

**A PROSPECTIVE ASSESSMENT OF  
GASTROINTESTINAL DISEASE AND  
NUTRITIONAL STATUS IN PATIENTS  
WITH SYSTEMIC SCLEROSIS**

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## Abbreviations

<sup>13</sup> C	<sup>13</sup> Carbon
ANOVA	Analysis of variance
ANS	Autonomic nervous system
AUC	Area under curve
BIA	Bioelectrical impedance
BMI	Body mass index
BP	Blood pressure
BSF	Biceps skinfold
CASS	Composite autonomic severity scale
CI	Confidence interval
COMPASS	Composite autonomic symptom scale
CSI	Cardiac sympathetic index
CRBSI	Catheter-related blood stream infection
CVC	Central venous catheter
CVI	Cardiac vagal index
DBP	Diastolic blood pressure
dcSSc	Diffuse cutaneous systemic sclerosis
DLCO	Diffusing capacity for carbon monoxide
DRV	Dietary reference values
ECG	Electrocardiogram
E:I	Expiratory : inspiratory
FEV	Forced expiratory volume
FVC	Forced vital capacity
GI	Gastrointestinal
GORD	Gastro-oesophageal reflux
GSR	Galvanic skin response
HAQ	Health assessment questionnaire
HPN	Home parenteral nutrition
HR	Heart rate

HR <sub>DB</sub>	Heart rate response to deep breathing
HR <sub>max</sub> /HR <sub>min</sub>	Heart rate maximum to heart rate minimum ratio
HRV	Heart rate variability
IBI	Inter-beat interval
ICC	Intraclass correlation coefficient
IF	Intestinal failure
lcSSc	Limited cutaneous systemic sclerosis
MAC	Mid-arm circumference
MAMA	Mid-arm muscle area
MAP	Mean arterial pressure
MET	Metabolic Equivalent of Task
MUAA	Mid-upper arm anthropometry
NN	Normal-to-normal
PhA	Phase angle
PN	Parenteral nutrition
SBP	Systolic blood pressure
SD	Standard deviation
SDNN	Standard deviation of normal-to-normal intervals
SHAQ	Scleroderma health assessment questionnaire
SIBO	Small intestinal bacterial overgrowth
SISF	Supra-iliac skinfold
SSc	Systemic sclerosis
SSSF	Sub-scapular skinfold
TSF	Triceps skinfold
RNI	Reference nutrient intake
UCLA SCTC GIT	University College Los Angeles scleroderma clinical trials consortium gastrointestinal tract
VAS	Visual analogue scale
VM	Valsalva manoeuvre
VR	Valsalva ratio

## Abstract

**Submitted to the University of Manchester by Elizabeth Harrison for the degree of Doctor of Philosophy (PhD) December 2015, entitled A PROSPECTIVE ASSESSMENT OF GASTROINTESTINAL DISEASE AND NUTRITIONAL STATUS IN PATIENTS WITH SYSTEMIC SCLEROSIS**

**Background:** Malnutrition and gastrointestinal (GI) involvement are common in patients with systemic sclerosis (SSc). Despite malnutrition being common, little is known about its associations and predictors. Although patients are frequently screened and assessed for malnutrition, different clinically applicable assessment modalities in SSc have not been compared. An understanding of the relationship between dietary intake and energy expenditure is important for nutritional assessment and management. However, studies have not compared these. For many years, home parenteral nutrition (HPN) has been used in patients with intestinal failure, but little outcome data exists to support its role in SSc. GI involvement results in dysmotility, the underlying mechanism for the development of which is unknown. However, autonomic dysfunction has been proposed.

**Aims:** To explore aspects of the nutritional assessment and management of patients with SSc. To seek associations and predictors of nutritional decline. To investigate for a link between GI dysmotility and autonomic dysfunction.

**Methods:** **Study 1:** A retrospective review of the survival and outcome data of patients commenced on HPN over 22 years. **Study 2:** An assessment of 168 patients recruited over 12 months and restudied after approximately 1 year. Assessment included demographics, clinical data, GI and functional questionnaires, nutrition screening tool, oral aperture, mid-upper arm and 4-site anthropometry, bioelectrical impedance and biochemical testing. Re-study included weight change. **Study 3:** A 3 day assessment of dietary intake and energy expenditure using food record charts and SenseWear® Armband involving 36 patients recruited to Study 2. **Study 4:** Patients and matched controls completed GI and autonomic questionnaires, an autonomic battery, a gastric emptying study and postprandial cardiovascular measures and GI sensations and symptoms scores.

**Results:** **Study 1:** The cumulative probabilities of surviving on HPN at 2, 5 and 10 years were 75%, 37% and 23%. HPN-associated complication rates were low. **Study 2:** Nutritional screening failed to identify all patients who lost weight. Mid-arm circumference correlated with body mass index (BMI) and weight change. Four-site anthropometry correlated with BMI more strongly ( $r=0.65$  vs.  $r=0.49$ ) than bioelectrical impedance analysis. Small intestinal, but not oesophageal, involvement correlated with baseline nutritional status. No clear predictors of nutritional decline were identified. **Study 3:** Predicted energy intakes correlated with measured expenditures, but absolute values differed. Energy intakes did not correlate with expenditures. **Study 4:** Autonomic measures did not correlate with gastric emptying. However, autonomic results were hindered by patient-related and technical limitations.

**Conclusion:** Nutritional screening tools cannot be relied upon to detect all at risk patients. MAC and 4-site anthropometry may have a role in nutritional assessment. When an accurate appreciation of energy requirements is needed, kinematic monitors should be used rather than predictive equations. For those patients who progress to intestinal failure, HPN is safe and effective. Autonomic studies were inconclusive. However, the autonomic apparatus has been refined for utilisation in more definitive studies in younger patients.

## **Declaration**

No portion of the work referred to in this thesis has been submitted in support of an application for another degree or qualification of this or any other university or other institute of learning



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## **The Author**

In 2001, I was awarded the degree of Bachelor of Science (BSc) in Medical Science by the University of St Andrews. Following this, I continued my medical studies at the University of Manchester where I was awarded the degree of Bachelor of Medicine and Bachelor of Surgery (MBChB) in 2004.

My postgraduate medical training started with house officer posts in Wigan, before continuing in Stoke-on-Trent where I spent three years as a medical senior house officer. Whilst working in Stoke-on-Trent, I obtained Membership of the Royal College of Physicians (MRCP, Edinburgh).

In 2008, I was appointed to the position of Gastroenterology Speciality Trainee in the West Midlands Deanery and was able to begin developing my interests in luminal gastroenterology and nutrition. I was awarded the Speciality Certificate in Gastroenterology in 2010 and a Postgraduate Certificate in Clinical Nutrition from the University of Surrey in 2013. These interests propelled me to undertake the work which is presented in this thesis for consideration for the award of Doctor of Philosophy (PhD).

# **CHAPTER 1:**

# **BACKGROUND**

# 1 Background

## 1.1 Systemic sclerosis

Systemic sclerosis (SSc), or scleroderma, is a chronic autoimmune disease characterised by excessive fibroblast-mediated collagen deposition throughout the skin and internal organs.

Reported incidence and prevalence rates of SSc vary widely depending on the geographic region and the method of identifying cases. The reported prevalence ranges from 7 to 489 per million and the incidence from 0.6 to 122 per million per year [1969 to 2006] [1].

Geographically, the prevalence of SSc differs. The prevalence is higher in North America than in Europe, where a North-South gradient is also observed (i.e. higher in Greece than England and Iceland). The most recent figures for England are based on a 2004 study in northeast England, which reported an adjusted UK prevalence of 8.2/100,000 [2]. Women are more commonly affected. The UK study reported a female to male ratio of 5.2:1 [2].

Historical incidence and prevalence rates are influenced by the lack of a standardised classification system prior to 1980, when the American College of Rheumatology classification criteria were introduced [3]. The criteria aimed to establish a standard for definite disease in order to permit comparison of patients from different centres and to assist in the accurate evaluation of clinical and trial results. Fulfilling one major or 2 or more minor criteria was associated with a 98% specificity and 97% sensitivity for definite SSc [3].

<b>Major criterion</b>
Proximal cutaneous scleroderma
<b>Minor criteria</b>
1. Sclerodactaly
2. Digital pitting scars of fingertips or loss of substance of the distal finger pulp
3. Bilateral bibasilar pulmonary fibrosis

Table 1.1: American Rheumatism Association Criteria for the classification of SSc

However, these criteria fail to detect patients with early or limited disease. Recently, these criteria have been succeeded by the 2013 American College of Rheumatology and European League Against Rheumatism Collaborative Initiative classification criteria [4]. This new classification system, which has improved specificity and sensitivity, contains

the same 4 items as the 1980 ACR criteria and an additional 5 items, with different weightings.

### **1.1.1 Disease sub-types**

Cases of SSc are divided into two subgroups, based largely on the extent of their cutaneous involvement. The subgroups are diffuse (dcSSc) and limited (lcSSc) cutaneous SSc [5]. Limited disease is far more common. In the UK, the ratio of lcSSc to dSSc is 4.7:1 [2].

Clinically, patients with lcSSc have cutaneous involvement confined to their face, hands, feet, forearms and lower legs [5]. Patients with dcSSc may also have truncal and proximal limb involvement. However, differences between the subgroups are not confined to the extent of cutaneous involvement [6]. Both subgroups are likely to develop secondary Raynaud's phenomenon. However, in patients with lcSSc, Raynaud's phenomenon may predate the development of SSc by many years, while in patients with dcSSc it often develops shortly after the onset of SSc. In addition, patients with dcSSc are more likely to have a worse prognosis [7]. They are more liable to develop cardiac, renal and pulmonary manifestations early in the course of their disease than patients with lcSSc.

Gastrointestinal (GI) involvement may occur in both subgroups. Immunologically, lcSSc is associated with the anti-centromere antibody, while dcSSc is associated with the anti-topoisomerase I (anti-Scl-70) antibody.

### **1.1.2 Pathogenesis of systemic sclerosis**

The pathogenesis of SSc is complex and incompletely understood. The mechanisms involved include vascular damage, autoimmunity, inflammation and connective tissue fibrosis. The relative importance of each mechanism differs between patients and with different disease manifestations. Each mechanism is briefly discussed below.

#### **Vascular mechanism**

Vascular changes usually precede tissue fibrosis, suggesting that endothelial cell dysfunction may activate immunological and inflammatory processes leading to fibrosis

[8]. Vascular changes predominantly affect capillaries and small blood vessels. Capillary architecture becomes distorted (irregular capillary loops). Small arterioles develop intimal swelling. Microscopically, there is disruption of tight cellular junctions and later nuclear injury and cellular apoptosis [8].

Endothelial cells play an important role in many physiological pathways such as coagulation and fibrinolysis and, via the controlled releases of vasodilators and vasoconstrictors, the regulation of vascular tone. Endothelial cell dysfunction disrupts these control pathways [8]. This may lead to the activation of the coagulation systems and the development of microthrombosis, resulting in further vascular injury. In addition, as a consequence of vascular dysfunction, pro-fibrotic signal molecule production may increase, which may in turn increase fibroblast proliferation and thus fibrosis.

### **Immune dysfunction**

SSc is associated with specific autoantibodies that may be detected in serum samples. Different autoantibody profiles are associated with the different disease subgroups (lcSSc and dcSSc) and differ within the subgroup between patients with different disease phenotypes.

Patients with SSc have altered B and T cell activity [9]. Aberrant immune function leads to the generation of pro-inflammatory cytokines, which propagate the fibrotic process, and autoantibodies, which act against nuclear antigens. Some autoantibodies act against endothelial cells, thus fuelling endothelial cell dysfunction.

### **Fibrotic mechanism**

SSc is characterised by the abnormal deposition of collagen tissue throughout the body, resulting in disrupted structure and function. Normally, the action of fibroblasts, which have a key role in the remodelling of the extracellular matrix, is tightly controlled. However, in patients with SSc, fibroblast action is triggered in an uncontrolled manner by many stimuli, including cytokines, chemokines and tissue hypoxia [9]. In addition, there is a disruption of the normal anti-fibrotic control mechanisms, which further compounds the problem.

## **1.2 Structure and function of the gastrointestinal tract**

The GI system consists of the luminal tract and associated solid organs. The tract's different parts have common structural features but differing functions.

### **1.2.1 Structure**

The mucosa is the innermost layer, followed by the blood vessel and nerve containing submucosa, then the muscularis externa with its smooth muscle layers and finally the adventitia. The nerves include the Meissner's and Auerbach's plexuses, which are part of the enteric nervous system. These have a role in the regulation of GI motility and secretions.

### **1.2.2 Function**

The primary function of the GI tract is to digest and absorb ingested nutrients, for release into the blood stream. Thus, the GI tract must be able to breakdown food, propel contents along its lumen by peristalsis and absorb nutrients. Any diseases which affect the tract's ability to perform these functions may have a detrimental effect on nutritional status.

Different patterns of involuntary motility are characteristic of the GI tract's different anatomical regions. These patterns differ in the fed and fasted states. Once swallowed, a food bolus is propelled along the oesophagus by a peristaltic wave to the stomach where contractions break it down to chyme, and propel it through the pyloric sphincter. In the small intestine, luminal contents move primarily by segmentation aided by a short-range peristaltic wave. Unlike peristalsis, which only moves luminal contents in a caudal direction, segmentation also moves chyme cranially and caudally to maximise mixing, digestion and absorption. Segmentation also occurs in the colon to facilitate mixing. In addition, mass movements (waves of prolonged contractions) occur at intervals during the day to propel contents towards the rectum in preparation for defaecation.

In the fasted state, the predominant pattern of GI motility is that of the Migrating Myoelectric Complex. This consists of a slowly propagated, forceful contraction, which passes from the stomach to the terminal ileum. This sweeps any remaining small intestinal



contents into the terminal ileum and, as such, has been termed the ‘housekeeper of the small intestine’ [10].

### **1.2.3 Pathological gastrointestinal features in systemic sclerosis**

SSc results in similar histopathological changes throughout the GI tract. In the mucosal layer, there may be chronic inflammation of the lamina propria and fibrotic replacement of the muscularis mucosa [11]. However, the epithelium is normally unaffected [11, 12]. In the smooth muscle layers of the muscularis externa, there may be atrophy and fragmentation [13, 14]. Over time this progresses from a patchy distribution to one of a generalised fibrosis, associated with loss of gap junctions between smooth muscle cells [15]. Gap junctions aid the transmission of peristalsis. The outermost layer, the adventitia, also shows evidence of thickening and fibrosis.

As occurs elsewhere in patients with SSc, small intra-mural capillaries and arterioles are affected, but larger vessels and veins are relatively spared. Vascular changes include thickening of capillary basement membranes and distortion of arteriolar lumens [16].

Autonomic nerves within the GI tract may be affected. Dense collagen bundles surround the ganglia of the myenteric plexus and interstitial cells of Cajal, separating them from smooth muscle cells and blood vessels [14, 15]. In addition, neurons and nerve endings appear oedematous.

### **1.2.4 Possible mechanisms for gastrointestinal manifestations**

SSc disrupts GI motility. With disease progression, the smooth muscle of the GI tract becomes increasingly fibrotic and eventually atrophies. In early disease, the GI tract is still functional but, as fibrosis and atrophy progress, the smooth muscle of the GI tract becomes less responsive to stimulation, until it no longer responds [17].

GI tract contractility is primarily controlled by the myenteric plexus. The principal excitatory neurotransmitter is acetylcholine which stimulates the muscarinic-3 receptor. In animal studies, rats immunised with purified immunoglobulin G anti-myenteric neuronal antibodies from patients with SSc developed prolongation and disruption of intestinal myoelectrical activity [18]. However, no effect was seen when the rats were immunised

with immunoglobulin G from normal healthy controls. In addition, immunoglobulin G from patients with SSc inhibited muscarinic-3 receptor activation in rat internal anal sphincter smooth muscle cells and removal of the antibodies reversed this effect [19]. Subsequent studies involving healthy human smooth muscle tissue showed similar results [20]. High-titre antibodies directed against myenteric neurons can be found in almost half of all patients with SSc, but not patients with idiopathic dysmotility [21]. Patients with early SSc and severe GI tract involvement have higher titres of anti-muscarinic-3 receptor antibody than patients without severe GI tract involvement [22]. The importance of these antibodies has yet to be proven.

In addition, dysmotility may be, in part, due to neuronal dysfunction which renders the GI tract unable to respond to some stimuli. In studies, the oesophageal smooth muscle of patients with early SSc fails to respond to indirect stimulation (edrophonium and gastrin 1 acting via cholinergic nerves), but does respond to direct stimulation using methacholine [23]. Thus, in the early stages of SSc, denervation may impair normally signalled GI motility, but leave the GI tract capable of responding to direct stimulation (prokinetics). However, with increased disease duration, the GI tract fails to respond to direct stimulation, possibly due to smooth muscle atrophy.

### **1.3 Gastrointestinal manifestations and nutrition**

GI manifestations commonly affect patients with SSc. Indeed, reports suggest the GI tract to be affected in over 90% of patients with SSc [24, 25]. GI tract involvement may feasibly contribute to a person failing to meet their nutritional requirements and, as a result, developing malnutrition. The following sections describe possible SSc-related GI manifestations and any potential or proven nutritional consequences.

#### **1.3.1 Oropharyngeal involvement**

Patients with SSc have significantly worse dental health than healthy volunteers (tooth decay, tooth loss and periodontal disease), which could be related to their reduced saliva production and smaller oral aperture (microstomia) [26-28]. In addition, poor hand dexterity may hinder effective dental hygiene [29-31]. Furthermore, microstomia may complicate denture fitting, thereby hindering mastication [32].

Oral manifestations may influence food choice. Studies involving populations without SSc show tooth loss to result in dietary modification (reduced fruit and vegetables), possibly due to impaired mastication ability [33-35]. A study involving patients with SSc, reported an association between reduced oral aperture and malnutrition risk ('Malnutrition Universal Screening Tool' ('MUST')) [36].

### **1.3.2 Oesophageal involvement**

Normally, tightly co-coordinated peristaltic movements propel luminal contents towards the lower oesophageal sphincter which relaxes, allowing their passage into the stomach, before closing to prevent reflux [37, 38]. Disruption of this in patients with SSc leads to reflux and dysphagia [24, 39]. In one study, 85% of patients reported reflux, despite 71% taking an acid-suppressant [24].

Not all patients with evidence of involvement are symptomatic. In one study involving patients not taking anti-secretory medications, 61% (76/194) had oesophageal symptoms (48% reflux; 47% dysphagia, 35% both) [40]. Furthermore, reflux oesophagitis was identified in 17% of asymptomatic and 50% of symptomatic patients. In addition, an oesophageal motor disorder was proven in 92% of symptomatic and 63% of asymptomatic patients. Oesophageal involvement may be noted incidentally on cross sectional chest imaging. One study identified radiological oesophageal abnormalities in 80% of patients with SSc-lung disease, all of whom were asymptomatic [41]. A second study, showed a clinically significant correlation between delayed oesophageal transit and oesophageal dilation on cross-sectional imaging [42]. Delayed transit and reflux, aided by an incompetent lower oesophageal sphincter, increases oesophageal exposure to damaging gastric acid, which facilitates the development of oesophagitis and Barrett's oesophagus, and if untreated may lead to stricture formation [40, 43-45]. However, since the introduction of wide-spread prescribing of acid-suppressing medications, the reporting of strictures has fallen, but oesophagitis may still persist [46].

Individuals with reflux, unrelated to SSc, modify their diet in an attempt to minimise their symptoms. Patients with SSc-related oesophageal symptoms may adopt similar dietary changes. A dietary study comparing patients with SSc with GI symptoms (30 patients; 28 with dysmotility; 23 symptomatic) to healthy controls found that patients consumed less bread (significant) and fruit /vegetables (non-significant) than the healthy controls [47]. In

addition, patients with severely disturbed oesophageal motility had significantly lower intakes of carotene and pectin than patients with slightly disturbed motility. If intake is affected, then nutritional consequences may be anticipated in severe cases. Two studies involving patients with SSc did not detect any association between dysphagia or reflux and malnutrition risk [36, 48]. However, neither study accounted for symptom severity or frequency.

### **1.3.3 Gastric involvement**

SSc may disturb gastric motility. Delayed gastric emptying is reported in 32-50% of patients [49-52]. Delayed emptying is more common in patients with abnormal antro-duodenal manometry and correlates with vomiting, abdominal pain, bloating and tenderness [51].

SSc may also cause mucosal damage. Endoscopic assessment within the first year of diagnosis in patients without suggestive symptoms showed gastritis in 92% (12/13) [53]. Furthermore, another study of a highly selected group of patients with early dcSSc, and specific internal organ involvement considered for cyclophosphamide or stem cell transplant, showed gastric antral vascular ectasia in 22% (23/103) [54].

Gastroparesis can cause early satiety, nausea and vomiting, which might, if sufficiently severe, affect a person's nutritional status [55]. Two studies have reported a significant correlation between early satiety and risk of malnutrition [36, 48]. Prokinetics may be used to try to control symptoms, despite only a few, small studies showing benefit [49, 56, 57]. In some severe cases, involving patients with uncontrolled symptoms, distal feeding may be required.

### **1.3.4 Small intestinal involvement**

Patients may develop small intestinal manifestations. Manometric studies have shown abnormalities in 88% of unselected patients (n=17, 35% with GI symptoms) [58].

Orocaecal transit times are prolonged in patients with SSc in comparison to healthy controls, with greater delay in patients with SSc for more than 5 years [50]. Patients with severely delayed intestinal transit may develop the rare complication of intestinal pseudo-obstruction.

Structural signs of small intestinal involvement visible on contrast imaging include: small intestinal dilation, mega-duodenum, multiple contrast-filled diverticulae, pneumatosis intestinalis and the typical 'hide-bound' appearance [39, 59, 60]. SSc-related small intestinal dilation and dysmotility lead to stasis of luminal contents, which predisposes to small intestinal bacterial overgrowth (SIBO). Based on unselected patient studies, SIBO affects 43-55% of patients [61, 62]. Associated symptoms included abdominal pain, bloating, diarrhoea, constipation and abdominal tenderness. SIBO has not been linked to immunosuppressive medication [61].

SIBO may be treated using intermittent or cyclical antibiotics [61, 62]. Symptoms improve following eradication. However, eradication is not always successful [62]. In addition, somatostatin analogues may improve motility, a contributing factor [63, 64]. Probiotics may benefit patients with non-specific distension and bloating [65].

The small intestine plays a key role in the digestion and absorption of macro and micronutrients and, as such, its dysfunction may lead to nutritional deficiencies. One study reported an association between diarrhoea requiring antibiotics and malnutrition risk score, but this was not supported by a second study [36, 48]. However, effective bacterial eradication may feasibly negate any nutritional risk. Bacteria deconjugate bile salts which may lead to the impaired absorption of fat soluble nutrients [66]. In studies involving patients with SSc, non-significantly lower levels of vitamin B<sub>12</sub>, ferritin and folic acid have been found in patients with SIBO [61, 67].

The rare complications of acute and chronic intestinal pseudo-obstruction are clear causes of nutritional problems. Acute episodes are usually managed conservatively by keeping the patient nil by mouth, while supported by intravenous fluids and sometimes parenteral nutrition (PN) [68]. In some cases, PN is required for a prolonged period. Similarly, some patients with chronic intestinal pseudo-obstruction may, due to symptom exacerbation on feeding, be unable to meet their nutritional requirements orally. In these cases, patients may require home parenteral nutrition (HPN) in order to survive [69, 70].

### **1.3.5 Colonic and anorectal involvement**

Colonic involvement may result in chronic constipation. When questioned, approximately 45 to 59% of patients with SSc reported experiencing constipation [24, 25, 71]. Studies confirm delayed colonic transit to be a common finding in unselected patients [72].

Patients with SSc often develop dysfunction of the anorectal sphincter leading to faecal incontinence. When questioned, 57% of patients reported incontinence to gas, 33% to liquid stools and 9% to solid stools [73]. Studies assessing anal function reveal sphincter atrophy and low resting pressures [74, 75].

Constipation and faecal incontinence would not be anticipated to result in nutritional problems, as the colon has no significant role in nutrient absorption.

### **1.3.6 Hepatic involvement**

Primary biliary cirrhosis is a common cause of chronic liver which leads to cholestasis, cirrhosis and ultimately liver failure. Anti-mitochondrial antibodies have a high sensitivity and specificity for primary biliary cirrhosis. SSc affects approximately 3% of patients with primary biliary cirrhosis [76]. Primary biliary cirrhosis specific auto-antibodies are detectable in approximately 15% of patients with SSc [77]. However, only 2% of patients with SSc have confirmed primary biliary cirrhosis [78]. Primary biliary cirrhosis associated liver disease may have a slower progression in patients with SSc than in patients without [79]. Cholestasis may impair fat absorption. However, fat soluble vitamin deficiency is uncommon in patients with primary biliary cirrhosis [80].

Autoimmune hepatitis is a rare cause of liver disease, which can progress to cirrhosis and liver failure, which is associated with nutritional problems. Autoimmune hepatitis has been reported in patients with other autoimmune diseases [81]. Autoimmune hepatitis has been noted in association with SSc in small case series only [82, 83].

### **1.3.7 Pancreatic involvement**

The pancreas releases enzymes needed for the digestion of nutrients. Untreated pancreatic exocrine insufficiency leads to weight loss, malabsorption and steatorrhoea. Small studies have shown insufficiency to be common in patients with SSc, but in most patients this was

not clinically significant [84, 85]. In patients with SSc, steatorrhoea has not been shown to be associated with risk of malnutrition [36].

### **1.3.8 Assessment of gastrointestinal involvement**

The University College Los Angeles Scleroderma Clinical Trials Consortium Gastrointestinal questionnaire (UCLA SCTC GIT) is the only questionnaire validated for the assessment of GI symptoms in patients with SSc (Appendix 1). It was developed using a combination of focus groups, field testing, results of other patient-reported measures and psychometric analysis [86]. The reliability of version 2.0 has been assessed by test-retest and internal consistency.

Version 2.0 is a 34-item questionnaire which covers the preceding 7 days and can be completed without interviewer assistance [86, 87]. Questions are divided into 7 stems (reflux, distension/bloating, diarrhoea, faecal soilage, constipation, emotional well-being and social functioning). All questions, with the exception of diarrhoea and constipation, are scored 0-3 depending on the number of days that symptoms were experienced. Scores for each stem are averaged and reported on a scale of 0.0 (least symptomatic) to 3.0 (most symptomatic), except for diarrhoea (0.0-2.0) and constipation (0.0-2.5). Averages are compared to defined reference ranges of severity (Appendix 2). The total GI score is the average of all stems, excluding constipation. Thresholds for clinically significant differences between multiple scores have been defined (Appendix 3) [88].

To-date, the questionnaire has been used in several studies to survey GI symptoms and to assess responses to interventions [24, 65]. Its reliability and validity have been confirmed by comparing results to physician diagnoses and patient self-reported GI severity scores [86]. A subsequent validation study also supported its usefulness for assessing GI involvement [89].

## **1.4 Other disease-related manifestations and nutrition**

SSc is a multi-system connective tissue disease. Most patients have extra-GI involvement which may directly, or indirectly, affect their nutritional status. This section will discuss some non-GI manifestations which may affect a person's ability to meet their nutritional requirements or which have an association with nutritional impairment.

### **1.4.1 Depression**

Depression is common in patients with SSc. The incidence/prevalence varies depending on the assessment tool and patient cohort studied. A recent review quoted prevalence of 36-69% for mild to moderate depression and of approximately 20% for moderate to severe depression [90]. At present, no specific assessment tools exist to assess mood disorders in patients with SSc. However, questions regarding mood are included in other SSc-specific questionnaires (e.g. UCLA SCTC GIT 2.0).

In patients with SSc, depression may be associated with GI symptoms [91, 92].

Depressive symptoms are strongly associated with oesophageal and upper GI involvement [93, 94]. Depression is known to affect appetite and thus may impact on weight [95, 96].

In patients with SSc, poor appetite has been associated with malnutrition risk [36]. In another study, in comparison to healthy volunteers, patients with SSc were more likely to report a reduced appetite [97]. However, no link to depressive symptoms was identified.

### **1.4.2 Hand function**

SSc is associated with secondary Raynaud's phenomenon, digital ulceration, thickened skin, finger contractures and digital calcinosis, all of which may impair hand function.

Raynaud's phenomenon is characterised by recurrent episodes of digital vasospasm which may be triggered by a temperature drop. Patients describe digital pallor, followed by cyanosis and finally a painful erythematous phase. Thus, when exposed to cold patients may experience functional impairment. At any one time, approximately 10% of patients with SSc may have a painful digital ulcer, which is an important cause of functional impairment [98]. In addition, ulcers may lead to soft-tissue infection, osteomyelitis and



gangrene which can result in amputation. In one study, almost 5% of patients had undergone a digital amputation [99].

Most patients with SSc develop skin thickening, hardening and tightening, related to collagen fibre changes [100]. The severity of skin involvement is linked to the severity of other organ manifestations and ultimately prognosis [101]. Skin thickness is reported using the validated Modified Rodnan Skin Score (MRSS) [100, 102, 103]. Scores are reproducible between different, trained assessors [104-106]. The MRSS is the sum of 17 individual assessments from specified locations (face, anterior chest, abdomen and right and left upper arm, forearm, dorsum hand, fingers, thigh, lower leg and dorsum foot), with each site scored on a scale from 0 to 3 (Table 1.2) [100].

<b>Grade</b>	<b>Skin description</b>
0	Normal
1	Thickened skin
2	Thickened, unable to move
3	Thickened, unable to pinch

Table 1.2: Modified Rodnan Skin Score

Good hand function is essential in order to undertake many of the activities of daily living. Functional limitations may be self-reported via validated hand-specific or non-specific functional questionnaires which include hand related questions, such as the Health Assessment Questionnaire (HAQ) and UK functional score [107-109].

### **Health Assessment Questionnaire**

The HAQ was originally designed to assess functional impairment in patients with arthritis, but has since been extensively used in other rheumatological diseases, including SSc [108, 110, 111]. It may be used either in its full form, which assesses disability, discomfort/pain, medication side effects and cost implications, or its shortened form, which focuses on disability and discomfort/pain. The shortened version (20-question) only includes the HAQ Disability Index and pain scale (Appendix 4). It assesses disability across 8 domains (dressing, arising, eating, walking, hygiene, reaching, grip and activity) over the previous 7 days. When completing the questionnaire, patients rate their ability to perform certain tasks of daily living and indicate their need for aids or assistance. The

highest reported score, for each domain, is used to score the level of impairment. The questionnaire includes a standardised 15cm visual analogue (VAS) pain scale. All measurements are converted into a score between 0 and 3.0. Five SSc-specific VAS scores (scored 0-3; GI, lung, global assessment, vascular, digital ulcer) were subsequently added to produce the Scleroderma HAQ (SHAQ) [112].

Regional and total skin scores correlate with hand dexterity, such that better hand dexterity is reported by patients with less skin involvement [113]. In addition, patients with higher total skin scores have higher HAQ scores than those with lower skin scores [114]. However, no difference in functional disability (HAQ) or skin thickness has been shown between patients with and without disease-related malnutrition [48].

### **UK functional score**

The UK Functional Score is an 11-item self-administered questionnaire which includes 9 items relating to upper limb function (Appendix 5) [107]. Items are scored 0 to 3, to generate a total score ranging from 0 to 33. Scores show satisfactory test-retest reliability and results are strongly correlated to those of the HAQ-Disability Index [107, 115].

### **1.4.3 Other severe organ involvement**

SSc manifestations may affect an array of organs. Manifestations may have no perceivable effects or be associated with considerable morbidity. Severe involvement may influence nutritional requirements.

### **Cardiac manifestations**

Cardiac manifestations are common [116]. Manifestations may be due to SSc-related pathology of the cardiac structures or conducting systems, or as consequence of medication, respiratory or other organ involvement. Approximately 15% of patients develop severe cardiac involvement, [117]. Heart failure, especially when advanced, is well recognised to be associated with malnutrition [118].

### **Respiratory manifestations**

Respiratory manifestations include interstitial fibrosis and pulmonary arterial hypertension. Approximately 15% of patients develop pulmonary hypertension or pulmonary restriction [117]. In its severest form, patients can develop respiratory failure, requiring long-term home oxygen supplementation and sometimes transplantation. Malnutrition is common in patients without SSc but with end-stage lung disease referred for transplantation, and corrects following transplantation [119].

### **Renal manifestations**

Patients may develop acute renal crisis [120]. It is more common in patients with dcSSc. Approximately 12% of patients with dcSSc develop renal crisis in comparison to 3% of all patients [117]. In severe cases, patients may require long-term renal replacement therapy [121]. Malnutrition is common in patients with end-stage renal failure [122, 123].

### **Assessment of disease severity**

The severity of SSc-related disease affecting an organ is defined by the total effect (reversible and irreversible) on the organ's function. Organ damage is the end effect of irreversible damage. Disease activity, which is reversible, may result in future damage. The Medsger severity scale for SSc rates organ systems individually [124]. Its 9 organ-specific severity scales are each scored 0 to 4, representing progression from normality to end-stage disease [124, 125]. For grades defined by 2 or more categories, the individual need only have one to be classed to meet that grade. Organ scores are not summative.

<b>Organ System</b>	<b>0 (normal)</b>	<b>1 (mild)</b>	<b>2 (moderate)</b>	<b>3 (severe)</b>	<b>4 (endstage)</b>
1. General	weight loss <5% PCV>37% Hb >12.3gm/dl	weight loss 5.0-9.9% PCV 33.0-36.9% Hb 11.0-12.2gm/dl	weight loss 10.0-14.9% PCV 29.0-32.9% Hb 9.7-10.9gm/dl	weight loss 15.0-19.9% PCV 25.0-28.9% Hb 8.3-9.6gm/dl	weight loss >20% PCV <25.0% Hb <8.3gm/dl
2. Peripheral Vascular Disease	No Raynaud's or Raynaud's not requiring vasodilator	Raynaud's requiring vasodilators	Digital pitting scars	Digital tip ulcerations	Digital gangrene
3. Skin	MRSS 0	MRSS 1-14	MRSS 15-29	MRSS 30-39	MRSS>40
4. Joint / Tendon	FTP 0-0.9cm	FTP 1.0-1.9cm	FTP 2.0-3.9cm	FTP 4.0-4.9cm	FTP >5.0cm
5. Muscle	normal proximal muscle strength	mild proximal weakness	moderate proximal weakness	severe proximal weakness	ambulation aids required
6. GI Tract	normal oesophagram normal small bowel series	distal oesophageal hypoperistalsis abnormal small bowel series	antibiotics required for bacterial overgrowth	malabsorption syndrome episodes of pseudo-obstruction	hyperalimantation required
7. Lung	DLCO >80% FVC >80% no fibrosis on x-ray PAP <35mmHg	DLCO 70-79% FVC 70-79% basilar rales fibrosis on x-ray PAP 35-49mmHg	DLCO 50-69% FVC 50-69% PAP 50-64mmHg	DLCO <50% FVC <50% PAP >65mmHg	oxygen required
8. Heart	ECG normal LVEF >50%	ECG conduction defect LVEF 45-49%	ECG arrhythmia LVEF 40-44%	ECG arrhythmia requiring treatment LVEF 30-40%	Chronic heart failure LVEF <30%
9. Kidney	no history of SRC with serum creatinine <1.3mg/dl	history of SRC with serum creatinine <1.5mg/dl	history of SRC with serum creatinine 1.5-2.4mg/dl	history of SRC with serum creatinine 2.5-5.0mg/dl	history of SRC with serum creatinine >5mg/dl or dialysis required

Table 1.3: Medsger SSc severity scale

PCV = packed cell volume; Hb = haemoglobin; FTP = finger-to-palm; SRC = scleroderma renal crisis; PAP = pulmonary artery pressure; DLCO = Diffusing capacity of the lung for carbon monoxide; FVC = forced vital capacity; LVEF = left ventricular ejection fraction; MRSS = modified Rodnan skin thickness score; ECG = electrocardiogram

## **1.5 Assessment of nutritional status**

The assessment of nutritional status is important in order to detect and assess malnutrition.

### **1.5.1 Malnutrition**

Malnutrition is a state of nutrition in which a deficiency, excess or imbalance of energy, protein and/or other nutrients causes measurable adverse effects on body shape, size, composition and/or function and clinical outcome [126]. It includes both under and overnutrition.

Undernutrition may occur either due to one, or to a combination of factors, including inadequate intake, increased requirements, impaired absorption, impaired nutrient transport and/or impaired nutrient utilisation. It may result in a wide range of negative physical, physiological and psychological consequences, such as muscle weakness and wasting (mobility, cardiac, respiratory), delayed healing, impaired immune function and apathy and depression. In addition, it increases mortality [127].

Overnutrition places individuals at risk. Long-term, it is associated with a variety of health complications, including diabetes mellitus, cardiovascular disease and osteoarthritis. During periods of severe acute illness, obese or overweight individuals may develop nutritional deficiencies requiring intervention.

### **1.5.2 Sarcopenia**

Sarcopenia is the progressive age-related decline of muscle mass and function [128, 129]. The functional impairment may be greater than predicted for any given change in muscle mass [128]. This functional decline, which leads to inactivity, is the prime focus of many clinical sarcopenia studies. Muscle mass and function may be measured by a variety of techniques. However, the interpretation of these measurements is often complicated by the absence of consensus agreed quantitative thresholds for sarcopenia.

To-date, no studies have assessed for age-related decline in muscle function in patients with SSc. In addition, to the best of my knowledge, only one nutritional study has included an assessment of skeletal muscle function [130]. This may be due in part to the

possibility that, in patients with SSc, any assessment of any functional decline may, in theory, be complicated by disease-specific manifestations.

In patients with SSc, muscle manifestations are common. In one study, 43% of patients had muscle manifestations in the form of muscle weakness, muscle atrophy and/or creatinine phosphokinase elevation [131]. Proximal muscle weakness may be due to myositis. Proximal weakness is reported to affect 16% of all patients with SSc and 24% of patients with dcSSc [117]. Any myositis-related weakness would confound the detection of sarcopenia through muscle strength testing. In addition, impaired hand function may have a confounding effect on grip strength testing. For these reasons, the assessment of sarcopenia was not included in this thesis.

### 1.5.3 Body mass index

The most commonly used tool to describe nutritional status is body mass index (BMI), which incorporates weight (kilograms) and height (metres). Clinically, this may be used alone or as part of a screening tool. Values are divided into qualitative categories, with set cut-offs (Table 1.4) based on the effects of obesity-associated morbidity [132]. Different cut-off ranges exist for different ethnicities. Caution must be used when interpreting BMIs due to the influences of other factors, such as age and body composition. A high BMI fails to differentiate between individuals with high muscle mass, high fat mass or gross oedema.

