A STUDY OF GASTROINTESTINAL DISEASE IN SYSTEMIC SCLEROSIS AND THE EFFECT ON ANORECTAL FUNCTION AND NUTRITION

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Statement of Contribution

I, Nora Thoua, confirm that the work presented in the thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the thesis.

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Abstract

This thesis investigates the prevalence and pathophysiology of gastrointestinal involvement in systemic sclerosis (SSc). The primary pathologies within the gastrointestinal tract affect the mucosa, vasculature, smooth muscle and enteric nervous system. The aim of this thesis was to conduct experiments to assess these pathologies within a well-characterised SSc patient cohort.

Introduction: A review of the current understanding of the pathophysiology of gastrointestinal disease in systemic sclerosis.

Prevalence of GI symptoms: A prospective questionnaire study of 400 patients in order to assess gut disease burden and review of patient disease characteristics.

Anorectal involvement: Extensive anorectal physiological assessment of symptomatic and asymptomatic systemic sclerosis patients compared with incontinent controls in order to assess aspects of neuropathy and myopathy.

Nutritional effect as an assessment of mucosal involvement: Nutritional assessment of patients with and without gastrointestinal symptoms through anthropometric assessment, indirect calorimetry and bioelectrical impedance.

The pathophysiology of gastrointestinal involvement in systemic sclerosis was further investigated in an established mouse model of scleroderma. This transgenic mouse model expresses a kinase deficient type II TGFβ receptor (TβRIIΔk) in fibroblasts and the mice develop skin fibrosis as well as pulmonary fibrosis and a structural vasculopathy. Gastrointestinal tissue from these mice was examined histologically and the contractile activity of gut tissue was examined in vitro.

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Ethical Approval

All the studies involving patients contained in this thesis had been previously granted full ethical approval by the National Hospital for Neurology and Neurosurgery & Institute of Neurology Joint research ethics committee (University College London Hospital). Informed written consent was obtained from all participants and all subjects were given a full information sheet pertaining to the study in which they were participating.

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Home Office approval was obtained prior to all animal procedures.

Publications Arising from this Thesis

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Presentations at International and National Conferences

1. Gut fibrosis with altered colonic contractility in a mouse model of scleroderma. American College of Rheumatology annual meeting, 2011

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4. Bioelectrical impedance is a useful adjunctive tool in assessing nutritional status in systemic sclerosis patients with gastrointestinal symptoms. BAPEN 2009 – Cardiff October 2009.

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CHAPTER 1:

PATHOPHYSIOLOGY OF GASTROINTESTINAL INVOLVEMENT IN SYSTEMIC SCLEROSIS

1.1. Introduction

Systemic sclerosis (SSc) is a chronic autoimmune disorder of unknown aetiology. The prevalence of SSc in UK is 8 per 100,000 of population although this varies in different studies (1-3). It is 3-5 times more common in women than in men, the peak age of onset is 30-50 years and it is twice more common in afro-caribbeans than in caucasians. Systemic sclerosis is characterised by proliferative vascular lesions and fibrosis of the skin and multiple internal organs, such as lungs, kidneys, heart and the gastrointestinal tract (GIT). There are 2 main SSc subsets, diffuse cutaneous or limited cutaneous based on the extent of skin involvement. Skin thickening above the elbows or knees is classified as diffuse cutaneous SSc and skin tightness limited to areas distal to elbows or knees is classified as limited cutaneous, the face can be affected in both subsets. The importance of this classification is that these 2 subsets have differing natural histories, organ involvement, prognoses and autoantibody associations (4).

After the skin the GIT is the most commonly affected organ in SSc. GIT involvement is reported in up to 90% of SSc patients in both diffuse and limited cutaneous forms. It can occur at any stage of the disease and can be slowly or rapidly progressive. Severe GI involvement occurs in about 8% of patients and is associated with increased mortality (5). Like with other severe organ involvement it is more likely to develop early – in the first 3 years – in the course of the disease (5), but involvement of any part of the GIT can occur at any stage.

1.2. Microvascular changes in SSc

One of the prominent early clinicopathological features of SSc is involvement of the vasculature. In the periphery this manifests as Raynaud's phenomenon, cutaneous and mucosal telangiectasia and nailbed capillary abnormalities. Telangiectasias found anywhere along the GI tract from the oral cavity to the rectum are the most common cause of blood loss which occurs in SSc with a frequency of 15% (6). Gastric antral vascular ectasia (GAVE) is a specific vascular abnormality seen in the gastric antrum and is associated with SSc (7). It is characterised by large, longitudinal, convoluted vessels in the gastric antrum converging towards the pylorus. These are highly prone to bleeding but often easily amenable to endoscopic treatment with laser therapy and argon plasma coagulation. Other than bleeding and probably more important, in the viscera involvement of the vasculature can lead to pathologies such as renal disease and pulmonary arterial hypertension (8;9).

Vascular change is thought to not only represent a common pathological manifestation but also to be the initiating event in the pathogenesis of GI involvement in SSc. Vascular abnormalities mainly affect small arteries, they are characterised by endothelial cell swelling and myointimal proliferation resulting in narrowing of the lumen and disruption of the internal elastic lamina (10). The consequence of this is recurrent episodes of vasoconstriction and tissue ischaemia. Vascular changes have been demonstrated along different parts of the gastrointestinal tract. For example reduced blood flow, determined endoscopicaly by laser Doppler or endoscopic oxygen electrode sensors has been found in the

stomach and duodenum in SSc patients (11;12). Rectal biopsies from patients with SSc show enteric vessels with thickened basal lamina, hypertrophied endothelial cells and partially obliterated lumen (13). Not only is a vascular event thought to be the initiating factor in the pathogenesis of GI manifestations in SSc but the vascular insufficiency and ischaemia has a perpetuating effect on smooth muscle atrophy and intestinal secretory and absorptive function.

Microvascular dysfunction and features such as Raynaud's phenomenon may also be secondary to a dysfunction in the autonomic control of microcirculation (14). Autonomic dysfunction is common in SSc and is characterized by marked adrenaline-mediated sympathetic overactivity and instability and impaired parasympathetic activity. This has been demonstrated in a number of studies in SSc patients mainly by means of conventional autonomic tests such as measurement of heart rate variability and cardiorespiratory reflex tests (15-17). Dysautonomia may contribute to intestinal ischaemia by inducing vasoconstriction as demonstrated by decreased superior mesenteric arterial blood flow (18). A number of studies have investigated the association between autonomic dysfunction and gastrointestinal impairment. Dessein *et al* showed that clonidine, an α2 adrenergic agonist, increased lower oesophageal sphincter pressure in patients with oesophageal symptoms and adrenaline-mediated sympathetic overactivity suggesting a potential role of autonomic dysregulation on oesophageal dysfunction (19). Iovino *et al* showed a positive correlation between gastric compliance and autonomic nerve function (20). In contrast di Ciaula *et al* did not find any association between

autonomic neuropathy and dyspepsia, gastric and gallbladder motility and intestinal transit (21).

1.3. Smooth muscle changes in SSc

The pathological effect of SSc in the GI tract is not confined to the vasculature. Several studies have demonstrated oesophageal, intestinal and colonic dysmotility in patients with SSc. The pathophysiology of this is similar throughout the GI tract; the mucosa is largely uninvolved, with the structure and function of epithelial cells and villi usually being normal. Findings are histologically localised to the submucosa and muscular layer. There is inflammatory cell infiltration in the lamina propria and collagen deposition in the submucosa. In the muscularis propria there is fibrosis and atrophy of the smooth muscle, the circular layer being more markedly affected that the longitudinal (22). Atrophy is usually patchy, but as the disease progresses becomes more extensive and often associated with marked fibrosis. There is also progressive collagen deposition and fibrosis of the serosa contributing to the attenuated motility and reduced distensibility of the viscera.

In ultrastructural studies of the muscle coat wide areas of marked focal fibrosis, characterised by collagen and elastic fibre depositions, are seen surrounding smooth muscle cells. Intercellular spaces are widened and gap junctions between smooth muscle cells are decreased which may account for impaired smooth muscle cell contraction and reduced transmission of peristalsis (23). Enteric neurons and satellite cells have been shown to be structurally normal except for cytoplasmic

vacuolisation but the surrounding axon terminals have a pale axoplasm with decreased number of vesicles and dense bodies and without cytoskeletal elements (13). Furthermore, abundant elastic and collagen fibres envelope nerve endings, separating them from smooth muscle cells and there are signs of degeneration of unmyelinated fibres.

The most accepted theory regarding the pathophysiology of GI dysmotility in SSc is that it is a progressive process with initial neural dysfunction followed by smooth muscle atrophy and eventually muscle fibrosis. The initial neural dysfunction has been hypothesised to be secondary to arteriolar changes in the vasa nervorum although an alternative explanation is direct nerve fibre compression by collagen deposits. A number of autoantibodies are found in patients with SSc but their involvement in the pathophysiology is still unknown. Certain autoantibodies are associated with specific organ involvement and disease characteristics. There is increasing evidence that autoantibodies in SSc play a pathogenic role (24). Antimyenteric neuronal antibodies have been found in SSc and may contribute in the process either as an initiating or concomitant event (25). This is supported by the finding that IgG from patients with SSc when injected in immunosuppressed rats induced alteration in intestinal myoelectic activity (26). This was further supported by the findings of Goldblatt *et al* of antibodies found in the serum of SSc patients inhibiting muscarinic receptor-mediated enteric cholinergic neurotransmission (27). In their study the patients that demonstrated those antibodies also reported more severe gastrointestinal symptoms. Singh *et al* showed that IgGs isolated from SSc patients (but not from normal volunteers)

attenuate muscarinic receptor activation by bethanechol in isolated smooth muscle cells of the rat internal anal sphincter (28). These antibodies are not yet checked routinely in SSc patients, nor have been tested in large patient cohorts.

During the neuropathic phase there is dysmotility but the muscle is still responsive to methacholine and symptoms tend to respond to prokinetic agents. Cisapride a known gastrointestinal prokinetic agent mediates its effects by the release of acetylcholine in the myenteric plexus. It has been shown to increase oesophageal and gastric transit (29), enhance gastric fundal contractions (30) and increase colonic transit (31) in patients with SSc. Chronic neural dysfunction perpetuates smooth muscle atrophy with gut muscle being less contractile but still responsive. Patients are more likely to become symptomatic at this point and treatment with prokinetics may still be partially successful. With disease progression fibrosis is superimposed on smooth muscle atrophy, rendering the muscle unresponsive to methacholine and patients no longer respond to prokinetics (32).

GI involvement in SSc manifests as a spectrum of disorders of mainly motility and transit time with specific abnormalities observed in the different parts of the GI tract. An interesting aspect of this spectrum is that the clinical manifestations can be progressive but the progression can be slow or rapid and can occur at any age. By understanding the disease process and pathophysiology further it may be possible to arrest this disease progression at an early stage and before significant symptoms develop.

1.4. Neuropathy

Oesophagus

Oesophageal dysmotility is the most common GI manifestation in SSc, being present in 80-90% of patients. Normal oesophageal function is characterised by tonic lower oesophageal sphincter (LOS) pressure being higher than gastric pressure, thus preventing significant gastro-oesophageal reflux. In a normal swallow, the oesophageal body contracts in a peristaltic manner and the contraction is propagated towards the stomach at a rate of 3-5 cm/sec. The LOS relaxes at the start of the peristaltic wave to allow the bolus to empty into the stomach. Peristalsis is the net result of the coordinated relaxation and contraction mediated by the inhibitory and excitatory myenteric plexus neurons along the length of the oesophagus. The excitatory neurons release acetylcholine while the inhibitory neurons release nitric oxide (NO) and vasoactive intestinal polypeptide (VIP) resulting in oesophageal and LOS contractions and relaxations, respectively. Defective excitatory innervation may partly explain low amplitude or ineffective oesophageal peristaltic contraction (33). Typical findings in SSc are a decrease in the amplitude and spread of the oesophageal body contractions in the distal two thirds (smooth muscle portion) of the oesophagus associated with reduced LOS pressure which relaxes variably to accommodate the bolus. With progression, the amplitude of contractions and LOS pressure decrease further and there is often lack of peristalsis and impaired coordination between the LOS and distal oesophagus. These abnormalities result in prolonged oesophageal transit time and delayed oesophageal emptying causing dysphagia. Furthermore the reduced LOS pressure

predisposes to gastro-oesophageal reflux. The dysmotility exacerbates the resulting reflux symptoms since there is impaired acid clearance.

Autonomic dysfunction, with high circulating adrenaline levels, has been shown to correlate with decreased distal oesophageal contraction amplitude and with reduced LOS pressure suggesting some causality of dysautonomia and dysphagia. In one study treatment with clonidine improved the autonomic dysfunction and also improved both oesophageal symptoms and oesophageal physiology in some patients (19).

Stomach

The stomach has been reported to be involved in at least 50% of patients with SSc. Normal antral and duodenal manometry in the fasting state is characterised by the migrating myoelectrical complex (MMC) which is a cyclical pattern of contractile activity which repeats at intervals of 1.5-2 hours and follows 3 cyclical phases. Phase I of the MMC lasts 5-20 minutes and is characterised by absence of contractions. Phase II lasts 10-40 minutes and consists of intermittent contractions on a background of slow waves. Phase III lasts 3-6 minutes and consists of bursts of regular rhythmic contractions at the same rate as the slow waves. This sequence of peristaltic activity progresses through the intestines in a regular cycle during fasting state and its purpose, in conjunction with an increase in gastric, biliary and pancreatic secretions, is to clear remnants of digestion and prevent excessive bacterial colonisation in the stomach and small intestine. Initiation of the MMC is thought to be partially controlled by motilin in response to vagal stimulation and propagation is coordinated by the enteric nervous system. Eating induces a marked

increase in contractile activity in the lower body and antrum, with strong peristaltic waves that increase in amplitude as they propagate towards the pylorus. This serves in grinding the food into chyme and forcing it through the pyloric canal into the small intestine. In patients with SSc the MMC in the fasting state occurs less frequently, is of low amplitude or even absent (34) and there is evidence of gastric dysrrhythmia (35). In the fed state there is again decreased frequency and amplitude of antral contractions. This type of abnormality is also observed in patients with diabetic neuropathy, supporting the hypothesis of gut dysfunction relating to a neuropathic process in SSc patients. The result is delayed gastric emptying and often exacerbation of gastro-oesophageal reflux. Autonomic dysfunction in the stomach causes impaired gastric accommodation and compliance. Increased proximal stomach compliance has also been shown in diabetic patients with autonomic neuropathy (36). In a study of SSc patients, those with higher scores of autonomic dysfunction showed greater impairment of gastric compliance compared with patients with normal autonomic scores (20). This abnormality can delay gastric emptying and induce more dyspeptic symptoms and bloating.

Small intestine

The motility pattern in the normal small intestine is similar to the stomach. Fasting small bowel motility follows the 3 cyclical phases of the MMC as described above. After a meal the contents of the small intestine are slowly transported distally through irregular vigorous contractions initiated at the small intestine. As in the stomach, small intestinal motility is abnormal in SSc, with a neuropathic pattern

early in the disease. In the fasting state the MMC is often absent and there are uncoordinated contractions, whereas in the post-prandial state there are reduced contractions and there is diminished response to distension of the duodenum resulting in slow intestinal transit and intestinal dilatation (37). The hypomotility, poor intestinal clearance and also reduced gastric acid secondary to acid suppressant therapy can lead to small intestinal bacterial overgrowth and consequent malabsorption of many substances such as carbohydrates, proteins, lipids and vitamins. Furthermore, alterations in the gut flora can affect the gut mucosa and its immune function as has been shown in other gastrointestinal disorders such as inflammatory bowel disease (38).

Colon and anorectum

The colon and the anorectum is the second (after the oesophagus) most commonly affected region of the GI tract in SSc. The motility of the normal colon is different to the small intestine with no cyclic pattern. It consists of either segmental or propagated (low amplitude or high amplitude) contractions. Segmental contractions, typically at a frequency of 3 cycles/minute, induce a slow aboral propulsion. Low amplitude propagated contractions occur about 50 times per day and function to transport fluid chyme and flatus through the colon, high amplitude propagated contractions occur only about 5 times per day and function to propel large volumes of colonic content. Post-prandially the motor activity rises sharply for 30-60 minutes, this constitutes the gastrocolic response and is mediated by a cholinergic pathway, this is often absent in SSc, colonic motility is reduced and there is prolonged colonic transit (39;40).

Anorectal function is impaired in between 50 and 70% of patients with SSc (32). Colonic peristalsis delivers content episodically to the rectum where it is retained until a socially opportune time for defaecation. When faeces or air arrive at the rectum, the rectum is stretched; the IAS relaxes reflexively (rectoanal inhibitory reflex) and at the same time the EAS contracts voluntarily to maintain continence. The maintenance of faecal continence is dependent on complex voluntary and involuntary coordination between anal and colorectal activity. The IAS receives both an autonomic and enteric innervation that contributes to resting tonic anal canal pressure (41). Resting anal tone reflects primarily (80%) IAS function, with a minority contribution from the EAS (42). Disordered anorectal function occurs frequently in SSc and is a major factor in the development of faecal incontinence. The rectoanal inhibitory reflex (RAIR) has been reported to be diminished or absent in approximately 50% of patients studied, and this occurs even in the early stages of SSc. Heyt *et al* assessed 35 patients with SSc and found an absent RAIR in 25 (43). In a study by Jaffin *et al* RAIR was absent in 8 out of 10 patients (44). This abnormality is thought to be secondary to a neural defect in the myenteric plexus possibly related to vascular ischaemia and fibrosis. Diminished or absent RAIR has been found to correlate to symptoms, especially faecal incontinence (43). More extensive anorectal dysfunction occurs in the later stages of the disease when smooth muscle atrophy and fibrosis become more prominent. Correlation between oesophageal dysmotility and anorectal abnormalities have been reported (45) but not consistently (46).

1.5. Myopathy

Oesophagus

Smooth muscle atrophy and collagen deposition are the pathological hallmarks found in SSc (47). Gut smooth muscle becomes weak and there is progressive muscle fibrosis ultimately resulting in complete lack of smooth muscle function. This can occur throughout the GI tract. In the oesophagus muscle atrophy and fibrosis affect the smooth muscle portion (distal two thirds) resulting in abnormal and often absent peristalsis. Myoelectrical studies of oesophageal smooth muscle show absent myoelectrical activity (48) and more frequent involvement of the circular compared to the longitudinal muscle (10). In late disease the LOS pressure is virtually absent meaning dysphagia may actually not be as prominent despite the deterioration in body contractions but the reflux symptoms may deteriorate. There is also shortening of the oesophagus which may contribute to the formation of hiatus hernia and this can exacerbate gastro-oesophageal reflux. Very late in the disease the oesophageal skeletal muscle (proximal third) and the upper oesophageal sphincter may also become involved which in combination with gastro-oesophageal reflux predisposes SSc patients to aspiration.

Stomach and small intestine

The stomach and the small intestine are similarly affected by smooth muscle atrophy and fibrosis. The MMC is absent and there is dramatic hypomotility. There is also no contractile response to a meal with only very few and low amplitude contractions (37). The response of the muscle to hormones such as gastrin and

secretin is also attenuated and may become absent (22). Pedersen *et al* and Gregersen *et al* in 2 studies investigating the mechanical properties of duodenal wall in SSc patients showed that the duodenum was stiffer and muscle function impaired (49;50). Zuber-Jerger *et al* in an endoscopic ultrasound study showed that involvement of the upper GI tract in patients with SSc leads to a thickening of the mucosa, submucosa and muscularis, secondary to accumulation of extracellular matrix in the oesophagus, stomach and duodenum (51). Apart from the effect on motility, collagen deposition also contributes to malabsorption as it may interfere with intestinal wall permeability. The weakness of the intestinal smooth muscle predisposes to the formation of multiple small bowel diverticulae which are characteristic in SSc and are one of the causes of small bowel bacterial overgrowth.

Marie *et al* in their series of 8 SSc patients who underwent 24hr small bowel manometry initially and at 5 year follow-up demonstrated a high frequency (75%) of intestinal motor disturbance at initial evaluation and myogenic abnormalities at 5-yr follow-up in all SSc patients (52). Their findings support the theory that neurogenic abnormalities precede myogenic disturbances in small-bowel of SSc patients.

Colon and anorectum

Colonic motility is further diminished in the late stages of SSc both in the fasting and the postprandial phase. In the absence of significant small bowel involvement and associated diarrhoea, the resulting slow transit leads to worsening constipation with often associated abdominal pain and bloating.

The anorectum is more often affected. There is already neural dysfunction as demonstrated by a diminished or absent RAIR. Smooth muscle atrophy and fibrosis affect the IAS (and less commonly the EAS). The sphincters become thin and atrophy can be demonstrated on anal ultrasound (53). In addition atrophy can be identified manometrically demonstrated by low resting pressure (IAS) but also low maximal squeeze pressure (EAS). Chiou *et al* studied 17 patients, 9 with faecal incontinence and 8 asymptomatic and found reduced resting pressure compared to controls and absent RAIR in 12 of the patients (54). Herrick *et al* found low resting and squeeze pressure in SSc patients with constipation compared to controls (55) and in another study of 6 patients with constipation resting pressures were decreased in all patients (40). Another study of 8 patients with faecal incontinence, four of them with prolapse, showed low resting pressure in all patients and especially those with prolapse and also reduced squeeze pressure and reduced rectal capacity (56). The length of the fibrosed atrophic anal canal is shortened. All these factors contribute to the development of faecal incontinence. Interestingly manometric abnormalities have also been noted in asymptomatic SSc patients (43;44). Furthermore collagen deposition leads to reduced rectal capacity and compliance which in turn contributes to faecal urgency (56). Chronic constipation or diarrhoea, which can result from the abnormal colonic dysmotility described previously, as well as the observed neural dysfunction and reduced rectal compliance predispose patients with SSc to develop rectal prolapse. This can contribute further to reduced anal sphincter pressures and incontinence (56). Treatment of these symptoms should focus on reversing physiology (anti-diarrhoeals, laxatives and biofeedback) or be more generic such as lifestyle advice

and diet alteration. Increasingly modalities applied to patients with idiopathic pelvic floor dysfunction and faecal incontinence are being offered to patients with systemic sclerosis (SNS, rectal prolapse surgery) (57).

1.6. Summary

GI tract involvement in SSc can occur with both limited and diffuse disease, at any time from diagnosis and at any age. It can range from minimal to extensive and cause a variety of symptoms from minimal heartburn to chronic pseudo-obstruction and malnutrition. Although there are valid theories, as described above, on the pathogenesis of GI involvement, there are still many questions. If the initial event is immunological, is GI involvement associated with certain disease subtype or autoantibodies, same way as pulmonary arterial hypertension? Is a vascular event and resulting ischaemia the main causative factor leading to all subsequent abnormalities? Is there a true progression from the neuropathic to the myopathic stage or do they occur independently? Are there any predictive factors for severe GI involvement or rapid disease progression? Treatment of GI involvement is so far largely symptomatic with no pharmacological or other agents known to arrest disease progression. Understanding the pathogenesis further may facilitate better treatment options and possibly preventative measures.

1.7. Thesis intention

This chapter has reviewed the established data on aetiopathogenesis of gut involvement in SSc. The primary pathologies within the GI tract affect the mucosa and vasculature, the smooth muscle and the enteric nervous system (Figure 1). The aim of this thesis was to conduct experiments to assess these pathologies within a well-characterised SSc cohort.

The first requirement was to characterise the gut symptom burden of the patients using the best available instruments. To that end I undertook a study of 402 patients in the Royal Free cohort using the Scleroderma Gastrointestinal 1.0 questionnaire (Chapter 2).

To assess aspects of neuropathy in the gut is difficult owing to the complex interaction between enteric and extrinsic nervous systems. There is also the problem of access to gut nerves and the interpretation of the neurophysiology. I have conducted a study applying best available anorectal neurophysiological techniques in SSc patients, comparing symptomatic with asymptomatic individuals to establish pathophysiological implications (Chapter 3).

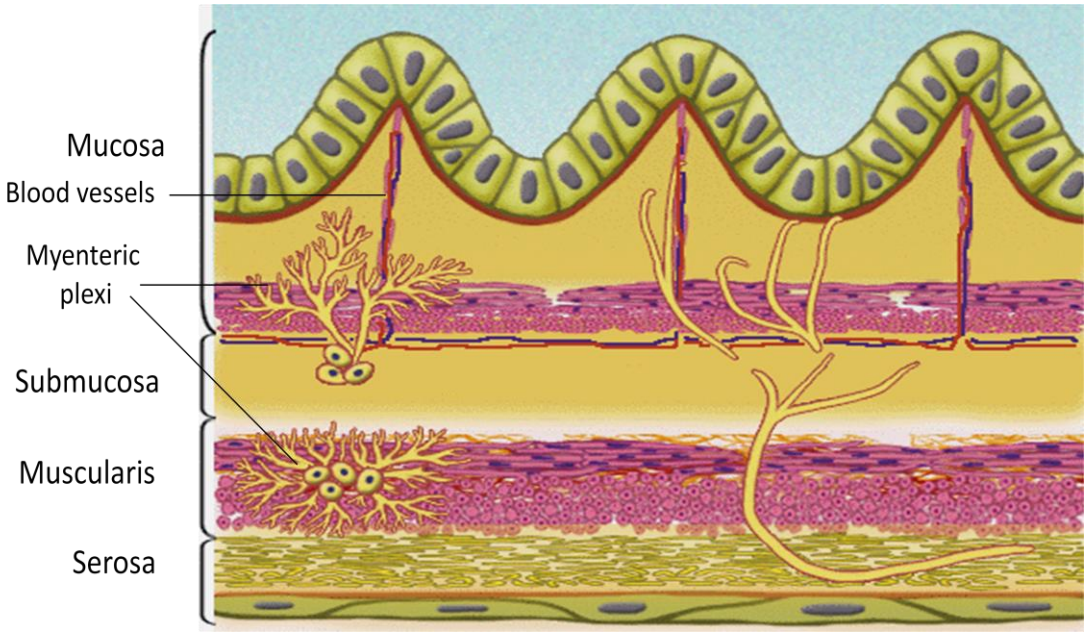
In these same patients I chose to assess the contribution of anal sphincter myopathy. The anal sphincter is an obvious choice of study since it has both an involuntary (autonomically innervated) smooth muscle component and a somatic striated one (Chapter 3).

Nutritional insufficiency is a significant co-morbidity in a sub-group of SSc patients. Whilst there may be contributory factors related to a generalised inflammatory

process, I hypothesised that gut infiltrative mucosal involvement would be a measurable contributor. So, in Chapter 4 I undertook definitive nutritional assessment by indirect calorimetry and bioelectrical impedance in symptomatic and non-symptomatic SSc individuals. Again the intention was to speculate on the pathophysiological contribution of these processes in SSc gut dysfunction.

Finally, Chapter 5 reports on an animal model of SSc, the transgenic mouse strain TβRIIΔk-fib with fibroblast specific TGF-β signalling activation. This mouse strain replicates skin, vascular and some pulmonary aspects of SSc. Gut involvement in this model has not been previously studied. It is known that these mice develop skin fibrosis and systemic vasculopathy as well as lung fibrosis, and by assessing the intestinal tract by both structural and physiological means I intended to demonstrate aetiopathogenic processes that may be relevant to human SSc.

Figure 1.1: Schematic representation of the intestinal tract layers depicting the structures affected in systemic sclerosis.



CHAPTER 2:

PREVALENCE OF GASTROINTESTINAL SYMPTOMS IN PATIENTS WITH SYSTEMIC SCLEROSIS.

2.1. Introduction

As I have considered in the previous chapter, the gastrointestinal tract (GIT) is affected in approximately 90% of patients (22;58) and gut involvement is the leading cause of morbidity and the third most common cause of mortality in patients with SSc (5). Any part of the GIT can be affected leading to a wide range of symptoms from gastro-oesophageal reflux to faecal incontinence (54;59-61). In clinical practice, skin, cardiorespiratory and renal involvement remain the focus of clinical assessment, despite the frequency of GI symptoms. In view of the sometimes embarassing nature of GI symptoms or the perception that they are not as important as other symptoms, GI involvement is frequently under-assessed. Compounding on that is the fact that treatment for GI symptoms is largely based on symptomatic control and there is no evidence to date that immunosuppression helps with GI disease progression. Unfortunately symptom control is often inadequate resulting in a significant effect on health-related quality of life (62). Health related quality of life (HRQOL) instruments are often used to assess disease activity and symptom burden in chronic rheumatological disorders such as rheumatoid arthritis with the RAQoL (63) and SSc with HAQ-DI (64;65) and also GIT related disorders such as inflammatory bowel disease with the IBDQ (66;67). HRQOL instruments can be generic illness instruments measuring general health status such as SF-36 and EuroQoL or disease specific providing a more detailed assessment of the specific illness and its impact. Recently Khanna *et al* in the USA developed a disease-specific HRQOL instrument for patients with SSc to assess their GIT-related activity and severity, which can be used in clinical trials and day-to-day

care (68). The SSC-GIT 1.0 is a 52-item questionnaire that has 7 scales: 5 aspects of GI dysfunction encountered in SSc patients (namely reflux, distension, diarrhoea, constipation and pain) and two generic aspects (emotional well-being and social functioning). The instrument has been shown to have satisfactory reliability (Cronbach's alpha 0.69-0.93 for the different scales, test-retest correlation coefficient ≥0.69) (68). This was the first questionnaire, specifically addressing GI symptoms in SSc. The patients self-rated their GI symptom severity as mild in 36%, moderate in 44% and severe in 20 %. The patients studied were not an unselected SSc patient group but patients with known GI involvement.

The aim of the study in this chapter was to validate this questionnaire in a UK population of patients with SSc and to assess GIT-related disease activity in unselected patients attending the scleroderma outpatient clinic at Royal Free Hospital, a tertiary referral centre.

2.2. Methods

The SSC-GIT 1.0 questionnaire was used with permission from Dr D. Khanna (Appendix 1). This questionnaire assesses the frequency of 5 categories of symptoms: reflux, distension, diarrhoea, constipation, abdominal pain and the effect of symptoms on social functioning and emotional well-being. The questionnaire assesses the number of days (divided in 4 scales: 0 days, 1-2 days, 3-4 days, 5-7 days) during the previous week that patients had specific symptoms, thus assessing frequency rather than severity of symptoms. In view of the wide range of

symptoms and the nature of the disease this is more relevant and preferable in assessing disease burden. Patients were advised to answer the questions and report on their symptoms whilst on their current medications, including those for GI symptoms such as acid suppressants, prokinetics, loperamide or laxatives. This allowed me to assess both the frequency of GI symptoms but also the symptom control achieved by standard treatment methods.

The Royal Free Hospital Scleroderma Unit is a tertiary referral centre. Approximately 1200 SSc patients are under regular follow-up, the majority of patients usually seen every 6-12 months. A third of this patient cohort was estimated to be a representative sample and therefore I aimed to collect 400 completed questionnaires. Patients were approached whilst attending for their outpatient appointment from December 2007 to June 2008. All patients attending the outpatient clinic were approached and encouraged to answer the questionnaire irrespective of whether they had a history of GI symptoms. Patients that did not speak English were excluded. Questionnaires were checked directly and if not filled in completely patients were encouraged to do so. Despite these efforts, some questionnaires were incomplete. Any questionnaires with less than 50% of questions answered, for any of the categories, were excluded. The questionnaires were scored as indicated in the original paper (68). Each item was rescaled to a range of 0 to 100, where 100 indicated better health. Scoring for the 4-item questions (comprising all except two questions) was as follows: 1=100, 2=66.6, 3=33.3, 4=0. The two 2-item questions were scored as follows: 1=0, 2=100. For each category the average score was calculated. For each patient with a completed

questionnaire, the patients' notes and/or the Royal Free Hospital Scleroderma patient database were reviewed to establish disease subtype, disease duration, presence of autoantibodies and evidence of pulmonary, cardiac or renal involvement. Pulmonary fibrosis was defined as predicted forced vital capacity (FVC) or carbon monoxide diffusing capacity (DL_{CO}) of <55% or a 15% decline from baseline in FVC or DL_{CO}, with fibrosis confirmed on high-resolution CT. Pulmonary arterial hypertension had to be confirmed by right heart catheterization with mean pulmonary arterial pressure of >25 mmHg and normal pulmonary capillary wedge pressure. Scleroderma renal crisis was defined as new-onset systemic hypertension >150/85 mmHg and a documented decrease in estimated glomerular filtration rate ≥30%. Cardiac involvement was defined as haemodynamically significant cardiac arrhythmias, pericardial effusion or congestive heart failure requiring specific drug treatment. Any previous clinical record of gastrointestinal involvement, as patient symptoms or positive tests, was recorded. Drug history was also recorded, with special reference to immunosuppression or use of drugs for GI symptoms.

Khanna *et al* in their study found that the SF-36 was not as good at discriminating GI symptom severity as the SSC-GIT 1.0. In order to assess if there is any correlation between the 2 questionnaires a small proportion of patients (approximately 10%) both with and without significant GI symptoms also filled in the SF-36 questionnaire (Appendix 2). Correlations between the total SF-36 score and total SSC-GIT 1.0 score, SF-36 physical health score and total of the 5 symptom categories of the SSC-GIT score and SF-36 mental health score and the category for emotional well being of the SSC-GIT 1.0 score were calculated.

Summary statistics were computed for each GIT scale. Data were non-normally distributed so it is presented in medians and interquartile ranges, however to enable comparison with previous work in this area, means and standard deviations are also provided. The proportion of patients scoring the maximum possible value for each scale (ceiling effect) and the lowest possible value for each scale (floor effect) was computed. Matrices of pairwise correlation coefficients were computed to assess the associations between GI symptoms and to examine the associations between GI symptoms and: 1) presence of other internal organ involvement, 2) presence of autoantibodies, 3) previously documented gastrointestinal disease and reported symptoms. No adjustment was made for multiple testing since the analysis was exploratory. I investigated associations in all patients and then in patient subgroups although the power was limited for the subgroup analysis. All statistical analyses were conducted using Stata 10 Intercooled (StataCorp LP, Texas, USA) statistical software.

2.3. Results

430 questionnaires were collected and analysed at a later date. Twenty-eight (6.5%) questionnaires had less than 50% of questions in one or more of the categories answered and were therefore excluded from the analysis. 402 completed questionnaires were the total included in the analysis. All the patients' notes and/or Scleroderma database were reviewed and demographics, disease characteristics and other internal organ involvement were recorded. The patients' demographics and disease characteristics are shown in table 2.1. Thirty nine per cent of patients

were on immunosuppressants (mycophenolate mofetil 16%, methotrexate 12%, azathioprine 8%) and 72% were on medications for GI symptoms.

The majority of patients were female (88.8%) with a mean age of 55 years and median disease duration of 10 years (range 1-52 years). 69% of the patients that completed the questionnaire had limited cutaneous (lcSSc) SSc and 30% diffuse cutaneous (dcSSc) SSc. There were 2 patients with autoimmune Raynaud's and 1 patient with scleroderma sine scleroderma. Patients with overlap syndromes were categorised according to the SSc disease subtype. The disease subtype and autoantibody profile reflected the overall Royal Free patient cohort (69).

