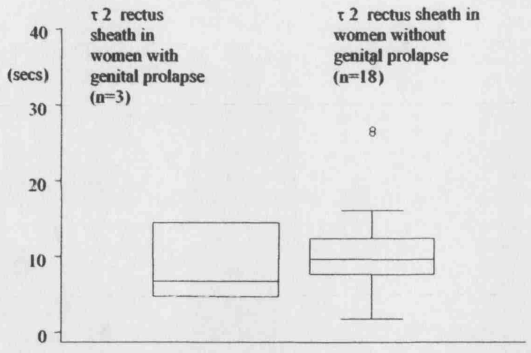


Fig 4.31 Box plot for the values of (τ2) in seconds of the rectus sheath amongst women with or without genital prolapse. (χ^2 =0.65; p=0.42)

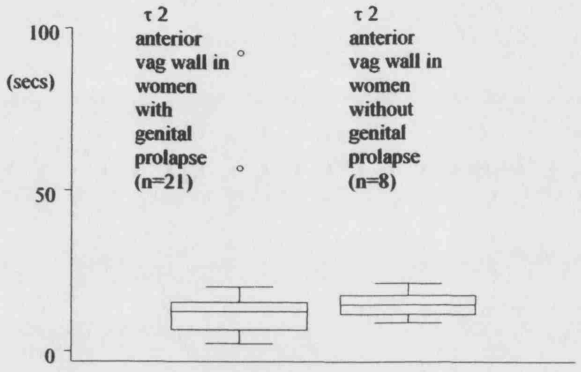


Fig 4.32 Box plot for values of (τ2) in seconds of the anterior vaginal wall amongst women with or without genital prolapse. (χ^2 =1.87; p=0.17)

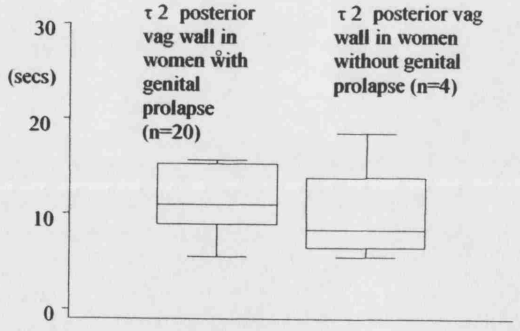


Fig 4.33 Box plot for values of (τ2) in seconds of the posterior vaginal wall amongst women with or without genital prolapse. (χ^2 =0.87; p=0.35)

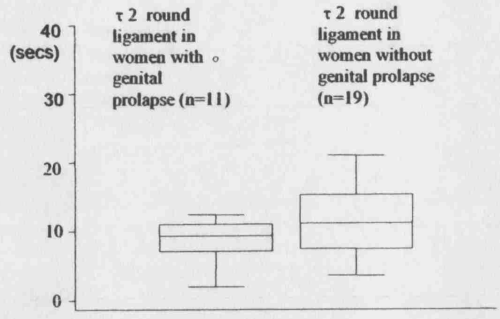


Fig 4.34 Box plot for values of (τ2) in seconds of the round ligament in women with or without genital prolapse. (χ^2 =3.28; p=0.07)

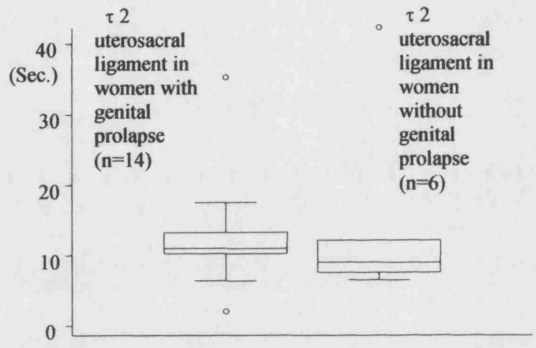


Fig 4.35 Box plot for values of (τ2) in seconds of the uterosacral ligament in women with or without genital prolapse. (χ^2 =0.31; p=0.58)

In summary, there was no statistically significant difference between the values of (Tss) or (τ2) in any of the tissues in parous women over 40 years of age with or without genital prolapse. There was a statistically significant difference between the values of (A) and (B) in the round ligament biopsies in these 2 groups of women (n =11,19). It is a consistent finding that the difference in one variable is similar to the other, since (A) and (B) are mirror images of each other. The values of (τ1) of the rectus sheath in women with and without genital prolapse were also different but this was not reflected in the values of (τ2), as expected. One must reiterate that only 3 women with genital prolapse, had their rectus sheath biopsied and this number could have been too small to notice a difference.

4.2.5 *The analysis of different tissue properties against different clinical variables in women over 40 years of age*

Testing the Null hypothesis: In-vitro biomechanical tissue properties reflect clinical in-vivo connective tissue markers.

The biomechanical tissue parameters were analysed against parity, ethnicity as well as mobility score 0 to 3 as specified by Norton et al. [77] The Kruskal-Wallis test was used.

Rectus Sheath:

There was no evidence of a difference between any of the biomechanical properties of the rectus sheath in the 18 patients without genital prolapse and their different ethnicity, parity and mobility scores- table 4.12. As only 3 patients with genital prolapse had their rectus sheath biopsied, analysis for this small group of patients was not performed.

Table 4.12 Rectus sheath properties amongst women without genital prolapse with different ethnicity, parity and mobility scores

	Ethnicity χ^2 (p)	Parity χ^2 (p)	Mobility score- 0 to 3; χ^2 (p)
Tss rectus sheath-no gen prolapse	0.13(0.72)	1.46(0.69)	1.91(0.59)
A rectus sheath -no gen prolapse	0.39(0.53)	1.65(0.65)	1.07(0.78)
B rectus sheath - no gen prolapse	0.64(0.42)	1.21(0.75)	2.79(0.42)
τ1 rectus sheath- no gen prolapse	2.73(0.10)	4.74(0.31)	0.74(0.95)
τ2 rectus sheath- no gen prolapse	0.84(0.36)	3.73(0.44)	3.44(0.49)

There was no association between ethnicity, parity and mobility scores and any of the biomechanical properties of the rectus sheath in women over 40 years of age without genital prolapse.

Anterior Vaginal wall:

Using the Kruskall Wallis or Mann Whitney tests; amongst patients *with* genital prolapse (n=21), there was evidence of a difference in the values of (A) of the anterior vaginal wall (ie. the time-dependent component of stress- during tissue loading) between 4 AfroCaribbean/African patients and 17 Caucasian/Asian patients (χ^2 = 4.24; p=0.04). It is to be noted that with the probability of 1 in 20 chance of the p value showing significance and the number of tests which were performed in this analysis, the results should be interpreted with caution. This is discussed further in section 5.5.2. There was no evidence of a difference between any of the biomechanical properties of the anterior vaginal wall in patients *without* genital prolapse (n=8) and their different ethnicity, parity and mobility scores- table 4.13.

Table 4.13 Anterior vaginal wall properties amongst patients with different ethnicity, parity and mobility scores

	Ethnicity; χ^2 (p)	Parity; χ^2 (p)	Mobility score 0 to 3; χ^2 (p)
Tss anterior wall-gen prolapse	4.24(0.40)	3.70(0.59)	0.51(0.78)
Tss anterior wall-no gen prolapse	1.35(0.24)	2.83(0.73)	0.22(0.89)
A anterior wall-genital prolapse	4.24(**0.04**)	1.93(0.86)	0.25(0.88)
A anterior wall- no genital prolapse	0.15(0.70)	6.58(0.25)	1.36(0.51)
B anterior wall- genital prolapse	0.80(0.37)	1.20(0.94)	2.03(0.36)
B anterior wall- no genital prolapse	0.15(0.70)	4.92(0.43)	1.14(0.57)
τ1 anterior wall- genital prolapse	0.29(0.59)	8.70(0.12)	0.08(0.96)
τ1 anterior wall-no genital prolapse	0.60(0.44)	5.50(0.36)	5.14(0.08)
τ2 anterior wall- genital prolapse	0.12(0.72)	4.99(0.42)	0.73(0.69)
τ2 anterior wall-no genital prolapse	2.40(0.12)	3.58(0.61)	0.56(0.76)

Posterior vaginal wall:

There was no evidence of a difference between the biomechanical properties of the posterior vaginal wall in patients with (n=20) or without genital prolapse (n=4) and their different ethnicity, parity and mobility scores - table 4.14.

Table 4.14 Posterior vaginal wall properties amongst patients with different ethnicity, parity and mobility scores

	Ethnicity; χ^2 (p)	Parity; χ^2 (p)	Mobility score- 0 to 3 ; χ^2 (p)
Tss posterior wall-genital prolapse	0.04(0.84)	4.45(0.49)	0.04(0.84)
Tss posterior wall-no genital prolapse	1.80(0.18)	2.40(0.30)	1.80(0.18)
A posterior wall- genital prolapse	0.25(0.62)	6.80(0.24)	1.24(0.54)
A posterior wall-no genital prolapse	1.80(0.18)	3.60(0.16)	1.40(0.50)
B posterior wall- genital prolapse	0.25(0.62)	3.18(0.67)	0.16(0.92)
B posterior wall-no genital prolapse	0.20(0.65)	3.60(0.16)	2.40(0.30)
τ1 posterior wall- genital prolapse	2.89(0.09)	3.73(0.59)	3.58(0.17)
τ1 posterior wall-no genital prolapse	0.60(0.44)	5.50(0.36)	1.14(0.57)
τ2 posterior wall- genital prolapse	2.52(0.28)	3.75(0.58)	2.52(0.28)
τ2 posterior wall-no genital prolapse	0.20(0.65)	0.00(1.00)	3.00(0.22)

Round Ligament:

Amongst the control group of patients (n=19), there was evidence of a difference in $\tau 2$ (time constant during unloading) of the round ligament between AfroCaribbean/African patients and Caucasian/Asian patients (χ^2 =8.18; p=0.004). There was no similar difference in $\tau 1$ values.

There was no evidence of a difference between any other biomechanical properties of the round ligament in patients with or without genital prolapse and their different ethnicity, parity and mobility scores – table 4.15. The number of patients with genital prolapse was eleven.

Table 4.15 Round ligament properties amongst patients with different ethnicity, parity and mobility scores

	Ethnicity; χ^2 (p)	Parity; χ^2 (p)	Mobility score-0 to 3 χ^2 (p)
Tss round ligament- genital prolapse	1.92(0.16)	6.81(0.23)	0.18(0.91)
Tss round ligament-no genital prolapse	0.34(0.56)	4.96(0.42)	2.77(0.25)
A round ligament - genital prolapse	0.42(0.52)	7.17(0.21)	0.18(0.91)
A round ligament –no genital prolapse	0.14(0.71)	3.66(0.60)	0.58(0.75)
B round ligament - genital prolapse	1.03(0.31)	5.78(0.33)	0.73(0.69)
B round ligament –no genital prolapse	1.23(0.27)	6.51(0.26)	0.88(0.64)
$\tau 1$ round ligament - genital prolapse	1.04(0.31)	5.93(0.31)	1.56(0.46)
$\tau 1$ round ligament- no genital prolapse	0.10(0.75)	6.85(0.23)	0.12(0.94)
$\tau 2$ round ligament - genital prolapse	0.42(0.52)	3.78(0.58)	6.73(0.69)
$\tau 2$ round ligament- no genital prolapse	8.18(**0.004**)	10.23(0.07)	0.25(0.88)

Uterosacral Ligament:

There was no evidence of a difference between the biomechanical properties of the uterosacral ligament in patients with or without genital prolapse and their different parity and mobility scores - table 4.16. There were no Caucasian patients amongst the control group and therefore ethnicity was not included in the analysis. The number of patients with and without genital prolapse was 14 and 6 respectively.

Table 4.16 Uterosacral ligament properties amongst patients with different ethnicity, parity and mobility scores

	Parity;χ^2 (p)	Mobility score-0 to 3;χ^2 (p)
Tss uterosacral lig -genital prolapse	1.90(0.86)	0.21(0.90)
Tss uterosacral lig-no genital prolapse	4.43(0.25)	0.81(0.67)
A uterosacral lig - genital prolapse	2.32(0.80)	0.21(0.90)
A uterosacral lig- no genital prolapse	4.43(0.35)	1.24(0.54)
B uterosacral lig - genital prolapse	3.38(0.64)	0.43(0.81)
B uterosacral lig- no genital prolapse	4.43(0.35)	0.43(0.81)
τ1 uterosacral lig - genital prolapse	6.73(0.24)	2.91(0.23)
τ1 uterosacral lig - no genital prolapse	4.43(0.35)	3.09(0.21)
τ2 uterosacral lig - genital prolapse	8.94(0.11)	1.94(0.38)
τ 2 uterosacral lig – no genital prolapse	4.86(0.30)	1.95(0.38)

In summary, although 2 parameters showed a correlation with ethnicity, there was no consistent difference between the biomechanical tissue properties and parity, ethnicity or the mobility score.

4.2.6 The effect of age in women without genital prolapse on connective tissue

The in-vitro tissue parameters

Investigating the effect of age on tissue biomechanical properties was not part of the original hypothesis but in view of recent reports by Goh et al [66], the data from this

study were analysed accordingly. Women with no genital prolapse who were under 39 years or over 40 years of age were compared together. The demographic data of these women are shown in the flow diagram in section 4.0.

The values for Tss

Table 4.17 shows a summary of the raw data for the values of (Tss) in the different tissue biopsies in both women ≤ 39yrs and ≥ 40yrs without genital prolapse. The values for women ≥ 40yrs are in bold face type.

Fig 4.36 to fig 4.40 show a comparison between data for women ≤ 39yrs and ≥ 40yrs without genital prolapse. The χ^2 test and p values from the Kruskal-Wallis test are presented. Outliers are shown as open circles.

Table 4.17 The effect of age in women without genital prolapse – a comparison of (Tss) in different tissues amongst women ≤ 39 years and ≥ 40yrs of age.

	n=	Median	25th centile	75th centile	**n=**	**Median**	**25th centile**	**75th centile**
Tss rectus sheath ($N.mm^{-3}$)	11	4.34	2.81	12.76	**24**	**4.91**	**3.13**	**9.38**
Tss ant. vag. wall ($N.mm^{-3}$)	6	3.30	2.43	4.50	**9**	**3.60**	**2.28**	**4.85**
Tss poster. vag. wall ($N.mm^{-3}$)	3	2.00	1.14	3.07	**5**	**3.92**	**3.31**	**3.11**
Tss round lig ($N.mm^{-3}$)	5	3.25	1.68	3.75	**17**	**9.42**	**4.80**	**16.55**
Tss uterosac. lig ($N.mm^{-3}$)	3	7.41	0.81	16.43	**5**	**2.48**	**2.31**	**16.27**

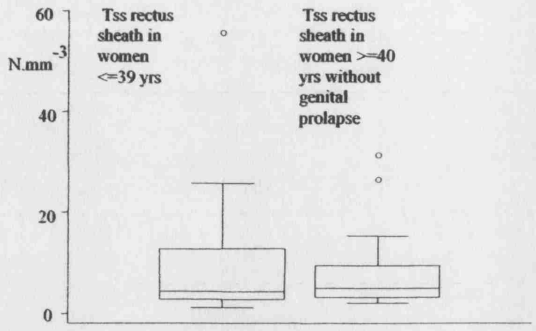


Fig 4.36 Box plot for Tss in $N.mm^{-3}$ of the rectus sheath amongst women ≤ 39 years and ≥ 40 years of age. (χ^2 <0.001; p=0.98)

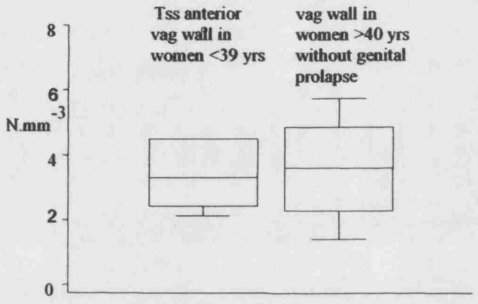


Fig 4.37 Box plot for Tss in $N.mm^{-3}$ of the anterior vaginal wall amongst women ≤ 39 years and ≥ 40 years of age. (χ^2 <0.001; p=0.98)

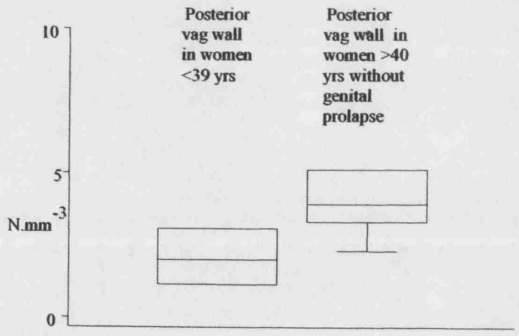


Fig 4.38 Box plot for Tss in $N.mm^{-3}$ of the posterior vaginal wall amongst women ≤ 39 and ≥ 40 years of age. (χ^2 =3.76; p=0.05)

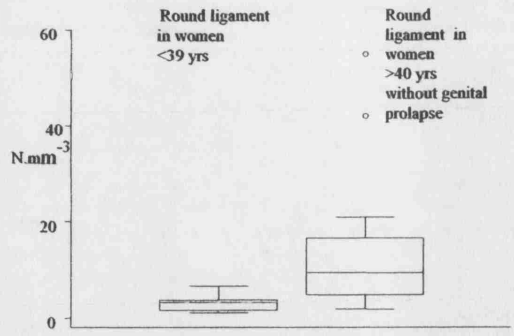


Fig 4.39 Box plot for Tss in $N.mm^{-3}$ of the round ligament amongst women ≤ 39 and ≥ 40 years of age. (χ^2 =6.05; p=0.01)

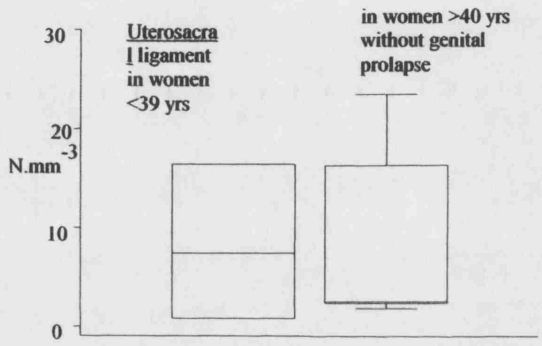


Fig 4.40 Box plot for Tss in $N.mm^{-3}$ of the uterosacral ligament in women ≤ 39 and ≥ 40 years of age. (χ^2 =0.27; p=0.60)

Values of (A) in different tissues

Table 4.18 shows a summary of the raw data for values of (A) in the different tissue biopsies in both women ≤ 39 years of age and ≥ 40 years of age. The data for women ≥ 40years of age are in bold face type.