<b>BMI (kg/m<sup>2</sup>)</b>	<b>Classification</b>	<b>Risk of obesity-associated co-morbidities</b>
<18.5	underweight	low (but risk of other clinical problems increased)
18.5 – 24.9	healthy range	average
>25 – 29.9	overweight	increased
>30 – 34.9	obese (class i)	moderate
>35 – 39.9	obese (class ii)	severe
≥40	obese (class iii)	very severe

Table 1.4: World Health Organisation BMI classification

#### **1.5.4 Body composition**

Caution must be used when using BMI alone, as it fails to account for the relative contribution of fat and lean tissues. Individuals with low BMIs may have as much relative fat as individuals with high BMIs. Thus, body composition assessment is important.

##### **Body composition models**

Tools to assess body composition are based on theoretical models of the composition of the human body. Models divide the body into components.

Two-component models merely divide the body into fat and fat-free body compartments. The fat-mass includes all extractable lipids from adipose and other body tissues. Meanwhile, fat-free mass consists of all residual chemicals and tissues, including water, bone, connective tissues and internal organs. Equations, such as that by Siri, deduce percentage body fat from total body density [133]. However, equations assume that any difference from the reference body is in triglyceride content, that the proportions of water, minerals and protein in the fat free body are constant and that the densities of the fat and fat-free components are additive. Siri et al estimated the total error to be 3.9% for a population [133]. However, this may be higher for population subgroups. In an attempt to improve this, multi-component models were proposed.

Three-component models, such as the 1961 Siri equation, adjust for the relative proportion of water in the body and may be more accurate in population sub-groups, whose fat-free body water content is likely to differ from the reference [134]. They require measurement of the individual's body density and total body water.

There also exists 4 and 6 component models. Four-component models assume that the body is composed of fat, water, bone mineral and protein [135]. Six-component models require direct in vivo chemical analysis and are based on the assumption that the body is composed of total body water, nitrogen, calcium, potassium, sodium and chloride [136].

## Measurement of body composition

A number of tests are available to assess body composition (Table 1.5) [137]. Some are used primarily as research tools, while others may be used in routine clinical practice.

Test	Measures	Advantages	Disadvantages
Hydrodensitometry	Total body volume	Traditional ‘gold-standard’	Prone to errors; time-consuming; large specialist equipment
Air displacement plethysmography	Total body volume	Quick; simple procedure	Expensive
Hydrometry	Body water	Excellent for total body water assessment	Long equilibrium time; difficult analysis
Dual energy x-ray absorptiometry	Total body bone mineral and bone mineral density	Simple for participant; quick; capable of regional assessment	Expensive; requires radiographer
Bioelectrical impedance analysis	Total body water and fat free mass	Quick; easy capable of compartmental analysis	Stringent hydration guidelines
Anthropometry	Body dimensions	Relatively accurate for lean participants	Less accurate in obese; technical skill required

Table 1.5: Summary of body composition measurement methods

### Hydrodensitometry

Hydrodensitometry is also known as underwater weighing [137]. The body is fully immersed in water to provide a measure of total body volume via the volume of water displaced, based on Archimedes’ principle. This can be used to calculate body density (2-compartment models) after taking into account residual lung volumes. Hydrodensitometry is generally considered to be the gold-standard method. However, it is not practical and, as such, largely considered to be a research tool. Accuracy of measurements may be influenced by confounding variables (e.g. recent diet, subject compliance, equipment).



### Air displacement plethysmography

This is similar to hydrodensitometry [137]. However, instead of water displacement it uses air displacement to calculate body volume and thus density. Two-component models are used to deduce percentage body fat. This method is used by the commercially available BodPod systems. BodPod systems are user friendly, and assessments are normally relatively quick to perform. However, systems are expensive to purchase and not portable.

### Hydrometry

Hydrometry is the measurement of water, which is predominantly associated with the fat-free tissue [137]. This involves administering an isotope, allowing it to reach equilibrium and then measuring the concentration achieved in biological tissue. Percentage body fat is calculated using a modified 2-component approach. This assumes that the tracer is distributed only in body water and that this distribution is equal. It also assumes that tracer equilibrium is rapidly achieved and that the tracer is not metabolised before this occurs. This is primarily a research tool for total body water.

### Dual-energy x-ray absorptiometry

Dual-photon absorptiometry measures the attenuation of x-rays to assess total body bone mineral and bone mineral density [137]. The attenuation of x-ray energies differs through fat, lean and bone. It is assumed that attenuation is the same in all individuals, that measurements are unaffected by body thickness and that the amount of fat over bone is the same as that over bone free tissue. This method may be used to assess the whole body or regions. It requires no special preparation by the subject and is relatively quick to perform.

### Bioelectrical impedance analysis

All biological tissues conduct electricity to a different extent [137, 138]. Bioelectrical impedance analysis (BIA) devices pass a low-level electrical current through the body and measure the opposition (impedance). As fat is a poor conductor, individuals with a higher relative fat content generate more resistance to current flow than individuals with a lower relative fat content. BIA measures the resistance (pure opposition to current flow of the

tissues) and reactance (opposition to current flow due to capacitance of cell membranes and tissue interfaces).

BIA makes a number of assumptions. It assumes the body is a perfect cylinder with constant conductivity, and that therefore resistance is directly proportional to the length (height) and inversely proportional to cross-sectional area. However, instead, the human body is more like a series of cylinders with differing conductivities.

Traditional BIA devices use a current with a frequency of 50kHz, which passes through the extracellular water and, to a variable extent, the intracellular water compartments. Equations allow calculation of fat and fat-free body mass from the relationship between the intracellular and extracellular water compartments. However, intracellular water is variably measured and the accuracy of BIA is influenced by body hydration and fluid distribution [139]. In oedematous states, extracellular water increases relative to intracellular water leading to reporting errors.

Lower frequency (0-5kHz) currents are unable to penetrate the cell membrane, and thus only assess the extracellular water [140]. Conversely, at higher frequencies (>100kHz) the body behaves like a perfect capacitor allowing better assessment of both intra and extracellular body water, by allowing cell membrane penetration. Many higher frequency BIA devices purport to give insight into cellular 'wellness' by assessing intracellular water changes. Other lower frequency devices report phase angle (PhA), which is a function of resistance and reactance [141].

PhA is considered to be a marker of cell membrane function and, as such, is often regarded as a surrogate marker of nutritional and disease status [142, 143]. Higher values reflect greater cell membrane integrity and thus better cellular health. Normal, healthy values are suggested to lie between 5 and 7 [144]. Lower values may represent malnutrition, age, inflammation and disease states [143].

A limitation is that most equations disregard the reactance component of the voltage signal [141]. Furthermore, equations differ between manufacturers and specific study populations. Validation studies have compared equations and devices against other assessment methods. However, no validated equations exist for patients with SSc.

BIA analysers measure across different contact points [145]. They may be bipolar with 2 contact points (foot-to-foot; hand-to-hand) or tetrapolar with four (hand, wrist, foot,

ankle). With foot-to-foot analysers the current only flows through the lower half of the trunk and lower limbs and upper body composition is extrapolated. Similarly, hand-to-hand analysers only measure across the upper limbs and extrapolate the lower limbs. Some analysers may allow segmental analysis across small sections of the body (e.g. forearm). When undertaking BIA measurements, it is important to place electrodes accurately as the position affects the recorded impedance [138, 146].

Many BIA devices are portable and thus are suitable for use in clinical settings. However, when doing so, confounding variables must be considered. Numerous variables other than body composition affect BIA. Variables include hydration status (dehydration, eating/drinking pre-study, oedema, electrolyte derangements), recent aerobic exercise, room (and thus skin) temperature, other body fluid (bladder fullness), body position (supine versus ambulatory), abnormal body shape (amputations, hemiplegia, dystrophy), health and medications which influence fluid status (e.g. steroids, diuretics) [146-151].

### Anthropometry

Anthropometry is the measurement of body dimensions, from which estimates can be made about body composition. Many different anthropometric measures of varying complexity exist. Types of measures include, height, weight, segment length, body breadth, circumference and skinfold thickness [152]. Circumferences involve placing a tape measure around specific body segments. Skinfolds (Figure 1.1) are an indirect measure of the thickness of subcutaneous adipose tissue measured using hand-held calipers. Thus, the benefit of anthropometry is that it can be performed quickly in clinical settings with relatively little equipment.

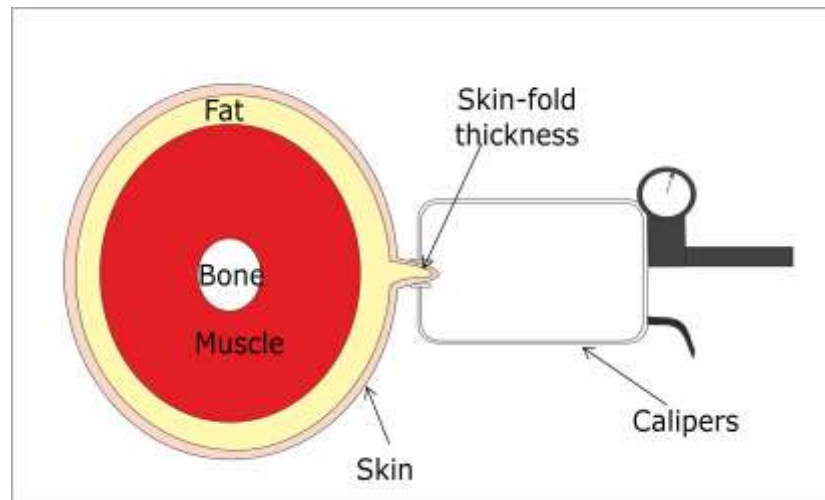


Figure 1.1: Skinfold measurement

Like all body composition measures, anthropometric measurements are prone to error. Operator skill is required [153]. Recommendations exist regarding the location of measurement sites. However, in some instances these recommendations conflict. The Anthropometric Standardization Reference Manual defines the mid-upper arm position as being half-way between the tip of the acromion and the olecranon process of the ulnar [152]. In contrast, the International Society for the Advancement of Kinanthropometry Manual positions it half-way between the superior part of the acromion border and the lateral border of the head of the radius [154]. Another author, on whose work the key mid-arm anthropometry reference centiles are based, defined it as halfway between the acromion process and the end of the humerus [155]. However, despite potential sources of error, studies of both inter and intra observer reproducibility have shown reasonable results [156]. Intra-observer reliability is better than inter-observer [157]. Skinfold measures are also affected by the ease of separation of subcutaneous fat from underlying tissues and the thickness of the layer of adipose tissues. Age and gender affect distribution of body fat, thus affecting interpretation of total body fat from selected measures

#### *Mid-upper arm anthropometry*

The most easily performed form of anthropometry is mid upper arm anthropometry (MUAA). It is simple, non-expensive and quick to perform. It assumes that the arm is a perfect cylinder, composed of only muscle and fat (Figure 1.2).

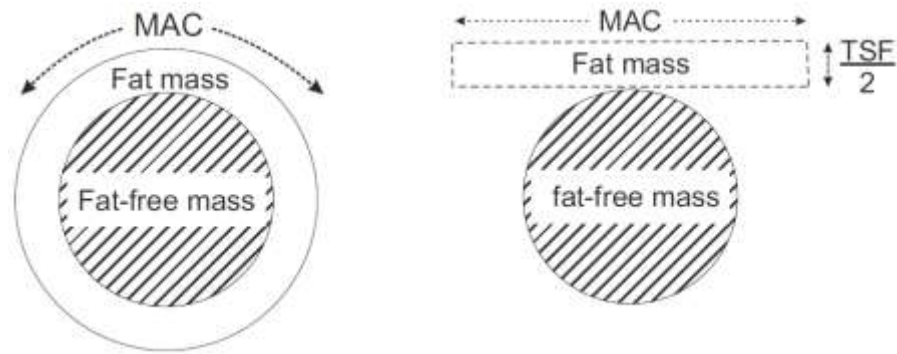


Figure 1.2: Cross-section through the upper arm

MUAA has been assessed in different population sub-groups and compared to other methods of body composition assessment. From measures of mid-arm circumference (MAC) and triceps skinfold thickness (TSF), it is possible to calculate mid-arm muscle circumference (MAMC), which is an estimate of muscle mass [155].

Age and gender specific normal MAC, MAMC and TSF results have been defined for healthy populations (aged 18-74), based on the measurement of 13,671 free-living Americans (Appendix 6) [155]. Measures can also be used to calculate the mid-upper fat-free (or muscle) area [158, 159]. Comparisons of anthropometric and CT-derived mid-upper arm muscle areas show variable agreement. However, one study, involving non-obese subjects with muscle atrophy, found it to be a reliable indicator of muscle mass [160].

Much of the early work was done using the right arm [155]. However, recent publications use the non-dominant arm. A comparison study, in healthy volunteers, identified no significant differences between right and left MAC and TSF measurements, with the exception of TSF in left-handed individuals and MAC in individuals performing predominantly right-handed sport/work-related activities [161]. Evidence also shows little difference between seated and standing measurements [156].

#### *Percentage body fat using skinfold thickness*

Mid-upper arm anthropometry does not give an overall estimate of percentage body fat. However, this may be achieved by using multiple skinfold measurements to determine body density, which can be converted into percentage body fat using the Siri equation

[133]. Body density may be calculated using Durnin and Womersley's equation and four specific skinfold measurements [162].

It is generally accepted that anthropometry, which is a field method, provides a less accurate assessment of percentage body fat than the previously described research tools [163, 164]. Studies conflict over the best formula to use. One involving normal and overweight females, which compared several equations (Durnin and Womersley and 3 versions of Jackson, Pollock and Ward) to air-displacement pleythsmography, reported the Durnin and Womersley equation to be the most accurate [165]. Different equations may give more accurate results in selected subject groups [166].

## **1.6 Assessing energy requirements**

Energy is required for a multitude of functions. At rest, it is used performing a range of functions which are essential for maintenance. The absolute minimum energy needed to maintain the most basic of physiological functions when at complete rest, not having consumed food and in a thermoneutral environment, is termed the basal metabolic rate. It is influenced by body composition, being higher in individuals with a greater relative fat-free mass (i.e. more metabolically active tissue).

Eating causes a small increase in energy expenditure, termed diet-induced thermogenesis. The resting energy requirement is the energy expended when at rest. It is similar to basal metabolic rate, but not the same. Movement further increases energy expenditure. It may be due to intended heat production (non-shivering thermogenesis), activity at rest (e.g. fidgeting, increased work of respiration) or purposeful activity (e.g. exercise). Energy requirements increase in disease states to cope with the extra metabolic challenges. Total energy expenditure takes into account all expended energy.

A number of methods are available to measure / estimate energy expenditure (Table 1.6). Some are used primarily in research, while others are used in clinical practice.

<b>Test</b>	<b>Measures</b>	<b>Advantages</b>	<b>Disadvantages</b>
Indirect calorimetry	Oxygen consumption and/or carbon dioxide production	Clinical and research applications	Expensive
Direct calorimetry	Heat loss	Mainly a research tool	Very expensive, not habitual activity
Double labelled water	Labelled carbon dioxide and water	Can be used in free-living individuals	Expensive
Kinematic monitors	Steps or displacement	Can be used in free-living individuals	Relatively cheap
Calculations	Estimated based on prediction equations	Easy to perform, quick, no specialist equipment	Less accurate as based on assumptions

Table 1.6: Summary of techniques for assessing energy expenditure

### **1.6.1 Indirect calorimetry**

Indirect calorimeters measure oxygen consumption and/or carbon dioxide production and converts this into energy expenditure [167]. Devices are based on the principle that food is oxidised to produce energy, and that by measuring consumed oxygen, energy produced can be determined. Different types of apparatus exist, ranging from some which are easily portable and suitable for clinical use, to respiration chambers for use in research. Some systems can be used during exercise.

### **1.6.2 Direct calorimetry**

Direct calorimeters measure heat loss from the body [167]. Direct calorimeters are very expensive, complex pieces of equipment that require a large amount of space and a trained technician to operate. They consist of a large insulated chamber, that is capable of measuring all heat generated by an individual who remains inside for 24 hours. Therefore, direct calorimetry is a research tool. As the subject is confined within an insulated chamber during the assessment, it cannot determine habitual energy expenditure.

### **1.6.3 Doubled-labelled water studies**

This method measures carbon dioxide produced [167]. It involves drinking water labelled with deuterium ( $^2\text{hydrogen}$ ) and  $^{18}\text{oxygen}$ , which then mixes with the body's water. The body generates carbon dioxide (labelled with  $^{18}\text{oxygen}$ ) and water (labelled with  $^2\text{hydrogen}$  and  $^{18}\text{oxygen}$ ). The difference between the rates of  $^2\text{hydrogen}$  and  $^{18}\text{oxygen}$  loss reflects carbon dioxide production, from which energy expenditure can be deduced. This method is expensive and requires specialist equipment and serial sampling. It is suitable for assessing free-living individuals over days to weeks.

### **1.6.4 Kinematic monitors**

Kinematic monitors assess an individual's movements to allow quantification of energy expenditure during free-living. Devices range from simple pedometers to more complex accelerometers. Pedometers merely record the number of strides, but not the stride length or speed. Accelerometers electronically detect body displacement in either one or three different axis. One comparison study showed undercounting of steps with the pedometer compared to the accelerometer, which was greatest during moderate and vigorous activity [168]. Little difference has been noted between uni- and tri-directional accelerometers [169]. More complex devices include other energy expenditure measures and advanced software. One such multi-sensor device (SenseWear® Armband) also monitors heat emitted, skin temperature and sweating. It has specialist software to deduce expended energy. Unlike traditional sensors which are worn at the hip, the SenseWear® device is worn on the arm. Studies comparing it to indirect calorimetry report it to be a valid tool to estimate energy expenditure associated with performance of the activities of daily living [170, 171]. Furthermore, SenseWear® devices may be more accurate than other accelerometers for assessing energy expended during light to moderate intensity activity [172].

### **1.6.5 Estimations of energy requirements**

In situations where energy expenditure cannot be measured, predictive equations can be used [173]. The most commonly used equation is the Schofield, followed by the Harris-Benedict equation [174]. Both are estimations of basal metabolic rate and generate different results for an individual.



The Schofield method, based on the results of 5000 adults, is based on body weight and age and gender-specific coefficients [175]. This basal metabolic rate can be adjusted to estimate energy requirements. Adjustments can be made for stress factors, activity and thermogenesis and desired weight modification (gain or loss) [173]. Similarly, the Harris-Benedict basal metabolic rate equation was derived from the study of healthy adults [176]. This equation also takes into account age, gender and height.

A factor may then be added to convert basal metabolic rate to resting energy expenditure. Stress factors may also be added. Habitual energy expenditure exceeds resting energy expenditure, as voluntary activities are not included. These can be included using a factorial approach. Exertion allowances exist for the time spent performing one of the many different activities of daily life or recreation. To determine total energy expended over a set time period, the basal metabolic rate for that period can be multiplied by a physical activity ratio. Alternatively, total daily energy expenditure may be estimated by applying a physical activity level ratio to the whole day. A physical activity level ratio of 1.4 is recommended for the UK population engaged in light work and non-active non-occupational activity [177]. Estimations are crude even when a subject's daily activity is clearly mapped.

## **1.7 Assessment of nutritional intake**

In order to assess nutritional intake, it is necessary to record normal daily diet, analyse the nutritional composition of that diet and to compare it to population or sub-group reference values. It is also possible to assess dietary adequacy using biochemical indicators.

Methods of dietary assessment are discussed.

### **1.7.1 Recording dietary intake**

Dietary intake may be recorded quantitatively and/or qualitatively using one of a variety of assessment methods. Each method has advantages and disadvantages, and may be appropriate for different durations and types of study (e.g. population versus individual assessment). Recording methods are summarised (Table 1.7). As well as considering the method of recording, it is also necessary to consider the optimal period.

<b>Test</b>	<b>Measures</b>	<b>Advantages</b>	<b>Disadvantages</b>
Weighed food record	Exact food/drink weights	More accurate than other methods	Burdensome
Estimated food record	Estimated food/drink portion sizes	Lower respondent burden	Relies on ability to estimate
24 hour food recall	Recall of consumption over preceding 24 hours	Possible to complete quickly by telephone	Memory dependent
Food frequency questionnaire	Recalled frequency of consumption of common foods from a list	Good for large scale surveys	Memory dependent

Table 1.7: Summary of dietary assessment methods

### **Weighed food record**

Weighed records are considered the most accurate dietary assessment method [178]. They require individuals to weigh and record every item of food and drink consumed using a specially designed diary. Intake may be recorded for 3 to 7 days. Seven days is generally regarded as ideal. However, it can become burdensome and lead to mis-reporting. Therefore, shorter periods are often used. A recorded assessment of intake over 3 days, including 1 weekend day, is considered by many to be adequate for the assessment of energy intake [179]. The weekend day is included as weekend consumption differs from that of weekdays [180].

### **Estimated food record**

These are similar to weighed records with the exception that the weighing is not required. Instead, individuals estimated portion sizes. Thus, accuracy is limited by the ability to estimate portion size [181]. Accuracy can be improved by including validated photographs illustrating food portion sizes [182-184]. Groups differ in their recommendation as to the appropriate number of sample photographs to include. In comparison to weighed food records, estimated diaries have a lower perceived burden which may improve participation.

### **Twenty four hour recall**

The interviewee is asked, by a trained interviewer, to recall details of the food and drink they have consumed in the preceding 24 hour period. This method relies on ability to recall both the items of food and the amounts consumed. Recall may be assisted by prompting about situations (e.g. work, eating out) and food groups. Portion size photographs may be used to improve the accuracy of portion size estimation. This method only provides information about a single 24 hour period, which is unlikely to be representative of habitual diet. This may be improved by extending the recording interval.

### **Food frequency questionnaire**

This is more suited to population studies. It allows assessment of types of food consumed, but cannot provide information about absolute quantities of nutrient intake. Participants are asked to estimate the frequency of selected foods consumed within a defined period (days, weeks or months). Food lists may be large or small depending on the study's goal. Questionnaires may be completed with or without the assistance of an interviewer.

### **General limitations to dietary assessment**

Dietary assessment may be affected by participant bias leading to under or over-reporting or dietary modification during the recording period [185, 186]. Under-reporting is greater in individuals with a higher BMI, social desirability score, body dissatisfaction score and lower income [187].

Nutrient composition is analysed using food composition tables. In the UK, the compositional data of food are recorded in a National Nutrient Databank maintained by the Food Standards Agency. A limited sub-set is periodically published (McCance and Widdowson's *The Composition of Foods*) [188]. Additional food items are included in separate supplements. This dataset is the best widely available resource to interpret food diaries. However, its use is not without limitation. Compositional data are obtained from samples of food. Therefore, accuracy relies upon consumed foods having the same composition as that sampled, which is unlikely to be true, as processed products differ between manufacturers. Similarly, unprocessed food may differ in their composition. Also, domestic preparation of food differs between households, with regard to the method

and duration of cooking. Finally, manufacturers intermittently alter the composition of prepared foods.

### **1.7.2 Nutrient requirements**

Dietary adequacy is assessed against the Dietary Reference Values, which are a series of estimates of the nutritional requirements of a particular population [177]. For each nutrient, an expert panel has defined a Recommended Daily Amount, which is ‘the average amount of the nutrient which should be provided per head in a group of people if the needs of practically all members of the group are to be met’ [189]. When comparing actual intakes to Recommended Daily Amounts, it is important to consider several factors. Recommended Daily Amounts were established using the best available evidence at the time. However, in many cases numerous assumptions were made as data were insufficient.

Recommended Daily Amounts were established to avoid deficiency. However, for any one nutrient, actual requirements differ between individuals based on the efficiency of absorption, etc. It is assumed that within a population, inter-individual variability in requirements displays a normal distribution. The Estimated Average Requirement is that intake believed to meet the needs of 50% of the UK population, but at which 50% of individuals will still be deficient. The Lower Reference Nutrient Intake is 2 standard deviations below this value, while the Reference Nutrient Intake (RNI) is 2 standard deviations above. An individual meeting the RNI for a nutrient has a 97.5% probability of meeting his/her requirements. However, actual requirements differ with age, but Dietary Reference Values are established for populations. When sufficient evidence was available, the panel established separate values for the elderly, children and pregnant or lactating women.

### **1.7.3 Nutritional biochemistry**

Biochemical parameters can provide insight into nutritional status [190]. Tests measure absolute nutrient concentrations or the consequences of deficiency. Concentrations are indicators of recent supply and uptake. Clinical manifestations only arise once a lower threshold has been passed. Examples are listed (Table 1.8) [191].

<b>Trace Element / Vitamin</b>	<b>Function</b>	<b>Symptoms and Signs</b>
Iron	Blood formation	Fatigue, pallor, dizziness, nail changes, sore mouth/tongue
Magnesium	Bone development, metabolism, DNA synthesis, conduction	Neuromuscular abnormalities, loss of appetite, nausea and vomiting, diarrhoea, numbness, tingling
Zinc	Metabolism, cell membranes	Poor growth, impaired wound healing, poor hair growth, infection risk, diarrhoea
Selenium	Antioxidant	Muscle weakness, cardiomyopathy
Copper	Healthy skin, hair and red blood cells	Metabolic and muscle problems
Calcium	Bone and teeth development, muscle contraction	Muscle aches, pain, twitching, spasm, cramps, tetany, loose teeth, infection
Phosphate	Bone development, energy release	Anorexia, lethargy, bone pain, soft tissue calcification
Folate	Cell growth	Anaemia, neural tube defects (newborns)
Vitamin B <sub>12</sub>	Cell synthesis, nerve myelination	Anaemia, fatigue, brain deterioration
Vitamin C	Immunity, collagen formation, wound healing	Bleeding gums, scurvy, bruising, fatigue, depression
Vitamin D	Development of teeth and bones, enhances calcium absorption	Rickets (children), osteomalacia (adults), reduced teeth and bone development

Table 1.8: Consequences of micronutrient and vitamin deficiencies

Serum proteins (e.g. albumin, pre-albumin, transferrin) are often considered to be markers of visceral protein status. Serum concentrations are however mainly reduced due to other reasons, e.g. sepsis, trauma, liver disease. Serum albumin has a long half life (14-20 days) [192]. It is not sensitive to short-term nutritional problems and concentrations may be preserved in the presence of severe malnutrition including total starvation [193]. Pre-albumin has a shorter half-life (<2days), and thus some view it as a more sensitive nutritional marker [194]. However, its levels may also be affected by hydration, liver disease and inflammation. In a study involving patients with SSc (n=299), pre-albumin was reported to be an independent predictor of mortality [195]. During the median 48

months follow-up, 11% of patients died. Significant predictors of mortality were identified as age, male gender, respiratory involvement, GI involvement,  $\geq 2$  visceral organ involvements and serum pre-albumin. However, nutritional statuses were not quantified.

Serum trace element concentrations only fall once body stores are depleted. Compensatory homeostatic mechanisms may mask nutritional deficiency until it is severe. Thus, periodically measuring serum trace elements may detect a chronic deficiency and guide long-term nutritional management, but will not reflect recent dietary consumption.

## **1.8 Nutrition screening tools**

Screening tools are designed to facilitate the identification of individuals who are undernourished or at risk of becoming undernourished. Screening tools should be easy to interpret, quick and easy to administer, acceptable to the individual being screened, reliable and cost-effective. They should be applicable to individuals of different ages, genders and ethnicities and be able to be used in a variety of settings.

Over the years numerous screening tools have been developed. A recent systematic review identified 32 screening tools intended for hospital use [196]. These tools use a variety of different measures to assess risk, including BMI, recent unintentional weight loss, dietary intake, GI symptoms, functional capacity, etc. The commonest screening tool used in the UK is 'MUST'. This fulfils the National Institute for Health and Care of Excellence's recommendations that 'screening should assess BMI and percentage unintentional weight loss and should also consider the time over which nutrient intake has been unintentionally reduced and/or the likelihood of future impaired nutrient intake' [197].

'MUST' was developed by the British Association of Parenteral and Enteral Nutrition. It was intended to be a simple, rapid tool for the identification of those medical patients at risk of malnutrition [198, 199]. The initial validation studies, using medical inpatients, compared 'MUST' to dietetic assessment and to TSF, MAC and MAMC. A subsequent study compared 'MUST' to other tools in surgical and community environments, and confirmed it to be a suitable tool, displaying fair to excellent agreement with the other methods [200]. 'MUST' has been used in studies involving patients with SSc [36].

Calculation of the 'MUST' score (Figure 1.3) and the instigation of the appropriate clinical response is divided into 5 steps. Step one involves the calculation and scoring of the individual's BMI. Step 2 involves the assessment and scoring of any recent unplanned weight loss in the past 3-6 months. Step 3 involves the assessment of acute disease effect.. Step 4 involves the addition of all scores to calculate the overall risk of malnutrition. Individuals scoring 0 are deemed to have low risk, while individuals scoring 1 are deemed to be at medium risk and those scoring  $\geq 2$  are thought to be at high risk. The final fifth step involves making the appropriate management recommendations.

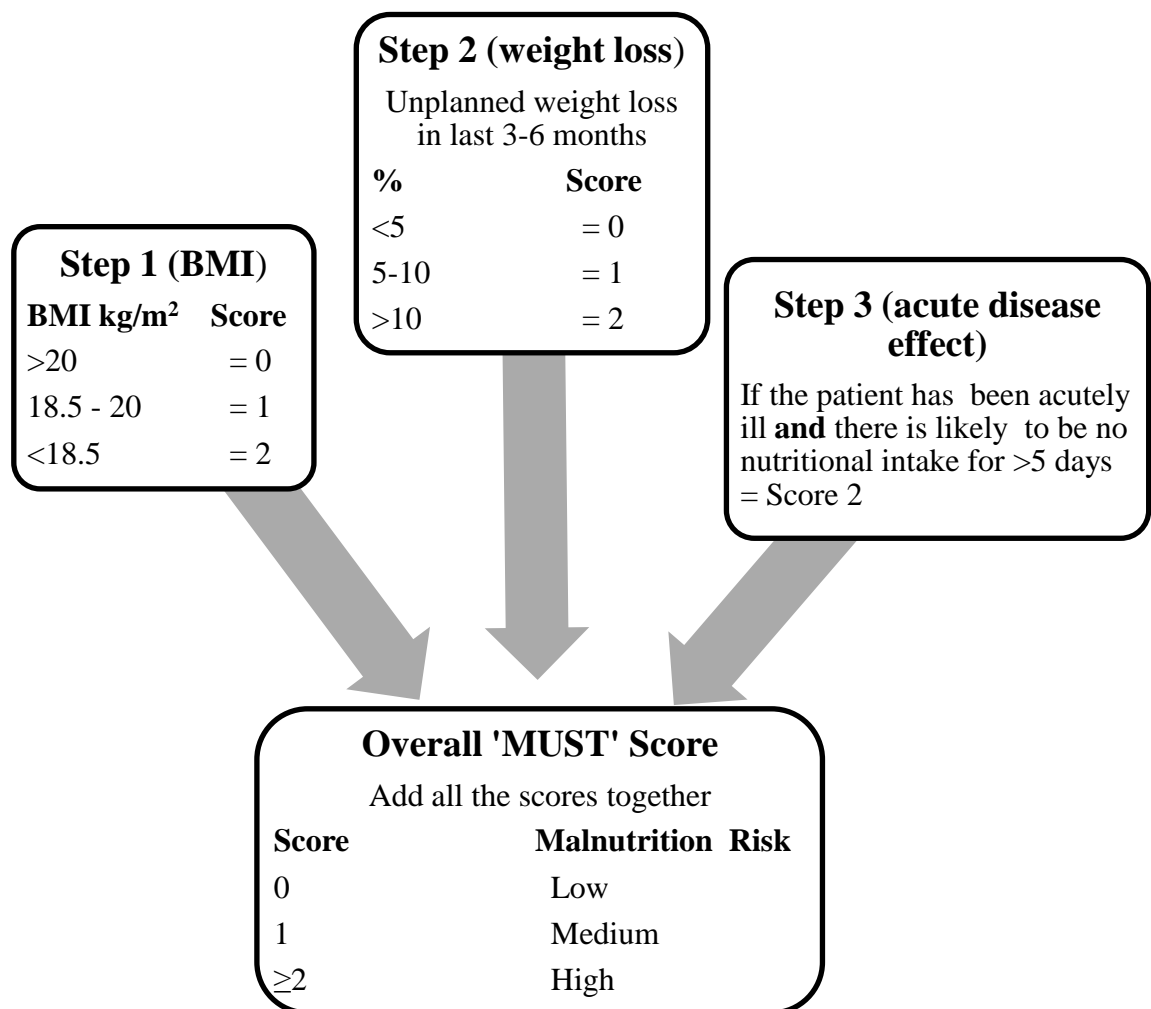


Figure 1.3: 'MUST'

## 1.9 Malnutrition in patients with systemic sclerosis

Between 1972 and 2002, the 10 year survival of patients with SSc rose significantly [201]. Over this 30 year period, the frequency of deaths due to SSc-related GI disease did not change significantly. However, the proportion of all deaths from GI disease did fall from 12% to 4%. Other studies have shown that deceased patients with SSc are significantly more likely to have had GI involvement and that a low BMI in patients with early SSc may be predictive of mortality [202, 203].

The following sections will discuss the evidence of the prevalence of nutritional problems in patients with SSc from screening, clinical assessment and dietary studies.

### 1.9.1 Studies of malnutrition in patients with systemic sclerosis

Several groups have reported on the prevalence of nutritional problems in unselected outpatients with SSc. Classification methods have differed between studies (Table 1.9).

Study	Number of patients	Prevalence of nutritional problems
Baron et al. [36]	586	17.4% at high 'MUST' risk 10.8% at medium 'MUST' risk
Krause et al. [204]	124	13.7% with BMI <19
Murtaugh et al. [205]	24	37.5% at medium or high 'MUST' risk
Caporali et al. [48]	160	15% with BMI <20 and/or spontaneous weight loss of ≥10% of body weight in the previous 6 months
Cereda et al. [206]	160	9.4% at high 'MUST' risk 24.4% at high risk and 30% at medium risk with modified 'MUST'
Ortiz-Santamaria et al. [207]	72	12.5% at medium or high 'MUST' risk

Table 1.9: Summary of malnutrition studies



The largest study conducted to-date identified significant correlates between high risk of malnutrition and number of GI complaints, disease duration, diffuse disease, physician global assessment of disease severity, haemoglobin, oral aperture, abdominal distension on physical examination, and physician assessed possible malabsorption [36]. However, GI complaints were only classed as present or absent.

One study compared BIA measures in patients with SSc to age, gender and BMI matched controls [204]. Patients were then divided into 'nutritional status' groups according to BIA (PhA) results and other measures compared between groups. However, as described earlier, PhA values do not solely reflect nutritional status.

The smallest study listed in the table compared patients identified as being at nutritional risk by 'MUST' to those identified by an alternative screening tool (Subjective Global Assessment of nutritional status) [205]. The alternative tool also included information regarding dietary intake, GI symptoms and functional capacity and a physical examination. Using this, 3/24 patients were considered to be severely malnourished (all high risk using 'MUST') and 9/24 were scored as suspected or moderately malnourished. Of these patients, 5 were scored as low risk using 'MUST'. It also compared 'MUST' and GI symptom scores (UCLA) and found no correlation. However, some differences between patients with differing levels of malnutrition risk was noted when using Minimally Important Difference cut-offs. Patients with moderate risk (n=3) scored higher on soilage, social functioning, emotional well-being and total GI than patients with low risk (n=15), but lower (i.e. somewhat better) on distension /bloating. Similarly, patients with high risk (n=6), scored higher on reflux, soilage, diarrhoea, emotional well-being and total GI than patients with low risk (n=15), but again lower on distension /bloating. This decrease in bloating and distension in patients at greater malnutrition risk is difficult to explain, and suggests the need for a larger study.

Another study reported malnutrition to be independently associated with disease activity and low serum pre-albumin [48]. Active disease was defined as a score of  $\geq 3$  on the Valentini Disease Activity Index [208]. They did not detect a significant association with GI involvement, but did detect a trend. They also found no association with nutritional intake. A second paper described 92% of these patients follow-up after a median of 46 months (25-75<sup>th</sup> percentile = 31-54) [206]. It scored baseline nutritional risk using a modified version of 'MUST'. In this modified version the third step, which is normally the

‘acute disease effect’ score, was replaced by a score of  $\geq 3$  on the Valentini Disease Activity Index. When using this modified form of ‘MUST’, 24.4% of patients had high risk and ‘MUST’ significantly predicted mortality. However, no such relationship to mortality was found when using only the weight loss and BMI components.

The most recent study, which identified 12.5% of patients to be at risk of malnutrition (‘MUST’), was designed to investigate the effects of nutritional intervention in a subgroup rather than to define the characteristics of the patients screened [207].

### **1.9.2 Studies of body composition in patients with systemic sclerosis**

A number of nutritional studies involving patients with SSc have sought to measure body composition using methods other than a simple BMI. The earliest study to do this involved 30 patients with SSc (17 dcSSc; 19 female) and pronounced GI manifestations[47]. It compared the results of MUAA to other clinical and nutritional measures and to the MUAA of healthy controls. BMI was not significantly different between male or female patients and controls. However, compared to the healthy controls, both male and female patients had a significantly lower MAC and MAMC, and the female patients also had a significantly higher TSF. The lower arm muscle circumference may be, in part, due to the higher TSF (SSc-skin involvement). On multiple regression analysis, TSF was lower in patients with lcSSc and dysphagia. Also, MAMC was higher in patients with dysphagia, short disease duration and a high triolein breath test result (i.e. no fat malabsorption). A very low TSF and MAMC may together reflect poor nutritional status as 2 patients, who both had a TSF and MAMC below the fifth centile, also had BMIs  $< 18.5 \text{ kg/m}^2$  (14.8 and 16.2), severe GI involvement (oesophageal stricture with intestinal stasis and severe diarrhoea) and later required enteral or parenteral nutritional support.

Another study compared bone mineral density between 43 postmenopausal patients with SSc and healthy controls, but excluded patients with intestinal malabsorption, renal failure, smoking history and osteoporotic drug usage [209]. Patients with SSc had significantly lower BMI, lean mass and fat mass.

An unpublished study, communicated in 2008, described serial use of BIA in 2 patients with dcSSc [210]. In both patients, there was a progressive increase in the recorded percentage body fat (less conductive tissue), but the patients’ weights decreased as their

disease progressed. It was hypothesised that the increase in resistance and decrease in reactance and PhA were related to disease progression.

Another study assessed 124 patients with SSc using BIA and compared findings to 295 healthy volunteers [204]. Of the 124 patients, only 84 could be age, gender and BMI-matched to healthy volunteers. Comparison of these matched subjects showed the patients with SSc to have significantly higher extracellular mass and lower PhA than the healthy controls despite being BMI-matched. However, there was no significant difference in lean body or fat mass. This study divided patients according to PhA cut-offs. The lower PhA brackets were associated with dcSSc, reduced forced vital capacity and increased ESR, skin score, SHAQ, PPI or prokinetic use and cardiac involvement. However, PhA was not compared to BMI or any other non-BIA body composition assessment.