The SSC-GIT 1.0 questionnaires were scored as described above. The findings are summarised in table 2.2. Overall there was a high prevalence of GI symptoms. Eighty-five percent of patients reported reflux symptoms and 87% reported distension at least 1-2 days per week, whereas 50% and 47% of patients reported diarrhoea and constipation, respectively, 1-2 days per week. Figure 2.1 depicts the percentage of patients reporting symptoms for each frequency category. Ninety-seven percent of patients reported symptoms at least once a week, and 15% reported daily symptoms; only 3% of patients reported no symptoms. 72% of patients were taking medication for GI symptoms, predominantly acid suppressants (71%), prokinetics (13%) and laxatives (4%). Despite this, 94% reported upper and 79% lower GI symptoms. The use of medication did not prevent the occurrence of symptoms. In fact, the patients on acid suppressants or prokinetics (AS/P) reported more frequent reflux and distension symptoms than the patients that were not on acid suppressants or prokinetics (reflux score: AS/P: 70.36 ± 1.47 vs no AS/P: 83.49

± 1.67; distension score: AS/P: 66.39 ± 1.44 vs no AS/P: 76.42 ± 1.93; p<0.001). 157 patients (39%) were on immunosuppressants: mycophenolate mofetil (16%), methotrexate (12%), azathioprine (8%), cyclosporine (2%), other (1%); there was no difference in GI symptoms with immunosuppressant use.

Table 2.1: Patient demographics and disease characteristics

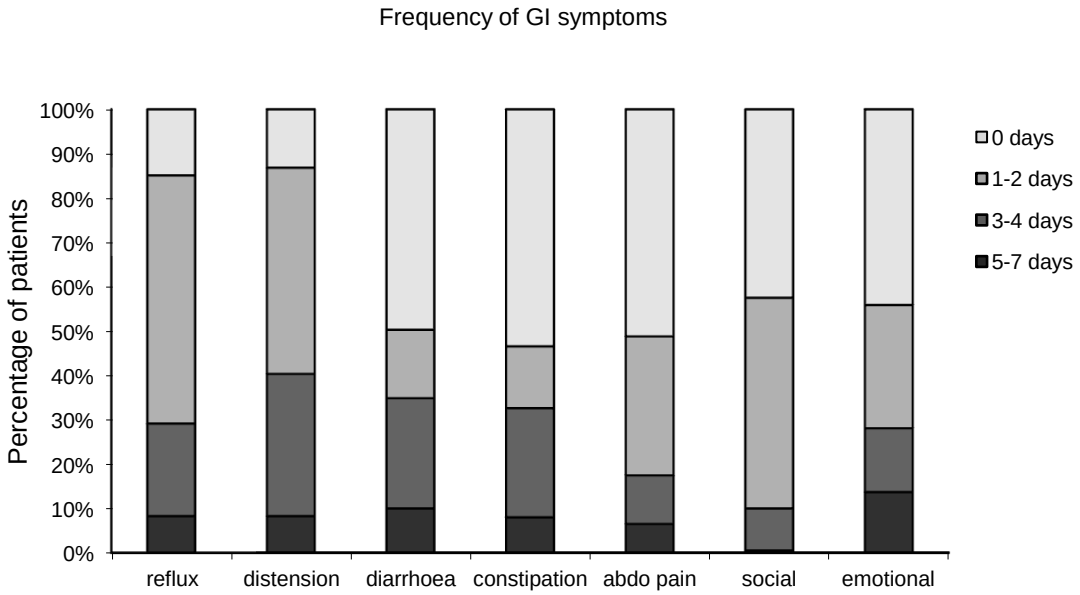
Age (mean + sd)	55.4 + 12.1
Sex - Female/Male (%)	357/45 (88.8/11.2)
Disease duration - median (range)	10 years (1-52 years)
Disease subtype – lcSSc/dcSSc (%)	277/122 (68.9/30.3)
Internal organ involvement	*n (%)*
Cardiac	25 (6%)
Pulmonary	168 (42%)
Renal	24 (6%)
Gastrointestinal	345 (86%)
Autoantibodies	*n (%)*
ANA	382 (95%)
ACA	128 (31.8%)
SCL-70	88 (21.9%)
U3-RNP	13 (3.2%)
RNAP	32 (8%)
U1-RNP	21 (5.2%)
other	45 (11.2%)

Abbreviations: lcSSc - limited cutaneous systemic sclerosis, dcSSc – diffuse cutaneous systemic sclerosis, ACA – anti-centromere antibody, Scl-70 – anti-topoisomerase antibody, RNAP – anti-RNA polymerase

Table 2.2: Descriptive statistics of SSC-GIT 1.0 questionnaire scores

SSC-GIT 1.0 scales	Sample size	No. of Items	Median score (IQ range)	Mean score ±SD	Min score	Max score	Floor effect %	Ceiling effect %
Reflux	402	9	81.5 (59.3-92.6)	73.99 ± 23.92	7.4	100	0	14.9
Distension	402	6	72.2 (50-88.9)	69.16 ± 23.84	5.6	100	0	13.2
Diarrhoea	402	3	88.9 (44.4-100)	75.17 ± 30.39	0	100	1.5	49.8
Constipation	401	3	100 (55.5-100)	76.63 ± 29.95	0	100	2.7	53.2
Abdominal Pain	401	2	100 (66.7-100)	80.59 ± 26.68	0	100	3.5	51
Social functioning	401	20	96.7 (85-100)	89.73 ± 15.69	12	100	0	42.3
Emotional well-being	401	9	88.9 (57.4-100)	76.19 ± 30.64	0	100	1	43.8

Figure 2.1: Percentage of patients for each symptom and frequency category.



The mean scores +/- standard deviation (possible score 0-100) ranged from 69.16 ± 23.84 for distension to 89.73 ± 15.69 for social functioning (Table 2.2). I found that distension and reflux were the categories with the lowest scores, meaning that patients complain of these symptoms most frequently. All 7 scales showed a ceiling effect (percentage of patients that reported no symptoms for that scale thus scoring 100 [highest score] for the scale). This ranged from 13.2% and 14.9% for distension and reflux scales respectively to 53.2% for the constipation scale. The lower ceiling effect on the reflux and distension scales reflects the higher prevalence of these symptoms but also the fact that there were more questions in these domains than in the diarrhoea and constipation domains. Only 3 of the 7 scales showed a floor effect (percentage of patients having the lowest score [0] for a category) which was small, ranging from 1.5% for diarrhoea to 3.5% for abdominal pain.

There was a high correlation between most symptom categories (table 2.3). The highest was between reflux and distension (r=0.89). There was no significant correlation between diarrhoea and constipation. The effect on emotional well being was significant, with an average score of 76.2, and was associated with low scores in all other symptom categories. The strongest correlation was with reflux (r=0.47) with distension being second (r=0.46) and diarrhoea third (r=0.41) whereas the weakest one was constipation (r=0.18). There was also significant correlation between all symptom categories and the effect on social life (r=0.23 to 0.63). The correlation between reflux and effect on social life was the greatest (r=0.63) with emotional well being being second (r=0.58).

Forty-four patients, 24 with and 20 without significant GI symptoms completed the SF-36 questionnaire. There was a positive correlation between the total SF-36 score and the total SSC-GIT 1.0 score (r=0.41). There was also a positive correlation between the physical health SF-36 score and the total of the 5 symptom categories score (reflux, distension, diarrhoea, constipation and abdominal pain) (r=0.48). There was significant correlation between the social function scores of SF-36 and SSC-GIT 1.0 (r=0.42) and between the SF-36 mental health score and the score of emotional well-being category of the SSC-GIT 1.0 (r=0.29; p=0.55) but the latter did not reach statistical significance (figure 2.2).

Table 2.3: Correlation matrix of all SSC-GIT 1.0 scores

(r=Pearson correlation coefficient):

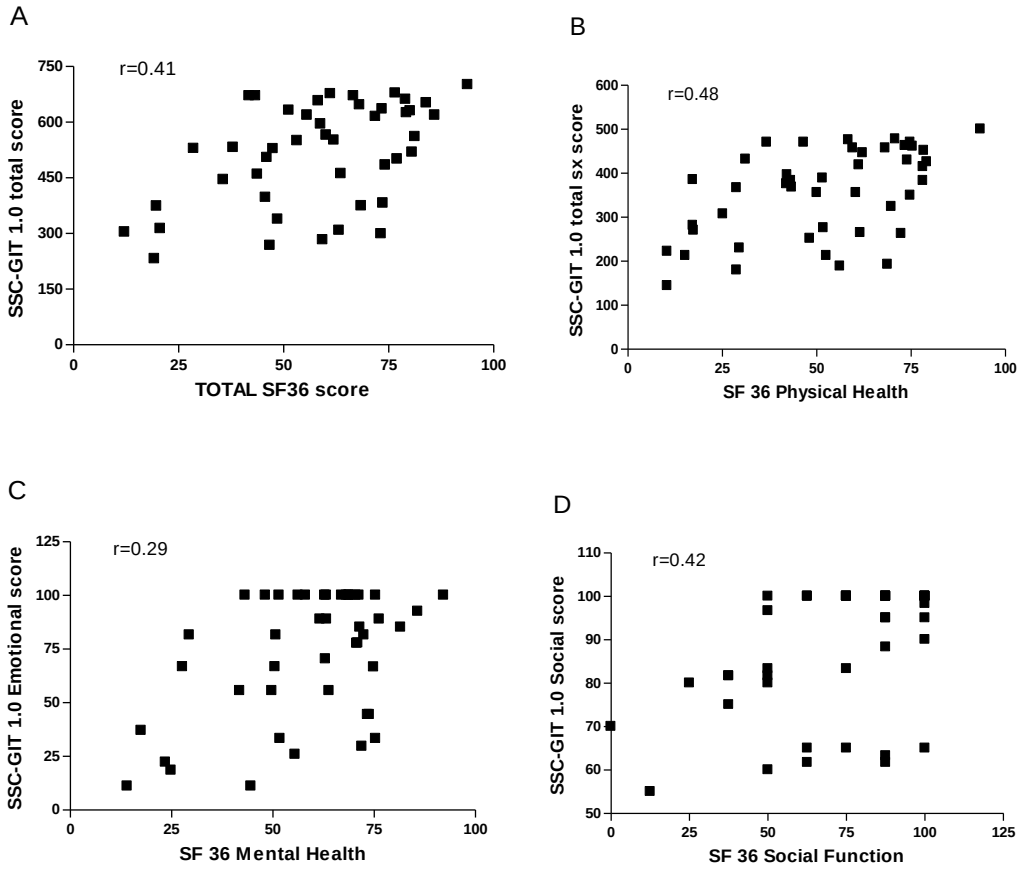
	Reflux	Distension	Diarrhoea	Const^{n}	Abdo pain	Emotional	Social
Reflux	r=1.0000						
Distension	r=0.8913 p<0.0001	r=1.0000					
Diarrhoea	r=0.3539 p<0.0001	r=0.3675 p<0.0001	r=1.0000				
Const^{n}	r=0.3529 p<0.0001	r=0.3340 p<0.0001	r=-0.470 p=0.3479	r=1.0000			
Abdo pain	r=0.5018 p<0.0001	r=0.4857 p<0.0001	r=0.2729 p<0.0001	r=0.4261 p<0.0001	r=1.0000		
Emotional	r=0.4716 p<0.0001	r=0.4612 p<0.0001	r=0.4118 p<0.0001	r=0.1767 p=0.0004	r=0.4007 p<0.0001	r=1.0000	
Social	r=0.6312 p<0.0001	r=0.4329 p<0.0001	r=0.4781 p<0.0001	r=0.2341 p<0.0001	r=0.4789 p<0.0001	r=0.5772 p<0.0001	r=1.0000

Table 2.4: Correlation table of SSC-GIT 1.0 scores and internal organ involvement

(r= Pearson correlation coefficient).

	Pulmonary fibrosis	Pulmonary hypertension	Cardiac involvement
Reflux (all SSC-GIT scores)	r=0.0132 p=0.7919	r=-0.0501 p=0.3161	r=-0.0325 p=0.5163
Diarrhoea (all SSC-GIT scores)	r=0.1347 **p=0.0068**	r=-0.0522 p=0.2965	r=0.0635 p=0.2036
Constipation (all SSC-GIT scores)	r=-0.0960 p=0.0547	r=0.0181 p=0.7184	r=0.0175 p=0.7274
Severe diarrhoea (SSC-GIT score <66.6)	r=0.0766 p=0.2184	r=-0.0086 p=0.8907	r=0.0461 p=0.4591
Severe reflux (SSC-GIT score <66.6)	r=0.1026 p=0.2212	r=-0.0529 p=0.5291	r=-0.0601 p=0.3365
Severe constipation (SSC-GIT score <66.6)	r=0.0277 p=0.6509	r=-0.0714 p=0.2434	r=0.0073 p=0.9052

Figure 2.2: Correlation between SF 36 scores and SSC-GIT 1.0 scores in 44 patients with and without GI symptoms. A) SF-36 total score and SSC-GIT 1.0 total score, B) SF-36 Physical health score and SSC-GIT 1.0 total symptom score, C) SF-36 mental health score and SSC-GIT 1.0 emotional score, D) SF-36 social functioning score and SSC-GIT 1.0 social score.



There was little evidence of any difference in symptom scores in any of the categories between dcSSc and lcSSc. With regards to autoantibody profile, U3RNP antibodies were associated with more frequent diarrhoea symptoms (p=0.015). There was a trend towards less frequent reflux symptoms in PM-SCL positive patients, but this was not statistically significant. There was no significant association with U1RNP and GI symptoms or any other significant association between autoantibody profile and GI symptoms.

I looked for an association between GI symptoms and the presence of other internal organ involvement (table 2.4). We found an association between diarrhoea scores and pulmonary fibrosis (r=0.135, p=0.0068), in that patients with pulmonary fibrosis were less likely to have diarrhoea. In contrast there was a negative correlation between constipation scores and pulmonary fibrosis although this did not reach statistical significance (r=0.096, p=0.0547). Specifically we did not find significant association between cardiac disease and GI symptoms, although case reports have suggested that diarrhoea and malabsorption can exacerbate cardiac complications, most likely secondary to electrolyte imbalances. Investigating whether more frequent GI symptoms were associated with other internal organ involvement, the groups were split in two groups according to scores, those with score less than 66.6 and those with scores more or equal to 66.6. The former group identifying patients with more frequent symptoms, equivalent according to the questionnaire to symptoms equal to or more than 3-4 days/week as an average for the category, suggestive of more severe GI involvement. No associations were found between either of the two severity groups and evidence of other internal

organ involvement. Interestingly although it has been previously suggested that severe gastro-oesophageal reflux may contribute to pulmonary fibrosis (70;71), such an association was not evident from our data (r=0.1). Patients with pulmonary fibrosis were more frequently taking acid suppressants than patients without pulmonary fibrosis (78% vs 66%; p=0.01) but no association was found between frequency of reflux symptoms and documented pulmonary fibrosis or pulmonary hypertension.

In order to assess whether the questionnaire scores reflected previously documented gastrointestinal disease and reported symptoms, patients' notes were reviewed and any gastrointestinal diagnosis or report of symptoms noted, for example gastro-oesophageal reflux, distension, endoscopic findings such as GAVE, small intestinal bacterial overgrowth, diarrhoea, constipation, anorectal symptoms (prolapse or faecal incontinence). Patients with documented gut involvement had overall significantly higher respective questionnaire scores for all the questionnaire categories (reflux, distension, diarrhoea, constipation) than those patients without previously documented gut involvement. There was no available documentation on the severity of GI involvement and therefore we could not correlate this to questionnaire scores. Unfortunately GI investigations results were often not available as tests were frequently done at patients' local hospitals. It was not possible therefore to correlate symptoms with objective measures of GI involvement.

2.4. Discussion

The development of SSC-GIT 1.0 by Khanna *et al* provided the first HRQOL instrument to capture GIT involvement in patients with SSc (68). HRQOL questionnaires are widely used both in clinical practise and as research tools both in rheumatological and gastrointestinal diseases. For example the inflammatory bowel disease questionnaire (IBDQ) is a 32-item questionnaire that assesses bowel function, general systemic health and social and emotional impact. It has been validated cross-culturally and is a regularly used tool in clinical studies. It has been shown to be responsive to changes in bowel function and detect differences in severity of disease activity (72-74). The SSC-GIT 1.0 assesses a variety of GI symptoms and the effect on social and emotional functioning in a similar way. In contrast though to inflammatory bowel disease for which there are objective markers, both laboratory and endoscopic, indicative of disease activity, gut involvement in SSc is often difficult to assess.

In developing this questionnaire Khanna *et al* assessed 88 SSc patients with GI involvement. In his patient cohort the questionnaires answered by the patients indicated a variety of GI problems. I have confirmed a similar burden of disease in a UK patient cohort to that reported in the USA population that the questionnaire was developed in. The high frequency of GI involvement in SSc is well documented and it has a major impact on the quality of life. A number of studies have demonstrated GI involvement of both the upper and lower GI tract based on endoscopic findings or physiological studies as well as reported symptoms (56;61;75). This questionnaire is designed to provide information not only about

symptoms but also about the psychological impact and the effect on the patients' social life. The questionnaire analysis showed that patients often reported both upper and lower GI symptoms although upper GI symptoms such as reflux symptoms and dysphagia occur more frequently. There is a significant effect of gut symptoms to patients' emotional and social well-being.

The SSC-GIT 1.0 has seven scales: reflux, distension, diarrhoea, constipation, pain, emotional well-being, and social functioning. Khanna *et al* had joined the reflux and indigestion scales in one bigger category as they found a strong correlation between the 2 categories (68). The 2 categories were kept separate in the current analysis in order to distinguish between reflux symptoms and distension as this is indicative of gastroparesis and small bowel involvement. There was though a high correlation between those 2 categories as reported previously. All seven scales showed some ceiling effect, ranging from 13-15% in reflux and indigestion to 50-53% in diarrhoea, constipation and abdominal pain. The higher ceiling effect in the latter three symptoms may be explained by the lower prevalence of lower GI symptoms. Furthermore, less items in the questionnaire were dedicated to those symptoms (three for diarrhoea, three for constipation and two for pain). Khanna *et al* found much lower ceiling effect in the reflux/indigestion scale than was found in our patient cohort. This is most likely secondary to the much large number of patients but more importantly secondary to the fact that asymptomatic patients were included in the 400 patients studied. Overall the mean questionnaire scores documented were comparable although a bit higher than those published by Khanna *et al* (68). Higher scores reflect lower symptom burden and this may well be

again a reflection of the larger sample size, including patients with no GI symptoms. Additionally a large percentage of patients were on regular medication for their GI symptoms.

General GI involvement has not been associated previously with a specific disease subtype (76), unlike other internal organ pathology such as pulmonary hypertension or renal crisis that are associated with lcSSc and dcSSc respectively. Nishimagi *et al* investigated 302 patients with SSc and found that in patients with early severe GI involvement, defined as malabsorption, pseudo-obstruction or need for hyper-alimentation, the ratio of dcSSc to lcSSc was higher than in patients without severe GI involvement (77). They also found that these patients were less likely to suffer from interstitial lung disease. In that study the presence of anti-centromere (ACA) or SCL-70 antibodies was less frequent in patients with severe GI involvement and there was a higher frequency of anti-U3RNP, anti-U1RNP and other anti-nucleolar antibodies (77). However, Steen investigating features associated with specific autoantibodies found that subjective gastrointestinal involvement occurred in more than 80% of patients without association with specific disease subtype or autoantibodies, but severe GI involvement was significantly greater in patients with anti-U3RNP and also Th/To and anti-U1RNP (5). In my study no association was found between general GI involvement and disease subtype or specific autoantibodies. This was though a study based on subjective symptoms and not proven GI involvement. The symptom category of this questionnaire most likely to indicate severe GI involvement is diarrhoea as it is often secondary to small intestinal involvement and bacterial overgrowth. A higher

incidence of more severe diarrhoea symptoms was found in patients with anti-U3RNP antibodies, but not so with anti-U1RNP. The frequency of other autoantibodies such as anti-KU, Th/To was too small for any meaningful associations.

Regarding association between the gastrointestinal tract and other organ involvement, previous studies have shown that there may be a causative link between gastro-oesophageal reflux and pulmonary fibrosis (70). Anecdotal evidence suggests a link between cardiac involvement and GIT involvement, possibly secondary to electrolyte disturbances often associated with diarrhoea and malabsorption. In this study there was an inverse relationship between symptoms of diarrhoea and pulmonary fibrosis. It may be that treatment for pulmonary fibrosis has some protective effects against intestinal involvement although review of patients' medications did not reveal any association between specific medications or immunosuppression and GI symptoms. Patients with pulmonary fibrosis were, as expected, more frequently on immunosuppressants but interestingly there was no difference in the frequency of diarrhoeal symptoms with immunosuppressant use. There was no evidence of more severe reflux symptoms in patients with pulmonary fibrosis. An explanation for this may be that reflux symptoms often do not represent severity of oesophageal disease, for example patients with Barrett's oesophagus often have less severe symptoms (78). Another explanation is that patients with pulmonary fibrosis have had their reflux symptoms treated more aggressively as suggested by the fact that these patients were more frequently on acid suppressants. This could indicate more severe or more frequent

symptoms but it could also indicate that physicians are more likely to prescribe / encourage use of PPI in patients with documented pulmonary fibrosis. It would be worth assessing in future studies oesophageal involvement in specific patient groups with pulmonary fibrosis, for example patients with dcSSc who are SCL-70 negative and in whom epithelial injury is more strongly implicated or patients with lcSSc, ACA positive. Furthermore it is worth exploring specific autoantibody and disease subtype association with site-specific gut involvement, rather than overall gut involvement.

One of the major limitations of using a subjective symptom questionnaire is that this may not accurately depict actual disease activity and severity. Previous studies have shown that asymptomatic patients did have abnormal oesophageal motility when investigated (79), so disease activity may actually have been underestimated using subjective measures. Furthermore patients were often on drugs such as proton pump inhibitors, prokinetics and laxatives as treatment for their gastrointestinal symptoms. On the other hand, GI symptoms may occur irrespective to actual SSc gut involvement, as for example bloating is often a symptom of irritable bowel syndrome, a condition occurring in up to 20% of the Western population, especially women (80). Additionally some of the drugs used commonly in SSc patients can have significant gastrointestinal side effects such as mycophenolate, bisphosphonates, opiates, antibiotics.

One of the main drawbacks of this questionnaire is that it is using symptom frequency only and not severity to assess symptom burden, unlike other quality of life questionnaires. In view of the multi-organ gastrointestinal involvement and the

variety of symptoms, the frequency of these symptoms is likely to be indicative of symptom burden. Furthermore, Khanna *et al* showed that the SSC-GIT 1.0 scales were able to discriminate the self-rated severity of GI symptoms and found a significant association between this questionnaire and other quality of life questionnaires (68) such as the SF-36 and health assessment questionnaire (SHAQ) (81). This association was confirmed in my study as there was positive correlation between the SSC-GIT 1.0 scores and related SF-36 dimensions. In some instances, for example SF-36 mental health score and the emotional effect category of the SSC-GIT 1.0 questionnaire, the correlation was lower than it would be expected. This could be explained by the fact that the SF-36 assesses general health and well being whereas the SSC-GIT 1.0 assesses specifically GI symptoms. In SSc, a disease that affects multiple organs, general health and well-being is likely to be affected by a variety of factors and therefore it is not surprising that the correlation between the 2 questionnaires is not greater than was shown. The fact that there is significant correlation between the SF-36 and SSc-GIT 1.0 supports further the importance and significant effect of GI involvement in SSc.

Another limitation of this questionnaire is that there are symptoms that are not explored adequately, in general lower GI symptoms compared to upper GI symptoms are less assessed, specifically for example constipation and faecal incontinence (43;44). The importance of bowel function in quality of life is demonstrated in neurological disorders such as spinal cord injury and multiple sclerosis. The neurogenic bowel dysfunction score was devised to assess colorectal and anal dysfunction in spinal cord injury patients and has been used in other

neurological disorders (82-84). Nonetheless, with a disease that can affect the whole of the GIT, there must be a balance between assessing adequately all different symptoms and avoiding a questionnaire that is too long, too time-consuming and therefore less practical for use on a regular basis in order to monitor disease progression. In fact the 52-item SSC-GIT 1.0 questionnaire was felt to be too long and a shorter version of this questionnaire has now been developed (85). Ideally the use of objective measures or tests to assess GI involvement should be used but these tests are often invasive and although more accurate in diagnosing GI involvement in SSc they are probably less useful in assessing disease progression. Whilst treatment for GI involvement remains symptomatic a dedicated GI questionnaire gives an accurate assessment of symptom burden and can be used to assess this at different times and be used as a guide to adjust treatment.

In summary, patients with SSc have an extremely high burden of both upper and lower gut symptoms. There is no strong association of gut symptoms with other organ involvement or disease profile. The degree of symptoms demonstrated is more than would be expected from the symptoms documented in the clinical notes suggesting that patients usually under-report these symptoms. This is regrettable since many of the symptoms may be amenable to treatment and therefore routine assessment of GI symptoms is recommended.

Having established gut disease burden in patients with SSc and in order to investigate the pathophysiology of gut disease in SSc I examined the anorectum, an area of the gut easily accessible and which allows assessment of the main pathologies recognised in systemic sclerosis.

CHAPTER 3:

ANORECTAL PHYSIOLOGY AND ENDOANAL ULTRASOUND IMAGING IN SYSTEMIC SCLEROSIS PATIENTS

Publications generated from this chapter can be found in Appendix 9

3.1. Introduction

The exact pathophysiology of GIT involvement is not known but it is related to both neurogenic and myogenic abnormalities (37;58). Physiologically the typical resulting abnormality is dysmotility such as low amplitude contraction in the distal oesophagus and decreased or absent migrating motor complexes in the intestine (37;60;86). Anatomical changes such as intestinal dilatation usually occur as the disease progresses and are thought to relate to severe dysmotility. Histologically the main changes seen in the GIT are patchy atrophy of the muscularis propria and varying degrees of fibrosis as the disease progresses (47). The smooth muscle atrophy seen is thought to be secondary to vascular ischaemia and neural plexus dysfunction (10;13). Reduced blood flow has been demonstrated in the stomach and duodenum (11;12).

In health faecal continence is maintained by the co-ordinated function of the pelvic floor, the rectum and the anal sphincters. The smooth muscle internal anal sphincter (IAS) is primarily responsible for the anal resting tone, contributing about 85% of the anal resting pressure. The striated external anal sphincter (EAS) contributes a small part towards the resting anal pressure but is primarily responsible for the voluntary contraction of the anal sphincter (42). Disruption or weakness of the IAS typically leads to passive faecal incontinence, whereas that of the EAS leads to urge faecal incontinence (87;88). The IAS being a smooth muscle is more likely to be affected in SSc (53) and it has been suggested that the changes are similar to those seen in the lower oesophageal sphincter.

A number of observational studies have investigated anorectal problems in SSc. The anorectum is affected in 50-70% of patients with SSc with more than 20% of patients developing faecal incontinence (32;89). The underlying mechanisms have not been systematically addressed though, and are still unclear, although there is evidence of smooth muscle atrophy and fibrosis but also of neural dysfunction. Most studies have shown a reduced resting anal pressure but a normal maximal squeeze pressure and an absent or impaired rectoanal inhibitory reflex (RAIR) (40;44;54;56). Apart from a strong correlation between an absent RAIR and faecal incontinence (43), the other physiological measurements have not been shown to correlate with symptoms. Imaging of the anal sphincters suggests that the IAS is thinned and hyperechoic on ultrasound in the majority of patients with SSc and faecal incontinence (43;90;91), although some patients may have a thickened hypoechoic sphincter (90). Even less is known about patients with SSc and no faecal incontinence. If the pattern is similar to that seen in oesophageal involvement, we would expect to see structural and functional changes even in the absence of symptoms (79).

The aim of this study was to undertake a comprehensive analysis of anorectal function, including neurophysiological and sonographic assessment, of patients with SSc with and without faecal incontinence in order to understand further the pathophysiology of anorectal dysfunction in these patients.

3.2. Methods

3.2.1. Patients

The study environment was a tertiary referral gastrointestinal physiology unit in collaboration with a tertiary referral scleroderma unit. Forty four patients with systemic sclerosis, 24 with anorectal symptoms and 20 patients without anorectal symptoms were recruited to the study. Patients were recruited whilst attending the Rheumatology outpatients department at Royal Free Hospital. Consecutive patients were approached to take part in the study. 30 patients with history of faecal incontinence were approached and 24 agreed to take part, whereas 42 patients with no known history or complaints of anorectal symptoms were approached and 20 consented to take part in the study. Patients were recruited over a period of 12 months. Twenty age and sex matched patients referred to our tertiary GI physiology unit, over approximately the same period of time, for investigations of faecal incontinence were used as incontinent controls. Patients with diabetes mellitus and patients with neurological disorders were excluded. All patients gave informed consent to participate in the study, which was approved by the National Hospital for Neurology and Neurosurgery & Institute of Neurology Joint research ethics committee.

3.2.2. Symptom assessment

Anorectal symptoms were assessed by history taking and review of notes. Faecal incontinence was assessed using the Wexner incontinence score (a validated 5-item

self-completed instrument with a score of 0 representing no incontinence and a score of 20 representing total incontinence) (92) (Appendix 5). Constipation was assessed using the Wexner constipation score (an 8-item self-completed instrument with a score of 0 representing no symptoms and maximum score of 32 representing severe constipation) (93) (Appendix 4).

Systemic sclerosis patients were asked to complete the following questionnaires on the day of their assessments:

1. Scleroderma GI tract 1.0 questionnaire: A 52 item self-completed instrument that provides a profile for GI symptoms in 5 broad categories and their effect on social and emotional well-being (68) (Appendix 1).

2. SF-36 general health questionnaire: a multi-purpose, short-form health survey with 36 questions that yields an 8-scale profile of functional health and well-being scores (94) (Appendix 2).

3. EuroQol: a 6-item self-completed instrument that provides a simple descriptive profile for health status by asking if the patient has no problems, some problems or significant problems in domains of: mobility, self care, usual activities, pain/discomfort, anxiety/depression (scoring 1,2 or 3 respectively) (95) (Appendix 3).

SSc patients' notes were reviewed for data on SSc subtype, disease duration and other internal organ involvement. The cause of faecal incontinence in incontinent controls was established on review of their notes and investigations.

3.2.3. Anorectal manometry

No bowel preparation was given prior to testing. An 8 channel radial water-perfused manometry system with a perfusion rate of 0.6 ml/minute (MMS, Enschede, Netherlands) was utilised. A latex-free balloon with 500mls capacity was attached at the end of the manometry catheters (*External Diameter 3.9mm*) (Ardmore Healthcare Limited, Amersham, UK). With the subject in the left lateral position, the station pull-through technique (catheter inserted to 5 cm and pulled back in 0.5 cm steps) was employed to assess anal canal length, anal resting pressure and anal squeeze pressure. Resting tone was determined as the highest average (of the 8-channel measurements) pressure at rest. The voluntary contraction squeeze pressure was determined as the highest pressure attained on 3 consecutive tries. The normal ranges quoted are the ones that are used at the GI physiology Unit at University College Hospital.

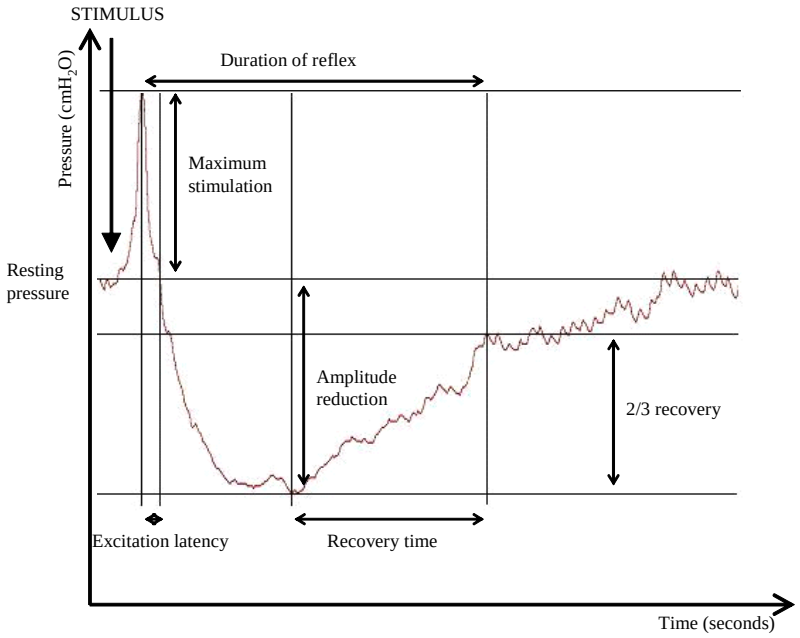
3.2.4. Rectoanal inhibitory reflex (RAIR)

Rapid distension of the rectum elicits an intrinsic reflex that produces relaxation of the IAS. The RAIR was elicited by rapid inflation and deflation of the latex balloon at the end of the anal manometry catheter using 50ml of air. The parameters of the RAIR analysed were as shown in figure 3.1. The RAIR was accepted to be present if the amplitude reduction was at least 25% of resting anal pressure. The process was repeated 3 times and the greatest response was used for the measurements. The RAIR was considered absent if the amplitude reduction was less than 25% of the

resting pressure in all 3 attempts. The point at which anal pressure returned to ⅔ of its original resting value was deemed to be the end of the reflex (96). The excitation latency was measured as being the time taken from maximal stimulation to when the anal pressure returned to its resting level. The beginning of the recovery time was when the amplitude reached its nadir. The total duration of the reflex was measured from the point of maximal stimulation to the point at which the amplitude returned to ⅔ of the original resting pressure (96).

Figure 3.1: RAIR parameters analysed.

The point of maximal stimulation was the start-point of the RAIR. The end-point was when the anal pressure had recovered to ⅔ of the original resting pressure; this was the reflex duration. Amplitude reduction was measured from resting pressure to lowest point of the RAIR. Percentage amplitude reduction was calculated with resting pressure being set at 100%. Time taken for the pressure to return to resting from maximal stimulation was termed the excitation latency. Recovery time began as soon as the RAIR reached maximal relaxation.