Fig 4.41 to fig 4.45 show comparison between data for women ≤ 39 years of age and ≥ 40 years of age. The χ^2 test and p values from the Kruskal-Wallis test are presented. Outliers are shown as open circles.

Table 4.18 Comparison of the values of (A) in the different tissue biopsies in women ≤ 39 years of age and ≥ 40 years of age

	n=	Median	25th centile	75th centile	**n=**	**Median**	**25th centile**	**75th centile**
(A) of rectus sheath ($N.mm^{-3}$)	11	2.07	1.60	4.65	**24**	**1.87**	**1.44**	**2.99**
(A) of anterior vag wall ($N.mm^{-3}$)	6	1.57	1.07	2.07	**9**	**1.23**	**0.93**	**1.52**
(A) posterior vag wall ($N.mm^{-3}$)	3	1.09	0.81	3.11	**5**	**1.74**	**0.83**	**1.96**
(A) round ligament ($N.mm^{-3}$)	5	1.76	1.00	1.81	**17**	**3.72**	**2.43**	**5.52**
(A) uterosacral ligament ($N.mm^{-3}$)	3	2.63	2.10	5.00	**5**	**2.36**	**0.58**	**4.00**

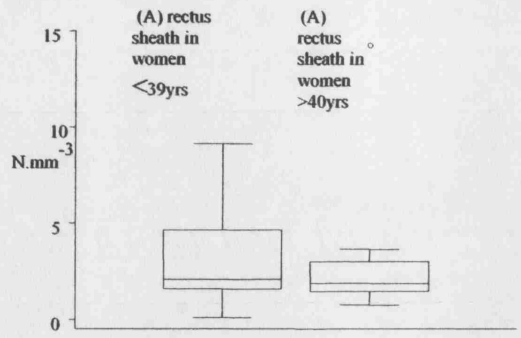


Fig 4.41 Box plot for the values of (A) in $N.mm^{-3}$ of the rectus sheath in women $\leq$ 39 and $\geq$ 40 years of age (χ^2 =0.46; p=0.50)

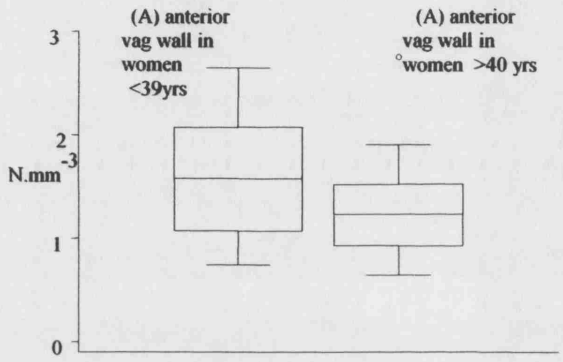


Fig 4.42 Box plot for values of (A) in $N.mm^{-3}$ of the anterior vaginal wall in women $\leq$ 39 years and $\geq$ 40 years of age (χ^2 =0.68; p=0.41)

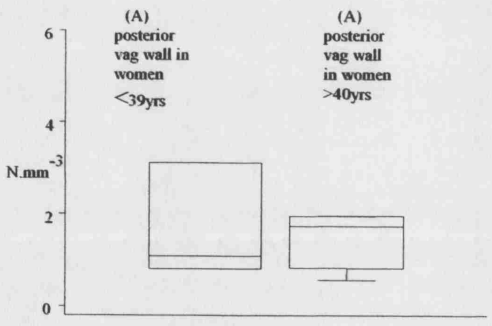


Fig 4.43 Box plot for values of (A) in $N.mm^{-3}$ of the posterior vaginal wall amongst women $\leq$ 39 and $\geq$ 40 years of age (χ^2 =0.02; p=0.88)

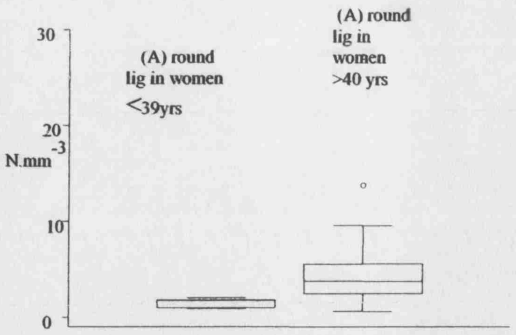


Fig 4.44 Box plot for values of (A) in $N.mm^{-3}$ of the round ligament in women ≤ 39 and ≥ 40 years of age ($\chi^2 = 6.42$; p=0.01)

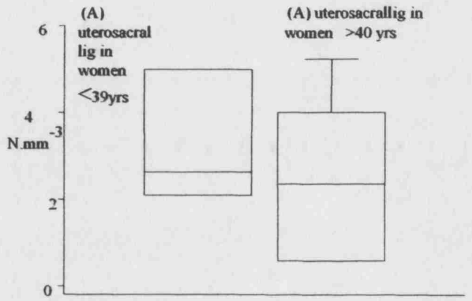


Fig 4.45 Box plot for values of (A) in $N.mm^{-3}$ of the uterosacral ligament amongst women $\leq$ 39 and ≥ 40 years of age ($\chi^2 < 0.001$; p=0.98)

Values of (B) in different tissues

Tables 4.19 shows a summary of the raw data for values of (B) in the different tissue biopsies in both women $\leq$ 39 years of age and ≥ 40 years of age. The data for women $\geq$ 40years of age are in bold face type.

Fig 4.46 to fig 4.50 show comparison between data for women $\leq$ 3 9 years of age and ≥ 40 years of age. The χ^2 test and p values from the Kruskal-Wallis test are presented. Outliers are shown as open circles.

Table 4.19 Comparison of the values of (B) in the different tissue biopsies in women ≤ 39 years of age and ≥ 40 years of age

	n=	Median	25th centile	75th centile	**n=**	**Median**	**25th centile**	**75th centile**
(B) rectus sheath ($N.mm^{-3}$)	11	1.15	0.77	3.12	**24**	**0.99**	**0.79**	**1.57**
(B) anterior vaginal wall ($N.mm^{-3}$)	6	0.93	0.89	1.18	**9**	**0.73**	**0.59**	**1.10**
(B) posterior vag wall $N.mm^{-3}$)	3	0.68	0.60	2.32	**5**	**0.97**	**0.57**	**1.60**
(B) round ligament ($N.mm^{-3}$)	5	0.83	0.82	0.92	**17**	**3.65**	**1.62**	**5.11**
Uterosacral lig.($N.mm^{-3}$)	3	2.94	1.70	3.81	**5**	**1.00**	**0.76**	**3.50**

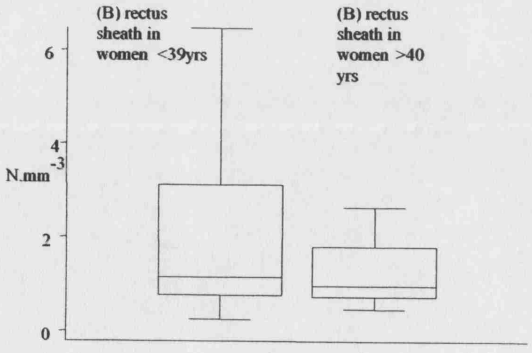


Fig 4.46 Box plot for the values of (B) in $N.mm^{-3}$ of the rectus sheath amongst women ≤ 39 years of age and ≥ 40 years of age (χ^2 =1.10; p=0.29)

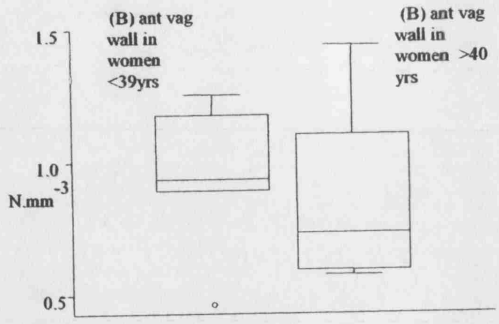


Fig 4.47 Box plot for values of (B) in $N.mm^{-3}$ of the anterior vaginal wall in women ≤ 39 and ≥ 40 years of age. ($\chi^2 = 0.68$; $p = 0.41$)

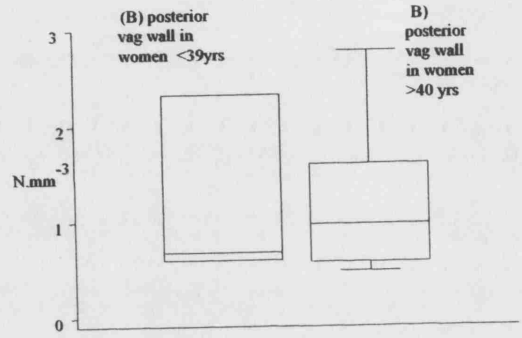


Fig 4.48 Box plot for values of (B) in $N.mm^{-3}$ of the posterior vaginal wall amongst women ≤ 39 and ≥ 40 years of age. ($\chi^2 = 0.02$; $p = 0.88$)

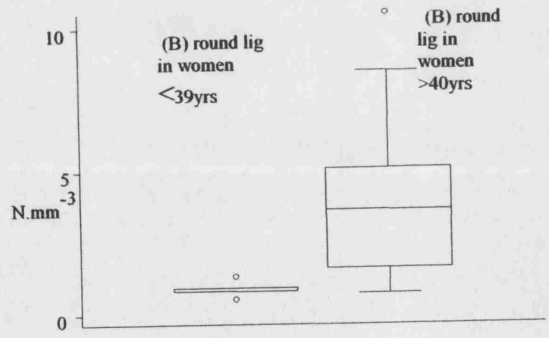


Fig 4.49 Box plot for values of (B) in $N.mm^{-3}$ of the round ligament amongst women ≤ 39 and ≥ 40 years of age. ($\chi^2 = 6.42$; $p = 0.01$)

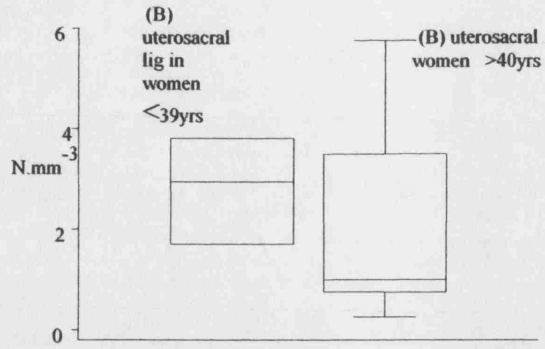


Fig 4.50 Box plot for values of (B) in $N.mm^{-3}$ of the uterosacral ligament amongst women $\leq$ 39 and $\geq$ 40 years of age. (χ^2 =0.07; p=0.80)

The values for (τ1)

Table 4.20 shows a summary of the raw data for the values of (τ1) in the different tissue biopsies in women with or without genital prolapse. The data for women $\geq$ 40years of age are in bold face type.

Fig 4.51 to fig 4.55 show comparison between data for women $\leq$ 39 years of age and $\geq$ 40 years of age. The χ^2 test and p values from the Kruskal-Wallis test are presented. Outliers are shown as open circles.

Table 4.20 Comparison of the values of (τ1) in the different tissue biopsies in women ≤ 39 and ≥ 40 years of age.

	n=	Median	25th centile	75th centile	**n=**	**Median**	**25th centile**	**75th centile**
(τ1) rectus sheath (secs)	11	5.96	4.60	8.75	**24**	**8.28**	**6.49**	**11.06**
(τ1) anterior vag wall(secs)	6	10.12	6.21	10.66	**9**	**12.45**	**10.00**	**13.20**
(τ1) posterior vag wall(secs)	3	3.55	0.09	10.95	**5**	**8.55**	**6.42**	**8.58**
(τ1) round ligament(secs)	5	9.40	7.78	11.80	**17**	**7.24**	**5.03**	**9.35**
(τ1) uterosacral ligament(secs)	3	3.22	0.65	12.23	**5**	**6.77**	**5.80**	**7.43**

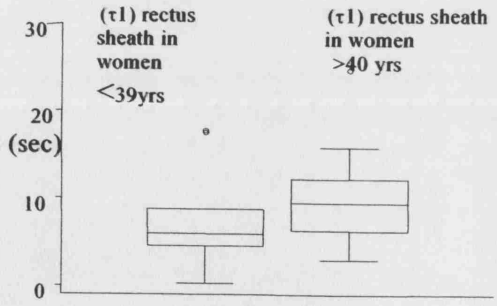


Fig 4.51 Box plot for the values of (τ1) in seconds of the rectus sheath in women ≤ 39 and ≥ 40 years of age. (χ^2 =2.33; p=0.13)

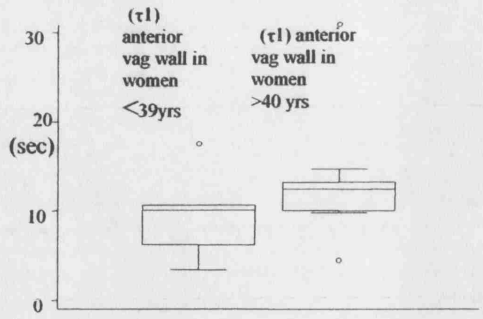


Fig 4.52 Box plot for values of (τ1) in seconds of the anterior vaginal wall amongst women ≤ 39 and ≥ 40 years of age. (χ^2 =1.12; p=0.29)

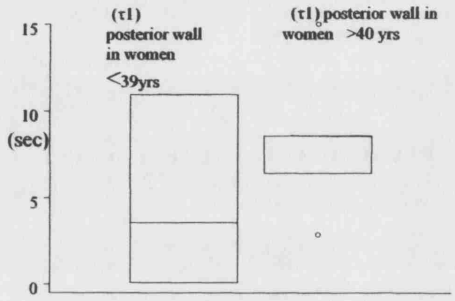


Fig 4.53 Box plot for values of (τ1) in seconds of the posterior vaginal wall amongst women ≤ 39 and ≥ 40 years of age. (χ^2 =0.56; p=0.45)

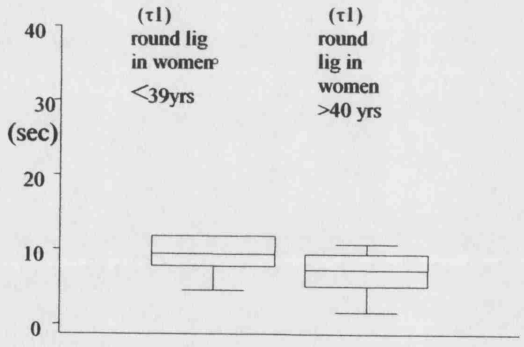


Fig 4.54 Box plot for values of (τ1) in seconds of the round ligament in women ≤ 39 and ≥ 40 years of age. (χ^2 =1.61; p=0.20)

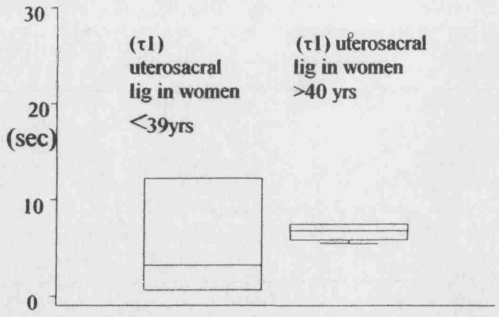


Fig 4.55 Box plot for values of (τ1) in seconds of the uterosacral ligament amongst women ≤ 39 and ≥ 40 years of age. (χ^2 =1.07; p=0.30)

The values for (τ2)

Table 4.23 shows a summary of the raw data for the values of (τ2) in the different tissue biopsies in women ≤ 39 years of age and ≥ 40 years of age. The data for women ≥ 40years of age are in bold face type.

Fig 4.56 to fig 4.60 show comparison between data for women ≤ 39 years of age and ≥ 40 years of age. The χ^2 test and p values from the Kruskal-Wallis test are presented. Outliers are shown as open circles.

Table 4.21 Comparison of the values of (τ2) in the different tissue biopsies in women ≤ 39 and ≥ 40 years of age.

	n=	Median	25th centile	75th centile	n=	**Median**	**25th centile**	**75th centile**
(τ2) rectus sheath (secs)	11	5.50	3.80	7.80	**24**	**9.63**	**7.64**	**12.35**
(τ2) anterior vag wall(secs)	6	11.06	8.23	15.36	**9**	**13.26**	**12.40**	**16.60**
(τ2) posterior vag wall (secs)	3	6.97	0.38	14.65	**5**	**8.68**	**7.46**	**9.15**
(τ2) round ligament (secs)	5	9.94	8.00	12.35	**17**	**11.20**	**8.95**	**15.30**
(τ2) uterosacral ligament (secs)	3	9.46	0.49	14.78	**5**	**10.00**	**8.03**	**12.16**

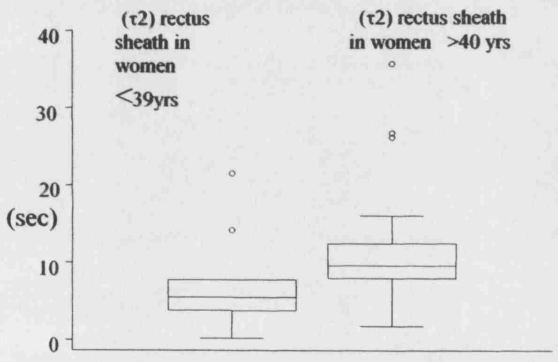


Fig 4.56 Values of (τ2) in seconds of the rectus sheath amongst women ≤ 39 and ≥ 40 years of age((χ^2 =5.46; p=0.02)

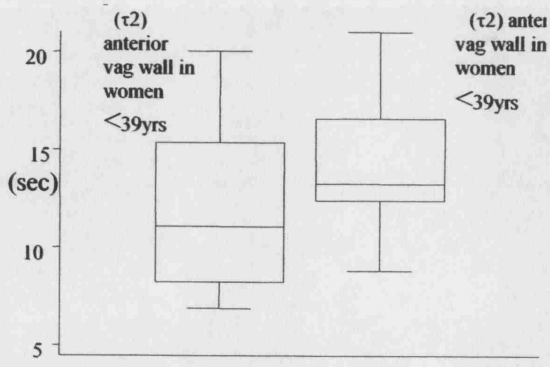


Fig 4.57 Box plot for values of (τ2) in seconds of the anterior vaginal wall amongst women ≤ 39 and ≥ 40 years of age. (χ^2 =1.12; p=0.29)

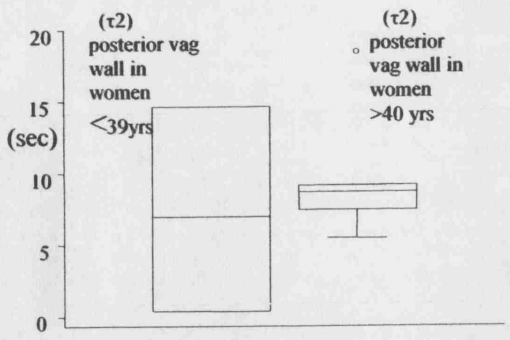


Fig 4.58 Box plot for values of (τ2) in seconds of the posterior vaginal wall amongst women ≤ 39 and ≥ 40 years of age. (χ^2 =0.55; p=0.46)

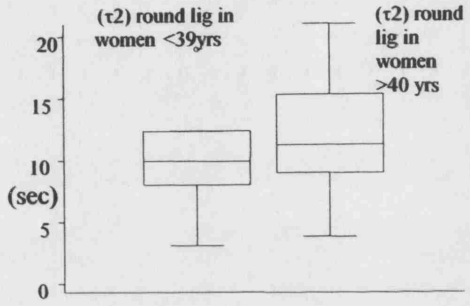


Fig 4.59 Box plot for values of (τ2) in seconds of the round ligament amongst women ≤ 39 and ≥ 40 years of age. (χ^2 =0.36; p=0.55)

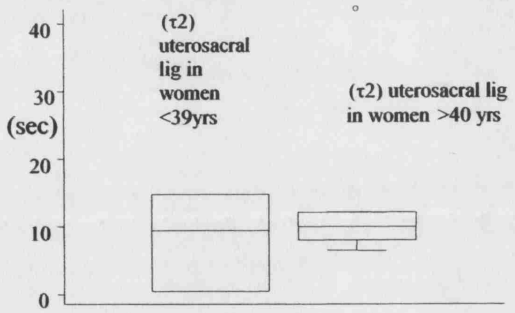


Fig 4.60 Box plot for values of (τ2) in seconds of the uterosacral ligament amongst women ≤ 39 and ≥ 40 years of age. (χ^2 =0.07; p=0.80)

In summary, Tss, (A) and (B) of the round ligament in women ≤ 39 (n=5) and ≥ 40 years (n=17), without genital prolapse, were statistically different. This will be

discussed further in chapter 5. The values of ($\tau 2$) were different in the rectus sheath but there was no similar expected difference in the values of ($\tau 1$); them being mirror images of each other. It is therefore concluded that this was probably an incidental finding.