One study, compared 61 females with SSc (31 lcSSc) to 67 age-matched healthy controls, using dual energy x-ray absorptiometry and BMI [211]. When compared to healthy controls and patients with lcSSc, patients with dcSSc had lower BMIs, total lean masses and appendicular lean masses. However, no difference in BMI or absorptiometry results was noted between patients with and without GI involvement.

Thus, despite several studies having been conducted, there are still many unanswered questions, in particular relating to the usefulness of BIA and MUAA in patients with SSc.

### 1.9.3 Dietary and exercise studies in patients with systemic sclerosis

Many studies, using a range of methods, have sought to assess dietary intake and energy expenditure in patients with SSc (Table 1.10).

Study	Number of patients	Dietary assessment method	Other nutritional measures
Lundberg et al. [47]	30 with GI manifestation	4-day diary and recall	Nutritional biochemistry; MUAA
Herrick et al. [212]	12	7-day weighed record	Serum antioxidants
Krause et al. [204]	86	'nutritional protocol'	BIA; calculated requirements
Marighela et al. [211]	61	3-day diary	Dual energy x-ray absorptiometry Physical activity questionnaire
Caporali et al. [48]	160	3-day diary with recall	Pre-albumin; disease activity; nutritional screening; GI symptoms and involvement; calculated energy expenditure
Battaglia et al. [213]	27	None	SenseWear® Armband; BIA; Lung function tests

Table 1.10: Summary of dietary studies

The earliest study was conducted by Lundberg et al., who compared 30 patients with SSc (17 dcSSc) and pronounced GI manifestations (proven oesophageal dysmotility or fat malabsorption) to 30 age and gender matched controls [47]. GI symptoms included non-specific oesophageal symptoms (23/30), vomiting at meals (6/30) and intermittent diarrhoea (5/30). Dietary assessment was by means of a 4-day estimated food diary with assisted recall. Intakes were compared to those of controls and to results of selected nutritional biochemistry and anthropometric measures. The mean total energy, fat and vitamin intakes were found to not differ between all patients and controls. However, patients had significantly lower intakes of fibre and female patients had a significantly lower intake of selected trace elements (zinc, copper, magnesium). In addition, patients with severe oesophageal dysmotility had significantly lower intake of carotene and pectin than patients with minimally disturbed oesophageal motility. This may be due to peri-oral

and oesophageal manifestations hindering the consumption of bulky foods, with a large total volume that required chewing.

In another study, 12 patients with SSc and healthy controls with similar BMIs completed a 7-day weighed record and had their serum antioxidants levels measured [212]. The majority of patients had minimal GI symptoms. Patients had a significantly higher energy intake, but no difference in consumed antioxidants. However, the patients had lower serum selenium (significant) and vitamin C (non-significant). Thus, the reduced blood levels could not be attributed to dietary deficiency.

In the study by Krause et al. patients completed 'nutritional protocols' which showed 50% to have a lower energy intake than required [204]. Requirements were based upon calculations of basal metabolism adjusted according to body cell mass. Energy intakes did not differ between patients with different PhA nutritional statuses. However, PhA has multiple limitations.

In the study by Caporali et al. patients completed 3-day food diaries which were clarified by interview. Predicted energy expenditure was taken to be 1.5 times the resting energy expenditure (Harris-Benedict equation). No difference in energy (total or <75% predicted) intake was evident between patients with and without malnutrition.

Marighela et al. compared 3-day estimated food records to body composition and physical activity [211]. Mean energy intake was similar between patients and controls. There was also no significant difference in energy or macronutrient intake between patients and controls, but patients did have lower intakes of fibre unrelated to GI involvement. Physical activity was assessed via the short version of the International Physical Activity Questionnaire. Individuals were classed as either active (>150 min of exercise per week) or inactive. There was also no significant difference in the percentage of patients and controls classed as physically active. However, patients did have a significantly lower BMI, percentage total fat mass and bone mineral density. Thus, this suggests the patients' abnormal body composition to be disease-related rather than diet or exercise related. However, a limitation was the method of activity assessment.

The most recent study by Battaglia et al. also assessed energy expenditure [213]. The energy expended by 27 stable patients with SSc and 11 matched healthy controls was measured over 6 to 8 days using a SenseWear® Armband. Measures were compared to

body composition and lung impairment. Patients and controls had similar BMIs and body compositions. However, patients had significantly reduced energy expenditures (duration and activity level). Reduced expenditures correlated with single breath diffusing capacity for carbon monoxide (DLCO), but not other lung function measures. This was interpreted to show a reduction in physical activity with early respiratory disease. Therefore, this predated disease-related malnutrition. However, no assessments were made of energy intake.

#### **1.9.4 Nutritional biochemistry in systemic sclerosis**

Many studies involving patients with SSc have included one or more nutritional blood markers and have compared serum concentrations to other disease manifestations or nutritional status (Table 1.11). As a result of such studies, Consensus Group recommendations have been developed for the nutritional screening of patients with SSc [214].

<b>Study</b>	<b>Number of studied</b>	<b>Nutritional blood tests</b>
Bluestone et al. [12]	21 SSc	Folate: lower with more extensive radiological small bowel involvement
Lundberg et al. [47]	30 SSc with GI involvement vs. healthy controls	Selenium, $\alpha$ -tocopherol, ascorbic acid and carotene: significantly lower Cobalamine (Vitamin B <sub>12</sub> ) and calcium: no difference
Herrick et al. [215]	18 SSc vs. healthy controls	Selenium: reduced in dcSSc compared to controls Vitamin E and $\beta$ -carotene: no difference to controls Ascorbic acid: reduced in dcSSc and lcSSc
Herrick et al. [212]	12 SSc vs. healthy controls	Selenium: significantly lower in SSc Ascorbic acid and $\beta$ -carotene: no significant difference
Tikly et al. [216]	15-30 SSc vs. healthy controls	Selenium: significantly lower in all SSc and dcSSc than controls Copper, iron and zinc: no significant difference
Marie et al. [61]	22 SSc with SIBO vs. 29 SSc without SIBO	Ferritin, folic acid and vitamin B <sub>12</sub> : lower in SIBO but not significant
Baron et al. [67]	258 SSc	Albumin: weak but significant association with 'MUST' and low predictive value for malnutrition on multivariate analysis
Murtaugh et al. [205]	24 SSc	Albumin: non-significantly lower in patients with poor nutritional status (Subjective Global Assessment)
Caporali et al. [48]	160 SSc	Pre-albumin: significantly lower in subjects with malnutrition (BMI<20 and/or spontaneous weight loss $\geq$ 10%)
Marie et al. [51]	27 with gastric delay; 30 without gastric delay	Vitamin B <sub>12</sub> and folic acid: no significant difference
Ruiter et al. [217]	47 with and 122 without pulmonary hypertension	Iron: deficiency (raised soluble transferrin receptor) more common in patients with pulmonary hypertension

Table 1.11: Summary of nutritional biochemistry studies

### **Albumin and pre-albumin**

Several studies have investigated the relationship between albumin or pre-albumin and nutritional status and/or GI manifestations. One study found a low serum albumin concentration in 2% (4/258) of patients studied [67]. All 4 patients had a high risk of malnutrition ('MUST  $\geq 2$ ). However, the other 51 patients with high risk had normal serum albumin concentrations. On multivariate analysis, 'MUST' scores were found to be independently associated with serum albumin, but this model only explained 7% of the variance in serum albumin. Thus, it was concluded that albumin was not a useful marker for malnutrition in patients with SSc. Similarly, another smaller study found a trend towards lower albumins in patients with a worse nutritional status, but this did not achieve statistical significance [205]. In contrast, another study, which studied pre-albumin, showed evidence of an association with malnutrition (BMI < 20 kg/m<sup>2</sup> and/or spontaneous weight loss  $\geq 10\%$ ) which was independent of disease activity [48]. Thus, although serum concentrations appear lower in patients with SSc and malnutrition, poor nutrition is not the sole contributor and the failure to detect all patients with malnutrition renders it a poor tool to detect nutritional risk.

### **Vitamins and trace elements for synthesis of haemoglobin**

Vitamin B<sub>12</sub> is absorbed in the terminal ileum bound to intrinsic factor, which is released in the stomach. Folic acid is absorbed in the small intestine. Some iron complexes require modification by acid in the stomach to a form which is mainly absorbed in the proximal small intestine. Several small studies have investigated those nutrients needed for haemoglobin synthesis in patients with GI involvement.

One study reported lower serum folate concentrations in patients with more severe radiological small bowel disease [12]. Similarly another study, involving patients with SIBO, reported non-significantly lower serum folic acid, vitamin B<sub>12</sub> and ferritin, which were attributed to SIBO-associated malabsorption [61]. However, a further study by this group showed no significant difference in serum vitamin B<sub>12</sub> and folic acid between patients with and without delayed gastric emptying [51]. In addition, a study comparing patients with SSc and GI involvement (13/30 fat malabsorption) to healthy controls showed no difference in serum vitamin B<sub>12</sub> [47]. Thus, there is some evidence of a link between small intestinal involvement (SIBO) and nutrient deficiency.



## Antioxidants

Several small studies have shown low serum selenium concentrations in patients with SSc [47, 212, 215, 216]. However, one study showed patients not to have correspondingly significantly lower intakes [212]. Thus, the cause for this deficiency is unknown.

A study involving patients with GI involvement showed patients to have significantly lower serum concentrations of  $\alpha$ -tocopherol, ascorbic acid and carotene [47]. However, other studies comparing unselected patients to healthy controls have shown no difference in serum vitamin E or  $\beta$ -carotene, and no difference in ascorbic acid [212, 215]. In addition, another small study showed no difference in serum copper or zinc between unselected patients with SSc and healthy controls [216]. Thus, many questions remain as to whether deficiencies exist.

## Vitamin D

Vitamin D deficiency is common in the UK [218]. Most vitamin D is produced via the action of ultraviolet light on a vitamin D precursor in the skin. However, some may be from diet. Vitamin D deficiency has been studied in patients with SSc (Table 1.13). However, not all deficiency will be nutritional in origin.

Study	Number of patients	Vitamin D Findings
Vacca et al. [219]	90 Northern France; 66 Southern Italy	France: deficiency 32%; insufficiency or deficiency 82%; Italy: deficiency 23%; insufficiency or deficiency 86%
Krause et al. [204]	124	No significant association with nutritional status
Caramaschi et al. [220]	65	Deficiency: 30%; insufficiency: 66% ; normal: 5%
Arnson et al. [221]	327	Significantly lower vitamin D Cutaneous fibrosis inversely related to vitamin D
Murtaugh et al. [205]	24	No association with nutritional status

Table 1.12: Summary of studies of vitamin D status

Vitamin D deficiency has a high prevalence in patients with SSc. Furthermore, very high levels of insufficiency or deficiency occur irrespective of latitude and thus level of ultra-violet light exposure [219]. In addition, vitamin D supplementation does not protect everyone against deficiency. A high prevalence was also detected in other studies, one of which showed only 3% of patients to have normal serum vitamin D concentrations, despite none having overt signs of malabsorption [220]. While another large, pan-European study showed patients to have a significantly lower mean vitamin D concentrations than healthy volunteers [221]. In this study, negative correlations were noted between age and vitamin D and between MRSS and vitamin D concentration.

Other studies have investigated the relationship between vitamin D deficiency and malnutrition. One reported a high prevalence of vitamin D deficiency, which was greatest in patients with a worse PhA-based nutritional status (non-significant) [204]. Meanwhile, another smaller study also found no significant association between vitamin D and nutritional status [205].

Thus, although vitamin D deficiency is common in patients, as in the general UK population, no clear nutritional links have been proven.

### **1.10 Nutritional interventions in patients with systemic sclerosis**

The studies described so far have sought to understand the extent and nature of nutritional problems. However, once detected, steps must be taken to intervene in order to correct any deficiencies and to prevent further deterioration. Possible nutritional interventions range from simple dietary advice to oral supplementation, enteral tube feeding and finally long-term PN (i.e. HPN). Preference is given to the simplest interventions that produce the desired nutritional improvement. Patients may use more than one feeding modality. The available supportive evidence for each modality of nutritional intervention is described.

### **1.10.1 Dietary and oral supplementation**

Despite dietary advice and oral supplementation being the commonest modalities to be used, little evidence exists to support their nutritional benefit in patients with SSc. However, their use in patients without SSc is well established.

In a recent study, the effectiveness of a dietitian-delivered dietary intervention was evaluated in 9 patients with SSc at medium risk of malnutrition ('MUST' $>1$ ) [207]. The intervention comprised of tailored nutritional advice regarding a balanced diet and, where appropriate, oral nutritional supplements (2 patients). In this small study, dietary intervention led to an increased or maintained body weight and to an increased energy and protein intake, but did not result in a significant improvement in quality of life.

The success of tailored dietary interventions has also been shown in another small study which followed up 14 stable patients with SSc, GI involvement and unintentional weight loss (83% malnourished) after implementing nutritional therapy [222]. Nutritional therapy emphasised increased calorie and protein intake and modifications to texture and lifestyle. After only 6 weeks, patients had significant decreases in their nutrition symptom scores and increases in their appendicular lean heights.

Thus, as would be anticipated, individualised nutritional oral support appears beneficial. However, larger studies over longer durations are required to determine if benefits are sustained.

### **1.10.2 Enteral tube feeding**

Enteral tube feeding is considered in patients who are unable to meet their nutritional requirements orally. Short-term feeding may be delivered via the naso-enteric route. However, for long-term feeding the percutaneous route is preferred. Feeding may be into the stomach or post-pyloric. Gastric feeding is only advisable in patients with normal gastric emptying. To-date, the only publications describing successful enteral tube feeding in patients with SSc are in the form of case reports or cases included in other nutritional (PN) series. Two publications report the successful use of percutaneous endoscopic gastrostomies in patients with SSc and symptomatic oesophageal or pharyngeal dysphagia, in the absence of other GI involvement [223, 224]. Another published case describes the failure of gastrostomy feeding in the presence of delayed gastric emptying and the

subsequent successful use of jejunal feeding [225]. Thus, enteral tube feeding appears beneficial in carefully selected patients. However, evidence is limited.

### 1.10.3 Parenteral nutrition

When enteral methods fail, patients may proceed to PN, which has potential complications. In one nutritional study, 1.9% of patients with SSc (11/586) were receiving long-term PN [36]. However, despite this, few case series specifically report on the outcome of patients with SSc on HPN (Table 1.13).

Study	Number of patients	Years	Location	HPN duration
Levien et al. [226]	2	unknown	USA	Unknown
Grabowski et al. [225]	4 HPN 1 enteral	unknown	USA	Range: 12-86 months (PN)
Ng et al. [227]	15	1979-1987	USA	Range: 2 months to 7.5 years Mean: 2.9 years
Brown et al. [69]	8	1993-2006	UK	Range: 0.8 to 192 months Median duration: 40 months
Jawa et al. [228]	12	1998-2010	Canada	Range: 5 to 270 months Mean survival: 229 months
Stanga et al. [229]	5	2008 - 2013	Switzerland	Duration: 12 months

Table 1.13: Summary of SSc only HPN series

The earliest case report describing the use of HPN in a single patient was published in 1974 and described the development of copper deficiency [230]. A subsequent single case report described a patient with SSc as an example of a patient group unable to manage HPN without nurse support, due to the nature of their underlying disease [231]. Subsequently, a 2 patient series also described the commencement of HPN in patients with SSc and severe GI involvement [226]. However, both patients developed catheter-related

blood stream infections requiring catheter replacement. A later series included 4 patients, with small intestinal dysmotility, who had been commenced on HPN [225]. By the time of publication, 3 patients had died (range 12-17 months) while one survived (86 months). None of the patients developed catheter-related complications, with the exception of the surviving patient who required hub replacements due to wear. None of the patients were self-caring, but all improved nutritionally (BMI) with careful feeding. Thus, HPN improved nutritional status, but did not prevent deaths due to other non-nutritional SSc related causes.

A subsequent larger HPN series, which included 15 patients with SSc and severe GI involvement, reported an improvement in quality of life on HPN (11 patients) [227]. However, similar to the previous study, 7 patients died from complications unrelated to HPN, but none died due to HPN-related complications. Complications noted were catheter related blood stream infections (CRBSI; n=2), central venous thrombosis (n=2) and mechanical catheter problems (n=3). A subsequent, smaller UK study also reported the safe use of HPN in SSc, and again highlighted the need for family or nursing support for catheter management and HPN delivery [69]. This was also confirmed by a very recent study including 12 patients, which reported an improvement in nutritional status on HPN, but could not demonstrate an improvement in functional status [228].

A recent retrospective study described the improvement in nutritional and quality of life outcomes in 5 patients commenced on HPN [229]. Over the 12 months following commencement, mean nutritional risk screening scores decreased and the mean BMI increased. In addition, there was evidence of improved physical and mental quality of life.

Other non-SSc specific PN series have included patients with SSc, but not all have compared the outcomes of subjects with SSc to those without [70, 232]. One study, which did compare outcomes of patients on HPN for chronic intestinal pseudo-obstruction, reported a higher mortality in patients with SSc than with other conditions [70].

Thus, HPN would appear to have benefit in selected patients with SSc. However, given the recent advances in HPN delivery, there still exists the need for more, up to-date information on the outcome of patients with SSc commenced on HPN.

## 1.11 The autonomic nervous system

The autonomic nervous system (ANS) is largely regarded as being part of the peripheral motor system, with the other part being the distinctly different somatic system.

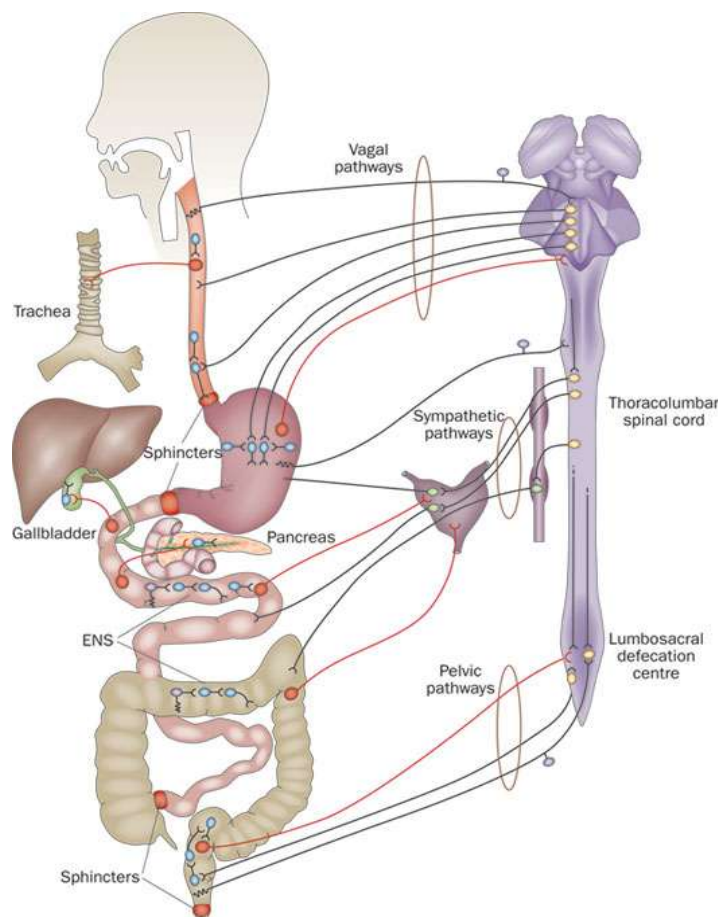


Figure 1.4: Autonomic nervous system

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Furness, J. B. (2012) The enteric nervous system and neurogastroenterology.

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The function of the ANS is to maintain a constant, optimal, internal environment in the face of internal and external stimuli which act against this. The ANS achieves this homeostatic control through numerous reflex arc pathways. Reflex arcs are composed of sensory receptors, which detect changes and transmit signals centrally via afferent pathways and central processing centres. These subconsciously determine the appropriate response and send signals via efferent pathways to effector organs, such as the heart, smooth muscle and glands, which produce the appropriate response.

The ANS has two efferent divisions, namely the sympathetic and parasympathetic nervous systems. These work in a co-ordinated fashion to achieve autonomic regulation. In some circles, the enteric nervous system is considered a third ANS division. However, unlike the sympathetic and parasympathetic nervous systems which operate under central nervous system control, the enteric nervous system operates independently [233]. Thus, it is often considered a separate entity.

### **1.11.1 Autonomic regulation of heart rate and blood pressure**

#### **Regulation of heart rate**

Both the sympathetic and parasympathetic nervous systems control heart rate (HR). The parasympathetic nervous system decreases HR. In response to acetylcholine release, the HR quickly slows, due to the short latency period of the synaptic channels. Likewise, once parasympathetic stimulation ceases, released acetylcholine is rapidly broken down, and HR increases. Thus, changes in vagal stimulation are rapidly translated into changes in HR, allowing beat-to-beat regulation.

The sympathetic nervous system increases HR. When stimulated, the nerve terminals slowly release noradrenaline which travels, via slow-mediated pathways, to increase HR. Upon the cessation of the sympathetic signal, the nerve terminals take up most of the released noradrenaline. Thus, HR responses to sympathetic stimulation occur much slower than those of the parasympathetic nervous system. This difference in neurotransmitter response times makes it possible to determine whether HR changes were predominantly sympathetic or parasympathetic mediated.

#### Heart rate variability

The variation in the interval between consecutive normal heart beats is termed heart rate variability (HRV). This variation is a consequence of the dynamic relationship between sympathetic and parasympathetic influences. High HRV is considered to be an indicator of a well functioning ANS, with a good ability to adapt to both internal and external stimuli. Conversely, reduced HRV may reflect an underlying ANS abnormality, which limits its ability to adapt. However, HRV is also affected by other factors, such as posture, age (decreases with age) and respiration. HRV is greater when stood than when supine [234].

Thus, to allow comparison of results, participants should adopt the same test position and absolute HRV comparisons should be confined to 5 year age groups [235].

### Sinus arrhythmia

At rest, the HR alters with respiration in healthy individuals [236]. During inspiration sympathetic activity is increased, while during expiration parasympathetic activity is increased. The parasympathetic nervous system is capable of rapid beat-to-beat regulation of the HR, while changes due to sympathetic stimulation are slower. Thus, the rapid HR changes associated with respiration (termed respiratory sinus arrhythmia) are predominantly due to alterations in parasympathetic stimulation. In disease states, this may be impaired.

### **Blood pressure**

Blood pressure (BP) is a function of vascular compliance, cardiac output and peripheral vascular resistance. Measurements fluctuate in response to discrete stimuli, but over a prolonged period are tightly regulated. Short term changes are influenced by the ANS, while long term control involves other systems, such as the renal renin-angiotensin system.

The ANS controls cardiac output and peripheral vascular resistance. Cardiac output is a function of end-diastolic volume, myocardial contractility and HR. End-diastolic volume is affected by venous pressure, which is linked to blood volume and venous smooth muscle tone, both of which are under sympathetic control. Myocardial contractility and HR are controlled by the sympathetic and parasympathetic divisions of the ANS.

Short-term control of BP is instigated by the baroreceptor reflexes. Should arterial BP increase, then baroreceptors signal an increase in parasympathetic activity, which slows HR, and a reduction in sympathetic stimulation, which causes vasodilation. Acting together these restore normal BP. Conversely, a reduction in BP results in reduced parasympathetic and increased sympathetic stimulation, which increases HR and cardiac contraction and causes vasoconstriction.



### Postprandial cardiovascular response

After eating, vasodilatory gut peptides are released which cause blood to be redirected to the GI tract [237, 238]. The enteric nervous system communicates with the central nervous system via the autonomic pathways. In order to compensate for this splanchnic pooling of blood, sympathetic outflow increases which leads to a compensatory increase in HR and cardiac output. Thus, systemic BP remains stable during the postprandial period.

#### **1.11.2 Sympathetic skin innervation**

Sweat is produced by specialist eccrine sweat glands [239]. Sweat glands have their greatest density on the palms and soles. They secrete a clear, fluid composed primarily of water (99%) and sodium chloride in response to environmental (thermoregulatory sweating), emotional (plantar and palmer only), intellectual or gustatory stimuli [240]. These stimuli trigger the transmission of signals via specialist acetylcholine-mediated sympathetic pathways. Pathways involve central sweat centres, preganglionic nerve fibres, unmyelinated class C postganglionic nerve fibres and sweat glands. The ability to sweat in response to sympathetic activation requires an intact pathway.

#### **1.11.3 Nerve supply to the gastrointestinal tract**

##### **Enteric nervous system**

The enteric nervous system is composed of over overlapping plexuses lying within the walls of the GI tract [241]. It has 2 divisions, namely the submucosal and the myenteric plexuses [233, 242]. The submucosal plexus regulates glandular secretion and intestinal water/ion transportation. Pathways link it to the myenteric plexus to allow co-ordination. The myenteric plexus controls GI motility. This plexus is capable of functioning even when independent from the body [233]. However, for normal functioning, it requires central nervous system regulation. Under the influence of the ANS noradrenergic containing postganglionic sympathetic nerve fibres, intestinal motility is reduced. Meanwhile, other central signals regulate blood flow and intestinal secretion.

## **Interstitial cells of Cajal**

First discovered in 1911, the interstitial cells of Cajal are specialist neurones, lying throughout the GI tract, which have an important role in GI motility [243]. There are different sub-sets of cells. Some are capable of initiating slow depolarization waves, which regulate the frequency of the regular phasic contractions of that part of the GI tract [244]. These cells are referred to as pacemaker cells. Pacemaker cells are found throughout the stomach with the exception of the fundus. However, the dominant pacemaker cells lie in the proximal part of the body. The importance of these cells for normal gastric motility is demonstrated by the association of their absence with dysmotility [245].

### **1.12 Autonomic dysfunction**

Autonomic dysfunction refers to the effects of any damage to autonomic pathways. Damage is normally, but not necessarily, permanent and may be localised or generalised. Numerous causes exist. Damage may be primary (e.g. pure autonomic failure) or secondary to other diseases including trauma/surgery, toxins, inflammation, infections, genetic and metabolic.

Dysfunction may affect any autonomic function. The following sections will focus on cardiovascular, sudomotor and GI autonomic dysfunction.

#### **1.12.1 Cardiovascular autonomic dysfunction in systemic sclerosis**

Autopsy series involving patients with SSc have shown evidence of pathological changes to the cardiac microcirculation together with myocardial fibrosis [13]. These may disrupt cardiac electrical conduction systems, leading to arrhythmias.

Early, often sub-clinical cardiac conduction defects are common in patients with SSc. In one series (n=436), approximately 25% had conduction defects (e.g. PR prolongation, left anterior fascicular block) on their resting electrocardiogram (ECG) [246]. Such dysfunction is clinically important as it may be associated with increased risk of arrhythmias and malignant arrhythmias, leading to sudden cardiac death.

A study involving 45 unselected patients with SSc showed the patients to have significantly impaired heart rate turbulence [247]. Heart rate turbulence is the

acceleration-deceleration response to a ventricular premature beat. Impairment of this is, in non-SSc post-infarction studies, associated with increased risk of malignant ventricular arrhythmia and sudden cardiac death [248].

Patients with SSc may also have reduced HRV. The clinical significance of disturbances in HRV were first recognised in the mid-1960s [249]. Since then, numerous more studies have investigated their relevance. In patients without SSc, reduced HRV has been linked to increased risk of death and arrhythmias [250]. One study involving patients with SSc (n=30), showed evidence of highly significant impairments in all HRV parameters [251]. In addition, low HRV correlated with preclinical cardiac involvement. Furthermore, patients with SSc have been shown to have evidence of cardiovascular autonomic dysfunction when performing provoking autonomic manoeuvres [252-254].

In the presence of autonomic dysfunction, there may be disruption to the compensatory changes that normally follow the ingestion of food in order to maintain BP. In the absence of this, patients may develop a postprandial hypotension. This has been shown in patients with autonomic dysfunction, but without SSc, when compared to controls [237]. Studies investigating this in patients with SSc are still awaited.

### **1.12.2 Sudomotor autonomic dysfunction in systemic sclerosis**

Disruption of the skin's sympathetic innervation disrupts sweat responses. A wide range of diseases may cause this. One study showed almost 69% of patients with SSc (n=32) to have abnormal sympathetic skin responses [255]. There was no correlation between the abnormality and the localisation, degree or character of skin changes, disease duration or autonomic symptoms. However, another study which analysed sweat production over the dorsum of the foot (sweat-spot-test) detected disruption to cholinergic fibres [52].

### **1.12.3 Gastrointestinal autonomic dysfunction in systemic sclerosis**

The GI tract has an abundant supply of autonomic nerves which are involved in the coordination of GI motility. Histological studies show evidence of involvement of gastric nerves in SSc disease processes. Dense collagen bundles envelope the myenteric plexus and neurones and nerve fibres are oedematous [15]. Nerve fibres, nerve endings and the interstitial cells of Cajal are enveloped by elastic and collagen fibres. Thus, they are

separated from the smooth muscle cells which they must innervate. Gastric autonomic involvement is supported by the detection of anti-myenteric neuronal antibodies in the sera of patients with SSc [21]. When injected into rats these antibodies evoke alterations in intestinal myoelectric activity [18].

Autonomic dysfunction is associated with disordered GI motility. Possible gastric manifestations include both delayed and more rapid gastric emptying. Rate of gastric emptying may impact upon systemic BP. Post-vagotomy patients with early dumping (rapid gastric emptying) often report symptoms of sweating and palpitations and may have evidence of a postprandial fall in BP [256]. Similarly, in studies involving patients with diabetes, the postprandial fall in systemic BP was greatest in those patients with the most rapid gastric emptying [257].

Studies involving patients with SSc have sought correlations between GI dysmotility and cardiovascular autonomic dysfunction. Reduced oesophageal contraction amplitudes have been linked to cardiovascular and pupillary autonomic dysfunction [258, 259]. Similarly, proximal stomach function has been shown to correlate with cardiovascular autonomic dysfunction [260]. Thus, these studies suggest a relationship between autonomic dysfunction and GI dysmotility. However, the exact nature of this relationship is unclear as other studies have offered conflicting results.

One such study comparing patients with SSc (n=38) to both normal and dyspeptic controls assessed GI symptoms, rate of liquid gastric emptying measured using functional ultrasound and cardiovascular autonomic function (using a battery of tests) [52]. This study found no evidence to support an association between dyspeptic symptoms and autonomic neuropathy, or between rate of gastric emptying and autonomic neuropathy. This is in-line with another earlier study which also showed no evidence of an association between cardiac autonomic dysfunction and gastric motor dysfunction [261].

### **1.13 Assessment of the autonomic nervous system**

The integrity of the ANS may be assessed using a variety of different methods. These include questionnaires for the presence of suggestive symptoms and specialised autonomic tests (the autonomic battery). These methods are discussed in the following sections.

### **1.13.1 Assessment of autonomic symptoms**

Symptoms may be assessed using validated, self-reporting questionnaires containing questions pertaining to autonomic involvement of a spectrum of organ systems. The Autonomic Symptom Profile, which was first described in 1999, contained 169 questions and covered demographics and 11 symptom domains [262]. Subsequent to this, the Composite Autonomic Symptom Score (COMPASS) was developed based on clinically important responses and validated by comparison to the Composite Autonomic Severity Score (CASS). This COMPASS questionnaire contains 84 questions, covering 11 autonomic domains (orthostatic intolerance, secretomotor, male sexual dysfunction, urinary, gastroparesis, constipation, diarrhoea, pupillomotor, vasomotor, reflex syncope and sleep) and 12 additional questions required to generate a validity score. To-date, this questionnaire has been utilised in several studies investigating autonomic involvement in a variety of different diseases but not, to the best of our knowledge, SSc.

More recently, this 84-item COMPASS questionnaire has been refined to produce a 31-item version, called COMPASS-31 (Appendix 11) [263]. This new version, which covers 6 autonomic domains (orthostatic intolerance, vasomotor, secretomotor, gastrointestinal, bladder, pupillomotor) is quicker to complete and simpler to analyse. Results for each domain are weighted accordingly, to produce an overall autonomic symptom score (range 0-100) (Appendix 12). COMPASS 31 has been shown to have better internal consistency than previous versions. However, it has yet to be tested on participants with a variety of different diseases with autonomic involvement.

### **1.13.2 Background to cardiovascular autonomic function tests**

The ANS acts to maintain a constant, optimal, internal environment. Therefore, by testing its ability to respond to these stimuli, assessments may be made of its function.

Assessments may be conducted when at complete rest or when a specific reflex arc is activated by a standardised external stimuli/manoeuvre. Numerous specific autonomic tests/manoeuvres have been devised [264]. Each test/manoeuvre assesses the whole, or part, of a specific reflex arc. In order for a test/manoeuvre to be clinically applicable, it must be easily reproducible and must generate a readily measurable response (i.e. BP, HR, sudomotor and pupillary). However, despite the apparent simplicity of this approach, the assessment of the ANS is complicated by the overlapping nature of the many reflex

pathways. Thus, individual tests should not be performed in isolation. Instead, a series of sympathetic and parasympathetic tests are combined together into a standardised ‘battery’ in order to give a global assessment of autonomic function [265].

### **Battery and scoring of autonomic tests**

Based upon diabetes mellitus research, a simple, reproducible, rapidly conducted ‘battery’ of 5 autonomic measures was proposed [266-268]. This first autonomic battery was ‘Ewing’s Battery’. It consisted of 3 manoeuvres assessing cardiac parasympathetic integrity and 2 measures assessing sympathetic integrity. Subsequently, the 5 tier Ewing’s Criteria was developed to classify the severity of autonomic dysfunction into normal, borderline or abnormal [266, 267]. However, this was based on the natural history of diabetes mellitus, and the sequence of autonomic dysfunction may not proceed similarly in other diseases.

Despite being reported to be a rapid measure taking only 20 minutes to complete, other authors report it to take longer in practice [269]. Thus, over time, other autonomic batteries were developed, e.g. ‘O’Brien’s Battery’ [270]. Thus, caution is required when comparing battery results. Recognising this difficulty, it was recommended that autonomic battery should be standardised [271]. Their consensus statement recommended the use of 5 autonomic tests (HR response to Valsalva manoeuvre (VM), standing and deep breathing and BP response to sustained handgrip and standing) and a test of sudomotor function. This battery is still widely used. However, over time, alternative scoring systems have evolved, to allow the inclusion of more modern analysis techniques.

One alternative scale is the 10 point Composite Autonomic Scoring Scale (CASS; Tables 1.14 to 1.16) [272]. It has a high sensitivity and specificity for the identification of autonomic failure in high scoring participants and corrects for the confounding effects of age and gender. Based upon the sum of their sudomotor (0-3), adrenergic (0-4) and cardiovagal (0-3) index results, participants are classified as having either mild (1-3), moderate (4-6) or severe (7-10) autonomic failure.

1 =	Single site abnormal on quantitative sudomotor axon reflex testing <u>OR</u> Length-dependent pattern (distal sweat volume < <sup>1</sup> / <sub>3</sub> of forearm or proximal leg values) <u>OR</u> Persistent sweat activity at foot [if thermoregulatory sweat test used substitute for anhidrosis present but <25%]
2 =	Single site <50% of the lower limit on quantitative sudomotor axon reflex test [if thermoregulatory sweat test used substitute for anhidrosis 25-50%]
3 =	Two or more sites <50% of lower limit on quantitative sudomotor axon reflex test [if thermoregulatory sweat test used substitute for anhidrosis >50%]

Table 1.14: CASS Sudomotor Index

1 =	HR <sub>DB</sub> or VR mildly decreased (above >50% of minimum normal value)
2 =	HR <sub>DB</sub> or VR decreased to <50% of minimum normal value
3 =	Both HR <sub>DB</sub> and VR decreased to <50% of minimum normal value

Table 1.15: CASS Cardiovagal Index

1 =	Phase II <sub>E</sub> reduction <40mmHg but >20mmHg mean BP <u>OR</u> Phase II <sub>L</sub> does not return to baseline <u>OR</u> Pulse pressure reduction to ≤50% of baseline
2 =	Phase II <sub>E</sub> decrease of <40mmHg but >20mmHg mean BP <u>AND</u> Phase II <sub>L</sub> or IV absent
3 =	Phase II <sub>E</sub> decrease of >40mmHg mean BP <u>AND</u> Phase II <sub>L</sub> <u>OR</u> IV absent
4 =	Criteria for 3 <u>AND</u> Orthostatic hypotension (SBP decrease of ≥30mmHg; mean BP decrease of ≥20mmHg)

Table 1.16: CASS Adrenergic Index

## **General Limitations of Autonomic Testing**

Over time, autonomic assessment methods have been extensively studied and refined. However, their results remain subject to numerous other confounding influences which may result in the measured outcome not being indicative of a participant's autonomic status. Potential confounding variables include: posture, ambient temperature, time of day, medications, exertion, diet, hydration, blood glucose, etc. [263-265]. In an attempt to control for these, most autonomic studies are performed in a highly standardised manner. For instance, participants normally lie supine, in a quiet, temperature controlled setting within a specified time-window [264]. Any potentially confounding medications and substances (e.g. alcohol, caffeine and nicotine) should be stopped [265, 273]. Thus, most protocols recommend that caffeine, alcohol, smoking and vigorous unaccustomed exercise be avoided for a pre-defined period and that any confounding medications be stopped for at least 5 half-lives [273]. Responses may also be influenced by participant specific characteristics, including level of exertion when performing manoeuvres, age and gender [274, 275]. Thus, age-standardised reference ranges are used when interpreting results.

Despite recommendations for standardized protocols, as yet, there remains no universally adopted approach. A study of European centres demonstrated a wide variation in equipment standards, test methods, source of normative values and staffing [276]. Furthermore, recent technological advances have led to some centres developing computerised analysis techniques. These will differ between centres. Thus, the inclusion of internal controls within centre protocols is highly recommended.

### **1.13.3 Assessment of heart rate variability**

HRV may be assessed by two different methods, namely time domain and frequency domain [277]. The accuracy of HRV assessment using these methods has several limitations. Both methods and their limitations are discussed in the following section.

#### **Time domain methods**

Time domain methods describe the variability in R-R intervals during a pre-specified time period in a continuous ECG [278]. A variety of outcome measures can be reported, such as normal-to-normal (NN) intervals, standard deviation of NN interval (SDNN), etc.



SDNN is recommended as the overall measure of HRV [277]. Time domain methods are used to assess HR variations when performing manoeuvres (VM, deep breathing, etc).

### **Frequency domain methods**

Frequency domain methods describe HRV in terms of its frequency components [279]. The high frequency component is thought to represent purely vagal tone, whilst the low frequency component is thought to be more representative of sympathetic tone. Thus, frequency domain methods may permit a more precise analysis of sympathovagal balance than time domain methods.