3.2.5. Anorectal sensory measurements

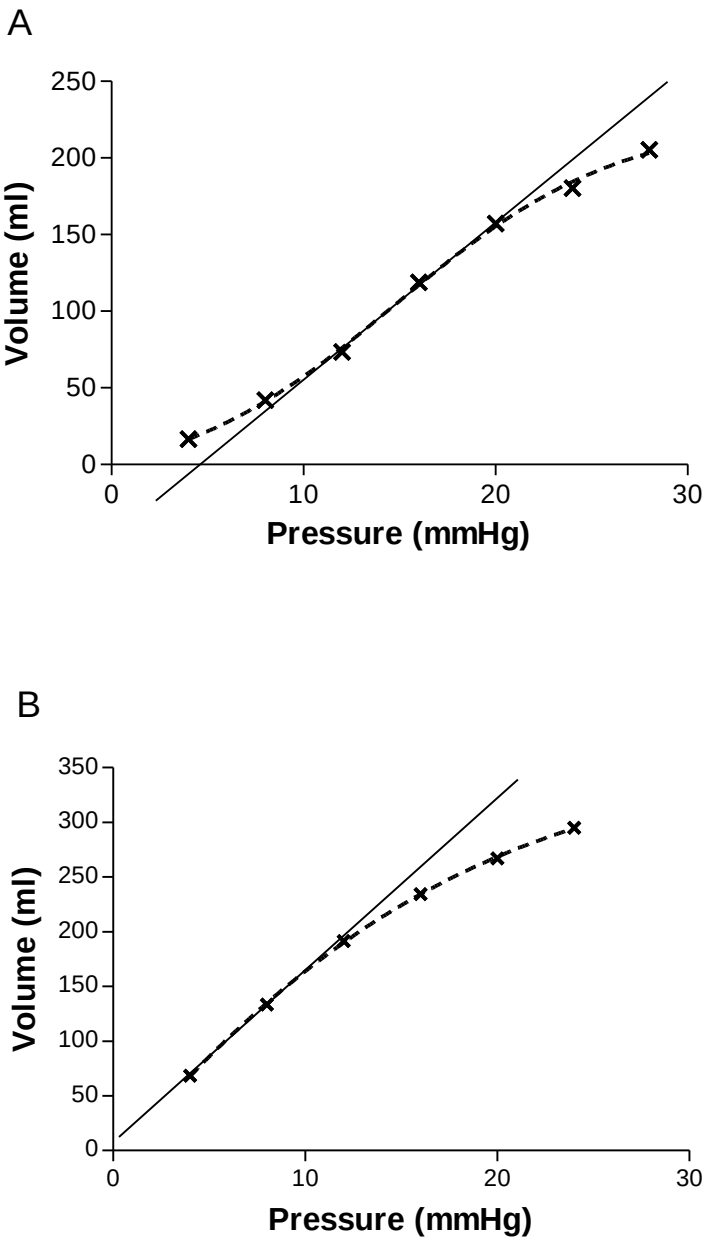
A bipolar electrode catheter (Galtec, Isle of Skye, Scotland, UK) was placed in the anal canal first and then the rectum to measure the anal and rectal sensitivities respectively. For determination of anal sensitivity electrical stimulation was applied at 5 Hz with a pulse width of 0.1 msec; the current was then incrementally increased to 20mA until the patient reported a change in sensation. In the rectum, electrical stimulation was applied at 10 Hz with a pulse width of 0.5 msec and increased to 50 mA until the patient reported a change in sensation.

3.2.6. Rectal Compliance

A mechanical barostat, insufflating air at 30ml/sec (Distender II, G & J Electronics, Ontario, Canada) was used for pressure-volume measurements. A 20cm x 15cm pillow shaped, polyethylene, over-sized, non-compliant bag (maximum volume 600ml) tied to ridges 10cm apart to a dual lumen silicon catheter was utilised to measure compliance (Mui Scientific Inc., Ontario, Canada). The catheter was placed directly into the rectum at least 5 cm from the anal verge. The minimal distending pressure (MDP) was determined by inflation at 1mmHg increments. The pressure was maintained for 15 seconds at each step. During each distension the subject was asked to breathe deeply to see if there were any pressure variations with respiration. If variations with respiration were not seen by 10mmHg, the MDP was set at 10mmHg. The basal operating pressure (BOP) was set at MDP + 2mmHg. By using the BOP as a baseline, the conditioning distension sequence was performed;

sequential 4mmHg staircase distensions were attained up to 20mmHg. Each step was maintained for 15s. Once the conditioning distension was completed, the index distension was performed; sequential 4mmHg stepwise distensions were attained up to a maximum of BOP + 40mmHg. Each distension step was maintained for 1 minute. The mean volume of air within the bag during the last 10s of each distension was calculated. This volume was then plotted against the pressure to produce a pressure-volume (P-V) curve. Compliance was measured as the gradient of the steep linear aspect of the curve (figure 3.2).

Figure 3.2: Examples of typical pressure/volume compliance curves. The corresponding mean volume of air within the bag during the last 10s of each distension (increasing pressure) was calculated (crosses) and plotted against the pressure to produce a pressure-volume (P-V) curve (dotted line). Compliance was measured as the gradient of the steep linear aspect of the curve (continuous line).



3.2.7. Laser Doppler Rectal Mucosal Blood Flow

Mucosal blood flow can be detected by Laser Doppler flowmetry (97). Studies in SSc patients suggest that the primary pathological lesion results from changes in the microvasculature producing a reduction in blood flow. Furthermore, rectal mucosal blood flow has been shown to be a measure of gut-specific autonomic innervation and altered by changes in that (98). A DRT4 laser Doppler flowmeter (Moor Instruments, Devon, UK) was used, this produces a low intensity beam almost exclusively of monochromatic coherent 780 nm light generated by a near infrared laser diode source and delivered by a fibreoptic probe. With patients in the same position the probe was placed against the mucosa 10 cm above the lower limit of the anal margin. A recording was taken for 20 seconds after a stable recording was obtained. Three recordings of blood flow were made at 120° circumferentially, and the mean of these was taken as the mucosal flux.

3.2.8. Endoanal ultrasound

Endoanal ultrasound was performed to assess the integrity of anal sphincters and possible presence of sphincter infiltration and atrophy. A conventional Hitachi EUB 8500 ultrasound machine was used with an EUP R54AW-19 endoanal probe covered with a lubricated condom inserted into the anus with the patient in the prone position. Conventional anal endosonography was performed according to a standard technique (99). Images of the anal sphincters were taken at proximal, mid, and distal canal levels. Images were acquired by a trained radiographer and

assessed independently by two experts. Where there was no agreement, a consensus was reached after discussion. The anal sphincter complex was examined and the integrity and atrophy of the internal and external sphincters were described as follows, and scored 1-3 for analysis purposes (100):

Integrity: Focal thinning: thinning in muscle thickness, but fibres in continuity.

Defect/scar: discontinuity of muscle fibres with or without replacement by lower echogenicity scar tissue. Integrity score: 1=intact, 2=focal thinning, 3=defect/scar.

Atrophy: Mild internal sphincter atrophy: abnormal increased echogenicity and/or measurement 1.5-2mm. Severe internal sphincter atrophy: measurement <1.5mm.

Mild external/puborectalis atrophy: muscle structure visible but abnormal high echogenicity suggestive of fatty replacement. Severe external/puborectalis atrophy: muscle structure very poorly or not defined suggestive of marked fatty replacement. Atrophy score: 1=normal, 2=mild atrophy, 3=severe atrophy.

The IAS was measured at the level of greater thickness at the 3, 6, 9 and 12 o'clock positions and the average thickness was calculated.

3.2.9. Correlation between physiological and structural parameters and symptoms

In order to establish which parameters most clearly contribute to symptoms the correlation coefficient between Wexner scores and physiology parameters and endoanal ultrasound findings in both the SSc patients and the incontinent controls was calculated.

3.2.10. Statistics

Statistical analysis was performed using GraphPad Prism 4 (California, USA). Data were expressed as mean (with 95% confidence intervals) or median (range), depending on whether the recorded values assumed a normal "Gaussian" distribution. A Kolmovgorov-Smirnov test was carried out to determine if data followed a normal distribution. Normally distributed values were compared between the three groups via one-way ANOVA, and for values not normally distributed the Kruskal-Wallis test was used. Bonferroni and Dunn's multiple comparison corrections were made respectively. For comparisons between the 2 SSc patient groups the Mann-Whitney test was used. The chi-squared test was used to compare proportions between groups. Pearson correlation was used to calculate correlation between Wexner incontinence and constipation scores and physiological parameters and Spearman correlation to calculate correlation between Wexner incontinence scores and structural parameters. Statistical significance was declared for p values ≤ 0.05.

3.3. Results

3.3.1. Patients

Forty four patients with SSc were studied. Twenty four patients reported faecal incontinence (symptomatic), of these patients 15 reported diarrhoea and 10 reported constipation, with one patient reporting both symptoms, a pattern commonly seen in systemic sclerosis. Twenty patients had no faecal incontinence

(asymptomatic), of these, 2 reported diarrhoea and 3 reported constipation. The mean disease duration was 11.8 (8.1-15.4) years in the symptomatic group and 8 (5.1-11.0) years in the asymptomatic group (p=NS). Twenty of 24 (83%) symptomatic patients and 13 of 20 (65%) asymptomatic patients had limited cutaneous SSc respectively (chi-squared 1.96, p=0.16). Sex and age matched patients with incontinence but no SSc were used as controls, matched to the symptomatic SSc group. The cause for incontinence in the control group was obstetric injury resulting in anal sphincter defects in 9 (45%) patients, obstetric injury but without demonstrable anal sphincter defect in 4 (20%) patients, neurological in 1 (5%) patient and idiopathic/functional pelvic disorder in 6 (30%) patients. There was no significant difference in parity between the two groups with incontinence but the asymptomatic SSc group had lower median parity. Table 3.1 summarises the demographics of the three patient groups. Wexner incontinence scores were higher in the incontinent control (IC) and symptomatic (Sx) groups compared to the asymptomatic (ASx) group. Although many patients reported both urge and passive faecal incontinence, urge incontinence was more common in the incontinent control group (IC) (16/20 IC patients vs 12/24 SSc Sx patients). SSc patients more commonly reported passive incontinence or soiling (20/24 patients). The Sx SSc patients also had higher constipation scores compared to the ASx patients. The GI questionnaire, EuroQol and SF-36 scores of the SSc patients are summarised in table 3.2.

Table 3.1: Demographic characteristics of the three patient groups.

	Asymptomatic SSc *Mean (95% CI)*	**Symptomatic SSc** *Mean (95% CI)*	**Incontinent Controls** *Mean (95% CI)*
Age	57.5 (53.3-61.7)	59.2 (55.4-63)	54.2 (47.9-60.4)
F/M ratio	20 / 0	20 / 4	20 / 0
Parity *(median- IQR)*	1 (0-2)	2 (1-2)	2 (2-3.5) *
Disease duration	8.0 (5.1-11.0)	11.8 (8.1-15.4)	N/A
lcSSc/dcSSc	13 / 7	20 / 4	N/A
Autoantibodies	**N (%)**	**N (%)**	N/A
-ACA	8 (40)	17 (71)	
-SCL-70	2 (10)	3 (13)	
Internal organ involvement	**N (%)**	**N (%)**	N/A
-Pulmonary	5 (25)	10 (42)	
-Cardiac	1 (5)	2 (8)	
-Renal	2 (10)	2 (8)	
-Oesophageal	14 (70)	23 (96)	
Wexner Incontinence *(median- IQR)*	2 (0.5-3.5)	10 (8-16) *	14 (11.5-16) *
Wexner Constipation *(median- IQR)*	3 (1.5-6)	10 (6-14) *	N/A

* $p<0.05$ compared to ASx

Abbreviations: lcSSc - limited cutaneous systemic sclerosis; dcSSc - diffuse cutaneous systemic sclerosis; CI - confidence interval, IQR – interquartile range

Table 3.2: Questionnaire scores in systemic sclerosis patients.

	Asymptomatic SSc *Median (IQ range)*	**Symptomatic SSc** *Median (IQ range)*
SF-36		
-Total score	62.43 (54.45-77.84)	54.01 (39.74-73.57)
-Physical Health score	60.4 (42.8-75.1)	50.8 (27-68.5)
-Mental Health score	65.15 (53.95-70.9)	62.35 (35.63-72.95)
Euroqol (EQ-5D)		
-Mobility	1 (1-2)	1 (1-2)
-Self-care	1 (1-1.5)	1 (1-2)
-Usual activities	1 (1-2)	2 (1-2)
-Pain/Discomfort	1.5 (1-2)	2 (2-2)
-Anxiety/Depression	1 (1-2)	2 (1-2)
-VAS score	80 (60-80)	62 (53-80)
SSC-GIT 1.0		
-Reflux	92.6 (81.5-96.5)	55.6 (42.55-77.75) *
-Distension	83.3 (72.2-94.35)	50 (36.1-63.85) *
-Diarrhoea	100 (77.75-100)	61.1 (27.75-100) *
-Constipation	100 (77.7-100)	66.65 (22.2-100) *
-Abdominal pain	100 (83.3-100)	66.7 (50-100) *
- Social	100 (100-100)	81.7 (65-95) *
-Emotional	100 (94.45-100)	55.6 (31.45-77.75) *

* $p<0.05$; Comparisons made using the Mann-Whitney test

3.3.2. Anorectal physiology measurements

The resting pressure was below the normal range (60-160 cmH_2O) in both the Sx and the IC group and was lowest in the IC group (IC: 42.2 [33-57.4] vs ASx: 63.8 [53.7-73.9]; $p<0.05$ vs Sx: 50.8 [40.7-61]; $p>0.05$) (Figure 3.3A). The squeeze pressure was lower in the IC group compared to both the ASx and Sx groups (IC: 46.95 [30-63.9]) vs ASx: 104.6 [81-128.3] vs Sx: 121.4 [101.3-141.6]; $p<0.05$) and below the normal range (50-180 cmH_2O) (Figure 3.3B). There was no significant difference in the mean squeeze pressure between the ASx and Sx groups. There was no significant difference in rectal compliance or rectal mucosal blood flow in the 3 groups.

Anal electrosensation threshold was highest in the Sx patients (Sx: 10.4 [8.8-11.4] vs ASx: 6.7 [5.7-7.7] vs IC: 8.5 [6.5-10.4]) (Figure 3.4A), the difference was statistically significant in comparison to ASx group, $p<0.05$. Although the anal sensory threshold was also higher in the IC patients compared to the ASx patients ($p>0.05$) this was still within the normal range (2.0-9.4) whereas this was not the case for the Sx patients. Rectal electrosensation thresholds were similar in the 3 groups (Figure 3.4B). The anorectal physiology results are summarised in Table 3.3.

Figure 3.3: Resting and squeeze pressure in the 3 groups.

(A) The resting pressure was lowest in the IC group 42.2 (33-57.4) vs Sx: 50.8 (40.7-61) vs ASx: 63.8 (53.7-73.9). (B) Squeeze pressure was significantly lower in the IC group (46.95 (30-63.9)) compared to the Sx (121.4 (101.3-141.6)) and ASx (104.6 (81-128.3)) groups. Results given as mean +/- standard deviation.

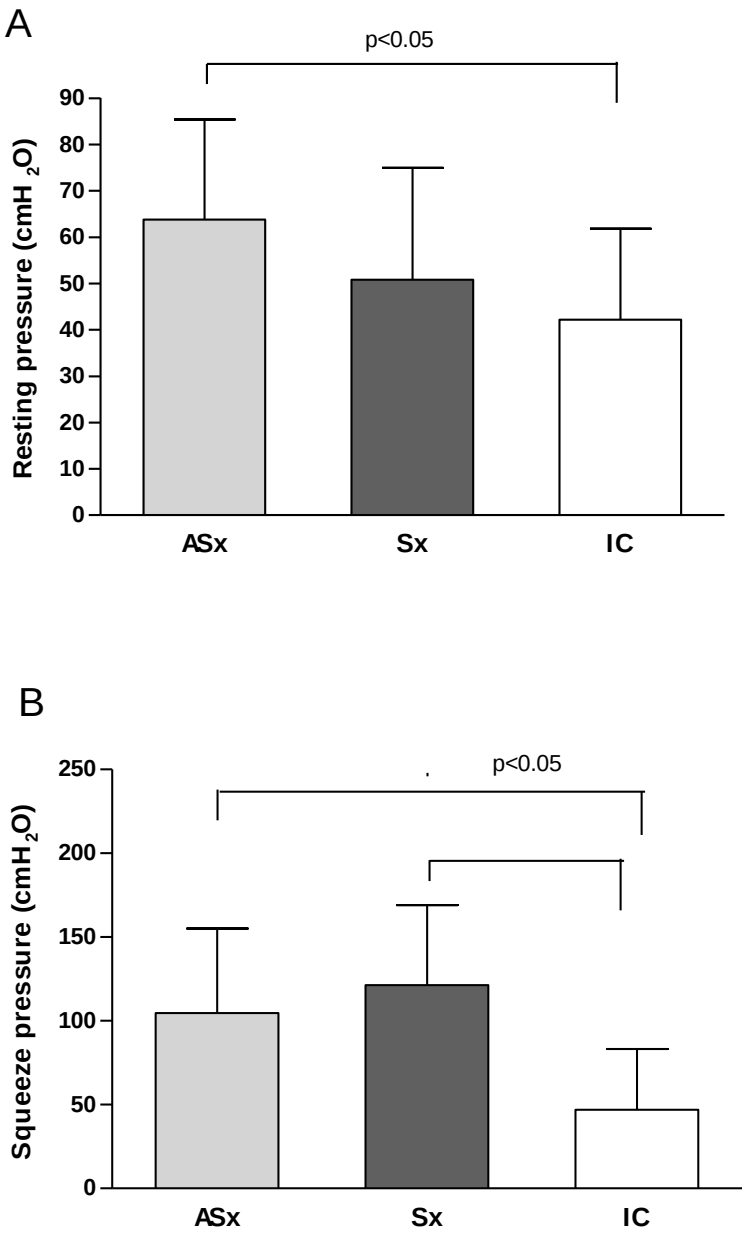


Figure 3.4: Anal sensory threshold and rectal sensory threshold in the 3 groups. (A) Anal sensory threshold was higher in the Sx (10.4 (8.8-11.4)) compared to the ASx group (6.7 (5.7-7.7)) and IC group (8.5 (6.5-10.4)). (B) Rectal sensory thresholds were not significantly different in the 3 groups. Results given as mean +/- standard deviation.

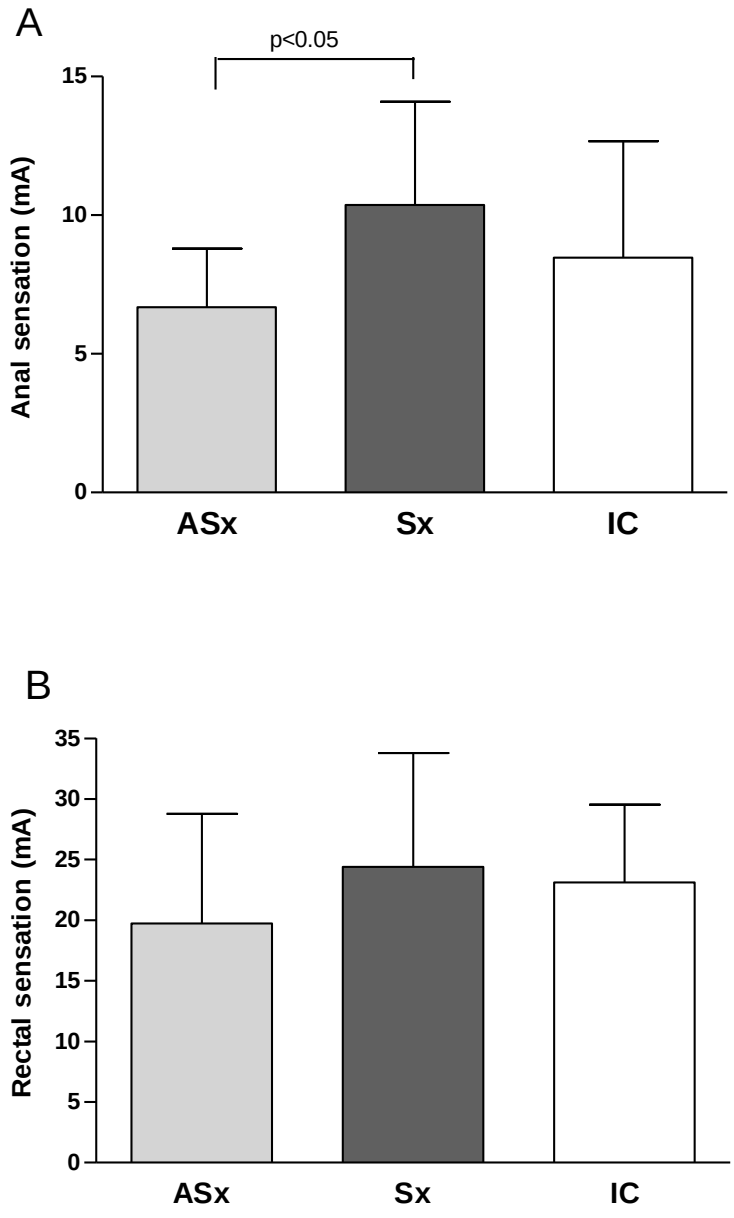


Table 3.3: Anorectal physiology measurements in symptomatic and asymptomatic SSc patients and incontinent controls.

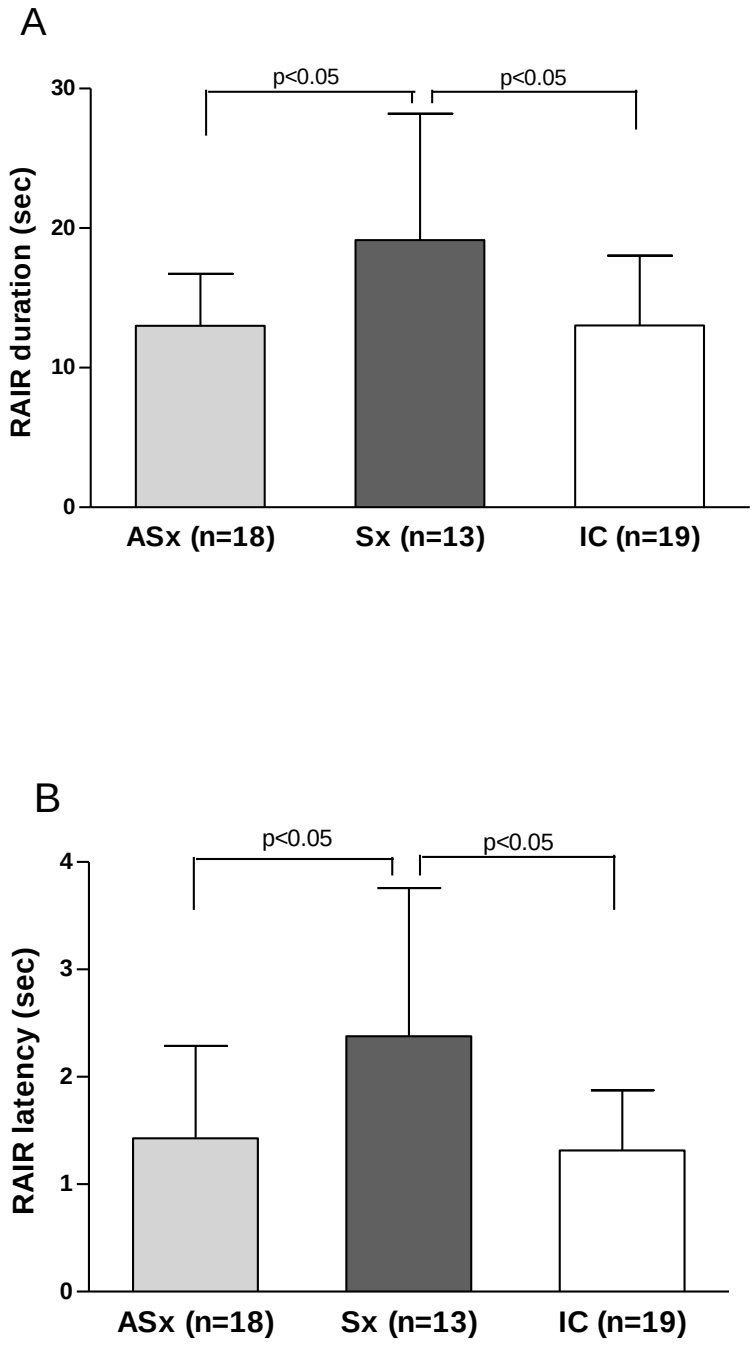
	Normal range	**Asymptomatic SSc** Mean (95% CI)	**Symptomatic SSc** Mean (95% CI)	**Incontinent Controls** Mean (95% CI)
Resting pressure (cmH_2O)	60-160	63.8 (53.7-73.9)	50.8 (40.7-61) *	42.2 (33-57.4)
Squeeze pressure (cmH_2O)	50-180	104.6 (81-128.3)#	121.4 (101.3-141.6)#	46.95 (30-63.9)
Rectal compliance (ml/mmHg)	11-15	10.9 (9.1-12.7)	10.6 (8.8-12.5)	11.9 (10.2-13.7)
RMBF (flux units)	90-250	158.4 (134.3-182.5)	150.1 (123.6-176.6)	136 (89.8-182.1)
Anal sensory threshold (mA)	2.0-9.4	6.7 (5.7-7.7)	10.4 (8.8-11.4) *	8.5 (6.5-10.4)
Rectal sensory threshold (mA)	7.0-36.0	19.7 (15.5-24)	24.4 (20.4-28.4)	23.1 (20.1-26.1)

* $p<0.05$ compared to ASx; **#** $p<0.05$ compared to IC

3.3.3. Rectoanal inhibitory reflex (RAIR) measurements

Eleven of 24 (46%) Sx SSc patients compared to only 2 of 20 (10%) ASx SSc patients and 1 of 20 (5%) IC demonstrated an absent RAIR, chi-squared 13.04, $p<0.01$. The total duration of the reflex was also longer in the Sx patients (Sx: 19.2 [13.7-24.6] vs ASx: 13 [11.2-14.9] vs IC: 13 [10.6-15.4] secs; p=0.001) (Figure 3.5A). The latency of the reflex was longer in the Sx patients compared to ASx and IC (Sx: 2.4 [1.5-3.2] vs ASx: 1.4 [1-1.9] vs IC: 1.3 [1.1-1.6] secs; p=0.006) (Figure 3.5B). There was a trend of lower amplitude (Sx: 42% [32.5-51.5] vs ASx: 57% [46.7-67.5] vs IC: 53.2% [45.7-60.7]; p=0.69) and longer recovery (Sx: 11.6 [7-16] vs ASx: 7.3 [5.6-9.1] vs IC: 7.6 [5.5-9.7] secs, p=0.055) of the reflex in the Sx patients compared to the ASx and IC but these differences were not statistically significant.

Figure 3.5: RAIR parameters in the 3 groups, (A) duration of RAIR and (B) latency. Sx group had longer total RAIR duration (Sx: 19.2 (13.7-24.6), ASx: 13 (11.2-14.9), IC: 13 (10.6-15.4)) and latency (Sx: 2.4 (1.5-3.2), ASx: 1.4 (1-1.9), IC: 1.3 (1.1-1.6)) compared to the other 2 groups.



This significant difference in the presence of RAIR between Sx and ASx SSc patients was investigated further in order to establish the association with faecal incontinence and any other factors that may be contributing to this by comparing patients with present RAIR with those with absent RAIR. The patients with absent RAIR had higher Wexner incontinence scores than those with present RAIR (10.3 [6.9-13.8] vs 6 [3.9-8]; p=0.023). Disease characteristics and the rest of the physiology parameters were compared in those patients. There was no difference between disease duration in the patients with and without elicited RAIR. There was no significant difference in other physiological parameters between the SSc patients that had a demonstrable RAIR and those patients that did not. Specifically the resting pressure between the SSc patients with present and absent RAIR was not significantly different (present 58.81 [51.1-66.6] vs absent 51.77 [3.1-69.4]). Nonetheless there was a difference in IAS atrophy scores between the 2 groups, the patients with absent RAIR having higher IAS atrophy score (median IAS-A: present RAIR: 2 [1-2] vs absent RAIR: 2 [2-3]; p=0.045). Despite the lower resting pressure in IC group only 1 patient had an absent RAIR.

3.3.4. Endoanal ultrasound

Anal sphincter atrophy and integrity scores are given as medians with interquartile range and shown in figure 3.6. EAS integrity scores: Sx: 1 [1-1], ASx: 1 [1-1], IC 1 [1-2] (figure 3.6A), IAS integrity scores: Sx: 1 [1-2], ASx: 1 [1-1], IC: 1 [1-3], EAS atrophy scores were 1 [1-1] for all 3 groups and IAS atrophy scores: Sx: 2 [1.5-3], ASx: 2 [1-2], IC: 1 [1-1] (figure 3.6B). IAS thickness: Sx: 1.85 [1.5-2.3], ASx: 1.8 [1.7-2.25], IC:

2.3 [2.05-2.85] (figure 3.6C). SSc patients had more atrophic IAS sphincter but largely intact IAS and EAS compared to incontinent controls. The age and sex matched incontinent controls were more likely to have EAS defects; they also had less IAS atrophy, evidenced by, most importantly, atrophy scores but also IAS thickness. Surprisingly there was no significant difference in atrophy scores or IAS thickness between the symptomatic and asymptomatic SSc patients. Examples of internal and external anal sphincter defect and IAS atrophy are shown in figure 3.7.

Figure 3.6: A) EAS integrity scores in asymptomatic (ASx), symptomatic (Sx) SSc patients and incontinent controls (IC); B) IAS atrophy scores in the 3 groups; C) IAS thickness in the 3 groups. (The horizontal line depicts the median, the rectangles the IQ range and the error bars the maximum and minimum values)

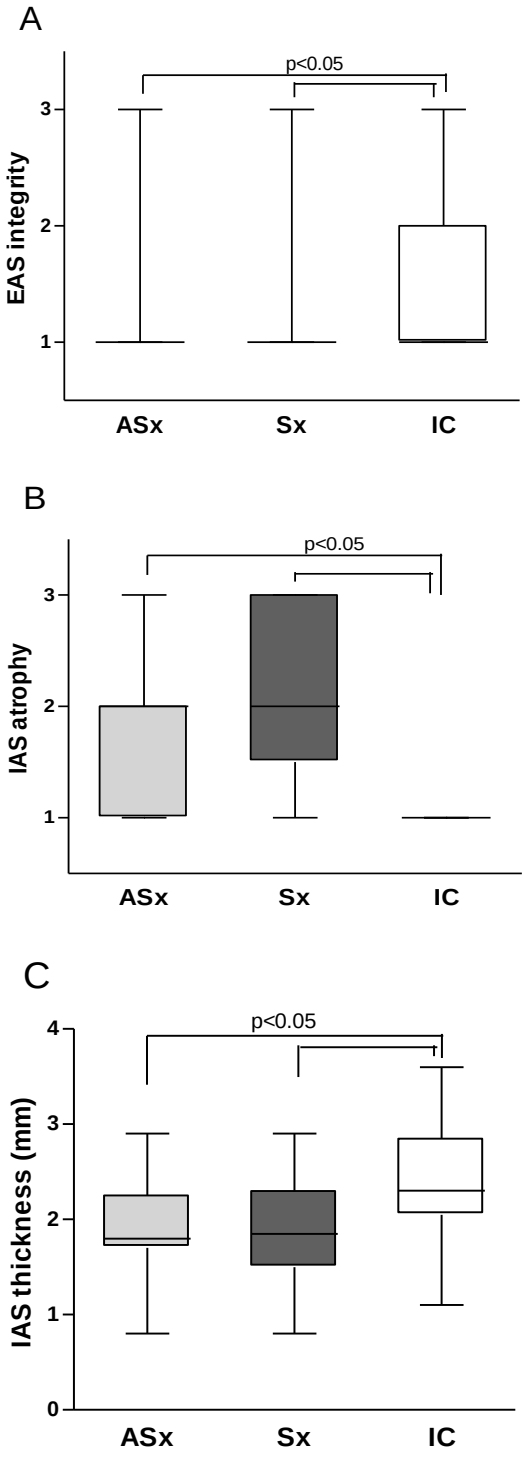
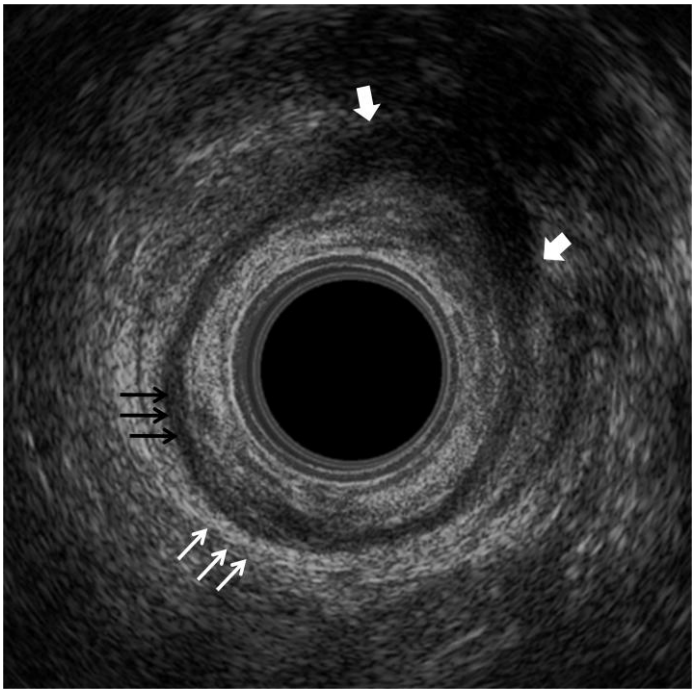
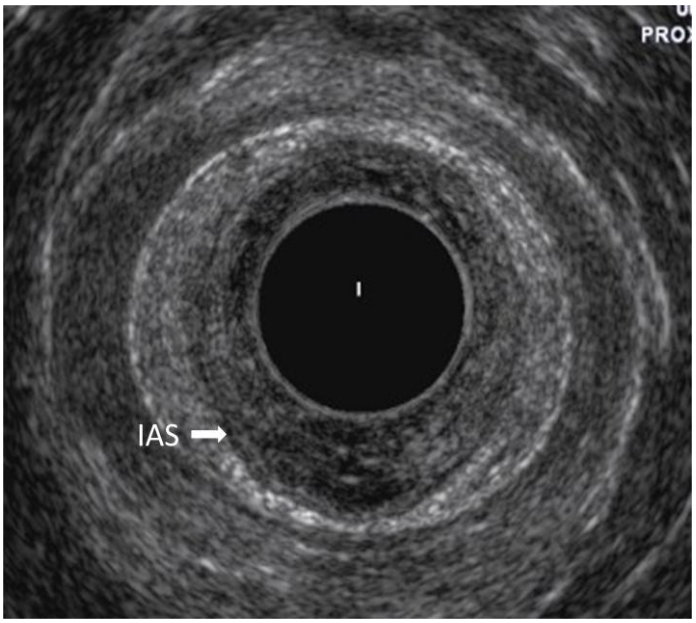


Figure 3.7: Endoanal ultrasound images showing A) EAS and IAS defect (thick arrows), IAS (black arrows), EAS (white arrows) and B) IAS atrophy (white arrow).

A)



B)



3.3.5. Correlation between physiological and structural parameters and symptoms

In the control group there was no significant correlation between Wexner incontinence score and anorectal physiology parameters. In the SSc patients there was a positive correlation with anal sensory threshold (r=0.5383, p=0.0002) (figure 3.8A) and negative correlation with resting pressure (r=-0.3433, p=0.0225) (figure 3.8B) and compliance (r=-0.3359, p=0.0258) (figure 3.8C). There was also a positive correlation of Wexner constipation score and anal sensory threshold (r=0.4286, p=0.0037) (table 3.4).

There was no significant correlation between incontinence scores and atrophy and integrity scores or IAS thickness. In subgroup analysis, there was a positive correlation between Wexner incontinence score and IAS atrophy in the SSc patients (r=0.3768, p=0.0117). There was no correlation between IAS thickness or IAS atrophy scores and disease duration in SSc patients. There was no correlation between disease subtype or disease duration and Wexner incontinence scores.