There was no association between the tissue biomechanical properties and the ethnicity, parity or joint mobility scores in women under 39 years of age and over 40 years of age; none of whom suffered from genital prolapse. For simplicity, the results have not been presented here.

4.3 The comparison of the biomechanical properties of tissues from the same patient

Testing the secondary Null hypothesis:

The biomechanical properties of the rectus sheath reflect the properties of pelvic connective tissue, represented by the round and uterosacral ligaments as well as the anterior and posterior vaginal walls. If the rectus sheath represents the pelvic tissue, further experiments could be carried out on the rectus sheath, which is much more accessible to biopsy during surgery.

The Wilcoxon signed rank sum (matched pairs) non-parametric test was used to compare tissues from the same patient.

Two women with genital prolapse had their rectus sheath and pelvic tissues biopsied at the same time. Tissue comparison in these women was not performed. The same applies for women under 39 years of age who had no genital prolapse.

4.3.1 Analysis of tissue biomechanical properties in women over 40 years of age without genital prolapse

Table 4.22 shows the results of the Wilcoxon signed rank sum (matched pairs) analysis on tissue from women older than 40 years of age, without genital prolapse. In this group of women, the values of (B) and (A) for the rectus sheath and the round ligament showed a statistically significant difference ($p=0.02$ and 0.03 respectively). There was a similar difference between the values of (A) of the rectus sheath and the anterior vaginal wall. All the other properties of all the other tissues were similar. Fig 4.61 to 4.63 represent the relationship of tissues which showed statistical significance. The confidence intervals of the paired medians are shown in brackets.

Table 4.22 Women over 40 years of age *without genital prolapse*: comparison of rectus sheath with the other tissues

	Tss: p	A: p	B: p	τ1: p	τ2: p
Rectus sheath/ Anterior wall (n=6)	0.25	**0.03**	0.17	0.46	0.75
Rectus sheath/ posterior wall (n=5)	0.34	0.68	0.89	0.22	0.22
Rectus sheath/round ligament (n=11)	0.21	**0.03**	**0.02**	0.07	0.42
Rectus sheath/uterosacral ligament (n= 4)	0.07	0.71	0.71	0.71	0.46

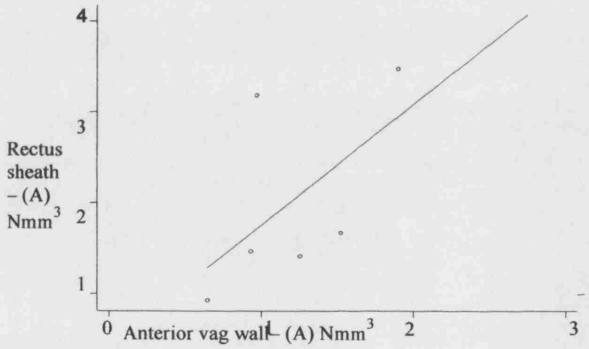


Fig 4.61 The association between (A) of the rectus sheath and (A) of the anterior vaginal wall in women without genital prolapse; p= 0.03 (CI: 0.14 to 2.21)

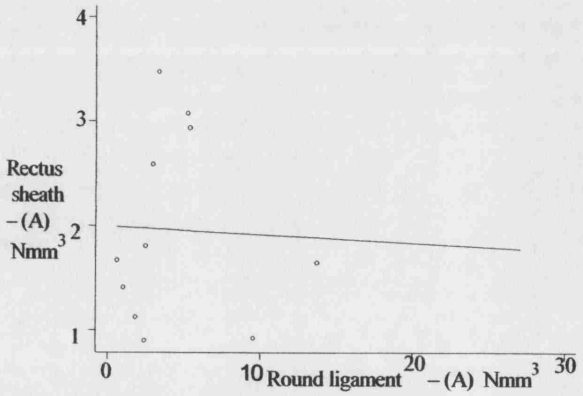


Fig 4.62 The association between (A) of the round ligament and (A) of the rectus sheath in women without genital prolapse; p= 0.03 (CI -5.84 to -0.16)

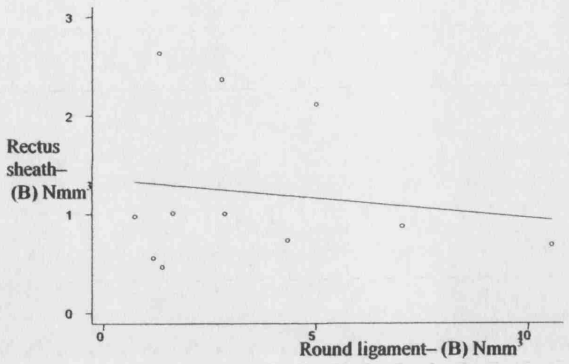


Fig 4.63 The association between (B) of the round ligament and (B) of the rectus sheath in women without genital prolapse; p=0.02(CI -4.87 to –0.25).

In summary, all the measured biomechanical properties of the uterosacral ligament as well as the posterior vaginal wall in women without genital prolapse, were similar to the rectus sheath properties in the same patient. The values of (B) and (A) of the rectus sheath and the round ligament showed a statistically significant difference (p=0.02 and 0.01 respectively). These 2 tissues are seemingly different. There was a similar difference between the values of (A) of the rectus sheath and the anterior vaginal wall but no expected difference was detected in the (B) values. Thus the similarity or difference amongst these 2 tissues is not certain.

In conclusion, the Null hypothesis was partly accepted. The biomechanical properties of the rectus sheath reflect those of the uterosacral ligament and posterior vaginal wall but not all the properties of the round ligament and the anterior vaginal wall.

4.3.2 The comparison of all the tissues of the pelvic floor together- in women over 40 years of age without genital prolapse

Comparison of all the other tissues together, was not part of the original hypothesis. In view of reports by other researchers [183], published at the time of the study, the median values of all tissues were compared together. Comparison of these results with those in the literature will be discussed further in chapter 5.

Using the Wilcoxon signed rank sum test for non-parametric data, the (B) values of the anterior vaginal wall and the round ligament (n=7) were statistically different; p=0.02 (CI -7.18 to -0.23) – fig 4.64. The confidence intervals of the paired medians are shown in brackets.

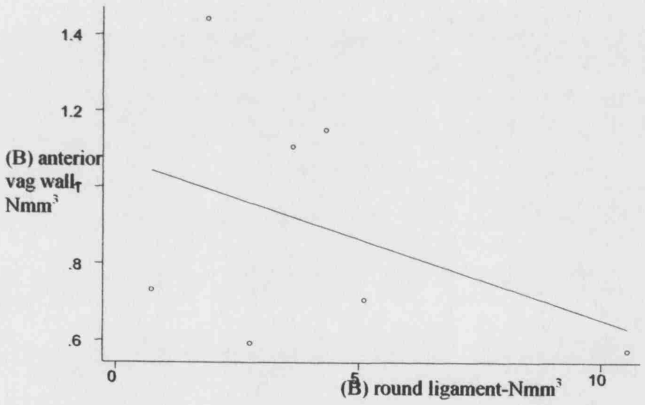


Fig 4.64 The values of (B) of the anterior vaginal wall and the round ligament were different in women without genital prolapse; p= 0.02(-7.18 to -0.23).

There was no difference between any of the properties of the anterior vaginal wall and and the posterior vaginal wall or the uterosacral ligament.

There was no difference between any of the properties of the round ligament and the uterosacral ligament or the posterior vaginal wall.

All the biomechanical properties of the round ligament were similar to those of the uterosacral ligament.

For simplicity, the results are not presented here.

In summary, amongst women without genital prolapse, only the values of (B) of the anterior vaginal wall and the round ligament showed a statistically significant difference. There was no similar difference in the values of (A). In conclusion, the biomechanical properties of the anterior and posterior vaginal wall as well as the round and uterosacral ligament, are similar in women over 40 years without genital prolapse.

4.3.3 The comparison of all the tissues of the pelvic floor together in women over 40 years of age with genital prolapse

In women with genital prolapse, values for Tss and (A) of the anterior vaginal wall showed a statistically significant difference with those of the uterosacral ligament table 4.23; figs 4.65 to 4.67. There was a difference in the values of $\tau 1$ of the anterior vaginal wall and the round ligament, but no such difference was noticed in $\tau 2$ values.

Table 4. 23 Women over 40 years of age *with genital prolapse*: comparison of anterior vaginal wall with the other tissues (r_s = Spearman coefficient)

	Tss: r_s(p)	A: r_s (p)	B : r_s (p)	τ1:r_s (p)	τ 2: r_s (p)
Anterior / Posterior vaginal wall =13)	0.45(0.12)	0.32 (0.29)	0.33 (0.30)	0.06(0.84)	0.25 (0.41)
Anterior vag wall / Round ligament (n=9)	0.43(0.24)	0.48(0.19)	0.65 (0.06)	0.81(**0.01**)	0.13(0.73)
Anterior vag wall/ Uterosacral lig.(n=12)	0.61(**0.04**)	0.62(**0.04**)	0.42 (0.17)	0.55(0.06)	0.50(0.18)

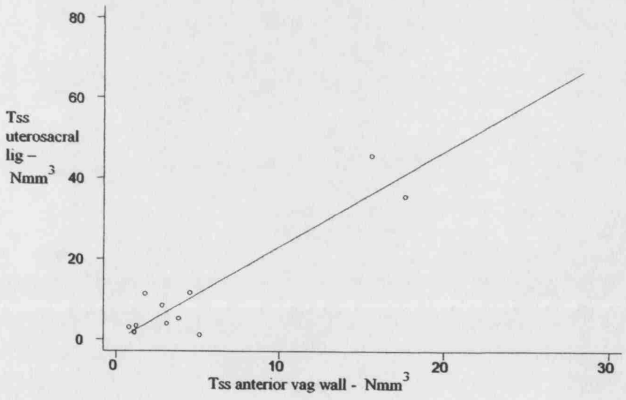


Fig 4.65 The correlation between (Tss) of the uterosacral ligament and (Tss) of the anterior vaginal wall in women with genital prolapse; r_s = 0.61(p=0.04)

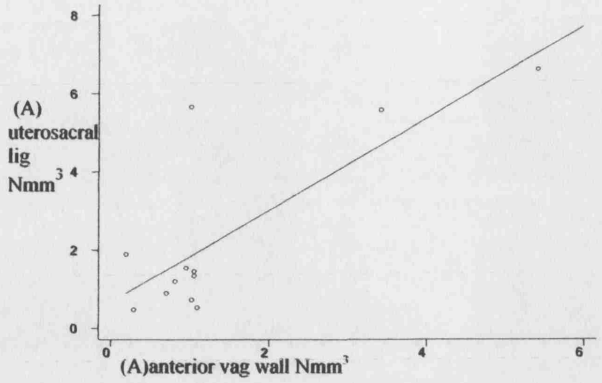


Fig 4.66 The correlation between (A) of the uterosacral ligament and (A) of the anterior vaginal wall in women with genital prolapse; $r_s = 0.62$(p=0.04)

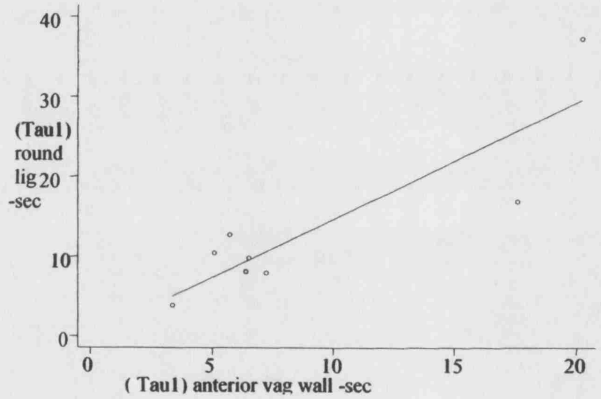


Fig 4.67 The correlation between (τ1) of the round ligament and (τ1) of the anterior vaginal wall in women with genital prolapse; $r_s = 0.81$(p=0.01)

All the biomechanical properties of the posterior vaginal wall were similar to those of the round and uterosacral ligaments in women with genital prolapse.

Similarly, all the properties of the round and uterosacral ligaments in this group of women, were similar.

For simplicity, the results are not presented here.

In summary, in women over 40 years of age, with genital prolapse, values for Tss and (A) of the anterior vaginal wall showed a statistically significant difference

with those of the uterosacral ligament. There were no other consistent differences. All the other tissues in the same patient, had similar biomechanical properties.

4.4 Summary of the in-vivo and in-vitro connective tissue marker study

No consistent differences were found in upper limb joint mobility amongst women with or without genital prolapse. There were no differences in biomechanical in-vitro properties between women of different ethnicity, parity or joint mobility when women with or without prolapse were analysed separately. Women over 40 years of age with genital prolapse had higher parity but were younger than women with prolapse.

In-vitro parameters: a wide biological tissue variability was noticed in every group of patients in the study. This variability together with the small number of patients, could have precluded us from noticing a difference in between groups during the analysis. However, viscous fraction of the elasticity of the round ligament (A and B), in parous women over 40 years of age, was different in women with (n=11) and without (n=19) genital prolapse. The round ligament's viscous fraction of elasticity (A and B) as well as the steady state tension (Tss) were also different in women under 39 (n=5) or over 40 (n=17) years of age who had no genital prolapse.

In a group of 11 women with no genital prolapse, the viscous fraction of elasticity in the rectus sheath (A and B) and the round ligament showed a significant difference. The values of (A) of the rectus sheath and the anterior vaginal wall were also different (n=6) but there was no similar difference in the values of (B). The rectus sheath and the uterosacral ligament as well as the posterior vaginal wall had similar properties.

In women *with* genital prolapse, the steady state tension (Tss) and the viscous fraction of elasticity (A only) of the anterior vaginal wall and the uterosacral ligament were different but the properties of all the other tissues were similar.

4.5 The Relationship of genital prolapse and the progress of the first labour/connective tissue markers

One hundred and ninety patients were recruited for this study; 94 women had undergone genital prolapse surgery and 96 women were in the control group. This was a cross-sectional study and therefore no life-time risk for genital prolapse could be assessed. Table 4.24 shows that there was no statistically significant difference in age at the time of the study, maternal age at time of the first delivery or BMI in the 2 groups, using the Mann Whitney test. The data for women with genital prolapse are in bold face type. There was a statistically significant difference in parity (0 to 7) amongst the 2 groups; χ^2=17.4, p=0.01. Two women amongst women with, and none without genital prolapse were nulliparous. Six and 4 women respectively, had 5 or more children amongst women with or without genital prolapse.

Table 4.24 A comparison of age and BMI in women without/ with genital prolapse surgery

	n=	Median	Rank sum	p value
Age (years)	96/**94**	45.0/**46.0**	9551/**8594**	0.31
Age at 1st delivery (yrs)	94/**92**	23/**25**	8097/**9293**	0.06
BMI (kg/m2)	91/**91**	25.6/**24.9**	9038/**7619**	0.06

A few data points only were missing but insufficient to make a material difference – table 4.24 and 4.27.

4.5.1 Type of surgery amongst women with prolapse surgery

Table 4.25 shows the type of surgery in 94 women who had undergone genital prolapse surgery. Thirty-nine women had undergone isolated anterior or posterior vaginal wall repair or both. Fifty-five women had undergone vaginal hysterectomy or Manchester repair with or without anterior and/or posterior repair. These women were compared to the other 96 women in the study who denied any symptoms of dragging sensation or bulges in the vagina and had never undergone surgery for genital prolapse.

None of the women in the control group and 5/94(5.3%) women with prolapse surgery had undergone a previous hysterectomy. One woman had undergone one previous prolapse surgery. Seven women in total were on HRT in this study.