### **Limitations**

#### ECG quality

A key limitation in the assessment of HRV is the requirement for an artefact-free ECG. Artefacts are often evident from the rate of change of HR, as normally beat-to-beat fluctuation is limited to 75-135% [280]. It is essential to remove artefacts as, even one, can result in a 3 natural log unit increase in the high frequency variability of spectral analysis, and thus invalidate any HRV assessment [281]. Therefore, it is recommended that inter-beat series, deduced by automatic detection programmes, be hand-corrected for artefacts (including ectopic beats) [278, 280]. An ectopic beat is one which does not originate from the sinoatrial node, and is thus not representative of ANS activity. Any R peak associated with an ectopic is normally manually excluded from analyses of inter-beat intervals (IBI).

#### Heart rate assessment period

HRV increases with the length of time over which HR is recorded [277, 282]. Therefore, participants should be studied over identical time periods. The optimal time period has not been fully established. Using spectral analysis, a fast 10 second (0.10Hz) frequency could be detected a maximum of 6 times within a one minute sample period, while a slower 25 second frequency could only be detected twice [235]. Therefore, the standard recommendation for short-term monitoring is 5 minutes [277]. However, more recently, studies have suggested that periods as short as 10 seconds may be used [283, 284].

#### **1.13.4 Assessment of beat-to-beat blood pressure**

To conduct a battery of autonomic tests, the ability to perform beat-to-beat assessments of BP is required. Traditionally, the gold-standard method relied upon invasive monitoring, the use of which is inappropriate for routine autonomic testing. Instead, a non-invasive approach is favoured. Non-invasive systems for the continuous recording of finger arterial BPs are widely used [273, 276, 285]. However, these systems have potential limitations for use in patients with SSc. Therefore, the mechanisms behind the most widely used, currently available, commercial system (Fina/Portapres™, Finapres Medical Systems Amsterdam, The Netherlands) are discussed in more detail.

#### **Finapres™**

Finapres™ (FINger Arterial PRESsure) technology was first developed in the early 1980s [286, 287]. Since this time, its technology has been refined and an ambulant version (Portapres with Finometer) developed [288].

#### Mechanism of action

The systems use the volume-clamp method to non-invasively measure finger arterial pressures [289]. Practically, this involves wrapping an inflatable cuff, which is controlled by a complex servo-controller system, around a digit. This cuff inflates and deflates in order to maintain the digital artery at a constant diameter, in spite of the changes in pressure associated with each heart beat. Pressure changes are detected by an infrared plethysmograph, which is built into the inflatable air bladder containing cuff. This works by emitting an infrared light which is absorbed by the haemoglobin, and the change in arterial diameter with each heart beat causes a pulsation in the light signal detector. However, as the unloaded diameter does not normally remain constant during a study, it must be intermittently reassessed by means of the Physiological procedure [290]. This Physiological procedure, which involves the regular checking of the set-up, by momentarily interrupting BP recordings, is initiated once a steady BP has been achieved. If needed, it will reset the set-point.

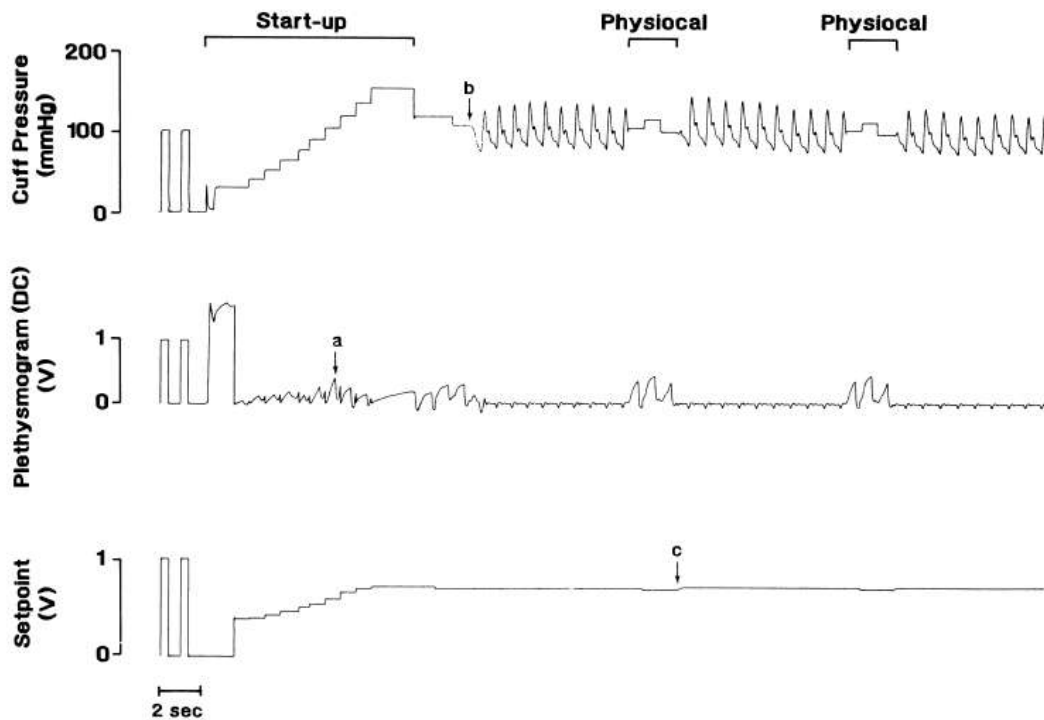


Figure 1.5: Finapres start-up and Physioical adjustments

The 3 simultaneous traces show finger cuff pressure, infrared plethysmogram light transmission and total amount of infrared light passing through the finger. Figure reproduced with permission from Oxford University Press (Ben et al. Fifteen years experience with finger arterial pressure monitoring. 1998, 36, 605-616).

#### Potential limitations in systemic sclerosis

Studies using this Finapres™ technology have reported it to be sufficiently accurate for BP tracking [291]. However, its use is not without limitations, some of which are more likely to occur in participants with microvascular involvement and hand deformity, such as those present in patients with SSc. One study involving patients with SSc has used Finapres™ technology for non-invasive BP assessment without reported difficulty [292]. However, in this study, half of the patients did not stop confounding medications.

Studies comparing Finapres™ and intra-arterial measurements show comparable measurements for mean arterial BPs (MAP) and DBPs, but a larger (not statistically significant) difference in SBPs [291]. For optimal performance, correct cuff application is crucial and the relative level of the finger to the heart must be kept constant [288, 293]. However, this is corrected in part by the in-built height correction unit within the

Portapres™ device [288]. This is especially important in patients with SSc as a study (n=7) has shown evidence of disordered digital blood flow autoregulation [294].

Theoretically, the use of Fina/Portapres™ systems may be limited in patients with SSc, due to the associated secondary Raynaud's phenomenon, microvasculature involvement, sclerodactyly and contractures. Classically, Raynaud's phenomenon causes digits to turn white (vasospasm), then blue (venous stasis) and finally red (reperfusion) in response to cooling [295]. Cold induced vasoconstriction interferes significantly with the assessment of finger arterial pressures in participants with primary or secondary Raynaud's phenomenon [296, 297]. Measurements fall on cooling, but thereafter increase to exceed upper arm pressures [297]. In addition, in participants with SSc with Raynaud's phenomenon, digital SBP is reduced at room temperature in comparison to participants without, and the proximal to distal phalanx difference is greater [298]. It is recommended that any vasoconstriction related SBP increase can be corrected by warming cold hands [291, 299].

It is recommended that the inflatable cuff be applied to the middle or ring finger [291]. However, early prototype thumb cuffs were also reported to work accurately, but the concept was not developed [300]. In patients with SSc, it may be preferable to use thumbs rather than fingers, as the thumb is less likely to be affected by secondary Raynaud's phenomenon [301-304].

### **1.13.5 Tests of the autonomic battery**

The standard autonomic battery consists of 4 manoeuvres, during which HR and BP responses are assessed.

#### **Deep breathing**

Rapid changes in HR in response to deep breathing ( $HR_{DB}$ ) are largely due to parasympathetic influences. The optimal breathing algorithm to generate the maximum changes in HR is 6 breaths per minute for 1min [266, 267]. Other algorithms that use a single deep breath produce responses which are poorly correlated with the standard deep breathing algorithm [270, 305].

HR response may be quantified by the respiratory sinus arrhythmia amplitude and/or the Expiratory:Inspiratory (E:I) ratio. The respiratory sinus arrhythmia amplitude is the mean change in HR (maximum minus minimum HR for each cycle; HR<sub>max</sub>/HR<sub>min</sub>) over the 6 respiratory cycles. Differences decrease with age. The lower (5<sup>th</sup>) percentile of normality falls with increasing age from 14 (10-29 year olds) to 7 (60-69 year olds) [275].

The E:I ratio is the mean of the longest R-R intervals during expiration to the mean of the shortest R-R intervals during inspiration. This ratio decreases with age; such that for a single breath, the lower (5<sup>th</sup>) percentile of normality falls from 1.23 in participants aged 16-20 to 1.05 in participants aged 76-80 [306].

In addition to age and parasympathetic activation, HR responses may also be influenced by potential confounders, such as posture and tidal volume. Larger volumes are associated with greater HRV. The breathing algorithm may be conducted with the participant seated or lying supine [264, 268]. A seated position may be preferable for participants with restricted ventilation [264]. In addition, results are strongly influenced by the participant's depth of breathing, which may be related to effort [307].

### **Sustained isometric handgrip**

Sustained isometric exercises increases HR and BP. Upon performing the manoeuvre, parasympathetic withdrawal leads to an increase in HR, while sympathetic activation leads to vasoconstriction, which increases BP, and contributes to the HR increase [308]. Most protocols involve a participant squeezing a handheld pressure gauge at 30% of their maximum achievable pressure for 5 minutes [268, 309]. However, some protocols advocate 50% for 3 minutes (Julu et al unpublished). Elevated tension, as that seen with the need to achieve an increased percentage of maximal grip strength, is associated with increased sympathetic outflow [310].

A normal HR increase to sustained grip test (50% for 3 min) is 22 to 45% (15-79 year olds, unpublished data) [311]. BP responses are assessed using DBP. If normal, DBP should rise by at least 16mmHg, if borderline by 11-15mmHg and if abnormal by less than 10mmHg (5min at 30%) [309]. Limitations for sustained grip testing are less well understood than with the other autonomic tests.

### **Valsalva manoeuvre**

A VM involves inhaling deeply, then blowing into a mouthpiece to achieve a pressure of 40mmHg for 15 seconds, whilst HR and BP are monitored [268, 272]. However, pressures of 30mmHg for more than 10 seconds are generally accepted [312, 313]. In order to capture the associated changes in BP and HR, recording continues for 1-3 minutes after cessation of blowing (strain period). To prevent the participant closing their glottis to maintain the pressure without blowing, the VM apparatus contains a small air leak [273]. Due to the effects of posture, many protocols recommend that the VM be performed while supine [264, 314]. However, others have the participant seated, or reclined at an angle [268, 315]. Participants studied at an angle of 20 degrees to supine, show reduced cardiac preload and improved VM morphology [315].

In health, the VM generates both sympathetic and parasympathetic responses. The BP and HR changes of a normal VM are divided into 4 phases [264, 316]. Phases I-II occur during the strain period. The 4 phases are described [264, 273, 317].

#### Phase I

This phase, which relates to the end of inspiration and the onset of strain, is primarily driven by mechanical forces. Straining causes an increase in intra-abdominal and intra-thoracic pressures which causes aortic compression. This forces the blood into the peripheral vessels to produce a rapid, but transient, BP rise. This rise in BP is accompanied by an increase in parasympathetic activity, which results in HR slowing.

#### Phase II

As the strain continues, cardiac output falls, due to a reduction in stroke volume and venous return (preload). This leads to a fall in BP, despite a withdrawal of parasympathetic stimulation which increases HR.

#### Phase III

In response to the hypotension, arterial baroreceptors trigger an increase in efferent sympathetic stimulation to the muscles and noradrenaline is released. Together, these

increase total peripheral resistance to rapidly halt the fall in BP and allow recovery, possibly to above baseline in a normal participant.

### Phase III

At the beginning of this phase, in normal participants, the MAP is at or above the baseline measurement. On cessation of straining, the intra-thoracic and intra-abdominal pressures suddenly fall, reducing pressure on the aorta and increasing pulmonary venous capacitance. This results in a transient fall in BP, which is accompanied by a reflex increase in HR, possibly due to a burst of efferent sympathetic activity.

### Phase IV

In this final phase, BP transiently rises above the baseline, due to the recovery of cardiac venous and cardiac output and the continued constriction of the arterial beds. This recovery is accompanied by a reduction in efferent sympathetic activity and increased parasympathetic activity. This increase in parasympathetic activity mediates a reduction in HR.

### Assessment of Valsalva manoeuvre

In the presence of autonomic dysfunction, normal HR and BP responses are affected. HR changes are reported using the Valsalva Ratio (VR), which is the maximum HR generated by the VM, divided by the lowest HR occurring within 30 seconds of the peak HR [318].

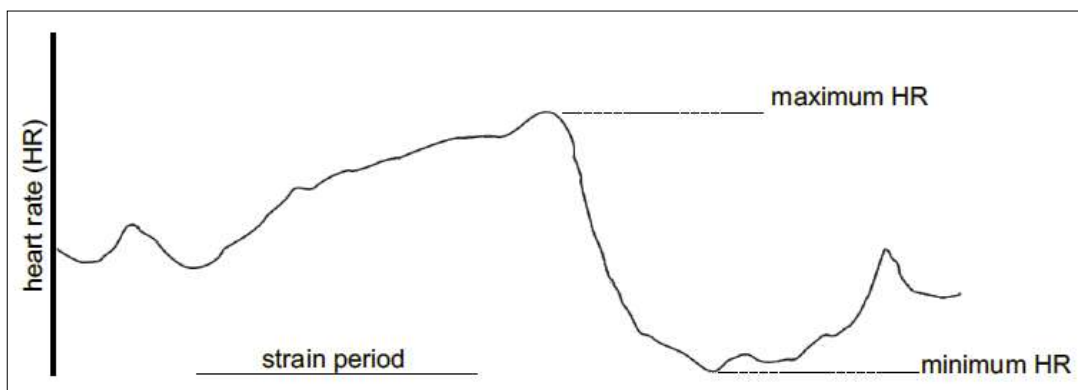


Figure 1.6: Change in HR changes during the VM

VR decreases with age [318]. However, for all ages, a normal VR is reported to be greater than 1.21 [267, 311, 318]. VRs are reduced with autonomic dysfunction [267].

Several measures of MAP may be measured to quantify the autonomic response to the VM. These include the maximum phase II fall in MAP, the peak MAP at the end of III, the phase IV MAP overshoot and pressure recovery time (time taken for phase III fall in SBP to return to baseline). Figure 1.7 illustrates the MAP measurements.

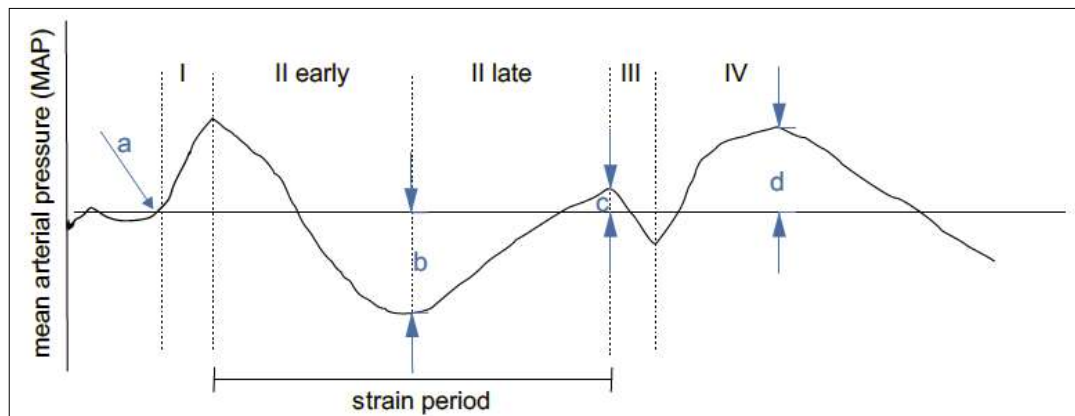


Figure 1.7: Normal MAP changes during the VM phases

(a = baseline; b = minimum phase II MAP; c = end of phase II recovery; d = peak overshoot)

In the presence of autonomic dysfunction, the VM induced BP responses may be absent or attenuated, such that a ‘flat-top’ response may be produced.

### Orthostatic response

Upon adopting an upright posture, gravitational forces exert a negative effect on the circulation which, unless compensated for, may result in collapse. The efficiency of the ANS to respond may be investigated using the head-up-tilt or the sit-to-stand test [264].

Ideally, responses should be assessed after resting supine for at least 10 minutes.

However, shorter periods are often used in practice. Postural adaptations to active standing are divided into those which are immediate (0-30 seconds), stabilized (30 seconds to 2 minutes) and prolonged (over 2 minutes) [319]. On standing, blood collects in the lower limbs, leading to a fall in venous return to the heart and thus stroke volume, cardiac output and BP. This fall is detected by baroreceptors, which trigger a compensatory increase in BP and HR. The HR peaks between approximately 3 and 12 seconds after standing. The 3



second (primary) peak is attributed to withdrawal of vagal tone. However, it may also be partly due to an exercise reflex from the voluntarily contraction of leg muscles in order to stand [320]. Meanwhile, the secondary 12 second peak (approximately 15<sup>th</sup> heart beat) is attributed to a further reflex inhibition of cardiac vagal tone, an increase in sympathetic outflow from the sinus node and a reduction in arterial baroreceptor activation due to a BP fall.

This is followed by an increase in venous return due, in part, to the active contraction of the limb muscles during active standing. As a consequence of this, the BP transiently overshoots its baseline leading to parasympathetic activation and a fall in HR. The HR reaches its minimum at approximately 20 seconds (30<sup>th</sup> heart beat) after standing. The ratio of the maximum to the minimum R-R interval is taken as a measure of vagal function [268]. In health, this is normally greater than 1.04. In the presence of autonomic dysfunction, this may be less than 1[307]. Postural hypotension is defined as a fall of 20mmHg in SBP or 10mmHg in DBP within 3 minutes of standing / head-up tilt [321].

### 1.13.6 Assessment of sudomotor function

A number of tests are available to assess sudomotor function (table 1.17) [322-324].

Test	Target sudomotor pathways	Advantages	Disadvantages
Thermoregulatory sweat test	Central and peripheral sympathetic sudomotor pathways	Shows distribution of disordered sweating	Cannot differentiate pre and post ganglionic lesions
Quantitative sudomotor axon reflex test	Postganglionic sympathetic cholinergic sudomotor function	Standardised sensitive and reproducible	Cannot assess preganglionic pathway
Sympathetic skin response	Surrogate marker of sudomotor function	Simplest to perform	High variability between subjects

Table 1.17: Summary of sudomotor assessment methods

### Thermoregulatory sweat test

This test is used to assess the integrity of the central and peripheral sympathetic sudomotor pathways. It involves the application of a sweat inducing stimulus, such as a rise in ambient room temperature and thus skin and blood temperature. This temperature rise is detected by central centres, which signal the sweat glands via pathways which include the sympathetic chain and sudomotor nerves. The resultant normal response is an increase in sweat production, which may be visualized by with an indicator dye. In the presence of autonomic dysfunction, sweat production is reduced or absent from the affected regions. Figure 1.8 shows the characteristic patterns of abnormal sweating in progressive autonomic failure and multiple system atrophy with autonomic failure.

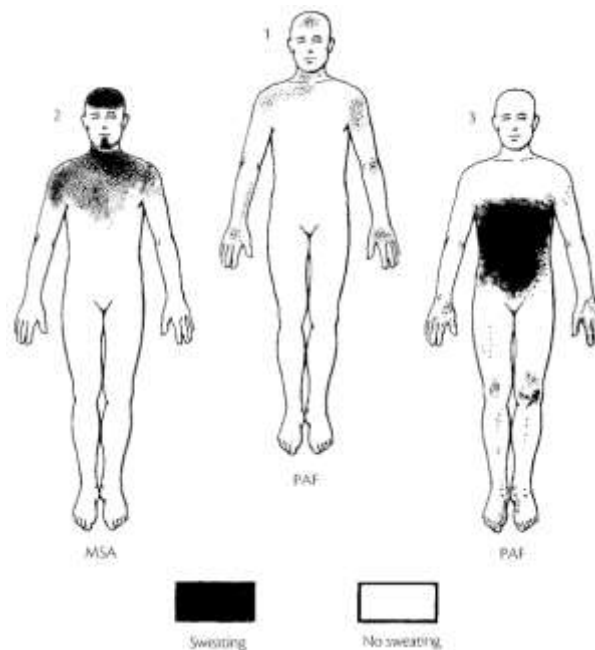


Figure 1.8: Thermoregulatory sweat test results

From Cohen J et al. Somatic and Autonomic Function in Progressive Autonomic Failure and Multiple System Atrophy. 1987. *Annals of Neurology*. Reproduced with kind permission from Wiley.

However, this test is time-consuming and requires careful control of the study environment and, in particular, a specialised temperature and humidity controlled laboratory. Thus, it is not widely available. In addition, although it can localise specific areas of sudomotor dysfunction, it cannot define whether the lesion is pre or postganglionic. Additional sudomotor tests are required for this.

### Quantitative sudomotor axon reflex test

This test is used to evaluate postganglionic sympathetic cholinergic sudomotor function. It does this by means of the axon-reflex mediated sweat response over time.

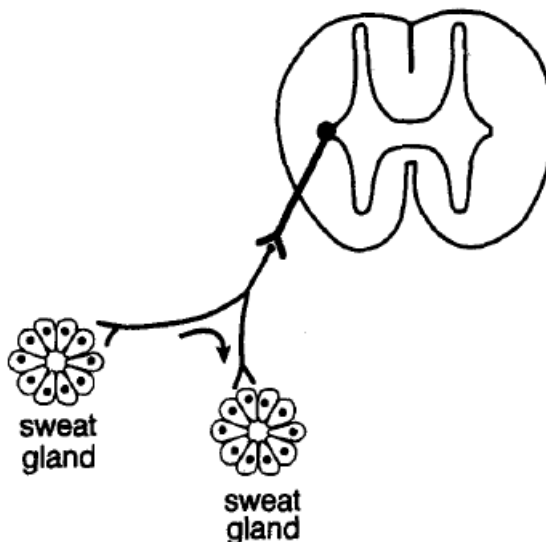


Figure 1.9: Sudomotor axon reflex pathway

From Greene et al. Sweating patterns in atopic dermatitis patients. 1989. Arch Dermatol Res 281:373-376. Reproduced with kind permission from Springer Science and Business Media

A cholinergic agent is used to directly stimulate the sweat glands via the process of iontophoresis. This leads to an increase in sweat production at a site separate to the area stimulated. This increase in sweat production leads to an increase in humidity which can be measured via a hygrometer. This test is reported to be sensitive and reproducible [325].

A limitation of this test is that it is only able to detect postganglionic lesions in the sudomotor pathway. However, by combining it with the thermoregulatory sweat test the site of lesions can be defined. In addition, as sweat volumes differ with age and gender, appropriate scoring systems are required [275]. Furthermore, it also requires specialist equipment which is not widely available.

### **Sympathetic skin response**

Sympathetic skin response (or galvanic skin response (GSR)) refers to a change in the electrical potential of the skin, due to a change in the level of sweat production, in response to a stimulus. Stimuli used include a standardised electric shock, a sudden loud noise or an inspiratory gasp. This test, which assesses a polysynaptic reflex containing a spinal, bulbar and suprabulbar component, is used to study the peripheral sympathetic system. It is only considered to be a surrogate marker of sudomotor functioning as, although the change in potential is assumed to be due to sweat gland secretion, responses are seen in patients with a congenital absence of sweat glands.

This test is relatively easy to perform and, in comparison to the other methods described, it requires minimal equipment. However, it has many limitations. Results are prone to considerable variability. The absence of a response is generally accepted as being abnormal. However, it may also be absent if stimulation is inadequate (e.g. inadequate gasp). In addition, responses are prone to habituation and are limb sensitive, with those evoked from hands generally being greater than those from feet, and are highly sensitive to any movement artefact. Also, as responses decline with age, and may be absent in healthy older adults, their absence may not be indicative of sudomotor dysfunction [326].

Sympathetic skin responses have been studied in patients with SSc. One study reported 77% to have an abnormal sympathetic skin response, with the abnormality being present most frequently in the lower limbs [327]. This agreed with the findings of an earlier study which reported abnormalities in 69% of patients (SSc, morphoea or mixed connective tissue disease with SSc-like features) [255]. However, another study only detected an abnormal sympathetic skin response in 25% [328].

## 1.14 Gastric emptying

Anatomically, the stomach is divided into the fundus, corpus and antrum. The normal functioning of each is required for coordinated gastric movement.

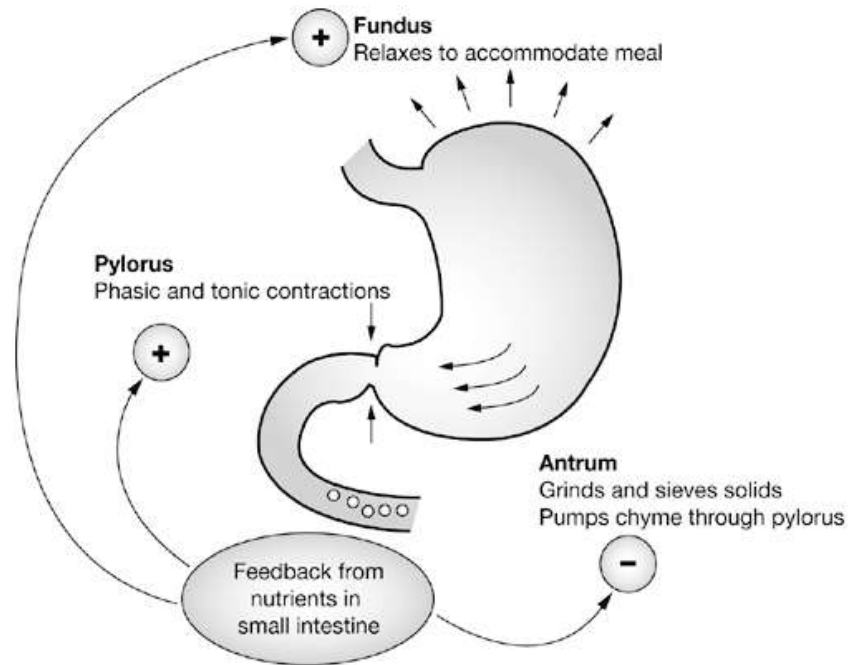


Figure 1.10: Motor events during normal gastric emptying

From Rayner et al. New management approaches for gastroparesis. *Nature Clinical Practice Gastroenterology and Hepatology*. 2005; 2 (10); 454-62. Reproduced with kind permission from Nature Publishing

The fundus acts as a reservoir. During the inter-digestive phase, it has a high muscle tone. Upon eating it relaxes to accommodate solids during the initial phases of liquid emptying [329]. Relaxation requires a reduction in smooth muscle tone, which occurs under the influence of parasympathetic stimulation and the enteric nervous system.

During the inter-digestive phase the distal stomach displays the migrating motor complex pattern of motility, which clears gastric secretions and debris. Postprandially, the antrum grinds and mixes food and liquids to produce chyme. These contractions are generated by electrical slow waves originating from the pacemaker interstitial cells of Cajal. These are independent of extrinsic autonomic nerve activity [330]. Chyme is then propelled into the small intestine, where it activates feedback mechanisms which reduce gastric emptying.

### **1.14.1 Influences of gastric emptying**

Gastric emptying rates differ between normal individuals, dependent on many factors.

#### **Individual-specific influences**

Numerous individual-specific factors may influence the speed of gastric emptying, including BMI, age, gender, blood sugar, smoking and medications. Increased gastric emptying of solids has been shown in morbidly obese ( $\text{BMI} \geq 40 \text{kg/m}^2$ ) individuals compared to non-obese ( $\text{BMI} < 30 \text{kg/m}^2$ ) individuals [331]. However, other studies have not confirmed this [332]. Males are reported to have slower gastric emptying than females which cannot be fully explained by sex hormone differences [332].

Hyperglycaemia may delay emptying even in non-diabetic individuals [333]. Prescribed and over-the counter medications may increase or decrease gastric emptying. For instance, opiate-based analgesics and anti-cholinergics may delay emptying while prokinetics may increase it. Nicotine is also reported to delay gastric emptying [334, 335].

#### **Meal-specific influences**

When comparing the results of different studies, it is important to consider the physical and chemical characteristics of the test meal. Liquid or solid meals may be used. Liquids leave the stomach more rapidly. For the assessment of gastroparesis, liquid meals have been shown to correlate well with solid meals [336]. In addition, liquid test meals identified delayed emptying in some patients, with early satiety and loss of appetite, who had normal gastric emptying with solids.

The test meal temperature influences gastric emptying. Both cold ( $4^\circ\text{C}$ ) and hot ( $50^\circ\text{C}$ ) drinks cause different patterns of antral motility to  $37^\circ\text{C}$  drinks [337]. Meal weight and composition also influence the speed of gastric emptying. Fat-rich meals empty slower than protein or carbohydrate-rich meals [338]. Larger meal volumes, which are associated with increased gastric distension, may lead to more rapid emptying [339]. Also, increasing the energy content of a fixed composition meal increases gastric emptying time [340].

### 1.14.2 Assessment of gastric emptying

A number of tests are available to assess gastric emptying (Table 1.18). Some are used primarily as research tools, while others are used in clinical practice.

Test	Gastric emptying measured via	Advantages	Disadvantages
Nuclear scintigraphy	Radioactivity remaining in stomach	Widely available Non-invasive	Radiation exposure
Breath test	Exhaled labelled carbon dioxide	Simple Radiation-free	Some isotopes affected by intestinal malabsorption
Ultrasonography	Change in antral area over time	Simple Non-invasive	Skilled operator Liquid test meals only
Magnetic resonance imaging	Gastric volume on magnetic resonance imaging	Also assesses motility and secretions	Expensive
Electrogastrography	Gastric myoelectrical activity	Non-invasive	Indirect measure of emptying
Antro-duodenal manometry	Pressure measurements	Distinguishes myopathy from neuropathy	Invasive Indirect measure of emptying Skilled operator
Wireless motility capsule	Ingestion to pH>3	Radiation-free Also assesses small bowel transit time	Large capsule Risk of capsule retention

Table 1.18: Summary of gastric emptying tests

#### Nuclear scintigraphy

Nuclear scintigraphy has become the standard method for assessing gastric emptying [338]. Following ingestion of a standardised radiolabelled test meal, the percentage retention of radiolabel in the stomach is measured. This is representative of the remaining volume of test meal. Studies last approximately 4 hours.

This test is widely available. Within centres, results are reproducible. However, differences occur between centres due to lack of standardisation. Furthermore, as it involves exposure to radiation, it is not the preferred method for healthy volunteer studies.

### **Gastric breath test**

Gastric breath tests involve the consumption of a liquid or solid test meal, labelled with a non-radioactive stable isotope. The  $^{13}\text{C}$  carbon does not dissociate in the stomach. It empties from the stomach at the same rate as the test meal and is then absorbed directly in the proximal small intestine to join the body's bicarbonate pool, before finally being exhaled as  $^{13}\text{C}$  carbon dioxide. Exhaled breath samples are collected at regular intervals. By measuring the ratio of  $^{13}\text{C}$  carbon dioxide /  $^{12}\text{C}$  carbon dioxide in exhaled breath, an indirect assessment can be made of the rate of gastric emptying.

Gastric breath tests are non-invasive and do not require ionising radiation. However, depending on the isotope used, results may be confounded by the need for digestion in patients with malabsorption. In addition, not all absorbed  $^{13}\text{C}$  carbon is ultimately exhaled. The model on which gastric emptying studies are based fails to account for other losses, including retention in the bicarbonate pool in organs and metabolic losses [341]. Studies suggest that up to 50% of the labelled  $^{13}\text{C}$  carbon is never exhaled [342].

### **Ultrasonography**

Trans-abdominal ultrasound scans, following a test meal, may be used to assess gastric emptying via serial assessments of the cross-sectional antral area. This is generally regarded as a research tool. It requires considerable operator skill and is only validated for liquid test meals.

### **Magnetic resonance imaging**

Magnetic resonance imaging may be used to assess gastric emptying using liquid test meals. It involves serial scans to assess gastric volume. It has the advantage of not requiring ionising radiation. However, it is time-consuming and very expensive to



perform. Also, gastric volumes may be confounded by gastric secretions. Thus, it is predominantly a research tool.

### **Electrogastrography**

This uses electrodes, placed over the anterior abdominal wall overlying the stomach, to measure gastric myoelectrical activity (slow waves). Abnormal patterns of slow wave activity are detected in patients with gastroparesis. However, it is not possible to directly assess gastric emptying rates.

### **Antro-duodenal manometry**

This uses an endo-luminal catheter or pressure transducer to measure gastric and duodenal contractions in the fed and fasted state. A disturbance in the relationship between antral, pyloric and duodenal contractions is seen in patients with gastroparesis. This test cannot provide a direct measure of the rate of gastric emptying, but can distinguish between myopathic and neuropathic causes of gastroparesis..

### **Wireless motility capsule**

This is an orally ingested, non-digestible, single use recording device. By measuring pH, pressure and temperature it is able to assess gastric, small bowel and colonic transit times. Gastric emptying time is measured from ingestion to a detectable pH>3. It has the advantages of not exposing the subject to radiation and can differentiate gastric transit from intestinal and colonic. However, capsules are large rendering them difficult to swallow. Risks include capsule retention if there is an unsuspected stenosing lesion.

### **1.14.3 Gastroparesis**

Gastroparesis a chronic motility disorder. It results in a notable delay in the emptying of liquids and solids from the stomach in the absence of any mechanical obstruction [343]. The underlying pathophysiology is poorly defined, but is thought to involve disturbances of the ANS, enteric nervous system and/or gastric smooth muscle cells.

Gastroparesis is estimated to affect 1.8% of free-living individuals [344]. However, less than 0.02% are diagnosed. Gastroparesis may develop as a consequence of many different disorders, including post-surgical, Parkinson's disease and SSc. However, the commonest is diabetes mellitus. Symptoms are often non-specific, but commonly include nausea, vomiting, bloating, abdominal pain, postprandial fullness and early satiety [345].

#### **1.14.4 Gastroparesis in patients with systemic sclerosis**

Generalised fibrosis is noted throughout all layers of the gastric wall of patients with SSc [15]. In addition, changes to the structure of the elastic and collagen tissues are present in the tissue surrounding the gastric nerve fibres, including the pacemaker interstitial cells of Cajal. In patients with diabetic gastroparesis, the loss of enteric nerves fibres and cells is linked to delayed gastric emptying [346].

Gastric involvement is common in patients with SSc. Multiple studies have sought to estimate the prevalence of delayed emptying using a variety of methods, some of which are summarised. As a consequence of the different methods used and patient groups studied, the prevalence of disordered gastric motility ranges from 38% to 82% [50, 347].

A recent retrospective study reported 38% of patients to have evidence of delayed gastric emptying. This study included a total of 99 patients, 45 of whom had had a <sup>13</sup>C octanoic breath test with a solid test meal, which showed delayed emptying in 38 patients (i.e. 38% of all patients, 84% of those who had had a gastric emptying study) [50]. This 38% is comparable to another recent prospective study, which also used <sup>13</sup>C octanoic breath test with a solid test meal (n=57), and reported delayed gastric emptying in 47% of patients with SSc [51]. In addition, it showed a significant correlation between selected GI symptoms (vomiting, postprandial bloating and abdominal tenderness, but not nausea) and delayed emptying when using the validated GI Global Symptoms Score Questionnaire.

Not all studies in patients with SSc have used breath testing. A recent study, which used ultrasonography within the first hour of ingestion of a liquid bolus, showed abnormal gastric emptying in 65-70% (n=20) [348]. Another study, which used a similar method, reported abnormal gastric emptying in 50% of all (n=38) patients with SSc and in 65% of patients an abnormal dyspeptic score [52]. In addition, an electrogastronomy study, involving 22 consecutive patients with SSc, reported disturbed gastric electrical activity in

81% [347]. However, this disturbance did not necessarily translate to gastric dysmotility as only 50% of patients had delayed emptying of pellets.

## **1.15 Regulation of food intake**

Regulation of food intake relies on the balance of numerous controlling sensations, which are generated via multiple complex brain-gut interactions. Sensations include hunger, satiation and satiety. Hunger is a physiological signal for the need to consume food. Satiation is the opposing signal, representing the disappearance of hunger as a consequence of eating. Meanwhile, satiety is the sensation of satisfaction which is present after eating. It suppresses hunger. The subsequent reduction in satiety leads to the re-emergence of hunger. These signals influence appetite, which is the desire to eat. However, appetite is also influenced by various non-physiological, learned and environmental factors.

### **1.15.1 Central mechanisms involved in the regulation of food intake**

Within the central nervous system, the hypothalamus has a pivotal role in the control of appetite. It receives information about food intake via hormonal (GI peptides), neural and nutrient signals and transmits an integrated assessment to the higher cortical centres for processing. These centres also take into account additional environmental and learned information to control food intake. Learned behaviours and environmental factors which promote overeating include the desirability of certain food (palatability and reward), other environmental factors (portion size, food availability) and emotional cues (e.g. mood). Thus, in response to these over-riding higher cortical influences, eating may continue in the absence of physiological need.

### **1.15.2 Gastrointestinal peptides involved in the regulation of food intake**

In response to eating, the stomach relaxes and distends. Distension, even in the absence of nutrients, leads to fullness and satiation [349]. Gastric distension, detected via specialised mechanoreceptors, leads to enteric and ANS activation which sends signals to higher centres. In addition, nutrient intake leads to release of satiety inducing GI peptides,

including cholecystikinin and glucagon-like peptide 1. Cholecystikinin, which is released by the cells of duodenum and jejunum in response to lipid and protein ingestion, delays gastric emptying and increases satiety via receptors on the vagal afferents, brainstem and hypothalamus [350, 351]. In addition, glucagon-like peptide-1 which is released from the ileum after meal ingestion acts as an ileal brake to reduce gastric emptying and acts centrally to reduce hunger and to increase perceptions of fullness.

In contrast, ghrelin which is released from the gastric fundus is a hunger stimulating peptide [351, 352]. Ghrelin plasma concentrations increase prior to the ingestion of a meal. Ghrelin acts via the hypothalamus to induce the sensation of hunger, thus promoting eating. Following the ingestion of food, plasma levels fall in relation to the number of calories consumed.

### **1.15.3 Assessment of appetite sensations**

Subjective sensations, such as those involved in appetite research, are difficult for individuals to quantify objectively. Visual analogue scales are often used to try to quantify them in order to allow comparison between different individuals and test meals. One such series of VASs has been validated for the quantification of hunger, satiety, fullness and prospective food consumption [353]. This validation study showed appetite scores to be sufficiently reproducible to allow their use in appetite research. However, comparisons within-subjects were noted to be more sensitive than those between-subjects.