Table 3.4: Correlation table of Wexner incontinence and constipation scores and anorectal physiological parameters and Wexner incontinence scores and structural parameters in systemic sclerosis patients

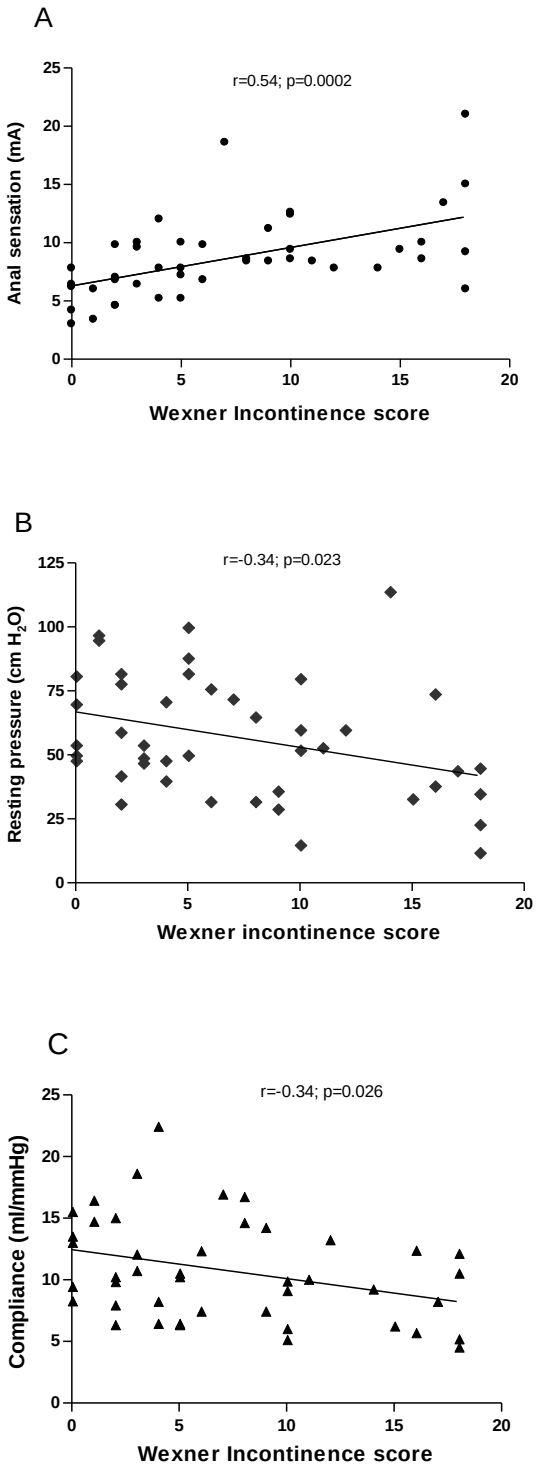
	Resting pressure	Squeeze pressure	Anal sensation	Rectal sensation	Rectal compliance
Wexner incontinence score	r=-0.3433 **p=0.0225**	r=-0.0038 p=0.9807	r=0.5383 **p=0.0002**	r=0.2922 p=0.0543	r=-0.3359 **p=0.0258**
Wexner constipation score	r=-0.3489 p=0.8221	r=0.0931 p=0.5476	r=0.4286 p=0.0037	r=0.0722 p=0.6416	r=-0.2486 p=0.1037

(r= Pearson correlation)

	IAS atrophy	IAS integrity	IAS thickness	EAS atrophy	EAS integrity
Wexner incontinence score	r=0.3768 **p=0.0117**	r=0.2235 p=0.1448	r=-0.1785 p=0.2837	r=0.3128 **p=0.0387**	r=-0.2982 p=0.9603

(r= Spearman correlation)

Figure 3.8: Correlation of Wexner incontinence score with anal sensory threshold (A), resting pressure (B) and rectal compliance (C). There is a positive correlation with anal sensory threshold and negative correlation with resting pressure and rectal compliance.



3.4. Discussion

Continence is maintained through the coordinated function of the pelvic floor, the rectum and the anal sphincters. Injury to components of this system either sensory or motor can therefore impair it (101). Anorectal involvement is reported in 50-70% of patients with systemic sclerosis (89). Faecal incontinence is probably underreported in view of the stigmatising nature of the symptom and its social implications. In an anonymous questionnaire study, 24% of patients reported faecal soiling (102). As the anorectum is the second most commonly affected site of the GI tract, after the oesophagus, a number of small studies have investigated anorectal symptoms through physiologic measurements such as anorectal manometry, sensation and reflexes. Despite those studies the pathophysiology of faecal incontinence in SSc remains largely unknown. The key pathophysiological elements of SSc, vascular involvement, neuropathy, smooth muscle atrophy and fibrosis are all candidates and most likely may all contribute at some point in the disease process. By trying to establish the sequence of events, if there is one, it may become possible to prevent either the onset or at least the progression of symptoms.

In this study of SSc patients with and without anorectal symptoms, SSc patients had higher mean resting pressure than the incontinent controls. This was the case for both Sx and ASx SSc patients although the mean resting pressure in Sx patients was lower (not statistically significant). A low mean resting pressure has been demonstrated in most studies to date (40;44;54;56) although 2 studies have found similar results to mine. Lock *et al* studied 26 patients, 3 with faecal incontinence,

and Heyt *et al* studied 35 patients, 13 incontinent. Both studies found that the mean resting pressure was within normal limits and not significantly different between patients with and without incontinence (43;46). In the latter study the mean resting pressure in SSc incontinent patients, although within normal limits, was lower than healthy controls. The patients' anorectal symptoms in these studies were quite variable which may explain the variability in the measurements of mean resting pressure. The mean squeeze pressure in all SSc patients was normal and higher than incontinent controls. This is in keeping with previous studies and expected as SSc is known to affect mainly smooth muscle, in the anal sphincter complex this being the internal anal sphincter rather than the striated external anal sphincter. In contrast faecal incontinence in other patients is most commonly secondary to obstetric injury to the EAS (103).

Despite the knowledge that the anal sphincters are affected in SSc there are only few studies which have assessed the anal sphincter structure. Engel *et al* were the first to report morphologic changes of the internal sphincter in SSc patients. They described two female patients with SSc and passive faecal incontinence with very thin, hyperechoeic internal anal sphincter (53). DeSouza *et al* in a MRI study of 18 patients with SSc and 19 controls with and without incontinence showed that the SSc patients with incontinence had thinner internal anal sphincters than controls, but not SSc patients without incontinence (91) suggesting that incontinence is related to smooth muscle atrophy in this group. They further found reduced gadolinium enhancement of the IAS in all SSc patients suggesting an ischaemic origin of IAS atrophy. They did not find any significant difference in the external

anal sphincter thickness in SSc patients (91). Koh *et al* in their study of 11 SSc patients with incontinence showed that there appeared to be two distinct types of morphologic changes in the IAS. Patients had either very thinned, difficult to delineate and hyperechoic internal sphincters or, less commonly, a thickened, homogeneous and uniformly hypoechoic internal anal sphincter (90).

In my study, SSc patients both those with faecal incontinence and the asymptomatic ones had thinned and atrophic IAS compared to the incontinent controls. In contrast the incontinent controls had more commonly evidence of external anal sphincter defects. I did not find any SSc patients with thickened IAS in our unselected sequential cohort of patients. Nonetheless the normal resting pressure often observed in SSc patients with faecal incontinence suggests that the mechanism for incontinence is more complex than purely structural or secondary to IAS dysfunction caused by smooth muscle atrophy and fibrosis.

The gastrointestinal smooth muscle may be involved early in patients with SSc, before the onset of clinical symptoms. This is seen in the oesophagus where physiologic, mainly motility abnormalities are seen even in patients with no oesophageal symptoms (75;104;105). The oesophageal abnormalities in SSc are low basal LOS pressure resulting in loss of competence and also lack of peristalsis of the lower oesophageal body (79;106;107). These abnormalities were thought to be secondary to collagen deposition resulting in smooth muscle atrophy and fibrosis (108). A more recent histological study of oesophageal tissue in SSc patients and controls found smooth muscle atrophy in 94% of SSc patients and only 5% of controls. The pathological findings seemed to be either secondary to loss of neural

function or a primary smooth muscle lesion (10). The IAS shares similarities with the lower oesophageal sphincter (LOS) in that they are both smooth muscle continuation of the circular muscle layer of the gut that maintains chronic tone. Engel *et al* showed thinning of the muscularis propria with fibrous replacement of both the circular and longitudinal layers of the smooth muscle (53). Although collagen deposition and smooth muscle atrophy are well recognised in GIT involvement in SSc, there is evidence that these abnormalities are preceded by vascular insufficiency and neural dysfunction (11;109).

Vascular involvement, especially affecting the small vessels is common in SSc. Decreased oesophageal blood flow has been found in patients with Raynaud's phenomenon and reduced gastric and duodenal blood flow has been demonstrated in SSc patients (11;12). In the anorectum deSouza *et al* showed that SSc patients with faecal incontinence had reduced gadolinium enhancement of the IAS (91). Histological studies of gastrointestinal mucosa show evidence of partially obliterated enteric vessels with hypertrophied endothelial cells and thickened basal lamina (13). In my study, through the use of laser Doppler probe the rectal mucosal blood flow was examined to assess for any obvious ischaemia. There was no demonstrable difference found in rectal mucosal blood flow between SSc patients, both symptomatic and asymptomatic, and incontinent controls. Although this finding is in contrast to the reduced gastrointestinal blood flow measured in the gastric antrum and duodenum by a similar technique there are a number of possible explanations for this disparity. Although the use of laser Doppler probe for measurement of blood flow in the rectal mucosa has been validated (97;98) there is

still some criticism of the method as there remains a risk of poor contact secondary to faeces. Nonetheless according to previous experience, if there is no adequate contact with the mucosa then no reading is obtained. On the other hand, other factors may also affect rectal mucosal blood flow. The rectal microcirculation is sensitive to menstrual cyclical changes (110). Although most patients in this study were post-menopausal women, I did not control for this in the remaining patients. Gastrointestinal mucosal blood flow is partly regulated by autonomic nerve supply (98). Autonomic dysfunction is well recognised in SSc, although not tested for in this study, and could therefore affect the findings.

The pathogenesis of neuropathy may be caused by compression of nerve fibres by collagen deposits or secondary to arteriolar changes in the vasa nervorum. Another possible mechanism is autoantibody related. Autoantibodies are seen in more than 97% of patients with SSc. Most of the SSc specific autoantibodies are associated with specific clinical characteristics. Although so far it is thought that autoantibodies are the result and not the cause of SSc, there is increasing amount of evidence pointing to a pathogenic role of some autoantibodies (24). With regards to neuropathy and gastrointestinal involvement, antibodies from SSc patient sera have been shown to specifically inhibit M3-muscarinic receptor–mediated enteric cholinergic neurotransmission (25-27). Furthermore Kawaguchi *et al* showed that anti-M3-muscarinic receptor (M3R) antibody frequently appeared in patients with SSc with severe GIT involvement, suggesting that M3R-mediated enteric cholinergic neurotransmission may provide a pathogenic mechanism for GIT dysmotility in SSc (111). With regards to anorectal dysfunction IgGs isolated from SSc patients

specifically interfered with the muscarinic receptor activation in smooth muscle cells of rat IAS (28). These autoantibodies are not routinely checked for as yet in SSc patients but if neuropathy is proven to be key to the development of GI / anorectal symptoms and M3 muscarinic receptor antibodies have a pathogenic role then there is a potential for new therapeutic agents. A neurogenic component in gut involvement in SSc is also supported by histological studies. Malandrini *et al* obtained deep rectal biopsies from 3 patients with SSc and showed structurally normal enteric neurons but abnormal axon terminals with axoplasm devoid of cytoskeletal elements suggestive of degeneration and depletion of neurotransmitters (13). This is supported by other studies that have shown depletion of the neuropeptides NY and YY in SSc (112).

My results suggest that neuropathy plays a key role in the development of faecal incontinence in SSc. The RAIR reflects a reflex relaxation of the IAS in response to rectal distension. It is a local intramural reflex within the wall of the rectum and anal canal and thought to be independent of spinal cord involvement (101) but it can be influenced by higher centres. It is absent in all patients with Hirschprung's disease where there is a congenital absence of ganglion cells in the rectum and colon (113). The RAIR facilitates an important sensory role requiring intact anorectal sensation, intact intramural nervous system and a coordinated sphincter response. The components of the reflex are: the latency period, the amplitude and the recovery time. The latency period reflects duration of neurological synaptic transmission, the amplitude reflects the muscular component and the recovery period reflects partly the neurological factors and partly the muscle tone. Impaired

RAIR secondary to neuropathy and correlation with faecal incontinence has been suggested in diabetic patients, specifically showing correlation between time to recovery and faecal incontinence scores (114). I demonstrated an absent RAIR in 46% of SSc patients with incontinence. In contrast, RAIR was absent in only 10% of SSc patients without incontinence and only one of 20 incontinent controls. Incontinent SSc patients had longer latency period which would suggest underlying neuropathy rather than reduction in muscle tone secondary to atrophy. Previous studies have demonstrated absent RAIR in SSc in 12-71% of patients. Basilisco *et al* observed that RAIR was absent in 5/6 patients with constipation but only 1 of 8 patients with normal bowel habit (40). Heyt *et al* showed a correlation between impaired RAIR and incontinence, 11 of 13 incontinent patients had absent or impaired RAIR whereas 14 of 22 continent patients had absent or impaired RAIR. The amplitude of the RAIR was lower in the incontinent patients (43). In their study the majority of patients reported at least one lower GI symptom which may explain why they found an impaired RAIR in a higher percentage of their continent patients, whereas in my study the asymptomatic patients had no or minimal lower GI symptoms. The lower amplitude noted in the incontinent patients may reflect either neuropathy or lower muscle tone. It is most likely that neuropathy plays a role in the development of other symptoms such as diarrhoea, constipation or evacuation difficulty and not just incontinence. Further evidence of neuropathy in incontinent SSc patients is demonstrated in my study by impaired anal sensation. Sensory testing by mucosal electrosensitivity is an indirect way of measuring the innervation of the anorectum. Roe *et al* have shown that patients with idiopathic faecal incontinence had sensory deficit as demonstrated by reduced anal sensory

threshold (115). In my study SSc patients with incontinence had higher anal sensory threshold than SSc patients without anorectal symptoms. Although the anal sensory threshold was also high in the incontinent controls this was within the normal range. Sensory information from the anal canal is carried by the somatic pudendal nerves whereas the pelvic visceral nerves are the main sensory pathways from the rectum. The anal canal is believed to have a greater variety of afferent nerve endings than the rectum (116;117) and this could explain the fact that anal sensory threshold and not rectal sensory threshold was affected.

These findings further support the hypothesis that anorectal symptoms and especially faecal incontinence in SSc patients is not exclusively secondary to smooth muscle atrophy. I did find evidence of IAS atrophy but this was seen in both the symptomatic and asymptomatic patients and there was not associated significant impairment of the resting tone. In contrast there were distinct neuropathic abnormalities in the symptomatic SSc patients not seen in the asymptomatic patients or in the incontinent controls.

The enteric nervous system consists mainly of the myenteric and submucosal plexi although other plexi have also been identified. The enteric plexi control a lot of the coordinated activity of the gastrointestinal tract in the absence of extrinsic innervation although a number of sympathetic and parasympathetic fibres terminate in the enteric plexi. Axons of plexus neurons innervate smooth muscle cells in the muscularis mucosae and muscularis externa as well as gland cells and exocrine and endocrine cells. Interneurons in the plexi connect afferent sensory fibres with efferent neurons to smooth muscle cells. Furthermore afferent fibres

from mechanoreceptors and chemoreceptors in the mucosa and gut wall synapse at the plexi thus allowing local reflex activity such as the RAIR. Neuropathy in SSc is multifactorial and is most likely affecting the enteric plexi. This would explain the findings of anal sensory neuropathy as well as the absent RAIR as both of these processes would depend on intact enteric plexi. The potential pathogenic role of autoantibodies to muscarinic receptors may still be due to effect on parasympathetic fibres in the intramural plexi. Studies to investigate these mechanisms further are likely to shed more light both in the pathogenesis of gastrointestinal involvement in SSc and the effect of treatments. With regards to management of anorectal symptoms it is possible that sacral neuromodulation may be more successful than other therapies and it may be worth considering treating early minimally symptomatic or even asymptomatic patients if anorectal physiology abnormalities are present.

In conclusion I have demonstrated a possible neuropathogenic mechanism underlying faecal incontinence in SSc, as suggested by the impaired RAIR and anal sensation. It may be that structural changes occur early in all SSc patients, but the development of faecal incontinence occurs with disease progression in some patients as neuropathy develops. This specific question will be addressed in further studies. Although the oesophagus and the anorectum are the most commonly affected areas of the GI tract, involvement of the rest of the GI tract often occurs but as it is less easily accessible, it is more difficult to study and assess. I hypothesised that not only neuropathy and myopathy but also an infiltrative

process earlier in the disease process may have an effect on absorption and therefore an effect on nutrition.

CHAPTER 4:

THE EFFECT OF GASTROINTESTINAL INVOLVEMENT ON NUTRITION – NUTRITIONAL ASSESSMENT IN SYSTEMIC SCLEROSIS

4.1. Introduction

Approximately 8% of patients with diffuse cutaneous SSc develop severe GI involvement described by Steen and Medsger as malabsorption syndrome, repeated episodes of intestinal pseudo-obstruction, or severe GI problems which require hyperalimentation (5). The mortality rate secondary to severe GI involvement has been reported to be 6-12% (118) and therefore early identification and prevention would be highly desirable.

SSc can affect any part of the GI tract and therefore the availability of nutrients is affected both by intake and absorption. Patients often have microstomia and xerostomia which makes the actual act of eating more difficult. Atrophic gingivae and papillae cause loss of taste and are associated with reduced oral intake. Oesophageal dysmotility and acid reflux cause dysphagia especially of coarser food, fruit and vegetables. Delayed gastric emptying and small intestinal dysmotility can cause bloating and patients often complain that they cannot have a normal size meal. Furthermore subjective intolerance of foodstuffs is common, and patients often follow a largely restricted diet.

Impairment of small bowel motility results in intestinal symptoms such as nausea, vomiting, bloating, distension, anorexia, abdominal pain, or even an overt malabsorption syndrome. The small intestinal hypomotility may provoke luminal dilatation and clinical pseudo-obstruction. Histological data and muscle mechanics data support the hypothesis that this is the result of abnormal collagen deposition as is seen in the oesophagus (50). Small intestinal bacterial overgrowth (SIBO) occurs because of impaired motility and intestinal stasis but also intestinal

diverticulae or sacculation (119). Malabsorption may be caused by SIBO, impaired blood supply but also decreased intestinal permeability secondary to intestinal mucosal fibrosis. The latter can cause malabsorption of fat and bile salts (59). This can accelerate transit and lead to steatorrhoea which can be exacerbated, although much less commonly by pancreatic insufficiency secondary to reduced pancreatic secretions (120). Involvement of the small intestine is a specifically difficult problem that can ultimately lead to intestinal failure.

Simple measures such as small but more frequent meals and artificial saliva or simple water can be effective, nevertheless patients often remain symptomatic. Medications such as proton pump inhibitors (PPI) and prokinetics may improve gastro-oesophageal reflux and delayed gastric emptying but when a patient is unable to maintain their nutritional status artificial feeding may be necessary. Oral nutritional supplements are the least invasive form of artificial feeding and the first line of management to boost calorific intake if tolerated by the patient. Nasogastric or percutaneous endoscopic gastrostomy (PEG) or jejunostomy feeding can be tried especially when intake is the main problem (118). In a small number of patients parenteral nutrition (PN) needs to be considered, most commonly when enteral feeding is not tolerated or adequate (59). However it can be associated with significant risks and complications such as central venous catheter infections, occlusions and fractures of lines, venous thromboses as well as complications of the PN itself such as hepatic disease.

Malnutrition has been defined as “a state of nutrition in which a deficiency, excess or imbalance of energy, protein, and other nutrients causes measurable adverse

effects on tissue/body form (body shape, size, composition) and function and clinical outcome" (121).

There have not been many studies looking at nutrition in patients with SSc but it is clear that both oral intake and absorption of nutrients can be affected by GI involvement (122). There are few studies that have looked into nutritional assessment in patients with SSc. One study comparing 30 patients with SSc versus matched healthy controls showed that although the intake of energy and nutrient distribution was not significantly different, there was lower intake of dietary fibre. Anthropometric assessment revealed lower arm muscle circumference and in 2 patients there was subnormal triceps skinfold thickness. 43% of patients were found to have fat malabsorption. There was no deficiency of trace elements apart from selenium which was lower in SSc patients. Overall this study showed that although the oral intake of nutrients is not different in SSc patients with significant gastrointestinal involvement there is decreased nutrient absorption and possibly selective nutrient loss leading to malnutrition (123). A recent multicentre study by the Canadian Scleroderma Research group showed that of 586 patients with SSc in the study screened using the malnutrition universal screening tool (MUST), 10.4% were at moderate risk and 17.4% at high risk of malnutrition (124). Overall it is recommended that patients with SSc should be screened regularly for malnutrition and that a multidisciplinary approach should be taken when there is such evidence (125). At the other end of the spectrum, there are a few case series that have looked at the outcome of SSc patients needing PN. A recently published retrospective review over a 13-year period described 8 patients with SSc requiring

PN. The median duration of PN was 40 months and overall there was a lower rate of line infections compared to other PN patients. 3 patients died, none of causes related to the PN. Hand function was a major factor for failure of the patients to manage the PN regime independently (126). Overall PN can be successfully used long-term for patients with SSc that are unable to maintain their nutritional status because of severe GI involvement. Nonetheless nutritional assessment and early treatment or supplementation may delay the need for PN and the morbidity associated with malnutrition.

The aim of this study was to perform detailed anthropometric and calorimetric assessments and hence establish any evidence of clinical or subclinical malnutrition and what features may be associated with that in SSc patients. As there have been no trials of bioelectrical impedance and indirect calorimetry with large numbers of SSc patients this was an exploratory study looking at the SSc patient cohort with and without GI symptoms previously studied.

4.2. Methods

Nutritional assessment incorporated conventional anthropometric measurements, indirect measurement of energy expenditure (indirect calorimetry) and indirect measurement of body composition (bioelectrical impedance). The MUST screening tool data was also collected for comparability with other subject/patient groups.

4.2.1. Patients

Patients attending the rheumatology outpatient department at Royal Free hospital were approached and invited to participate in the study. Verbal and written details about the study were provided and patients were contacted approximately 3-7 days later. Written consent was obtained when patients attended for the study day. Forty three patients with SSc were studied. 23 patients had at least moderate GI symptoms and 20 were either asymptomatic or had only mild GI symptoms on direct questioning and past medical history. All measurements were taken on one study day.

4.2.2. Questionnaires

Patients were asked to complete the following questionnaires on the day of their assessments.

1. Scleroderma GI tract 1.0 questionnaire: A 52 item self-completed instrument that provides a profile for GI symptoms and their effect on social and emotional well-being. The questions cover 7 broad categories: reflux, distension, diarrhea, constipation, abdominal pain, effect on social functioning and emotional well being (Appendix 1) (68).

2. SF-36 general health questionnaire: a multi-purpose, short-form health survey with 36 questions that yields an 8-scale profile of functional health and well-being scores (94) (Appendix 2).

3. EuroQol: a 6-item self-completed instrument that provides a simple descriptive profile for health status by asking if the patient has no problems, some problems or significant problems in the following domains: mobility, self care, usual activities, pain/discomfort, anxiety/depression (95;127) (Appendix 3).

4.2.3. Anthropometric assessments

1. Height, weight, body mass index (BMI)

Height was recorded to the nearest 0.5 cm. Weight was recorded to the nearest 0.5 kg. BMI was calculated using height and weight (weight (kg)/height^2 (m)).

2. Mid-arm circumference/area (MAC)

The patient was asked to bend the non-dominant arm at the elbow at a right angle with the palm up. The measurement was taken at the posterior surface of the arm. The mid-point between the acromial process of the scapula and the olecranon process of the elbow was measured and marked. The patient was then asked to stand with the non-dominant arm hanging loosely by the side. The circumference was measured at the previously marked mid-point with a tape tightened snugly (128).

3. Triceps skinfold thickness (TSF)

Measurement of this was taken on the upper non-dominant arm posterior surface. The patient was asked to stand with the non-dominant arm hanging loosely by the side. A vertical pinch of skin and subcutaneous fat was grasped between thumb and

forefinger, 1 cm above the midpoint mark. The skinfold was gently pulled away from underlying muscle tissue. The skinfold calliper (Gaiam Ltd, UK) jaws were placed over the skinfold at midpoint while maintaining grasp of the skinfold and a reading was taken to the nearest mm 2-3 seconds after applying the calliper. Three readings were taken and averaged.

The mid arm muscle circumference (MAMC) was calculated using the MAC and TSF measurements by the formula: MAMC = MAC - 3.14 x TSF (128).

4. Forearm muscle strength (handgrip test)

Muscle strength was assessed by recording the maximal voluntary contraction of the non-dominant arm using a handgrip dynamometer (DynEx1, MD Systems, Ohio, USA) with the subject standing and the hand held by the side. Three readings were taken and averaged.

For all the above measurements the values were compared to sex and age reference values (129) and are presented as percentiles of these values.

4.2.4. Malnutrition Universal Screening Tool (MUST)

This tool has been developed by the Malnutrition Advisory Group of BAPEN. It is called the 'Malnutrition Universal Screening Tool' ('MUST') to indicate that it can be applied to all types of adult patients in all care settings. It is a valid, reliable, and easy to use tool which can be applied to all adult patients (121).

Using the MUST, patients are scored to establish the malnutrition risk based on BMI (BMI >20 = 0, 18.5-20 = 1, <18.5 = 2), unplanned weight loss in last 3-6 months (<5% = 0, 5-10% = 1, >10% = 2), presence of acute illness and likely no oral intake for >5 days = 2. A total score of 0 suggests low risk, score of 1 medium risk and score of 2 or more high risk of malnutrition.

4.2.5. Indirect calorimetry (non-invasive measurement of resting energy requirements)

Indirect calorimetry measures energy expenditure (EE) by calculating the patient's metabolic rate through measurements of oxygen consumption (VO_2) and carbon dioxide production (VCO_2). It is based on the premise that gas volumes and concentrations exchanged at the mouth reflect cellular metabolic activity. By measuring the difference between inspired and expired levels of O_2 and CO_2, determinants of VO_2 and VCO_2 can be obtained. These values can then be converted to a resting energy expenditure (REE) (130). The respiratory quotient (the VCO_2:VO_2 ratio) varies from 0.67 (ketone body metabolism) in the fasting state, to a maximum of 1.3 (lipogenesis derived from glucose). And usually lies between 0.8 and 1.0 the former reflecting a fat rich diet and the latter a carbohydrate rich diet in health. The resting energy expenditure comprises 60-80% of the total daily energy expenditure with the rest made up of the specific dynamic action of nutrients and physical activity energy expenditure. The effect of disease on energy metabolism has been mostly investigated by studies on REE.

Patients were asked to fast for 4 hours prior to the assessment (except water) and asked to rest in a supine position for 10 minutes before the study. A canopy, connected via a pipe to the calorimeter (VMax Series 2130 System, Sensor Medics Corp, CA, USA) was placed over the patient's head (appendix 8.1) and measurements of inspired and expired levels of O_2 and CO_2 were taken in a quiet thermoneutral environment for 15-20 minutes provided that a steady state (defined as a period of five minutes in which variations of VO_2 and VCO_2 are <10%) was reached. Signals from the calorimeter were transmitted to a PC for online display and subsequent storage to hard disk. A dedicated software program (Viasys) was used for online monitoring and analysis purposes. REE was calculated by averaging measurements during steady state (minimum of 10 minutes). This was then given as a percentage of the basal metabolic rate (BMR) calculated based on sex, age, height and weight. The BMR based on predictive equations is an estimation of the basal metabolic rate of a healthy individual. The REE as calculated by indirect calorimetry data is a more accurate measure of the actual metabolic rate. The comparison with the predicted value allows an assessment on whether that individual is hyper or hypo-metabolic.

4.2.6. Bioelectrical Impedance

Bioelectrical impedance (BIA) is an indirect method for assessment of body composition (fat/muscle/water). The principle behind it is that living organisms consist of intra and extra-cellular fluids that behave as electrical conductors and cell membranes that act as electrical condensers (figure 4.1). Bioelectrical resistance is

the pure opposition of a biological conductor to the flow of an alternating current whereas reactance is the resistive effect due to capacitance produced by tissue interfaces and cell membranes.

Body impedance (Z) is defined as the opposition of a conductor to the flow of an alternating current, and consists of two components: resistance (R) and reactance (Xc). Resistance (R) is the major opposition of the conductor and at usual low frequency (50 kHz), the extra-cellular part of non-adipose tissue works as a resistor. Reactance is an additional opposition or the storage of an electrical charge by a condenser for a short period of time; the lipid component of the membranes of the body cell mass (BCM) behave as capacitors and reduce the flow of intracellular ions. In practice, impedance is the amount of dropped voltage when a small constant current with a fixed frequency passes between electrodes spanning the body. Lean tissue, which is rich in water and electrolytes, has minimal impedance and fat/adipose tissue has maximum impedance. Lean body mass and fat mass (FM) can therefore be calculated from the difference in conductivity. Capacitance, or the storage of electric charge by a condenser, causes the current to lag behind the voltage, creating phase shift. This shift is quantified as the phase angle. Phase angle has been shown to play an important role as a morbidity and mortality marker in several clinical conditions such as HIV, lung cancer, pancreatic and colorectal cancer (131).

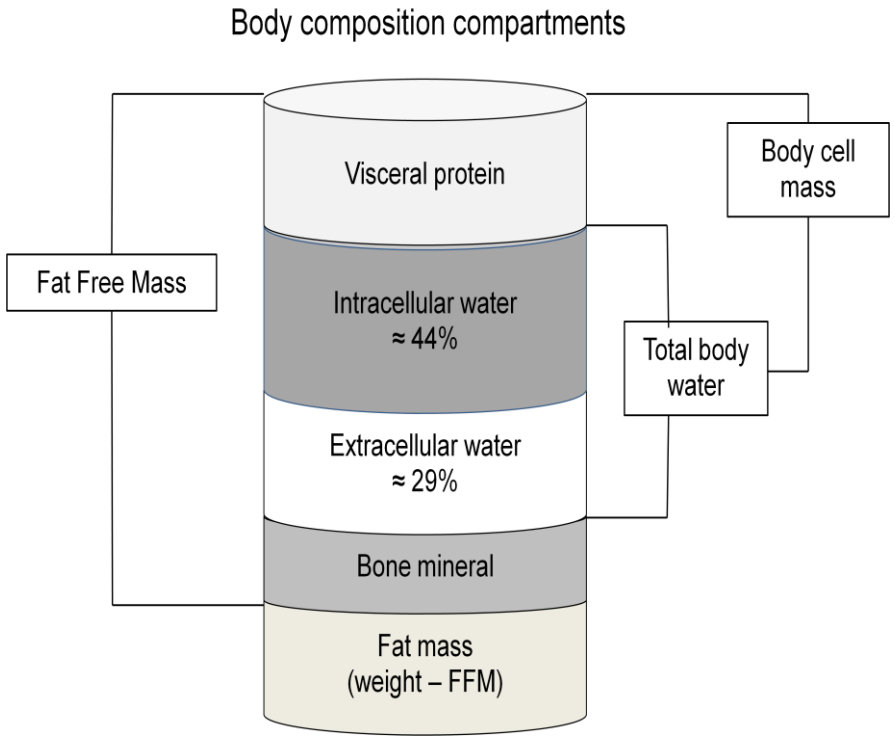
Each patient was asked to avoid caffeine containing drinks and alcohol for 24 hour prior to testing and to fast (except water) for 4 hours prior to testing. Patients rested flat for 5-10 minutes prior to testing. Two electrode pads were placed on the

dorsum of right hand (over the wrist and at the mid-point between the 2nd and 3rd metacarpals) and 2 on the dorsum of the right foot (over the ankle joint and at the mid-point between the 2nd and 3rd metatarsals) (figure 4.2). The area was cleaned with alcohol wipe prior to placement of the electrodes. The patient was then asked to lie flat and completely relaxed and remain still while the data was being processed. The data was collected using a hand-held monitor (Maltron Bioscan; Maltron Int ltd, Essex, UK). The measurements calculated were resistance, reactance, impedance, phase angle, fat mass, fat free mass, total body water, extracellular water, intracellular water, body cell mass.

4.2.7. Statistics

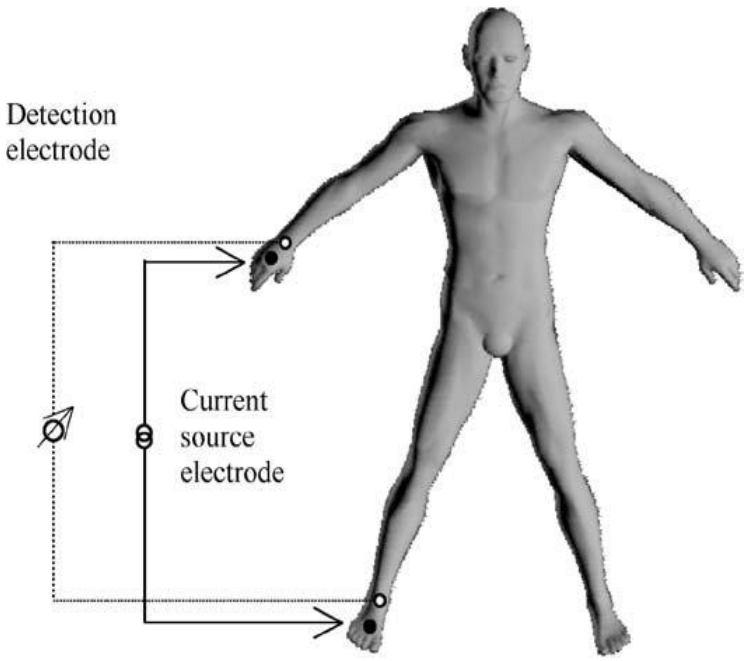
Statistical analysis was performed using GraphPad Prism 4. Data were expressed as mean (with 95% confidence intervals) or median (interquartile range), depending on whether the recorded values assumed a normal "Gaussian" distribution. A Kolmovgorov-Smirnov test was carried out to determine if data followed a normal distribution. Paired t test was used to compare the indirect calorimetry and bioelectrical impedance measurements in the 2 groups and the non-parametric Mann-Whitney test was used for comparison of anthropometric assessment values between the 2 groups. The chi-squared test was used to compare proportions between groups. Statistical significance was declared for p values ≤ 0.05.

Figure 4.1: Schematic representation of body composition compartments. (FFM=fat free mass)



Abbreviations: FFM – fat free mass

Figure 4.2: Placement of electrodes in hand and wrist and foot and ankle for tetrapolar bioelectrical impedance (132).



4.3. Results

4.3.1. Patients

Forty-three patients, 23 with moderate to severe GI symptoms and 20 patients with no or mild GI symptoms were studied. Forty patients were female, mean age 57.3 years and mean disease duration 10.3 years. 11 patients had lcSSc and 32 dcSSc. The demographic characteristics of the 2 patient groups are shown in Table 4.1. There was no significant difference in age, sex, disease subtype or disease duration between the 2 patient groups. One asymptomatic patient did not undergo indirect calorimetry due the patient's time constraints and 5 symptomatic and 4 asymptomatic patients did not have impedance measurements due to equipment failure.

Table 4.1: Demographic characteristics of symptomatic and asymptomatic patients.