Table 4.25 The type of prolapse surgery performed in 94 women

	n=
Anterior repair	11/94(11.7%)
Anterior & posterior repair	16/94(17.0%)
Posterior repair	12/94(12.8%)
Manchester/anterior posterior	1/94 (1.1%)
Vaginal hysterectomy	6/94 (6.4%)
Vaginal hysterectomy/anterior repair	17/94(18.1%)
Vaginal hysterectomy/posterior repair	3/94 (3.2%)
Vaginal hysterectomy/anterior/posterior repair	19/94(20.2%)
Manchester/anterior repair	5/94 (5.3%)
Manchester repair	4/94 (4.3%)

4.5.2 *Type of Delivery*

Amongst women with genital prolapse, 76/94 (80.8%) women had a normal vaginal delivery, whereas amongst the control group 87/96 (90.6%) had a normal delivery. In the same two groups, 18 and 9 women respectively had emergency caesarean section or instrumental delivery. The χ^2 test showed no evidence of a difference in the 2 groups (χ^2= 3.8; p= 0.15). The raw data are illustrated in table 4.26.

Table 4.26 The type of delivery amongst women in the study

	Instrumental delivery	Caesarean section	Spont. Vag. delivery
Women without Prolapse	2 (2.1%)	7 (7.3%)	87 (90.6%)
Women with Prolapse	5 (5.4%)	13 (13.8%)	76 (80.8%)

4.5.3 *Normality tests*

Shapiro-Francia W' test for normal data showed that age, body mass index (BMI), parity and maternal age at first delivery were not normally distributed – table 4.27. The first child's birthweight was normally distributed, as were left thumb and little finger mobility.

The duration of the first stage of labour was not normally distributed. It was skewed to the left, with 50% and 75% of women delivering within 6 and 9.8 hours, respectively. The duration of the second stage of labour was not normally distributed. It was also shifted to the left. In 50% and 75% of women, the second stage lasted 35 and 60 minutes, respectively.

Table 4. 27 Normality tests for continuous variables

	Mean	SD	n=	W' =	p=
Age	NA	NA	190	0.932	<0.001
Age 1st delivery	NA	NA	186	0.852	<0.001
BMI	NA	NA	182	0.711	<0.001
First stage of labour	NA	NA	182	0.837	<0.001
Second stage of labour	NA	NA	180	0.913	<0.001
Birthweight	3.44	0.46	180	0.986	0.05
Left thumb	4.14	2.20	186	0.986	0.06
Left little finger	66.15	17.00	187	0.987	0.07

4.5.4 Analysis of data for women with and without genital prolapse

Joint Mobility Findings:

The paired t test showed no difference in right and left thumb mobility. There was evidence that the range of movement of the left little finger was greater than that of the right little finger – table 4.28.

Table 4.28 A comparison between right and left-sided little finger and thumb mobility

	Difference in means	p	95% CI for diff in means
Thumbs(cms)	0.11	0.21	-0.28 to 0.06
Little fingers(degrees)	1.74	0.02	-3.18 to -0.29

Table 4.29 A comparison between right and left-sided knee and elbow mobility

	Proportion	Rt /Lt sides (χ^2)	P value
Rt Knee hyperextension	68/188 (36.1%)		
Lt Knee hyperextension	45/188 (23.9%)	83.7	<0.001
Rt Elbow hyperextension	18/188 (9.6%)		
Lt Elbow hyperextension	8/188 (4.3%)	78.9	<0.001

More women had right rather than the left elbow and knee hyperextension – table 4.29.

The left sided joints were used in the analysis.

Comparison of obstetric data and joint mobility in women with and without genital prolapse

The Kruskall Wallis test was used to analyse the distribution of continuous variables amongst women with and without genital prolapse - table 4.30. The data in bold face type represent the parameters for women without genital prolapse.

Table 4.30 A comparison of obstetric parameters amongst women with and without genital prolapse

	Median	Rank sum	χ^2	p value
Birthweight- 1st child (kg)	3.5 / **3.2**	6841/ **9448**	14.04	<0.001
First stage of labour (hrs)	6.0 / **6.0**	8373/**8280**	0.42	0.52
Second stage of labour(mins)	30.0/**40.0**	7020 /**9269**	7.35	0.007

There was no statistically significant difference in the first stage of labour amongst women with and without genital prolapse. However, there was a difference in the second stage of labour as well as the first child's birthweight.

The 2- sample t test was used to analyse thumb and little finger mobility as they were normally distributed. There was no evidence of a difference in the mobility of the left thumb; p=0.38 (CI -0.92 to 0.35). In contrast, there was evidence of a strong statistically significant difference in the mobility of the left little finger amongst the 2 groups; p=0.007 (-11.51 to -1.88).

Using the same test, there was a highly statistically significant difference in the first child's birthweight, amongst women with and without genital prolapse; $p<0.001$ (CI -0.40 to -0.14.

The χ^2 test showed that more women in the prolapse group had induction of labour with prostaglandins; 38/93(40.9%) as opposed to 23/91(25.3%) women in the no-prolapse group ($\chi^2= 5.8$; $p=0.02$). There was also evidence of a difference in the number of women who were induced or augmented with syntocinon in the 2 groups ($\chi^2=11.59$; $p=0.001$).

4.5.5 Symptomatology, social history, family history and Brighton criteria

Women in both groups showed a statistically significant difference in history of heavy lifting, smoking, backpain and constipation with straining. None of the women in the study had a history of repeated joint dislocation/subluxation or epicondylitis/ tenosynovitis and bursitis. None of them had drooping eyelids, myopia, an antimongoloid slant or marfanoid habitus described in Brighton criteria [119]. Response to local anaesthetic is one of the minor Brighton criteria. Four women in the study had a history of hernia repair and none had a history of rectal prolapse.

Table 4.31 Comparison of general parameters in women with or without surgery for genital prolapse

	No genital prolapse - Proportion of patients	Prolapse surgery - Proportion of patients	χ^2	p
H/O heavy lifting	41/94 (44%)	66/94(70.0%)	13.5	<0.001
Smoking history	19/92(20.6%)	32/94(34.0)	4.2	0.04
Backpain	37/91(40.7%)	30/94(31.9%)	1.5	0.22
Constipation/straining	22/94(23.4%)	37/93(39.7%)	5.8	0.01
Skin Striae	40/94(42.5)	32/92(34.8)	1.2	0.28
Varicose veins after pregnancy	25/94(26.6%)	33/92(35.9%)	1.9	0.17
Clicking joints	13/94(13.8%)	22/94(23.4%)	2.8	0.09
Palms to ground	21/94(22.3)	27/94(28.7%)	1.0	0.32
Local anaest. effective	87/94(92.5%)	72(77.4%)	8.4	0.004
F/H severe backpain	18/94(19.1%)	32/91(35.2%)	6.0	0.01
F/H prolapse	25/94(26.6%)	40/94(42.5%)	5.3	0.02
F/H stress incontinence	7/94(7.4%)	21/94(22.3%)	8.2	0.004
F/H varicose veins	31/94(33.0%)	45/94(47.9%)	4.3	0.04
Lt knee hyperextension	12/94(12.8%)	33/94(35.1%)	12.9	<0.001
Lt elbow hyperextension	3/94(0.03%)	5/94(0.05%)	0.52	0.47

Family history: there was evidence of a difference in family history of genital prolapse, stress incontinence, backpain as well as varicose veins in between the 2 groups.

4.5.6 *Further data analysis - Logistic regression*

Logistic regression was then performed for both binary and continuous variables which showed evidence of a difference. The odds ratios and CI are illustrated in table 4.32. A value of more than 1 means an increased odds and a value of less than 1.0 means a decreased odds of having genital prolapse.

Table 4.32 Logistic regression results of variables which showed a significant difference

	Odds ratio	p value	95% CI
Syntocinon use	2.79	0.001	1.53 to 5.08
Induction of labour	0.49	0.03	0.21 to 0.92
Smoking	0.50	0.04	0.26 to 0.98
Heavy lifting	3.15	0.002	1.67 to 5.56
Constipation /straining	2.16	0.02	1.14 to 4.07
Backpain	1.46	0.21	0.81 to 2.67
Local anaesthetic effective	0.27	0.006	0.11 to 0.68
Palms to ground	1.40	0.32	0.72 to 2.71
Lt knee hyperextension	3.70	0.001	1.76 to 7.74
FH of prolapse	2.04	0.02	1.11 to 3.77
FH of urinary stress incontinence	3.57	0.006	1.44 to 8.88
FH of varicose veins	1.87	0.04	1.03 to 3.37
Parity	1.03	0.80	0.80 to 1.32
Birthweight-1st child	3.90	< 0.001	1.93 to 7.87
2nd stage of labour	0.99	0.03	0.97 to 0.99
Lt little finger extension	1.02	0.008	1.01 to 1.04

Multiple logistic regression was then performed for variables which showed a significant difference. The adjusted odds ratios and 95% confidence intervals are presented in table 4.33.

The odds of having surgery for genital prolapse up to the age of 50 years was 7.78 times higher in women who gave a history of heavy lifting than women with no such history. For women who were current or ex-smokers the odds of having prolapse surgery was reduced by more than 4 times when compared to non-smokers. The use of syntocinon during the first childbirth was associated with 4.58 times increased odds and there was more than fivefold increased odds of genital prolapse surgery in later life for each 1kg increase in birthweight of the first child. Hyperextension of the left knee was associated with more than two and a half times increased odds (OR 2.71) of having genital prolapse surgery. Constipation was associated with genital prolapse (OR 3.79).

Table 4.33 – Multiple logistic regression analysis in women with or without prolapse surgery

Covariate	Odds ratio	P value	95% CI	LR- χ^2	LR- p value
Birthweight- 1st child	5.26	<0.001	2.14 to 12.92	14.96	0.0001
Syntocinon in labour	4.58	<0.001	2.70 to 16.70	12.13	0.0005
H/O Smoking	0.21	<0.001	3.16 to 19.18	15.52	0.0001
Lt knee	2.71	0.03	1.07 to 6.84	4.65	0.03
H/O heavy lifting	7.78	<0.001	3.16to 19.18	24.1	<0.0001
Constipation/straining	3.79	0.003	1.59 to 9.06	9.91	0.002

The prediction equation was statistically evaluated with the Homer and Lemeshow goodness-of-fit test and did not indicate a good fit ($\chi^2 = 22.50$, 8DF; p=0.004). This model lacks one or more variables to be able to predict the likelihood of any type of prolapse surgery in females by the age of 50 years.

The Null hypothesis was accepted as a difference in the duration of the first or the second stage of labour was not detected between women with or without genital

prolapse. Seven and 13 women respectively had caesarean section in the no-prolapse and prolapse groups. Since the difference in numbers is small, it is unlikely that the results could have been affected.

4.5.7 Secondary hypothesis

Multiple logistic regression was then performed to compare women who had middle compartment prolapse surgery with or without anterior or posterior vaginal wall repair (n=55) to women with no prolapse surgery (n=96) - table 4.34. The same process as above was repeated and the variables that continued to show a difference were analysed in a multiple logistic regression model.

As seen in table 4.34, some of the risk factors for having Manchester repair or vaginal hysterectomy with or without anterior or posterior repair up to the age of 50 years were the same as when any type of prolapse surgery was considered in the analysis. However constipation and straining no longer predicted the onset of middle compartment prolapse. A longer first stage of labour and increased BMI were additional contributing factors. Parity was not a contributing factor.

Thus, the Null hypothesis was partly rejected. For each one hour increase in the active first stage of labour, there was a 16% increased odds of middle compartment prolapse surgery by the age of 50 years. When analysed independently, the second stage of labour was significantly different in women with and without middle compartment prolapse surgery (regression coef = -0.02; p= 0.02; CI -0.03 to -0.003). This was not so in multiple logistic regression analysis.

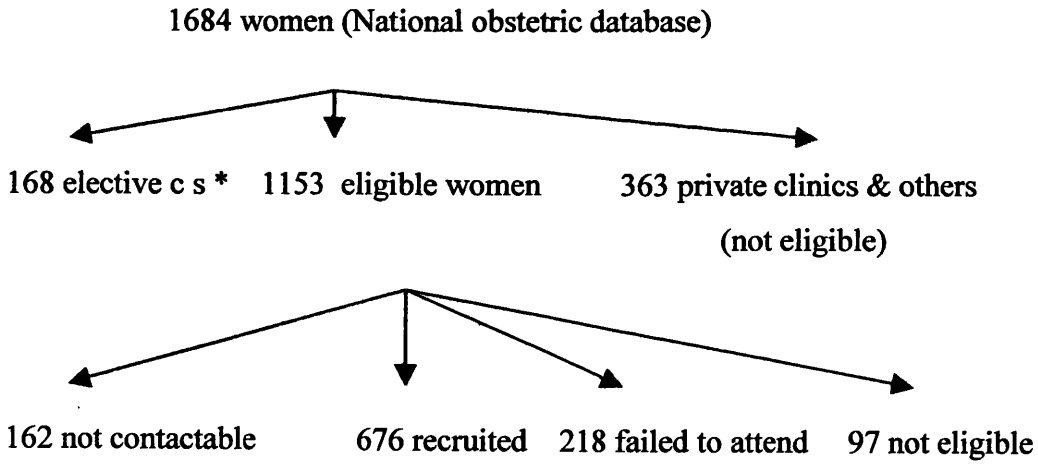
Table 4.34 A final model of multiple logistic regression for women with no prolapse and women who had middle compartment prolapse surgery. (LR=likelihood ratio)

	Odds ratio	p value	95% CI	LR- χ^2	LR- p value
Birthweight-1st child	13.78	<0.001	3.77 to 50.40	21.17	<0.001
BMI	1.14	0.002	1.05 to 1.24	11.38	<0.001
H/O Smoking	0.30	0.03	0.10 to 0.89	5.06	0.02
Syntocinon use	2.74	0.04	1.03 to 7.29	4.27	0.04
Lt knee hyperextension	4.90	0.006	1.58 to 15.19	7.98	0.005
H/O heavy lifting	9.00	<0.001	2.82 to 28.71	17.26	<0.001
Active 1st stage of labour	1.16	0.013	1.03 to 1.29	7.94	0.005

The prediction equation was statistically evaluated with the Homer and Lemeshow goodness-of-fit test and indicated a good fit ($\chi^2 = 6.33$, 8DF; p=0.61).

4.6 The Relationship of Connective tissue markers and the progress of labour

The national obstetric database in Malta detected 1684 nulliparous women over 18 years of age who had their first baby in 2001- flow diagram below. One hundred and sixty eight women (10%) had elective caesarean sections. Overall, in 2001, 10.5% of all women had their baby in private clinics but the proportion of nulliparous women could not be estimated separately. The presented national data does not distinguish between 'private' and 'NHS' patients. Women with a foreign citizenship could not be recruited as specified by the clinical research regulations on the islands.



(* cs: caesarean section)

For this cross sectional study, 1153 eligible women were detected from the labour ward registers. The survey was conducted between July and September 2002 and the short summer working hours on the Maltese islands as well as the good

weather probably encouraged women to attend. One hundred and sixty two women could not be contacted and were therefore excluded from the study. This will be discussed further in section 5.4.

On introducing the study to women, 97 subjects were not eligible to enter the study because they had one of the exclusion criteria discussed in section 3.4.

Six hundred and seventy six women were recruited and 665 had their obstetric history retrieved from their hospital notes. Eleven sets of notes could not be retrieved and therefore these women were excluded from the study.

Two hundred and eighteen women failed to attend mainly because of work commitments. Amongst 162 women who could not be contacted, 6 women had undergone an emergency caesarean section.

4.6.1 General characteristics

Table 4.35 shows data from this study and the national obstetric database. Less women had induction of labour as well as interventional deliveries in this study than nationwide.

The position of the presenting part was recorded as occipito-posterior in 15 patients (2.25%) whilst only 2 women had an occipito-transverse position. Because of the small numbers, this variable was not analysed.

In this study, 88/180 (48.8%) and 23/180(12.8%) of nulliparous women whose labour was induced, had an emergency caesarean section and an instrumental delivery respectively. National statistics for nulliparous women showed lower rates of interventional delivery; 33.8% (175/518) and 10.4% (54/518) respectively.

Amongst women with spontaneous labour, 2.1% (10/485) delivered by emergency

caesarean section and 0.02% (1/485) had an instrumental delivery. The corresponding national rates were 10.6% (106/998) and 4.4% (44/998).

Table 4.35 A comparison between this study and the national obstetric database

	This study (n=665)	National obstetric statistics (n=1507)	χ^2	p=
Age (years)	25.6	26.1	NA	NA
Spontaneous vaginal delivery	543 (81.6%)	1136 (75.4%)	10.35	0.001
Induction of labour	180 (27.1%)	518 (34.4%)	11.29	<0.001
Emergency caesarean section	98 (14.7%)	281 (18.6%)	4.90	0.03
Instrumental delivery	24 (3.6%)	98 (6.5%)	7.29	0.007

4.6.2 Normality Tests

The distribution of age, BMI, first and second stages of labour, infant's head circumference as well as gestation were not normally distributed- table 4.36. The infant's birthweight was normally distributed. The left thumb and left little finger mobility were not normally distributed.

Table 4.36 Normality Tests for continuous variables

	n=	W'	p=
Age	665	0.98	<0.001
BMI	665	0.84	<0.001
First stage of labour	572	0.91	<0.001
Active first stage	572	0.89	<0.001
Second stage of labour	572	0.87	<0.001
Birthweight	665	0.99	0.26
Head Circumference	665	0.61	<0.001
Left thumb mobility	662	0.99	<0.001
Left little finger mobility	661	0.98	<0.001

4.6.3 Joint Mobility

In this study, 187/665 (28.1%) women could touch the floor with the palms of their hands without bending their knees – table 4.37.

Table 4.37 Joint hypermobility amongst women in this study

	Proportion (%)
Left elbow hyperextension ≥ 190°	77 /660 (11.7%)
Left knee hyperextension ≥ 190°	195 /661 (29.5%)
Left little finger hyperextension ≥ 90°	268 /661 (40.5%)
Left thumb apposition to forearm	136 /662 (20.5%)
Ability to touch palms to ground	187 /665 (28.1%)

Knee mobility: 240 /660 (36.4%) and 195/661 (29.5%) women could hyperextend their right and left knees respectively ($\chi^2 = 263.9$; p<0.001).

Elbow mobility: 93 /661 (14.1%) and 77/660 (11.7 %) women could hyperextend their right and left elbows respectively ($\chi^2 = 330.3$; p= <0.001). The matched pairs Wilcoxon rank-sum test showed that the left little fingers were statistically more

hypermobile than the right but the right and left thumb mobility were not statistically different - table 4.38.

Table 4.38 The comparison of the right and left sided thumbs and little fingers

	Rank sums	p value
Thumbs(cm)	93109/87840	0.56
Little fingers(degrees)	43836/109614	**<0.001**

The overall Beighton score has been reported in population studies but because of reasons described in section 2.6, each joint was compared separately to the outcome variables. The left sided measurements were used in the analysis.

