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The Growth Hormone Axis as an Aetiological Factor in Adult Inguinal Hernia Development

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A thesis submitted for the degree of MSc to the University of Glasgow

From the Division of Cancer Sciences and Molecular Pathology

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Statement of Collaboration

The original concept for this thesis was conceived by Mr David Wright (Consultant Surgeon, Southern General Hospital, Glasgow). Otherwise, I developed the study design and protocols. Ethical approval for the project was sought and received by me. The hernia clinic was performed by myself and I personally recruited all of the patients in this study and collected all of the clinical information and blood samples.

The blood samples were spun and stored by the staff of the Southern General Biochemistry Department and analysed by Dr Christina Gray and staff of the Royal Infirmary Biochemistry Department.

The composition of this thesis is my own work and has not been submitted for a previous degree. The entire content of this thesis was typed by myself using Microsoft Word 2003 which I also used to collate all of the references. The anatomical figure was drawn by provided by Professor O'Dwyer. Other schematics were entirely drawn by myself. I entered all the data into a database and statistical analysis of this was performed by myself using SPSS v12.0 for windows. Graphs were drawn using SPSS v12.0. Tables were created personally using Microsoft Excel 2003 or Microsoft Word 2003. This project was undertaken whilst I was an SHO and SpR in General Surgery and was funded privately.
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Summary

Adult inguinal hernia development is a common general surgical problem with 85,000 inguinal hernia repairs being performed annually in the United Kingdom. An understanding of the aetiology is important as it may help improve operative repair or identify modifiable factors that could prevent the disease. The current understanding of adult inguinal hernia aetiology is complex and multi-factorial. The inguinal canal is a dynamic structure and paralysis of the deep inguinal ring or variations in parameters of the canal can predispose to hernia development. The presence of a patent processus vaginalis can also result in hernia development but cannot alone explain the aetiology. There is a genetic component with a number of hereditary connective tissue and metabolic storage diseases being associated with inguinal hernia development.

The extra-cellular matrix is a complex alloy of protein families that provides a structural framework for tissues. It is a dynamic environment whose composition and activity varies between tissues. Control of this environment involves both paracrine and autocrine regulation by a wide spectrum of growth factors, hormones and enzymes. The mechanical properties of tendon and interstitial tissue extra-cellular matrix is defined by the proportion of type I : type III collagen within it. Adult inguinal hernia patients have been found to have reduced ratios of type I : type III collagen caused by elevated expression and production of type III collagen, occurring in both direct and indirect hernias. Levels of matrix metalloproteinases, the degradative enzymes of the matrix, have been found to be variable but may have an influence in younger inguinal hernia patients.

The growth hormone axis in adults influences body composition including protein, fat, carbohydrate and bone metabolism. It works directly by the effects of growth hormone and indirectly via the mediator insulin-like growth factor-1. The axis activity declines with age and is associated with many of the changes in body habitus associated with ageing. The peak decline in this activity coincides with the peak incidence of adult male inguinal hernias. A variety of studies in the fields of dermatological, vascular and orthopaedic research have demonstrated that growth hormone, insulin-like growth factor-1 and age can modulate both collagen metabolism and MMP activity. Alterations in the ratio of type I : type III collagen
have also been noted. These observations are both time and tissue dependant. This study hypothesized that the growth hormone axis, in particular its age-related decline, could have a role in the aetiology of adult inguinal hernia development. Gonadal axis function also declines with age and this was assessed also.

47 adult male patients were recruited from 100 consecutive patients referred to the Southern General Hospital with inguinal hernias between 2003 and 2005. These were compared to 47 age-matched controls recruited from 850 patients reviewed with benign pathology in the general, orthopaedic and day-case clinics over a similar time period. Patients were excluded if they had any history of endocrine pathology, aneurysmal vascular disease, diabetes, liver or renal pathology or malignancy to exclude factors known to influence growth hormone axis activity. In addition, the control patients were examined by two independent clinical practitioners to exclude occult groin hernia and were also excluded if they had a history of ventral abdominal wall hernias. All other medical history, including smoking history, was noted and height/weight measurements were taken for calculation of body mass index. A single serum blood sample was taken for the analysis of IGF-1 (as a surrogate marker of GH/IGF-1 axis activity), testosterone and sex hormone binding globulin. The free androgen index was calculated from these results in order to exclude gonadal axis activity as a confounding factor.

Both groups were similar in baseline characteristics except for mean BMI (mean BMI hernia 25.1 vs control 26.6; p=0.035). IGF-1 negatively correlated with age in both groups. There was a negative correlation with testosterone and age also. Free androgen index displayed a significant negative correlation with age which was maintained when controlling for BMI and IGF-1. There was no significant difference between the mean IGF-1 concentrations of the hernia cohort compared to controls. The hernia cohort had significantly higher gonadal axis activity but this was within normal limits.

These results confirmed the recognized age-related declines in the growth hormone and male gonadal axis within this cohort. It did not show a significant difference between IGF-1 levels in the hernia and control groups. The noted elevated testosterone and free androgen binding index levels in the hernia cohort was
unexpected but certainly excluded reduced gonadal axis activity as an aetiological factor. This was the first study to investigate a possible relationship between adult inguinal hernia development and the growth hormone and gonadal axes. It suggests that the recognized hormonal changes associated with adult male ageing are not aetiological factors in adult male inguinal hernia development. Further research in this area is warranted to confirm these findings.
Section One: Review of Relevant Literature
Chapter One
The Aetiology of Adult Inguinal Hernia Development

1.1 - Introduction

Inguinal hernias are one of the most common general surgical conditions. They are 10 times more common in males than females. The lifetime risk of developing an inguinal hernia for a normal adult male is approximately 5%, with a peak incidence in the fifth and sixth decades [1]. 85 0000 inguinal hernia repairs are performed per year in the National Health Service, with a peak rate of 75 per 10 0000 population in the 55 – 85-year age group[2]. Inguinal hernias can be associated with significant morbidity and mortality, especially in the elderly population[3], and their operative repair is not without risk[4]. An understanding of the aetiology of adult inguinal hernia is important as not only might it assist with improving operative repair but identification and modification of risk factors could also help prevent development of the disease.

In the 19th century, the cause of inguinal hernia was thought to be simply mechanical, due to increased intra-abdominal pressure. Cooper identified ‘causative’ factors such as cough, obesity and constipation[5]. Over the last 100 years, our understanding of inguinal hernia development has expanded dramatically and the genetic, anatomical, physiological and biochemical factors that have been implicated in the aetiology of inguinal hernia will now be discussed.
1.2 - Anatomy and Physiology of the Inguinal Canal

The inguinal canal is a 4-6cm long oblique passageway through the anterior abdominal wall extending from the internal ‘deep’ ring to the external ‘superficial’ ring. The anterior wall is formed by the fibres of external oblique aponeurosis, reinforced by internal oblique fibres in its lateral third. The floor consists of the rolled edge of external oblique forming the inguinal ligament. The roof is made up of the arching fibres of internal oblique and transversus abdominus which arch from a position in front of the cord laterally, where they reinforce the anterior wall, to behind the cord medially, where their fibres condense to form the conjoint tendon. This structure reinforces the medial aspect of the posterior wall which is also formed from the transversalis fascia. In this way the deep and superficial rings, as potential weak points, are reinforced.

The myopectineal orifice of Fruchaud [6] has been well described and encompasses the region where all groin hernias occur (Fig.1). It comprises the arching fibres of transversus abdominis, internal and external oblique muscles superiorly, the ilium and pectineal ligament inferiorly, the rectus muscle medially and the iliopectineus muscle laterally. The inguinal ligament divides the area in two, allowing passage of the spermatic cord above and the femoral vessels below. Direct inguinal herniation occurs through the area called Hesselbach’s triangle, bound by the deep inferior epigastric vessels laterally, rectus muscle medially and iliopubic tract posteriorly. The floor of the triangle consists of the weakened transversalis fascia. By definition, indirect inguinal herniation occurs lateral to the deep inferior epigastric vessels through the deep ring.

It is important to note that the deep ring is not a fixed opening but moves superolaterally under the fibres of internal oblique and transversalis muscles on straining therefore increasing the obliquity of the opening at the deep ring [7,8]. This superolateral ring movement can be seen during local anaesthetic repair and has been described at laparoscopy [9]. In addition, the conjoint tendon approximates to the inguinal ligament to ‘close off’ Hessert’s Triangle; a triangle with the deep ring at its apex, rectus muscle at its base and conjoint tendon & inguinal ligament forming its sides (Fig.2). The canal thus has dynamic mechanical protective factors.
Figure 1. Schematic representation of the myopectineal orifice of fruchaud.
Figure 2. Schematic representation of Hessert’s triangle

The blue triangle bound by the conjoint tendon, inguinal ligament and rectus muscle is Hessert’s triangle. The arrow indicates the direction of movement of the conjoint tendon towards the inguinal ligament on straining thereby re-inforcing the underlying weak transversalis fascia.
against herniation. A recent study found the size of Hessert's triangle in patients undergoing hernia repair was significantly larger than compared with fresh cadavers. This difference was due to a higher intersection of internal oblique with the rectus muscle. It was suggested this may make occlusion of the triangle on straining incomplete and predispose to hernia formation [10].

It would certainly appear therefore that normal function of the lateral abdominal muscles and transversalis fascia are important factors in protecting the inguinal canal. An increased incidence of right inguinal hernia has been shown following appendicectomy [11,12] and this has been attributed to damage to the segmental nerves supplying the internal oblique and transversalis muscles paralysing the above protective mechanisms. This was demonstrated by Tobin and colleagues who described a case of right inguinal hernia developing six months following appendicectomy in whom laparoscopy showed normal deep ring movement on the left side but paralysis of the deep ring on the right [9].