	Symptomatic (n=23)	**Asymptomatic (n=20)**	**p value**
Age (mean & 95% CI)	56.6 (52.5-60.7)	58.2 (56.5-65.8)	0.58
Disease duration (mean & 95% CI)	11.3 (8-14.7)	9.1 (5.4-12.8)	0.35
Female : Male	20/3	20/0	0.24
LcSSc:DcSSc	19/4	13/7	0.29
Autoantibodies	**n (%)**	**n (%)**	
-ACA	16 (70)	9 (45)	0.13
-SCL-70	3 (13)	2 (10)	1.0
Internal organ involvement	**n (%)**	**n (%)**	
-Pulmonary	7 (30)	5 (25)	0.75
-Cardiac	5 (22)	1 (5)	0.19
-Renal	2 (9)	1 (5)	1.0

Abbreviations: 95% CI – 95% confidence interval, ACA – anti-centromere antibody, Scl-70 – anti-topoisomerase antibody.

4.3.2. Questionnaire scores

The SSC-GIT 1.0 scores for the 2 patient groups can be seen in figure 4.3 and table 4.2. The scores were statistically significantly lower in the symptomatic group for all categories. SF-36 and EuroQol scores can be seen in table 4.2. The SF-36 total score was not different between the 2 groups (Sx: 53.27 [35.67-73.27] vs ASx: 63.93 [53.47-77.84]) and neither was the mental health score (Sx: 61.70 [29.40-72.50] vs ASx: 65.15 [53.95-70.62]). The physical health score was lower in the symptomatic group (Sx: 48.20 [25.20-61.60] vs ASx: 61.70 [44.40-75.10]; $p<0.03$). A higher percentage of patients in the symptomatic group reported at least some problems for each of the EuroQol domains (figure 4.4).

Figure 4.3: SSC-GIT 1.0 scores for symptomatic and asymptomatic patients.

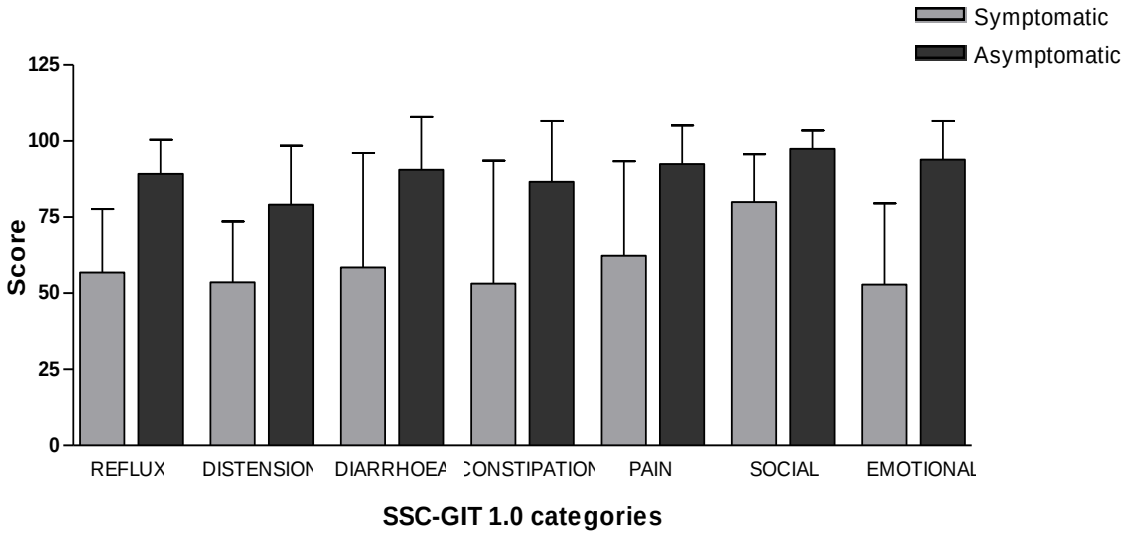


Figure 4.4: Percentage of patients that indicated at least some problems (scores 1 or 2) in the domains of the EuroQol questionnaire.

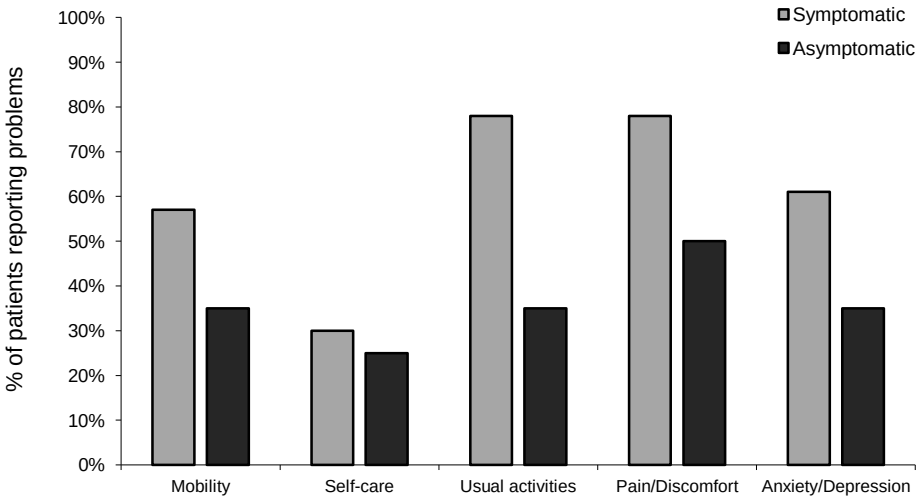


Table 4.2: Questionnaire scores in systemic sclerosis patients.

	Symptomatic SSc *Median (IQ range)*	***Asymptomatic SSc*** *Median (IQ range)*
SF-36		
-Total score	53.27 (35.67-73.27)	63.93 (53.47-77.84)
-Physical Health score	48.2 (29.4-72.5) *	61.7 (44.4-75.1)
-Mental Health score	61.7 (29.4-72.5)	65.15 (53.95-70.62)
Euroqol (EQ-5D)		
-Mobility	2 (1-2)	1 (1-2)
-Self-care	1 (1-2)	1 (1-1.5)
-Usual activities	2 (2-2) *	1 (1-2)
-Pain/Discomfort	2 (2-2)	1.5 (1-2)
-Anxiety/Depression	2 (1-2)	1 (1-2)
-VAS score	62 (53-80)	77 (60-80)
SSC-GIT 1.0		
-Reflux	55.6 (40.7-74.1) *	92.6 (83.35-96.3)
-Distension	50 (38.1-61.1) *	83.3 (72.2-94.35)
-Diarrhoea	55.5 (22.2-100) *	100 (88.9-100)
-Constipation	55.5 (22.2-100) *	100 (77.7-100)
-Abdominal pain	66.7 (50-100) *	100 (83.3-100)
- Social	81.7 (65-95) *	100 (100-100)
-Emotional	55.6 (29.6-81.5) *	100 (94.45-100)

* $p<0.05$; Comparisons made using the Mann-Whitney test

4.3.3. Anthropometric measurements

The weight, height and body mass index of the 2 patient groups were very similar. Mean BMI in symptomatic SSc patients was 22.9 (20.5-25.4) and in asymptomatic 22.7 (20.15-24.9). This was lower than in the general UK population as measured in a study of 989 women (mean BMI 26) (133) but within the 25-75th percentiles. The rest of the anthropometric measurements, mid-arm circumference, mid-arm muscle circumference and triceps skinfold thickness were also not significantly different between the 2 groups. The values are expressed as percentiles for sex and age matched controls. Values below the 5th percentile are indicative of nutritional depletion and values between the 10-15th percentiles of minimal depletion (128). The medians and interquartile ranges can be seen in table 4.3. The median percentile for mid-arm circumference was the 25th percentile in both groups and the mid-arm muscle circumference percentile the 25th and 50th respectively for the Sx and ASx group. There were 6 Sx and 6 ASx patients for who the MAC was below the 15th centile and 7 Sx and 8 ASx patients that MAMC was below the 15th centile. The mean triceps skinfold thickness was overall at the 10th percentile for both groups. There was no statistically significant difference between the 2 groups for these measurements. The handgrip measurements were 47% and 50.5% of the value predicted in the symptomatic and asymptomatic patients respectively. More than 50% of patients reported difficulty performing the handgrip test, 12 of 23 Sx patients and 12 of 20 ASx patients suffered from digital ischaemia or hand contractures.

Table 4.3: Anthropometric measurements of the 2 patient groups

	Symptomatic (n=23)		**Asymptomatic (n=20)**		
	Median	**IQ range**	**Median**	**IQ range**	**p value**
Weight	61	53-66	58.5	55-67	0.92
Height	158	150-169	163	158.5-168	0.98
BMI	22.9	20.5-25.4	22.7	20.15-24.9	0.8
MAC cm	27.5	26.5-29.5	27.65	25.75-29	0.63
MAC centile	25	10-50	25	10-37.5	0.52
MAMC cm	23.1	21.3-24.95	23.15	20.58-24.7	0.67
MAMC centile	25	10-50	50	10-75	0.63
TSF mm	16	12.5-18	16	13.75-21.5	0.39
TSF centile	10	5-25	10	5-25	0.63
Handgrip	47	36-72	50.5	37.5-66.75	0.78

Abbreviations: BMI – body mass index, MAC – mid-arm circumference, MAMC – mid-arm muscle circumference, TSF- triceps skinfold thickness

4.3.4. Malnutrition Universal Screening Tool

The majority of patients, both Sx and ASx scored 0 on the MUST scoring. One ASx patient scored 2 secondary to BMI<18.5 and one Sx patient scored 1 secondary to BMI of 19. Another Sx patient scored 1 secondary to weight loss and lastly one Sx patient scored 2 for both low BMI and weight loss.

4.3.5. Indirect calorimetry measurements

The indirect calorimetry results are shown in table 4.4. Due to time restraint of the patient on the day of the tests, there was no data obtained from one of the asymptomatic patients. There was no significant difference in the resting energy expenditure (REE) between the 2 patient groups, both as the measured value and as a percentage of the predicted value for the age, sex and BMI of each patient. Of the Sx patients 8 (44%) were hypometabolic (REE <90% predicted), 1 (6%) hypermetabolic (REE >110% predicted) and 9 (50%) normometabolic (REE 90-110% predicted); of the ASx patients 4 (25%) were hypometabolic, 1 (6%) hypermetabolic and 11 (69%) normometabolic. Typical traces of indirect calorimetry measurements from symptomatic and asymptomatic patients are shown in appendix 8.2.

4.3.6. Bioelectrical Impedance measurements

The results obtained from bioelectrical impedance measurements are shown in tables 4.5 and 4.6 and figure 4.5. Bioelectrical impedance measurements were obtained from 18 Sx and 16 ASx patients. Impedance, resistance, reactance were all statistically significantly lower in the symptomatic compared to the asymptomatic patients. The phase angle, a potential marker of morbidity and mortality, was also lower in the symptomatic group (Sx: 8.35 [5.51-11.13] vs ASx: 16.39 [10.52-22.26], p=0.01. There was a negative correlation between the phase angle and ECM/BCM (r=-0.3, p=0.047), an index of early protein catabolism.

The body composition measurements, specifically fat and fat free mass are shown both as percentages and also as fat and fat free mass index (mass/height^2). These values have been shown to be significantly different in males and females (134;135). Similarly body water compositions are affected by sex, women generally having lower body water composition than men (136). There were 3 male patients in the symptomatic group and for more accurate comparison between the 2 groups the values from the 3 male patients were excluded from the analysis, in order to avoid falsely significant differences.

Symptomatic patients had higher percentage of fat free mass and lower percentage of fat mass than the asymptomatic patients but this difference was not statistically significant. The fat mass indices were not significantly different in the 2 groups. In contrast, the fat free mass indices were higher in the symptomatic group (figure 4.5). Measurements of water spaces are presented in table 4.6. The male patients are again excluded from this analysis as it is well recognised that there are sex

differences in body water spaces (136). The TBW percentage was significantly higher in the symptomatic group compared to the asymptomatic group. The ICW percentage was lower and the ECW percentage was higher in the Sx patients. The TBW/FFM was higher in the Sx group 75.84 vs 72.26, p=0.04. This ratio is thought not to be influenced by gender and therefore analysis including the 3 male patients was also performed, Sx: 76.02 (73.47-78.56) vs ASx: 72.26 (69.73-74.78); p=0.034. ICW/FFM is possibly a better indicator of hydration and also not affected by gender, analysis including the 3 male patients showed Sx: 44.03 (41.51-46.59) vs ASx: 45.86 (42.39-49.32); p=0.36. There was no significant difference in the Sx vs ASx patients in either analysis. These results suggest that the TBW is increased in the symptomatic group with an increase in ECW/ICW ratio.

Table 4.4: Indirect calorimetry measurements of the 2 patient groups

	Symptomatic (n=23)		**Asymptomatic (n=19)**		
	Mean	**95% CI**	**Mean**	**95% CI**	**p value**
BMR	1301	1219-1383	1271	1220-1322	0.54
REE	1221	1131-1311	1202	1098-1306	0.78
REE %predicted	94.3	89.1-99.4	94.8	87.5-102.1	0.9
Resp. Quotient	0.81	0.78-0.84	0.78	0.7-0.86	0.44

Abbreviations: BMR – basal metabolic rate, REE – resting energy expenditure

Table 4.5: Bioelectrical impedance measurements of the 2 patient groups

	Symptomatic (n=18)		**Asymptomatic (n=16)**		
	Mean	**95% CI**	**Mean**	**95% CI**	**p value**
Impedance	608.4	540.3-676.6	862.5	759.1-965.9	**<0.0001**
Phase angle	8.35	5.51-11.13	16.39	10.52-22.26	**0.01**
Resistance	602	531.5-672.6	804.8	727.8-881.9	**0.0002**
Reactance	78.85	63.84-93.86	269.4	159.1-379.7	**0.0005**

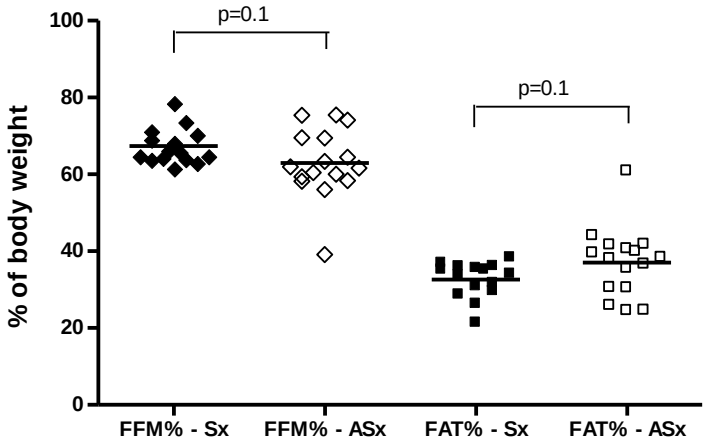
Table 4.6: Bioelectrical impedance measurements of body composition in the 2 patient groups, excluding the 3 male patients from the symptomatic group.

	Symptomatic (n=15)		**Asymptomatic (n=16)**		
	Mean	**95% CI**	**Mean**	**95% CI**	**p value**
FFM%	67.33	64.79-69.88	62.94	58.64-67.73	0.1
FFMI	15.87	14.96-16.77	14.33	13.78-14.88	**0.004**
FAT%	32.67	30.12-35.21	37.06	32.27-41.86	0.1
FMI	7.76	6.9-8.61	8.89	6.91-10.87	0.28
TBW%	51.11	48.68-54.15	45.34	41.83-48.84	**0.013**
ECW%	41.42	38.99-43.86	36.38	31.69-41.07	0.055
ICW%	58.57	56.13-61	63.61	58.92-68.31	0.055
BCM (Kg)	23.39	21.81-24.96	22.15	21.18-23.11	0.15
ECM (Kg)	17.99	16.76-19.21	16.92	15.62-18.22	0.21
ECM/BCM	0.77	0.74-0.81	0.76	0.71-0.82	0.78
TBW/FFM	75.84	73.39-78.29	72.26	69.73-74.78	**0.038**
ICW/FFM	44.47	41.87-47.07	45.86	42.39-49.32	0.5

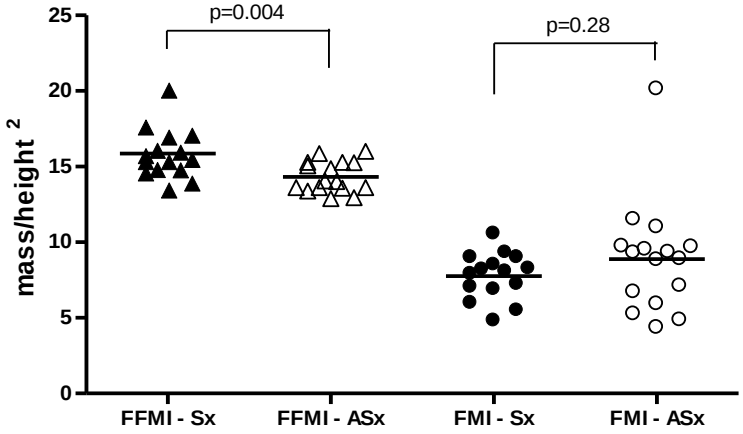
Abbreviations: FFM- fat free mass, FFMI – fat free mass index, FMI – fat mass index, TBW – total body water, ECW – extra-cellular water, ICW – intra-cellular water, BCM – body cell mass, ECM – extra-cellular mass

Figure 4.5: A. Fat mass and fat free mass percentages in the symptomatic and asymptomatic patient groups. B. Fat and fat free mass indices in the 2 patient groups.

A



B



4.4. Discussion

Several chronic inflammatory conditions are associated with changes in body composition and malnutrition. There is data suggesting that SSc patients are at a risk of malnutrition, especially those with GI symptoms (124). My study of 43 SSc patients, both with and without GI symptoms aimed to assess if there are significant differences in the 2 groups of patients by anthropometric measurements, indirect calorimetry and bioelectrical impedance measurements. This was an exploratory study collecting nutritional assessment data from SSc patients investigated for other aspects of gastrointestinal involvement. This study as an exploratory study was not powered to assess specific differences between the 2 groups. The data presented, especially the bioelectrical impedance data, adds to the existing limited data on SSc patients and can be used to plan further studies.

BMI is a commonly cited measurement but often not an accurate tool to assess malnutrition as shown in a number of studies (137;138). In this study BMIs in symptomatic and asymptomatic patients were similar (median <23) but lower compared to the general British population. In a large study that looked at self-reported weight and perception of weight in the British population in 1999 and 2007 it was shown that BMI increased over that period. The BMI of 1836 subjects studied in 2007 was 26.2, mean age 47.6. In the 989 women studied (mean age 46.7) BMI was 26.05. Although our patient group mean age was higher, it is well recognised that BMI tends to increase with age. This difference in BMI compared to that of the general British population does suggest that there is a degree of reduced nutrition in SSc patients. This is further supported by the measured TSF values.

Although none of the measurements indicated severe depletion, the median TSF lay below the 15th centile in both groups suggestive of mild depletion. TSF relies on measuring skin thickness which may be difficult to assess and less accurate in a disease that affects skin thickness although this would be expected to be increased rather than decreased. Lundberg *et al* had found increased mean TSF in their patients with SSc, of which 17/30 had dcSSc, compared to controls (123). The exact effect of increased skin thickness to TSF is not known. This is likely to be varied depending on the extent and severity of skin thickness and therefore it would be difficult to apply a correction factor for skinfold measurements in patients with dcSSc. Only eleven of 43 patients in this study had dcSSc, TSF measurements would not be expected to be affected in lcSSc as by definition the skin is affected below knees and elbows in lcSSc.

Although handgrip strength is normally a good measure of protein malnutrition, in SSc patients this may not be applicable. The handgrip measurements obtained were low, but in the case of SSc patients this is most likely a reflection of impaired hand function secondary to digital vasculopathy or hand contractures rather than malnutrition.

Surprisingly despite low BMI and anthropometric measurements there were only 4 patients that scored 1 or 2 in the MUST score, thus putting them at medium or high risk of malnutrition. This is in contrast to a Canadian study of 586 SSc patients that showed that 11% and 17% of patients were at medium and high risk of malnutrition

respectively (124). A possible reason for the lower rates of malnutrition based on the MUST scores in this study is the small size of the patient groups. According to

the Baron study, there was an association of MUST score with overall disease severity as indicated by the global physician's assessment. My patient cohort was in the majority patients with relatively stable disease, including their GI disease and this may be another reason for the lower number of patients with MUST scores 1 or 2. Nonetheless MUST is a useful and easy to calculate nutritional score capable of predicting GP visits, hospital admissions, length of hospital stay and mortality (121) and it or another screening tool should be calculated in SSc patients regularly both in the inpatient and outpatient setting.

Indirect calorimetry is a relatively easy and accurate way of measuring REE. There have been numerous studies using indirect calorimetry in cancer patients, critically ill patients in intensive care and a number of other conditions but there is little evidence of indirect calorimetry being used in SSc patients. A recent study of 714 cancer patients and 642 controls showed that a higher proportion of cancer patients were hypermetabolic compared to controls (139). This study used mREE/pREE (% predicted REE) to account for differences in height and weight as well as age and sex. Although SSc patients may be predisposed to malnutrition, this was not explained by differences in the metabolic rate. The indirect calorimetry measurements did not identify any difference in resting energy expenditure between the 2 SSc groups. Only 2 patients (1 Sx and 1 ASx) were hypermetabolic with the majority of patients being normometabolic. Although BMI was lower in SSc patients compared to British population the fact that MUST scores were largely 0 suggest that any weight loss was not recent. There is anecdotal evidence that patients lose weight shortly after diagnosis and their weight then stabilises thus

explaining my findings. Another possible explanation is that this is still a small patient group and therefore small differences are less likely to become evident in this sample size. Predicted REE is calculated using the Harris-Benedict equation. This equation has been based on normal healthy population. A number of studies have shown that predictive equations are not as accurate as indirect calorimetry in patients who are critically ill (140), malnourished (141) or possibly patients with alterations in body composition (142) as found in my study. Therefore the conclusion of whether patients were normometabolic or hyper/hypometabolic may not be accurate. Nonetheless the measured REE was not different between the symptomatic and asymptomatic groups in my study.

Another explanation is that the ensued malnutrition is secondary to malabsorption or energy deficiency rather than increased energy consumption. Marie *et al* in a recent study of 51 SSc patients showed that 43% had small intestinal bacterial overgrowth (SIBO) with associated GI symptoms (143). None of the patients in the asymptomatic group had SIBO, 3 symptomatic patients had antibiotics for bacterial overgrowth in the 3 months prior to the study but none on the day of the study. Krause *et al* showed that 50% of the patients they studied had lower energy intake than their energy requirements and that nutritional intervention had a significant effect on nutritional status (137). Although I did not assess the nutritional intake of the patients studied, the majority of patients had what they considered a normal diet; only one of the symptomatic patients and none of the asymptomatic were on nutritional supplements.

Bioelectrical impedance analysis measures the restriction of flow through the body of an electric current. The body offers 2 types of restriction of flow of the electric current, resistance and reactance. The resistance is primarily related to the amount of water present in the tissues and reactance is the resistive effect produced by cell membranes. In this study the Sx SSc patients had much lower impedance, secondary to both lower resistance and reactance, than the ASx patients. These differences may be the first, subtle changes in the body reflecting nutrition and hydration status. The relationship between resistance and reactance is measured as the phase angle and reflects different electrical properties of tissues and the relative contributions of fluid and cellular membranes which can be affected by various diseases. The phase angle has been described as a prognostic tool in various chronic diseases such as liver fibrosis and renal failure, motor neuron disease and also in malignancy such as lung, breast, pancreatic and colorectal cancers (144-148). Smaller phase angles suggest cell death. Sx SSc patients in this study did have a lower phase angle compared to ASx patients. In patients with advanced cancer, phase angle scores lower than 5.6 have been associated with shorter survival. In the Sx SSc patients in my study, although the phase angle scores were lower than those of the ASx patients, they were not as low as the above scores. Krause *et al* in their study of 124 SSc patients found poor nutritional status and lower phase angle values in patients with more active and severe disease. The overall phase angle values recorded in their study were much lower (mean 4.7). They also found that patients with malnutrition had a higher risk for SSc-related mortality and that phase angle was the best predictor for mortality (137). It is likely that other organ involvement, rather than just gastrointestinal involvement, contributes to the

phase angle values and therefore its prognostic potential. Although the groups of patients studied had largely similar pattern of other organ involvement, there were not enough patients to perform such subgroup analysis.

Bioelectrical impedance is becoming increasingly utilised for assessment of body compartments. It has been used to assess both fat free mass and body fat mass but also body water compartments. Fat free mass and fat mass are more qualitative tools than BMI in the evaluation of nutritional status and they have been shown to be predictors of outcome (149;150). Reference intervals for FFMI and FMI for Caucasian European population were recently published (134). A study comparing patients at the time of hospital admission compared to controls showed a higher prevalence of low FFM and high FM (151). COPD patients who had low FFM had worse outcome post lung reduction surgery (152) and HIV patients with higher FFM had better physical functioning. RA patients often display reduced FFM, regardless of disease activity, together with stable or increased FM, in keeping with the concept of rheumatoid cachexia (153). In this study of SSc patients, the ASx patients had lower FFMI than Sx patients. This finding is unexpected in that the Sx patients would be more likely to develop malnutrition. For the analysis, the 3 male patients in the symptomatic group were excluded as there are significant gender differences in FFMI and I did not want to bias the results. Two of the 3 male patients had moderate to severe GI disease and therefore the difference between the 2 groups may not be as significant. As fat free mass consists of total body water, this difference may reflect more the difference in total body water especially as body cell mass was not different between the 2 groups. Krause *et al* compared SSc

patients with age and sex matched controls and did not find a difference in lean body mass and fat mass between the 2 groups. They did though find higher ECM and a higher ECM/BCM ratio in SSc patients than controls (137), thought to reflect increased fibrosis present in SSc patients. I did not find a difference in ECM/BCM ratio between the symptomatic and asymptomatic SSc patients but this would be explained by the fact that there was a similar ratio dcSSc:lcSSc in both groups of patients and the amount of fibrosis present is unlikely to differ significantly.

Another important element of bioelectrical impedance is the calculation of body water compartments. Hydration disorders occur in both health and disease. In healthy people hydration affects cognitive function and exercise performance. In disease, over-hydration is frequently caused by heart, kidney or liver failure. In the SSc patients studied there was a significant difference in TBW between the Sx and ASx group. The percentages of ICW and ECW were also significantly different but not the ICW/FFM ratio. This has been suggested by Ritz *et al* to be a better indicator of cellular hydration and influenced less by gender and BMI (136). TBW/FFM was statistically higher in the Sx group, more so when the 3 male patients were included in the analysis. The fact that ICW/FFM ratio is not different in the 2 groups indicates that this difference in water spaces may not be secondary to differences in hydration. A possible explanation is that it is secondary to oedema, which would be indicated by the higher ECW%, although this would be subclinical as none of the patients had clinical oedema. Bioelectrical impedance seems to be a sensitive tool for evaluation of body water spaces as well as fat free and fat mass and is increasingly used in a number of chronic conditions (154). In a chronic disease such

as SSc which can affect multiple organs, it may prove to be a valuable tool in assessing not only nutritional status but hydration status also.

At the time of the study design and data collection there was no data on bioelectrical impedance in SSc and only a couple of studies that had assessed nutrition, one by anthropometric assessments and dietary assessment (123) and one using bone mass density scan (155). Baron *et al* demonstrated increased risk of malnutrition in SSc patients as assessed with the MUST (124) and Krause *et al* recently showed for the first time that bioelectrical impedance is a useful nutritional tool in SSc patients with differences observed between SSc patients and healthy controls (137). Although Baron *et al* assessed the impact of GI symptoms on MUST scores and risk of malnutrition, Krause *et al* assessed consecutive SSc patients without specific reference to GI involvement. My study was an exploratory study of the use of bioelectrical impedance in SSc and on whether GI involvement causes changes in body composition as well as assessment of other nutritional assessment tools such as indirect calorimetry. The patients that I studied were all except one outpatients and with generally stable disease severity. This may explain the lower proportion of patients at risk of malnutrition based on the MUST score. Nonetheless the mean BMI, was lower than that of the British population. Although more commonly used markers of malnutrition such as BMI, which is part of MUST, were not different I showed that there are differences in body composition between patients with moderate to severe GI symptoms and those without. It is clear that further, larger studies on nutritional assessment and the effect of GI symptoms in development of malnutrition in SSc patients are needed. This study, as

an exploratory study proved that a detailed nutritional assessment based on anthropometric measurements and bioelectrical impedance can be easily undertaken without being very time consuming for the patients, involving invasive procedures or a need for significant resources. This kind of assessment could be undertaken in the outpatient setting. Indirect calorimetry is more time consuming, needs more equipment and in the outpatient setting is less useful and should probably be reserved for patients with more severe GI involvement and malnutrition as a tool for more accurate assessment of nutritional need. Through studying a larger number of patients with a variable extent of gastrointestinal involvement, both in terms of site involved and severity, as well as patients without gastrointestinal involvement we would be able to assess in the future the risk of malnutrition, the disease pattern associated with higher risk and early identifying features so as to try and prevent its occurrence.

The findings of the studies undertaken as part of this thesis so far have helped define more the pathophysiological processes involved in gut involvement in SSc. I have also shown though how there are multiple processes involved and the difficulty in identifying single causative factors for symptoms thus also making it challenging identifying therapeutic options. In a number of autoimmune and inflammatory conditions including systemic sclerosis animal models have been used to help both understanding disease processes and in testing new therapies.

CHAPTER 5:

GASTROINTESTINAL MANIFESTATIONS IN A TRANSGENIC MOUSE MODEL OF SYSTEMIC SCLEROSIS

Publication presented from this chapter can be found in Appendix 2

5.1. Introduction

Animal models exist for a number of diseases and are widely used to understand disease processes in vivo. Animal models of systemic sclerosis have provided valuable insights into its pathogenenesis and have provided the means to test potentially useful therapeutic interventions. The majority of models are murine, and can therefore benefit from the large number of inbred mutant mouse strains with detailed genetic, cellular and molecular information available for these strains. Vasculopathy, activation of the cellular immune system and fibrosis are key features of the pathogenesis of SSc found in the skin but also internal visceral organs, including the gastrointestinal tract. Although none of the animal models reproduce all the pathogenetic components, several of them show some of the typical abnormalities of SSc. The changes seen in the gastrointestinal tract differ in these animal models. Cellular infiltration and extracellular matrix (ECM) deposition are the most commonly seen features. Some of these animal models and specifically those with gastrointestinal tract involvement are briefly described below.

University of California at Davis (UCD 200/206) chickens

This avian model of SSc derives from male Leghorn chickens. These chickens develop skin and systemic manifestations resembling human SSc. The chickens develop oedema and Raynaud's like changes in their combs shortly after hatching. In a percentage of animals, the skin of the neck and back becomes oedematous and indurated and ischaemic lesions of the toes may also develop. Internal organs, including the oesophagus, heart, lungs, and kidneys, become involved in the disease

process between 3 and 6 weeks (156). Histopathologically the lesions resemble SSc. There is perivascular mononuclear cell infiltration in all layers of skin and subcutaneous tissue, signs of an obliterative vasculopathy and endothelial cell apoptosis with loss of functional capillaries and ECM deposition by fibroblasts which leads to fibrosis of the skin and the combs (157). Apart from histopathological findings, UCD-200 chickens develop autoantibodies, including antinuclear antibodies with a centromeric staining pattern, antiphospholipid antibodies and rheumatoid factor (156). In a study investigating involvement of internal organs in this model Nguyen *et al* showed that the oesophagus of UCD 200 chickens, was the most affected internal organ, showing mononuclear cell infiltrations and increased deposition of collagen (158). UCD-206 chickens resemble the UCD-200 line but often have more severe disease manifestations.

Mouse model of bleomycin-induced dermal fibrosis

Bleomycin has strong anti-tumour effects but its use is limited by dose-dependent toxicity, mainly lung fibrosis but SSc-like skin fibrosis has also been reported. Due to its profibrotic effects bleomycin administration has been used to study murine pulmonary fibrosis. Yamamoto *et al* established a model of skin fibrosis by daily subcutaneous injection of bleomycin for 4 weeks (159). In addition to fibrotic changes of the skin, lung fibrosis with thickened alveolar walls and cellular infiltrates also developed. The heart, kidneys and gastrointestinal tract were not involved. Bleomycin induced fibrosis can occur in various mouse strains but there are variations between strains in the severity and length of bleomycin administration required. Bleomycin induced skin fibrosis mimics inflammatory

changes that occur early in the disease process of SSc. It induces the production of reactive oxygen species, endothelial cell damage and expression of adhesion molecules attracting inflammatory cellular infiltrate (leukocytes, mast cells, macrophages) which leads to activation of fibroblasts. Furthermore there is sustained activation of TGF-β and other inflammatory and pro-fibrotic cytokines (159). The fibrosis though is only local without a systemic effect and also without typical vascular phenomena. The bleomycin-induced skin fibrosis model has been used to evaluate anti-inflammatory and anti-fibrotic therapies.

Graft-versus-host disease (GVHD) model

These murine models reflect immunologically driven fibrosis in the skin and other target organs and have some similarity with human GVHD, which in its chronic form can replicate some important features of systemic sclerosis. Human chronic GVHD occurs following haematopoetic cell transplantation. There are 2 types, cytotoxic and sclerodermatous GVHD, the latter resembles clinically diffuse SSc. These similarities lead to the development of a mouse model of SSc by transplanting haematopoetic cells from donor to recipient mice mismatched for minor histocompatibility antigens (160). Approximately 2 weeks after transplantation, T cells, monocytes, mast cells, and other leukocytes infiltrate affected tissues and stimulate resident fibroblasts to release large amounts of ECM proteins (161). TGF-β has been shown to play a critical role in this process (162). Three weeks after bone marrow transplantation, sclerodermatous GVHD mice develop pulmonary and dermal fibrosis with loss of dermal fat and atrophy of appendages (160). Ruzek *et al* developed a modified GVHD model by using RAG-2KO mice genetically deficient in

mature T and B cells as recipients for transfer of donor B10.D2 spleen cells to induce the GVH SSc disease (163). This model demonstrates the fibrotic effects of dermal thickening of extremities and progressive fibrosis of internal organs as well as vasoconstriction and morphologic changes in smooth muscle cells surrounding vessels and evidence of early immune responses. There was also intestinal involvement in this model, affecting mainly the large intestine where accumulation of connective tissue is observed in the lamina propria and submucosa (163).

Type 1 and type 2 tight skin (Tsk-1 and Tsk-2) mouse strains

Tsk-1 is a spontaneous dominant mutation that occurred in the inbred B10.D2 (58N/Sn) strain. Homozygous animals die in utero whereas heterozygous animals develop SSc like features with skin fibrosis as well as visceral changes such as heart enlargement and lung abnormalities, namely distended lungs and emphysema like changes (164). Fibrosis in Tsk-1 mice arises from increased ECM production by activated fibroblasts, although the exact mechanisms remain unclear. Cellular immunity (in view of production of autoantibodies in the Tsk-1 mouse), inflammatory cells and the fibrillin-1 gene have all been implicated. The second tight skin mutation is an autosomal dominant mutation resulting from administration of the mutagenic agent ethylnitrosourea (165). Again only heterozygous animals survive. Tsk-2 mice develop fibrosis of the skin and internal organs. They also display inflammatory cell infiltrates in the affected tissue and autoantibodies such as anti-Scl-70, anti-centromere and anti dsDNA. The internal organ changes seen in these 2 models do not fully mirror the changes seen in SSc,

for example they develop emphysematous rather than fibrotic changes in the lungs. Intestinal involvement has not been reported in these mouse models.