4.6.4 Type of delivery

Chi squared test (χ^2) or Kruskal Wallis tests showed that there was no evidence of a difference between any of the other connective tissue markers and the type of delivery – table 4.39. A history of twisting joints showed borderline significance.

Table 4.39 Type of delivery and joint mobility

	χ^2	p value
Thumb mobility	2.8	0.25
Little finger mobility	1.6	0.41
Elbow hyperextension	3.4	0.20
Knee hyperextension	2.0	0.42
Palm to ground	2.75	0.25
Twisting of joints	5.9	0.05

Using the Kruskal-Wallis test, there was a difference with the child's birthweight and the maternal BMI – table 4.40. Simple regression showed similar results for these 2 variables- table 4.40

Table 4.40 The analysis of type of delivery and birthweight of the first child and maternal BMI

	Rank sum NVD/Inst D/ Emerg cs *	χ^2	p value	Regress. coefficient	p value	C I
Birthweight	175726 /9695 / 36023	7.74	0.02	0.15	0.02	0.02 to 0.28
BMI	177178 /6684 / 37582	0.40	0.009	0.02	0.002	0.006 to 0.03

* (NVD- normal vaginal delivery; Inst D-instrumental delivery; Emerg cs- emergency caesarean section)

Women who underwent emergency caesarean section or instrumental delivery for any reason were then grouped together (n=122) and compared to women having a spontaneous vaginal delivery (n= 543). Multiple logistic regression showed that the odds of having an interventional delivery increased by 4% for every unit increase in BMI (OR 1.04; p =0.02, CI 1.007 to 1.07) and by 80% for every 1 kg increase in birthweight (OR 1.8; p=0.01; CI 1.12 to 2.88). The Hosmer Lemeshow test showed a good fit; $\chi^2 = 9.2$; (DF8); p=0.66.

Failure to progress in labour and joint mobility

Emergency caesarean section was performed for failure to progress in 62 women. Nine caesarean sections were performed at full cervical dilatation. Twenty seven women in the study had a caesarean section for failure to progress during the latent

phase of labour. Amongst them, 18/ 27 women had been induced with prostaglandin and 4 other women had been induced with syntocinon after spontaneous rupture of membranes. Thus the indication for the caesarean section in these women was probable failed induction of labour. These women were excluded from the following analysis. Two women amongst this latter group had caesarean section for fetal distress. Failure to progress was considered as the indication for instrumental or caesarean delivery in the analysis when the recorded indication was combined poor progress and fetal distress.

The other 49 women with intervention delivery for failure to progress in the first or second stages of labour were grouped together and compared to women who achieved a spontaneous normal vaginal delivery – tables 4.41 and 4.42. These women were grouped together as the underlying indication was similar. It is to be reiterated that they are not strictly speaking similar and the underlying pathology might be different. There was no evidence of a difference in joints' mobility amongst the two groups of women. The Kruskall Wallis test was used to analyse the little finger and thumb mobility. The χ^2 test was used for the knee and elbow mobility.

Table 4.41 Failure to progress in labour versus spontaneous vaginal delivery

	n	Percentage
Spontaneous vaginal delivery	543	91.7%
Instrumental delivery or emerg c s for failure to progress	49	8.3%

Table 4.42 Failure to progress in labour and thumb/little finger mobility

	Mean rank sum in NVD/ failure to progress	χ^2	p value
Lt thumb	157888 /15867	1.53	0.21
Lt little finger	158941 /14225	0.03	0.82
Lt knee	NA	0.005	0.95
Lt elbow	NA	0.24	0.62
Palms to ground	NA	0.47	0.49

4.6.5 First and second stages of labour

Table 4.43 shows a summary of the duration of the first and second stages of labour amongst women who had a spontaneous or an instrumental delivery.

Table 4.43 The first and second stages of labour

	Median	25th percentile	75th percentile
First stage of labour(mins)	312	210	435
Second stage of labour (mins)	30	15	50

Spearman correlation r_s showed no association between the first stage of labour and the left thumb as well as the left little finger- table 4.43. There was a significant correlation between the active first stage of labour and thumb mobility - fig 4.68

A significant correlation was also present between the second stage of labour and the left thumb as well as the left little finger - table 4.44; Figs 4.69 and 4.70. As seen in the scatter plots, the biological variation was great and the clinical significance of the correlation is doubtful.

Table 4.44 Joint mobility and the duration of labour

	1st stage of labour		Active 1st stage		2nd stage of labour	
	r_s	p value	r_s	p value	r_s	p value
Lt thumb	0.07	0.09	0.11	**0.008**	0.19	**<0.001**
Lt little finger	0.003	0.94	0.02	0.65	-0.12	**0.003**

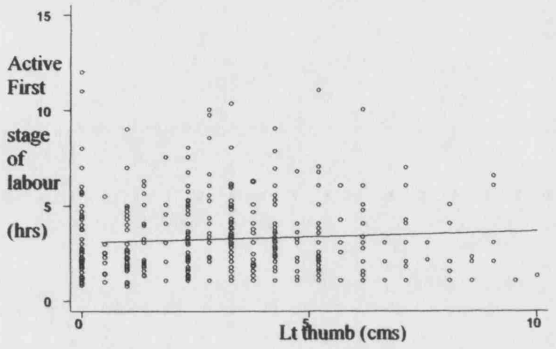


Fig 4.68 Comparison of the active first of labour and left thumb mobility
($r_s = 0.11$; p= 0.008)

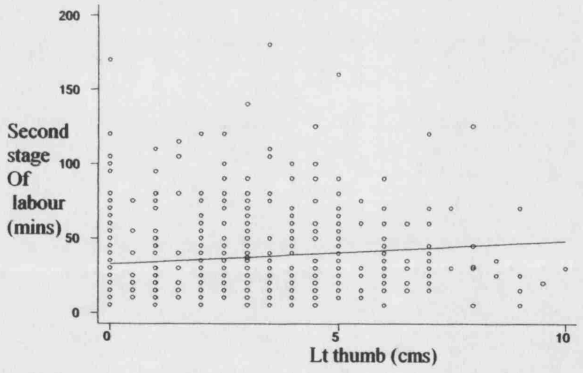


Fig 4.69 Comparison of the second stage of labour and left thumb mobility
($r_s = 0.19$; p<0.001)

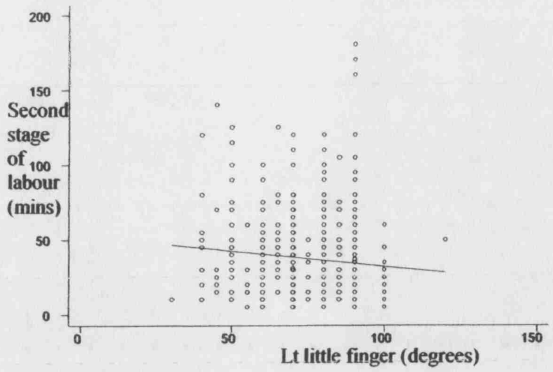


Fig 4.70 Comparison of the second stage of labour and left little finger mobility (r_s - 0.12; p= 0.003)

The Mann-Whitney test showed no evidence of a difference between the total length of the first stage of labour, the active first stage or the second stage of labour and left elbow mobility or the ability to touch the palms of the hands to the ground –table 4.45. Knee hyperextension correlated with the duration of the second stage of labour (p=0.04) in the initial independent analysis.

Table 4.45 The length of labour versus the left knee/elbow mobility and the ability to touch the palms of the hands to the ground.

	1st Stage of labour Sum ranks (p)	Active 1st stage Sum ranks (p)	2nd stage of labour Sum ranks (p)
Lt elbow extension (>180°)	142059 /18969(0.35)	143151 /18444(0.1)	143192 /17835 (0.07)
Lt knee extension (>180°)	113977 /47619 (0.92)	115722 /46442 (0.33)	117484 /44112 (**0.04**)
Palms to ground (yes/no)	163549 /50328 (0.17)	117080 /47370(0.75)	118195/45682 (0.23)

Factors affecting the duration of labour

Pearson's correlation *r* was used to find a correlation between birthweight of the first child and the duration of labour.

Spearman's r_s correlation was used to find a correlation between the head circumference of the first child, maternal BMI and age of women in the study with the duration of labour – table 4.46.

Table 4.46 The comparison of the length of labour with the first baby as well as maternal parameters.

	1st stage of labour		Active 1st stage		2nd stage of labour	
	r/r_s	p value	r/r_s	p value	r/r_s	p value
Birthweight(kg)	0.06	0.60	0.1	0.02	0.22	<0.001
Head circumference(cm)	0.02	0.66	0.06	0.13	0.19	<0.001
BMI	0.03	0.52	0.04	0.32	0.09	0.03
Age(years)	0.03	0.60	0.02	0.65	0.15	<0.001

Using the Mann-Whitney test, there was no difference in the duration of labour amongst women with or without several connective tissue markers- table 4.47. A family history of genital prolapse was associated with the duration of the active first stage as well as the total first stage but not with the second stage of labour.

Table 4.47 The comparison of connective tissue markers and duration of labour

	1st Stage of labour Mean rank sums (p)	Active 1st stage Mean rank sums (p)	2nd stage of labour Mean rank sums (p)
Constipation (no/yes)	107052 /56253 (0.47)	109329 /54540 (0.80)	108866 /54439(0.80)
Loc. anaes. effective (no/yes)	28939 /134367 (0.54)	29829 /134049 (0.24)	26312 /136994 (0.25)
Twisting joints (no/yes)	150634/12671 (0.93)	151716 /12162 (0.67)	151148 /12163 (0.69)
Clicking joints (no/yes)	106702/57176 (0.14)	105516 /58935 (0.72)	108494 /55383 (0.14)
Backpain (no/yes)	131307 /32571 (0.14)	130047 /34403 (0.58)	129137 /34741 (0.89)
Haemorrhoids (no/yes)	144353 /19525 (0.47)	144628 /19822 (0.35)	145536 /18342 (0.82)
Striae before preg. (no/yes)	145640/17666 (0.46)	143640 /20237 (0.20)	147138 /16167 (0.05)
H/O hypermobility (no/yes)	146275 /14753 (0.81)	147440 /14156 (0.75)	148477 /12551(0.08)
Varicose veins preg(no/yes)	140422 /22313 (0.37)	142181 /21124 (0.98)	141025 /21709 (0.66)
F/H constipation (no/yes)	3851848 /1755 (0.45)	90511 /73366 (0.67)	90842 /72464 (0.41)
F/H varicose veins(no/yes)	76892 /86985 (0.21)	78721 /85730 (0.59)	79773 /84105 (0.83)
F/H hypermobility(no/yes)	152378 /7517 (0.15)	153387 /7074 (0.05)	151569 /8325 (0.61)
F/H prolapse(no/yes)	131881 /28580 (**0.03**)	132828 /28199 (**0.01**)	130521 /29940 (0.18)

Simple regression was then performed with the logarithm of the second stage as the second stage of labour was not normally distributed- fig 4.71.

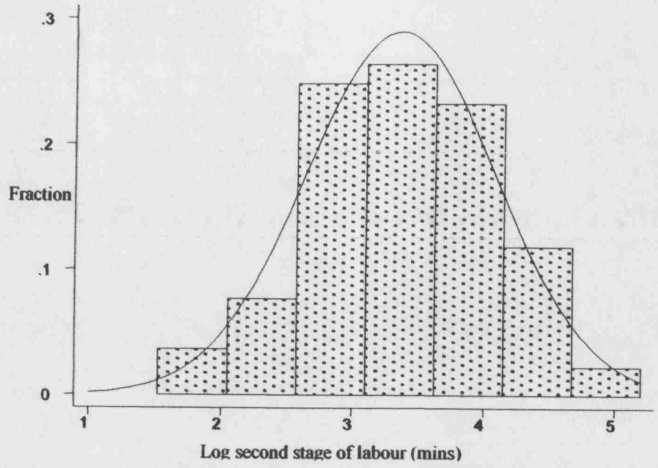


Fig 4.71 Log second stage of labour

Table 4.48 illustrates the simple regression coefficient, p values and CI of the variables that showed a significant correlation.

Table 4.48 Regression results – the second stage of labour

	Regression coeff.	P value	95% CI
Age	1.01	<0.001	0.45 to 1.57
Birthweight	14.2	<0.001	9.11 to 19.26
Head circumference	2.43	<0.001	1.14 to 3.72
Left little finger	-0.2	0.01	-0.36 to -0.05
Left knee	-0.17	0.01	-0.30 to -0.04
Left thumb	1.56	0.002	0.57 to 2.50
Skin striae before pregnancy	-5.9	0.09	-12.80 to 0.94

Simple regression of thumb mobility and a family history of genital prolapse with the first stage of labour, showed no statistical significance (regression coefficient of 0.32 and 0.13 ; p=0.08 and 0.87 ; respectively).

Multiple standard regression was then performed to analyse the association of statistically significant variables together with the duration of the second stage of labour- table 4.49.

Table 4.49 Multiple regression for the log. of the duration of the 2nd stage of labour

	Coefficient	P value	95% CI
Age (years)	0.27	<0.001	0.01 to 0.04
BMI	-0.01	0.03	-0.02 to -0.001
Birthweight (kg)	0.41	<0.001	0.24 to 0.58
Head circumference(cm)	0.01	0.47	-0.03 to 0.06
Skin striae before pregancy	-0.19	0.04	-0.37 to -0.01
Left little finger(degrees)	-0.005	0.04	-0.009 to -0.0002
Left knee ≥ 190°	-0.10	0.10	-0.23 to 0.02
Left thumb (cm)	0.04	0.01	0.008 to 0.06

Parameters which did not show any further significance were then eliminated and further multiple standard regression was performed for the duration of the second stage of labour to obtain a final model – table 4.50.

Table 4.50 Final multiple standard regression model for the logarithm of the duration of the second stage of labour

	Regression Coefficient	Standard error	P value	95% CI
Age (years)	0.03	0.007	<0.001	0.01 to 0.04
BMI	-0.01	0.005	0.01	-0.02 to -0.003
Birthweight (kg)-1st child	0.45	0.07	<0.001	0.31 to 0.58
Left little finger(degrees)	-0.005	0.002	0.04	-0.009 to -0.0002
Left thumb (cm)	0.04	0.01	0.01	0.009 to 0.07
Total	1.81	0.37	<0.001	1.08 to 2.55

In the final model, the baby's birthweight, maternal age and BMI together with the thumb and little finger mobility showed evidence of a difference with the second stage of labour.

The final regression equation was:

Log second stage = 1.81 + (0.03 x age in years) - (0.01x BMI) + (0.45 x birthweight in kilograms) + (0.04 x left thumb mobility in centimetres) - (0.005 x left little finger mobility in centimetres). The R-Sq for this equation was 0.12.

This equation attempts to provide a model for predicting the duration of the second stage of labour in nulliparous women. Important parameters must be missing as the R-sq value is low. This will be discussed further in section 5.7.

Back- transformation (anti-log) of the mean of the logarithm of the second stage of labour is necessary to obtain the geometric mean. This is essential to interpret the results of the model. The geometric mean was calculated as 28.79 minutes and is less than the mean (36.7 mins), but very similar to the median (30.0 mins) of the untransformed second stage data. Fig 4.72 shows the normal quantile plot for the model, showing that the residuals had a normal distribution. This assures us that the model estimates are correct.

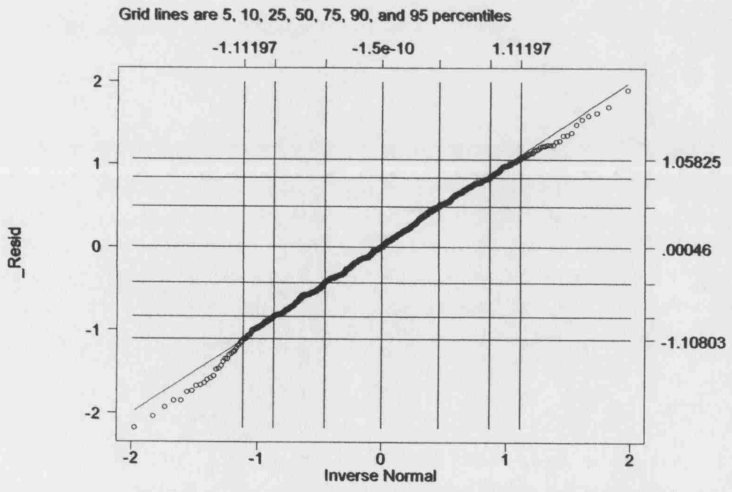


Fig 4.72 Normal quantile plot for the multiple regression model

CHAPTER V

DISCUSSION

5.1 Clinical Connective tissue markers:

The principal clinical connective tissue marker described in the literature is joint mobility.

5.1.1 The measurement of Individual joint Mobility

In section 3.1, I described how joint mobility was measured in the studies described in this thesis. Beighton et al remarked that joint angles were best measured using a goniometer [170]. They remarked that placing the arms of the goniometer can be subjective and suggested a pen mark over fixed anatomical points such as the lateral malleolus and greater trochanter for the knee. Alternatively, they placed the arms of the goniometer parallel to the main axis of the thigh/tibia or upper arm/forearm for the knee and elbow respectively. For the knee, patients could be asked to lie flat on their back, 'clamp' the thigh by firm pressure above the knee and lift the foot up with maximum effort to hyperextend the knee. This is the method used at the joint hypermobility clinic at The University College London Hospitals (personal communication). However, such patients can have extreme angles of hyperextension not seen in a general population. To measure large joint angles, a large joint goniometer is required. It was not practical to carry such an instrument from clinic to clinic or hospital to hospital in any one of the studies described here. As an alternative, a mark was made on cardboard alongside the main axis of the long bones adjacent to either elbow or knee joints. The small goniometer was then used to measure the angle made by extrapolation of these marks.

The Beighton score requirement is to record whether the thumb touches the forearm or not. It was felt more appropriate to record the actual thumb-forearm distance in a general population such as described in the studies described here.

History taking and examination: the study carried out in this thesis, could be criticised that I was not blinded to the patient's history when I examined the joints and this could have influenced the results in the genital prolapse study. However at the time of examination, I was not aware of the duration of labour.