Hahn-Pedersen and co-workers have used CT scanning to visualise the dilatation of the femoral vein during periods of increased abdominal pressure [13]. They claim that this dilatation acts to close the femoral canal and so protect against herniation into the space. Similar techniques were then used to investigate patients with inguinal hernias [14]. In the normal inguinal canal they have shown no change in the diameter of the pampiniform plexus veins lateral to the conjoint tendon. Medially, however, they have demonstrated distention of these veins. The same changes were demonstrated in patients who had direct inguinal hernias, but in patients with indirect hernias there was no demonstrable dilatation of the veins. The authors suggested that there may be a "vascular on-demand closing mechanism" which works along with the other protective mechanisms to prevent inguinal herniation. They speculated that a competent valve in the spermatic vein at the level of the deep ring would prevent filling and therefore predispose to inguinal hernia formation. It was acknowledged, however, that the phenomenon may simply represent a passive occupation of the gap between the conjoint tendon and the external oblique aponeurosis during periods of raised intra-abdominal pressure.
1.3 - Saccular Theory

In 1906 Hamilton Russell, an Australian paediatric surgeon, published the Saccular Theory of Hernia [15]. This theory "rejects the view that hernia can ever be "acquired" in the pathological sense" and maintains that "the presence of a congenital peritoneal sac is a necessary antecedent condition in every case of ordinary abdominal hernia." In the case of indirect inguinal herniation the congenital sac is formed by the failure of closure of the processus vaginalis. These congenital sacs have been found to contain smooth muscle within their walls which is thought to be a reason for failure of obliteration of the processus [16]. Russell states, "When the peritoneal tube is obliterated fairly up to the level of the opening in the transversalis fascia the occurrence of inguinal hernia in the future will be impossible." In this paper the presence of the 'sphincter' mechanism produced by the conjoint tendon apposing to the inguinal ligament is described, but Russell felt that the presence of a peritoneal sac in the canal was enough to render the muscular apparatus ineffective and allow herniation to occur.

Hammond, in 1923, criticised the Saccular Theory because it could not account for the delay in hernia formation until adult life [17]. Unlike Russell he felt that the sphincteric action of the conjoint tendon normally was able to protect the deep ring even with the presence of a patent processus vaginalis. Inguinal herniation was thought to occur when the muscular mechanism was overcome by severe or prolonged strain or there was a sudden inco-ordination of the muscular activity, in essence increased abdominal pressure. It is now known that up to 10% of male patients treated by continuous ambulatory peritoneal dialysis (CAPD) develop an inguinal hernia [18]. These hernias occur within a few months of commencing CAPD and all are of the indirect type [19]. A two litre intraperitoneal dialysate volume equates to a pressure of 30-40cm of water at the inguinal level when the patient is in the upright position [20]. It is thought that this pressure causes distention of a patent processus vaginalis leading to development of an indirect inguinal hernia [18,20]. Grosfeld and Cooney have suggested that the same mechanism is responsible for an increased incidence of indirect inguinal hernias in infants after ventriculo-peritoneal shunt for hydrocephalus [21].
Harrison observed that, while the muscles of the abdominal wall seemed to be normal in structure and development, the transversalis fascia was weak. He therefore concluded that the cause of inguinal herniation was a failure of the transversalis fascia to withstand the intra-abdominal pressure to which it was subjected [22]. This view was supported by Keith who argued that sac formation did not cease at birth but in fact could occur at any stage in life under suitable circumstances [23]. Keith stated that, "All femoral hernias are produced in this manner ....... and so are all direct inguinal hernias ....... for none of these is there a vestige of evidence that the sac was formed before birth and by developmental means." Like Harrison, Keith thought that the increased incidence of inguinal hernias in the middle aged and elderly was due to a pathological change occurring in the abdominal wall.

Further evidence against the theory of a purely congenital aetiology for groin herniation is found in the observation that 15-20% of healthy adult males [7,24] have a patent or partially patent processus vaginalis throughout life without ever developing an inguinal hernia. An indirect inguinal hernia has also been documented in a man proven not to have a patent processus by herniography prior to development of the hernia [25]. Similarly, Gullmo observed that 5% of young women who had incidental herniography after hysterosalpingography had an open processus vaginalis without clinical evidence of a hernia [26]. In most mammals the testes pass through the abdominal wall to reach the scrotum, and only in man and the gorilla is the processus vaginalis normally closed afterwards. Therefore, in most other mammals a patent processus vaginalis is always present and yet inguinal herniation is much less common at every stage of life [23].

Man’s predisposition to inguinal herniation has been attributed to the attainment of an upright posture, with the observation that quadrupeds have similar deficiencies in the inguinal region as erect mammals without the associated herniation [27]. However, Keith argued that it was the modifications of the pelvis for bipedal locomotion that weakened the inguinal region rather than simply having an upright posture [23]. Similarly, Parry argued that if upright posture alone was responsible femoral herniation should be more common in view of the dependant position of the femoral canal [28]. He observed that an action which quadrupeds cannot do, but
which man does frequently and forcibly, is rotation of the trunk. Parry associated this factor with the high incidence of inguinal hernia in man.
1.4 - Hereditary Factors

Inguinal hernia is a common feature in many hereditary diseases of connective tissue. In a review of 50 consecutive cases of Marfan's syndrome Pyeritz and McKusick described a 22% incidence of inguinal hernia [29]. In this paper it was also stated that inguinal hernias associated with this condition tended to recur after surgical repair. Types I and IX of Ehlers-Danlos Syndrome (EDS) are also associated with an increased incidence of hernias [30]. While the molecular defect in type I EDS is not known, a defect in copper metabolism is present in most patients with type IX EDS[31]. Copper is an essential co-factor for lysyl oxidase (see section 1.5). In view of the association between abdominal aortic aneurysm (AAA) and inguinal hernias (see section 1.6), it is interesting to note that AAA has also been associated with similar abnormalities in copper metabolism [32,33].

Reduced plasma and hernial sac copper levels have recently been found in a group of direct inguinal hernia patients[34].

Another group of hereditary disorders associated with an increased incidence of inguinal hernia are the mucopolysaccharidoses (including Hurler's Syndrome and Hunter's syndrome) [35]. In these conditions storage related abnormalities in connective tissue produce a laxity which is manifest by inguinal and umbilical hernias. A high incidence of adult hernia formation amongst the Inuit of Greenland has been linked with a high incidence of the HLA-B27 allele [36], which is also associated with a number of connective tissue diseases. Inguinal hernia has also been shown to occur more frequently in children with congenital dislocation of the hip [37].

Heredity has been included amongst the factors thought to predispose to paediatric inguinal hernia for many years [38-41]. Marshall and co-workers used the twin-study method to demonstrate a significantly higher concordance for hernia formation in monozygotic compared with dizygotic twins [42]. The nature of genetic transmission, however, has not yet been clearly defined. Hypotheses proposed include autosomal dominant with incomplete penetrance [39,41], autosomal dominant with sex influence^26^, X-linked dominant [38], and polygenic
from a study of 707 index cases of congenital inguinal hernia. Czeizel and Gardonyi produced data supporting a multifactorial threshold model involving dominance variance [44]. However, Gong and co-workers have recently reported on an analysis of 280 families with congenital indirect inguinal hernia in the Shandong province of China which does not support this model [45]. They found frequent vertical transmission and a high segregation ratio which suggest that an autosomal dominant pattern with incomplete penetrance and sex influence is the most likely mode of inheritance. Evidence supporting preferential paternal transmission of the disease gene was also found.
1.5 – Collagen and the Extra-cellular Matrix

The extracellular matrix (ECM) is a complex alloy of protein families that provides a structural framework for all types of tissue. ECM is composed of a variety of collagens, glycoproteins, proteoglycans and elastin and its structure varies depending on the site to provide specific micro-environments for cellular functions and interactions[46]. The collagens are the major components of ECM maintaining structural integrity. Far from being a static environment, the ECM is dynamic and undergoes continuous remodelling. Protein synthesis is balanced by a specific enzyme family called matrix metalloproteinases (MMP) that control protein degradation [47]. The action of MMPs can release biologically active molecules that influence the cellular micro-environment [48]. Furthermore, the ECM environment can be influenced by a variety of cytokines and growth factors that provide an additional layer of control. An understanding of both collagen and ECM biology is essential to both understanding and developing current knowledge on disordered ECM function in adult inguinal hernia development.

Collagen Metabolism

Collagen is defined as a structural protein of the extracellular matrix which contains one or more domains with the conformation of a collagen triple helix [49]. There are currently 26 collagen types described (table 1) which can be grouped depending on their molecular structure and supra-molecular organization [46,50]. It is the fibril-forming collagens that provide tensile stiffness and strength to tendons and interstitial tissues and are therefore of interest when discussing hernias. It is these collagens that will be focused upon in this section.

Regardless of type, all collagens contain at least one characteristic triple helix domain. This consists of three polypeptide chains (α-chains) each with a repeating Gly-X-Y- amino acid sequence with the X and Y positions most often being occupied by proline, hydroxy-proline and hydroxy-lysine residues [51]. Each of these chains forms left-handed helixes which coil around a central axis to form a
<table>
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<th>HyAl IVA AIX IVA XIX IIIA IIIA</th>
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<td>CLASSIFICATION OF COLLAGEN</td>
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right-handed triple helix. The glycine residue occupies every third position as it is the only amino acid small enough to fit in the centre of this helix. This also means that the other residues are always positioned on the outside of the structure to allow intra- and inter-molecular interactions to occur. The collagen domain can be a homo-trimer or hetero-trimer depending on whether the individual α-chain sequences are identical or not.