### **1.15.4 Appetite in patients with systemic sclerosis**

Surprisingly, very few studies have investigated appetite in patients with SSc. One study (n=38) which included postprandial VAS assessments of appetite, satiety, nausea, fullness and epigastric pain, found no difference in appetite and satiety between patients with SSc and either healthy or dyspeptic controls [52]. However, the patients with SSc did report greater fullness than healthy controls.

GI peptides may influence gastric motility and appetite. Thus, aberrant peptide responses may disrupt GI motility and reduce appetite. A few small studies report differences in the concentrations of certain GI peptides between patients with SSc and healthy controls.

Motilin which increases intestinal motility is reported to be increased in patients with SSc (n=43) compared to healthy controls [354]. However, tissue motilin concentrations appear reduced in patients with oesophageal and intestinal dysmotility (n=6) compared to those with oesophageal dysmotility alone (n=6) [355]. This suggests that motilin may be involved in SSc-related intestinal dysmotility, but larger studies are needed.

Few studies have investigated cholecystokinin in patients with SSc. One study (n=25) reported higher, fasting and postprandial, plasma cholecystokinin concentrations in patients with dcSSc than healthy controls [356]. However, another study (n=10) comparing patients with SSc and intestinal involvement (80% with pseudo-obstruction) to healthy controls did not demonstrate any significant difference in motility regulating peptide levels (including cholecystokinin) [357]. Thus, further studies are needed to clarify whether altered peptide responses are present and, if present, whether they produce or are as a result of dysmotility and whether they affect appetite.

## **1.16 Summary**

Despite nutritional and GI problems being common in patients with SSc, as highlighted in this review of the literature there are still many unknowns, some of which this thesis will endeavour to address.

With regard to those patients with IF, secondary to severe GI involvement, who are commenced on HPN, little is known about their long term nutritional outcome or their survival on HPN in comparison to patients without SSc. This is especially relevant given the recent advances in both HPN and in the management of other non-GI SSc related manifestations, which may have otherwise led to the demise of patients with SSc before GI manifestations progressed to IF.

Furthermore, despite several studies to-date showing malnutrition to be common in patients with SSc, none have sought to compare different, potentially clinically applicable assessment methods which, if proven useful, could be incorporated into routine clinical practice to facilitate the earlier detection of patients developing nutritional compromise. Rather, some studies have focused on BIA, which may have limitations, without comparing it to other body composition measures or simple screening tools for the detection of malnutrition. In addition, few nutritional studies have sought to identify

clinical manifestations which predict nutritional decline. If these could be identified, then their development in patients with SSc, might offer clinicians the opportunity for early intervention.

To-date, several studies have analysed the diet of patients with SSc with the intention of detecting energy and/or nutrient deficiencies associated with either malnutrition or clinical manifestations. However, despite one study showing reduced physical activity in patients with SSc, which is greater in some than others, none as yet have sought to compare actual energy expenditures to intakes or predicted requirements. Should the measurement of energy expenditure prove beneficial for assessing actual requirements, then it might assist in the clinical nutritional assessment and management of patients.

Finally, there still remains much which is unknown about the underlying pathophysiology of GI involvement in patients with SSc. Histological studies have shown pathological changes involving the nerves within the walls of the GI tract, including in the stomach. These nerves have a role in GI motility, which is disturbed in patients with SSc. Links have been shown between cardiovascular autonomic dysfunction and altered oesophageal motility. However, despite this known gastric wall involvement, similar studies have not detected links between cardiovascular autonomic dysfunction and gastric dysmotility. A better understanding of the basis of gastric dysmotility may assist in the development of future intervention.

Though this thesis cannot hope to address all of the unknowns with regard to nutritional and GI problems in patients with SSc, it can hope to make some advance on the current knowledge. With this goal in mind, this thesis puts forward the following hypotheses and aims.

### **1.17 Hypotheses**

The key hypotheses to be addressed by this thesis include:

- HPN in patients with SSc improves nutritional status and outcomes
- Clinical measures of nutritional status made using different assessment methods will concur

- Nutritional status and decline are linked to SSc-related manifestations and symptoms
- Active energy expenditure falls with worsening disease
- Predicted energy requirements are a poor estimate of expenditure
- GI symptoms are associated with gastric emptying
- Cardiovascular autonomic dysfunction is associated with GI symptoms and/or gastric emptying

### **1.18 Aims**

In order to address the above listed hypotheses, this thesis aims to:

- Review the outcome data of all patients with SSc commenced HPN and compare survival to that of patients without SSc
- To compare the results of individuals' measures of nutritional status using different assessment modalities
- To seek associations between nutritional status and decline and SSc-related manifestations and symptoms
- To seek associations between total/active energy expenditures and disease or nutritional status.
- To compare measures of energy intakes and requirements
- To seek any associations between GI symptoms and rate of gastric emptying
- To look for any associations between cardiovascular autonomic dysfunction and GI symptoms and/or gastric emptying

**CHAPTER 2:**

**RETROSPECTIVE REVIEW OF**

**PATIENTS ON HOME PARENTERAL**

**NUTRITION**



## **2 Retrospective review of patients on home parenteral nutrition**

### **2.1 Introduction**

For patients with malabsorption as a result of small intestinal involvement, HPN may be a viable option for maintaining their nutritional status. However, HPN is a complex intervention with significant associated risks and data evaluating the long-term outcome of HPN in the SSc population are sparse. Indeed, to-date, only 4 papers describe the short term outcomes of series of patients with SSc requiring HPN (8, 12, 15 and 5 patients followed for a median of 40, 16, 30 and 12 months respectively) [69, 227, 229, 358]. A fifth paper describes nutritional support in 5 patients with SSc (4 on HPN) [225]. Other evidence for HPN use derives from case studies of 1 to 2 patients [226, 230, 231]. Thus, little is actually known about the long-term outcome of patients with SSc on HPN. Furthermore, there are minimal data describing the complications associated with HPN provision in patients with SSc versus other groups of patients with IF.

### **2.2 Hypothesis**

Patients with SSc are hypothesised to have extensive intestinal involvement. Provision of HPN is hypothesised to be as safe in patients with HPN as in patients without SSc. HPN is hypothesised to improve nutritional status.

### **2.3 Aim**

We aimed to review the disease characteristics and survival and outcome data of all patients with SSc, who commenced HPN, at a national U.K. IF referral centre, over a 22 year period.

### **2.4 Ethical approval**

All surviving patients gave their consent for this review of their records (North West Ethics Committee 12/NW/0247).

## **2.5 Materials and methods**

This study was conducted at a centre which houses both a tertiary rheumatology referral centre for patients with SSc and a National IF Unit. The details of all patients requiring HPN are stored on a prospectively-maintained database. This database was used to identify all those patients with SSc who received HPN between May 1990 and October 2012. All available records were reviewed for each patient. Any information pertaining to demographics, SSc characteristics, GI manifestations and HPN characteristics (including survival, complications and management) were recorded. The functional ability of patients with SSc was assessed using a validated 11 item self-reporting questionnaire [107]. Results of this questionnaire from within 2 years of HPN initiation were also noted.

Survival data for all other patients on HPN for over 3 months, at the same national IF unit, were available for comparison to the SSc cohort.

### **2.5.1 Statistical analysis**

The difference between the mean SSc functional statuses, recorded within 2 years of HPN initiation, of patients who were trained to care for their own catheter and those who were not trained, was assessed using an unpaired Student's t test, with a significant difference accepted as a p value of  $\leq 0.05$ . The difference between the mean times from SSc diagnosis to HPN initiation of patients with different disease sub-types was assessed using an unpaired Student's t test, with a significant difference accepted as a p value of  $\leq 0.05$ .

The cumulative probability of survival for all patients with SSc was calculated using the Kaplan-Meier method (SPSS version 20), censoring patients upon discontinuation of HPN (weaning) or HPN continuance at the end of follow up.

Survival data were available for all other patients, at the same national IF unit, who had survived on HPN for more than 3 months. Data were unavailable for those patients who had received HPN for less than 3 months. Therefore, for the comparison of survival on HPN between the patients with and without SSc, any patients with SSc, surviving on HPN for less than 3 months, were excluded. All patients, who had received HPN for more than 3 months, were censored upon discontinuation of HPN (adaptation, surgical intervention or transplantation), loss to follow-up or HPN continuance at the end of follow up. A log rank test was performed in order to identify whether survival differed significantly

between the groups (patients with and without SSc). In addition, three Cox regression analyses were performed to compare survival between the groups after adjusting for differences in age and HPN start date. Fitting the models in a sequence is informative as it shows how the hazard ratio for the patient group changes after introducing each potential confounder, thereby highlighting possible explanations for any apparent differences in survival other than the underlying disease. The first Cox model included only the patient group (with or without SSc), giving an estimate of the hazard of death for patients with SSc compared to patients without SSc, unadjusted for other potential confounders. The second model extended this by introducing age, giving a hazard ratio for patients with SSc compared to those without, after taking age differences into account. The final model extended this further with the addition of HPN start date; HPN start date was coded as an integer, with the first year in the dataset [1978] set to zero and unit increases for each subsequent year. This adjustment for HPN start date was made in order to adjust for any underlying trend resulting from changes to the treatment over time.

Using the same data sets, a log rank test sub-analysis was conducted to determine whether survival significantly differed between the patients with SSc-related dysmotility and the patients with non-SSc related dysmotility. In addition, two Cox regression analyses (unadjusted and age-adjusted) were performed to estimate the hazard of death for patients with SSc compared to patients with non-SSc related dysmotility.

## **2.6 Results**

### **2.6.1 Demographics of patients with systemic sclerosis**

Twenty-five patients (5 (20%) male) with SSc commenced HPN during the 22 year period (1990-2012) and were managed on HPN over 37,200 intravenous central venous catheter (CVC) days. Early data from 7 of these patients were included in an earlier series publication [69]. The median age at HPN commencement was 55 years (range 24 to 76). HPN use has increased over time. During the periods 1990-1994, 1995-1999, 2000-2004, 2005-2009 and 2010-2012, the number of SSc patients commencing HPN was 2, 3, 6, 9 and 5 respectively.

### 2.6.2 Systemic sclerosis characteristics

Nineteen of the 25 patients (76%) had lcSSc and 6 (24%) had dcSSc [5]. Ten (40%) patients were anti-centromere positive and 4 (16%) were anti-topoisomerase positive, while 10 (40%) were anti-centromere and anti-topoisomerase negative. The autoantibody status was unavailable for one patient.

Thirteen (52%) patients had evidence of digital pitting, reflecting the severity of digital vasculopathy. Six patients were recorded as having had a digital amputation either before or after starting HPN. Ten patients had cardiac disease.

### 2.6.3 Time to commencement of home parenteral nutrition

The median interval from the onset of SSc, as defined by the patient's recall of the date of their first non-Raynaud's clinical feature, to HPN commencement was 102 months (range 14 to 389; n=23). There was no evidence of any difference between disease sub-types for mean interval to HPN commencement (dcSSc 101±77 months); lcSSc 116±100 months, p=0.74).

### 2.6.4 Gastrointestinal disease characteristics

All patients underwent multiple GI investigations, prior to, and following, commencement of HPN. All patients had evidence of small intestinal involvement. The proportions of patients with dysfunction involving the different parts of the GI tract are shown in Table 2.1.

Diagnosis	Number of patients (%)
Oesophageal dysfunction	24 (96)
Gastric dysfunction	14 (56)
Small bowel dysfunction	25 (100)
Colonic dysfunction	5 (20)

Table 2.1: Distribution of GI tract dysfunction

In addition, 4 (16%) patients had an oesophageal stricture and 3 of these patients required dilation of their stricture. Twenty (80%) patients had proven (SIBO, while there was evidence of at least one episode of pseudo-obstruction in 11 (44%) patients.

Eleven (44%) patients underwent at least one abdominal operation (median 2; range 1 to 4; all unrelated to enteral tube placement); an additional 2 patients required one or more surgical procedures as a consequence of enteral feeding tube placement. The main indication for the initial surgical procedure was abdominal pain or obstruction. Seven patients had one or more operation involving an intestinal resection; this included 4 small bowel resections (4 patients), 6 large bowel resections (4 patients) and one appendiceal resection; two patients had both small and large bowel resections. One patient underwent an elective colectomy for severe disease-related constipation. Pathological abnormalities noted included intestinal ischaemia (3 patients; 2 large and 1 small intestine) and small intestinal pneumatosis (1 patient). The majority of patients who underwent an intestinal resection had their first resection after being diagnosed with SSc (4/7), but before commencing HPN (7/7).

#### **2.6.5 Enteral nutrition prior to home parenteral nutrition**

Nine (36%) patients commenced HPN directly, primarily because of the severe nature of their small intestinal involvement. In the remaining 16 (64%) patients, enteral feeding was attempted prior to HPN. In 10 of these patients, a naso-enteral feeding trial was conducted, which 6 (60%) patients failed to tolerate (naso-gastric (n=2); naso-jejunal (n=4)). The remaining 4 patients who tolerated a naso-enteric feeding trial, together with another 6 patients, for whom no evidence could be found of a naso-enteric trial, proceeded to per-stomal feeding. Of these 10 patients receiving per-stomal feeding, 2 initially had a gastrostomy and 8 had a gastrostomy with jejunal extension.

However, per-stomal feeding was not without problems; 6 patients required a revision of their appliance and one patient developed volvulus at her gastrostomy with jejunal extension site. Of the 10 patients in whom gastric or jejunal feeding was attempted, 7 fed for less than 1 year, 3 of whom opted to proceed to HPN within one month. Two patients fed for >4 years, and in one the duration of per-stomal feeding was unknown. All patients, including those who tolerated per-stomal feeding for >4 years, reported worsening small

intestinal symptoms on feeding; these included nausea (n=3), vomiting (n=5) and bloating and/or abdominal pain on feeding which limited the rate of feeding (n=7).

### **2.6.6 Central venous catheter management**

All patients used a single lumen tunnelled CVC. Nine (36%) patients were fully trained to manage their CVC and self-administer HPN. However, a third of these ultimately needed assistance after a median of 9 months (7 to 145), as their overall condition deteriorated. The remaining 16 (64%) could not be trained due to lack of manual dexterity. Of these, 11 relied on nursing care, while 5 relied on their family (1 later converted to nursing support). None of the patients who were trained to manage their CVC were recorded as having had a finger amputation, either before or after starting HPN. SSc functional scores within 2 years (median 1.4 months; range minus 6 to 22) of HPN initiation were recorded for 13 patients [107]. Using this score, untrained patients had significantly worse baseline functional status (mean  $8.8 \pm 5.9$  versus  $19.6 \pm 9.0$ ;  $p=0.04$ ).

### **2.6.7 Effect of home parenteral nutrition**

On starting HPN, patients' median BMI was  $17.9 \text{ kg/m}^2$  (range 15.3 to 25.6; n=21). Initial and 12 month repeat BMIs were available for 13 patients; for these, the median BMI increased from  $18.5 \text{ kg/m}^2$  to  $21.3 \text{ kg/m}^2$ . The median initial BMI for those who died within the first year of receiving HPN was  $17.0 \text{ kg/m}^2$  (n=5; range 15.8 to 18.2).

### **2.6.8 Patients weaned from home parenteral nutrition**

Two patients returned to oral feeding. One patient had a history of severe constipation, depression and pharyngeal problems. Despite previously experiencing small bowel symptoms on enteral tube feeding, he returned to oral feeding after almost 8 months HPN, helped by improvement in constipation related-symptoms and psychological well-being; he remained PN-free for over 8 years. The other patient returned to oral feeding after 29 months; she had small bowel dysmotility and bacterial overgrowth, but was able to tolerate enteral feeding following intensive management of her bacterial overgrowth. She remains PN-free 7 years after returning to enteral feeding.

### **2.6.9 Parenteral nutrition regime**

All but one patient infused their HPN overnight. The median maintenance volume of HPN infused was 1600ml (range 1000 to 3000ml). Four patients with uncontrolled diarrhoea (SIBO-related) required 3000ml infusion volumes (later reduced in one patient).

### **2.6.10 Complications on home parenteral nutrition**

Seven episodes of CRBSI occurred in 5 patients, which equates to a rate of 0.19 episodes/1,000 CVC days. Five (71%) of the CRBSIs occurred >10 years ago. Of the 4 patients who experienced one CRBSI episode, 3 were managing their own CVC at that time. One patient, whose CVC was managed by nurses, experienced 3 CRBSIs. Causative organisms were identified in 6 episodes (5 coagulase negative Staphylococci; 1 gram negative Staphylococcus).

Four patients developed a central venous thrombosis (2 subclavian; 1 brachiocephalic; 1 superior vena cava) while on HPN, which equates to a rate of 0.11 episodes/1,000 CVC days in the whole SSc HPN cohort.

There were 26 episodes of non-thrombotic CVC occlusion in 12 patients, which equates to a rate of 0.70 episodes/1,000 CVC days. In 10 (38%) of these cases, the occlusion was managed using CVC clearing techniques, while in 16 (62%) a replacement CVC was required.

Six CVCs, in 3 patients, were reported to have been damaged to the extent of requiring repair or replacement. Two of these patients had HPN for >10 years. The third patient had HPN for >5 years. As described in our centre's earlier report, 2 patients developed significant calcification surrounding their tunnelled CVC, which hindered line removal [69]. One patient had a tendency to severe calcinosis and this, her third CVC, had been in-situ for 1 year. The CVC of the second patient, who had no clinical calcinosis, had been in-situ for 17 years. It was so severely calcified that it could not be removed. No patients were recorded as having IF-associated liver disease.

### **2.6.11 Survival on home parenteral nutrition**

Of the 25 patients, 8 were alive at the end of the study period. Fifteen patients died whilst still receiving HPN. One patient died 4.6 months after suspending HPN due the severity of her underlying SSc-related cardiac failure, as recommended by the attending cardiologist. Another patient died 100 months after resuming enteral nutrition.

Of the 17 deaths, 16 were attributed to SSc-related complications. In most cases, this related to cardiac or respiratory involvement. One patient died of an un-related cancer. No deaths were related to HPN. Seven of the 8 patients alive at the end of follow-up still required HPN. The median time for which these patients had received HPN was 40 months (range 9 to 178).

Using the Kaplan-Meier method, the cumulative probability of patients surviving on HPN was calculated for all patients with SSc. For this analysis, patients were censored upon weaning from HPN or termination of follow-up. Using this method, the cumulative probabilities of surviving on HPN at 2, 5 and 10 years were 75%, 37% and 23% respectively.

### **Comparison between of patients with and without systemic sclerosis**

Survival data were available for 507 patients without SSc who received HPN for >3 months (1978 to 2011). This group had a median age of 48 years (range 12-86) at the start of HPN. Patients had a variety of underlying diagnoses, with the most common being Crohn's Disease (31%), surgical complications (23%), mesenteric infarction (15%) and non-SSc related dysmotility (8%).

Figure 2.1 shows the unadjusted cumulative survival on HPN for all patients with and without SSc who survived more than 3 months on HPN. Log rank analysis showed a significant difference between the survival distributions of the two groups on HPN ( $p=0.02$ ). The first Cox regression gave an unadjusted hazard ratio (95% CI) for survival for the patients with SSc compared to those without of 1.86 (95% CI 1.08 to 3.22;  $p=0.03$ ), suggesting that the risk of death was higher for patients with SSc. After including age however, the hazard ratio (95% CI) for the patients with SSc was 1.48 (0.86 to 2.58;  $p=0.16$ ), indicating that the apparent difference in survival was at least partially attributable to differences in age between the patients with and without SSc. After



extending the model further to adjust for start date, the hazard ratio (95% CI) for the patients with SSc was 1.42 (0.82 to 2.48;  $p=0.21$ ), consistent with the previous estimate.

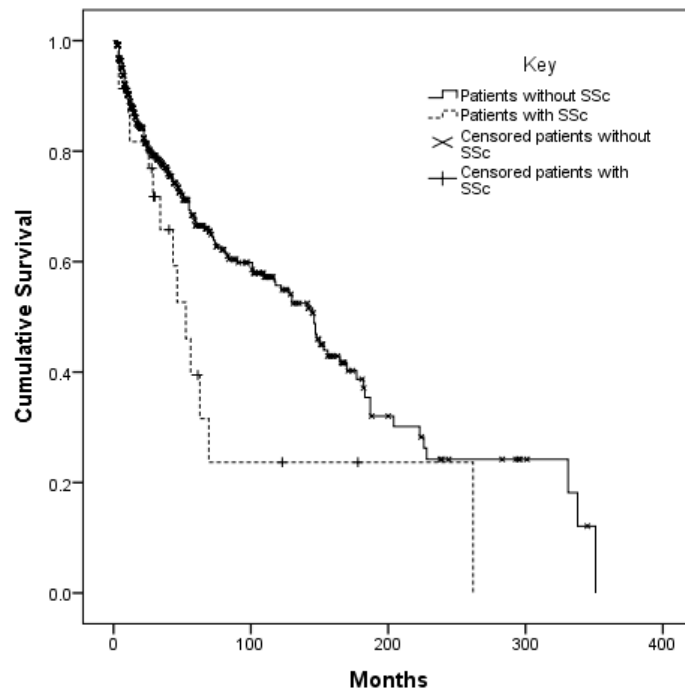


Figure 2.1: Kaplan-Meier cumulative survival on HPN

Log rank analysis showed a significant difference between the survival distributions of patients with SSc-related dysmotility and patients with non-SSc related dysmotility ( $p=0.04$ ). Using Cox regression, the unadjusted hazard ratio (95% CI) for the patients with SSc-related dysmotility compared to those with non-SSc related dysmotility was 3.79 (1.44 to 10.03;  $p=0.07$ ). After including age, however, the hazard ratio (95% CI) for the patients with SSc was 2.34 (0.85 to 6.427;  $p=0.10$ ), suggesting that part of this increased hazard is attributable to age.

## 2.7 Discussion

This study is the largest and most detailed series so far reported describing the use and experience of HPN in patients with SSc, and also the cohort followed over the longest period of time. The data highlight the difficulties associated with enteral feeding in those with severe SSc-related GI involvement but demonstrate that HPN is a relatively safe and effective feeding modality in this complex group of patients.

### **2.7.1 Gastrointestinal involvement**

Patients in this series had IF secondary to severe, widespread GI involvement, with all patients demonstrating small intestinal involvement. Small intestinal bacterial overgrowth, which has been reported to affect 40 to 50% of unselected patients with SSc, was proven in 80% of patients in this series [61, 62]. Similarly, chronic intestinal pseudo-obstruction has been reported to affect approximately 3.9% of unselected patients with SSc, whereas 44% of the patients included in this series had experienced at least one episode of pseudo-obstruction [117]. These data highlight the severity of GI involvement in this series of patients requiring HPN.

### **2.7.2 Mode of nutritional support**

When a patient with severe GI disease requires nutritional support, the modality of that support must be carefully considered. The optimal mode of artificial nutritional support in any patient with a functional GI tract is enteral nutrition. However, as demonstrated in this patient cohort, the efficacy and tolerance of enteral nutrition may be limited in patients with severe SSc-related small bowel involvement [359]. While this may reflect the severity of GI involvement seen in patients in this tertiary cohort of patients, other case series also report a high rate of progression from enteral to parenteral nutrition in patients with SSc-related small bowel dysmotility [226, 360]. Indeed, published reports of successful outcomes with enteral nutrition in patients SSc largely relate to patients without small bowel involvement [223-225]. Thus, early consideration should be given to PN in malnourished patients who have clinical evidence of severe small bowel disease.

### **2.7.3 Complications**

In this series, the use of HPN in patients with SSc has increased over time, mirroring the increase in HPN usage in non-SSc groups [361, 362]. However, HPN use is not without risk of complications. Repeated CVC complications may lead to the loss of vascular access and associated failure of HPN; this, in itself, can be an indication for small bowel transplantation, which may not be an ideal option for patients with multi-system diseases such as SSc [363]. However, rates of CRBSIs in this cohort of patients with SSc was remarkably low at 0.19 CVC infections/1000 catheter days, when compared to other patients at our centre (overall CVC infection rate of 0.39/1000 catheter days in all HPN

patients over this time period) [364]. Furthermore, while we are unable to provide the CVC-related thrombosis rate for our entire cohort of patients on HPN, the CVC-related thrombosis rate for this cohort of patients with SSc was comparable to that of other published HPN series [365, 366].

#### **2.7.4 Administration of nutrition**

Although self-administration of HPN is encouraged to promote patient autonomy, relatively few patients with SSc were able to manage this, largely as a result of hand deformity and/or functional impairment. Indeed, all patients with a digital amputation relied on others. Similarly, patients unable to be trained had a significantly worse SSc-specific functional ability index score, suggesting that such scores may be a useful guide to a patient's ability to self-administer HPN. Further on-going work at our centre into patient reported outcome measures for individuals with IF, will help evaluate any detriment that dependence on others to administer their HPN will have on associated long term quality of life.

#### **2.7.5 Survival**

Severe SSc-related GI involvement is defined by malabsorption, repeated episodes of pseudo-obstruction or HPN dependence [124]. Severe GI involvement affects approximately 5% (95% CI 3 to 6) of all patients with SSc [117]. In patients with dcSSc, severe GI involvement affects 4% of patients by 3 years and 8% by 9 years, and within 3 years of the diagnosis of severe GI involvement most patients die [367]. Approximately 3-4% of all patients die from GI-related causes, including malnutrition [201, 368]. Thus, severe GI involvement is not uncommon, and can result in death. In these patients, the use of HPN may offer some benefit. In our series of patients with severe GI involvement who were unable to meet their nutritional requirements enterally, we identified 2, 5 and 10-year cumulative survivals of 75%, 37% and 23% respectively. Although few patients in our series survived long-term, many did survive for more than 2 years on HPN. Deaths were not related to HPN but instead to other SSc-related manifestations. Without a direct comparison to patients with SSc and severe GI involvement, who did not receive HPN, it is not possible to conclude whether patients receiving HPN would have died earlier without nutritional support, but this seems highly likely.

Patients with SSc who survived for more than 3 months on HPN, had an increased likelihood of death compared to patients who required HPN but had diagnoses other than SSc [364]. However, the hazard ratio had a very wide CI, most likely due to the small number of patients with SSc studied. The validity of the comparison was also influenced by the heterogeneous nature of the group without SSc. Only 8% had IF secondary to dysmotility (unrelated to SSc), introducing the effects of differing disease courses. The survival of patients with SSc-related dysmotility differed from those with non-SSc related dysmotility but the hazard ratio had a very wide confidence interval which, after adjusting for age, crossed zero. This probably likely reflects the relatively small number of cases. Furthermore, patients with SSc had a higher median age. Predictably, increasing age at HPN initiation was associated with decreased survival. When age and HPN start date were taken into account, the certainty about the increased risk of death for patients with SSc was lost, as the hazard ratio was attenuated and the 95% CI spanned one. Our series supports the concept that HPN may offer patients with SSc and severe GI involvement a chance of long-term survival.

### **2.7.6 Limitations**

The main limitation to this study is its retrospective nature. Nonetheless, it remains the largest and longest study of HPN use in patients with SSc. It shows that patients with SSc can survive on HPN, but that their long-term survival on HPN may be worse than that seen in patients without SSc, due to concomitant and progressive multisystem disease. A larger, multi-centred, study may be needed to confirm this.

### **2.7.7 Conclusions**

In summary, our data demonstrate HPN to be an effective option for patients with SSc-related IF. It is highly likely that survival is significantly prolonged on HPN, with few complications. Clinicians who care for patients with SSc should not be deterred from referring these complex and difficult cases for expert assessment and intensive management.

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**CHAPTER 3:**  
**PROSPECTIVE STUDY OF**  
**NUTRITIONAL STATUS**

### **3 Prospective study of nutritional status**

#### **3.1 Introduction**

Malnutrition is reported to be common in patients with SSc, with one study finding approximately 28% of patients to be at medium or high risk of malnutrition, based on nutritional screening [36]. However, other studies, using a range of definitions have reported different values. In addition, few studies have assessed body composition in patients with SSc, and none of them used more than one method for comparison. Methods of body composition assessment used in patients with SSc include dual energy absorptiometry, BIA and MUAA [47, 204, 209, 211, 370]. Furthermore, to-date, no study involving patients with SSc has investigated changes in body composition over time by performing repeated measures in the same patients.

Despite malnutrition affecting a significant number of patients with SSc, few studies have identified any clear contributors. In the largest study by Baron et al., risk of malnutrition ('MUST') was associated with symptoms of poor appetite, early satiety, nausea, constipation and diarrhoea and with physician-assessed possible malabsorption [36]. However, this study included no formal assessments of malnutrition or severity of GI involvement or symptoms.

A subsequent, much smaller study by Murtaugh et al. did include an assessment of the severity of GI symptoms (UCLA questionnaire), but did not include any assessment of malnutrition other than 'MUST' [205]. It found no evidence of any correlations between 'MUST' and total UCLA scores, or any between 'MUST' and any of the UCLA domain sub-scores. However, patients at medium and high risk of malnutrition (n=9) were noted to have GI symptoms that were at least 'somewhat worse' than patients with no nutritional compromise (n=15) [88].

Another study, which also assessed systemic features, showed no significant relationships between malnutrition (BMI<20kg/m<sup>2</sup> and/or spontaneous weight loss of ≥10%) and functional disability (HAQ), disease duration, CRP, subset or presence of organ involvement (lung or GI) [48]. However, it did detect associations between malnutrition and anorexia, early satiation, heartburn, regurgitation and disease activity (Valentini criteria) [208]. This study did not quantify symptom severity.

Thus, the current study was conducted to try to clarify some of the points raised by these studies, relating to contributors to the development of malnutrition and whether there is an association between nutritional decline and GI involvement. It includes different methods of body composition assessment and assessment of both the severity of organ involvement and of patient-reported symptoms.

### **3.2 Hypotheses**

The first hypothesis was that, in patients with SSc, clinical measures of nutritional status would concur.

The second hypothesis was that patients' nutritional status would be linked to the nature and severity of their SSc-related manifestations and symptoms.

The third hypothesis was that deteriorating nutritional status would be experienced by a number of patients with SSc.

The fourth hypothesis was that this nutritional decline would be linked to GI and other SSc-related organ involvement.

### **3.3 Aims**

The first aim was to compare the results of individual patient's measures of nutritional status to determine their agreement.

The second aim was to identify any associations between nutritional status and SSc-related manifestations and symptoms.

The third aim was to identify the proportion of patients with a declining nutritional status.

The fourth aim was to identify any associations between nutritional decline and GI and other SSc-related organ involvement.

### **3.4 Ethical approval**

Ethical approval was granted from the North West Ethics Committee (12/NW/0247).

## **3.5 Materials and methods**

### **3.5.1 Patient recruitment**

The intention was for a single researcher to recruit, and assess, 170 patients with SSc. Patients were contacted by post prior to a scheduled attendance. Where possible, this was their annual assessment. Upon attendance, patients were invited to participate. If other commitments prevented this, they were invited to participate at their next attendance.

#### **Inclusion criteria**

Patients with either lcSSc or dcSSc were recruited through the SSc clinic at Salford Royal NHS Foundation Trust [5]. Patients were required to be aged 18 to 85 years and able to give informed consent.

#### **Exclusion criteria**

Patients with any history of an eating disorder, severe psychiatric illness or another GI disease associated with weight loss were excluded. Acutely unwell patients were excluded. Patients who were pregnant or had implanted electrical devices did not undergo BIA. Any patients who lost capacity prior to follow-up were excluded from re-assessment. Due to the questionnaires, non-English speaking patients were excluded.

### **3.5.2 Study environment**

The study was conducted in the outpatient department of Salford Royal NHS Foundation Trust, a secondary and tertiary referral centre for patients with SSc. All patients were under the care of a single rheumatologist.

### **3.5.3 Follow-up arrangement**

Follow-up assessments were planned for 10 to 14 months after recruitment at a scheduled SSc clinic attendance. However, as appointments were clinically scheduled, they did not necessarily fall within this interval. Therefore, follow-up assessments were not limited to this period.



### **3.5.4 Demographic and clinical data**

Demographic and clinical data were obtained by review of the Salford Royal NHS Foundation Trust electronic patient record, case note and SSc research database and through patient questioning.

Demographic details included date of birth, gender, handedness and smoking status. Clinical details included SSc disease duration, severity and disease characteristics, GI investigations and diagnoses, previous dietetic assessments and interventions and medication. Investigations were included if conducted within 6 months of recruitment.

#### **Disease sub-type**

Disease sub-type was recorded [5]. SSc onset was defined by the first non-Raynaud's symptom. The last available Modified Rodnan skin score was recorded [104]. All skin scores were assessed by the same consultant rheumatologist. When unavailable at patient recruitment, scores from within the preceding 6 months were used in stable patients.

#### **Medsgger systemic sclerosis severity scale**

Disease severity was defined using the Medsgger SSc Severity Scale (Table 1.3), which defines the severity of SSc-related disease affecting an organ by the total effect (reversible and irreversible) on the organ's function [125]. Organs are rated individually using 9 organ-specific severity scales which are graded from 0 to 4 (normality to end-stage disease). For grades defined by more than one marker, only one needs to be present to be classed as meeting it. For the purpose of this study, total weight loss was omitted from the general severity organ-specific category criteria as the category score was to be compared to BMI and 'MUST', both of which include an assessment of weight.

### **3.5.5 Questionnaires**

Patients completed the UCLA GIT 2.0 (GI symptoms) and SHAQ (functional disability) questionnaires at baseline and follow-up [86, 89, 107, 112]. The HAQ used was the shorter Disability Index questionnaire.

### **Gastrointestinal questionnaire**

Individual symptom domain and total GI scores were recorded. For patients restudied, changes in scores were calculated. Scores were compared to established cut-offs of significance [88].

### **Functional assessment**

Individual and total scores were recorded. Total scores were calculated using the ‘Standard Disability Index’ method, which adjusts category responses depending on use of aids or the need for assistance. For the SHAQ VAS component, only the scores for pain, GI, lung and global disability were analysed.

### **3.5.6 Clinical nutritional assessment**

This section describes the nutritional assessments (BMI, ‘MUST’, anthropometry, BIA) which were completed at baseline and recruitment.

#### **Body Mass Index**

Baseline height, without shoes, was recorded to the nearest 1cm using a calibrated measure (Seca 240 measuring rod), with the subject’s head in the Frankfort plane. If unable to stand, the last true measure was used. Weight, without heavy outdoor clothing, was recorded to the nearest 0.1kg using calibrated sitting scales (Charder Electronic Company Ltd, Taiwan). BMI was calculated.

$$\text{BMI (kg/m}^2\text{)} = \frac{\text{weight (kg)}}{\text{height (m)}^2}$$

Equation 3.1: BMI equation

For any change in BMI to be of nutritional significance, baseline height was re-used.

#### **Assessment of unintentional weight loss**

Patients were asked about any recent (preceding 3-6 months) unintentional weight loss. Supportive evidence (recorded weights) was sought. As recall is not exact for weight change, patients were only graded as having had <5%, 5-10% or >10% weight loss.

The above was repeated at follow-up assessment. In addition, percentage weight change was calculated (Equation 3.2). Intentional versus unintentional weight loss was noted.

$$\text{percentage weight loss (\%)} = \frac{(\text{baseline weight (kg)} - \text{followup weight (kg)})}{\text{baseline weight (kg)}} \times 100$$

Equation 3.2: Percentage weight loss equation

### **‘Malnutrition Universal Screening Tool’**

Nutritional risk was calculated using ‘MUST’ (Figure 1.3). The BMI, scores were 1 for 18.5-20kg/m<sup>2</sup> and 2 for <18.5kg/m<sup>2</sup>. For unplanned weight loss in the past 3-6 months, patients who reported losing 5-10% of their body weight were scored 1 and those who had lost >10% were scored as 2. As acutely ill patients were excluded, no one had a positive acute disease effect score. Scores were totalled. Patients scoring 0 had a low risk of malnutrition, patients scoring 1 had a medium and patients scoring ≥2 had a high risk.

### **Oral aperture**

Maximum inter-incisor distance was measured at baseline and follow-up using graduated calipers (Figure 3.1). Measurements were made with the patient lying on the examination couch with their mouth wide open. Distances were recorded to the nearest 1mm. Patients missing their incisors/dentures were excluded.



Figure 3.1: Graduated inter-incisor calipers

### **Non-dominant mid-upper arm anthropometry**

Mid-arm measurements were made at the mid-point of the non-dominant arm. The mid-point was defined as half way between the tip of the acromion process of the scapula and the inferior border of the olecranon process of the ulnar, with the arm flexed to 90 degrees at the elbow [152]. Measurements were made using a non-stretch tape measure. The mid-point was marked using a non-permanent pen. If possible, patients were standing.



Figure 3.2: MAC measurement

The MAC was measured, to the nearest 1mm, with the arm hanging freely and the tape measure centred over the mark.

For TSF, the researcher stood behind the patient. Patients stood with their arm hanging loosely. If unable to stand, measurements were made whilst seated. Skin and subcutaneous tissue were separated from the underlying triceps muscle using 2 fingers placed 1-2cm above the mid-point. Harpenden calipers (British Indicators, Weybridge, UK; Figure 3.3b) were applied perpendicular to the skin at the mid-arm point. Three pinch measurements were made, each for a count of 3 (1-2-3, approximately 2-3 seconds) (since longer measurements cause fluid displacement). Measurements were to the nearest 0.1mm. The final result was the average of the 2 closest measurements.



Figure 3.3: Harpenden calipers

MAMC was calculated from MAC and TSF (Equation 3.3). MAC, TSF and MAMC were compared to age and gender centiles [155]. However, these were only for individuals up to 74 years. Thus, patients >75 years were included in the 64 to 74 year bracket.

$$MAMC (cm) = MAC (cm) - [TSF (mm) \times 0.314]$$

Equation 3.3: MAMC calculation

#### **Four-site anthropometry**

During the study, concerns developed over the reliability of BIA. Therefore, in order to address this and hypothesis one, an additional measure of body composition (four site anthropometry) was introduced.

Right-sided BSF, TSF, SSSF and SISF were measured using the same equipment and principles as described above. If right arm TSF had already been assessed as the non-dominant limb, it was not repeated. BSF was measured at the mid-arm level over the most anterior part of the biceps, with the arm in a relaxed position and palm facing forwards [152]. To measure SSSF, the patient's right hand was placed on their back to demonstrate the right scapula's medial border [152]. The skinfold was measured approximately 1cm below the inferior angle of the scapula with the skinfold direction taking on the direction of the natural skin crease (approximately 45 degrees downwards from the spine). For SISF, the standardised SISF site was chosen [152]. The right arm was placed across the

chest. The site was approximately 1cm above the anterior superior iliac spine, in the midaxillary line. The fold assumed the natural line (slightly downwards).