5.1.2 *Joint Mobility – An overview of findings amongst all the patients in all the 3 studies in this thesis*

Joint mobility has long been used by rheumatologists as one marker in connective tissue disease. With time, it became evident that there was a range of joint mobility in the 'normal population'. In 1973, Beighton et al measured the mobility of joints amongst people of all ages in a rural village, 60 miles to the North West of Johannesburg in South Africa.[170] The score was validated and over the years, many researchers screened other populations in different countries using the same criteria as those used by Beighton et al (section 2.6). Beighton et al reported the actual extension angle of the right and left little finger although no other raw data were reported for any of the other joints. In our study, notification of the dominant side was not made. All other researchers used the Beighton score not the raw data of individual joints. The importance of joint mobility dominance was realised only after data had been collected and the left sided joint data were analysed assuming that all the population was right handed. This created an error in the results as up to 10% of the general poulation can be left-handed [182]. It is recommended that this is rectified in future studies.

The collective results from all patients participating in the three studies described in

Table 5.1 Little finger mobility- the results reported by Beighton et al (1973) and this thesis.

	This thesis In-vitro/in vivo connective tissue study-section 4.1	This thesis Gen. prolapse surgery and controls study – section 4.5 *(ages 40 to 51years)*	This thesis The progress of labour study – section 4.6 *(mean age=26 yr for women > 20 yrs)*	Beighton et al (1973 - South Africa)[170]
Right little finger (20 to 44yrs)	62.6°±17.7° (n=32)	65.0° ± 15.1° (n=71)	75.0° ± 15.0° (n=613)	58° (n=72)
Left little finger (20 to 44yrs)	66.6° ± 20.7° (n=32)	67.0° ± 16.6° (n=71)	77.0° ± 14.0° (n=612)	69° (n=72)
Mean little finger mobility (20 to 44yrs)	64.6° ± 19.3° (n=32)	66.2° ± 15.1° (n=71)	76.0° ± 14.1° (n=612)	60°±24° (n=101)
Right little finger (45 to 64yrs)	60°± 17.8° (n=29)	64.0° ± 17.4° (n=116)	NA	48° (n=72)
Left little finger (45 to 64yrs)	61.8° ± 17.7° (n=33)	65.5° ± 17.0° (n=116)	NA	64° (n=72)
Mean little finger mobility (45 to 64yrs)	60.1° ± 17.3° (n=29)	64.7° ± 16.6° (n=116)	NA	51°± 25° (n=107)

Although the standard deviations for the mean little finger mobility were reported by Beighton et al the standard deviations for the means of the right and left little fingers separately were not reported [170].

These researchers reported a bigger discrepancy between the right and left little fingers

than that found in this thesis. Their discrepancy for women aged between 20 to 44 years, was 11° and it was 16° for women aged 45 to 64 years. In the in-vitro tissue study, the discrepancy was 4° and in the other two studies, it was 2°. For women aged 45 to 84 years, the discrepancy was 1.8° and 1.5° - table 5.1. For the right little finger, a consistently greater angle of extension was found than that reported by Beighton et al. The reverse was true for the left little finger [170]. As 97% of females in their study were right-handed, one would have expected the right-sided muscles to be stronger than those of the left side. As discussed in section 2.6.2, increased muscle bulk, restricts joint movement. One would expect women in Beighton's study to have to do more manual work than any of the groups of women in the studies reported here. This might explain the difference between results.

This or any other group of researchers did not report the raw data for each of the other joints by and direct comparison is not possible.

The unpaired t-test was used to compare the mean little finger mobility in Beighton's study with the mean mobility in each of the studies. The results are shown in table 5.2. There was a highly statistically significant difference between the mean little finger mobility in women between 45 and 64 years of age in the genital prolapse study and that in Beighton's study. There was a similar difference in women aged 20 to 44 years in the progress of labour study.

Table 5.2 Mean little finger mobility- a comparison between the results reported by Beighton et al [170] and this thesis.

	In-vitro/in vivo connective tissue study-section 4.2 Beighton et al (1973) SE diff; 95% CI (p value)	Gen. prolapse surgery and progress of labour study - section 4.3 *(ages 40 to 51years)* Beighton et al (1973) SE diff; 95% CI (p value)	The progress of labour study section 4.4 *(mean age=26 yr for women > 20 yrs)* Beighton et al (1973) SE diff; 95% CI (p value)
Mean little finger mobil. (20 to 44yrs)	4.66; -4.6 to 13.82 (p=0.32)	3.22; -0.16 to 12.56 (p=0.06)	1.70; 12.65 to 19.35 **(p=<0.001)**
Mean little finger mobil. (45 to 64yrs)	4.94;-0.67 to 18.87 (p=0.07)	2.82; 8.14 to 19.26 **(p=<0.001)**	NA

The mean mobility for the right and left little fingers was 75° and 81° respectively in females under 19 years of age. In the study of nulliparous women and their progress of labour, the mean age for women was 26.2 years. The mean age for the retrospective genital prolapse study was 43 years. The mean ages were not reported by Beighton et al. Direct comparison is not possible but the increased mobility of the little fingers in these studies is likely to be secondary to a younger mean age than in the 2 groups in Beighton's study.

To enable comparison, the overall Beighton score was estimated for women in the second and third studies described here. Table 5.3 shows that there was no significant difference with the 9-point score reported by Beighton et al [170].

Table 5.3 Joint mobility- a comparison with the results reported by Beighton et al

	Genital prolapse and Progress of labour study (section 4.3)	Beighton et al (1973)	Estimate of risk diff. Fisher's exact test; p value (95% CI)
Mobility score (20-44 yrs)	0 to 2 – 73.6% 3 to 5 - 25% 6 to 9 - 1.4%	0 to 2 - 68% 3 to 5 - 25% 6 to 9 - 7%	0.06; p=0.44(-0.06 to 0.18) 0.00; p=1.00(-0.12 to 0.12) 0.06;p=0.06(-0.11to-0.006)
Mobility score (45 – 64 years	0 to 2 – 80.2% 3 to 5 –17.2 % 6 to 9 - 2.6%	0 to 2 - 83% 3 to 5 –16 % 6 to 9 - 1%	-0.03; p=0.72(-0.14 to 0.08) 0.01; p=1.00(-0.09 to 0.11) 0.02; p=0.62(-0.02 to 0.06)

Following Al Rawi et al [120], Norton et al also reported a strong correlation between genital prolapse and joint hypermobility.[77] However this group of researchers used a modified Beighton score as they assessed the mobility of the upper limb joints only. They also used 55 rather than 90 degrees as the cut-off point to diagnose little finger hypermobility. Joint hypermobility was diagnosed if two of the following 3 criteria were met:

1. Passive apposition of one or both thumbs to the volar aspect of the forearm
2. Passive hyperextension of one or both digits to greater than 55° from the horizontal
3. Active hyperextension of one or both elbows to greater than 190°

These criteria were used in the in-vivo/in-vitro clinical connective tissue study and women were divided into hypermobile or not hypermobile – table 5.4. The results are comparable as seen using Fisher's exact test when the 2 proportions for independent samples were compared.

Table 5.4 Hypermobility as compared to women in Norton's study

	This thesis:in-vivo/in-vitro connective tissue study	Norton et al study [77]	Estimate of risk diff. Fisher's exact test; p (95% CI)
Hypermobility (score 2 or 3)	24/ 70 (34.3%)	39/108 (36%)	0.02; p=0.37 (-0.16 to 0.47)
Normal mob. (score 0 or 1)	46/ 70 (65.7%)	69/108 (64%)	0.02; p=0.87 (-0.12 to 0.16)

In summary: Beighton's overall score has been used to describe joint hypermobility by many researchers. A consistently greater angle of hyperextension was found for the right little finger, than that reported by Beighton et al [170] and the reverse was true for the left little finger – table 5.1. This difference might be secondary to increased manual work by women in Beighton's study. It was also noted that in Beighton's study, women were younger but statistical comparison was not possible as these researchers did not report the mean age of women. Norton's criteria[77] were used to define joint hypermobility for the in-vitro and in-vivo connective tissue study. Findings were similar- table 5.4.

The dominant hand was not recorded in the studies reported here and an assumption was made that all women were right handed. This could have affected the results significantly and future studies *must* take this into account.

5.1.3 Joint Mobility and genital prolapse

As discussed in section 2.4, Al Rawi et al as well as Norton et al found increased joint mobility in women with genital prolapse [77;120]. In the second study described in this thesis, only knee hyperextension correlated with genital prolapse.

In the in-vitro and in-vivo connective tissue study, no difference was found in joint hypermobility amongst women with genital prolapse. In this study, women with prolapse (POP-Q stage II or III) were compared with those without (POP-Q stage 0 or I). Norton et al classified grade I prolapse as descent towards the introitus, grade II as prolapse at the introitus and grade III as prolapse that reached beyond the introitus [77]. According to the POP-Q staging system, genital prolapse reaching within 1 cm above or beyond the hymenal ring is stage II prolapse. Thus some women with stage II prolapse in the in-vivo/in-vitro connective tissue study, would have been classified as stage I and some as stage II or III by Norton et al. When women were reclassified according to Norton's classification, 4/17 women with stage II genital prolapse now had stage III prolapse. The 2 women with stage IV prolapse were now re-classified as stage III. Cross tabulation of this 'modified prolapse classification' with left elbow mobility in women over 40 years of age still did not show any correlation; $\chi^2 = 9.97$; p=0.13. Kruskal-Wallis test did not show a correlation between prolapse and the left little finger ($\chi^2 = 1.10$; p=0.78) or the left thumb mobility ($\chi^2 = 1.55$; p=0.67).

The prevalence of hypermobility amongst women in our study was high (34.3%) and compares well with that in Norton's study (36.0%) - table 5.4. Such a high prevalence of hypermobility might be inherent to women presenting to gynaecology clinics. In Norton's study, all subjects were recruited as they attended university gynaecology clinics and were reported to be middle class, white women between 49 and 59 years of age. The mean parity was 2.8 and 3.1 for women with hypermobility and normal

mobility respectively. All women but 3, in the control group of our in vitro/in-vivo connective tissue study were recruited prior to general gynaecology surgery and this might have caused further bias.

One should also note that in Norton's study, 15/39(38%) hypermobile women and 34/69 (50%) of women with normal mobility had undergone previous hysterectomy [77]. Similarly, 5/39 and 5/69 women in our 2 respective groups had also undergone previous genital prolapse surgery. If the hysterectomy was for genital prolapse, one would have been investigating a high risk population of recurrent prolapse rather than a more general gynaecology clinic population. In their study, 40% of women in the normal mobility group and 36% of the hypermobile group were on hormone replacement therapy (HRT) and 7% and 5% respectively were on steroid treatment [77]. Seven patients in the second study described here, were on HRT and none were on steroids. The difference in prevalence of HRT intake could have been secondary to a cultural difference or secondary to higher prevalence of osteoporosis or other indications for HRT, which on their own can predispose to genital prolapse. The effect of HRT on joint mobility is unknown.

Seventy six women had genital prolapse in Al Rawi's study [120]. Uterine prolapse was defined a first-degree descent if the prolapsed uterus did not reach the introitus. A second-degree descent was defined as the uterus reaching beyond the introitus on straining and third-degree descent was permanent descent of the uterus beyond the introitus. One must assume that 'uterus' refers to the most dependent part of the cervix. In fact their second-degree uterine prolapse could have been stage II, III or IV POP-Q stage. Vaginal wall prolapse was classified separately and no definition was made regarding the level of descent. Eighteen patients amongst 76 women in the prolapse group had uterine descent beyond the introitus, with or without anterior and posterior

vaginal wall prolapse. Eighteen percent (14/ 76) of women had isolated vaginal wall prolapse without uterine descent.

Thirty six (47.4%) women with genital prolapse group and 33/76 (44%) women without prolapse were under 39 years of age in Al Rawi's study [120]. In the genital prolapse/progress of labour study, 11/94 (11.7%) women with genital prolapse and none of the women in the control group were less than 39 years of age. Al Rawi et al reported that backpain was twice as common in the prolapse than in the control group and 34% of prolapse patients had joint pains compared with 24% in the control group. The inferences from these differences are that women could have had the benign joint hypermobility syndrome (BJHS) and were indeed at the upper range of the Gaussian distribution.

The varying rates of hysterectomy in different populations must also be emphasised. This can be a true difference secondary to cultural differences, or can be secondary to selection bias of the studied population.

In the genital prolapse/progress of labour study, 39/94 (41.5%) patients had isolated or combined anterior/posterior vaginal wall repair only. Ten patients (10.6%) underwent Manchester repair or vaginal hysterectomy only. All other patients underwent a combined repair. Five patients amongst women with surgery and none in the control group had undergone previous hysterectomy or any other prolapse surgery. The cervix reached beyond the hymenal ring in 10/29 women (34.5%) and none of the women with prolapse were under 39 years of age.

Thus there were major differences in demographic data as well as medical and surgical history of women in the genital prolapse/progress of labour study described here, and women in Al Rawi's and Norton's studies [77;120].

Both Al Rawi and Norton groups of researchers could have been investigating women at the upper extremes of the Gaussian hypermobility distribution.

An increased odds of 2.47 was found in undergoing prolapse surgery for women with knee hyperextension in the second study but no difference was found in any of the other joints amongst the 2 groups of women.

With increasing age, there is a decrease in joint mobility- section 2.6 and the rate of decrease can be higher in hypermobile joints. In fact, Al Rawi noted increased back and joint pains in hypermobile women. It is possible that as hypermobile women grow older, their inherent joint hypermobility becomes obscured with increased stiffening secondary to increased wear and tear. Performing a longitudinal study will confirm or refute this hypothesis.

5.1.4 Joint mobility and the progress of labour in nulliparous women

The Null hypothesis was partly rejected as thumb and little finger mobility correlated with the second stage of labour. The other joints assessed in Beighton score and the other clinical connective tissue markers did not show a significant correlation with the duration of the first and second stages of labour. As mentioned earlier, future prospective studies would need to consider which side is dominant. If these findings are confirmed, mobility of the hand joints might be used to predict the outcome of the second of labour. Consequently, patients' management could be affected accordingly with a better understanding of the intrinsic physiological behaviour of individual women.

5.2 Interview or questionnaire

The women in these studies were interviewed personally by me. The questions were standardised and were repeated when the patient did not understand the question. There might be criticism that women were not asked to fill in the questionnaires themselves. It has been demonstrated in the literature that postal questionnaire responses have a better relationship between urodynamic stress incontinence and detrusor overactivity and urodynamics, than interview-assisted questionnaire responses [184]. At the onset of the in-vitro/in-vivo study, patients were asked to fill in the questionnaire without interview assistance. However, most patients did not fill in the questionnaire completely and interviews were the better option for a better response rate. This was the experience of some other researchers [78]. It is possible that patients are keener to fill in questionnaires related to their symptoms than are women acting as controls for research studies.

5.2.1 Clinical connective tissue markers:

The questionnaire for the diagnosis of BJHS assesses clinical connective tissue markers. It was translated in Maltese and the questions in Interview No1 and II - Appendix I were asked in the second and third studies respectively. The English version has been well validated, however the translated version has not been [119]. Assessment of varicose veins and skin striae was subjective. The end results showed that there were no significant differences between groups and possible bias in the findings did not affect the results.

In summary, the revised Brighton criteria for the diagnosis of benign joint hypermobility syndrome, as well as bowel habit, haemorrhoids and back pain or relevant family history were not found to be different in women with or without genital

prolapse. They were not associated with a difference in the duration of labour in nulliparous women.

5.3 Personal and Family history of varicose veins, constipation and other pelvic floor symptoms

No internal consistency tests or test-retest reliability analyses and correlation with other validated symptom questionnaires were performed for bowel habit or family history. Questions were standardised. The end result showed no differences between women with or without genital prolapse or amongst nulliparous women with different duration of labour.

A family history of genital prolapse and urinary stress incontinence was included in the questionnaires. The stigma related to pelvic floor symptoms in previous generations, make family histories unreliable (see section 2.4). However, some researchers recognized a positive history of genital prolapse as a risk factor for genital prolapse [17]. There was no significant difference between women with or without genital prolapse in the second study. There was also no significant difference amongst younger women with different duration of labour in the third study.

Defaecation problems are present in a high proportion of women with genitourinary prolapse (section 2.4) [185]. However, it is difficult to determine whether a causal relationship exists. Weber et al found that the severity of posterior vaginal prolapse is not associated with the severity of bowel dysfunction [133]. Fialkow et al reported that only perineal descent rather than any of the POP-Q examination findings was strongly associated with bowel symptoms amongst women with genital prolapse [186].

A personal as well as a family history of constipation, haemorrhoids, backpain and varicose veins as well as all the symptoms assessed by the Brighton criteria were recorded in the second and third studies. Various studies have shown that constipation and varicose veins often coexist in the same patient with a family history of similar problems [187;188]. Thus a personal as well as a family history of the varicose veins as well as constipation were investigated in the questionnaires. No consistent differences were found amongst women with or without genital prolapse or amongst women with different duration of labour.

Backpain is a common symptom in the general population and more so in pregnancy - section 2.5.1. It is highly prevalent amongst subjects with joint hypermobility and there is a familial predisposition [119;189-191]. For these reasons, a personal and family history of sciatic backpain was included in the second and third studies. No significant differences were found amongst different groups of women in the second and third studies.

5.4 First and Second stages of labour

Estimates of the total first stage of labour can be highly inaccurate. In the studies in this thesis, data were collected from the obstetric records. Future studies should address this issue. Only an approximate estimate to the first stage of labour can be made for women who reach labour ward beyond 4 cm cervical dilatation. Although the number of such women would be small, these might be the women who could affect the results. Dietz et al attached specifically designed data sheets to the patients' antenatal records such that information was collected as accurately as possible [192]. Neither these researchers nor Chaliha et al [193] defined the first stage of labour.

Analysis of data in the second and third studies, showed that the first and second stages of labour were not normally distributed and non- parametric analysis was performed to test for a statistical significance. The mean values were reported by Chaliha et al[193] as well as by Dietz et al implying a normal distribution although no specific reference was made in the papers– table 5.5 [193;194].

The progress of labour is complicated. Many factors including a trusted companion in labour, play a role-see section 2.5. Epidural analgesia prolongs labour. The percentage of women who had an epidural in labour was 51.9% in the study reported by Chaliha et al [193] but was not mentioned by Dietz et al [195]. In these studies conducted in Malta, epidural analgesia was not used. Women would have commenced active pushing (active second stage) as soon as the first stage of labour finished. On its own, the use of epidural analgesia can explain the differences in duration of the first and second stages of labour. In both studies reported in this thesis, both the first and second stages of labour were significantly shorter than in the study reported by Chaliha et al, ($p<0.001$) using the 2 sample t-test – table 5.5. No statistical comparison could be performed with the study by Dietz et al because the minimum and maximum values but no standard deviations were reported in their study. Future studies in nulliparous women who are not offered epidural analgesia shall be very difficult. However, strict protocols for the management of labour in women using epidurals would enable researchers account for its confounding effect on final results.