Initial transcription and translation of these α-chains is highly complex but regardless of this, they all undergo important post-translational modifications that confer immense strength to the collagen triple helix:

1. **Proline Hydroxylation.**
Prolyl-4-hydroxylase converts proline residues to 4-hydroxyproline [52]. This is essential for intra-molecular hydrogen bond formation within the collagen molecule. This process is highly oxygen dependent. Studies of the melting temperature of unhydroxylated collagen (the temperature at which the protein molecule denatures) show that it is lower indicating the triple helix is markedly less stable[53]. The hydroxylation of proline residues is therefore important for the molecular stability and strength of collagen.

Prolyl-3-hydroxylase converts proline residues to 3-hydroxyproline but the functional significance of this is unclear[46].

2. **Lysine Hydroxylation.**
Lysine hydroxylation is catalysed by lysyl hydroxylase [54]. This allows subsequent formation of intermolecular cross-links by joining two hydroxylysine residues and one lysine residue through the action of lysyl oxidase. [51]. This helps provide stability to the matrix of extra-cellular collagen molecules. Hydroxylation of lysine residues also allows the residues to be further modified by other enzyme systems, such as hydroxylysyl galactosyltransferase, to allow addition of carbohydrates [55].
The fibrillar collagen molecules contain a triple helix 300nm in length which have non-helical domains, called C- and N-propeptides, at either end. Following excretion into the extra-cellular environment, these propeptides are cleaved by specific C- and N-proteinases respectively. This triggers the spontaneous assembly of the collagen molecules into highly ordered and orientated fibrils [50]. These fibrils are characterised by a striated appearance on electron microscopy that represents a staggered alignment of collagen molecules within the fibril [51]. It is at this point that the lysyl oxidase family of enzymes creates strong inter-molecular covalent bonds between aligned molecules [56].

It is important to note that, although each type of collagen can form fibrils on their own, they often bind with other collagen types like a molecular alloy [49]. It is this interaction that bestows specific physical and biological characteristics to different tissue sites. Type I collagen is the predominant collagen within connective tissue, bestows mechanical strength to those tissues and represents 90% of all collagen [46]. It is the predominant collagen within fascia, tendon and skin. On its own it would form thick, strong fibrils as seen by the number of covalent bonds between adjacent molecules. Type III collagen, in contrast, would form thinner fibrils with fewer bonds [57]. It provides a degree of elasticity and compliance to the tissues it is within. In vivo, Type I and Type III collagens form collagen fibrils together [58]. The ratio of the ‘strong’ Type I to the ‘weak’ Type III molecules within the fibrils therefore determine the strength and physical characteristics of the overall tissue. It is thought that a higher relative content of Type III collagen would create ‘weaker’ tissue by reducing the covalent bonding between molecules within the fibrils and thereby reducing their size and physical characteristics [59].

Matrix Metalloproteinases

The matrix metalloproteinases are a family of proteases that are integral to the regulation of the ECM. They can degrade any component of the ECM [60], release bio-active fragments including growth factors and inflammatory mediators from the ECM [48], stimulate cell migration through the ECM [47] and are increasingly implicated in a wide spectrum of human physiology. MMPs are zinc-dependent enzymes that have a distinctive domain structure including pro-peptide, catalytic
and substrate domains. They are secreted as inactive zymogens due to a characteristic 'cysteine switch' in which an unpaired cysteine in the pro-peptide interacts with the zinc ion in the catalytic domain to exclude an essential water molecule [61]. The proteolytic removal of this pro-peptide activates the enzyme. The exact mechanism varies with each MMP and is one of the key features in their regulation. Expression profiles vary between normal tissues with elevated activity seen in a variety of physiological and pathological states such as wound healing, arthritis or cancer [62-64]. Activity is influenced at translation, transcription and activation by a wide range of growth factors and cytokines as well as cell-cell and ECM-cell interactions [65]. A family of 4 inhibitors, tissue inhibitors of MMPs (TIMPs), can both inhibit and potentiate MMP action and provide a further layer of control [66].

28 MMPs have been identified currently and are described numerically based on the order they were discovered [47]. These can be categorised on the basis of their substrate specificity into collagenases, gelatinases, stromelysins and membrane-type MMPs (MT-MMP) [see table 2]. It is the collagenases that are of importance when considering the fibrillar collagens. Fibrillar collagens consist almost entirely of the collagen triple helix domain and are extremely resilient to enzymatic degradation. Only the MMP family can degrade the collagen triple helix within the different collagens and the collagenases have most specificity for the fibrillar collagens [65]. Therefore, MMP-1, MMP-8 and MMP-13 activities determine the rate of fibrillar collagen breakdown and could theoretically influence the tensile strength of tissues high in type-I and type-III collagens.
<table>
<thead>
<tr>
<th>Category</th>
<th>Enzyme Name</th>
<th>MMP number</th>
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Table 2. Classification of matrix metalloproteinases
(Source: MEROPS: the protease database [http://merops.sanger.ac.uk])
1.6 - Evidence for disordered collagen metabolism

Although Harrison in 1922 was the first to suggest that there may be a pathological change in the abdominal wall causing hernia formation, it was not until the 1970’s that the concept of defective connective tissue, specifically collagen deficiency, was introduced. Read noted the rectus sheath appeared thin in patients undergoing hernia repair, especially in those with direct defects [67]. Wagh and Read then demonstrated reduced levels of hydroxyproline in sheath biopsies of both direct and indirect hernia patients compared with controls, with the biggest reduction in direct herniation. They proposed the thinning of the rectus sheath was due to a deficiency in collagen and hypothesized this was likely to be a failure of hydroxylation of proline or lysine, or incomplete formation of the collagen molecules, rather than a defect of cross-linking. Reduced collagen synthesis by fibroblasts was subsequently demonstrated in hernia patients [68,69]. Connor and Peacock demonstrated in rats that a combination of a structural defect (enlargement of the deep ring) and a metabolic defect (chemically induced impaired cross linking of collagen) was required to reliably induce direct and indirect inguinal hernias [70]. These early studies suggested that a degree of ‘collagen deficiency’ was required for all types of inguinal herniation and that, from an aetiological perspective, the differentiation of direct and indirect defects was not relevant.

Connor and Peacock suggested that the abnormalities of collagen metabolism may be localised to the inguinal region. In contrast, Read’s group suggested inguinal hernia was a local manifestation of a systemic change in collagen metabolism. Read had noted developments in the understanding of pulmonary emphysema, previously thought to be due to mechanical pressure effects. In 1963, Laurell and Erikson reported an association between low levels of a protease inhibitor alpha-1-antitrypsin (α1AT) and an inherited form of pulmonary emphysema [71]. In 1965, Gross et al induced experimental emphysema in rats using a crude plant protease. From these papers developed the ‘protease-anti-protease’ theory of pulmonary emphysema in which an imbalance of protease activity and protease inhibition in the lung parenchyma mediated alveolar destruction. α1AT was found to be a specific inhibitor of neutrophil elastase (produced by polymorphonuclear
leukocytes), which on its own also induced experimental emphysema [72]. Subsequently, compounds within cigarette smoke were shown to inactivate α1AT and thus were implicated in an 'acquired' emphysema [73].

Read had noticed that many of his hernia patients were also smokers. In a paper titled “Metastatic Emphysema”, Cannon and Read demonstrated an increase in serum elastolytic activity and a decrease in serum alpha-1-antitrypsin activity in smokers with hernias when compared with non-smoking controls [74]. They claimed that an imbalance of blood proteases and antiproteases resulting from cigarette smoking could damage connective tissue in the inguinal region as well as in the lung. Jorgensen et al, using implanted ePTFE in human volunteers as a model of wound healing, subsequently demonstrated reduced levels of hydroxyproline deposition in healing wounds of smokers [75]. They related this to hypoxia at a cellular level caused by smoking both from nicotine induced vasospasm and carboxyhaemoglobin formation from inhaled carbon monoxide interfering with the activity of prolyl-4-hydroxylase. However, a wide variability in hydroxyproline deposition was also noted amongst the non-smoking controls. Recently the same group have demonstrated that smoking is a risk factor for groin hernia recurrence [76].

Read’s group noted a similar imbalance in blood proteases and antiproteases in patients with abdominal aortic aneurysm (AAA), but not in those with aorto-occlusive disease [77]. They therefore hypothesised that there should be an increased incidence of inguinal hernia in patients with AAA, in keeping with a ‘systemic’ disorder. In a retrospective study, Cannon and co-workers found that the frequency of inguinal hernia was higher in AAA patients (25.8%) compared with patients with Leriche syndrome (14.6%) [78]. Subsequent studies have shown an increased incidence of inguinal hernia in patients with AAA disease rather than aorto-occlusive disease, coronary atherosclerosis or peripheral vascular disease [79-81]. The prevalence of AAA has been shown to be 12.2% in male patients aged over 55 years having undergone hernia surgery compared with age-matched controls without such a history [82].
With increasing evidence of 'weakened' connective tissue associated with inguinal hernia development, Friedman et al looked at collagen synthesis in inguinal hernia patients by measuring pro-collagen secretion from cultured skin fibroblasts[83]. There was an increased expression of Type III procollagen mRNA with concomitant increased production of Type III collagen resulting in a relative reduction in Type I : Type III collagen ratio compared to controls. There was no difference between direct and indirect hernia subgroups. They hypothesized the collagen 'deficiency' was therefore a qualitative rather than quantitative one.