Estimated body density was calculated using the Durnin and Womersley equation and TSF, BSF, SSSF and SISF [162]. Age and gender specific co-efficients (C and M) are listed in Appendix 7 [162]. Then, percentage body fat was calculated using the Siri equation [133].

$$\text{Body Density} = C - [M \log_{10}(\text{triceps} + \text{biceps} + \text{subscapular} + \text{suprailiac})]$$

Equation 3.4: Durnin and Womersley 4 skin-fold measurement equation

$$\% \text{ Body Fat} = \left[ \left( \frac{4.95}{\text{Body Density}} \right) - 4.5 \right] \times 100$$

Equation 3.5: Two component Siri equation

## **Bioelectrical impedance analysis**

### Equipment

There are many different BIA devices of differing design and complexity. Given the rapidly evolving nature of devices, and the commercial secrecy surrounding their underlying equations, it is difficult to compare devices. Bodystat<sup>®</sup> has existed for several years and over time its products have undergone considerable development [371]. Several different analysers are currently commercially available. All are based on the early Bodystat<sup>®</sup> validation studies [372].

Bodystat<sup>®</sup> 1500MDD (Bodystat Inc, Douglas, UK), a dual frequency (5kHz and 50kHz), tetrapolar analyser capable of measuring impedance (range 20 to 1,300ohms), resistance (at 50kHz) and reactance (at 50kHz) was chosen for this study. It uses a two-compartment body composition model.



Figure 3.4: Bodystat® 1500MDD analyser, leads and long electrodes

The Bodystat® 1500MDD unit consisted of an analyser, 2 leads (foot and hand) and long electrodes (4 per study) (Figure 3.4). It measured impedances (5kHz and 50kHz), resistance and reactance. In-built software used specialist (non-published) equations to calculate percentage fat and body water. It also deduced phase angle (at 50kHz). Calibration was checked regularly.

### Procedure

BIA was intended to be assessed as a clinically applicable field method, with assessments conducted in clinic, rather than as a research tool. Patients were not fasted or asked to avoid exercise, skin creams, caffeine or alcohol. To maintain normal hydration, patients were allowed to drink up to the test. Medications were not withheld. Time of assessment was not standardised. Instead, it was dictated by clinic scheduling. Measurements were at room temperature.

Patients removed their right footwear and lay supine on a non-conductive examination couch. If unable to lie flat, due to their disease manifestations, they lay as flat as tolerable. Limbs were abducted sufficiently to prevent inner thighs from meeting and upper arms from touching the trunk.

As advised by the manufacturer, the electrode site skin was cleaned with an alcohol skin preparation and allowed to dry. Long electrodes were applied overlying imaginary lines

bisecting identifiable anatomical points (Figure 3.5). Upper limb electrodes were applied between the styloid processes of the ulnar and radius (proximal electrode) on the dorsal surface of the right wrist and across the base of the metacarpal-pharyngeal joints (distal electrode). Lower limb electrodes were applied to the right ankle across the medial and lateral malleoli (proximal electrode) and across the bases of the metacarpal-pharyngeal joints on the dorsal surface of the foot. To prevent current arcing, there was  $\geq 3$ cm between electrodes.

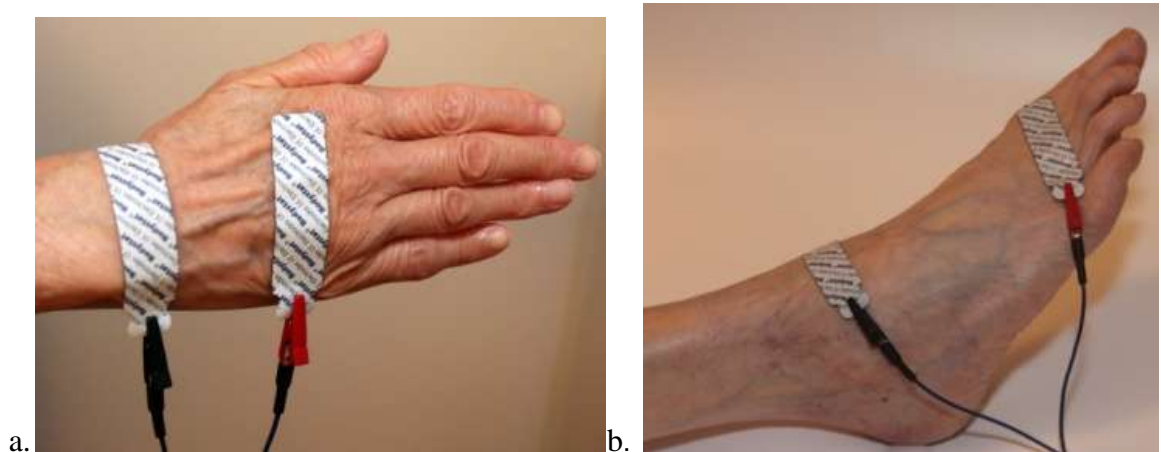


Figure 3.5: Electrode positions on the hand (a) and foot (b)

The red leads were attached to the distal electrodes and the black leads to the proximal electrodes. Gender, age, weight and height were entered into the analyser. Measurements were made after lying flat for 4 minutes. Results were later exported for analysis.

### 3.5.7 Blood tests

All samples were collected by nursing staff using standard techniques. All samples were analysed by the Biochemistry and Haematology Departments at Salford Royal NHS Foundation Trust. Routine bloods (including: full blood count, inflammatory markers, bone profile) were analysed using standard laboratory methods and reported using clinical reference ranges (manufacturer recommended and/or in-house assay). Research bloods (vitamin D, zinc, selenium, iron studies (serum iron, iron saturation and total iron binding capacity) and magnesium) and routine bone profile were analysed as described below:



## **Vitamin D**

Samples were extracted using a liquid to solvent extraction method and analysed on a Quattro Premier XE mass spectrometer using an ultra performance liquid chromatography-tandem mass spectrometry method. This allowed measurement of both 25-hydroxyvitamin D2 and 25-hydroxy vitamin D3 from a single serum sample. Samples were compared to in-house assays. Reference ranges for total vitamin D were as determined by the Greater Manchester Medicines Committee.

## **Zinc**

Serum zinc was requested from the first 100 patients (unselected). Clinical samples were also available from other patients. Samples were analysed using flameless furnace atomic absorption mass spectrometry (Varian AA Duo system) with a Zeeman-effect background correction. Reference ranges were based on in-house values.

## **Selenium**

Serum selenium was requested from the first 25 patients (unselected). Clinical samples were also available from other patients. Samples were analysed using flame atomic absorption spectroscopy (Varian AA Duo system) with a Zeeman-effect background correction. Normal reference ranges were obtained from the literature.

## **Iron studies**

Iron studies were requested from the first 100 patients (unselected). Clinical samples were also available from other patients. Samples were analysed using the Cobas kit on the Roche Modular P automated analyser using a FerroZine colorimetric method. Manufacturer advised reference ranges were used.

## **Magnesium**

Serum magnesium was requested from the first 100 patients (unselected). Clinical samples were also available from other patients. Samples were analysed using the Cobas kit on the

Roche Modular P automated analyser using a Xylidyl blue assay. Manufacturer advised reference ranges were used.

### **Calcium**

Samples were analysed using the Cobas kit on the Roche Modular P automated analyser with a Colorimetric assay (o-cresolphthalein complex one reagent). Reactions produce a photometrically measured colour change. This method was fully validated by the manufacturer and further validated by in-house kit. The total calcium was adjusted for albumin.

### **Phosphate**

Samples were analysed using the Cobas kit on the Roche Modular P automated analyser using a colorimetric assay (ammonium molybdate reagent). Reactions produce a photometrically measured colour change. This method was fully validated by the manufacturer and further by an in-house kit.

#### **3.5.8 Statistical analysis**

Data were analysed using Microsoft Excel and SPSS (version 20.0).

Student's t-test was used to compare mean BMIs, discrepancies between BIA and anthropometric percentage body fats, changes in weight and serum nutritional biochemical results between selected patient groups (e.g. clinical manifestations, survival, dietetic intervention). Associations between 'MUST' and demographic and clinical manifestations were sought using linear-by-linear analysis.

Correlations (Pearson's (r) or Spearman's (s)) were sought between the baseline nutritional measures (e.g. BMI, weight, percentage body fat) and the changes and discrepancies in nutritional measures. In addition, correlations were sought between these and patient demographics, clinical manifestations and symptoms. Agreement analyses were also conducted between the different measures of body composition.

For all analyses, a p value of  $\leq 0.05$  was considered significant.

## 3.6 Results

### 3.6.1 Patients and demographics

170 patients were recruited from between 8<sup>th</sup> May 2012 and 8<sup>th</sup> May 2013 (12 months), but 2 were later excluded.

The median age was 60.7±11.5 years (mean 59.2; range 25.3 to 81.1). Thirty one (19%) were male. Forty five (27%) had dcSSc. Twenty seven (16%) were anti-topoisomerase 1 and 57 (34%) were anti-centromere antibody positive. The median intervals from onset of Raynaud's was 167±156 months (range 0 to 778; n=167) and SSc was 133±110 months (range 0 to 742).

### 3.6.2 Baseline nutritional assessment

The mean BMI was 24.6±4.9kg/m<sup>2</sup> (range 15.6 to 39.8; n=168; Figure 3.6). Based on WHO classifications, 6.5% were underweight, 52.4% were a healthy weight, 29.8% were overweight, 7.7% were class I obese and 3.6% were class II obese.

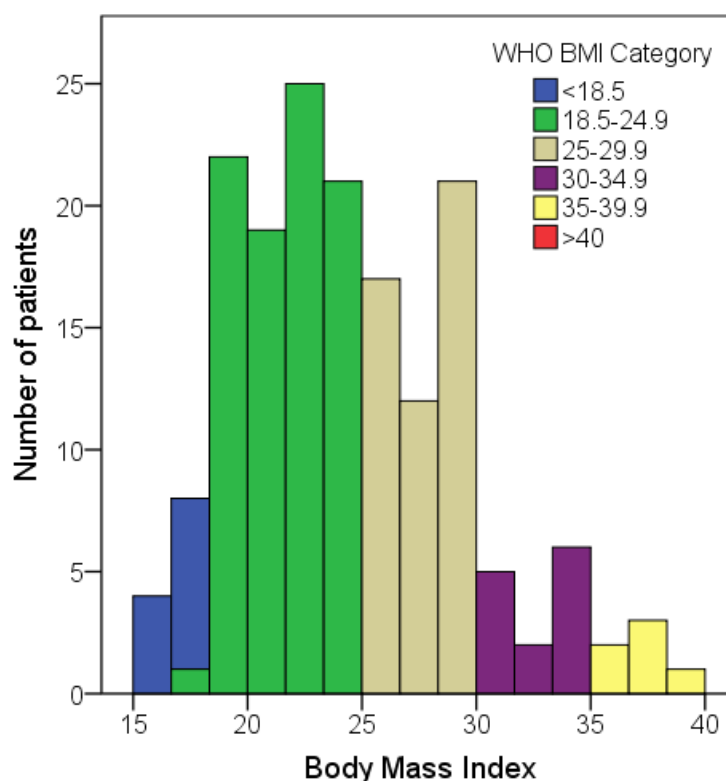


Figure 3.6: Distribution of BMIs and WHO category

### ‘Malnutrition Universal Screening Tool’

Total and category scores are shown in Table 3.1.

‘MUST’ component	Patients (%)
<b>‘MUST’ BMI score</b>	
‘MUST’ = 0 (BMI >20 kg/m <sup>2</sup> )	134 (80%)
‘MUST’ = 1 (BMI = 18.5-20 kg/m <sup>2</sup> )	22 (13%)
‘MUST’ ≥ 2 (BMI <18.5 kg/m <sup>2</sup> )	12 (7%)
<b>Unintentional weight loss in past 3-6 months ‘MUST’ score</b>	
‘MUST’ = 0 (0-5% weight loss)	148 (88%)
‘MUST’ = 1 (5-10% weight loss)	16 (10%)
‘MUST’ ≥ 2 (>10% weight loss)	4 (2%)
<b>Total ‘MUST’ score (risk of malnutrition)</b>	
‘MUST’ = 0 (Low)	125 (74%)
‘MUST’ = 1 (Medium)	23 (14%)
‘MUST’ ≥ 2 (High)	20 (12%)

Table 3.1: ‘MUST’ score

### Non-dominant mid-upper arm anthropometry

MAC, TSF and MAMC were available for 168, 167 and 167 patients respectively. Scores were compared to age and gender specific 5<sup>th</sup> centile lines (Table 3.2). The number of patients in each BMI category with MUAA the ≤5<sup>th</sup> centile were tabulated.

	All patients	BMI		
		>20kg/m <sup>2</sup>	18.5-20 kg/m <sup>2</sup>	<18.5kg/m <sup>2</sup>
MAC (%)	28 (17%)	4 (3%)	13 (59%)	11 (92%)
TSF (%)	37 (22%)	15 (11%)	12 (55%)	10 (83%)
MAMC (%)	16 (10%)	3 (2%)	6 (27%)	7 (58%)

Table 3.2: MUAA ≤5<sup>th</sup> centile versus BMI

There were significant differences (t-test; p<0.01) between the mean BMIs of patients with MAC >5<sup>th</sup> (25.7±4.5kg/m<sup>2</sup>) and ≤5<sup>th</sup> (18.8±1.6kg/m<sup>2</sup>) centiles, with TSF >5<sup>th</sup> (25.9±4.7kg/m<sup>2</sup>) and ≤5<sup>th</sup> (20.2±2.6kg/m<sup>2</sup>) centiles and with MAMC >5<sup>th</sup> (25.2±4.7kg/m<sup>2</sup>) and ≤5<sup>th</sup> (18.8±1.8kg/m<sup>2</sup>) centiles.

### Four-site anthropometry

Anthropometric percentage body fat was attempted in 102 patients, but could not be calculated for 4 patients due to an inability to measure at least one skinfold. The mean percentage body fat was  $29.5 \pm 5.9\%$  (range 15.4 to 45.5;  $n=98$ ; Figure 3.7).

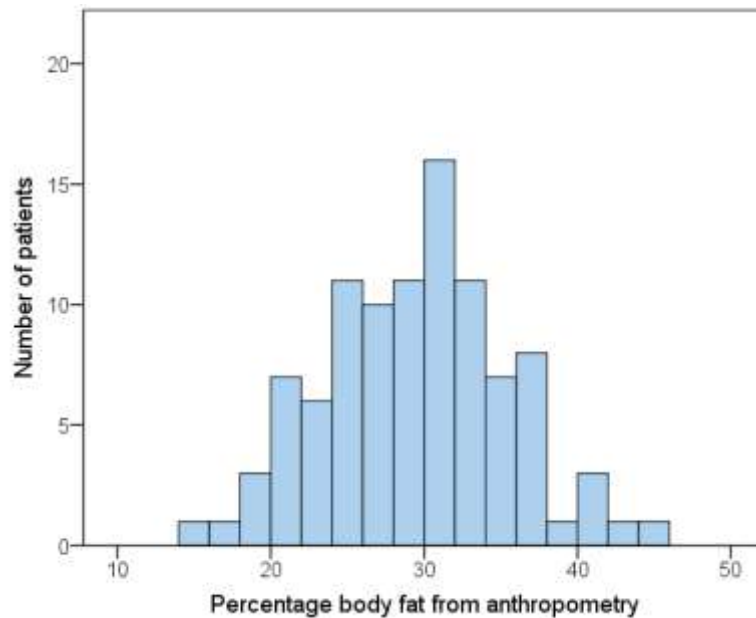


Figure 3.7: Percentage anthropometric body fat

There was a significant correlation between percentage body fat and BMI ( $r=0.645$ ;  $n=98$ ;  $p<0.01$ ; Figure 3.8).

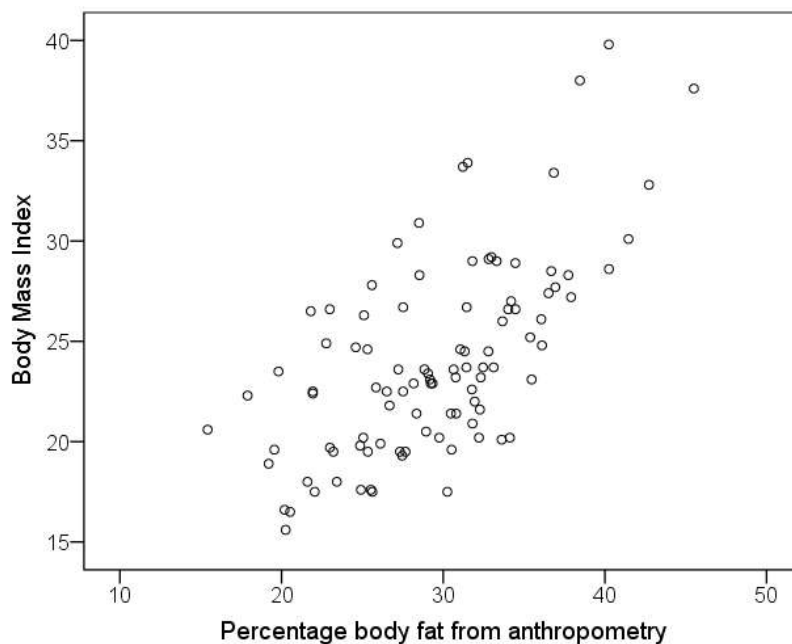


Figure 3.8: BMI against anthropometric percentage body fat

### Bioelectrical impedance analysis

Three patients were excluded due to implanted electrical devices (1 pacemaker; 1 sacral stimulator for incontinence; 1 spinal cord stimulator for Raynaud's). Forty six (28%) patients were taking steroids.

Mean body fat was  $34.9 \pm 7.6\%$  (range 11 to 52). Mean phase angle was  $4.5 \pm 1.0$  (range 1.8 to 6.8). There were significant correlations between BMI and percentage body fat ( $r=0.491$ ;  $p<0.01$ ; Figure 3.9) and BMI and phase angle ( $r=0.354$ ;  $p<0.01$ ; Figure 3.9).

There was also a significant correlation between PhA and anthropometric percentage body fat ( $r=0.224$ ;  $n=96$ ;  $p=0.03$ ).

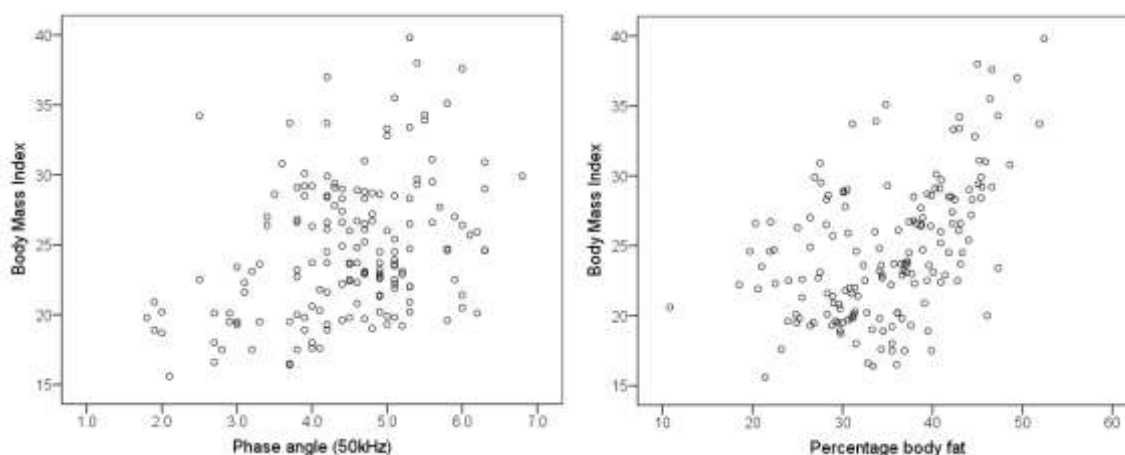


Figure 3.9: BMI against BIA phase angle and percentage body fat

### Comparison of 'MUST' percentage body fats

There were significant correlations between total 'MUST' scores and anthropometric ( $s=-0.479$ ;  $n=168$ ;  $p<0.01$ ) and BIA ( $s=-0.162$ ;  $n=165$ ;  $p=0.04$ ) percentage body fats.

### Comparison of BIA and anthropometric percentage body fats

Ninety six patients had both BIA and anthropometric percentage body fats. Individual measures showed a positive correlation ( $r=0.683$ ;  $p<0.01$ ), but absolute values often differed (Figure 3.10). The mean discrepancy was  $4.4 \pm 5.5\%$ . The intraclass correlation coefficient (ICC) was 0.51 (95% limits of agreement -15.1 to 6.4). The discrepancy between measures correlate with age ( $r=0.295$ ;  $p<0.01$ ).

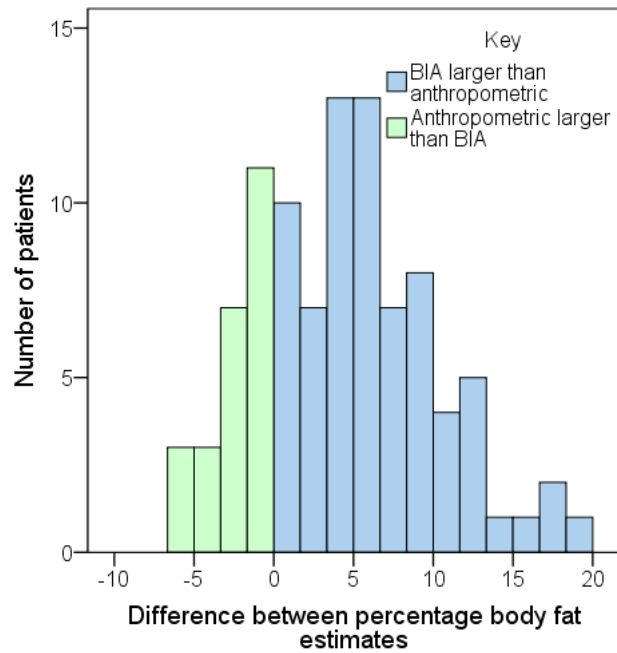


Figure 3.10: Difference between BIA and anthropometric percentage body fats

There were no correlations between the percentage fat discrepancy and BMI ( $r=-0.147$ ;  $n=96$ ;  $p=0.15$ ), total skin score ( $s=-0.182$ ;  $p=0.08$ ) or skin involvement at BIA sites ( $s=-0.158$ ;  $p=0.13$ ), but there was with involvement at anthropometric sites ( $s=-0.273$ ;  $p<0.01$ ). There were no differences in the mean of body fat discrepancies of patients taking or not taking steroids (t-test;  $p=0.40$ ) or with or without lung fibrosis (t-test;  $p=0.53$ ). There were differences in the mean of body fat discrepancies of patients with and without oesophageal involvement (t-test;  $p=0.05$ ) and with small intestinal (t-test;  $p=0.01$ ) involvement.

### 3.6.3 Systemic sclerosis organ-specific manifestations

This section describes the allocation of patients to Medsger Severity Scale categories.

#### **Medsger general severity score**

Haemoglobin was  $>12.3\text{gm/dl}$  in 65%. Haematocrit was  $\geq 0.37$  in 75%. Based on the published criteria (excluding weight loss), 61% of patients were normal, while 24% had mild, 9% had moderate, 1% had severe and 1% had end stage disease. 4% were unknown.

### **Medsger peripheral vascular**

99% had Raynaud's phenomenon. 51% were taking oral vasodilators, 34% had received intravenous vasodilators and 3% had a sympathectomy. 46% had digital pitting, while 51% had experienced digital ulceration. At least 14% had undergone  $\geq 1$  debridement and 4% had an amputation. Based on Medsger criteria, 16% were normal and 19% had mild, 14% had moderate and 51% had severe disease.

### **Medsger skin scores**

The mean total skin score was  $7 \pm 8$  (range 0 to 39; n=166). Fingers (right 84%; left 80%) were most commonly involved, followed by the feet (right 40%; left 40%) and face (40%). Based on Medsger criteria, 13% were normal and 70% had mild, 15% had moderate and 1% had severe disease. 1% was unknown.

### **Medsger joint and tendon scores**

Data was unavailable for 14% of patients. 68% had either no or minimal hand joint restriction. Based on Medsger criteria, 59% were normal and 3% had mild, 12% had moderate, 7% had severe and 5% had end stage disease. 14% were unknown.

### **Medsger muscle scores**

Muscle assessments were recorded for 82%. 27% used a walking aid. Based on Medsger criteria, 55% were normal while, 18% had mild and 13% had end stage disease. 14% were unknown.

### **Medsger gastrointestinal scores**

4% of patients required HPN (included in Chapter 2). GI involvement is discussed in section 3.5.4. Based on GI criteria, 47% were normal and 40% had mild disease, 7% had moderate, 2% had severe and 4% had end stage.



### **Medsger lung scores**

Spirometry was unavailable for 1% of patients and >2 years old for 4%. Forced vital capacity was  $\geq 80\%$  predicted in 68% and  $< 50\%$  in 5%. Diffusion capacity was unavailable for 23%. Of all patients, 30% had a diffusing capacity  $\geq 80\%$  predicted, while in 7% it was  $< 50\%$ . Interstitial fibrosis was present in 40%. Pulmonary artery pressures were unavailable for 15%. Estimated pressures were  $\geq 35\text{mmHg}$  in 8%. Two patients had home oxygen. Based on Medsger criteria, 31% were normal and 28.5% had mild disease, 28.5% had moderate, 10% had severe and 1% had end stage. 1% was unknown.

### **Medsger heart scores**

An ECG was available for 94%. 17% had a conduction abnormality, 2% had an arrhythmia and 1% had an arrhythmia requiring treatment. 94% had an echocardiogram. Only 6% had LV dysfunction or an ejection fraction  $\leq 50\%$ . Based on Medsger criteria, 77% were normal and 19% had mild disease, 2% had moderate, 1% had severe disease. 1% was unknown.

### **Medsger kidney scores**

Six patients had experienced renal crisis. Based on Medsger criteria, 94% were normal and 1% had mild disease, 2% had moderate and 1% had end stage. 2% were unknown.

### 3.6.4 Gastrointestinal involvement

A gastroenterologist had reviewed 46% of patients. GI manifestations are described.

#### Oesophageal and gastric involvement

Upper GI investigations: gastroscopy in 77%; barium swallow in 61%; CT reporting oesophageal diameter in 26%; oesophageal manometry in 9%; pH study in 5% and gastric emptying study in 5%. Based on these, 52% had evidence of oesophageal and 7% had gastric dysmotility. In addition, 86% of patients were taking at least one medication for acid suppression (Table 3.3).

Medication	Patients
Proton pump inhibitor only	74%
Histamine H2 receptor antagonists only	2%
Proton pump inhibitor and histamine H2 receptor antagonists	8%
Prokinetic	20%

Table 3.3: Upper GI medications

#### Small intestinal involvement

Small intestinal investigations included barium follow-throughs (20%), abdominal CT or MRI scan (11%) and breath tests for overgrowth (34%). Imaging showed small intestinal involvement in 14%. SIBO was identified in 15%. Regular or intermittent antibiotics for SIBO were used by 10%. In summary, 18% had small intestinal dysmotility.

#### Colonic involvement

Colonic investigations included sigmoidoscopy (8%), colonoscopy (22%), CT colon (13%), barium enema (3%) and transit studies (1%). Transit studies showed severe constipation. Other investigations excluded alternative pathology. Regular or intermittent medications with an effect on colonic transit include laxatives (15%), loperamide (5%) and opiates (23%).

### 3.6.5 Baseline oral aperture

Mean inter-incisor distance was  $34.0 \pm 8.4$  mm (range 12 to 50;  $n=162$ ; Figure 3.11).

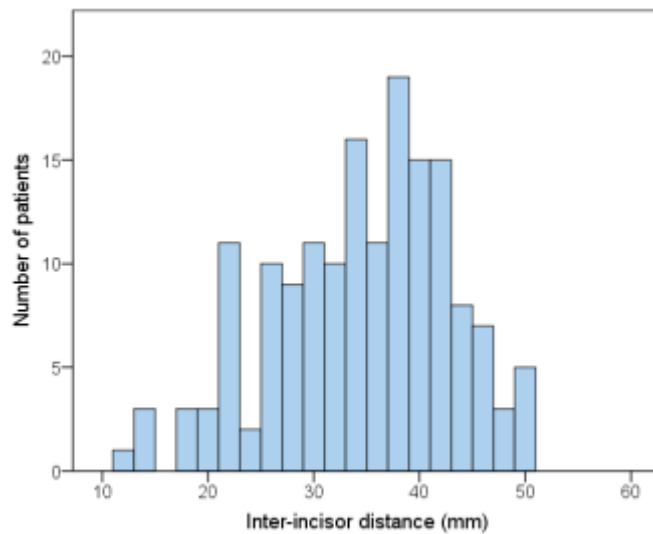


Figure 3.11: Oral apertures

### 3.6.6 Baseline scleroderma health assessment questionnaire

HAQs were incomplete for 3 patients. The mean HAQ was  $1.4 \pm 0.84$  (Figure 3.12).

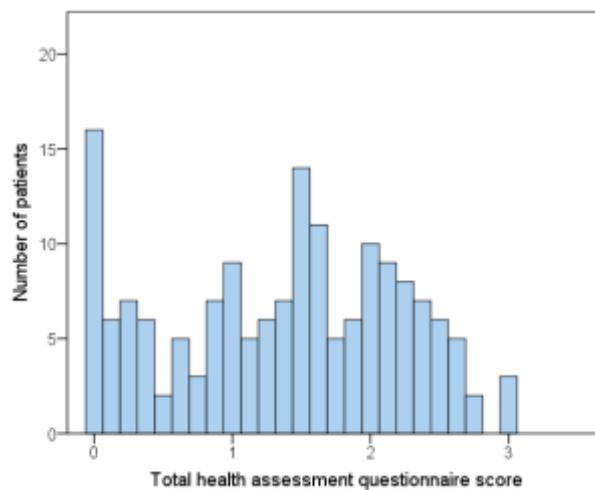


Figure 3.12: Distribution of total HAQ scores

VAS scores were incomplete for 2 patients. Mean VAS scores were  $1.08 \pm 0.81$  (range 0 to 2.9) for pain,  $0.91 \pm 0.86$  (range 0 to 3) for GI,  $0.98 \pm 0.87$  (range 0 to 3) for respiratory and  $1.27 \pm 0.83$  (range 0 to 3) for global disability (Figure 3.13).

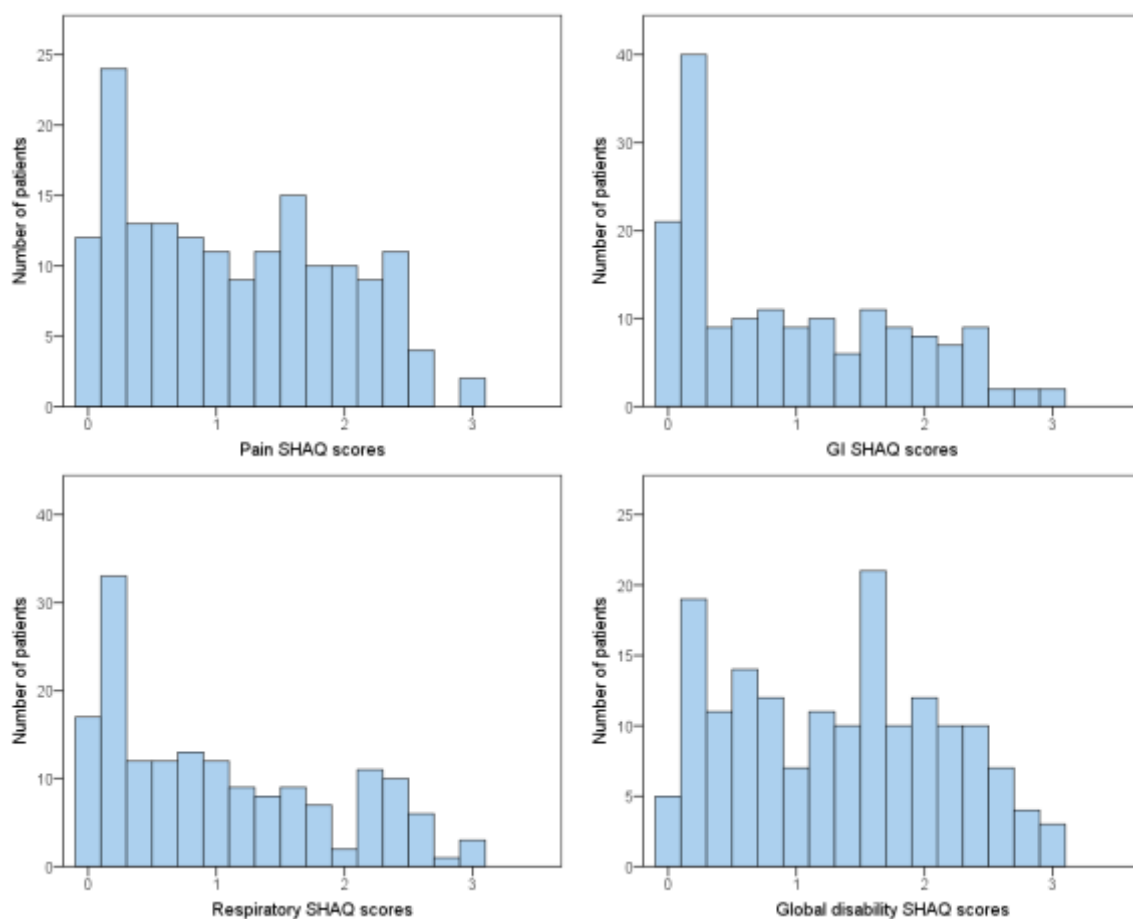


Figure 3.13: SHAQ VAS scores

### 3.6.7 Baseline gastrointestinal symptom scores

Symptom domain	Mean	Range	IQR	Percent. scored minimum	Percent. scored maximum
Reflux	0.70	0.0 - 2.88	0.13 - 1.13	20%	0%
Distension / bloating	1.13	0.0 - 3.0	0.25 - 1.75	18%	7%
Faecal soilage	0.42	0.0 - 3.0	0.00 - 0.75	75%	6%
Diarrhoea	0.46	0.0 - 2.0	0.00 - 1.00	56%	5%
Social functioning	0.41	0.0 - 3.0	0.00 - 0.66	50%	1%
Emotional well-being	0.52	0.0 - 3.0	0.00 - 0.77	46%	1%
Constipation	0.41	0.0 - 2.5	0.00 - 0.75	49%	2%
Total GI score	0.61	0.0 - 2.26	0.17 - 0.96	10%	0%

Table 3.4: UCLA scores

Patients were allocated to validated symptom severity categories (Table 3.5). Of the patients with 'none to mild' reflux, 73% were taking a proton pump inhibitor and/or

histamine H2 receptor antagonist in comparison to 96% of patients with ‘severe to very severe’ symptoms.

<b>Symptom domain</b>	<b>None to mild (%)</b>	<b>Moderate (%)</b>	<b>Severe to very severe (%)</b>
Reflux	47%	25%	28%
Distension / bloating	57%	11%	32%
Faecal soilage	89%	5%	6%
Diarrhoea	57%	25%	18%
Social functioning	65%	24%	11%
Emotional well-being	67%	12%	21%
Constipation	61%	29%	10%
Total GI score	54%	25%	21%

Table 3.5: UCLA severity categories

In addition, 9% of patients with ‘none to mild’ distension/bloating took prokinetics and 7% needed regular or intermittent SIBO antibiotics. In comparison, 35% of patients with ‘severe to very severe’ distension/bloating took prokinetics and 15% needed antibiotics.

### **3.6.8 Baseline nutritional biochemistry**

Unselected micronutrient concentrations were requested for the first 100 patients, with the exception of selenium (first 25). Subsequent patients were tested based on clinical need only. Results were compared to reference ranges (Table 3.6).

Test	Total number	Mean	Standard deviation	Range	Percent. outside reference limit
Haemoglobin (g/L)	162	126	13.3	80 - 167	21% ▼
Albumin (g/L)	163	43	3.5	31 - 50	1% ▼
CRP (mg/L)	157	6.6	9.8	0 - 62	17% ▲
ESR (mm/hr)	145	18	16.8	2 - 104	32% ▲
Calcium (mmol/L)	145	2.38	0.10	1.89 – 2.76	2% ▼
Magnesium (mmol/L)	111	0.82	0.11	0.24 – 1.06	7% ▼
Zinc (µmol/L)	107	10.4	1.71	4.3 – 14.5	39% ▼
Selenium (µmol/L)	45	0.87	0.23	0.28 – 1.45	9% ▼
Vitamin D (nmol/L)	155	49	26.1	7.7 - 114.1	54% ▼
Iron (µmol/L)	109	11.7	5.6	2.5 – 37.7	49% ▼
Iron saturation (%)	108	21.9	12.4	5.5 – 82.0	53% ▼
Total iron binding capacity (µmol/L)	108	52.9	12.8	8.6 – 90.0	4% ▲

Table 3.6: Biochemical and haematological results

Below (▼) or above (▲) reference limit

Of the patients tested, 42% had a low iron and iron saturation, while 1% also had raised total iron binding capacity. Anaemia was present in 21%; of whom 56% had a low iron and serum iron, but none had a raised total iron binding capacity. However, 12% of patients were taking oral iron or had recently received intravenous iron. Of those receiving iron, anaemia was present in 37% and low serum iron and iron saturation in 50%.

Oral (n=4) or intravenous (n=1) magnesium had been prescribed for 3%. Of these, only 80% had serum magnesium tested at Salford Royal. Only 25% of these were magnesium deficient on testing. As a consequence of the unselected testing, 3 patients were admitted for replacement therapy following the study.

Vitamin D deficiency was detected in 21% of patients tested and insufficiency in 34%. However, 41% of tested patients were prescribed vitamin D. Of these, only 9% were deficient and 23% insufficient compared to 29% and 41% of patients not receiving vitamin D respectively. For the 92 patients not prescribed vitamin D, there was no correlation between season and serum vitamin D (s=0.088; p=0.40).

Test	Selected (clinical) results			Unselected (study) results		
	Number	Mean (range)	Percent outside reference limit	Number	Mean (range)	Percent outside reference limit
Magnesium	18	0.80 (0.49–1.06)	17% ▼	92	0.82 (0.24–0.99)	5% ▼
Zinc	15	10.4 (7.0–14.5)	53% ▼	92	10.4 (4.3–13.8)	37% ▼
Selenium	21	0.84 (0.28–1.20)	10% ▼	24	0.89 (0.35–1.45)	8% ▼
Iron	17	10.3 (3.1–21.1)	59% ▼	92	11.9 (2.5–37.7)	46% ▼
Iron saturation	16	19.8 (8.2–44.382.0)	69% ▼	92	22.3 (5.5–82.0)	50% ▼
Total iron binding capacity	16	51.6 (10.5–65.3)	0% ▲	92	52.9 (8.6–90.0)	4% ▲

Table 3.7: Micronutrient values of selected and unselected patients

Below (▼) or above (▲) reference limit

There were no significant differences (t-test) between the mean serum values of selected and unselected patients for selenium ( $p=0.56$ ), zinc ( $p=0.96$ ), magnesium ( $p=0.69$ ), iron ( $p=0.27$ ), iron saturation ( $p=0.45$ ) or total iron binding capacity ( $p=0.55$ ).