Transgenic and knockout mouse models

Improved genetic techniques have allowed the development of specific transgenic and knockout animals that enabled further characterisation of the signalling pathways, cytokines and inflammatory mediators involved in the disease process. Examples of such mouse models are the caveolin knock-out mice and mice with mutations involving the platelet-derived growth factor receptors and TGF-β signalling. Some of these mouse models have demonstrated gastrointestinal involvement.

Caveolin knock-out mice

Caveolin is a plasma membrane-associated protein responsible for caveolae formation, flask-shaped membrane invaginations. Caveolins modulate receptor complexes and signal transduction. Radiation induced lung fibrosis has previously been found to be associated with down-regulation of caveolin-1 in alveolar epithelium. This was confirmed in more recent studies and caveolin-1 knockout mice were found to induce a systemic fibrotic disease affecting the lungs and other organs (166). Alveolar septae are thickened due to hypercellularity and increased deposition of ECM. In addition to lung fibrosis, caveolin knockout mice develop skin fibrosis with a marked increase in collagen deposition. These mice also display vascular changes with altered vessel tone and permeability as well as pulmonary

hypertension on histological analysis (167). In the intestine caveolin knockout mice show abnormalities in pacing and contractile activity of the small intestine (168).

Platelet derived growth factor (PDGF) receptor mutations

PDGF signalling regulates the development of mesenchymal cell types including proliferation, survival, migration, and control of differentiation. PDGF receptor alpha (PDGFRα) signalling serves critical functions during embryo development. Aberrant PDGFR signalling has been implicated in diverse fibrotic conditions (169) where fibroblasts proliferate and deposit excessive connective tissue matrix, leading to progressive scarring and organ dysfunction. The overactivation of PDGFRα signalling has been investigated in knockin mouse lines with mutations of PDGFRα. These mice developed tight skin that adhered strongly to underlying muscle. Connective tissue fibrosis was noted in heart muscle, around bronchioles, in skeletal muscle and in the kidneys as well as the gastrointestinal tract (170).

$TBRI^{CA}$; Cre-ER mice

Overactivity of TGF-β leads to extracellular matrix overproduction and promotes myofibroblast differentiation. It is thought to play a key role in the development of systemic sclerosis. Bou Gharios *et al* had previously identified a largely fibroblast specific, transcriptional enhancer far upstream of the transcription start site of the pro-a2(I) collagen gene (171). Sonnylal *et al* using a Cre/loxP system in which tamoxifen-inducible recombinase was activated after birth, generated transgenic mice in which TGF-β signalling was disrupted after birth (172). The $TBRI^{CA}$; Cre-ER mice when injected with tamoxifen which activated TGF-β signalling developed

marked skin fibrosis and thickening of the walls of the small arteries of the lungs and kidneys, key pathological features of SSc. They also showed increased collagen accumulation and abnormalities in extracellular matrix deposition. These mice though did not show fibrotic lesions of the lungs or other internal organs (172).

TβRIIΔk mice

Denton *et al* generated another transgenic mouse strain with fibroblast specific TGF-β signalling activation (173;174). They used the same promoter of the pro-a2(I) collagen gene to express a kinase deficient type II TGF-β receptor (TβRIIΔk) in fibroblasts. Despite TβRIIΔk having previously been characterised as a dominant negative inhibitor of TGF-β signalling, adult mice expressing this construct demonstrated TGF-β overactivity and developed skin fibrosis. A proportion of animals also developed significant lung fibrosis paralleling the human disease (173). Further experiments from the same group examined the effect of experimental injury to alveolar epithelial cells and showed that even minor epithelial injury induced significant lung fibrosis (175). More recently Derrett-Smith *et al* showed diminished aortic ring contractility and relaxation in this mouse model compared to wild-type controls and associated aortic adventitial fibrosis and smooth muscle attenuation in the thoracic aorta as well as cardiac fibrosis (176). This mouse model was used in the experiments described in this chapter.

The development of novel animal models of SSc is an ongoing process, and, particularly, the repertory of genetic mouse models is expanding (177). Nonetheless, no animal model so far exhibits all aspects of the disease process. The GI tract has not been the focus in these animal models but is affected in some of

them mainly characterised by fibrosis. As the TβRIIΔk mice show skin and pulmonary fibrosis as well as a structural vasculopathy it was felt that vascular and fibrotic changes may also occur in the gastrointestinal tract of these mice. The aim of this study was to assess histologically and physiologically the gastrointestinal tract and specifically the lower GI tract of the transgenic TβRIIΔk-fib mouse strain to see if any of the key features of SSc seen replicated in other organs are also evident in the lower GI tract.

5.2. Methods

5.2.1. Animals

5.2.1.1. Generation of genetically modified mouse model TβRIIΔk-fib

The transgenic (TG) TβRIIΔk-fib strain was generated and characterized by Professor Christopher Denton at University College London and University of Texas, and has been described previously (173;174). In brief, a fibroblast-specific expression cassette was devised in which there was expression of the target cDNA but also co-expression of a marker gene. The fibroblast-specific expression cassette was subcloned from the upstream region of the proα2(I)collagen gene. This incorporates a fragment between -19.5 and -13.5kb upstream of the transcription start site that, when linked to an endogenous minimal promoter drives gene expression at high levels in fibroblasts, but not in other type I collagen producing cells. Reporter genes linked to this promoter-enhancer show high level fibroblast-specific expression in embryonic development and postnatally. The mouse strain

TβRIIΔk-fib was generated by subcloning the cDNA encoding the extracellular and transmembrane portion of the human type II TGFβ receptor into the Sal1 site of the pCD3 expression vector. A bacterial β-galactosidase marker gene (LacZ) was co-expressed from a dicistronic transgene mRNA product via an encephalomyocarditis virus internal ribosome entry site sequence (IRES) (178). Neonatal pups were genotyped by PCR analysis of genomic DNA extracted from tail-biopsy specimens, by using primers specific for the β-galactosidase reporter gene (5'-CGGATAAACGGAACTGGAAA- 3' and 5'-TAATCACGACTCGCTGTATC-3') (Sigma-Genosys, Haverhill, UK). Littermate wild-type (WT) C57/B6 mice were used as control animals.

5.2.1.2. Animal housing

The mice examined were both male and female transgenic mice and littermate wild-type controls. Animals were housed in a clean conventional colony, with access to food and water *ad libitum*. Strict adherence to institutional guidelines was practiced, and full local ethics committee and Home Office approval were obtained prior to all animal procedures.

5.2.2. Histological Analysis

5.2.2.1 Sample collection

Animals were sacrificed by cervical dislocation following narcosis with carbon dioxide. The stomach and the small and large intestine were dissected en bloc and

immersed in 10% formal saline containing 4% formaldehyde (CellPath, UK) for histology.

5.2.2.2. Tissue processing and preparation

For formalin-fixed paraffin-embedded (FFPE) specimens, gut tissue was fixed overnight in 10% formal saline containing 4% formaldehyde (CellPath, UK). Fixed tissues were processed and dehydrated overnight, and embedded in molten paraffin wax. For routine histology and immunohistochemistry, 3µm sections were cut using a Leica UK microtome, and mounted onto polylysine coated microscope slides (VWR, UK).

5.2.2.3. Routine histology

Prior to staining, sections of stomach, small and large intestine were de-waxed in xylene (Genta Medical, UK), and passed through graded alcohols to water. Gastrointestinal tract architecture was determined by staining with haematoxylin and eosin (H&E) as per standard protocols. Non fibrillar collagen deposition was detected by sequential staining with Weigert's iron haematoxylin followed by picrosirius red. Following staining, sections were dehydrated with alcohol, cleared in xylene, and mounted with a permanent mount (DPX). Unless stated, reagents were purchased from Raymond Lamb Ltd, UK.

5.2.2.4. Immunohistochemistry

Immunohistochemical analysis was performed on FFPE gastrointestinal sections. Sections were pre-treated with methanol and 0.5% hydrogen peroxide (VWR, UK) for 10 minutes to quench endogenous peroxidase activity, followed by antigen

retrieval in citrate buffer 10mM (pH 6) by microwave for 5-20 minutes. Samples were washed x 3 between steps with phosphate buffered saline (PBS; Oxoid, UK). Endogenous biotin activity was blocked using an avidin/biotin blocking kit (Vector Laboratories, US) according to the manufacturer's instructions. Primary antibodies used were: 0.7μg/ml mouse monoclonal anti α-smooth muscle actin (α-SMA; Sigma-Aldrich, St. Louis, MO), 0.66μg/ml of the proliferation marker rat anti-Ki67 Tec-3 (; Dako, Denmark), and 100μg/ml of rabbit phospho-SMAD2/3 (pSMAD2/3) (Santa Cruz Biotechnology, Santa Cruz, CA) a marker of TGF-β pathway activation. For neural staining 10μg/ml of protein gene product (PGP) 9.5, a general neural tissue marker and 2μg/ml of anti-S-100 (a glial cell marker) were used. A mouse-on-mouse immunodetection kit was used with the mouse monoclonal primary antibodies, in order to reduce non-specific binding. After washing with PBS, sections were sequentially incubated for 30 minutes with a 1/200 dilution of a biotinylated secondary antibody, followed by Vectastain avidin biotin complex (ABC) - peroxidase conjugate, (or Vectastain ABC - alkaline phosphatase for α-SMA). Sections were developed using chromogen DAB (3,3' diaminobenzidine tetrachloride). α-SMA was developed using alkaline phosphatase substrate with levamisole solution to block endogenous alkaline phosphatase activity. Finally, sections were briefly counterstained with Meyer's haematoxylin, and prepared for permanent mounting. Unless stated, reagents were purchased from Vector, USA.

All histology and immunohistochemistry sections were viewed and photographed using a Zeiss Axioscope light microscope (Carl Zeiss, Germany) with Axiovision software. Sections were photographed at x100 and x200 magnifications.

The images of large and small bowel stained with Ki-67 and pSMAD2/3 antibodies were further analysed for quantification of positively stained cells. At x200 magnification, positively stained cells (brown) and negatively stained cells were counted in up to 10 crypts and results given as a proportion of all cells counted. Thickness of muscularis propria (both longitudinal and circular muscle) was assessed in tissue stained with α-SMA. The muscularis propria was measured in representative sections of large intestine at x100 magnification using the Axiovision software.

Representative small and large bowel sections stained with picrosirius red were photographed at x25 magnification thus allowing the whole bowel section to be photographed in one field and the extent of collagen deposition seen was quantified using the NIS Elements BR 2.30 system (Nikon, Japan). The colour wavelengths of the image were transformed into digital readings, allowing for quantification of the various colour wavelengths with pixels as the unit of measure. The colour spectra were analyzed and those corresponding to collagen (red wavelengths) were quantified as percentage of the whole tissue. Comparison was made between transgenic mice and wild-type controls. Results are given as mean percentage of tissue +/- standard deviation.

5.2.3. Isometric tension measurement in isolated large intestine

Transgenic mice (6.5-7.5 month old) and sex and age matched wild-type littermate controls were sacrificed by cervical dislocation following narcosis with carbon dioxide. The distal large intestine was dissected and immersed in 10% formal saline containing 4% formaldehyde (CellPath, UK) for histology. The proximal large

intestine was dissected and immersed in fresh Krebs buffer (119 mmol/L NaCl, 4.7 mmol/L KCl, 1.2 mmol/L $MgSO_4$, 1.2 mmol/L KH_2PO_4, 11 mmol/L glucose, 25 mmol/L $NaHCO_3$, 2.5 mmol/L $CaCl_2$). Large intestine, specifically the caecum, was washed in aerated fresh Krebs buffer, and the loose connective tissue removed. It was then cut longitudinally in 2-3 mm wide rings and the rings cut along the mesenteric border to form strips of tissue 2-3 mm x 7-10 mm long. These strips were tied at each end with thread and mounted on the transducers in 7 ml organ baths containing Krebs buffer at 37°C, continuously oxygenated with 95% O_2/5% CO_2. Isometric tension was measured with force-displacement transducers (Grass Instruments, Quincy, MA), and digitized using a multichannel recording system (Grass Instruments, Quincy, MA) with MP100 acquisition unit and AcqKnowledge software (Biopac Systems, Goleta, CA). A resting tension of 500-550 mg was applied to the strips, which were then allowed to equilibrate for 60 minutes. In this period, tissues were washed out with Krebs buffer, and the applied tension readjusted at 15-minute intervals (179;180).

After the equilibration period, the intestinal strips were contracted with cumulative doses of potassium chloride (KCl) (30 mmol/L and 80 mmol/L) until a stable contraction plateau was reached. Contractile responses were measured by recording changes in tension (milligrams). After washout, the tissues were allowed to re-equilibrate for 30 minutes, and contractile dose-response curves were constructed using cumulative doses of carbachol (10^{-9} to 10^{-4} mol/L) and 5-hydroxytryptamine (5-HT; 10^{-9} to 10^{-4} mol/L) (181) with washout and equilibration after each dose response curve. As a negative control 1 strip was pre-treated for 20

minutes with 10μM atropine before contractile responses to carbachol were measured. The gut contractile response of 6 mice (3 transgenic and 3 wild-type) was examined on 2 experiments (3 mice, mix of wild-type and transgenic) performed on consecutive days. The scale of contractile responses was different in the 2 experiments and therefore if the results were expressed in milligrams it would not be possible to combine the 2 experiments. Therefore the maximum contractile response was established for each experiment and the rest of the contractile responses are expressed as percentage of the maximal contraction. Data are expressed as mean ± SEM.

5.2.4. Statistics

Student t-test was used to compare quantitative values obtained between transgenic and wild-type animals described above. A value of $p<0.05$ was considered significant.

5.3. Results

5.3.1. Animals

The mice examined were both male and female transgenic mice and equal number sex and age matched littermate wild-type controls. Initially 2 age groups of mice were sacrificed, 3 transgenic and 3 wild-type controls aged 11-13 months and another 6 (3 transgenic and 3 wild-type) aged 5.5-6.5 months. Fibrosis may develop in gut tissue with age and therefore 2 age groups of mice were examined. Colonic

tissue from another 3 transgenic and 3 wild-type mice (6.5-7.5 months old) was used for the isobaric tension measurements of large bowel and other histological analysis.

5.3.2. Histological analysis

5.3.2.1. Routine histology

The gastrointestinal tract architecture was examined by H&E staining. Figure 5.1 shows large intestinal mucosa with the anatomical layers identified. Figure 5.2 shows representative examples of H&E staining from large intestine, small intestine and stomach. There was no difference in the gastrointestinal tract architecture between the transgenic TβRIIΔk-fib strain and wild-type mice and between the 2 age groups. Picrosirius red staining was used to assess collagen deposition. Representative examples are shown in figure 5.3. The amount of collagen was estimated as percentage of tissue stained red with picrosirius red for small and large intestine, results given as percentage of tissue. Collagen deposition was higher in the large intestine (% of tissue stained red: WT: 6.4 ± 1 vs TG: 9.2 ± 0.9; p=0.059) and the small intestine (WT: 4.6 ± 2 vs TG: 6.17 ± 1.5; p= 0.23) of the transgenic mice compared to the wild-type controls although this did not reach statistical significance.

5.3.2.2. Immunohistochemistry

Smooth muscle cells were stained with α-SMA antibody. There was no obvious smooth muscle atrophy observed in the mouse GIT of transgenic TβRIIΔk-fib strain

or wild-type control mice. Although previously smooth muscle attenuation has been reported in the aorta of this transgenic mouse model, there was no obvious difference in smooth muscle thickness in small and large intestine between transgenic and wild-type mice. Representative examples of α-SMA staining are shown in figure 5.4.

Cellular proliferation was assessed using the proliferation marker Ki-67, a nuclear protein associated with the cell cycle and expressed from late G_1 through to M phase (182). There was no difference in the number of positively stained cells between the transgenic mice and wild-type controls both in the small (WT: 67.8 ± 15.2 vs TG: 80.8 ± 3.2; p=0.68) and large intestine (WT: 43.9 ± 9.8 vs TG: 41.5 ± 10.2; p=0.63). TGF-β pathway activation was assessed through staining for pSMAD2/3. No difference in positively stained cells was seen between transgenic and wild-type mice in the small (WT: 41.7 ± 18 vs TG: 42 ±17.9; p=0.97) or large (WT: 34.2 ± 15.6 vs TG: 39.3 ± 16.5; p=0.51) intestine. Representative examples of Ki-67 and pSMAD2/3 staining are shown in figures 5.5 and 5.6.

5.3.3. Isometric tension measurement in isolated large intestine

In systemic sclerosis, gastrointestinal involvement has been attributed to dysmotility and smooth muscle atrophy and fibrosis. In order to investigate whether gut dysmotility occurs in the TβRIIΔk-fib mouse strain I examined responses of strips of proximal large intestine in isolated organ bath experiments. Colon smooth muscle contraction was induced with potassium chloride (KCl) which

directly causes smooth muscle cell contraction and then with carbachol a known cholinergic agonist. Contractile responses to KCl were significantly reduced in transgenic mice at 80mM concentration (WT: 53.5 ± 14.2 vs TG: 13.5 ± 12.8; p=0.022) but not at the lowest 30mM concentration (WT: 48.9 ± 30.5 vs TG: 33.5 ± 29.6; p=0.56) (figure 5.7and 5.8A). Contractile responses to carbachol were reduced in transgenic mice compared to wild-type controls. The difference was significant in the higher carbachol concentrations 10^{-5} (WT: 90.4 ± 8.7 vs TG: 50.6 ± 17.8; p=0.025) and 10^{-4} (WT: 64.6 ± 7.2 vs TG: 31.97 ± 13.9; p=0.023) (figure 5.7 and 5.8B). The addition of atropine to the organ bath after the initial contractions to KCl abolished any contractile response to carbachol in both experiments. 5-HT has been shown to be an intestinal neurotransmitter and therefore response to 5-HT was also investigated. No significant contractile response was observed following 5-HT addition in either wild-type or transgenic mice.

In order to further investigate the reduced contractile response of colon smooth muscle observed in transgenic TβRIIΔk-fib strain mice, the remaining colon was used as described above for histology and immunohistochemistry. H&E and picrosirius red staining was performed as described earlier for the 2 age groups of mice. No difference was seen between transgenic and wild-type mice in large intestinal architecture and there was no evidence of increased inflammatory cells. There was higher collagen deposition in the colon of transgenic mice compared to wild-type controls as estimated by the percentage of tissue staining red on picrosirius red staining (% of tissue stained red: WT: 3.95 ± 1.2 vs TG: 9.7 ± 3.5; p=0.05). Immunohistochemical staining with α-SMA did not reveal any difference in

smooth muscle cell staining between transgenic and wild-type mice. There was no difference in the percentage of positively stained cells for Ki-67 (WT: 47.3 ± 11.9 vs TG 42 ± 13.9; p=0.64) and pSMAD2/3 (WT 26.3 ± 16.2 vs TG 26.7 ± 4.5; p=0.97) between the transgenic and wild-type mice. There was no obvious difference in the neural cells stained between the transgenic and wild-type mice to explain the difference in contractile responses (figure 5.9).

In summary, there was no major architectural differences in mucosa and smooth muscle of the intestinal tract between wild-type and transgenic mice. There was evidence of increased fibrotic connective tissue in the large intestine of the transgenic mice. The function of the large bowel as demonstrated in the isobaric tension experiments was altered in transgenic mice with reduced contractile responses seen in the transgenic mice.

Figure 5.1: Haematoxylin and eosin staining of 2 different segments of large intestine in wild-type and transgenic mice (magnification x100). The different layers of the intestine are depicted.

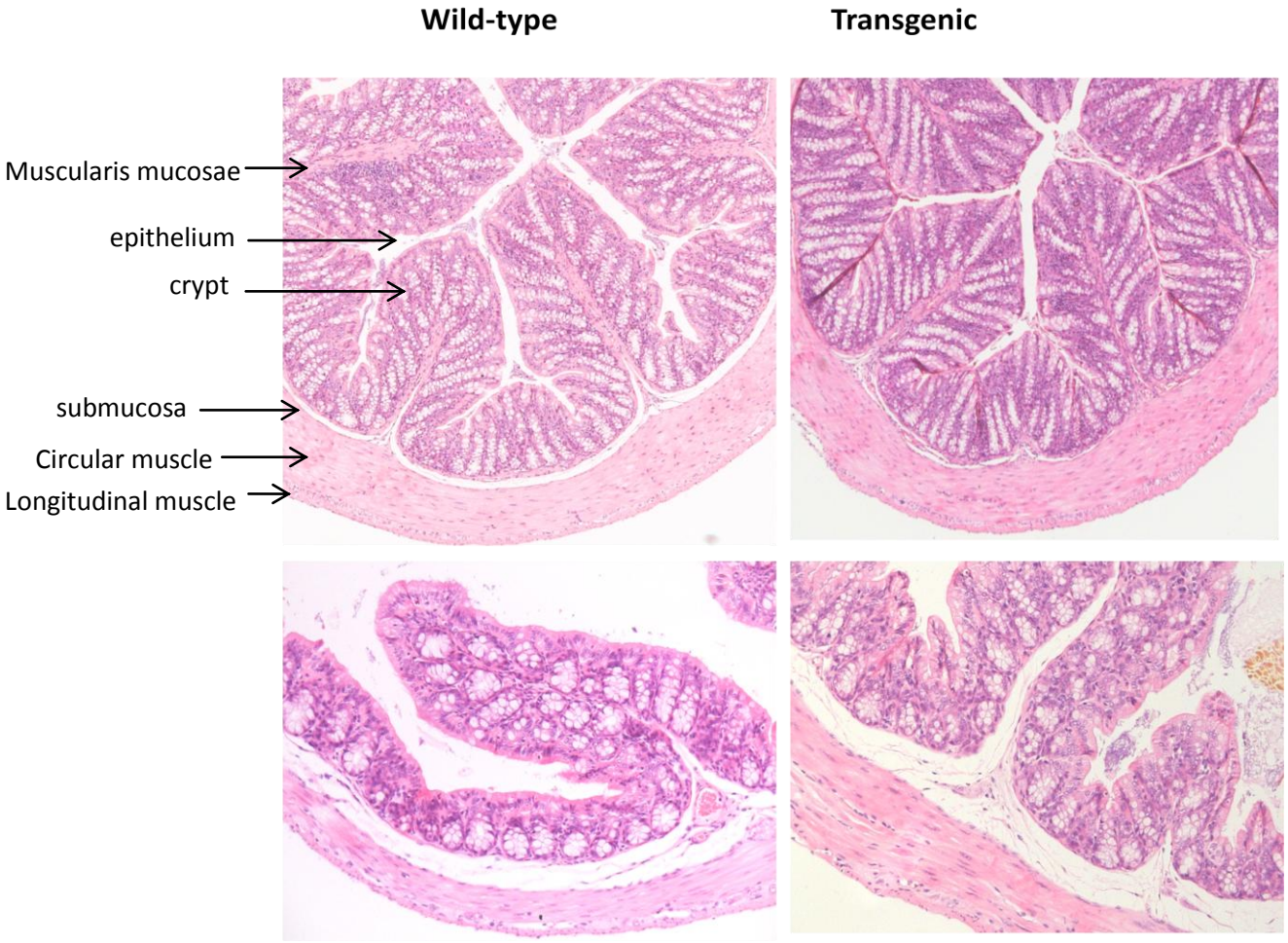


Figure 5.2: Haematoxylin and eosin staining of representative sections of large intestine, small intestine and stomach in wild-type and transgenic mice (magnification x100).

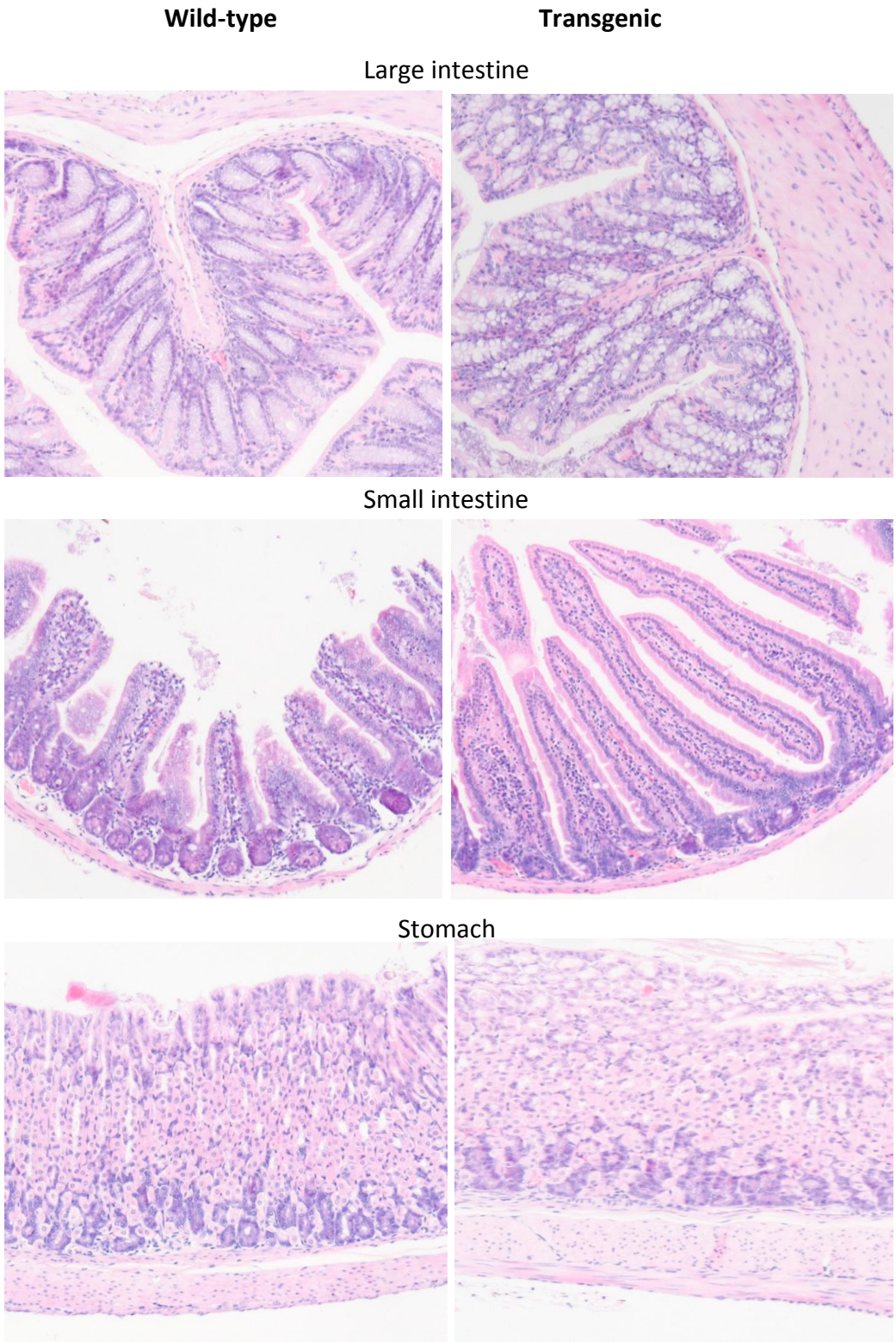


Figure 5.3: Picrosirius red staining of representative sections of large intestine, small intestine and stomach in wild-type and transgenic TβRIIΔk-fib strain mice (magnification x100).

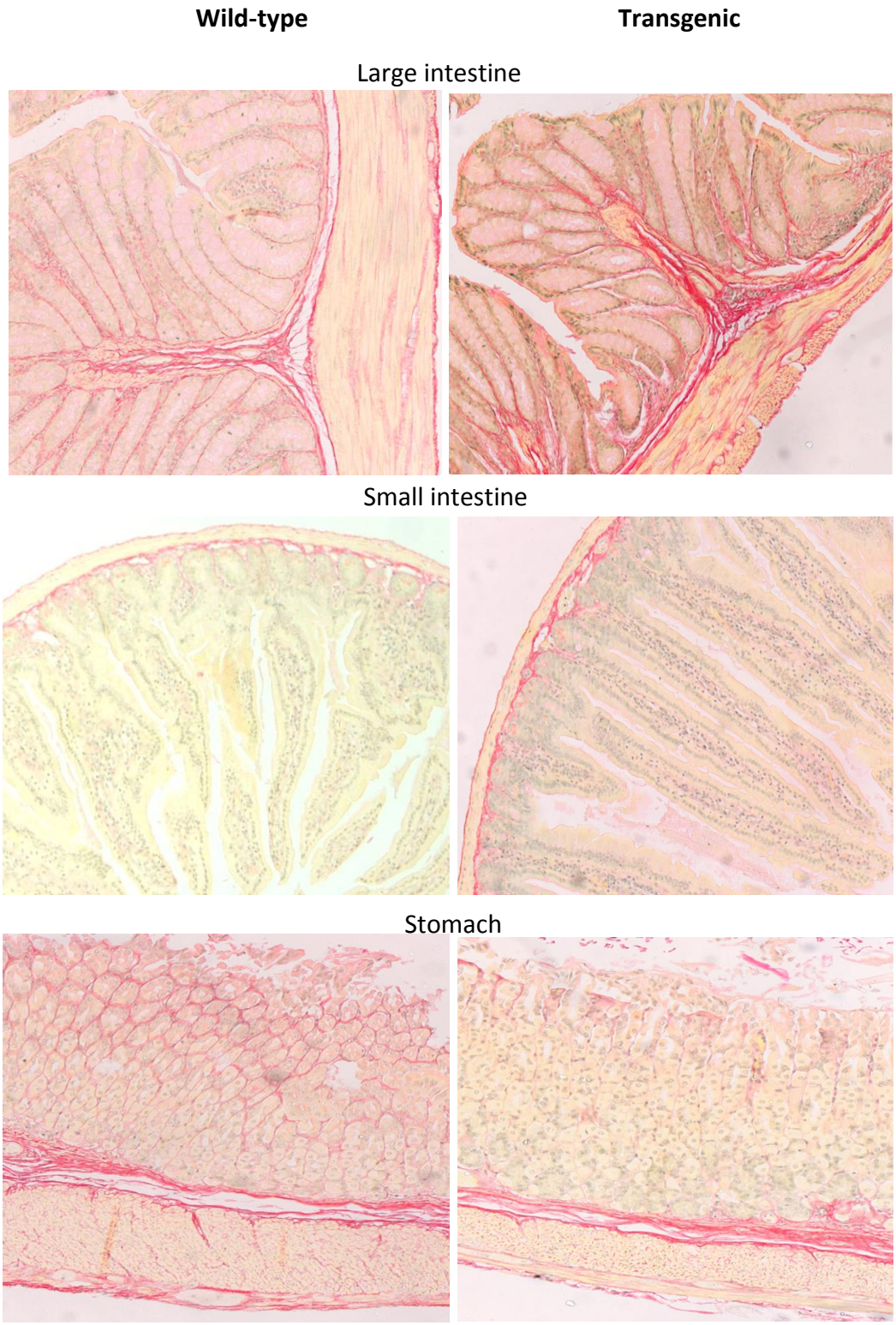


Figure 5.4: alpha-smooth muscle actin (SMA) staining of representative sections of large intestine, small intestine and stomach in wild-type and transgenic mice (magnification x100).

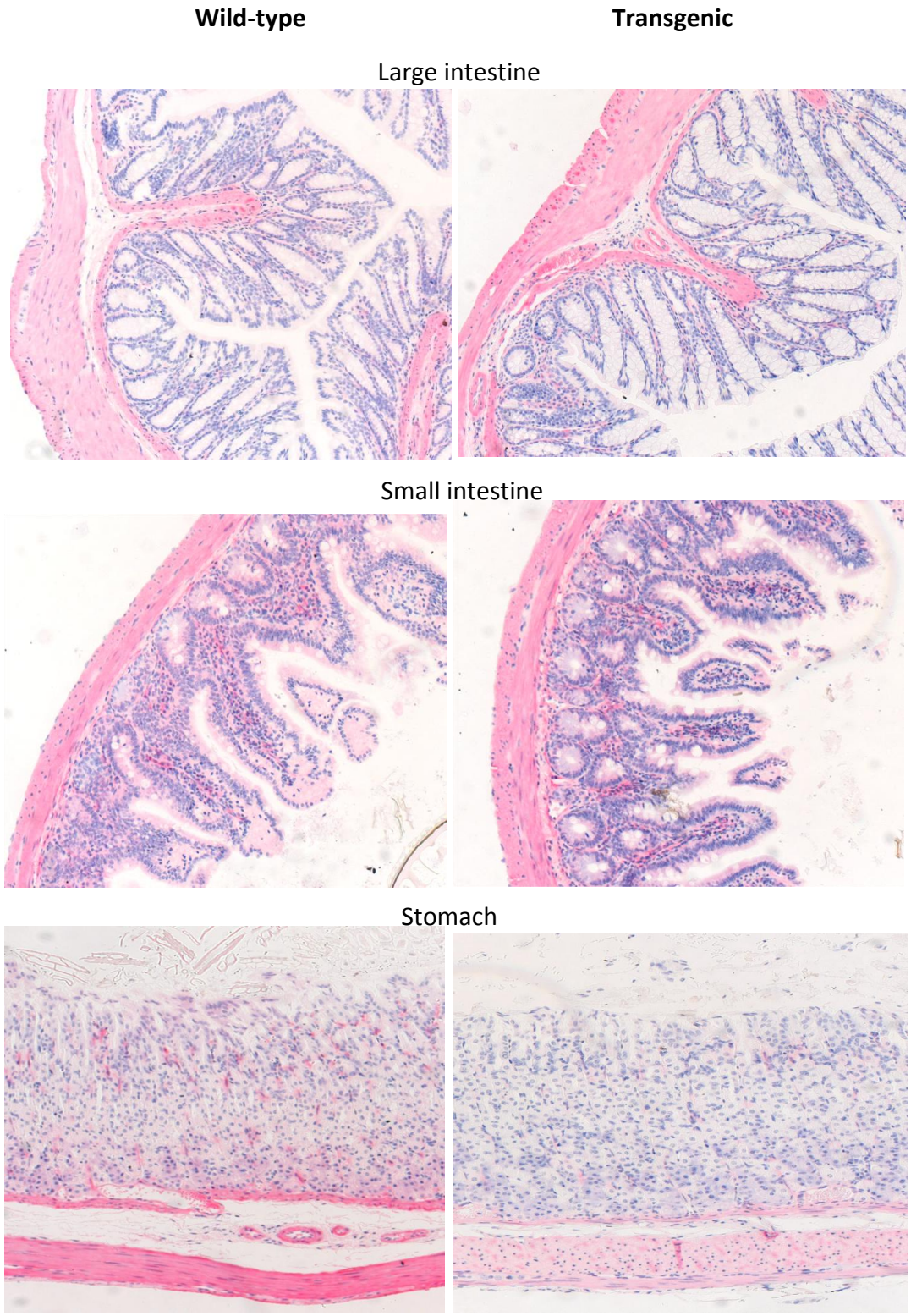


Figure 5.5: Ki-67 staining of representative sections of large intestine and small intestine in wild-type and transgenic mice (magnification x100).