Schumpelick et al revealed similar alterations in collagen ratios in skin and hernia sac samples. They also analyzed for MMP-1 and MMP-13 and found no difference between hernia patients and controls suggesting the collagen disorder was more likely of synthesis, rather than degradation[59,84]. Subsequently, using oligonucleotide primers for the published gene sequences for Type I / Type III collagen and MMP-1 / MMP-13, the same team have demonstrated similar results for the transcription of the collagen and MMP genes with a relatively reduced Type I : Type III collagen ratio[85]. Similar collagen ratios were found with incisional hernias[86] and recurrent inguinal hernias[87] but the latter was associated with elevated levels of MMP-1 and MMP-13 compared to those seen in 'normal' scar tissue.

In another study, Bellon et al analyzed collagen ratios, proline & lysine hydroxylation, MMP-1 and MMP-2 expression in the transversalis fascia of hernia patients[88]. Again, there was no difference in collagen levels between indirect and direct hernia patients but elevated MMP-2 expression in young (20-40 years) patients with direct hernial defects was noted. Altered lysine hydroxylation was noted in the older (21-60 years) patients but the study was hampered by small numbers and lack of control group. The same team, however, subsequently found increased fibroblast MMP-2 expression in young direct hernia patients compared to indirect and control patients[89] with reduced MMP-2 levels in the older patients. As MMP-2 degrades non-fibrillar components of the ECM (including elastin) this raises the possibility that the hernial process may be different between young and old patients. Recently, elevated TIMP-2 levels have been found in transversalis
fascia samples from direct inguinal hernia patients and could be a factor in the increased MMP-2 expression seen in the bellon studies[90].

Harrison commented on the fact that the peak incidence of inguinal hernias in adults occurs at a time of life when physical activity may be reduced as men come towards the end of their working life [22]. Similarly, lack of physical exercise has been associated with development of inguinal hernias [91]. Prolonged reduction in muscular activity has been shown to reduce the tensile strength of tendons. [92]. Karpakka and co-workers have further shown that muscle contractile activity and tension seem to be positive regulators of prolyl 4-hydroxylase and galactosylhydroxylsine glucosyltransferase. [93]. These factors could also contribute to altered collagen synthesis at a local level.
1.7 - Conclusions

Initially, inguinal hernia development was related to anatomical and mechanical factors with a raised intra-abdominal pressure and patent processus vaginalis overcoming the physiological protective mechanisms of the inguinal canal. Early observations of the qualitative deficiencies of tissue within the groin were not heeded. The association of inguinal hernia disease with a number of metabolic and connective tissue diseases, and experiments suggesting a systemic ‘protease/antiprotease imbalance’ in patients with inguinal hernia, emphysema and aortic aneurysm, again focused concentration on the fascia of the canal and the extra-cellular matrix within it. Adult inguinal hernia patients have been found to have reduced ratios of type I : type III collagen caused by elevated expression and production of type III collagen, occurring both in direct and indirect hernias. The role of MMPs in hernia development is not clear. The collagenases do not appear to be elevated in primary inguinal herniation but MMP-2 activity has been found in some hernia patients, with elevated MMP-1 and MMP-13 seen in recurrent hernia. MMP expression, however, is under tight control and ‘snapshot’ levels may not represent overall activity over a period of time. Also, both collagen synthesis and MMP expression / activation are influenced by a variety of cytokines and hormones systemically and at a local level. Further understanding of the physiology of the ECM environment is required to fully explain the reasons behind adult inguinal hernia development.
Chapter Two
The Adult Growth Hormone / IGF-1 Axis

2.1 – Introduction

The somatotrophin axis comprises the hypothalamus, pituitary gland and numerous target tissues. Via the actions of Growth Hormone (GH) and Insulin-like Growth Factor-1 (IGF-1), and under tight neuro-hormonal control, the axis is an important determinant of body composition. During puberty, it is responsible for axial somatic growth. In adulthood, it continues to be an essential anabolic pathway for the control of body composition and is intimately involved with changes in body habitus associated with ageing [94]. A complex topic in its own right, this chapter will provide an overview of the somatotrophin axis prior to focusing on the alterations of the axis associated with ageing and ultimately the potential influence the pathway has on collagen metabolism.
2.2 – The Somatotrophin Axis

**Growth Hormone**

Growth hormone (GH) is a 191 amino acid protein produced by the anterior pituitary somatotropes. It does not have a specific target organ but exerts a variety of effects either directly or via the Insulin-like Growth Factor system (IGF-1, IGF-2)[94]. GH is secreted in pulsatile bursts and is subject to diurnal variation, with peak secretion occurring nocturnally during deep phase III/IV sleep [95,96]. GH circulates bound to GH binding protein (GHBP) which is derived from the extracellular domain of the GH receptor[97,98] and complexes around 90% of circulating GH[99].

The growth hormone receptor (GHr) is expressed by most cells of the body. GH has two binding sites for the GHr with its activation requiring formation of a dimer between two receptors and one GH molecule [100]. This results in activation of Janus Kinase 2 (JAK2) which leads to phosphorylation of ‘Signal Transducers and Activators of Transcription’ or STAT proteins [101]. These STAT proteins activate gene transcription and generation of proteins such as IGF-1 [102]. GHr activation can also activate the Insulin Receptor Substrate (IRS) proteins [103] which are responsible for many of the direct metabolic effects of GH.

**IGF-1**

IGF-1 is the mediator for the indirect actions of GH. The majority of circulating IGF-1 is hepatocyte-derived [104] and correlates well with GH axis activity. However, animal models have demonstrated that normal growth and development can occur in the absence of hepatic IGF-1 production [105] and both animal and human studies have shown that local tissue concentrations of IGF-1 can differ significantly from serum concentrations [106,107]. Autocrine and paracrine production of IGF-1 is therefore just as important for its action. Not all production requires the influence of GH and both the sex steroids and glucocorticoids can affect the production and activity of IGF-1 and its binding proteins [108,109].
IGF-1 has a family of six IGF-binding proteins (IGFBP) to which it can bind [110]. These not only serve as carrier proteins, IGFBP-3 binding 70% of serum IGF-1, but also function to regulate the actions of IGF-1. Although poorly understood, this is not simply achieved by controlling the amount of free IGF-1 available for interaction at a local tissue level. Experimental studies have suggested there is a complex interplay between the IGFBPs and IGF-1. IGFBPs can influence cellular responsiveness to IGF-1 [111,112] as well as IGF-1 availability [107,110]. In return, IGF-1 can modulate IGFBP expression [109]. Some of the effects of IGF-1 require synergistic action with IGFBPs [113]. The IGFBPs can also act independently as growth factors, directly stimulating transcription and activation of genes [110].

IGF-1 mediates its effects via the IGF-1 receptor (IGF-1r) or the receptor for Insulin (to which it is structurally related) [114]. Activation of the IGF-1r results in a complex activation of multiple intra-cellular signalling pathways, including the IRS proteins and the mitogen activated protein (MAP) kinase signalling pathway [115]. These eventually result in the stimulation of transcription and the various effects of IGF-1.

**Neuro-hormonal control of Growth Hormone**

GH synthesis and release is predominantly controlled by two opposing hypothalamic hormones – Growth Hormone Releasing Hormone (GHRH) and Somatostatin (SN). GHRH promotes the synthesis and release of GH whereas SN inhibits it [94]. The majority of GH release is determined by resting SN inhibitory tone with the interplay of this and GHRH determining the pulsatile nature of GH release [94]. In addition to hypothalamic control, a hormone called Ghrelin has been identified [116] that is produced predominantly by stomach endocrine cells and stimulates GH release in response to nutritional intake [117]. IGF-1 helps to regulate GH release both at the hypothalamic and pituitary levels via a negative-feedback loop [94] (fig.3).
Figure 3. Schematic diagram of growth hormone / IGF-1 axis regulation. GH denotes Growth Hormone; IGF-1, Insulin-like Growth Factor-I; GHRH, GH-Releasing Hormone.
Hypothalamic control can be further modulated by endocrine factors (thyroxine, glucagon, sex steroid and adrenal hormones), metabolic factors (glucose, free fatty acids) and psychological status (stress, depression) [94]. Free fatty acids and glucose concentrations also influence GH release at the pituitary level. Additionally, adiposity, lean body mass, exercise and hepatic and renal pathology can also affect the activity of the somatotrophin axis [93,118,119].

Clinical Effects of the Axis in Adults

GH and IGF-1 have a variety of independent and linked effects on:

1. Nitrogen Balance
Both GH and IGF-1 stimulate protein synthesis with or without inhibition of proteolysis dependent on the patho-physiological state of the subject [120]. This results in an overall positive nitrogen balance.

2. Lipid Metabolism
GH directly influences adipocytes by increasing catecholamine receptor sensitivity and number and thereby stimulating lipolysis [121]. Induction of hepatic LDL receptors results in an improved serum lipid profile [122]. GH differentially both inhibits and stimulates lipoprotein lipase activity in adipocyte and striated muscle respectively, resulting in an overall increase in serum Free Fatty Acids (FFA) [121,123].

The above actions promote increased lean body mass and reduction and redistribution of fat mass from central to peripheral areas.

3. Carbohydrates (CHO)
GH can increase hepatic gluconeogenesis, decrease CHO breakdown and stimulate insulin resistance [124] but none of these are particularly clinically significant within normal physiology. IGF-1 has beneficial effects on CHO metabolism including increased insulin sensitivity [125], especially on striated muscle. It can also induce insulin-like effects by direct stimulation of the insulin receptor [115].
4. Bone and Cartilage

Growth hormone stimulates the differentiation of pre-chondrocytes and increase the sensitivity of chondrocytes to IGF-1 as well as stimulating production of IGF-1 mRNA [126,127]. IGF-1 stimulates proteoglycan, glycosaminoglycan and collagen synthesis by chondrocytes [128] and has also been shown to increase IGF-1 mRNA expression by osteoblasts. The clinical significance of the GH / IGF-1 axis in healthy subjects is unclear but its' importance is seen in growth hormone deficient patients with reduced bone mass and increased risk of fractures [129].