Table 5.5 The duration of the first and second stages of labour (± SD)

	Chaliha et al [193] (n=503)	Genital prolapse and 1st labour (n=180)	Progress of labour (n=665)
First stage of labour (mins)	591 ± 363	431 ± 311	353 ±194
Passive second stage(mins)	37 ± 33	NA	NA
Active 2nd stage of labour (mins)	55 ± 37	43 ± 28	37 ± 26

Comparison with other studies is difficult. Thus, in 1999 Albers reported a mean length of 462 ± 828 minutes for the active-phase of the first stage of labour in 806 nulliparous women who did not receive epidural analgesia during labour. The mean length of the second stage was 54± 64 minutes [196]. This study was conducted in nine midwifery-led units across the US during 1996. Women came from multicultural backgrounds. Thus 51.3% were Hispanic and 30.4% were non-Hispanic white. In their study, 161 women arrived 'late in labour' (more than 4cm cervical dilatation), 578 had an interventional delivery, 932 women received epidural analgesia and 1336 received syntocinon during labour. All these women were excluded in the analysis. Women who were less than 37 weeks or more than 40 weeks gestation or had ruptured their membranes for more than 24 hours were also excluded. Thus, the reported results were on a very selected population.

Similarly, Duignan et al reported a mean duration of 336 minutes for the first stage of labour in nulliparous women [197]. This was estimated from the time of admission to the labour ward to full dilatation of the cervix. The mean duration of the active second stage of labour was 41.5 minutes. In summary, direct comparison of the duration of labour is not possible because studies are heterogenous.

Of note is the duration of labour in both the studies reported here. Women in the first study (1970's) had a significantly longer labour (7.18; SD 5.2 hours) than women in the second study in 2001 (5.88; SD 3.23 hours). The difference in means for the first stage of labour in the 2 studies was 1.3 hours (CI: 0.66 to 1.93) and that for the second stage of labour was 6.22 minutes(CI: 1.70 to 10.74). Syntocinon was used in 391/665(58.8%) women during the first stage of labour in the 2001 study and in 80/186 (43.0%) women in the 1970's study (CI: 0.08 to 0.24). This difference could possibly be secondary to a lower threshold to use syntocinon and could have contributed to the shortening of labour. The discrete use of syntocinon in low-risk populations of women, was illustrated by the wide variations in patterns of its use across geographic areas in the different units [196;198]. It could also reflect the selected population of women in the second study. At recruitment, these women were attending gynaecology clinics albeit half of them only attended for a cervical smear test.

5.5 In-vivo and in-vitro connective tissue markers and Genital Prolapse

All the work described in this first study relates to experiments performed on human female tissue biopsies taken during surgery. Examination for genital prolapse was performed using the standardised POP-Q classification. Since lithotomy examination beds were not available, patients were examined in the standing position to ensure the full extent of their genital prolapse was being estimated (section 3.1.2; page 75). Although this method has been acknowledged in the literature, it is not the standard method and comparison with other literature becomes difficult.

The Null Hypothesis:

The first part of the Null hypothesis was rejected. In this study no consistent correlation between biomechanical tissue properties and clinical markers in women with and without genital prolapse was detected. There was also no consistent correlation between joint mobility, parity or ethnicity and genital prolapse.

5.5.1 In-vitro parameters - Methods

Recruitment of patients: in this study, 70 patients underwent gynaecological and 3 patients underwent general surgery. It proved difficult to recruit general surgery patients because most patients did not fulfil the entry criteria. It was not possible to recruit patients undergoing emergency surgery as it was not ethical to contact patients immediately prior to their surgery. Thus only 3 women with genital prolapse had their rectus sheath biopsied.

Tissue biopsy sampling: Biopsies were obtained by seven different gynaecologists. All gynaecologists used diathermy to open the abdominal wall. The rectus sheath biopsies were sampled as far as possible from the site of diathermy but some degree of desiccation could have affected the results. However, since all the surgeons used diathermy and sampling was performed in a standardised way for all women, this is unlikely to have affected the comparison of results.

I sampled the pelvic tissue biopsies from the uterus once it was excised. Following excision of the uterus, some pedicles retracted and it was impossible to retrieve the required biopsies without traumatising the tissue; hence they were not taken. Direct sampling could have supplied me with a greater number of adequate tissue biopsies. This was not possible for most of the hysterectomy specimens as the majority of the

procedures, were performed by trainees under tuition. Direct sampling would have added the surgical risks to the patients.

Preparation of tissue strips:

There is a learning curve for carrying out any experiment. There was a high intra-experimental variation amongst the first 9 patients in the study as assessed by the sample variation test (page 100). These experiments were not included in the analysis. It was necessary to prepare tissue strips of restricted diameter for each experiment to ensure diffusion of Tyrode's solution throughout the whole cross section of the biopsy. A thin strip and an adequate flow of superfusate ensured tissue viability. Tissue strips could easily be retrieved from the rectus sheath but for the other specimens, the identification of fibre orientation and trimming of a thin strip necessitated greater dissection. This sampling of the specimens and their trimming would have caused some disruption of the connective network, probably affecting the mechanical properties of the specimens. However, the trimming was performed in a standardised way for all groups of women. Specimens of varying lengths and cross-sectional areas are able to support different forces or loads and cause variations in total elongation of the specimen. This was taken into account by standard biomechanical calculations; with stress adjusted per unit cross sectional area and deformation allowed for the initial length of the biopsy strip. This enabled tissue strips of varying length and thickness to be compared together. As mentioned earlier, theoretically the smallest rather than the mean diameter of the strip should have been used in the analysis. The difference was was not statistically different and the end results would not have been changed.

Number of patients: For some groups of women, only a small number of biopsies from some sites could be obtained. However, other researchers also compared relatively

small numbers of women with and without genital prolapse. Thus Norton et al found a difference in collagen content amongst 8 women with recurrent genital prolapse and 15 women without prolapse [61]. Jackson et al found a difference in collagen content amongst 8 premenopausal women with genital prolapse and 10 women without [62].

5.5.2 In-Vivo parameters - The results

Parity and ethnicity were analysed for a correlation with the POP-Q examination findings.

Parity: Landon et al studied the biomechanical properties of the rectus sheath in 24 pregnant and 92 non-pregnant women in relation to urinary incontinence[199]. The rectus sheath was less stiff and stretched more than that in non-pregnant women. The tensile strength was reduced and less stress was required to result in tissue failure. No consistent relationship was found between the biomechanical parameters and parity in the in-vitro/in-vivo connective tissue study. In conclusion, the measured biomechanical parameters did not reflect the effect that parity might have on connective tissue.

Ethnicity: as discussed in section 2.4, Caucasian women have been shown to have an increased incidence of genital prolapse when compared to African women. There was no consistent association in this study as women were subdivided into Caucasian or Asian and compared with Afro-Caribbean or African women. Afro-Caribbean women might well have different connective tissue properties than African women. Patients were only recruited in this study if they consented to have rectus sheath or pelvic floor tissue biopsies. I found it difficult to obtain consent for biopsies in African women who were undergoing surgery namely for fibromyomata. This seems to be a cultural issue, with these women being very resistant to give away anything related to their uterus

which represents their womanhood. This explains the small number of African women in this study. One must also note that mixed ethnicity of many patients make such analysis difficult.

5.5.3 Biomechanical properties:

The properties of connective tissue are influenced by all its components including its collagen content. As discussed in section 2.2.2, its water content and the 'buckling function' of the ground substance affects its mechanical properties. The mechanical strength and distensibility/ elasticity of tissues from pelvic floor, skin as well as the rectus sheath have been investigated by other researchers to investigate different pathology of the pelvic floor [66;199] [66;183]. Analysis using the load-elongation curves with ultimate tensile strengths, stress at maximum or breaking load as well as the elastic modulus were measured biomechanical parameters- section 2.2.3. In clinical practice, it is unusual to submit tissue to high mechanical stress. In the in-vitro/in-vivo connective tissue study, changes on strain during imposition of stress on the tissue was investigated The objective of these experiments was to find a biomechanical model that explains any biomechanical differences in tissue amongst women with and without genital prolapse. The wide biological inter-experimental variability amongst patients, prevented the formulation of such a model. Although direct comparison of the results with published data is not possible, in the following sections, attempt will be made to compare the observations with published findings.

Biomechanical Results:

Following some publications whilst this study was being conducted, extra tissue analysis was performed to compare women according to age. Comparisons between different pelvic floor biopsy specimens were also performed.

Due to the wide inter-experimental biological variability and the small number of patients, the differences in the in-vitro tissue properties amongst different groups of women must be interpreted with caution. However the differences in the round ligament biomechanical properties were consistent.

The values of (Tss), (A) and (B) of the round ligament were significantly higher in women over 40 years of age without genital prolapse than in women under 39 years of age (n=5) - table 5.6. Amongst women over 40 years of age, values of (A) and (B) of the round ligament were significantly higher in women without genital prolapse (n=19) than in those with prolapse (n=11)- table 5.6. The values of Tss of the round ligament in women over 40 years, with and without prolapse were not significantly different. This implies that the round ligament becomes more elastic and has a larger steady state tension with increasing age. Amongst women with genital prolapse this increased visco- elastic component is lost although the steady state elasticity is retained. Whether this is a cause or the effect of genital prolapse remains to be investigated in well designed studies.

As noted in table 4.4, there was no plastic change in any of the tissues as the resting state tension before (T1) or after (T5) stretching was not significantly different.

In practice, the overall thickness of the round ligament in women varies greatly and a very thin strip is then used during the individual experiments. The outermost layers were used to sample the tissue strips in this study. As explained in section 2.1.3, the

round ligament consists of an inner core of unstriated muscle separated from an outer thin layer of muscle by connective tissue and covered by peritoneum. The inner core was avoided entirely in the sampling of the biopsies. The thickness of the peritoneal layer also varies amongst women, and in some biopsies could have formed a main constituent of the tissue strip. Thus further experimentation on the round ligament as well as the peritoneum are warranted. Both are relatively easy to biopsy during surgery. Histological analysis would determine which constituents of the round ligament were being sampled.

If the round ligament parameters truly represent intrinsic connective tissue properties, the round ligament can be used to study the changes and effects of modulators in genital prolapse.

Table 5.6 The comparison of round ligament properties
(data from tables, 4.8, 4.9, 4.17, 4.18, 4.19)

	Median Tss (Nmm^{-3})	Median A (Nmm^{-3})	Median B(Nmm^{-3})
Women <39 years (no genital prolapse)	3.25	1.76	0.83
Women > 40 years no genital prolapse (*)	9.40 (9.42)	3.72 (3.72)	2.84 (3.65)
Women > 40 years (with genital prolapse)	6.45	1.79	1.04

(*) Nulliparous women included

Adams et al found no difference in strength or elasticity, in any of the parameters of the round, uterosacral or cardinal ligaments or the abdominal wall and vaginal skin

amongst pre and post menopausal women [200]. These researchers measured the elasticity per unit weight of collagen rather than per unit change in length of the tissue strip. They also measured the 'load bearing ability' in Newtons/mg of collagen.

The Secondary Null hypothesis: in this study, only 2 women over 40 years of age with genital prolapse had their rectus sheath biopsied and therefore comparison with biopsies from other sites was not possible.

To our knowledge, no study in the literature compared biomechanical properties of the rectus sheath representing systemic connective tissue with biopsies taken from the pelvic floor. The rectus sheath is easier to biopsy and if its characteristics reflect those of the pelvic floor, further experimentation to study pelvic floor tissue could be performed on the rectus sheath.

The results showed that in women *without* genital prolapse, the values of (B) and (A) of the rectus sheath and the round ligament were statistically different (table 4.22, page 150). The rectus sheath had a smaller viscous component of elasticity than the round ligament in women without genital prolapse – table 5.7. There were no other consistent differences. The uterosacral ligament and the posterior vaginal wall had similar biomechanical properties as the rectus sheath. A difference in properties between the rectus sheath and the anterior vaginal wall is questionable as the statistical difference seen in (A) was not reflected in (B). There was no difference in Tss or steady state tension within the tissues.

In conclusion, the rectus sheath can be used for further experimentation to study the biomechanical properties of the pelvic floor. The round ligament however, has different

elasticity properties and should be studied separately. In this study, no in-vitro parameters of any of the tissues, reflected in-vivo clinical connective tissue markers.

Table 5.7 The comparison of round ligament and rectus sheath properties in women without genital prolapse (data from tables 4.8, 4.9)

	A (Nmm^{-3})	B (Nmm^{-3})
Round ligament	3.72	2.84
Rectus sheath	1.77	0.96

When the biopsies from the pelvic floor of each patient were compared together amongst women without prolapse, the values of (B) of the anterior vaginal wall (median of 2.84 $Nmm^{-3)}$ were statistically different from the (B) values (median of 0.72 Nmm^{-3}) of the round ligament. The corresponding values of (A) were 3.72 Nmm^{-3} and 1.24 Nmm^{-3} but statistically there was no difference. All the other pelvic floor tissues were similar. Adams et al found that the elasticity of the vaginal wall (1.92 mm/mg collagen) was significantly different from that of the round ligament (3.12 mm/mg collagen) in women without genital prolapse [200]. They found no other significant differences in the elasticity of the uterosacral, round and cardinal ligaments or the vaginal wall and abdominal skin amongst women without genital prolapse. Overall, it is likely that the round ligament has different biomechanical properties from the anterior vaginal wall in women without genital prolapse, but the other tissues have similar properties.

In women *with* genital prolapse: Jurnalov et al (1999) at the Mayo clinic, found increased tensile strength and stiffness of the uterosacral ligaments when compared to the anterior as well as the posterior vaginal wall in women with genital prolapse [183]. For

women without genital prolapse, the reverse was true. Twenty nine of 34 women in their study underwent vaginal hysterectomy and none of their patients had undergone previous pelvic floor surgery. POP-Q stage 0 or I was classified as no prolapse and stage II and III was considered as prolapse.

The in-vitro and in-vivo connective tissue study: The findings from this study show some differences. In women with genital prolapse, the values of (A) and (Tss) of the anterior vaginal wall were significantly lower than the corresponding values of the uterosacral ligaments. For the same group of women, the median value of (B) for the anterior vaginal wall was 0.71 and that for the uterosacral ligament was 0.79, (tables 4.8 and 4.9) but this difference did not reach statistical significance. It is therefore likely, that in women with genital prolapse, the anterior vaginal wall is stiffer (lower elasticity). The raw data are replicated in table 5.8.

In women without genital prolapse, the anterior vaginal wall and the uterosacral ligaments had statistically similar baseline tension and viscous component elasticity.

It is difficult to hypothesise the mechanism that triggers genital prolapse. Jurnalov et al felt that failure and prolapse of the posterior vaginal wall puts excessive strain on the uterosacral ligaments supporting the uterus higher up. These in turn lose their tensile strength genital prolapse is the end result. However the noticed differences might be the effect of prolapse rather than a causative factor. These findings warrant further investigation. Since our own as well as other studies in the literature were cross-sectional studies, the longitudinal effects on tissue cannot be extrapolated.

Overall, it is possible different types of prolapse are secondary to different tissues properties. Analysis should be based on the type of prolapse present, in order to gain a clearer understanding of the biological events that trigger weakening of the pelvic floor.

Table 5.8 Tss-steady state tissue stress and (A) change in stress during loading (data from tables 4.19, 4.20)

	A (Nmm^{-3}) Uterosacral lig - median	A (Nmm^{-3}) Anterior vag wall - median	Tss (Nmm^{-3}) Uterosacral lig - median	Tss(Nmm^{-3}) Anter. vag wall - median
Women with genital prolapse	1.4	1.00	4.53	3.12
Women without genital prolapse	3.18	1.24	9.37	4.17

In summary, from this study, we suggest further longitudinal biomechanical and possibly other connective tissue studies of the round ligament. There seems to be a significant difference betwee women with and without prolapse but only longitudinal studies can confirm or refute this data. Studies of the peritoneum amongst young women undergoing any type of abdominal surgery is also suggested to correlate with clinical connective tissue markers. Any significant differences car be used to help us understand the effects of labour on the pelvic floor as well as genital prolapse i middle and older age.

5.6 The Relationship of Genital Prolapse and the progress of the first labour/connective tissue clinical markers

Increased parity has long been associated with genital prolapse and urine incontinence but the obstetric history has not been well investigated [2;4]. There are questions as to whether it is pregnancy, the delivery process or the size of the baby delivered vaginally, that are most responsible for damage to the pelvic support. This study was designed to investigate obstetric parameters during the first labour amongst women with or without genital prolapse surgery. Patients were recruited after genital prolapse surgery but this resulted in selection bias that is likely to have affected the results. Different surgeons have varying threshold for surgery. Random selection of women in the general community and a POP-Q prolapse examination would have excluded this bias but would have led to difficulties in recruitment.

It has been noted that there is a doubling of the incidence of genital prolapse for every decade of life[79]. In this study, women of the same age group were compared together to eliminate age as an independent risk factor. All women had the same ethnicity and were delivered in the same hospital; hence other confounding factors were excluded. This implies that the findings could be specific to this population of women, only.

Women in the control group, were recruited in smear test clinics if they denied any genital bulge in the vagina or a history of genital prolapse. The symptoms of genital prolapse vary, as does the perception of bother. Pelvic heaviness, the presence of genital bulge and use of fingers on the perineum or in the vagina to facilitate defaecation have been used by researchers as confirmatory symptoms for genital prolapse[78;201]. Other symptoms, such as sexual and incontinence problems are other common but non-specific symptoms of genital prolapse [202]. However, a genital bulge or protrusion seems to be the symptom showing the best correlation with the prevalence of genital prolapse

on examination [4;11;203]. Other researchers did not specify subjective symptoms for a diagnosis of genital prolapse.[204] Premenstrual pelvic congestion or uterine fibroids can result in a dragging sensation and therefore 'vaginal heaviness or dragging' as a diagnostic symptom was avoided. Only women whose obstetric notes (from the 1970's) could be found in the NHS hospital records, were included. The outcome data are unlikely to have been affected as selection of patients was similar in both groups. Precise data on the proportion of women who had their babies in private hospitals in the 1970's are not available, but more than 90% of births on the islands occurred in the NHS hospital where this study was conducted.