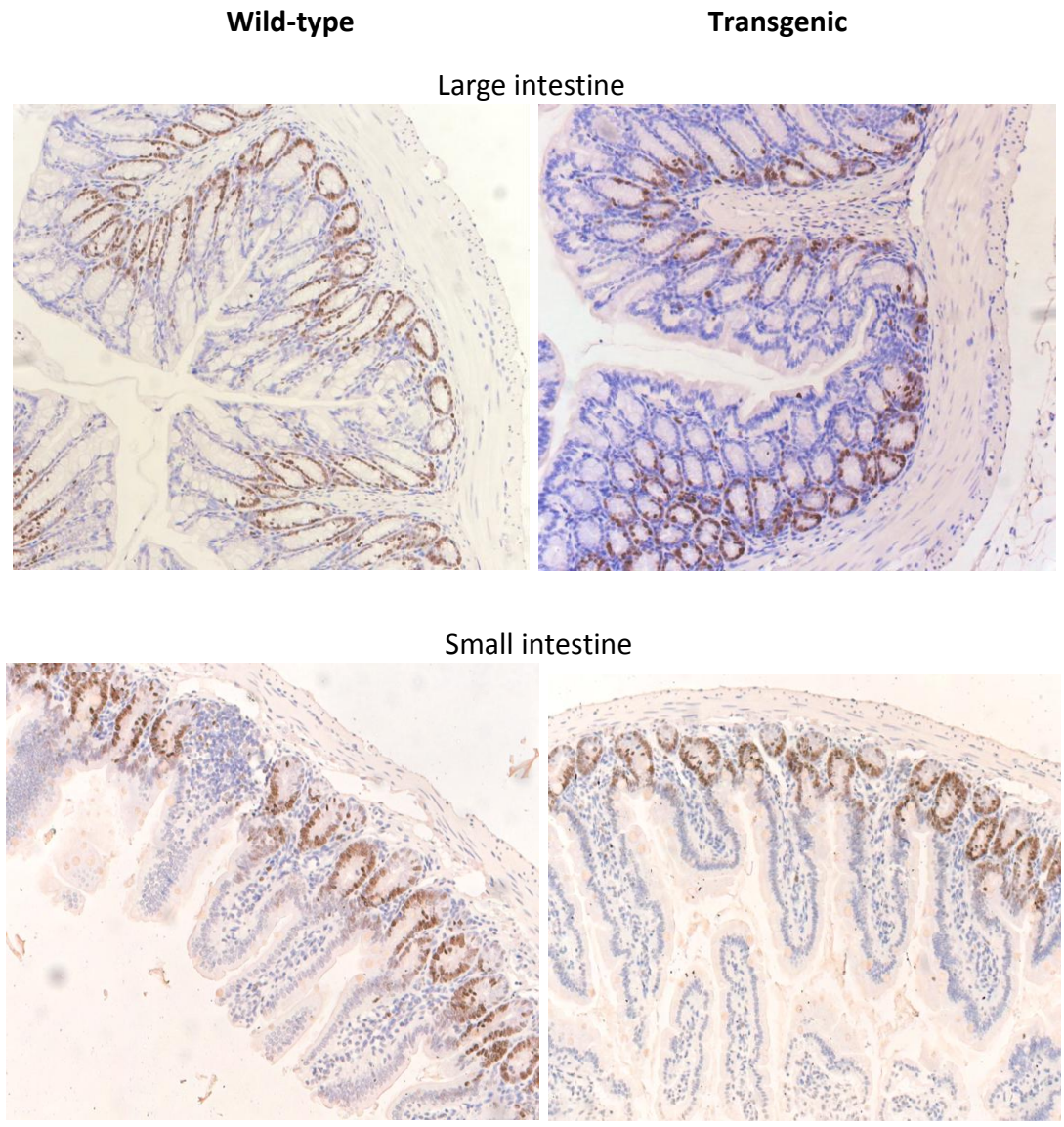


Figure 5.6: phospho SMAD2/3 staining of representative sections of large intestine and small intestine in wild-type and transgenic mice (magnification x100).

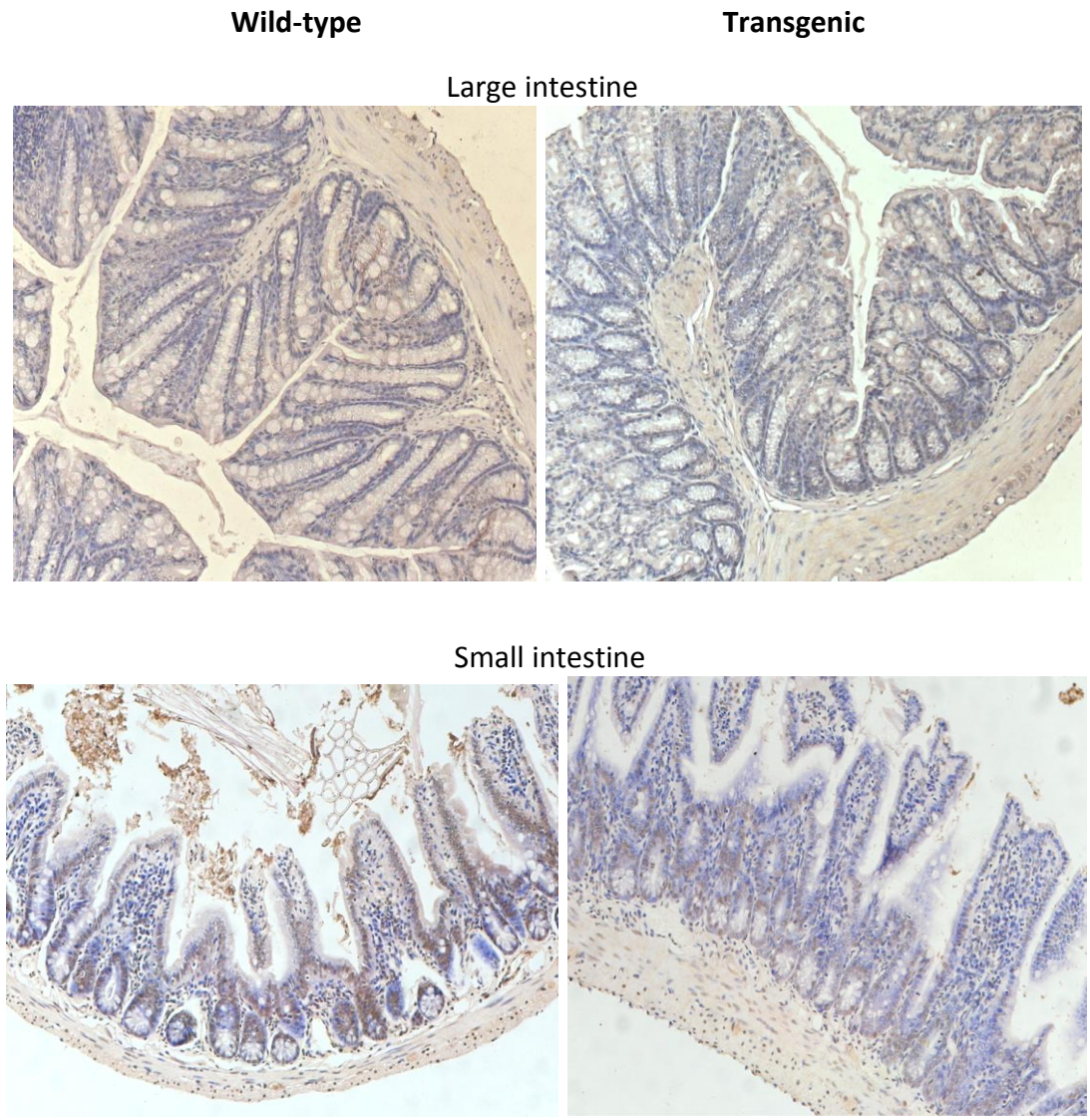


Figure 5.7: Traces of colonic strips' contractile responses in 3 transgenic and 3 wild-type mice. Responses to KCl and high doses of carbachol are shown.

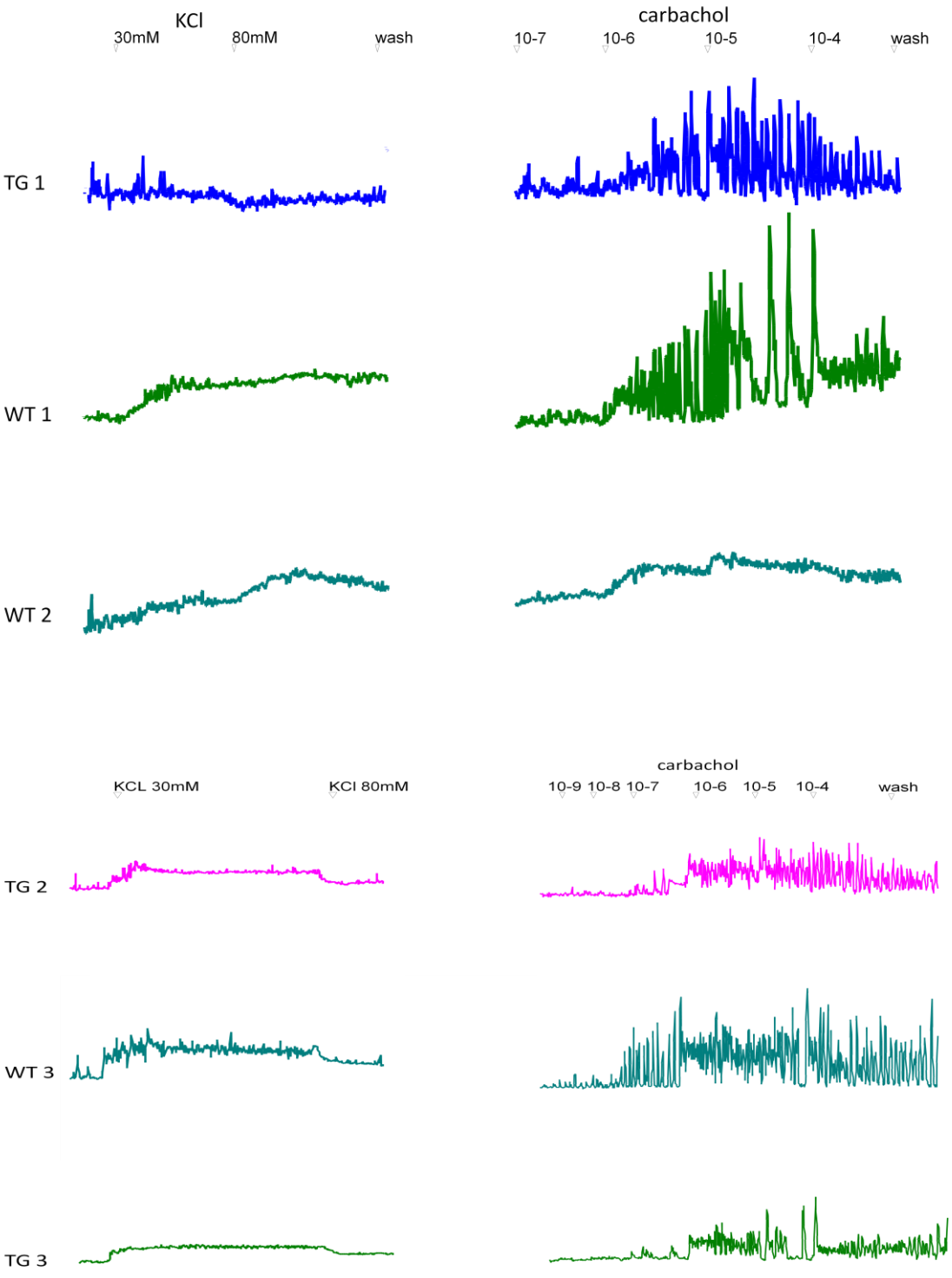
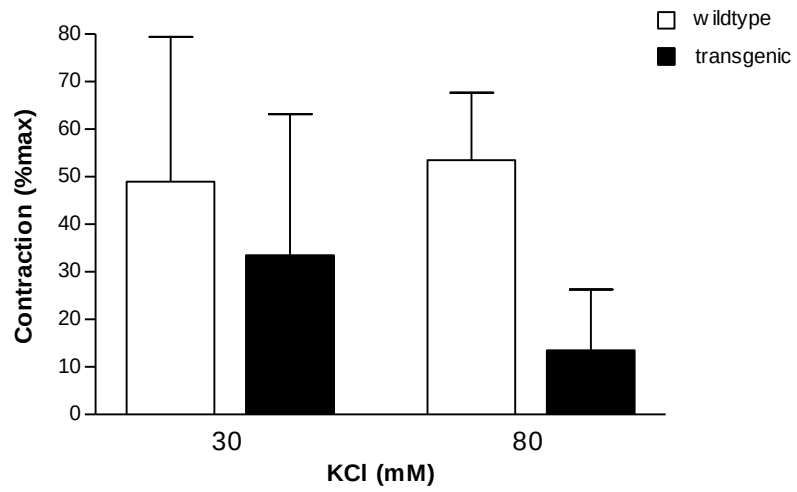


Figure 5.8: Summary data of colonic contractile responses to A) KCl and B) carbachol in wild-type and transgenic mice. Results are expressed as % of maximal contraction (+/- standard error of mean).

A



B

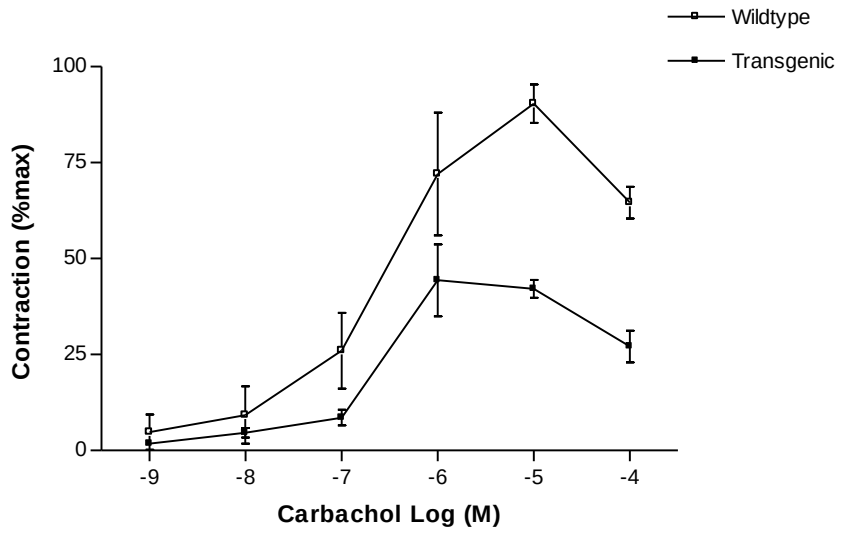
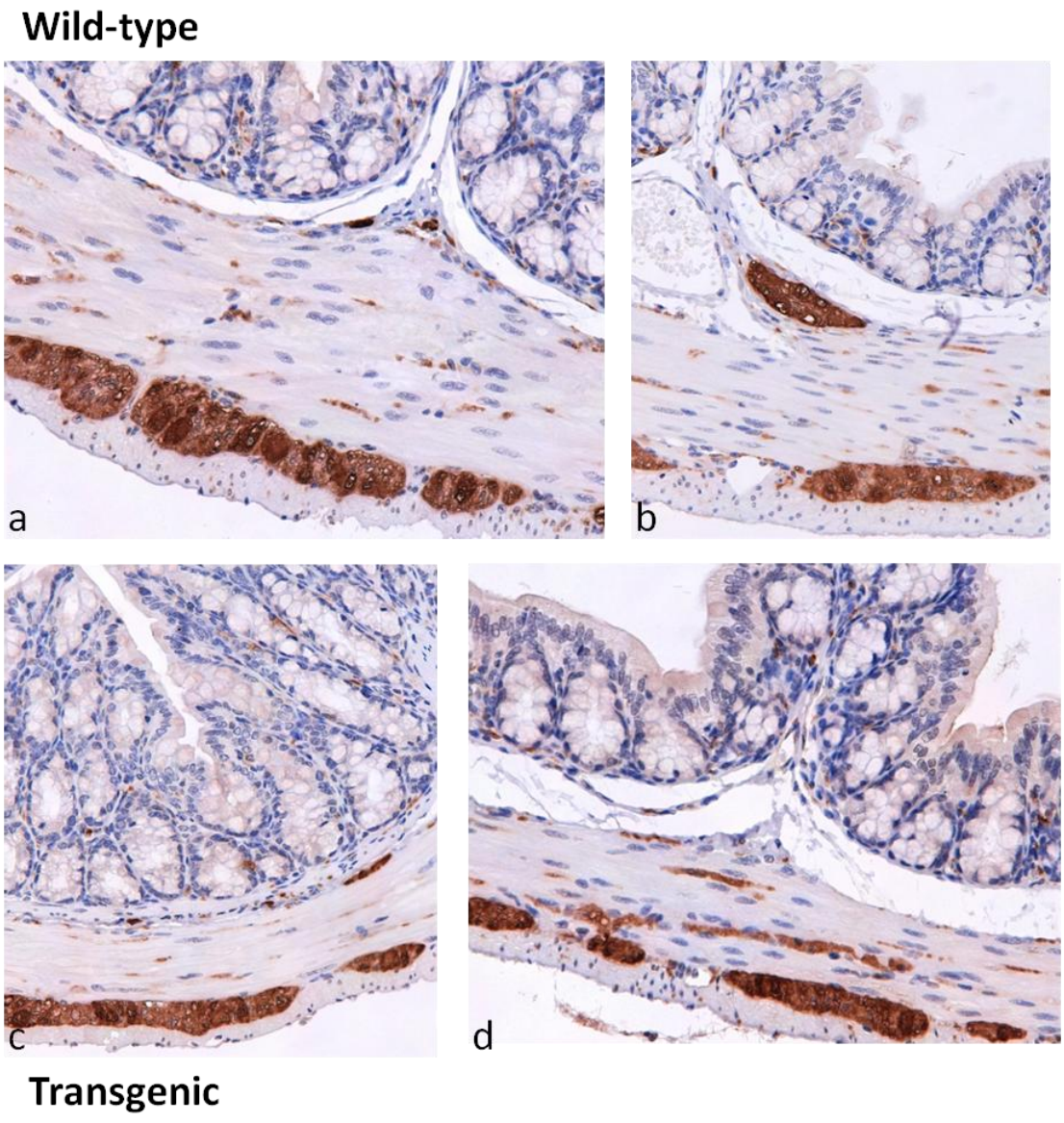


Figure 5.9: Protein gene product (PGP) 9.5 staining of large intestinal mucosa in wild-type (a and b) and transgenic mice (c and d), magnification x100 (a and c) and x200 (b and d).



5.4. Discussion

The transgenic TβRIIΔk-fib mouse strain is a mouse model of SSc in which the primary defect is fibroblast-specific perturbation of TGF-β signalling. This mouse model replicates some of the hallmark features of SSc. These transgenic mice have been shown previously to have abnormal wound healing with failure of wound contraction and scar formation. They develop skin fibrosis and sporadic lung fibrosis (173;174). They have also been shown to develop exaggerated lung fibrosis after bleomycin treatment (175). In more recent experiments Derrett-Smith *et al* described in this strain diminished aortic ring contractility and relaxation and associated aortic adventitial fibrosis as well as cardiac fibrosis (176). The aim of this study was to investigate if there are histological or functional features of colonic involvement in this mouse model.

Although oesophageal involvement is more common than colonic involvement in SSc patients, the upper GI tract has been more extensively investigated and therefore there is overall better understanding of the pathophysiological processes occurring in the oesophagus. Different processes may operate in different parts of the GI tract and therefore I chose to concentrate on the lower GI tract in order to potentially enhance our understanding of the pathophysiology of colonic involvement.

Although other models of systemic sclerosis such as the GVHD mouse model and PDGF knock-in mouse model have been shown to develop gastrointestinal involvement, this has not been the focus of experiments previously except in the UCD chickens (157). In a study to investigate the initial disease phase, the

oesophagus of UCD chickens which was the most affected internal organ, showed endothelial cell apoptosis followed by mononuclear cell infiltrations and increased deposition of collagen (158). The intestine was not found to be affected in the UCD chickens.

Gastrointestinal dysmotility is common in systemic sclerosis but the underlying pathogenesis remains only partially understood. Histological studies of the gastric wall in systemic sclerosis patients have shown prominent fibrosis and severe ultrastructural alterations of smooth muscle cells and nerve fibres (183) and a similar picture has been described in the rectum (13). These studies were based on full thickness or deep biopsies which are not easily obtained. An animal model exhibiting intestinal involvement could significantly enhance our understanding of the disease process as well as the effect of treatments.

Our results showed that the gastrointestinal tract of this transgenic TβRIIΔk-fib mouse strain was structurally normal on light microscopy but there is increased collagen deposition observed in mainly the submucosa and the muscularis mucosa. The fibrosis observed in this transgenic mouse strain is likely to be related to altered TGF-β bioactivity as has been observed in lung tissue in these mice. A role of TGF-β has been shown in other experimental models of intestinal fibrosis such as the TNBS colitis mouse model. In the TNBS colitis model acute and then chronic inflammation precede the development of fibrosis. In the intestine of the TβRIIΔk-fib mouse model there was no evidence of increased inflammation. This is consistent with the findings in lung tissue although injury with bleomycin has been shown to induce inflammation to both skin and lung tissue in these mice. No

intestinal injury was induced in these experiments. Another reason for the absence of inflammation may be that this occurs earlier in the disease process and the mice examined were all at least 5-6 months old. An inflammatory response at an earlier time will be investigated in future studies.

Transgenic TβRIIΔk-fib strain mice were shown to have reduced colonic contractility to both direct non-receptor mediated muscle stimulation with KCl and to carbachol, a cholinergic agonist which causes contraction via direct activation of muscarinic receptors. As contractions to both these agents are through direct activation of the smooth muscle the most likely cause for the reduced contractions in transgenic mice is either smooth muscle atrophy or fibrosis of the smooth muscle. No contractions were induced in transgenic or wild-type mice with 5-HT. 5-HT is found in only about 1% of enteric neurons in the mouse but is an enteric neurotransmitter which has been shown to increase colonic motor migrating complexes and induce colonic contractions. As 5-HT was added to the organ bath after the KCl and carbachol it is possible that the tissue's contractile response had diminished. Alternatively the contractions induced by 5-HT may be much finer compared to the actions of KCl and carbachol which act directly onto the smooth muscle. A third explanation is that there is an underlying neuropathic process which prevented the effect of this neurotransmitter.

Dysmotility is one of the key features of gut involvement in systemic sclerosis. Although the pathogenesis of this remains uncertain neuropathy and smooth muscle atrophy have both been demonstrated (37;53;60;86). Fibrosis and collagen deposition can disrupt neural pathways causing neuropathy. There was no evidence

of degeneration of neural tissue histologically in the transgenic TβRIIΔk-fib strain mice but as mentioned above, changes may only be demonstrable on electron microscopy. One histological study showed structurally normal enteric neurons but morphological abnormalities in the axon terminals and degeneration of unmyelinated fibres in rectal tissue (13) and Manetti *et al* found similar features in gastric tissue (183). Other causes for neuropathy are neurotransmitter depletion that has been previously demonstrated in SSc (112) and autoantibodies. Howe *et al* found myenteric neuronal antibodies in patients with SSc (25). Goldblatt *et al* in a later study showed that functional antibodies from SSc patients inhibited M3-muscarinic receptor mediated enteric cholinergic neurotransmission in mouse colon (27). Although there was no difference in the number of myenteric and submucosal plexi neurons a neuropathic cause for the observed reduced colonic contractility cannot be excluded.

This transgenic mouse model develops skin, lung and adventitial fibrosis which can be spontaneous in a proportion of mice but is more pronounced after tissue injury. There was increased colonic but not small bowel fibrosis seen in a proportion of the transgenic TβRIIΔk-fib strain mice. This is spontaneous fibrosis as there was no intestinal injury induced. To investigate further the intestinal involvement in this mouse model the effect of tissue injury to the development of fibrosis will be investigated in future studies. Although fibrosis may be a late development, at least in human intestinal involvement it is also the point where intestinal dysfunction becomes irreversible. It is possible therefore, that anti-fibrotic agents may have an effect on gut dysfunction. So far no drugs are effective in preventing or reversing

gut involvement and any treatment is aimed at symptom control. The TβRIIΔk-fib mouse strain may in the future be a useful tool in testing the effect of such treatments in gut involvement.

In conclusion, I have shown that this transgenic mouse model previously shown to develop skin, lung and cardiac fibrosis also develops colonic fibrosis with associated effect in colonic tissue contractility. Although fibrosis is not the only pathophysiological process underlying gut involvement in systemic sclerosis, this mouse model may offer further insight in pathologic processes leading to the development of gut fibrosis as well as providing a potential experimental platform for exploring therapies targeting gastrointestinal manifestations of SSc.

CHAPTER 6:

DISCUSSION AND CONCLUSIONS

The aim of this thesis was to conduct experiments in order to assess the primary pathologies affecting the gastrointestinal tract in systemic sclerosis (SSc), specifically pathologies affecting the mucosa, vasculature, smooth muscle and enteric nervous system in systemic sclerosis. To do this, the intention was to use the best currently available validated techniques.

In the first instance I wanted to establish the prevalence of GI symptoms in UK patients with SSc attending a tertiary care rheumatology outpatient department (Chapter 2). I used a contemporary disease specific questionnaire about gastrointestinal symptoms created by Khanna *et al* (68) and validated it in a UK patient cohort. I showed that gastrointestinal symptoms are common and they have a significant effect on patients' quality of life. The questionnaire used was the only available GI questionnaire specifically for SSc patients at the time, but had not been validated against objective measures of gut involvement. Ideally GI involvement should be diagnosed based on findings on investigations such as oesophageal manometry, endoscopy or imaging but as these are often invasive procedures, development of symptoms is often used as a marker to signify GI involvement.

This is both one of the aims of this study, but also one of the limitations: a subjective symptom questionnaire is not an accurate measure of disease. It may both underestimate (due to asymptomatic or more stoical patients that may not report symptoms) and over-estimate (due to GI symptoms being secondary to non-SSc related GI disease or side effects of drugs) GI involvement both in extent and

severity. Patients' notes were reviewed for evidence of GI investigations and documented GI involvement. A lot of patients have not had any invasive gastroenterological investigations and for those that had, these were often performed at patients' local hospitals and therefore unfortunately the findings were not always available. Another limitation of this questionnaire is that although it covers a wide range of symptoms it does not cover anorectal symptoms. A unique feature of this questionnaire is that it enquires about symptom frequency, and gastrointestinal disease burden is derived by that, rather than by symptom severity which is more commonly assessed in disease assessment and quality of life questionnaires. The benefit of using symptom frequency is that frequency (in this case, number of days in a week) is a more objective measure than severity. Furthermore this questionnaire addresses a multitude of symptoms thus providing information about the range of gastrointestinal sites (apart from the anorectum) that may be affected in SSc.

A second version of this questionnaire has included questions on anorectal symptoms and is also shorter and easier to complete but at the same time keeping the wide range of symptoms being assessed. Validation of this second version of this questionnaire in a UK population needs to be undertaken and is planned in future studies. This questionnaire should also be validated against objective evidence of GI involvement. Questionnaires are often used as indicators of disease activity for other organ involvement, such as pulmonary involvement in SSc. The use of a GI questionnaire such as the one used in this thesis or its second version may have a role as part of routine assessment of GI involvement in SSc patients

both on diagnosis and in subsequent visits both to assess progression of disease and response to treatment: this needs to be addressed in future long-term prospective studies.

In order to establish any association between GI involvement and other SSc characteristics patients' notes were reviewed. Gastrointestinal involvement was mainly based on the presence of symptoms with the limitations of this approach as previously discussed and although no specific associations were found this is based on possibly inaccurate assessment of GI involvement. Furthermore, the definition of severe GI involvement based on higher frequency of GI symptoms (3 or more days/week on the questionnaire used) is an arbitrary cut off that evolved from Khanna *et al*'s original description of their survey instrument. The implications of my findings are that in future studies, GI involvement as well as severity, should be proven by a GI history allied to endoscopic, physiological or radiological studies. Specific autoantibody profile associated with GI involvement needs to be further assessed with extensive autoantibody profiling of patients including myenteric autoantibodies: it may be that certain aspects of GI involvement, such as dysmotility or vascular changes or sites affected are associated with certain autoantibody profile or other disease characteristics. With the increasing recognition of GI involvement as well as the greater availability of GI investigations, such involvement may need to become better characterised as routine in the future.

More than 50 percent of SSc patients suffer from anorectal symptoms including faecal incontinence. This is often under-reported by the patients but also possibly due to lack of effective treatment options not specifically inquired about by physicians. Studies to date have mainly shown that patients with SSc have low anal sphincter resting pressure and imaging of the anal sphincters has classically shown internal anal sphincter (IAS) atrophy. I studied patients with and without faecal incontinence performing more extensive anorectal assessment than previously published, and importantly also studied GI asymptomatic individuals (Chapter 3). A key finding was that endoanal ultrasonography revealed IAS atrophy not only in the SSc patients with faecal incontinence but also in the SSc patients without such symptoms. Furthermore the resting pressure in these asymptomatic SSc patients was not necessarily increased compared to those patients with faecal incontinence. A new finding in this study and I would contend a key factor in symptom development is evidence of neuropathy most likely affecting the myenteric plexi. This is shown not only by absent rectoanal inhibitory reflex (RAIR, an intrinsic enteric mediated reflex) but also attenuated anal sensation. Anorectal sensation can be measured by mucosal electrosensation but also by sensation to rectal distension. Such rectal distension was not undertaken in this cohort, as I felt it was invalid in a disease that causes fibrosis and it could confound the interpretability of the results. Sensitivity to electrical stimulation is not affected by this process and furthermore allows study of both rectal and anal sensitivity.

The RAIR has previously been shown to be absent or impaired in patients with SSc and this was confirmed in this study. I speculated that one possible confounding

factor to the loss of this reflex is the lower resting pressure already observed in the SSc patients with faecal incontinence, which could impair the ability to demonstrate a RAIR. A further key result therefore was the observation that the absence of RAIR did not correlate with the resting pressure or the degree of IAS atrophy. Furthermore it was the latency period component of the RAIR which was longer in Sx SSc patients, suggesting a delay in synaptic transmission. Another method of assessing neuropathy is pudendal nerve terminal latency measurements The pudendal nerve is a mixed nerve providing efferent and afferent pathways to the EAS, perineal musculature and mucosa of the anal canal amongst other structures. In keeping with current best practice, I chose not to use pudendal nerve terminal latency measurements as this method has been shown to be inconsistent, with normal latencies recorded even in a damaged nerve as long as some fast fibres remain intact. The test has a low sensitivity and specificity and it is very operator dependent.

Neuropathy seems to be a key factor in symptom development. One implication of my results is that neuropathy and its pathogenesis needs to be investigated further in the future, especially the significance of autoantibodies as well as therapeutic interventions for its treatment such as sacral nerve stimulation and posterior tibial nerve stimulation.

The structure of the anal sphincters was assessed by endoanal ultrasound (EAUS). An alternative method would have been endoanal MRI. This is much more time consuming and can be more uncomfortable for the patients in addition to being more expensive. More importantly the internal sphincter is very well seen on EAUS

and thickness and atrophy accurately assessed. Atrophy and thinning of the EAS may be more difficult to assess on EAUS but there is still good correlation with MRI which is more sensitive (184;185). Muscle integrity of both the internal and external sphincter is also very accurately assessed by EAUS. As it is mainly the IAS which is affected in SSc I felt that EAUS was the best method to assess the sphincter complex in these patients.

One limitation of this study was the choice of the control group. I made the decision to use patients with faecal incontinence as I felt that if there are features distinct to SSc patients for the development of incontinence such a control group would be better able to demonstrate that. The main cause of incontinence in that control group was obstetric injury which may lead to more complex pathophysiological processes than mere sphincter tear, such as occult nerve injury.

I excluded patients with diabetes from this study in order to avoid this confounding factor as diabetic neuropathy could lead to a similar clinical picture. Studying patients with diabetes and anorectal symptoms and comparing them with patients with SSc could provide further information in the pathophysiology of anorectal symptoms in SSc as the underlying pathophysiological processes of diabetic vasculopathy and neuropathy may be similar to those seen in systemic sclerosis.

In the 4th chapter I described the effect of gut symptoms on nutritional status of SSc patients. Severe gastrointestinal involvement can lead to malnutrition and ultimately even the need for parenteral nutrition. Hence there is a need for

accurate nutritional assessment and identification of patients at risk of developing malnutrition. This was an exploratory study using nutritional assessments such as bioelectrical impedance and indirect calorimetry. There are very few previous nutritional studies in SSc patients and my aim was to try and establish if there are any differences between SSc patients with and without gut symptoms. The findings of this study could then be used to plan future studies. Even with the small number of patients studied I did find some significant differences between the groups in bioelectrical impedance findings. These differences were found in the impedance measurements as well as body composition such as fat free mass and total body water. The clinical significance of these findings is not known but early changes in body composition may identify patients that are at risk of developing significant malnutrition. The other significant difference found between SSc patients with and without gut symptoms is a higher phase angle in the patients without gut symptoms. Phase angle has been shown in other patient groups to be a prognostic indicator. In order for the importance of these findings to be assessed further a prospective longitudinal study of a larger number of patients should be planned in the future. This study assessed a number of nutritional assessments and I showed that a thorough and comprehensive nutritional assessment can be performed in a short period of time in the outpatient setting. The weight, height and therefore BMI as well as calculation of the MUST score can be easily undertaken. Anthropometric assessments are probably less useful in SSc patients as measurement of triceps skinfold thickness and handgrip can be altered by the cutaneous manifestations of SSc. Indirect calorimetry involves expensive equipment, operator expertise and is time consuming but bioelectrical impedance can be assessed easily. Therefore

assessing BMI, MUST score and bioelectrical impedance in a prospective and longitudinal study is planned for the future.

There are a number of limitations of this initial, observational, study. This study, being exploratory, was not powered to show specific differences and therefore these results need to be reproduced in a larger study. The second limitation of this study was that I did not use a control group. In future studies a healthy age and sex matched control group should be included.

Studies investigating patients with and without gastrointestinal involvement with physiological, radiological and endoscopic assessments have provided a wealth of information regarding the pathophysiology of gastrointestinal disease in SSc. This has been supplemented by histological findings from surgical specimens as well as post-mortem examinations. Nonetheless there were still unanswered questions. I showed that neuropathy is a key feature in the aetiopathogenesis of gastrointestinal involvement and more specifically in the development of anorectal symptoms. Gastrointestinal involvement leads to differences in body composition and there may be early evidence of nutritional deficiencies.

Other aspects of SSc such as skin involvement, vasculopathy and pulmonary hypertension have been investigated through the use of animal models. These animal models, especially more recently transgenic and knock-out mouse models, have provided much more detailed understanding of the fibrotic and inflammatory

processes and the relative contributions of specific ligands, receptors and their signalling pathways.