5. Immune System

The GH / IGF-1 axis has been shown to influence the immune system. IGF-1 acts as a differentiation factor encouraging B-cell maturation [130] and can stimulate immunoglobulin production [131]. GHr are expressed by immunological cells which can also produce GH [132]. The clinical significance of axis activity on immune function is not clear.

Growth hormone deficiency states have also suggested a role for GH / IGF-1 in neuro-psychiatric status and cardiac and renal function [94].
2.3 – The Somatopause

Following the growth spurt in puberty and early adulthood, the activity of the GH/IGF-1 axis declines with age. It has been estimated that with each decade, GH synthesis declines by 14% and GH half-life by 6% [133]. This is associated with a gradual decline in serum IGF-1 levels from the third decade onwards [134] with peak decline during the fifth and sixth decades. This age-related decline in the somatotrophin axis has been termed the ‘somatopause’ and is now well recognized as one of the endocrinological hallmarks of ageing [135-137].

The somatopause has been associated with the changes in adult body habitus and function that occur with ageing. These are strikingly similar to those changes seen with adult growth-hormone deficiency syndromes (AGHD) [94] and include:

- Increased fat mass, esp. in the abdonimo-visceral compartment [138-140]
- Reduced lean body mass [138-140]
- Reduced muscular strength and exercise capability [141]
- Reduced bone mineral density [129]
- Altered lipoprotein and carbohydrate metabolism [142,143]
- Impaired renal and cardiac function [94,144,145]
- Impaired psychiatric wellbeing [146,147]

As well as the GH/IGF-1 axis, gonadal sex steroid function also declines with age [135]. It is difficult to separate some of the effects of this decline from those associated with the GH/IGF-1 axis. Both hormonal axes can also influence each others’ activity and actions [136,148]. Although testosterone levels remain within low-normal levels with age [135], and the evidence for its effects on body habitus is less compelling [149], sex steroid status should be taken into account when considering the effects of the somatopause and human ageing.

Fat mass has been shown to be inversely proportional to GH and IGF-1 activity independent of age [150,151]. Body mass index (BMI) can result in false findings of growth hormone deficiency on provocative testing (although does not seem to alter
IGF-1 findings) [152]. Animal studies have suggested adiposity may alter noradrenergic neuronal regulation of the hypothalamus, alter the expression of ghrelin receptors and modulate GHRH cells within the hypothalamus [153-155]. Thus, it is not clear whether the noted changes in adiposity with age are a cause or effect of concomitant declining GH/IGF-1 activity.

The mechanism underlying the somatopause has yet to be fully elucidated. Both human and animal studies have demonstrated a reduction in GHRH synthesis [156], reduced pituitary response to GHRH [157] and elevation of somatostatin tone [158] with age. Other studies have suggested that the GHRH response is maintained in elderly adults [159]. Similarly, the literature is divided as to whether altered feedback mechanisms may contribute to the decline in the GH/IGF-1 axis [160,161]. Although testosterone can influence the activity of the axis, it is not thought to be the primary cause of the somatopause either [162]. Recently, studies have focused on the potential role of ghrelin in the aetiology of the somatopause. Experimental animal studies have suggested that the age-related decline in the GH/IGF-1 axis is not due to a reduction in the release or effects of ghrelin [163]. In contrast, human studies have suggested a decline in ghrelin secretion and function with age [164,165]. Plasma Ghrelin concentrations also positively correlate with serum IGF-1 concentrations in elderly human subjects [166]. Overall, it would seem that complex alterations in the stimulation, production and release of GHRH, Sn and ghrelin as well as altered sensitivity of the hypothalamus and pituitary to these hormones contribute to the development of the somatopause.

Whether the somatopause is a pathological or physiological adaptive response to ageing is a point of controversy. The observed increase in central adiposity and proatherogenic lipid profile with age is associated with increased risks of coronary atherosclerosis and hypertension [167]. Reduced IGF-1 levels in elderly individuals have been associated with increased risks of congestive cardiac failure and stroke [168,169]. Retrospective studies in hypopituitary patients have suggested increased risks of cardiovascular mortality and reduced life expectancy with AGHD [170,171], although this has not been shown in prospective studies [172]. It was hypothesized that increased life expectancy might be expected to occur with higher GH/IGF-1 activity but this has not been proven. Patients with acromegaly have been shown to
have increased cardiovascular risks and reduced life expectancy [173] and very old age is not associated with elevation of IGF-1 [174]. Experimental animal studies in species with congenital reductions in GH/IGF-1 axis activity appear to show increased life expectancy with reduced axis activity [175,176]. Anecdotal reports of human populations with untreated congenital GH deficiencies vary with regards to survival [177,178]. In addition, IGF-1 has been implicated as a risk factor in prostate, breast and colorectal cancers [179-181]. Thus, it has been postulated that the age related decline in the GH/IGF-1 axis may be an adaptive response to reduce cancer risk, improve insulin sensitivity and prolong longevity [182] but this would appear to be at the expense of increasing other intercurrent pathologies.
2.4 - GH / IGF-1 and Collagen Metabolism

The effects of GH and IGF-1 on collagen metabolism and their implications on normal and pathological skin, cardiovascular, orthopaedic and respiratory physiology have been investigated. The effects of axis activity and age have also been the subject of investigation with regards to collagen production.

Early studies by Jorgensen et al using rat models looked non-specifically at the effects of growth hormone on granulation tissue and skin. They found GH increased granulation tissue collagen content and tissue strength but not in a dose dependent manner. They could not repeat the findings in a similar, later study [183,184]. In contrast, the same team found a dose dependent increase in both collagen content and strength of intact rat skin to GH administration [185]. Avian skin fibroblast models suggested GH and IGF-1 had a synergistic action on collagen production and could actually inhibit type I procollagen gene expression, with IGF-1 also stimulating collagenase activity [186].

Ghahary et al, investigating post-burn hypertrophic scar (Hsc) tissue, found increased expression of type I and type III procollagen mRNA in both normal and Hsc human skin fibroblasts exposed to IGF-1 [187]. The Hsc tissue was found to contain higher levels of IGF-1 compared to normal skin [187]. They also demonstrated Hsc fibroblasts had lower levels of collagenase mRNA and collagenase activity and that exposure to IGF-1 reduced these collagenase levels in both normal and Hsc fibroblasts [188]. The same team found that IGF-1 stimulated TGFβ-1 expression (an important growth factor in fibroblast proliferation and function) in dermal fibroblasts and hypothesized that this may be one of the mechanisms by which IGF-1 mediated its effects [189]. IGF-1 has also been shown to stimulate IGFBP-3 release from Hsc fibroblasts but in a heterogeneous manner, with the release dependant on the dermal layer the cell was originally derived from [190].

Keloid scars (Ksc) are characterized by abnormal fibroblast proliferation and overproduction of collagen [191]. Ksc fibroblasts are known to overexpress IGF-1r
Quercetin is a plant-derived compound that is a potent inhibitor of the enzymes involved in IGF-1 intra-cellular signalling pathways [191]. Phan et al found quercetin significantly reduced collagen type I and collagen type III expression by human Ksc fibroblasts [191]. Although the exact mechanism by which these changes were induced was not known, it was speculated that inhibition of IGF-1 induced MAP kinase activity, and subsequent phosphorylation of transcription factors such as Elk-1, was the most likely reason. Dahlgren et al, using a collagenase induced equine model of flexor tendinitis, demonstrated elevated hydroxyproline content of healing tendon treated with intra-lesional injection of IGF-1 associated with increased fibroblast proliferation [193]. However, there was no change in the proportion of collagens, unlike previous in-vitro studies [194], and it was noted that both control and IGF-1 tendons had virtually healed by the end of the study.

Bruel and Oxlund, investigating the effects of GH on the cardiovascular system using a rat model, found increased concentrations of collagen in the aorta, with a relative increase of type I collagen, in response to an 80 day course of GH [195]. Using a novel technique to assess in-vivo collagen deposition, they found GH increased collagen deposition in both the aorta and the left ventricle. The left ventricular deposition rate was noted to be five times greater than the aorta [196]. In contrast, the same group found in another study that although overall collagen content was increased, this was in proportion to the growth of the heart and GH actually reduced the concentration of collagen in the ventricles by seventeen percent [197]. There was no change in the relative amounts of type I or type III collagen either. These findings reflect the conflicting results in the literature [198,199]. Recently, IGF-1 has been shown to promote collagen synthesis by cardiac fibroblasts [200].

With age and senescence, the physical characteristics of the aorta change with increased stiffness and reduced elasticity. Bruel and Oxlund demonstrated reduced aortic collagen content with increasing age [201]. This has been confirmed by other studies revealing reduced aortic type I procollagen mRNA and collagen synthesis with age [202-203]. GH was found to increase the collagen content, concentration and relative ratio of type I to type III collagen in the aorta of old rats as well as
young rats but the magnitude of the response was diminished [204]. The authors of this paper suggested the observed decline in collagen turnover was related to the age related decline in the GH/IGF-1 axis. They noted, however, that the increased collagen observed with GH administration did not alter the physical properties of the aged aorta.

Other studies have also shown altered fibroblast function with age. Some ageing human fibroblasts have been shown to have elevated collagenase mRNA expression and activity with reduced procollagen type I mRNA and this was suggested to contribute to poor wound healing in the elderly [205]. Although the number of IGF-1r expressed in young and aged human fibroblasts is the same, aged fibroblasts do not respond as vigorously to IGF-1 [206]. Elevated IGFBP-3 expression has also been observed in senescent fibroblasts and has been found to inhibit IGF-1 mediated DNA synthesis [207].