### 3.6.9 Baseline nutritional status compared to outcome/manifestations

This section investigates possible correlations, associations or difference in means between nutritional status ('MUST' or BMI) clinical manifestations/assessments.

#### Demographics

There were no correlations between 'MUST' and age ( $s=0.32$ ;  $p=0.68$ ) or time from onset of Raynaud's ( $s=0.028$ ;  $p=0.72$ ) or SSc ( $s=0.076$ ;  $p=0.33$ ). There were no linear-by-linear

associations between 'MUST' and gender ( $p=0.06$ ), sub-type ( $p=0.83$ ) or anti-topoisomerase ( $p=0.12$ ) or anti-centromere ( $p=0.72$ ) antibody status.

There were correlations between BMI and time from onset of Raynaud's ( $r=-0.162$ ;  $p=0.04$ ) and SSc ( $r=-0.159$ ;  $p=0.04$ ) but not between BMI and age ( $r=0.38$ ;  $p=0.62$ ). Mean BMIs did not differ with gender (males  $26.0\pm 4.3$  vs. females  $24.3\pm 5.0$ ;  $p=0.08$ ), disease-subtype (lcSSc  $24.5\pm 5.0$  vs. dcSSc  $24.7\pm 4.7$ ;  $p=0.87$ ) or anti-topoisomerase (positive  $25.2\pm 4.9$  vs. negative  $24.5\pm 4.9$ ;  $p=0.51$ ) or anti-centromere (positive  $25.0\pm 5.6$  vs. negative  $24.4\pm 4.6$ ;  $p=0.44$ ) antibody status.

### **Disease severity**

Correlations were detected between 'MUST' and the general ( $s=0.236$ ;  $p<0.01$ ), muscle ( $s=0.178$ ;  $p=0.03$ ), GI ( $s=0.195$ ;  $p=0.01$ ) and lung ( $s=0.263$ ;  $p<0.01$ ) Medsger categories, but not between 'MUST' and the peripheral vascular disease ( $s=0.071$ ;  $p=0.36$ ), joint/tendon ( $s=0.158$ ;  $p=0.06$ ), skin ( $s=0.061$ ;  $p=0.44$ ), heart ( $s=0.058$ ;  $p=0.46$ ) or renal ( $s=-0.113$ ;  $p=0.15$ ) categories.

Correlations were also detected between BMI and the general ( $s=-0.211$ ;  $p<0.01$ ), joint/tendons ( $s=-0.193$ ;  $p=0.02$ ), skin ( $s=0.514$ ;  $p=0.05$ ), lung ( $s=-0.172$ ;  $p=0.03$ ), heart ( $s=-0.163$ ;  $p=0.04$ ) and GI ( $s=-0.241$ ;  $p<0.01$ ) categories, but not peripheral vascular disease ( $s=-0.122$ ;  $p=0.11$ ), muscle ( $s=-0.067$ ;  $p=0.43$ ) or renal ( $s=-0.50$ ;  $p=0.53$ ).

### **Gastrointestinal manifestations**

An association (linear-by-linear) was shown between 'MUST' and small bowel involvement ( $p=0.01$ ) but not between 'MUST' and oesophageal involvement ( $p=0.18$ ). 65% of patients with high risk of malnutrition had small intestinal involvement in comparison to 14% of patients with low risk. Mean BMIs of patients with ( $24.0\pm 4.8\text{kg/m}^2$ ;  $n=88$ ) and without ( $25.2\pm 5.0\text{kg/m}^2$ ;  $n=80$ ) oesophageal involvement did not differ significantly ( $p=0.11$ ). However, mean BMIs of patients with (mean  $22.0\pm 3.9\text{kg/m}^2$ ;  $n=30$ ) and without (mean  $25.1\pm 4.9\text{kg/m}^2$ ;  $n=138$ ) small bowel involvement did ( $p<0.01$ ).



### Scleroderma health assessment questionnaire

No correlations were found between ‘MUST’ and total HAQ ( $s=0.135$ ;  $p=0.08$ ) or pain ( $s=0.020$ ;  $p=0.80$ ), GI ( $s=0.004$ ;  $p=0.96$ ), lung ( $s=-0.030$ ;  $p=0.70$ ) or global disability ( $s=0.087$ ;  $p=0.27$ ) SHAQs. There were also no correlations between BMI and total HAQ ( $s=0.012$ ;  $p=0.89$ ) or pain ( $s=0.105$ ;  $p=0.18$ ), GI ( $s=-0.040$ ;  $p=0.61$ ), lung ( $s=0.069$ ;  $p=0.38$ ) or global disability ( $s=-0.069$ ;  $p=0.38$ ) SHAQs.

### Gastrointestinal symptoms

Symptom domain	Mean Domain score		
	‘MUST’ = 0	‘MUST’ = 1	‘MUST’ $\geq$ 2
Reflux	0.72 $\pm$ 0.70	0.61 $\pm$ 0.64	0.71 $\pm$ 0.57
Distension / bloating	1.14 $\pm$ 0.98	1.02 $\pm$ 0.83	1.19 $\pm$ 0.87 (#)
Faecal soilage	0.44 $\pm$ 0.88	0.43 $\pm$ 0.84	0.30 $\pm$ 0.57
Diarrhoea	0.48 $\pm$ 0.64	0.35 $\pm$ 0.53	0.53 $\pm$ 0.71 (#)
Social functioning	0.42 $\pm$ 0.57	0.35 $\pm$ 0.71 (*)	0.38 $\pm$ 0.67
Emotional well-being	0.52 $\pm$ 0.71	0.57 $\pm$ 0.87	0.43 $\pm$ 0.88
Constipation	0.41 $\pm$ 0.61	0.49 $\pm$ 0.51	0.35 $\pm$ 0.42
Total GI score	0.62 $\pm$ 0.55	0.55 $\pm$ 0.53	0.57 $\pm$ 0.60
*somewhat better than ‘MUST’=0; # somewhat worse compared to ‘MUST’=1			

Table 3.8: UCLA scores for ‘MUST’ categories compared to MID

No significant correlations were detected between ‘MUST’ and the reflux ( $s=0.002$ ;  $p=0.98$ ), distension/bloating ( $s=0.014$ ;  $p=0.86$ ), soilage ( $s=-0.006$ ;  $p=0.94$ ), diarrhoea ( $s=-0.075$ ;  $p=0.33$ ), social functioning ( $s=-0.101$ ;  $p=0.20$ ), emotional well-being ( $s=-0.11$ ;  $p=0.16$ ), constipation ( $s=0.052$ ;  $p=0.50$ ) or total GI ( $s=-0.050$ ;  $p=0.52$ ) scores.

Similarly, no significant correlations were detected between BMI and the reflux ( $s=0.003$ ;  $p=0.97$ ), distension/bloating ( $s=0.00$ ;  $p=1.00$ ), soilage ( $s=-0.059$ ;  $p=0.45$ ), diarrhoea ( $s=0.010$ ;  $p=0.90$ ), social functioning ( $s=0.038$ ;  $p=0.62$ ), emotional well-being ( $s=0.023$ ;  $p=0.77$ ), constipation ( $s=-0.133$ ;  $p=0.09$ ) or total GI ( $s=0.015$ ;  $p=0.84$ ) scores.

### Oral aperture

There were correlations between inter-incisor distance and both ‘MUST’ ( $s=-0.157$ ;  $p=0.05$ ) and BMI ( $r=0.191$ ;  $p=0.02$ ).

### **Skin involvement**

There were no correlations between skin score and ‘MUST’ ( $s=0.086$ ;  $p=0.27$ ) or BMI ( $r=0.023$ ;  $p=0.77$ ).

### **Nutritional biochemistry**

Significant correlations were found between ‘MUST’ and serum albumin ( $s=-0.285$ ;  $p<0.01$ ), ESR ( $s=0.207$ ,  $p=0.01$ ) and zinc ( $s=-0.428$ ;  $p<0.01$ ), but not CRP ( $s=0.097$ ;  $p=0.23$ ), magnesium ( $s=-0.113$ ;  $p=0.24$ ), selenium ( $s=-0.167$ ;  $p=0.27$ ) and vitamin D without oral supplementation ( $s=0.087$ ;  $p=0.41$ ). Significant correlations were found between BMI and serum albumin ( $r=0.236$ ;  $p<0.01$ ), ESR ( $s=-0.163$ ;  $p=0.05$ ), zinc ( $s=0.212$ ;  $p=0.03$ ) and vitamin D without oral supplementation ( $s=-0.244$ ;  $p=0.02$ ), but not CRP ( $s=0.066$ ;  $p=0.41$ ), magnesium ( $r=0.016$ ;  $p=0.87$ ) or selenium ( $r=0.153$ ;  $p=0.15$ ).

### **3.6.10 Deceased patients**

Thirteen (8%) patients died before follow-up, after a median of 9.0 months (range 1.2 to 19.9). Their median age was 66.4 years (range 43 to 78). 30% were male. 38% had dcSSc. 8% were anti-topoisomerase and 46% were anti-centromere antibody positive. Their median time from onset of Raynaud’s was 216 months (range 14 to 522) and of SSc was 169 months (range 21 to 366).

Their mean BMI at recruitment was  $20.6\pm 3.5\text{kg/m}^2$ . In comparison, surviving patients had a mean recruitment BMI of  $24.9\pm 4.9\text{kg/m}^2$ . There was a significant difference between means recruitment BMIs of surviving and deceased patient (t-test;  $p<0.01$ ).

At recruitment, 3 patients had a BMI  $<18.5\text{kg/m}^2$ , 4 had a BMI of 18.5 to  $20.0\text{kg/m}^2$  and 6 had a BMI  $>20\text{kg/m}^2$ . In addition, during the 3 to 6 months before recruitment, 3 patients reported unintentional weight losses of 5 to 10% and 2 patients of  $>10\%$ . In summary, 46% were at high, 15% were at medium and 39% were at low risk of malnutrition. A significant association (linear-by-linear) was shown between ‘MUST’ and death ( $p<0.01$ ).

### 3.6.11 Re-studied patients

Between 1<sup>st</sup> March 2013 and 26<sup>th</sup> June 2014 (16 months), 127 (76%) patients were followed up after a median of 14±2.5 months (range 9 to 23) (Figure 3.14).

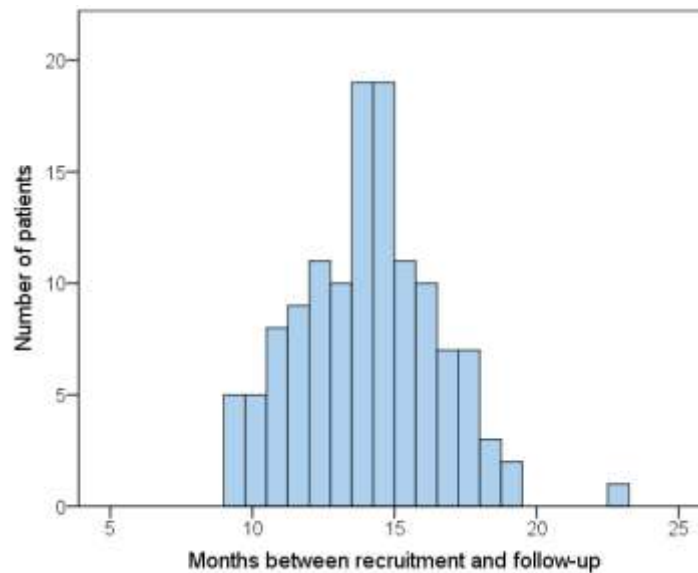


Figure 3.14: Range of follow-up intervals

Twenty six (20%) patients were followed-up after more than 16 months. Reasons for delay included clinic scheduling and lack of time at attendance. Forty one patients were not restudied. Reasons included death (n=13), insufficient time at clinic review (n=10), lost to follow-up (n=12) and researcher unavailability (n=6).

There were no significant differences in patient demographics at recruitment and follow-up.

### 3.6.12 Change in nutritional scores

Changes in nutritional scores for followed up patients were calculated and compared.

#### Weight loss over study period

Seven patients intentionally lost weight during. The mean unintentional percentage weight change was 0.51±6.5% (inter-quartile range -3.0% to 3.1%). Absolute unintentional weight change ranged from an 11.0kg (17.5%) loss to a 15.8kg (28.1%) gain over the whole follow-up period (n=120).

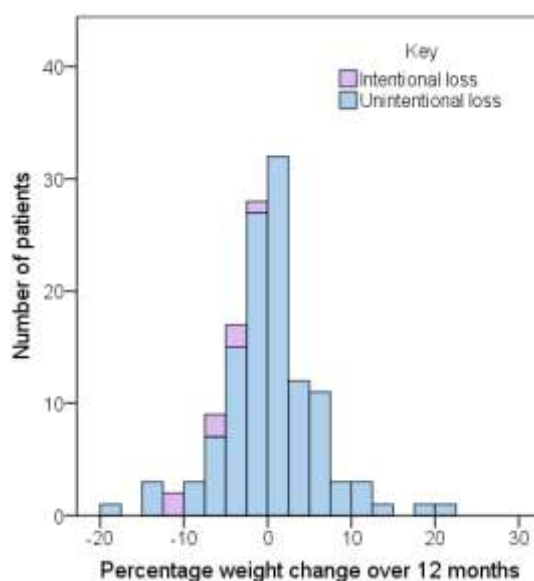


Figure 3.15: Percentage weight change over 12 months

However, the follow-up interval varied. Therefore, it was not sufficient to merely compare total weight changes. Using the assumption that weight change trajectory was constant, weight changes were adjusted to represent 12 months. Mean percentage unplanned weight change for 12 month was  $+0.3 \pm 5.7\%$  (range 19.4% loss to 21.4% gain). The weight of 54% of patients fluctuated by  $<3\%$  over 12 months (Figure 3.15).

### ‘Malnutrition Universal Screening Tool’

The mean BMI of all patients was  $24.8 \pm 5.1 \text{ kg/m}^2$  (range 15.6 to 39.1). Excluding patients with intentional weight loss, the mean BMI was  $24.6 \pm 5.1 \text{ kg/m}^2$  (range 15.6 to 39.1).

‘MUST’ scores were calculated for all patients (Table 3.9).

‘MUST’ component		Patients (%)
‘MUST’ unplanned weight loss	0-5%	118 (93%)
	5-10%	6 (5%)
	>10%	3 (2%)
‘MUST’ BMI	>20 $\text{kg/m}^2$	104 (82%)
	18.5-20 $\text{kg/m}^2$	13 (10%)
	<18.5 $\text{kg/m}^2$	10 (8%)
Total ‘MUST’ score	‘MUST’ = 0	101 (80%)
	‘MUST’ = 1	13 (10%)
	‘MUST’ $\geq 2$	13 (10%)

Table 3.9: Follow-up ‘MUST’ score

Recruitment and follow-up ‘MUST’ scores were compared (Table 3.10).

Baseline ‘MUST’	Follow-up ‘MUST’		
	‘MUST’ = 0	‘MUST’ = 1	‘MUST’ ≥ 2
‘MUST’ = 0	87	7	3
‘MUST’ = 1	10	4	3
‘MUST’ ≥ 2	4	2	7

Table 3.10: ‘MUST’ scores at baseline and follow-up

The mean 12 months adjusted unintentional weight change of patients with low baseline risk (‘MUST’=0) was  $-0.1 \pm 5.2\%$  (n=90; -18.8% to 19.6%), medium baseline risk (‘MUST’=1) was  $-1.7 \pm 7.3$  (n=17; -21.4 to 13.0%) and high baseline nutritional risk (‘MUST’≥2) was  $-0.1 \pm 6.6\%$  (n=13; -12.6 to 13.1%).

### Non-dominant mid-arm anthropometry

Absolute MUAA changes were compared to absolute percentage weight changes (Figures 3.16-3.18). Mean change in MAC was  $0.72 \pm 1.62$ cm (n=125; range -3.9 to 5.9), in TSF was  $1.47 \pm 4.0$ mm (n=123; range -6.3 to 24.1) and in MAMC was  $0.27 \pm 1.5$ cm (n=123; range -3.9 to 4.2).

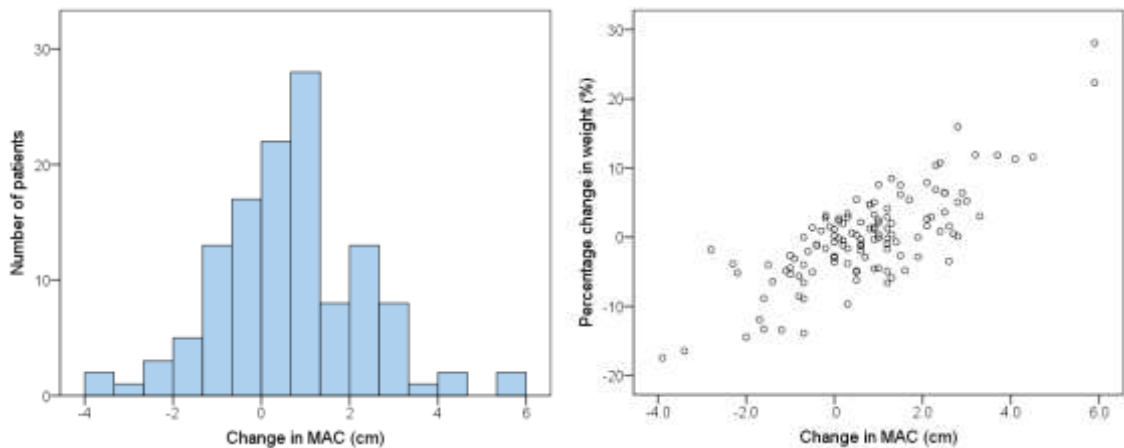


Figure 3.16: Changes in MAC

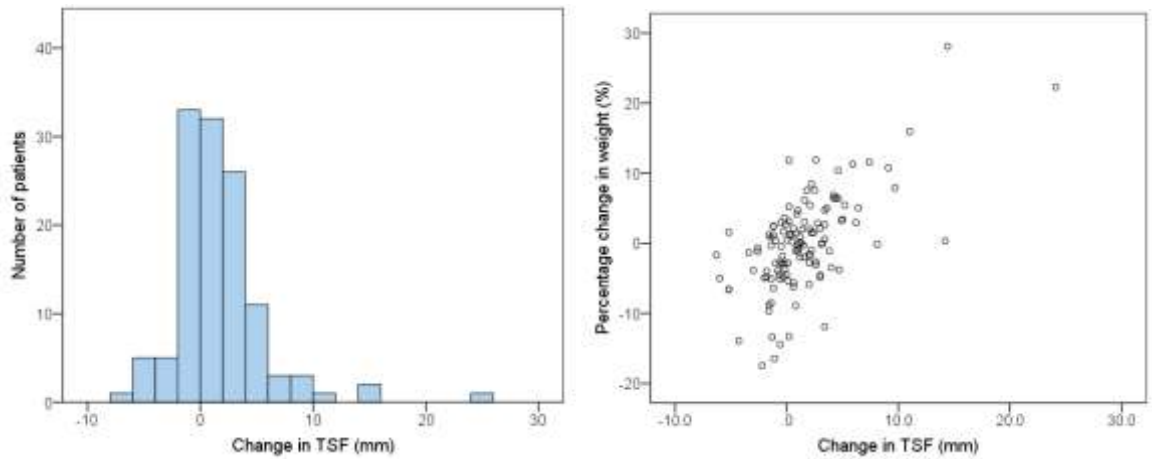


Figure 3.17: Changes in TSF

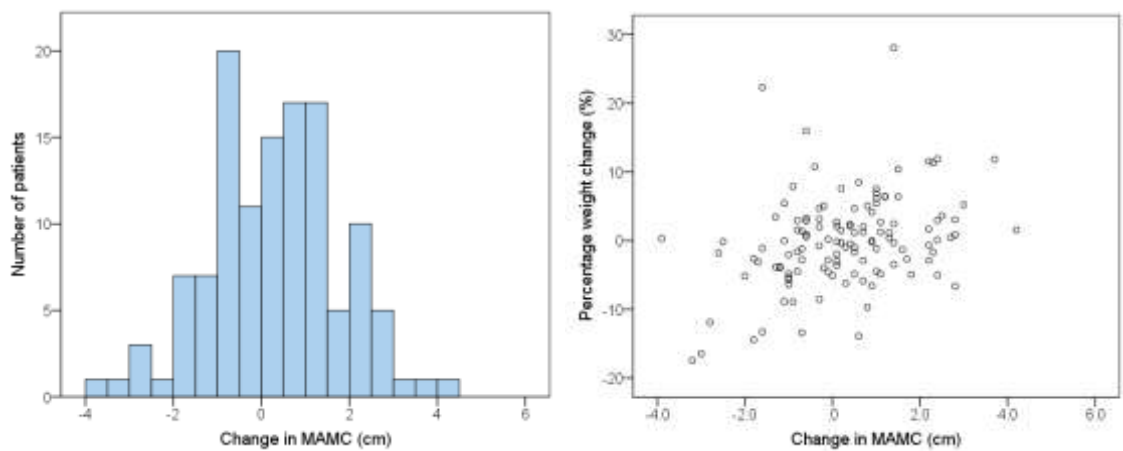


Figure 3.18: Changes in MAMC

As expected from the plots (Figures 3.16-3.18), there were significant correlations between absolute percentage weight change and change in MAC ( $r=0.783$ ;  $p<0.01$ ), TSF ( $r=0.634$ ;  $p<0.01$ ) and MAMC ( $r=0.340$ ;  $p<0.01$ ).

#### Four-site anthropometry

Mean change in anthropometric percentage body fat was  $1.1\pm 2.5\%$  (range -4.6 to 8.2;  $n=69$ ; Figure 3.19). There was a significant correlation between change in body fat and weight ( $r=0.753$ ;  $p<0.01$ ; Figure 3.19).

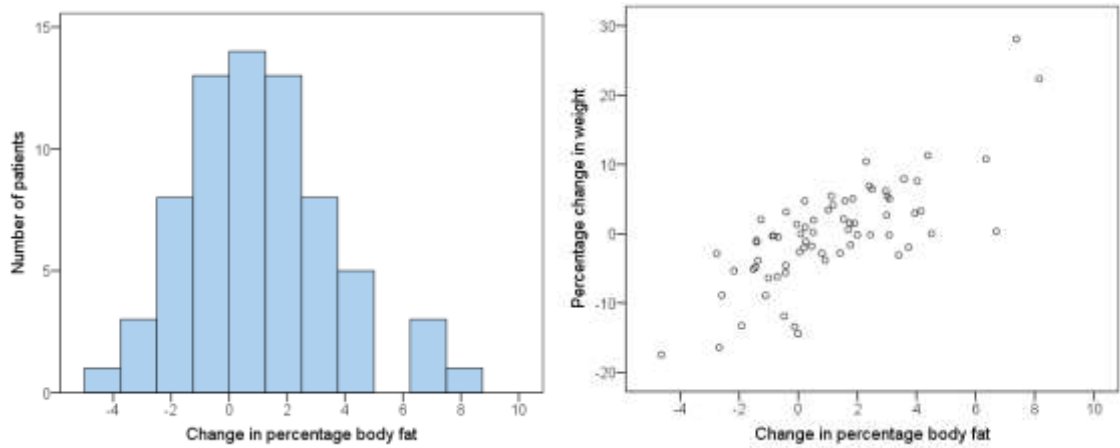


Figure 3.19: Change in anthropometric percentage body fat

### Bioelectrical impedance analysis

The mean change in BIA percentage body fat was  $0.54 \pm 2.8\%$  (range -8.2 to 10.1%;  $n=122$ ; Figure 3.20). There was a significant correlation between change in body fat and weight ( $r=0.633$ ;  $p<0.01$ ; Figure 3.20).

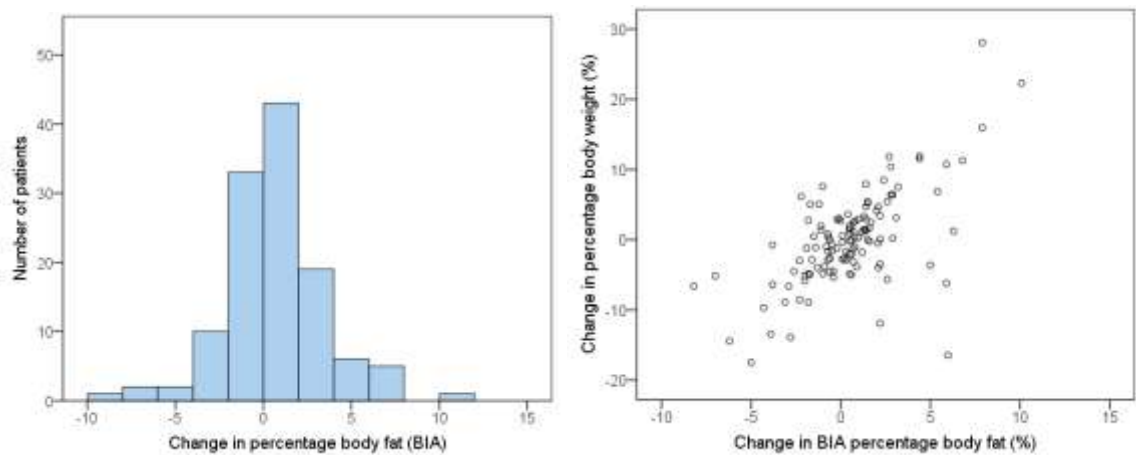


Figure 3.20: Change in BIA percentage body fat

The mean change in the phase angle was  $0.03 \pm 0.39$  (range -0.9 to 1.5;  $n=122$ ; Figure 3.21). There was a significant correlation between changes in weight and phase angle ( $r=0.420$ ;  $n=122$ ;  $p<0.01$ ; Figure 3.21).

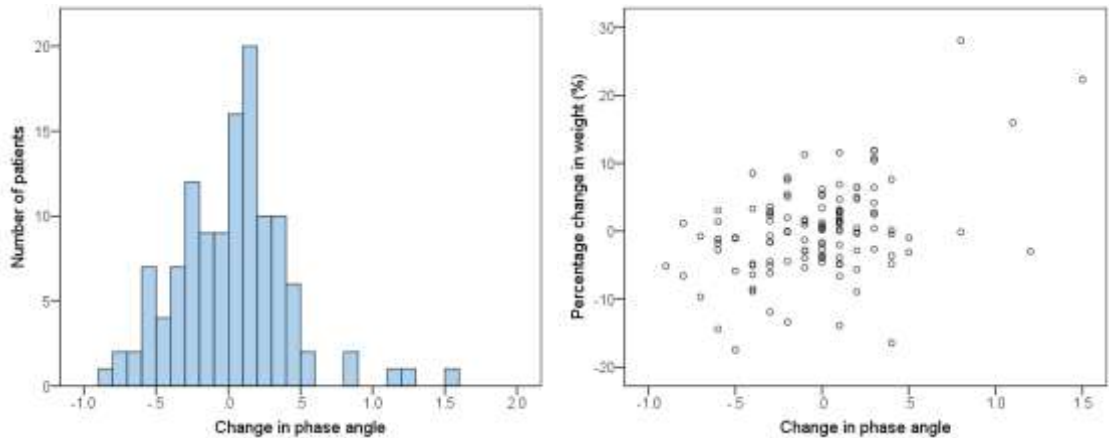


Figure 3.21: Change in phase angle

### Change in BIA compared to anthropometric percentage body fat

Repeat BIA and anthropometric percentage body fats were available for 66 patients. For these patients, the mean anthropometric change in body fat was  $0.8 \pm 2.9$  (range -6.2 to 10.1) and the mean change in BIA body fat was  $1.2 \pm 2.5$  (range -4.6 to 8.2). There was a significant correlation between changes in BIA and anthropometric body fats ( $r=0.487$ ,  $p<0.01$ ; Figure 3.22). Agreement analysis showed an ICC of 0.48 (95% limits of agreement = -16.8 to 8.0).

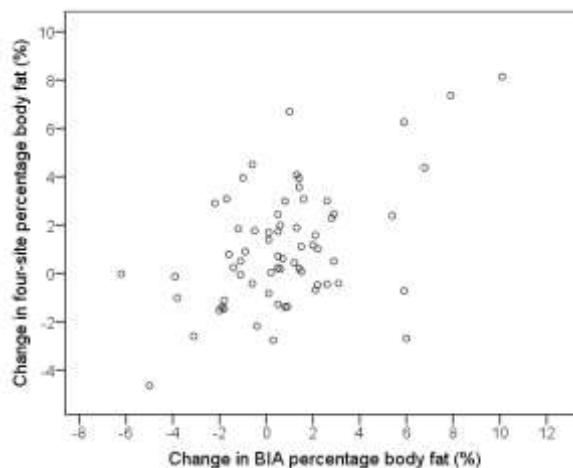


Figure 3.22: Changes in anthropometric and BIA percentage body fat

There was also a correlation ( $r=0.897$ ;  $n=66$ ;  $p<0.01$ ) between the difference in BIA and anthropometric body fats at recruitment and follow-up. Thus, the biggest discrepancies were in the same patients. The ICC was 0.89 (95% limits of agreement = -5.0 to 5.7). The 3 patients with the greatest increase in discrepancy all had a substantial weight loss. There



was no significant relationship between changes in weight and discrepancies in BIA and anthropometric measurements ( $r=0.046$ ;  $p=0.71$ ).

### 3.6.13 Change in non-nutritional assessments

This section describes the change in follow-up non-nutritional measures.

#### Skin score

The mean total skin score was  $6.8\pm 7.3$  (range 0 to 34;  $n=125$ ). The mean change was  $-0.5\pm 2.6$  (range -12 to 6;  $n=124$ ). Skin score was unchanged in 41% of patients.

#### Oral aperture

The mean inter-incisor distance was  $36.3\pm 8.43$ mm (range 14 to -54,  $n=117$ ). Mean change was  $2.32\pm 2.0$ mm (range -8mm to 12mm;  $n=117$ ).

#### Scleroderma health assessment questionnaire

The mean HAQ score was  $1.27\pm 0.81$  (range 0 to 3;  $n=127$ ). The mean change was  $-0.63\pm 0.40$  (range -1 to +1;  $n=127$ ; Figure 3.23).

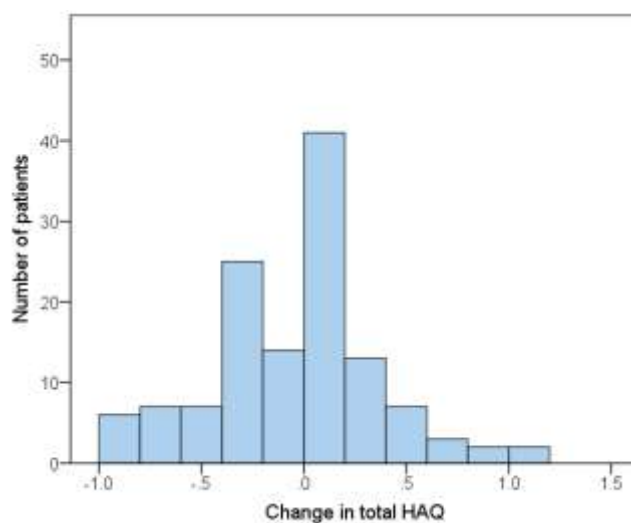


Figure 3.23: Change total HAQ

The mean pain score was  $1.04 \pm 0.91$  (range 0.0 to 2.9;  $n=127$ ) and the mean change was  $-0.04 \pm 0.73$  (range -2.9 to 2.2;  $n=125$ ). The mean GI score was  $0.89 \pm 0.91$  (range 0.0 to 2.9;  $n=127$ ) and the mean change was  $0.07 \pm 0.75$  (range -2.1 to 2.1;  $n=125$ ).

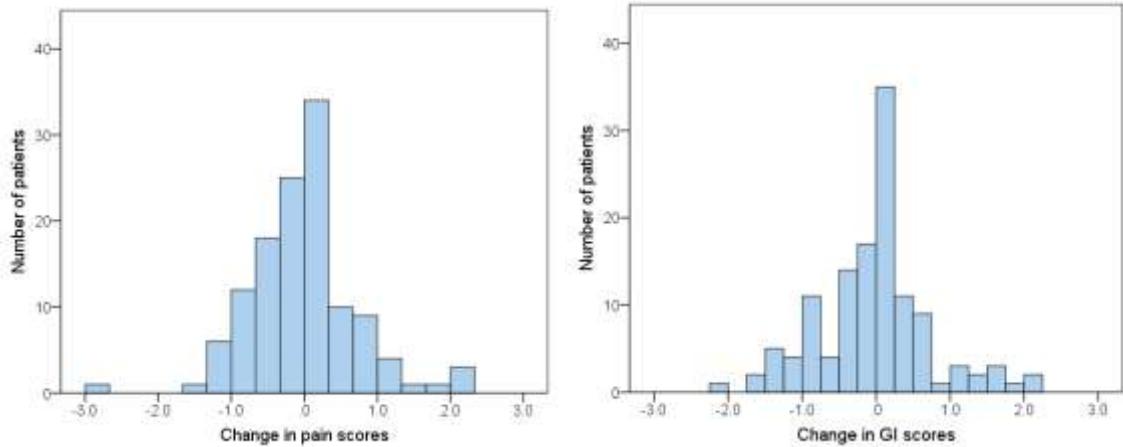


Figure 3.24: Changes in pain and GI SHAQ scores

The mean respiratory score was  $1.05 \pm 0.90$  (range 0.0 to 3.0;  $n=127$ ) and the mean change was  $0.07 \pm 0.65$  (range -1.7 to 2.1;  $n=124$ ). The mean global disability score was  $1.29 \pm 0.91$  (range 0.0 to 3.0;  $n=127$ ) and the mean change was  $0.04 \pm 0.62$  (range -2.9 to 1.7;  $n=125$ ).

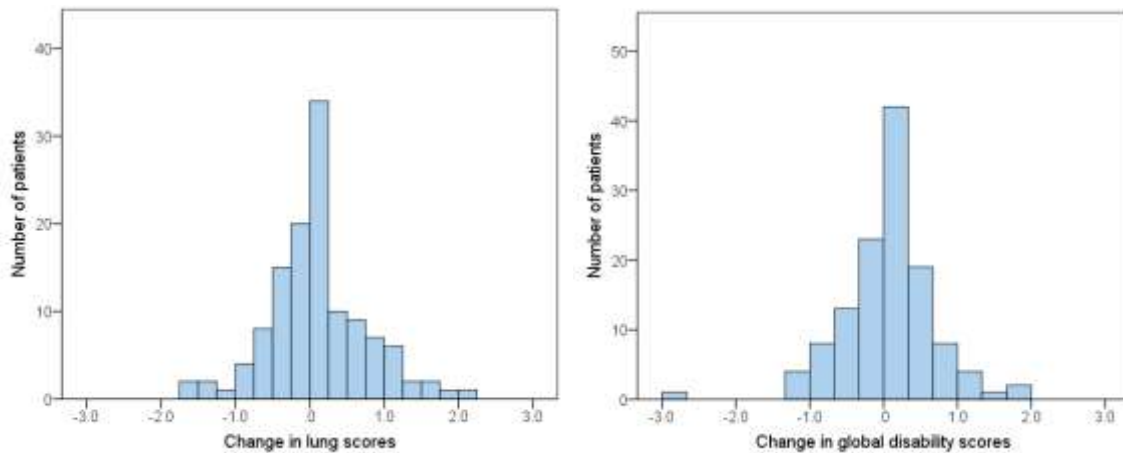


Figure 3.25: Changes in lung and global disability SHAQ scores

### **Gastrointestinal symptom score**

Most patients' scores did not change significantly between recruitment and follow-up (Figures 3.26-28). The mean change in reflux was  $-0.03 \pm 0.47$  (range -1.38 to 1.63). The mean change in distension/bloating was  $-0.08 \pm 0.64$  (range -2.75 to 2.25).

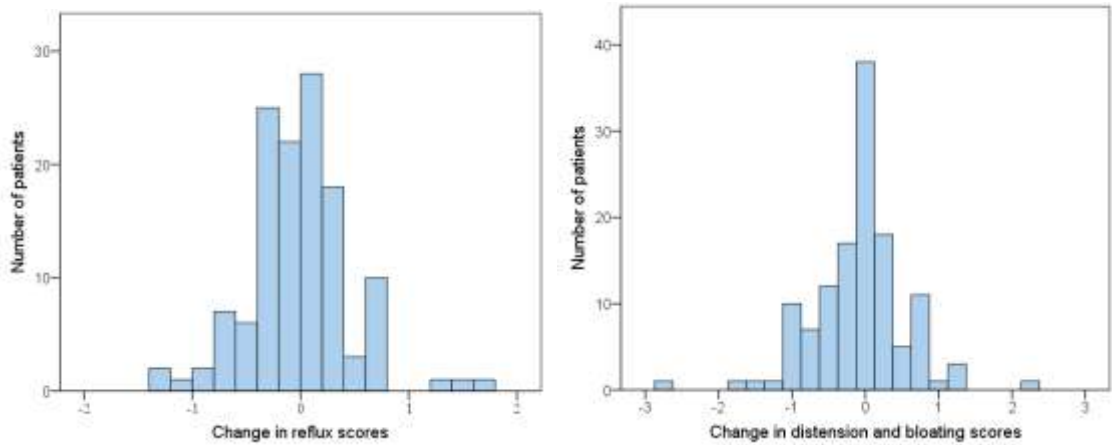


Figure 3.26: Changes in reflux and distension/bloating scores

The mean change in the faecal soilage was  $-0.01 \pm 0.65$  (range -2.00 to 3.00). The mean change in diarrhoea was  $0.07 \pm 0.65$  (range -1.50 to 2.00).

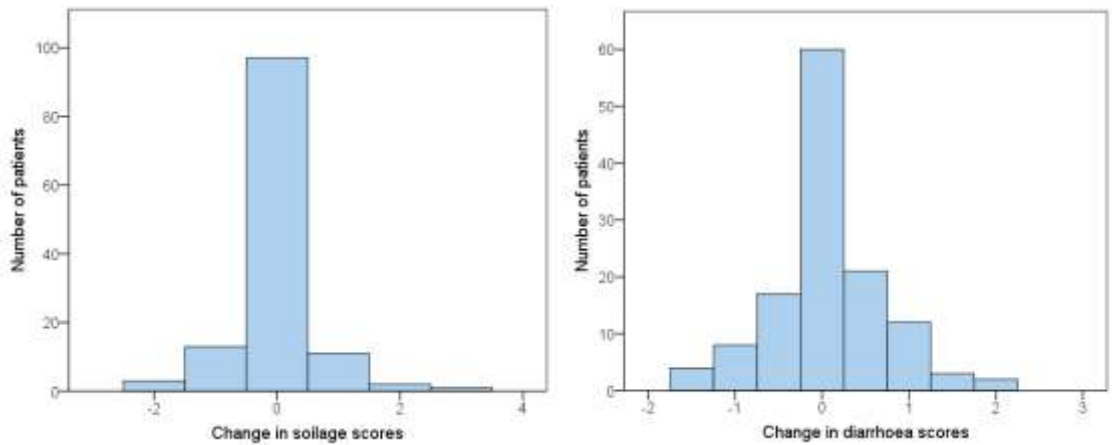


Figure 3.27: Changes in soilage and diarrhoea scores

The mean change in social functioning was  $-0.03 \pm 0.46$  (range -1.50 to 1.34). The mean change in emotional well-being was  $0.05 \pm 0.68$  (range -1.88 to 3.00).