5.6.1 Parity, Pregnancy and mode of delivery

The caesarean section rate (20/190; 10.5%) in the genital prolapse study was comparable to that in the UK and USA in the 1970's [205;206]. The rate of instrumental delivery in this study was 3.7% (7/190). Chaliha et al reported a 15.8% rate of emergency caesarean section and in Dietz's study it was 18% [165;193]. Dietz et al reported a 17% rate of instrumental delivery and in Chaliha's study it was 22.7%. The differences in instrumental delivery, reflect the use of epidural analgesia by women in these studies. The progress of labour study will be discussed later but the rate of emergency caesarean section was 14.7% and the instrumental delivery rate was 3.6% (24/665).

In recent years, urine incontinence has been given ample attention in relation to the mode of delivery. One must reiterate that the underlying pathology of urinary incontinence and genital prolapse are likely to be different and extrapolation is wrong. Chaliha et al reported an 8.4% prevalence of stress incontinence soon after elective or

emergency caesarean section and Iosif et al reported an 8.8% prevalence soon after elective caesarean section [193;207]. This suggests that for some women, a mechanism other than trauma sustained during vaginal delivery is responsible for urinary stress incontinence. The association with genital prolapse is less clear as the condition becomes evident at a later stage in life. In the genital prolapse study, there was no statistically significant difference in instrumental or caesarean delivery amongst women with or without genital prolapse, but the number of women was small. One should note that the cesarean section rate has increased over the last few decades and this could have an effect on long-term pelvic floor function.

Chiaffarino reported that forceps delivery was not associated with risk of prolapse after taking into account the effect of the number of vaginal deliveries [17]. Carley et al found that caesarean section delivery was associated with a reduced prevalence of incontinence and pelvic organ prolapse [12].

MacLennan et al investigated long-term pelvic floor dysfunction in a survey in Australia that included women between 15 and 97 years of age [11]. Pregnancy regardless of the mode of delivery, greatly increased the prevalence of major pelvic floor dysfunction defined as any type of incontinence, symptoms of prolapse or previous pelvic floor surgery. When compared with nulliparity, pelvic floor dysfunction was significantly associated with caesarean section (OR 2.5), spontaneous vaginal delivery (OR 3.4) and at least one instrumental delivery (OR 4.3). The difference between caesarean and instrumental delivery was significant ($p < 0.03$) but was not for caesarean and spontaneous delivery. They did not differentiate between emergency and elective caesarean sections.

All these studies are retrospective case-control studies and the question as to how much pregnancy and vaginal delivery contribute to genital prolapse remains to be answered in a longitudinal prospective study.

5.6.2 The Null hypothesis

The primary Null hypothesis of this study was accepted, as there was no difference in the first or second stage of labour in women with and without any type of genital prolapse surgery, when other factors were taken into account by logistic regression. A longer second stage of labour was associated with genital prolapse when assessed independently but not in multiple logistic regression, when account was taken of the other factors. The power of this study might not have been enough to detect a difference in the duration of labour. At the onset of this study, the number of patients required to detect a 20-minute difference in the duration of labour was calculated to be 90 in each arm. This estimate was based on the duration of the second stage of labour reported in the literature. As explained earlier, the duration of labour varies in each unit and in each study, as obstetric practice and the population of women studied are different.

In order to detect a difference in the duration of the second stage of labour in the obstetric unit where this study was conducted, a sample size of 145 women would have been required in each arm, for the study to have an 80% power at 0.05 significance level. This calculation was based on the findings of a mean value of 38.2 (SD: 27.6) and 47.4 (SD: 28.0) minutes for the second stage of labour in women with and without genital prolapse, respectively.

Secondary hypothesis: the duration of the active first stage of labour correlated with middle compartment prolapse surgery. It is possible that the pathology of the 3 compartments of the pelvic floor is different. A large study with an adequate number of women suffering from different compartment genital prolapse is required to confirm or refute these results.

5.6.3 Other results

Initial analysis showed that there were correlations of several variables and genital prolapse. However, the ultimate regression analysis model showed that only a history of heavy lifting, knee hyperextension, the use of syntocinon in the first labour and the birthweight of the first child were associated with the odds of having genital prolapse surgery up to the age of 50 years. The association of genital prolapse with birthweight and heavy lifting has been reported by other researchers.- section 2.5.2g. This study reports similar findings.

Smoking

In this study, smokers had a significantly decreased odds of having genital prolapse surgery. For genital prolapse as well as incontinence surgery, Samuelsson reported that smoking increased the odds ratio by 1.9 times [204]. Olsen et al noticed that amongst women undergoing genital prolapse sugery, (patients detected from a hospital admission database in Denmark), nearly half were current or former smokers and one-fifth had chronic lung disease. The overall recurrent prolapse surgery rate was 29.2% [2]. One would expect an increase in coughing causing increased intra-abdominal pressure, to increase the incidence of genital prolapse in these women. Mant reported on a cohort of women originally recruited in their 20's in family planning clinics [4]. There was a

borderline significant trend for heavier smokers to be at lower risk of prolapse surgery [4]. Ex-smokers at entry, had a 26% higher risk of prolapse surgery than non-smokers. These researchers remarked that one possible explanation might be that women who had some degree of stress incontinence at a younger age, had stopped smoking to control their coughing, in the awareness that their condition could deteriorate. Other changes in their lifestyle could have helped to safeguard the pelvic floor from prolapse. Another explanation might be that surgeons are more reluctant to perform surgery on a smoker and Mant's study as well as the one presented in this thesis, only recruited women who had undergone prolapse surgery. A longitudinal study of women in the general community would give unbiased conclusions.

Infant's birthweight and Syntocinon

The increased odds for genital prolapse surgery with increased use of syntocinon needs to be confirmed in a prospective study. The risk was lower in women with middle compartment prolapse than that in women without any type of prolapse. A low threshold for caesarean section might be justified for women with delayed progress of labour and a suspected large baby, as a prophylactic measure against genital prolapse later on in life.

5.7 The Relationship of Connective tissue markers and the progress of labour

The objectives of this study were to investigate the relationship of the mode of delivery as well as the duration of labour to connective tissue markers. Recruited women were of the same ethnicity and their parameters cannot be generalised. It was a well conducted survey and every attempt was made to recruit a large number of women who represented the general population.

One hundred and sixty two amongst the 1153 eligible women could not be contacted and were excluded from the study. Attempt was made to contact women at different times of the day. Initially, I called them during daytime hours. If there was no answer, women were contacted either that same evening or between 7.15hr and 8.00hr, the following morning. The local office hours in Malta often start at 7.30 or 8.00 o'clock and a journey to or from work often does not last longer than 30 minutes. If unsuccessful, I phoned again the next day and over the weekend. The same process was repeated several times during the following three weeks. Only then, were women believed to be not contactable. Sixty two women in this group, were from holiday resort areas. Couples in these areas often live in temporary accommodation, and move house after short periods of time.

This study was conducted between July and September 2002. This was advantageous because short summer working hours on the islands as well as good weather, probably encouraged more women to attend. Some women however, must have been living in summer residences and were not contactable.

After introduction of the study, 97 women had one or more of the exclusion criteria. Amongst the remaining 885 women eligible for the study, 218 women (25%) failed to attend for interview.

Mode of delivery

The odds of having an interventional delivery was 1.8 times for every 1 kg increase in birthweight. BMI also correlated with the mode of delivery but joint mobility and other clinical connective tissue markers were not. Birthweight was also strongly predictive of the duration of labour. This is in keeping with the current literature (section 2.5) but the extent varies in different studies.

One should note that the proportion of women who had interventional deliveries in the study, was smaller than that registered in the national obstetric database. (Table 4.35; page 170). This might reflect the lower prevalence of women who had induction of labour in the study. Chaliha et al investigated joint mobility in 549 nulliparous women at St. George's teaching hospital, London [193]. There was no difference in the rate of interventional delivery amongst women with different joint mobility. Dietz et al, also found no correlation between the mode of delivery and joint mobility [165].

5.7.1 *The Null hypothesis*

The Null hypothesis was rejected. Increased little finger and thumb mobility was associated with decreased duration of the second stage of labour. One must note that the lower the thumb/forearm distance, the greater the joint mobility. Joint mobility may provide a measure of the 'elasticity' of the pelvic girdle - the 'passages'. In this study, left knee and little finger mobility increased with increased BMI ($\chi^2 = 10.5$; p=0.001, and $r_s = 0.08$; p=0.04 respectively). There was no statistically significant difference with any other joints.

In the current literature, Dietz et al are the only researchers who compared upper limb joint mobility as well as pelvic floor ultrasound findings to the duration of labour.[165] This study was described in page 62. Pelvic organ mobility as detected on perineal

ultrasound, was significantly associated with the total length of the second stage of labour. This was mainly due to the length of passive, not active second stage. There was also a statistically significant correlation between delivery mode and pelvic organ descent, with the lowest mobility seen in women who required a Caesarean section in the second stage. Joint mobility did not correlate with delivery data. Women with caesarean section for failure to progress in the active first stage of labour were excluded in the analysis and epidural analgesia was not accounted for in this study which included 160 nulliparous women. The larger number of women in the study described in this thesis might account for the observed difference.

Final Regression Model – besides studying the association of clinical connective tissue markers to the progress and outcome of labour, an attempt was made to generate a predictive model equation for the progress and outcome of labour in nulliparous women.

A final standard multiple regression equation could only be generated for the second stage of labour:

Log second stage = 1.81 + (0.03 x age in years) - (0.01x BMI) + (0.45 x birthweight in kilograms) + (0.04 x left thumb/forearm distance in centimetres) - (0.005 x left little finger mobility in centimetres).

This signifies that a longer second stage of labour would be expected with increased maternal age and baby's birthweight but the reverse would be expected in women with increased thumb as well as little finger mobility and increased BMI. It should be reiterated that the thumb-forearm distance is represented in the equation and the smaller it is the greater the thumb's mobility.

The above variables in the equation explain 12% (R-sq) of the variation in the duration of the logarithm of the second stage of labour and although statistically significant, this model is not highly predictive. Other obstetric factors as mentioned in section 5.7 should be investigated in future prospective studies. The position of the presenting part during labour is known to have a great impact on the progress and outcome of labour but unfortunately it was recorded as occipito-anterior in all but a few patients. It is important to distinguish between quantitative estimation and hypothesis testing. The effect of joint mobility on the duration of the second stage of labour although statistically significant, is small and on its own is unlikely to be clinically useful.

CHAPTER VI

6.0 CONCLUSION

Genital prolapse can severely impact on quality of life and is likely to become more common as the population ages in the coming years. Vaginal deliveries have the strongest link to genital prolapse but vaginal delivery is a poor predictor for genital prolapse. The pathophysiological mechanisms of prolapse have not been fully elucidated but multiple factors must play a role in the progression of the disease. In this thesis, the aim was to investigate further, the role of connective tissue in genital prolapse as well as during the first childbirth when it is likely that most of the damage to the pelvic floor occurs.

Collagen studies in genital prolapse have been reported in the literature. However connective tissue has other constituents and for this thesis it was decided that the biomechanical study of connective tissue gave a more holistic approach. Tissue specimens were subjected to physiological ranges of stretching and response was measured by purpose-built apparatus. Such studies were not reported previously.

In the *in-vitro biomechanical tissue study*, recruitment of women with genital prolapse, requiring abdominal surgery was difficult. Thus, I only obtained 3 rectus sheath (representing systemic connective tissue) biopsies from women with genital prolapse and comparison in women with and without prolapse was impossible. Recruitment of women undergoing emergency surgery can increase the chances of recruiting an adequate number of such women.

Albeit several limitations which have been discussed in the previous chapter, the round ligament showed interesting differences. The round ligament warrants further biomechanical and other investigations as a marker for intrinsic connective tissue properties. Histology analysis might be helpful to ascertain which of its layers are

present in the tissue biopsy being tested and concurrent biopsies of the peritoneum for similar experiments might be helpful. *Diathermy* is best avoided to prevent desiccation of tissue, which is likely to affect the results. Our findings point to the round ligament as an important marker for further investigation of the aetiology in genital prolapse. *Connective tissue* is a complex structure and the changes that occur throughout a women's life is essential for the prevention and adequate treatment of female pelvic floor dysfunction.

The association of joint mobility as a clinical marker of connective tissue and the progress as well as the outcome of labour was also investigated. This was done amongst women who underwent genital prolapse surgery as well as in young primiparous women. This had not been done previously although at the time of the study, Dietz et all reported a similar study [165]. Perineal injuries were also investigated but as no association was found with connective tissue markers and the results were not presented here.

The objective validated assessment of skin elasticity is still being investigated [208-210]. Skin elasticity is a long-standing, well-described marker of abnormal connective tissue and a validated assessment would be another useful marker of connective tissue function in future studies.

Connective tissue affecting the progress of labour and both affecting the onset of genital prolapse is likely.

To our knowledge, the first retrospective study was performed to investigate the progress of the first labour in women with and without different types of genital

prolapse. A large prospective study with an adequate number of women suffering from different compartment genital prolapse is required to confirm or refute our results.

The essential use of validated questionnaires cannot be over-emphasised for future studies. The results presented from the second and third studies were derived from a population of women in the Maltese islands. Connective tissue properties in this population might well be different from other populations and the conclusions cannot be generalised. However, an association was found between mobility of the hand joints and the progress of labour. A similar association was found between apical genital prolapse and knee mobility but none was found when all types of prolapse were analysed together. Future studies should distinguish between prolapse of different compartments as the aetiology is likely to be different. Longitudinal prospective studies will confirm or refute our findings.

The properties of connective tissue must influence all organs of the body including those of a baby during birth. The interaction of the moulding of the bones of the newborn's head during birth as well as the compliance of the maternal pelvic wall and pelvic floor must interact in a complex manner. It is essential to investigate this most natural phenomenon by all possible means and from all possible aspects. In practice, one must investigate the physiological effect of the fetus on the pelvic floor during pregnancy and labour. Obstetric practice has changed over the last few decades, with an increase in epidural analgesia as well as instrumental delivery rates. The duration of the first and second stages of labour have increased with a possible impact on pelvic floor tissues. Epidemiological changes of genital prolapse are possible. It has been noted in the genital prolapse study, that syntocinon increases the odds of genital prolapse surgery. This might imply, that in women with a tight cephalo-pelvic fit or a

prolonged labour, enhancing uterine contractions with syntocinon to achieve a vaginal delivery might damage the pelvic floor. The introduction of active management of labour in the 1970's might have increased the use of syntocinon. It must be reiterated that comparing obstetric practice, in two studies 30 years apart, is not appropriate. However, although epidural analgesia was not used by women in either study, syntocinon was used in 59% of women in the third study (2001) and in 43% of women in the second study (1970's). This change in practice might affect the long-term function of the pelvic floor.

The maternal age at the first delivery has also increased in the western world. Although the parity amongst women in the developed world has declined and the caesarean section rate continues to rise, other precipitating factors secondary to changes in obstetric practice could counteract the otherwise possible decline of genital prolapse. Further epidemiological studies are required to observe the trend amongst different populations of women using the standardised POP-Q classification for genital prolapse.

The seemingly protective effect of smoking on genital prolapse might be secondary to other unknown factors and a longitudinal study would be required to assess its true role in genital prolapse.

Longitudinal studies are required to confirm these relationships as well as the relationship of the duration of labour and development of genital prolapse later on in life. The role of pregnancy, management of labour as well as the ultimate mode of delivery with their effect on the pelvic floor later on in life warrant well-designed longitudinal cohort and observational studies. Nulliparous young women, could have

their pelvic floor and joint mobility assessed at a young age prior to their first pregnancy. Their obstetric history could be followed up and the pelvic floor would need to be examined at intervals until after menopause. Pelvic floor symptom questionnaires and POP-Q examination of the pelvic floor at the onset as well as during critical stages of the study, would be essential to investigate anatomical and functional changes in the pelvic floor. MRI studies would be essential for anatomical detail.

It is unknown whether there is real benefit from intensive physiotherapy programmes in the prevention of genital prolapse. Once women at high risk of developing genital prolapse are identified, a longitudinal study in which young women are randomly assigned either to intensive physiotherapy or to expectant management would be necessary to provide evidence.

Such longitudinal studies are expensive and take a long time to complete. Relatively stable populations would need to be studied to minimise the inability to follow- up patients. Surrogate studies with short-term follow up are more practically possible but would not give as reliable conclusions as long-term follow up studies.

APPENDIX I

A. Interview No. 1 / Connective tissue signs

1. How many pregnancies have you had beyond 24 weeks gestation?
2. What do you do for a living? What other occupations did you have in the past? Has heavy lifting been an issue during any period in your life?
3. Have you ever smoked? How many? For how many years have you smoked?
4. How often do you open your bowels per day/week? Do you have to strain at stools? Was straining ever a problem? Have you ever suffered from haemorrhoids?
5. Has backpain been a regular problem in your life? Do/did you require frequent painkillers to relieve the pain? If there was a positive answer to the question, the response was recorded as positive.
6. Have you had joint pains for longer than 3 months in 4 or more joints.
7. Have you ever dislocated/subluxated any joint in your body, eg shoulder or knee. If so, how many times?
8. Have you ever suffered from rheumatism (e.g., epicondylitis, tenosynovitis, bursitis). If so, how many times?
9. Marfanoid habitus (tall, slim, span/height ratio > 1.03)
10. Eye signs: drooping eyelids or myopia or antimongoloid slant.
11. Varicose veins or hernia or uterine/rectal prolapse.
12. Have you ever suffered from varicose veins? Can I have a look please?
13. Have you ever had abundant stretch marks? Can I have a look please?
14. Family history of: constipation, haemorrhoids, sciatica/severe low back pain, herniae, varicose veins, prolapse, stress incontinence.

For all questions involving family history:

Are there members in your family who suffer from .....? Who are they? Is there anybody else who suffers from...on your mother or your father's side of the family?

If two or more first or second degree relatives had the condition, the response was considered positive.

B. Interview No. 2 / Connective tissue signs

1. Have you ever smoked? How many? For how many years have you smoked?
2. Before the pregnancy how often did you open your bowels per day/week? Did you have to strain at stools? Was straining ever a problem? Have you ever suffered from haemorrhoids?
3. Has backpain been a regular problem before or during the pregnancy? Did you require frequent painkillers to relieve the pain?
4. Have you suffered from varicose veins prior to or related to the pregnancy? Can I have a look please?
5. Have you had abundant stretch marks prior to or related to the pregnancy? Can I have a look please?
6. Family history of: constipation, haemorrhoids, sciatica/severe low back pain, herniae, varicose veins, prolapse, stress incontinence. *For all questions involving family history*:

Are there members in your family who suffer from .....? Who are they? Is there anybody else who suffers from...on your mother or your father's side of the family?

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