GH administration to adults with GH deficiency states results in a variable rise in serum markers of type I and type III procollagen depending on the dosage administered [208]. This is associated with proportionately higher levels of type III procollagen propeptides. IGF-1 administration to adults with primary growth hormone resistance (Laron syndrome) also results in elevation of serum type I and type III procollagen propeptides [209,210]. In contrast to GH in GH deficiency, a proportionately greater rise in type I procollagen is observed but this is from a lower level in the untreated disease state. Although administration of both hormones resulted in apparent alterations of the procollagen ratios, this was observed in serum samples and reflected global connective tissue synthesis. Tissue-specific collagen ratios and the effect of GH/IGF-1 administration on them has not been investigated.
2.5 - Conclusions

It is clear that the GH/IGF-1 axis continues an important role in adulthood beyond the pubertal growth spurt. It influences bodily functions including protein, carbohydrate, bone and fat metabolism. There is an age-related decline in the GH/IGF-1 axis that is one of the endocrinological hallmarks of ageing. This can result in undesirable effects such as increased cardiovascular morbidity but also appears to be a protective response reducing oncogenic risk and promoting longevity. The GH/IGF-1 axis influences collagen metabolism and the effects are both time and tissue dependant. GH, IGF-1 and IGFBPs can all affect collagen production and collagenase activity. The ratios of type I:Type III collagens can be altered also. Age has been shown to alter fibroblast production of collagens, collagenases and fibroblast responsiveness to IGF-1 separate from the effects of the somatopause related GH/IGF-1 decline. Although alterations in collagen metabolism have already been observed in skin, tendon, aorta and heart tissue, it is likely that the GH/IGF-1 axis has a role in collagen metabolism at many other sites.
Section Two:
A Prospective Case-Control Study of Growth Hormone Axis
and Activity in Adult Inguinal Hernia Patients
Chapter Three

Methods

3.1 – Introduction

Adult inguinal hernia development has been found to be a disease of collagen metabolism with altered ratios of collagens, altered MMP activity and recently TIMP activity being found to varying degrees in the skin, scar, fascia and hernial sacs of patients with primary, recurrent and incisional hernias. Altered fibroblast activity has also been observed. Due to the complex control mechanisms of the ECM environment, the reasons behind this altered metabolism have not been fully elucidated.

The GH/IGF-1 axis, and the somatopause, have been shown to influence collagen metabolism, collagen ratios and MMPs in skin, bone and vascular tissues. The peak decline in activity occurs in the fifth and sixth decades which coincides with the peak incidence of adult inguinal hernia development. It was therefore hypothesized that the GH/IGF-1 axis, in particular the somatopause, may have a role in the aetiology of adult inguinal hernia development. As aforementioned in Section 1, the gonadal axis also declines with age and influences body habitus. It was hypothesized that this could be a ‘confounding’ aetiological factor and was therefore investigated also.
3.2 – Aims

1. To assess if men with inguinal hernia had reduced growth hormone (GH) / 
   insulin-like growth factor-1 (IGF-1) axis activity (represented by lower IGF-
   1 concentrations) compared to age-matched controls without such a history.

2. To determine if reduced gonadal axis activity, as estimated by testosterone 
   and free androgen index, was present in men with inguinal hernia compared 
   to age-matched controls.
3.3 – Subjects

Adult male patients referred to the Southern General Hospital with an inguinal hernia were reviewed in a dedicated clinic. All hernia patients over 35 years old were considered for inclusion in the study. Exclusion criteria included current or past medical history of endocrine pathology, aneurysmal vascular disease, diabetes, liver or renal pathology and malignancy to exclude factors known to influence GH / IGF-1 axis activity. All other medical history, including smoking history, was noted. Height/weight measurements were taken for calculation of body mass index (BMI) by the formula \[ \text{BMI} = \frac{\text{weight}}{\text{height}^2} \] (see appendices 6.1-6.3). A single venous blood sample was taken in the morning between 9am and 12pm from the antecubital fossa for evaluation of serum IGF-1 (as a surrogate marker of GH / IGF-1 axis activity). Testosterone and sex hormone binding globulin (SHBG) were also evaluated to allow calculation of the Free Androgen Index (as a marker of androgen activity). The subsequent operative findings were reviewed to delineate the proportion of direct and indirect hernias.

Healthy controls were sought from patients attending for minor and day case operative procedures and patients attending surgical and orthopaedic outpatient clinics for the follow-up of benign disease. Controls were age-matched within one year to the hernia patients and as close as possible for BMI. In addition to the above exclusion criteria, controls were not included if a current or past history of any type of ventral abdominal wall hernia was noted. Clinical examination was performed by two independent fully-qualified medical practitioners to exclude undiagnosed groin hernias.

Informed consent was obtained from all participants. The study was approved by the Southern General Hospital ethics committee.
3.4 - Blood Sample Assays

Once taken, blood samples were placed in a centrifuge at 3000rpm for 10 minutes and frozen at -20°C for later batch analysis.

**IGF-1**

A 2-site immunoenzymometric (IEMA) assay was performed using the Nichols Advantage IGF-1 assay (Nichols Institute Diagnostics, California, USA). Pretreatment acid-ethanol extraction was performed to avoid interference from binding proteins. The detection limit was 10 ug/L. The inter-assay coefficient of variation using quality control pools at 80 & 260 ug/L was 7%.

**Testosterone**

A commercial competitive chemiluminescence immunoassay was performed using the Bayer Centaur analyser (Bayer Healthcare, Leverkusen, Germany). A pretreatment step with a testosterone releasing agent released bound testosterone from endogenous binding proteins. The detection limit was 0.5 nmol/L. The inter-assay coefficient of variation using quality control pools at 3, 21 & 44 nmol/L was 9%.

**SHBG**

A 2-site chemiluminescent immunometric assay was performed using the DPC Immulite 2000 analyser (Diagnostic Products Corporation, California, USA). The detection limit was 1 nmol/L. The inter-assay coefficient of variation using quality control pools at 5, 38 & 80 nmol/L was 4%.

**Free Androgen Index**

This was calculated using the formula:

\[
\text{Free Androgen Index} = \left[ \frac{\text{testosterone (nmol/L)}}{\text{SHBG (nmol/L)}} \right] \times 100
\]
3.5- Statistical Analysis

Baseline characteristics were expressed as median (interquartile range) or proportions as appropriate. Serum hormone assays were expressed as mean +/- standard deviation. Differences between baseline characteristics were analyzed using chi-squared or Mann-Whitney U-tests where appropriate. Comparison of means between groups was performed using one-way analysis of variance (ANOVA). Simple linear correlation was performed using bivariate Pearson's correlation and the F-test was used to assess significance.

P<0.05 was considered statistically significant.

Statistical analysis was performed using Statistical Package for the Social Sciences v10.0 for Windows (SPSS, Chicago, Illinois, USA).
Chapter 4
Results

4.1 Recruitment and baseline characteristics

47 hernia patients were recruited from 100 male patients referred to the southern general hospital with a diagnosis of inguinal hernia between 2003 and 2005. 47 control patients were recruited according to the above criteria from 850 patients reviewed attending the southern general hospital daycase surgery, endoscopy, general surgical and orthopaedic outpatient departments over the period 2004 to 2005. 3 hernia patient serum samples were not available for processing at the time of batch analysis. These patients and their corresponding age-matched controls were therefore excluded leaving 88 subjects for analysis.

Table 3 summarises the characteristics of these patients. There were no significant differences in age, height, weight, smoking history or presence of non-excluding co-morbidity between the two groups. There was a statistically significant difference in mean BMI (mean BMI hernia 25.1 vs control 26.6; p=0.035) but when the BMI was considered in terms of the recognized healthy (18.5-25), overweight (25.1-30) and obese (>30.1) ranges there was no difference between the hernia and control patients.

Table 4 summarises the clinical characteristics of the hernias identified. 57% (25) were right-sided with a median history of 4 months. Of the 44 patients recruited, 3 patients subsequently declined surgery, 2 were known still to be on the waiting list and 7 cases were unavailable for review at the time of batch analysis. This left 32 patients with full operative details. 22.7% (10) were direct hernias (defined as occurring lateral to the deep inferior epigastric artery), 31.8% (14) were indirect hernias (defined as occurring medial to the deep inferior epigastric vessels), 13.6% (6) had combined defects, 2.3% (1) involved recurrent hernias and 2.3% (1) were not defined.
### Table 3. Characteristics of Patients. (n=44 per group)

Continuous data expressed as median with interquartile range with mean and standard deviation in brackets beneath. Categorical data expressed as percentage with absolute value in brackets. n.s.=non-significant.
<table>
<thead>
<tr>
<th>Hernia Characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Side</strong></td>
</tr>
<tr>
<td>Right</td>
</tr>
<tr>
<td>Left</td>
</tr>
<tr>
<td>Bilateral</td>
</tr>
<tr>
<td><strong>Duration</strong></td>
</tr>
<tr>
<td>Mean 12.8mths(1-120mths)</td>
</tr>
<tr>
<td><strong>Operative Type</strong></td>
</tr>
<tr>
<td>Direct</td>
</tr>
<tr>
<td>Indirect</td>
</tr>
<tr>
<td>Combined</td>
</tr>
<tr>
<td>Recurrent</td>
</tr>
<tr>
<td>Not Defined</td>
</tr>
<tr>
<td>Not Known</td>
</tr>
</tbody>
</table>

Table 4. Clinical Characteristics of Hernias.
4.2 IGF-1 Assay Results

**Comparison of IGF-1 assays with age in hernia and control groups**
Both hernia and control groups displayed a negative correlation between age and serum IGF-1 concentrations with Pearson's correlation coefficients of -0.176 and -0.166 respectively. These were not statistically significant (See Fig.4 and Table 5). Controlling for BMI did not alter these findings but controlling for FAI weakened the correlation findings in both groups.