In chapter 5 I investigated the gastrointestinal tract of a transgenic mouse strain with fibroblast specific TGF-β signalling activation gene to express a kinase deficient type II TGF-β receptor (TβRIIΔk) in fibroblasts. The TβRIIΔk transgenic mice develop skin fibrosis with a proportion of animals developing significant lung fibrosis and also diminished aortic ring contractility and cardiac fibrosis. Fibrosis is believed to be a late stage of the disease process and once it has developed changes are not reversible. In this thesis I have shown the first evidence of an animal model of gut fibrosis in SSc. I have demonstrated that this transgenic mouse strain develops fibrosis in the large intestine and this is associated with dysmotility. The major limitation of this study is the small number of mice examined but this is unavoidable with transgenic mice in view of the expense associated with the generation, breeding and maintenance of the mice. Another limitation is the quantification of fibrosis done by quantifying the amount of staining in sections of the GI tract. This semi-quantitative method has been used in a number of studies assessing collagen deposition in the gastrointestinal tract. Further evaluation of fibrosis will need to be performed in future studies. This should include performing a Sircol assay and studying mice of different ages in order to investigate at what time point gut fibrosis develops in this mouse model. It is possible that a degree of fibrosis develops even in control mice with increasing age which would explain the slight differences in fibrosis noted between younger and older mice. The colonic dysmotility observed in this mouse strain is likely secondary to increased fibrosis as

no other structural or immunohistochemical differences were demonstrated between the transgenic and wild-type mice. More specifically there was no evidence of difference in neural staining although this does not exclude neuropathy which could be secondary to circulating autoantibodies or neural changes only visible on electron microscopy. Nonetheless this is a fibrotic mouse strain as demonstrated in skin, lung, aorta and heart and now also in intestinal tissue. The importance of this finding is that although a number of mouse models have shown fibrosis in skin and lung there are only a couple of other mouse models that demonstrate fibrosis in the gut. Further studies with larger number of mice and further histological examination, including electron microscopy and physiological studies investigating other neurotransmitters should be planned in the future to further characterise gut involvement in this mouse strain. Development of a mouse model of SSc that demonstrates gut involvement will allow further research into the mechanisms of gut fibrosis and its treatment.

CHAPTER 7:

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CHAPTER 8:

APPENDICES

Appendix 1: The Scleroderma gastrointestinal tract 1.0 questionnaire

THE SCLERODERMA GASTROINTESTINAL TRACT 1.0 QUESTIONNAIRE

SSC GIT 1.0

CORRESPONDENCE TO:

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Khanna D, Hays RD, Park GS, Braun-Moscovici Y, Mayes MD, McKearney TA et al.
Development of a preliminary scleroderma gastrointestinal tract 1.0 quality of life instrument.
Arthritis Rheum 2007; 57(7):1280-1286.

The next few questions ask about your general health in the past 1 week. Answer every question by selecting the answer as indicated. If you are unsure about how to answer a question, please give the best answer you can.

In the past 1 week, how often did you ...		(CIRCLE ONE RESPONSE FOR EACH QUESTION)			
		No Days[1]	1-2 Days[2]	3-4 Days[3]	5-7 Days[4]
1.	... have difficulty swallowing solid food?	1	2	3	4
2.	... have an unpleasant stinging or burning sensation in your chest (heartburn)?	1	2	3	4
3.	... have a sensation of bitter or sour fluid coming up from your stomach into your mouth (acid reflux)?	1	2	3	4
4.	... wake up at night with a cough?	1	2	3	4
5.	... wake up at night with choking sensation?	1	2	3	4
6.	... have an acid taste in your mouth?	1	2	3	4
7.	... have heartburn on eating 'acidic' foods such as Tomatoes & Oranges?	1	2	3	4
8.	... regurgitate (throw up or bring up small amounts of previously eaten food)?	1	2	3	4
9.	... sleep in a 'raised' or an 'L shaped' position?	1	2	3	4
10.	... feel bloated (a sensation of gas or air in the stomach)?	1	2	3	4
11.	... feel like vomiting or throwing up?	1	2	3	4
12.	... vomit or throw up?	1	2	3	4
13.	... notice an increase in your belly, sometimes requiring you to open your belt, pants or shirt?	1	2	3	4
14.	... feel full after eating a small meal?	1	2	3	4
15.	... have watery or loose stools (diarrhea)?	1	2	3	4
16.	... pass excessive gas or flatulence?	1	2	3	4

In the past 1 week, have you noticed your stools becoming...	(CIRCLE ONE RESPONSE FOR EACH QUESTION)	
	Yes[1]	No[2]

17.	... harder?	1	2
18.	... watery?	1	2

In the past 1 week, how often ...		(CIRCLE ONE RESPONSE FOR EACH QUESTION)			
		No Days[1]	1-2 Days[2]	3-4 Days[3]	5-7 Days[4]
19.	... were you constipated or unable to empty your bowels?	1	2	3	4
20.	... did you have hard stools?	1	2	3	4
21.	... did you have discomfort or cramping in your abdomen relieved by a bowel movement?	1	2	3	4
22.	... did you have pain while passing your stools?	1	2	3	4

In the past 1 week, how often did the following symptoms delay or prevent you from leaving your home?		(CIRCLE ONE RESPONSE FOR EACH QUESTION)			
		No Days[1]	1-2 Days[2]	3-4 Days[3]	5-7 Days[4]
23.	Nausea	1	2	3	4
24.	Vomiting	1	2	3	4
25.	Stomach ache or pain	1	2	3	4
26.	Diarrhea	1	2	3	4
27.	Difficulty swallowing food	1	2	3	4
28.	Worry you would accidentally soil your underwear	1	2	3	4
29.	Bloated sensation	1	2	3	4

In the past 1 week, how often did the following limit your ability to work outside the home or do housework?		(CIRCLE ONE RESPONSE FOR EACH QUESTION)			
		No Days[1]	1-2 Days[2]	3-4 Days[3]	5-7 Days[4]
30.	Nausea	1	2	3	4
31.	Vomiting	1	2	3	4
32.	Stomach ache or pain	1	2	3	4
33.	Diarrhea	1	2	3	4
34.	Difficulty swallowing food	1	2	3	4

35.	Worry you would accidentally soil your underwear	1	2	3	4
36.	Bloated sensation	1	2	3	4

In the past 1 week, how often did the following interfere with social activities (such as visiting friends or relatives)?		(CIRCLE ONE RESPONSE FOR EACH QUESTION)			
		No Days[1]	1-2 Days[2]	3-4 Days[3]	5-7 Days[4]
37.	Nausea	1	2	3	4
38.	Vomiting	1	2	3	4
39.	Stomach ache or pain	1	2	3	4
40.	Diarrhea	1	2	3	4
41.	Difficulty swallowing food	1	2	3	4
42.	Worry you would accidentally soil your underwear	1	2	3	4
43.	Bloated sensation	1	2	3	4

In the past 1 week, how often did you …		(CIRCLE ONE RESPONSE FOR EACH QUESTION)			
		No Days[1]	1-2 Days[2]	3-4 Days[3]	5-7 Days[4]
44.	… feel worried or anxious about your bowel problems?	1	2	3	4
45.	… feel embarrassed because of your bowel symptoms?	1	2	3	4
46.	… have problems with sexual relations because of your bowel symptoms?	1	2	3	4
47.	… fear not finding a bathroom?	1	2	3	4
48.	… feel depressed or discouraged due to your bowel symptoms?	1	2	3	4
49.	… avoid or delay traveling because of your bowel symptoms?	1	2	3	4
50.	… feel angry or frustrated as a result of your bowel symptoms?	1	2	3	4
51.	… have problems with your sleep as a result of your bowel symptoms?	1	2	3	4
52.	… feel 'stress' or an upset mood worsens your bowel symptoms?	1	2	3	4

Thank you for completing the questionnaire

Appendix 2: SF-36 questionnaire

Name: **Hospital No:**

SF-36 HEALTH SURVEY

INSTRUCTIONS: This survey asks for your views about your health. This information will help keep track of how you feel and how well you are able to do your usual activities. Answer every question by marking the answer as indicated. If you are unsure about how to answer a question, please give the best answer you can.

1. In general, would you say your health is:

(circle one number **only**)

Excellent 1

Very good 2

Good 3

Fair 4

Poor 5

2. Compared to one week ago, how would you rate your health in general now?

(circle one number **only**)

Much better now than one week ago 1

Somewhat better now than one week ago 2

About the same as one week ago 3

Somewhat worse now than one week ago 4

Much worse now than one week ago 5

3. The following questions are about activities you might do during a typical day. Does <u>your health now limit you</u> in these activities? If so, how much?

(circle one number on each line)

<u>ACTIVITIES</u>	**Yes, Limited A Lot**	**Yes, Limited A Little**	**No, Not Limited** At All
Vigorous activities, such as running, lifting heavy objects, participating in strenuous sports	1	2	3
Moderate activities, such as moving a table, pushing a vacuum cleaner, bowling, or playing golf	1	2	3
Lifting or carrying groceries	1	2	3
Climbing **several** flights of stairs	1	2	3
Climbing **one** flight of stairs	1	2	3
Bending, kneeling, or stooping	1	2	3
Walking **more than a mile**	1	2	3
Walking **half a mile**	1	2	3
Walking **one hundred yards**	1	2	3
Bathing or dressing yourself	1	2	3

4. During the past week, have you had any of the following problems with your work or other regular daily activities as a result of your physical health?

(circle one number on each line)

	YES	NO
Cut down on the **amount of time** you spent on work or other activities	1	2
Accomplished less than you would like	1	2
Were limited in the **kind** of work or other activities	1	2
Had **difficulty** performing the work or other activities (for example, it took extra effort)	1	2

5. During the past week, have you had any of the following problems with your work or other regular daily activities as a result of any emotional problems (such as feeling depressed or anxious)?

(circle one number on each line)

	YES	NO
Cut down on the **amount of time** you spent on work or other activities	1	2
Accomplished less than you would like	1	2
Didn't do work or other activities as **carefully** as usual	1	2

6. During the past week, to what extent has your physical health or emotional problems interfered with your normal social activities with family, friends, neighbours, or groups?

(circle one number **only**)

Not at all 1

Slightly 2

Moderately 3

Quite a bit 4

Extremely 5

7. How much bodily pain have you had during the past week?

(circle one number **only**)

None 1

Very mild 2

Mild 3

Moderate 4

Severe 5

Very severe 6

8. During the past week, how much did pain interfere with your normal work (including both work outside the home and housework)?

(circle one)

Not at all 1

A little bit 2

Moderately 3

Quite a bit 4

Extremely 5

9. These questions are about how you feel and how things have been with you during the past week. For each question, please give the one answer that comes closest to the way you have been feeling. How much of the time during the past week.

(circle one number on each line)

	All of the Time	Most of the Time	A Good Bit of the Time	Some of the Time	A Little of the Time	None of the Time
Did you feel full of life?	1	2	3	4	5	6
Have you been a very nervous person?	1	2	3	4	5	6
Have you felt so down in the dumps that nothing could cheer you up?	1	2	3	4	5	6
Have you felt calm and peaceful?	1	2	3	4	5	6
Did you have a lot of energy?	1	2	3	4	5	6
Have you felt downhearted and low?	1	2	3	4	5	6
Did you feel worn out?	1	2	3	4	5	6
Have you been a happy person?	1	2	3	4	5	6
Did you feel tired?	1	2	3	4	5	6

10. During the past week, how much of the time has your physical health or emotional problems interfered with your social activities (like visiting with friends, relatives, etc.)?

(circle one)

All of the time 1

Most of the time 2

Some of the time 3

A little of the time 4

None of the time 5

11. How TRUE or FALSE is <u>each</u> of the following statements for you?

(circle one number on each line)

	Definitely True	**Mostly True**	**Don't Know**	**Mostly False**	**Definitely False**
I seem to get ill a little easier than other people	1	2	3	4	5
I am as healthy as anybody I know	1	2	3	4	5
I expect my health to get worse	1	2	3	4	5
My health is excellent	1	2	3	4	5

Thank you

Appendix 3: EuroQol questionnaire

EuroQoL

Patient number……

Patient initials……Date ……..

By placing a tick in one box in each group below, please indicate which statements best describe your own health state today.

Mobility

I have no problems in walking about ☐

I have some problems in walking about ☐

I am confined to bed ☐

Self-Care

I have no problems with self-care ☐

I have some problems washing or dressing myself ☐

I am unable to wash or dress myself ☐

Usual Activities *(e.g. work, study, housework, family or leisure activities)*

I have no problems with performing my usual activities ☐

I have some problems with performing my usual activities ☐

I am unable to perform my usual activities ☐

Pain/Discomfort

I have no pain or discomfort ☐

I have moderate pain or discomfort ☐

I have extreme pain or discomfort ☐

Anxiety/Depression

I am not anxious or depressed □

I am moderately anxious or depressed □

I am extremely anxious or depressed □

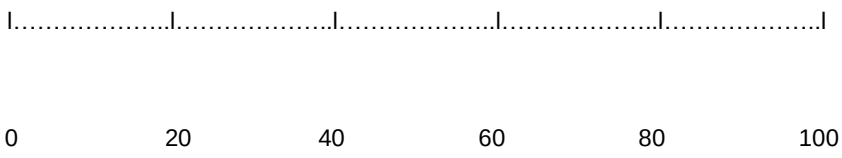
Linear analogue scale

Indicate for today, where

100 is the best state you can imagine

0 is the worst state you can imagine

Draw mark on the scale



Appendix 4: Wexner constipation score

Name:Hospital No:

Wexner Constipation Score

Please answer the following questions that relate to the emptying of your bowels. Circle the most appropriate number that applies to you.

1). Typically how often do you empty your bowels?	Score
1-2 Times per 1-2 days	0
2 times per week	1
Once per week	2
Less than once per week	3
Less than once per month	4

2). How often do you have to strain to empty your bowels?

Never	0
Rarely	1
Sometimes	2
Usually	3
Always	4

3). How often do you feel you have not fully evacuated your rectum when you empty your bowels?

Never(always feel empty)	0
Rarely	1
Sometimes	2

Usually	3
Always (never feel empty)	4

4). How often do you suffer with abdominal pains due to your bowel evacuation problem?

Never	0
Rarely	1
Sometimes	2
Usually	3
Always	4

5). Typically how long do you spend in the lavatory per attempt?

Less than 5 Mins	0
5 – 10Minutes	1
10 – 20 Mins	2
20 – 30 Mins	3
More than 30Mins	4

6). Which of the following do you need, to help with the emptying of your bowel?

No help	0
Laxatives	1
Digital assistance, suppositories, or enema	2

7). Typically how often do you attempt to empty your bowels **WITHOUT** a result in a 24hr period?

Never	0
1-3	1
3-6	2
6-9	3
More than 9	4

8).How long have you had these bowel symptoms?

0 Years	0
1-5 Years	1
5-10 Years	2
10-20 Years	3
More than 20 Years	4

Thank you

To be completed by Unit

Total score ……

Appendix 5: Wexner faecal incontinence score

Name:Hospital No:

Wexner Faecal Incontinence Scoring System

Please note that all questions below refer to your **bowels** and not to your bladder.

Tableau I. – *Anal incontinence scoring system according to Jorge and Wexner [4].*
Score d'incontinence anale [4].

Type of incontinence	Frequency				
	Never	Less than once per month	Greater than once per month, Less than once per week	Greater than once per week, Less than once per day	Greater than or equal to once per day
Solid	0	1	2	3	4
Liquid	0	1	2	3	4
Gas	0	1	2	3	4
Requires pad	0	1	2	3	4
Lifestyle alteration	0	1	2	3	4

0 = normal continence
20 = total incontinence

Thank you

To be completed by Unit

Total ……..

Appendix 6: Copy of consent form

University College London Hospitals NHS

NHS Foundation Trust

Physiology Unit

Podium Level 2

University College Hospital

235 Euston Road

London NW1 2BU

Unit Director: Dr Anton Emmanuel BSc, MD, FRCP

Unit Consultants: Mr Richard Cohen MD, FRCS

Dr Laurence Lovat BSc, PhD, FRCP

CONSENT FORM

Title of project: Quantifying anorectal function and malnutrition in systemic sclerosis (SSc)

Name of Principal investigator: Dr Anton Emmanuel

Please initial box

1. I confirm that I have read and understood the information sheet dated 04/02/08 (version 02) for the above study and have had the opportunity to ask questions. ☐

2. I confirm that I have had sufficient time to consider whether or not I want to be included in the study ☐

3. I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason, without my medical care or legal rights being affected. ☐

4. I understand that sections of any of my medical notes may be looked at by responsible individuals or from regulatory authorities where it is relevant to my taking part in research. I give permission for these individuals to have access to my records. ☐

5. I agree to take part in the above study. ☐

Continued on next page/

(1 form for Patient, 1 to be kept as part of the study documentation, 1 to be kept with hospital notes)

University College London Hospitals NHS
NHS Foundation Trust

Physiology Unit

Podium Level 2

University College Hospital

235 Euston Road

London NW1 2BU

Unit Director: Dr Anton Emmanuel BSc, MD, FRCP

Unit Consultants: Mr Richard Cohen MD, FRCS

Dr Laurence Lovat BSc, PhD, FRCP

CONSENT FORM

Title of project: Quantifying anorectal function and malnutrition in systemic sclerosis (SSc)

Name of Principal investigator: Dr Anton Emmanuel

_____

Name of patientDateSignature

_____ _____

Name of Person taking consentDateSignature

(if different from researcher)

_____ _____

Researcher (to be contacted DateSignature

if there are any problems)

Comments or concerns during the study

If you have any comments or concerns you may discuss these with the investigator. If you wish to go further and complain about any aspect of the way you have been approached or treated during the course of the study, you should write or get in touch with the Complaints Manager, UCL hospitals. Please quote the UCLH project number at the top this consent form.

(1 form for Patient, 1 to be kept as part of the study documentation, 1 to be kept with hospital notes).

Appendix 7: Copy of patient information leaflet

University College London Hospitals NHS
NHS Foundation Trust

Physiology Unit

Podium Level 2

University College Hospital

235 Euston Road

London NW1 2BU

Unit Director: Dr Anton Emmanuel BSc, MD, FRCP

Unit Consultants: Mr Richard Cohen MD, FRCS

Dr Laurence Lovat BSc, PhD, FRCP

Quantifying anorectal function and malnutrition in systemic sclerosis (SSc)

PATIENT INFORMATION LEAFLET

Introduction

You are being invited to take part in a research study. Before you decide it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully and discuss it with others if you wish. Ask us if there is anything that is not clear or if you would like more information. Take time to decide whether or not you wish to take part.

What is the purpose of the study?

The purpose of the study is to investigate gut function and nutritional status in patients with Systemic Sclerosis (SSc). Both patients with gastrointestinal symptoms and asymptomatic patients will be asked to take part. The results of this study will help us understand further why patients with SSc develop gastrointestinal symptoms and enable us to tailor their treatment. Assessing both symptomatic and asymptomatic patients will also help us identify any specific features associated with gastrointestinal and nutritional problems.

Why have I been chosen?

You have Systemic Sclerosis and may have gut symptoms associated with this. We want to assess patients like you so that we can understand better why patients with SSc develop gut problems. The more patients we have to study SSc will allow us to get more accurate results and this will lead to a more accurate understanding. Other patients (approximately …..) with SSc are also included in the study.

Do I have to take part?

It is up to you to decide whether or not to take part. If you do decide to take part you will be given this information sheet to keep and be asked to sign a consent form. If you decide to take part you are still free to withdraw at any time and without giving a reason. A decision to withdraw at any time, or a decision not to take part, will not affect the standard of continuing care you receive.

What is involved in the study?

The study will involve two sets of investigations (anorectal assessment and nutritional assessment) which we will be performed on the same day.

Anorectal assessment will involve:

1) Answering 3 questionnaires assessing lower gut symptoms and the effect of SSc on your quality of life (Wexner constipation score, St Mark's faecal incontinence score and SF-36). On average they take about 10 minutes to complete.

2) Anorectal assessment: anorectal physiology and endoanal ultrasound
Anorectal physiology tests take approximately 30-40 minutes to perform. During ano-rectal physiology the strength, sensitivity, and integrity of reflexes of the back passage and pelvic floor muscles will be tested and also the compliance (elasticity) of the bowel wall. Testing for strength is done by inserting a small catheter, "the size of a straw", no further than 7cm into the back passage to measure pressures on squeezing and resting. Testing compliance will be done by inflating a small inflatable balloon inserted in the back passage. At the same time we will also test sensation and reflexes. Sensation will also be tested by a small electrically stimulating catheter.

The endo-anal ultrasound scan will involve a small probe the size of your index finger being placed no further than 7 cm into the back passage. On ultrasound the muscles will be looked at for tears and quality. The actual scan should not take more than 5 minutes. None of these tests have been reported as being painful although some patients have reported that they can be uncomfortable a times.
The results from the questionnaires, endo-anal ultrasound and the anorectal

physiology will allow for better understanding of the effect of SSc on lower gut function.

3) Nutritional assessment

This assessment takes approximately 45 minutes. You will be asked to fast for 4 hours (except water) and to avoid caffeinated drinks and alcohol the day before. The weight and height, arm circumference and skinfold thickness of the back of your arm will be measured. The latter will be measured by gently pinching the skin at the back of your arm and measuring it with a calliper. Your hand-grip strength will also be measured. In the second part of the assessment you will be asked to rest for 30 minutes and then two small electrode pads will be applied on your foot and hand and whilst you're lying completely relaxed a measurement will be taken. Following this and whilst still lying down you face will be covered by a plastic 'bubble' connected by a plastic tube to a computer, this is quite spacious and you will only be asked to stay relaxed and breath normally for 15 minutes. This test gives us an estimation of how much energy you need during the day.

What are known risks of the study or the side effects of any treatment received?

Anorectal physiology, endo-anal ultrasound and the nutritional assessment are safe procedures. There are no risks associated with any of these procedures.

What are the possible benefits of taking part?

Your referring doctor is involved in the study and will have full access to the investigation results and these may inform further treatment plan. However it may be that this study will have no direct benefit to you, but we hope the information we gather from this study will benefit patients with SSc in the future.

What happens to the data collected about me?

We will create a database for this study. The data will be annoymised such that your name, hospital number date of birth and address will be fully removed and you will be given a unique trial identification number. The list of identification numbers will

be held on a password protected secure computer separate from the database controlled by trial principal investigator (Dr Anton Emmanuel). We will store basic information such as your age, as well as results of your endo-anal ultrasound, anorectal physiology, nutritional assessment and data from questionnaires. The database will be stored on a password protected computer drive held by UCL who will collect, store, handle and process the data. Only the trial principal investigators (Dr Anton Emmanuel, Prof Alastair Forbes) and study co-ordinator (Dr Nora Thoua) will have access to the database and will be responsible for the safety and security of the data. With your permission we may use your data for future studies, although again it will be anonymised when handled as explained above. For this reason we expect to keep the database for 3 years.

What will happen to the results of the research study?

The information regarding your studies will be held in the department of GI physiology, UCH and will be kept safely in this department. Dr Anton Emmanuel is the principal investigator and will ensure the safety and security of your information. All information which is collected about you during the course of the research will be kept strictly confidential. Any information about you which leaves the hospital will have your name and address, date of birth and all identifiable information (including patient/hospital/NHS number) removed so that you cannot be recognised from it.

Will my GP be informed?

Studies not being conducted by your GP require us to inform them regarding your participation in our study, but only if you agree for us to do so.

What happens when the research study stops?

Once we have completed our study, we will examine the data we have collected and publish our results in journals for other doctors to read and thus benefit other women in the future, with similar problems to yours.

What will happen if the findings affect me?

A report of the findings of your investigations will be sent to your referring doctor in the usual manner; who will decide on the treatment you need. This study does not affect your results or treatment.

What if something goes wrong?

If you are harmed by taking part in this research project, there are no special compensation arrangements. If you are harmed due to someone's negligence, then you may have grounds for a legal action but you may have to pay for it. Regardless of this, if you wish to complain, or have any concerns of this study, the normal National Health Service complaints mechanisms should be available to you.

What will happen to the results of the research study? What will happen to the results of the research study?

We will publish the results so that as many of our findings as possible will be made available to the medical and scientific community. You will not be personally identified in any publication. The timing of any publication will depend mostly on the speed with which we collect data but we hope will take no more than a year. Your doctor at UCH will be able to tell you about the results when they are published so you can get a copy if you wish.

Who is organising and funding the research?

This study is being organised by the doctors at the bottom of this information sheet in collaboration with Prof. Chris Denton and Dr Geraldine Brough at Royal Free Hospital. Funding for this study is provided by the Scleroderma Society and Royal Free and University College Hospitals. The doctors are not paid for including you in this study.

Withdrawal from the project

Your participation in the trial is entirely voluntary. You are free to decline to enter or to withdraw from the study at any time, without having to give a reason. If you choose not to enter the trial, or to withdraw once entered, this will in no way affect your future medical care. All information regarding your medical records will be treated as strictly confidential and will only be used for medical purposes. Your medical records may be inspected by competent authorities and properly authorised persons, but if any information is released this will be done in a coded form so that confidentiality is strictly maintained. Participation in this study will in no way affect your legal rights.

Who has reviewed the study?

This study has been reviewed by the UCL/UCLH Research Ethics Committee.

Contact for further information

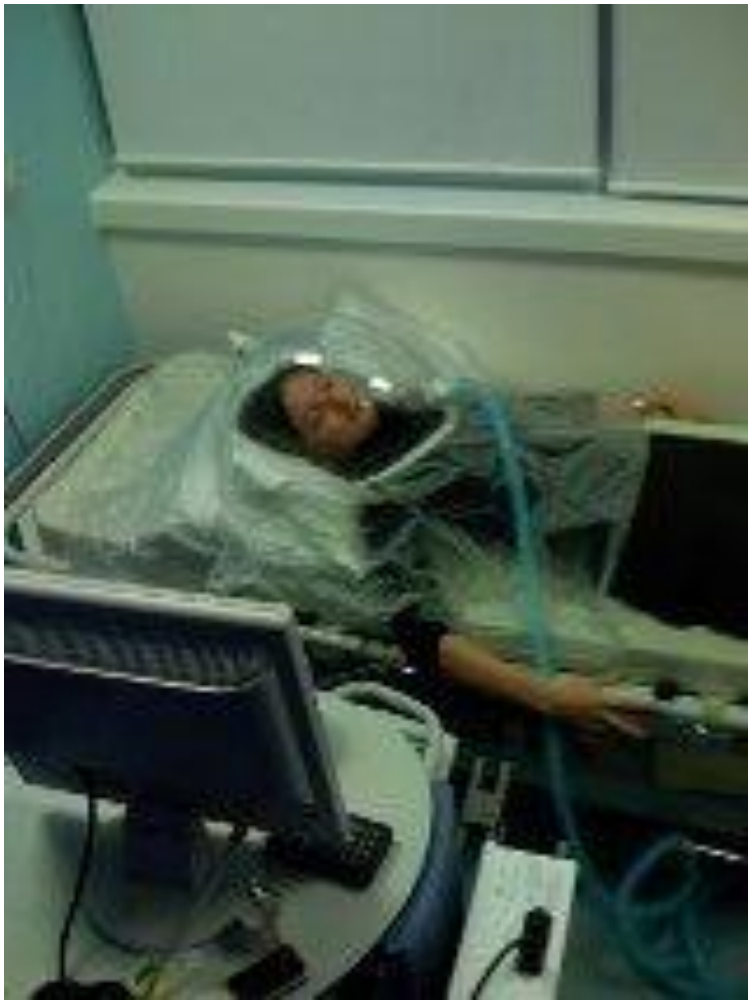
Dr Nora Thoua, Gastroenterology Research Registrar, University College Hospital London,
Tel: 0845 155 5000 Ext: 73208
Email: nora.thoua@uclh.nhs.uk

Dr. Anton Emmanuel, Senior lecturer in Neurogastroenterology and Consultant Gastroenterologist, University College Hospital London,
Tel: 0845 155 5000 Ext: 73208

Professor Alastair Forbes, Professor of Gastroenterology and Nutrition, University College Hospital London,
Tel: 0845 155 5000 ext

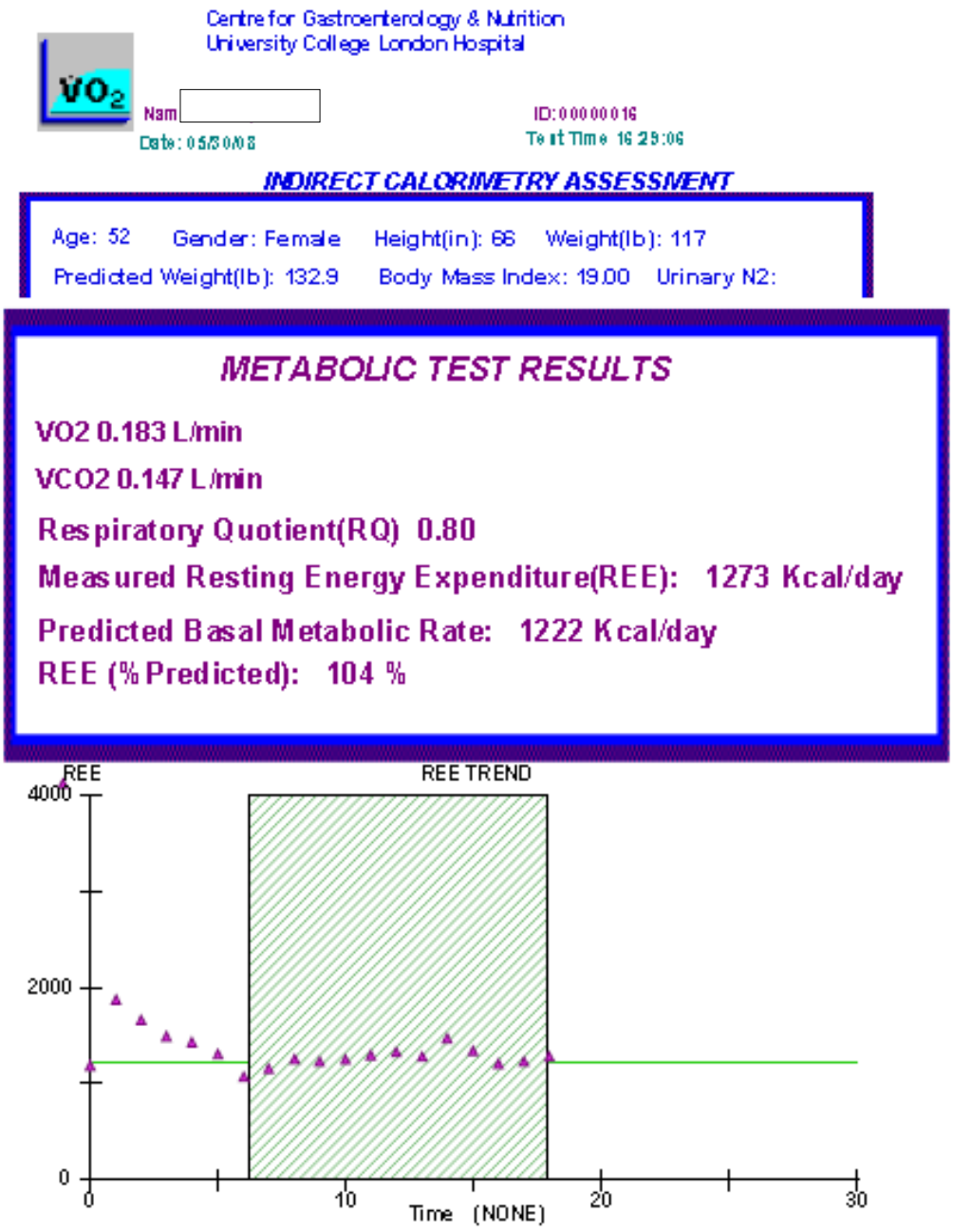
Appendix 8:

8.1. Photograph of NT demonstrating the indirect calorimetry assessment set up.

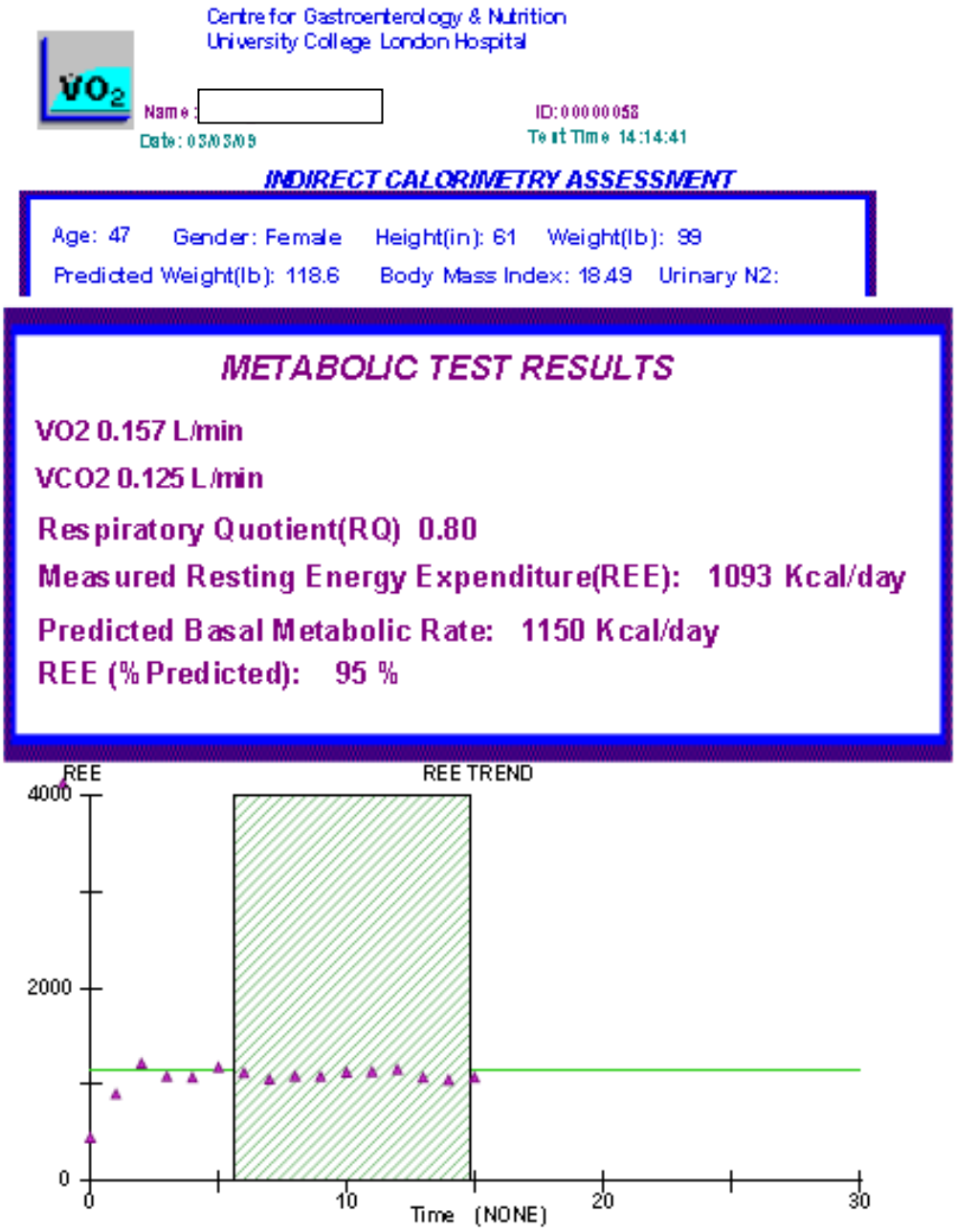


8.2. Indirect calorimetry traces from A) a symptomatic patient and B) an asymptomatic patient.

A) Symptomatic patient



B) Asymptomatic patient



Appendix 9: Copies of publications arising from this thesis