**Comparison of IGF-1 assays between groups**
Figure 5 presents simple boxplots displaying the IGF-1 concentrations of both cohorts of patients. The hernia group’s mean IGF-1 concentration was lower at 125.7\(\mu\)mol/L +/- 47.7 compared with 135.0\(\mu\)mol/L +/- 50.9 of the control group but this did not reach statistical significance (p=0.374, see Table 6). Subgroup analysis of the direct and indirect hernia cohorts did not reveal a significant difference either.
Figure 4a / 4b. Correlation between IGF-1 and Age in hernia and control groups.

Pears Coeff = Pearson’s Correlation Coefficient
<table>
<thead>
<tr>
<th>Correlation</th>
<th>Pearson’s Co-efficient</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>All subjects</td>
<td>IGF-1-Age</td>
<td>-0.168</td>
</tr>
<tr>
<td>Hernia</td>
<td>IGF-1-Age</td>
<td>-0.176</td>
</tr>
<tr>
<td>Control</td>
<td>IGF-1-Age</td>
<td>-0.166</td>
</tr>
</tbody>
</table>

* = statistically significant

Table 5. Pearson’s correlation co-efficients for IGF-1 vs age.
Figure 5. Comparison of serum IGF-1 levels between hernia and control groups.

<table>
<thead>
<tr>
<th></th>
<th>Hernia</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>All Cases</td>
<td>125.7 +/- 47.7 (111.1-140.1)</td>
<td>135.0 +/- 50.9 (19.6-150.5)</td>
</tr>
<tr>
<td></td>
<td>*F=0.80 p=0.374</td>
<td></td>
</tr>
<tr>
<td>Direct sub-group</td>
<td>117.2 +/- 36.3 (15.2-139.1)</td>
<td>131.5 +/- 45.2 (104.1-158.8)</td>
</tr>
<tr>
<td></td>
<td>*F=0.80 p=0.383</td>
<td></td>
</tr>
<tr>
<td>Indirect sub-group</td>
<td>135.8 +/- 58.0 (91.3-180.3)</td>
<td>143.2 +/- 61.6 (95.9-190.6)</td>
</tr>
<tr>
<td></td>
<td>*F=0.70 p=0.795</td>
<td></td>
</tr>
</tbody>
</table>

* = statistically significant

Table 6. Comparison of IGF-1 assays between hernia and control groups.
Values expressed as mean with standard deviation and 95% confidence intervals in brackets. Statistical analysis by ANOVA.
4.3 Testosterone Assay and Free Androgen Index Results

Comparison of Testosterone assays with age in hernia and control groups
The hernia group displayed a negative correlation between age and serum testosterone concentrations with a Pearson’s correlation coefficient of -0.175.
There was no correlation between serum testosterone and age in the control groups (see Fig.6 and Table 7). Controlling for BMI weakened the hernia group correlation findings.

Comparison of Free Androgen Index with age in hernia and control groups
Both hernia and control groups displayed a statistically significant negative correlation between age and calculated FAI (see Fig.7 and Table 7).
The hernia group Pearson’s correlation was -0.366 (p=0.015), the control group Pearson’s correlation was -0.313 (p=0.038).
Controlling for BMI strengthened these findings with a p-value of 0.002 and significance was also maintained when controlling for IGF-1 (hernia p= 0.032, control p= 0.070).

Comparison of Testosterone assays between groups
Figure 8 presents simple boxplots displaying the testosterone concentrations of both cohorts. The hernia group had a higher mean testosterone concentration of 19.4nmol/L +/- 6.4 compared with 14.8nmol/L +/- 6.6 of the control group. This was statistically significant (p=0.001, see Table 8). This significant difference was also reflected in subgroup analysis of direct and indirect hernias (direct p=0.004, indirect p=0.034).

Comparison of Free Androgen Index between groups
Figure 9 presents simple boxplots displaying the calculated FAI of both cohorts. The hernia group had a higher mean FAI of 50.8 +/- 15.9 compared with 43.1 +/- 19.4 of the control group. This was statistically significant (p=0.044, see Table 9). This result was not reflected in subgroup analysis.
Figure 6a / 6b. Correlation between Testosterone and Age in hernia and control groups.

Pears Coeff = Pearson’s Correlation Coefficient
Figure 7a / 7b. Correlation between FAI and Age in hernia and control groups.

Pears Coeff = Pearson’s Correlation Coefficient
<table>
<thead>
<tr>
<th>Correlations</th>
<th>Pearson’s Co-efficient</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>All subjects</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Testosterone-Age</td>
<td>-0.040</td>
<td>0.709</td>
</tr>
<tr>
<td>FAI-Age</td>
<td>-0.331</td>
<td>0.002*</td>
</tr>
<tr>
<td>Hernia</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Testosterone-Age</td>
<td>-0.175</td>
<td>0.256</td>
</tr>
<tr>
<td>FAI-Age</td>
<td>-0.366</td>
<td>0.015*</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Testosterone-Age</td>
<td>-0.097</td>
<td>0.531</td>
</tr>
<tr>
<td>FAI-Age</td>
<td>-0.313</td>
<td>0.038*</td>
</tr>
</tbody>
</table>

* = statistically significant

Table 7. Pearson’s correlation co-efficients for Testosterone and FAI vs Age.
Figure 8. Comparison of serum Testosterone levels between hernia and control groups.

<table>
<thead>
<tr>
<th></th>
<th>Hernia</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole Group</td>
<td>19.4±/-6.4 (17.5-21.3)</td>
<td>14.8±/-6.6 (12.8-16.8)</td>
</tr>
<tr>
<td></td>
<td>( F=11.2 ) ( p=0.001^* )</td>
<td></td>
</tr>
<tr>
<td>Direct sub-group</td>
<td>18.9±/-6.1 (15.2-22.6)</td>
<td>11.7±/-5.4 (8.4-15.0)</td>
</tr>
<tr>
<td></td>
<td>( F=9.98 ) ( p=0.004^* )</td>
<td></td>
</tr>
<tr>
<td>Indirect sub-group</td>
<td>20.0+/4.9 (16.2-23.8)</td>
<td>15.0+/4.27 (11.7-18.3)</td>
</tr>
<tr>
<td></td>
<td>( F=5.37 ) ( p=0.034^* )</td>
<td></td>
</tr>
</tbody>
</table>

* = statistically significant

Table 8. Comparison of Testosterone assays between hernia and control groups.
Values expressed as mean with standard deviation and 95% confidence intervals in brackets. Statistical analysis by ANOVA.
Figure 9. Comparison of FAI calculations between hernia and control groups.

<table>
<thead>
<tr>
<th></th>
<th>Hernia</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole Group</td>
<td>50.8±/-15.9 (46.0-55.7)</td>
<td>43.1±/-19.4 (37.2-49.0)</td>
</tr>
<tr>
<td></td>
<td>F=4.17  p=0.044*</td>
<td></td>
</tr>
<tr>
<td>Direct sub-group</td>
<td>45.0±/-9.4 (39.3-50.7)</td>
<td>44.0±/-15.6 (35.1-54.0)</td>
</tr>
<tr>
<td></td>
<td>F=0.01  p=0.928</td>
<td></td>
</tr>
<tr>
<td>Indirect sub-group</td>
<td>50.7±/-17.6 (37.7-64.2)</td>
<td>46.9±/-21.1 (30.6-63.1)</td>
</tr>
<tr>
<td></td>
<td>F=0.17  p=0.686</td>
<td></td>
</tr>
</tbody>
</table>

* = statistically significant

Table 9. Comparison of FAI calculations between hernia and control groups.
Values expressed as mean with standard deviation and 95% confidence intervals in brackets. Statistical analysis by ANOVA.
Chapter 5

5.1 Discussion

The main aim of this study was to assess if there was an association between reduced growth hormone axis activity and inguinal herniation. Section 1 of this thesis clearly demonstrated the potential influence of the GH/IGF-1 axis on collagen metabolism and the importance of altered collagen metabolism in the aetiology of inguinal herniation. To the author's knowledge, the hypothesis that the two may be linked has never been suggested or investigated before. This research was therefore novel and original.

Serum IGF-1 assays within this cohort negatively correlated with age in keeping with the established age-related decline in axis activity seen in the literature [134-137]. Although the median IGF-1 concentration was lower in the hernia group, and the negative correlation appeared to be greater, this did not reach statistical significance. There are reasons that a significant association was not found. It is possible that patients with occult endocrine or renal disease could have influenced these findings. Also, clinical examination may not have fully excluded occult hernias in controls. Even though this cohort was carefully selected, a degree of biological variability in serum hormone activity was observed in both groups that could have affected analysis. It is clear that, although there is a recognized age-related decline in GH/IGF-1 axis activity and the axis can influence collagen metabolism, this activity and influence at the tissue level can differ from site to site and does not necessarily always reflect 'global' axis activity. It is possible, therefore, that the reduced IGF-1 concentrations and effects may be more evident within the tissues of the inguinal canal. This is an area that would benefit from further assessment.

Serum testosterone and free androgen index within this cohort also negatively correlated with age in keeping with the literature [135,137]. The findings of significantly elevated testosterone and free androgen index levels in the hernia patients compared to the control patients was unexpected. Age-related decline in the male gonadal axis is a well documented, but not universal, phenomenon in adult
males [135,137]. Similar to the GH/IGF-1 axis, this decline has been associated with changes in body habitus including reduced lean body mass and reduced bone mineral density. The evidence for this is, however, inconsistent within the literature [135,137,148,149]. Whether these effects are independent of, or additive to, the GH/IGF-1 axis is also not clear [148,149,212]. Figure 6b initially appeared to suggest that the control group were not behaving as expected of a normal population, with no correlation between age and testosterone levels. The FAI, however, did correlate and is a much more sensitive indicator of gonadal axis activity. Appendix 6.6 displays the origins of the control patients. They all were attending hospital with benign conditions that were not known to influence the gonadal axis. In addition, none of the controls had a history of current or previous ventral abdominal wall or groin hernias either as adults or children. It is likely, therefore, that Figure 6b represents a random sampling effect.

Much of the research in the influence of the gonadal axis on collagen metabolism is in the field of bone metabolism. Although conflicting, results here have shown either no influence or a positive influence of testosterone on collagen metabolism [213-216]. In vascular research, testosterone has been found to be a positive regulator of lysyl oxidase activity [217] and can influence MMP activity. One study, by Natoli et al using an experimental human model of aortic smooth muscle, found that testosterone reduced extra-cellular matrix collagen deposition (types I, II and III) by 30%. They also found testosterone positively regulated MMP-3 gene expression [218].

There is therefore evidence within the current literature that the male gonadal axis can regulate the extracellular matrix. The exact role, however, at different sites is controversial. The male gonadal axis assays in this study were performed to take into account reduced testosterone levels as a confounding factor when considering the GH/IGF-1 axis in inguinal hernia development. The elevated levels found in the hernia patients were still within normal limits and certainly excluded reduced axis activity as an aetiological factor.
The ideal method to have investigated whether an aetiological relationship existed between age-related hormonal changes and inguinal hernia development would have been to perform a large scale epidemiological study with a longitudinal nested case-control study. The cost of analysis (see appendix 6.4) would have been prohibitively expensive and the study logistically challenging. The case-control design chosen represented a realistic and pragmatic approach that was both affordable and achievable in the given time. The tight exclusion criteria selected, although challenging to employ in the West of Scotland population, were aimed at reducing the impact of confounding variables on this smaller sample size. Initially, only direct herniation was to be assessed but further review of the literature indicated that classification of inguinal hernias was less relevant to the aetiology of adult inguinal herniation (see chapter 1.6). This opened the study to all adult males presenting with inguinal herniation.

The gold standard for assessing GH axis activity is an insulin tolerance test [211]. The potential risks of this procedure made it inappropriate to be used in this setting. Serum IGF-1 assays were used as they have been shown to correlate well with axis activity and are not subject to the same diurnal variation as growth hormone [211]. Serum IGF-1 binding proteins were not measured as these have not been shown to correlate as well with axis activity [211], were expensive to assay and did not offer any advantage in addition to serum IGF-1.
5.2 Conclusions

The aetiology of adult inguinal hernia development is multi-factorial. Although genetic, anatomical and physiological factors are involved disordered extra-cellular matrix and collagen metabolism is a key feature. Reduced ratios of type I : type III collagen have been noted related to increased expression of type III collagen and possibly to altered MMP mediated collagen degradation. The GH/IGF-1 axis also influences collagen and the extra-cellular matrix in a heterogeneous fashion dependant on time and location.

This study did not reveal a significant association between reduced somatotrophin axis activity and adult male inguinal hernias. Male gonadal axis activity was elevated in this group (the significance of which was unclear) but there was no association between reduced gonadal axis activity and hernia development either. Overall, the results suggest that the recognized hormonal changes associated with adult male ageing are not aetiological factors in adult male inguinal hernia development. Further research in this area is warranted to confirm these findings.
Chapter 6

Appendices
Appendix 6.1 – Hernia Data Collection Form

G.H. in Inguinal Hernia Study

PATIENT DETAILS

Hospital Number:
Surname:
Forename:
Date of Birth: / /

Inclusion Criteria (all answers should be yes)

>35yrs YES / NO
Inguinal Hernia YES / NO
Consent YES / NO
Bloods taken IGF-1 YES / NO
GH YES/ NO
TESTOSTERONE YES / NO
FREE ANDROGEN BINDING INDEX YES / NO
DETAILS OF HERNIA

Is hernia Primary? yes / no
   Recurrent? yes / no

Duration of Hernia ___ mths

Side of Hernia left / right / bilat.

Clinical Type of Hernia Direct / Indirect / Combined / Recurrent

Operative Findings Direct / Indirect / Combined / Recurrent

PATIENT DEMOGRAPHICS

Height: __ cm Weight: __ Kg

Smoker: Yes / No

If yes,
   Duration yr mth
   Amount cigs / wk

Vascular Disease: Yes / No

If yes, PVD / Aneurysm / IHD / Other (specify)

Endocrine Disease: Yes / No

If yes, Specify

Diabetes: Yes / No

All other PMH:
Appendix 6.2 – Control Data Collection Form

G.H. in Inguinal Hernia Study

CONTROL FOR HERNIA PATIENT No.

PATIENT DETAILS

Hospital Number:
Surname:
Forename:
Date of Birth: / / 

Inclusion Criteria (all answers should be yes)

Matched for Age, BMI & Co-morbidity YES / NO

Clinical Absence of Inguinal Hernia YES / NO
(2 clinicians in accordance)

Clinician 1 _____________________________ Date ______
Clinician 2 _____________________________ Date ______

Consent YES / NO

Bloods taken IGF-1 YES / NO

GHI YES/ NO

TESTOSTERONE YES / NO

FREE ANDROGEN BINDING INDEX YES / NO
PATIENT DEMOGRAPHICS

Height: cm
Weight: Kg

Smoker: Yes / No
If yes,
Duration yr mth
Amount cigs / wk

Vascular Disease: Yes / No
If yes,
PVD / Aneurysm / IHD / Other (specify)

Endocrine Disease: Yes / No
If yes,
Specify

Diabetes: Yes / No

All other PMH:
Appendix 6.3 – Patient Information and Consent Forms

Is growth hormone associated with the development of inguinal hernia?

Inguinal hernia are a common surgical problem requiring treatment. Understanding why they develop is very important in order to help us treat them effectively. Many different factors have been implicated including inherited factors, differences in our tissue and muscle production and the strength of those muscles. Growth hormone is a natural body hormone that helps control the production and make up of our body tissues. It is possible that naturally lower levels of this hormone may contribute to the development of inguinal hernia. We are currently investigating whether this is true by comparing growth hormone levels in patients with and without inguinal hernia to see if there is a connection.

You have been identified as a patient who could assist in this study. This would involve taking one sample of blood for analysis and collecting some information from your medical case notes. All information would be anonymous and you would not be identified personally.

There is a consent form to sign if you are interested in assisting in this study. You do not need to take part and your care will not be affected at all if you choose not to participate.

You can contact Mr Paul Witherspoon at the Southern General Hospital if you have any queries on 0141-201-1100.
SOUTHERN GENERAL HOSPITAL
CONSENT FORM - Growth Hormone Study

PATIENT NAME........................................DATE OF BIRTH......................
To be completed by the Patient

Please Tick

Yes  No

. Have you read the Patient Information?

. Have you had an opportunity to ask questions and discuss this study?

. Have you received satisfactory answers to all your questions?

. Have you received enough information about the study?

Who have you spoken to?
Dr/Mr/Ms_____________________________________________________

Do you understand that you are free to withdraw from the study -

. at any time

. without having to give a reason

. and without affecting your future medical care?

. Do you agree to take part in this study?

Do you have any reason to believe you are or may be pregnant?

YES, I may be pregnant    □  
NO, I am not pregnant    □

Signed.......................................................... Date.......................  

Name in Block Letters.........................................................

Date......................
Appendix 6.4 – Cost Analysis

Analysis

IGF-1 - £7.89 per sample
Testosterone - £11 per sample
Sex Hormone Binding Globulin - £5 per sample

Technician Support / Lab costs

£22 per sample

Total cost per patient - £45.89

Grand Total for 94 subjects = £4,313.66
Appendix 6.5 – Case Summaries

<table>
<thead>
<tr>
<th></th>
<th>Age</th>
<th>Height</th>
<th>Weight</th>
<th>BMI</th>
<th>Smoker</th>
<th>Comorb</th>
<th>IGFl</th>
<th>Testosterone</th>
<th>SHBG</th>
<th>FAI</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>78</td>
<td>170.1</td>
<td>87.2</td>
<td>30.2</td>
<td>ex</td>
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<td>99.00</td>
<td>13.50</td>
<td>55.00</td>
<td>25.00</td>
</tr>
<tr>
<td>2</td>
<td>62</td>
<td>171.5</td>
<td>64.8</td>
<td>22.0</td>
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<td>75.00</td>
<td>20.40</td>
<td>48.00</td>
<td>43.00</td>
</tr>
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<td>Orthopaedic Clinic</td>
<td>33</td>
<td>Osteoarthritis</td>
</tr>
<tr>
<td>General Surgery</td>
<td>8</td>
<td>Benign ano-rectal (5)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Benign skin lesions (3)</td>
</tr>
<tr>
<td>Endoscopy Suite</td>
<td>3</td>
<td>Dyspepsia</td>
</tr>
<tr>
<td>Day Surgery</td>
<td>3</td>
<td>EUA Rectum (1)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Varicose Veins (1)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Arthroscopy (1)</td>
</tr>
</tbody>
</table>

There were no noted histories of current, previous or childhood hernias.

There were no noted histories of liver, renal, endocrine, diabetic, aneurysmal or malignant pathology.
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