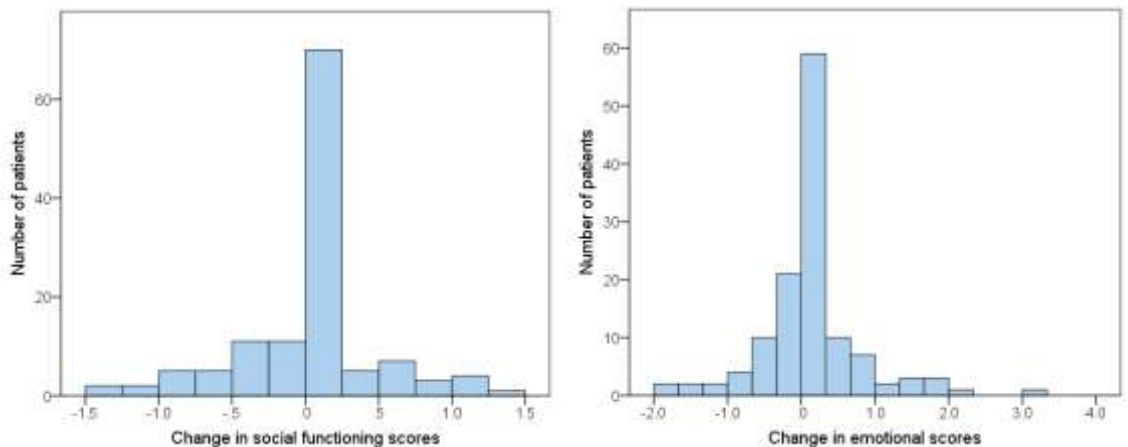


Figure 3.28: Changes in social functioning and emotional well-being scores

The mean change in constipation was  $-0.06 \pm 0.56$  (range -2.00 to 1.50). The mean change in total GI score was  $0.00 \pm 0.34$  (range -1.17 to 1.15).

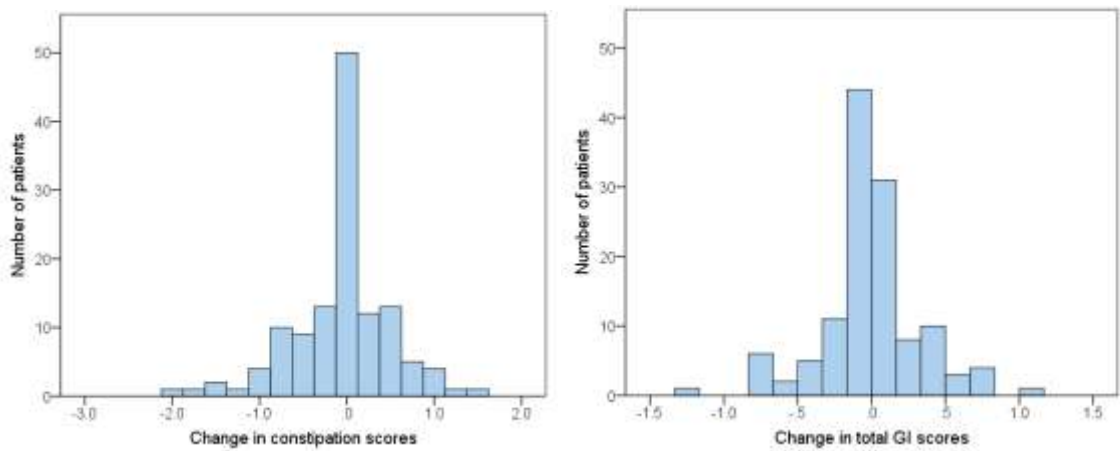


Figure 3.29: Changes in constipation and total GI scores

### 3.6.14 Change in nutritional status compared to baseline measures

This section compares actual percentage unintentional weight change to baseline measures. Patients with intentional loss were excluded as their weight loss was not disease-related.

### Systemic sclerosis demographics

There was a significant correlation between weight change and age ( $r=-0.216$ ;  $n=120$ ;  $p=0.02$ ), but not time from onset of Raynaud's ( $r=-0.020$ ;  $n=119$ ,  $p=0.83$ ) or SSc ( $r=-0.083$ ,  $n=120$ ,  $p=0.37$ ).

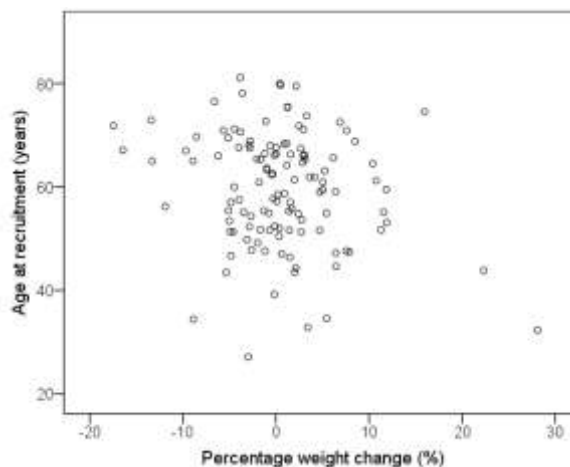


Figure 3.30: Age against unintentional weight change

There were no significant differences (t-test) in mean weight change with gender (males  $0.6\pm 5.7\%$ ,  $n=17$  vs. females  $0.5\pm 6.6\%$ ,  $n=103$ ;  $p=0.95$ ), sub-type (lcSSc  $0.7\pm 4.9\%$ ,  $n=87$  vs. dcSSc  $0.1\pm 9.6\%$ ,  $n=33$ ;  $p=0.73$ ), anti-centromere antibody (positive  $-0.2\pm 4.6\%$ ,  $n=38$ ; vs. negative  $0.9\pm 7.3\%$ ,  $n=81$ ;  $p=0.31$ ), anti-topoisomerase antibody (positive  $1.0\pm 6.5\%$ ,  $n=18$  vs. negative  $0.5\pm 6.5\%$ ,  $n=100$ ;  $p=0.79$ ) or steroid usage (taking  $2.2\pm 9.0\%$ ,  $n=29$  vs. not taking  $-0.03\pm 5.4\%$ ,  $n=91$ ;  $p=0.21$ ).

### **Medsger disease severity**

There were no significant correlations between weight change and general ( $s=-0.030$ ;  $n=125$ ;  $p=0.74$ ), peripheral vascular disease ( $s=0.044$ ;  $n=127$ ;  $p=0.53$ ), skin ( $s=0.077$ ;  $n=126$ ;  $p=0.39$ ), joint/tendon ( $s=0.028$ ;  $n=112$ ;  $p=0.77$ ), muscle ( $s=-0.10$ ;  $n=107$ ;  $p=0.31$ ), GI ( $s=-0.037$ ;  $n=127$ ;  $p=0.68$ ), respiratory ( $s=0.055$ ;  $n=126$ ;  $p=0.54$ ) or cardiac ( $s=-0.036$ ;  $n=125$ ;  $p=0.69$ ) Medsger organ severity, but there was between weight change and Medsger renal organ severity ( $s=-0.22$ ;  $n=126$ ;  $p=0.01$ ).

### **Gastrointestinal manifestations**

There were no significant differences (t-test) in mean weight change with oesophageal (with  $0.6\pm 5.3\%$ ,  $n=63$  vs. without  $0.4\pm 7.6\%$ ,  $n=57$ ;  $p=0.91$ ) or small intestinal (with  $-0.9\pm 6.3\%$ ,  $n=21$  vs. without  $0.8\pm 6.5\%$ ,  $n=99$ ;  $p=0.26$ ) involvement.

### **Scleroderma health assessment questionnaire**

There were no correlations between weight change and total HAQ ( $s=-0.018$ ;  $n=117$ ;  $p=0.85$ ) or SHAQ pain ( $s=0.028$ ;  $n=118$ ;  $p=0.77$ ), GI ( $-0.033$ ,  $n=118$ ;  $p=0.72$ ), lung ( $-0.040$ ;  $n=117$ ;  $p=0.67$ ) or global disability ( $-0.034$ ;  $n=118$ ;  $p=0.72$ ) scores.

### **Gastrointestinal symptoms**

There were no correlations between weight change and UCLA reflux ( $s=0.036$ ;  $n=120$ ;  $p=0.70$ ), distension/bloating ( $s=-0.040$ ;  $n=120$ ;  $p=0.67$ ), soilage ( $-0.007$ ;  $n=120$ ;  $p=0.94$ ), diarrhoea ( $s=-0.138$ ;  $n=120$ ;  $p=0.13$ ), social functioning ( $s=-0.126$ ;  $n=120$ ;  $p=0.17$ ),

emotional well-being ( $s=-0.106$ ;  $n=120$ ;  $p=0.25$ ), constipation ( $s=0.080$ ;  $n=120$ ;  $p=0.39$ ) or total GI ( $s=-0.037$ ;  $n=120$ ;  $p=0.69$ ).

### **Oral aperture**

There was no correlation between weight change and inter-incisor distance ( $s=-0.061$ ;  $n=115$ ;  $p=0.52$ ).

### **Skin involvement**

There was no correlation between weight change and skin score ( $s=0.070$ ;  $n=117$ ;  $p=0.45$ ).

### **Nutritional biochemistry**

There were no significant correlations between biochemical results and weight change.

<b>Test</b>	<b>Patients</b>	<b>Pearsons (r)</b>	<b>p value</b>
Albumin	119	0.280	0.77
Corrected calcium	106	0.060	0.54
Magnesium	112	-0.061	0.52
Zinc	77	-0.125	0.28
Selenium	36	0.149	0.39
Vitamin D without medications	67	-0.002	0.99
Iron	78	0.001	0.99
Iron saturation	77	-0.009	0.94
Total iron binding capacity	77	-0.014	0.90

Table 3.11: Blood results versus unintentional weight change

### **3.6.15 Change in nutritional status compared to change in outcome**

This section compares percentage unintentional weight change to changes in disease. Intentional weight changes were excluded.

### **Scleroderma health assessment questionnaire**

There were no correlations between weight change and change in HAQ score ( $s=-0.009$ ;  $n=120$ ;  $p=0.92$ ) or pain ( $s=0.018$ ;  $n=118$ ;  $p=0.84$ ), GI ( $s=0.037$ ;  $n=118$ ;  $p=0.69$ ), lung ( $s=-0.085$ ;  $n=117$ ;  $p=0.36$ ) or global disability ( $s=-0.069$ ;  $n=118$ ;  $p=0.46$ ) SHAQ scores.

### **Gastrointestinal symptoms**

There were no correlations between weight change and change in UCLA distension/bloating ( $s=0.048$ ;  $n=120$ ;  $p=0.60$ ), soilage ( $s=0.027$ ;  $n=120$ ;  $p=0.77$ ), diarrhoea ( $s=0.012$ ;  $n=120$ ;  $p=0.90$ ), emotional ( $s=-0.107$ ;  $n=120$ ;  $p=0.25$ ), constipation ( $s=-0.106$ ;  $n=120$ ;  $p=0.25$ ) or total GI ( $s=0.79$ ;  $n=119$ ;  $p=0.39$ ) scores. However, there were significant correlations between weight change and change in reflux ( $s=0.195$ ;  $n=120$ ;  $p=0.03$ ) and social functioning ( $s=0.212$ ;  $n=119$ ;  $p=0.02$ ) scores.

### **Skin involvement**

There was no correlation between changes in weight and skin score ( $s=0.053$ ;  $n=117$ ;  $p=0.57$ ).

### **Oral aperture**

There was a significant correlation between changes in weight and inter-incisor distance ( $s=0.327$ ;  $n=111$ ;  $p<0.01$ ; Figure 3.31).

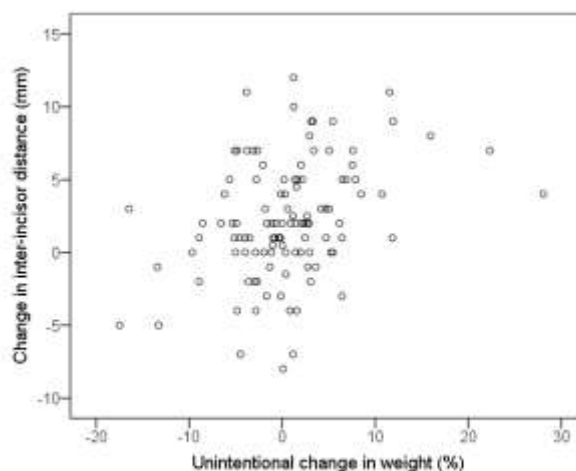


Figure 3.31: Inter-incisor change against unintentional weight change

### **3.6.16 Nutritional interventions**

#### **Dietitian**

Excluding obesity management, at recruitment 46 patients had been reviewed by a dietitian. Time since the last review was unknown. Dietetic contact was either a single or multiple consultations. Nine patients were cared for by the Intestinal Failure Team.

During follow-up, 16 patients were reviewed by a dietitian. Five were new referrals. Twenty three patients who had previously seen a dietitian were not re-reviewed.

#### **Oral supplement**

At recruitment, 11 patients took daily oral nutritional supplements and 5 took them intermittently. At follow-up, 13 patients had daily oral supplements and 4 intermittently.

#### **Enteral tube**

At recruitment, 1 patient received supplemental nutrition (600kcal/day) via a post-pyloric feeding tube due to persistent vomiting. This patient was followed-up. One more patient commenced gastrostomy feeding (6 night/week) shortly after recruitment.

Prior to recruitment, 8 patients had previously received percutaneous enteral tube feeding. Four of these subsequently proceeded to HPN due to a failure to tolerate enteral feeding. Four patients had required enteral tube feeding due to dysphagia. Three of these had overlap myositis and 3 were also fed percutaneously for less than 2 years.

#### **Parenteral nutrition**

At recruitment, HPN (3 to 7 nights/week) was prescribed for 8 patients. One patient had previously needed HPN. All were included in the retrospective review (Chapter 2). No new patients commenced HPN during follow-up. Of the 8 patients on HPN, 3 died before follow-up, 2 were lost to follow-up and 3 were restudied.

Excluding those patients on HPN, at recruitment no one recalled having inpatient PN within the last 5 years. However, during follow-up, one patient required inpatient PN for acute bowel obstruction.



### **Change in weight and nutritional interventions**

Of the 16 patients reviewed by a dietitian during follow-up, 3 were on HPN and 1 had percutaneous feeding. There was a significant difference (t-test;  $p=0.04$ ) in the mean percentage unintentional weight change between those patients who had received dietetic review ( $-2.5\pm 6.7\%$ ;  $n=16$ ) and those who had not ( $1.0\pm 6.4\%$ ;  $n=104$ ). After excluding patients receiving HPN or tube feeding, the difference in mean percentage unintentional weight loss of patients reviewed ( $-3.5\pm 7.2\%$ ;  $n=12$ ) and patients not reviewed ( $1.0\pm 6.3\%$ ;  $n=104$ ) increased in significance ( $p=0.02$ ).

In total, 16 patients were supported by HPN, percutaneous enteral feeding or daily oral supplements. However, there was no statistically significant difference ( $p=0.40$ ) between the mean percentage weight change of those patients receiving ( $-0.8\pm 7.6\%$ ;  $n=16$ ) and not receiving ( $0.7\pm 6.3\%$ ;  $n=104$ ) support.

## **3.7 Discussion**

This is the first study to evaluate different methods of anthropometry over time with a view to identifying accurate tools for clinical use. It has also been an observational tool over time to assess changing clinical manifestations with baseline and changing nutritional status.

### **3.7.1 Baseline BMI and 'MUST'**

Only 6.5% of patients were underweight (World Health Criteria - BMI below  $18.5\text{kg/m}^2$ ). However, 14% of patients were at medium and 12% at high risk of malnutrition. These figures are lower than those from Baron et al, but greater than those from Cereda et al. [36, 206]. This difference may be due to the differing study burdens and patient cohorts studied. The least burdensome study by Baron et al, may have had the most representative recruitment. Patients recruited to that study were younger and more likely to have dcSSc than in the present study.

Similar to other studies, almost 30% were overweight and 11% were obese [211, 370]. These patients may develop nutritional problems, but will be less easily identified by nutritional screening.

### **3.7.2 Baseline MUAA**

The greatest proportion of patients with a BMI less than 18.5kg/m<sup>2</sup> had a low MAC ( $\leq 5^{\text{th}}$  centile), followed by TSF and MAMC. Thus, of the MUAA measures, low MAC showed greatest concordance with low BMI. However, MAC did not detect all patients with a low BMI. Conversely, not all patients with a low MUAA had low BMI, with TSF being the least specific. MAC, in contrast to TSF and MAMC, does not require skinfold thickness measurement, and is hence not impeded by cutaneous involvement or fibromyalgia. It is quick and simple to perform and could aid clinical assessment.

### **3.7.3 Baseline BIA and 4-site anthropometry**

BMI showed a stronger correlation with anthropometric percentage body fat than BIA. Both percentage body fats were strongly correlated with each other, but their absolute values differed. BIA body fats often exceeded those of anthropometry. The variation may have been due to inaccuracies in one or both measurements. As with MUAA, anthropometric measures may have been affected by cutaneous involvement. However, the discrepancy did not correlate with cutaneous involvement. Potential BIA confounders included steroids, hydration status and fibrosis. Previously, worsening skin fibrosis has been linked to increasing BIA body fat despite weight loss [210]. The present study was unable to detect any effect of lung fibrosis or steroid use, but did note an association with small intestinal involvement.

PhA is influenced by disease and nutrition [373]. PhA had a weaker correlation with BMI than BIA or anthropometric body fats. Thus, this disagrees with previous reports that malnutrition in SSc is best reflected by PhA [204].

In summary, for clinical use BIA, with its additional costs and time requirements, offers little benefit over that of 4-site anthropometry.

### **3.7.4 Change over time**

Despite 26% of the original cohort being at risk of malnutrition, few of the followed up patients had unintentional weight loss at 12 months (mean change  $+0.3 \pm 5.7\%$ ). Efforts made to maintain weight were not quantified. A higher proportion of patients at high risk were lost to follow-up. These and other patients lost to follow-up may have had weight

loss, but this cannot be confirmed. There was a subtle suggestion that a higher proportion of patients at high risk may have died. Previous studies have not noted this despite their longer follow-up [206]. Of the patients with low risk, several developed medium or high risk. Their nutritional decline had not been predicated.

With regard to serial MUAA assessment, MAC showed the greatest correlation with percentage weight change. It would have been least affected by SSc cutaneous manifestations. However, TSF also showed a strong correlation, suggesting that when confounding skin influences remain stable, change in TSF may be clinically useful.

Change in weight showed a stronger correlation with change in anthropometric than BIA body fat. Analysis showed reasonable agreement between changes in individuals' BIA and anthropometric values. Baseline and follow-up discrepancies also showed strong agreement. However, as BIA and anthropometric repeat values still differed, for clinical use, temporal patient assessments should use only one method.

There was a weaker correlation between changes in PhA and weight. Thus, PhA was not solely influenced by weight. Substantial changes may be more indicative of disease status.

### **3.7.5 Demographics and nutritional status**

Baseline nutritional status, as defined by BMI and/or 'MUST', showed no associations with age, gender, subtype or antibody status. BMI, but not 'MUST', showed negative correlations with time from Raynaud's phenomenon and SSc onset. However, there was no association between weight change and time from onset, but there was with age. Few patients studied had early dcSSc. Thus, there is insufficient evidence to dispute a previously shown link between shorter disease duration in patients with dcSSc and increased nutritional risk [36]. Thus, clinically, greater nutritional attention should be directed towards patients with early dcSSc, long disease durations and advancing age.

### **3.7.6 GI manifestations and nutritional status**

Oral involvement may, in theory, affect dietary choices and hinder nutrient intake. Smaller baseline oral apertures were significantly linked to worsening baseline nutritional status, but not changing nutritional status. The link to worse baseline status has been previously

detected [36]. In addition, changing oral apertures showed a significant positive correlation with weight change. Thus, perhaps clinically, inter-incisor distance could be an easily performed indirect measure of nutritional status.

Oesophageal involvement causes dysphagia and reflux. Despite this study including many patients with oesophageal involvement, there was no association evident with baseline nutritional status or follow-up weight change. As disease was only classed as present or absent, effects of severity were lost. Despite patients reporting a range of symptoms, there were also no associations with baseline UCLA reflux domain scores. This is not the first study to find no association between baseline 'MUST' and oesophageal symptoms [36]. Unexpectedly, there was a weak positive correlation between changes in both weight and reflux score. Thus, perhaps the subjectively completed UCLA questionnaire hindered the detection of associations between individuals, but was a better tool when used for intra-individual comparisons. Or perhaps the questionnaire's questions are not sufficiently weighted to detect those aspects of oesophageal involvement which affect nutrition. However, unless dietary adaptations have occurred, these theories fail to account for the converse relationship to that expected being found. Thus, clinically, clinicians should not rely on UCLA reflux score to exclude nutritional detriment from oesophageal involvement.

Worse overall GI involvement (Medsger) was associated with worse baseline nutritional status, but not weight change. Patients with only oesophageal involvement have lower Medsger graded severity than those with small intestinal involvement [124]. Small intestinal involvement can result in abdominal distension and malabsorption. Small intestinal involvement was associated with a worse baseline nutritional status, but not weight change. The association with 'MUST' has been previously shown [36]. Despite this, there were no correlations between baseline UCLA diarrhoea or distension/bloating scores and baseline nutritional status (BMI, 'MUST') or follow-up weight change. However, at baseline, high nutritional risk patients had 'somewhat worse' diarrhoea and distension/bloating than those with medium, but not low, risk. Furthermore, there were no correlations between changes in UCLA scores and weight. This absence of associations is in spite of a previous study showing associations between 'MUST' and patient reported diarrhoea and requirement of antibiotics for diarrhoea [36]. Thus, it would appear that the ULCA questionnaire lacks the sensitivity to detect those aspects of small intestinal involvement associated with nutritional risk. However, the relatively short follow-up may

have also hindered significant comparisons. Thus in summary, despite small intestinal involvement being associated with nutritional risk, clinicians should not rely upon the small intestinal domains of the ULCA questionnaire to screen for those patients at risk of nutritional compromise.

### **3.7.7 Non-GI manifestations and nutritional status**

Theoretically, worsening disease-related manifestations would be associated with worsening nutritional status. This study showed significant associations between worsening baseline nutritional status ('MUST' and BMI) and the worsening general (haemoglobin and haematocrit only) and respiratory statuses (Medsger). Similar correlations were also detected between 'MUST' and muscle involvement, and between BMI and cardiac and skin scores. The Medsger skin correlation was not supported by any correlations with Rodnan skin scores. All associations were relatively weak. Despite this, the links to both cardiac and respiratory disease are supported by other studies [370, 374]. Associations may have been weakened by the limited number of patients allocated to some Medsger and 'MUST' categories. With the exception of renal disease, no associations were detected between baseline Medsger scores and follow-up weight change. This was likely due to the very low number of patients with renal involvement leading to an underpowered analysis. This absence of any other associations may be due to the relatively short follow-up interval.

Despite patients reporting a range of HAQ and SHAQ responses, there were no significant associations with baseline nutritional status or change in weight. In addition, there were no associations between changes in weight and functional scores. This concurs with the findings of a previous study [48].

Thus, worsening SSc-related respiratory and cardiac involvement appears associated with nutritional decline, and therefore clinicians should monitor these patients more closely, to facilitate its early detection. Also, there is no evidence to support the HAQ and SHAQ for the detection or prediction of nutritional compromise.

### **3.7.8 Nutritional biochemistry and nutritional status**

There were significant correlations between albumin and baseline nutritional status (BMI and 'MUST'), but not weight change. This supports the finding of other studies, indicating albumin to be a poor marker of malnutrition [67]. Instead it is acting as an acute phase protein falling in the presence of inflammation and/or infection rather than nutritional decline.

CRP was raised in 17%, but no correlations were detected between CRP and either baseline nutritional status (BMI or 'MUST') or weight change. Thus, like other acute phase proteins, the rise in CRP was linked to inflammation/infection rather than nutrition. Indeed, previous studies have confirmed CRP to be elevated in a quarter of patients, especially in early disease, and to be correlated with disease activity [117].

ESR was elevated in 32% and correlated with worse baseline nutritional status (BMI and 'MUST'), but not weight change on follow-up. ESR is known to independently correlate with disease activity in patients with both lcSSc and dcSSc [208]. Thus, it is linked to disease activity, which may be linked to nutrition [206].

Vitamin D deficiency and insufficiency were common. In unsupplemented patients, vitamin D negatively correlated with BMI. As the primary source is not dietary, the cause is unlikely to be nutritional. Deficiency has previously been linked to other disease manifestations including cutaneous involvement [221].

Thus, albumin, ESR, CRP, iron studies and vitamin D are all poor indicators of nutritional status and should not be used for this indication.

### **3.7.9 Nutritional interventions**

There was no detectable correlation between change in weight and change in UCLA emotional well-being scores, despite nutritional interventions being known to improve quality of life in patients with severe GI involvement [229]. However, for most patients changes in weight may have been insufficient to identify significant changes in quality of life.

There was a significant difference in weight changes between patients who had and had not seen a dietitian during follow-up. Patients under dietetic review experienced a mean

weight loss, while patients not under review did not. Though not proven, weight losing patients were likely being appropriately referred. The benefit of appropriate dietetic intervention is understood [207, 222].

### **3.7.10 Summary**

This study was conducted with the hope of improving the nutritional management of patients with SSc. It was envisaged that this could be accomplished by having a better understanding of clinically applicable methods of nutritional assessment and follow-up and by improving understanding of the predictors of nutritional decline.

Through this study, it has been noted that 'MUST' has a role, but due to its failure to identify all patients, should not be used in isolation. It has also intimated that MAC, 4-site anthropometry and oral aperture may have a role in the SSc clinic to facilitate nutritional assessment and follow-up. BIA with its additional costs and complexity has been shown to offer little additional benefit over that of 4-site anthropometry. In addition, with regard to nutritional assessment, it has confirmed that albumin and other acute phase proteins have no role. Finally, it has highlighted the need for clinicians to have increased vigilance for nutritional decline in the presence of small intestinal involvement and worsening cardiac and respiratory manifestations.

## **CHAPTER 4:**

# **ASSESSMENT OF DIET AND ENERGY**

## **EXPENDITURE**



## **4 Assessment of diet and energy expenditure**

### **4.1 Introduction**

Malnutrition is common in patients with SSc. However, the factors leading to the development of malnutrition have not been clearly defined. In particular, at the time this study was commenced, it had yet to be explored whether weight loss is due to a single driving force or was multi-factorial in origin. In particular, is weight loss purely due to reduced oral intake, perhaps secondary to GI manifestations, or is there also increased energy expenditure, and if so, is it active, resting, or both.

A recent cross-sectional study compared 61 patients with SSc to age and gender matched healthy participants, with statistically different BMIs [211]. It identified similar total energy, macronutrient and essential amino acid intakes and similar proportions that were physically active for at least 150 minutes per week (questionnaire assessed). Thus, patients' lower BMI were concluded to be despite, them having comparable energy intakes and active expenditures to controls. In addition, dietary intakes did not differ with GI involvement. Soluble fibre intake was noted to be significantly lower in patients with lcSSc than in healthy controls. However, intakes and expenditures were not compared between patients.

Lower total fibre intake was also demonstrated in smaller study comparing 30 patients with SSc and pronounced GI manifestations to matched healthy participants [47]. However, there was no difference between soluble and insoluble fibre. In addition, this study also failed to show any difference in energy intakes between patients and healthy participants, despite patients having lower MAMC. Another study, which compared patients with (n=24) and without (n=136) disease-related malnutrition (BMI<20kg/m<sup>2</sup> and/or spontaneous weight loss ≥10%) found no significant difference in energy intakes [48]. Energy intakes recorded via 3 day dietary records were also compared to predicted energy expenditures (Harris-Benedict equation) and intake was considered 'adequate' if ≥75% of predicted expenditure. No difference was found in the percentage of patients with and without disease-related malnutrition with 'adequate' intake. With the exception of anorexia and early satiety, there were no significant differences in the frequency of GI complaints between the groups. Thus, this study was unable to attribute disease-related malnutrition to dietary inadequacy. However, estimated requirements were solely derived from a generic equation.

Hence, the impact of dietary intake versus energy expenditure on the nutritional status of patients with SSc remains unclear. Thus, giving rise to the need to compare dietary intakes to measured expenditures. To-date, only one recent study has investigated expenditure in patients with SSc, and it did so using the SenseWear® Armband. That study compared the physical activity of 27 patients with SSc and preserved nutritional status to 11 matched healthy participants over at least 6 days [213]. Diet was not assessed. This study found that the patients with SSc performed less daily physical activity than the healthy participants, and that this reduction in activity (duration and level) occurred even in patients with very early respiratory involvement affecting DLCO. However, no associations were found between physical activity and other lung function markers (FVC, FEV, fibrosis on imaging) or nutritional status. There was also no difference in these variables between patients and volunteers, suggesting that patients may not have had sufficient involvement to detect associations.

The present study was conducted to further the understanding of the relationship between energy intakes and predicted and measured energy expenditures in patients with SSc. It was hoped that, by including patients with differing nutritional statuses and GI manifestations, this would help to define influence of these factors in the development of malnutrition.

## **4.2 Hypothesis**

The first hypothesis was that, in patients with SSc, dietary macronutrient and energy intakes would be related to GI symptoms (reflux and/or distension/bloating) and to GI involvement (oesophageal and small intestinal).

The second hypothesis was that dietary micronutrient deficiencies would be associated with biochemical deficiencies.

The third hypothesis was that active energy expenditure (i.e. physical activity) would be inversely linked to markers of worsening disease severity.

The fourth hypothesis was that predicted energy requirements, which have been used for comparison to intake in published studies, would be a poor estimate of expenditure in patients with SSc.

The fifth hypothesis is that energy expenditures and intakes would be similar in weight stable patients, and different in patients losing weight, and that these differences may be related to GI or other clinical manifestations.

### **4.3 Aims**

This study aimed to address the above listed hypotheses.

### **4.4 Ethical approval**

This study was granted ethical approval by the North West Ethics Committee (12/NW/0247).

### **4.5 Materials and methods**

#### **4.5.1 Study subjects**

Patients with SSc were recruited through the parallel-running GI involvement and nutritional status study (Chapter 3). The same inclusion and exclusion criteria applied.

#### **4.5.2 Subject selection**

Recruitment occurred during either the baseline or follow-up GI involvement and nutritional status study attendance. Initially, consecutive patients were approached when the SenseWear® Armband was available. Later, to optimise the recruitment of patients with a range of nutritional statuses and GI symptoms, patients with more pronounced GI symptoms and/or greater nutritional risk were preferentially approached.

#### **4.5.3 Study period**

Patients were studied over 3 consecutive days (two weekdays and one weekend day). This is the standard approach to improve assessment of normal intake and activity by including typical working and non-working days.

#### **4.5.4 Demographics, clinical details and nutritional status**

Demographic and clinical details (e.g. SSc disease characteristics) were recorded as per Chapter 3. Clinical assessments (height, weight, BMI, nutritional status ('MUST', BIA, MUAA) and oral aperture were made as per Chapter 3.

#### **4.5.5 Gastrointestinal symptoms**

The UCLA questionnaire was completed within 2 weeks of the planned study start date.

#### **4.5.6 Functional status**

The SHAQ was completed within 2 weeks of the planned study start date. The HAQ used in this was the shorter Disability Index questionnaire.

#### **4.5.7 Predicted energy expenditure**

Basal Metabolic Rate was calculated using Schofield's equation which includes age and gender specific co-efficients (Appendix 8) [175].

$$\text{basal metabolic rate (kcal/day)} = (A \times \text{weight (kg)}) + B$$

Equation 4.1: Schofield equation

Values were multiplied by 1.4, which is the agreed UK average Physical Activity Level. No allowances were made for diet-induced thermogenesis or additional stress factors [375].

#### **4.5.8 Measured energy expenditure**

##### **Equipment**

The intention was to measure energy expended whilst completing daily activities. The SenseWear® Armband (BodyMedia Inc, Pittsburgh, PA; Figure 4.1) was chosen as it is a specialist ambulatory monitor which has been extensively validated in healthy adults, with differing levels of exertion and of differing ages, and in the presence of chronic diseases (including rheumatoid arthritis and COPD) [376, 377]. In addition, studies in healthy

adults have shown an acceptable level of agreement with indirect calorimetry [378-380]. At the time that this study was conducted, the Armband had not been used in studies involving patients with SSc.

The Armband is worn on the upper left arm, irrespective of handedness (Figure 4.2). This position limits discomfort and does not impede normal activities [381]. It calculates energy expended using measures made by multiple specialist sensors, from which it records 32 times per second, using in-built software. It contains a 3-axis accelerometer which measures motion referenced against gravity. In addition, this accelerometer counts the number of steps by detecting and measuring the distinct patterns of movement made by walking and running. A heat flux sensor measures heat lost through convection to the external environment. A GSR sensor uses a low level voltage between the two electrodes to measure conductivity, which is affected by skin moisture (sweating). Skin temperature is measured.



Figure 4.1: SenseWear® Armband (a) and Armband skin contacts (b)

The Armband's associated software (SenseWear® Basic software version 7.0) reports the percentage of time that the Armband was worn during the recording period. Report intervals may be adjusted to encompass the whole study period or any sub-interval (e.g. 24 hours).

For the time within the recording period that the device was not worn, the software estimates the expended energy. The number of steps is reported. The software deduces total energy expenditure and the energy expended at different intensities (Metabolic Equivalent of Task (METs)). A MET is the ratio of a person's working metabolic rate

relative to their resting metabolic rate. One MET is the energy cost of sitting still at rest and is equivalent to 3.5ml of oxygen per kg body weight per minute (approximately 1.2kcal/min for a 70kg person) [382]. Sedentary activities are defined as  $\leq 1.5$  METs. Light intensity activity is classed as 1.1 to 2.9 METs, moderate intensity as 3.0 to 5.9 METs and vigorous intensity as  $>6.0$  METs. Light intensity activities include light housework and slow walking ( $<2.5$  miles/hour on the flat).

### **Protocol**

Prior to recording, weight, height, handedness, gender and smoking status were entered into the Armband. Participants were instructed to wear the Armband correctly orientated on their left upper arm, with the metal contact against their clean, dry skin.



Figure 4.2: SenseWear® Armband on the left upper arm

Patients were requested to start the Armband recording period on the morning of the dietary recording and to finally take it off at least 72 hours after they first placed it on their arm. As it was not water-proof, they removed it when bathing.

### Analysis

Upon completion of the study, the Armband was returned and the data downloaded to the analysis programme (SenseWear® 7.0, BodyMedia Inc). The maximum period analysed

was 3 full days (4320 minutes). Any data recorded after 4320 minutes from first application was discarded. For patients who had stopped prematurely (<4320), the whole time recording was analysed. The time (minutes) and the percentage of the 3 days that the Armband was worn were recorded. Average daily energy expenditure was calculated to allow comparison to daily predicted requirements. Average times spent expending energy at METs of  $\leq 1.0$ , 1.1 to 2.9, 3.0 to 5.9 and  $>6.0$  were noted. Any time within the recording period when the Armband was not worn was assumed to be at 1.0 METs.

#### **4.5.9 Dietary intake assessment**

##### **Assessment method**

The 3 day estimated food diary method was chosen as it is less burdensome than a weighed food diary, whilst still offering acceptable results [179, 383]. However, this method relies on individuals' ability to estimate portion sizes. To facilitate this, a selection of validated food portion images, illustrating portion sizes of known weights, was provided [384]. Images were used under licence of Crown copyright (Appendix 9).

##### **Protocol**

Patients were asked to record their food and drink consumption, as accurately as possible, over the same 3 day period that they were wearing the SenseWear® Armband. Patients were asked not to modify their normal diet.

Patients were given an instruction sheet, sample and blank diet diary and sample portion size pictures (Appendix 9). They were requested to record the day and time of consumption and to be as descriptive as possible when documenting the preparation (e.g. fried, baked, boiled) and item of food or drink. To improve accuracy, they were asked to record items in their diary as soon as possible. Patients were asked to record the quantity or volume as accurately as possible, without weighing. If weights were easily available from packaging, they were asked to document these. If not available, and if relevant, they were asked to refer to the food portion images. If more than one estimate of portion size was given for a single food item, the most accurate was used. Completed diaries were returned via post with the Armband.

### **Nutritional biochemistry**

Biochemical results (zinc, magnesium, selenium, iron studies, calcium, phosphate and vitamin D) were available for some patients from their baseline assessment in the GI involvement and nutritional status study (Chapter 3). Analysis methods are described in Chapter 3. Results were compared to dietary intake.

### **Dietary analysis**

Diet diaries were analysed for their nutritional content using Microdiet version 2.0 (Downlee Systems Ltd, High Peak, UK). It used nutrient data from validated food composition tables which describe the composition of standard foods [385]. When nutrient data were unavailable for any comparable food item, commercial values were used (e.g. for nutritional supplements and uncommon processed foods). However, commercial values did not normally include fluid and micronutrient details. Qualitative portion size estimates (e.g. large, average, medium, small) were interpreted using agreed estimated food portion sizes [386].

The percentage of daily energy intake from different nutrient groups was determined. Daily micro- and macronutrients were compared to UK RNIs [177].

#### **4.5.10 Data handling and statistical methods**

Data was compiled using Microsoft Excel. Total dietary nutrients and expended energies were converted into daily averages for comparison to predicted and reference requirements. Statistical analysis was performed using SPSS (Version 22) and StatsDirect (Version 3). Reference values used are listed in Appendix 10.

Correlations (Spearman (s) and Pearson (r)) and agreement (ICC) between energy intakes, predicted requirements and expenditures were sought. Micronutrient intakes were compared to RNIs using Chi-squared.

Student's t-test was used to test for any significant differences in mean expended energies between patient sub-groups (e.g. sub-type, gender, with and without weight loss).

Correlation (Spearman or Pearson) tests were used to identify any significant relationships



between total expended energy or physical activity and clinical manifestations, nutritional measures (i.e. BMI, 'MUST', body composition) or functional or symptom scores.

The discrepancy between dietary and expended energies was calculated. Relationships between this discrepancy and a range of clinical manifestations and measures of nutritional status were sought using either Student's t-tests or correlation (Spearman or Pearson) tests.

For all statistical analyses, the cut-off for a significant difference was accepted as a p value of  $\leq 0.05$ .

## 4.6 Results

### 4.6.1 Patients recruited

Forty two patients were recruited (18<sup>th</sup> May 2012 to 30<sup>th</sup> May 2014). However, only 36 were included (Figure 4.3).

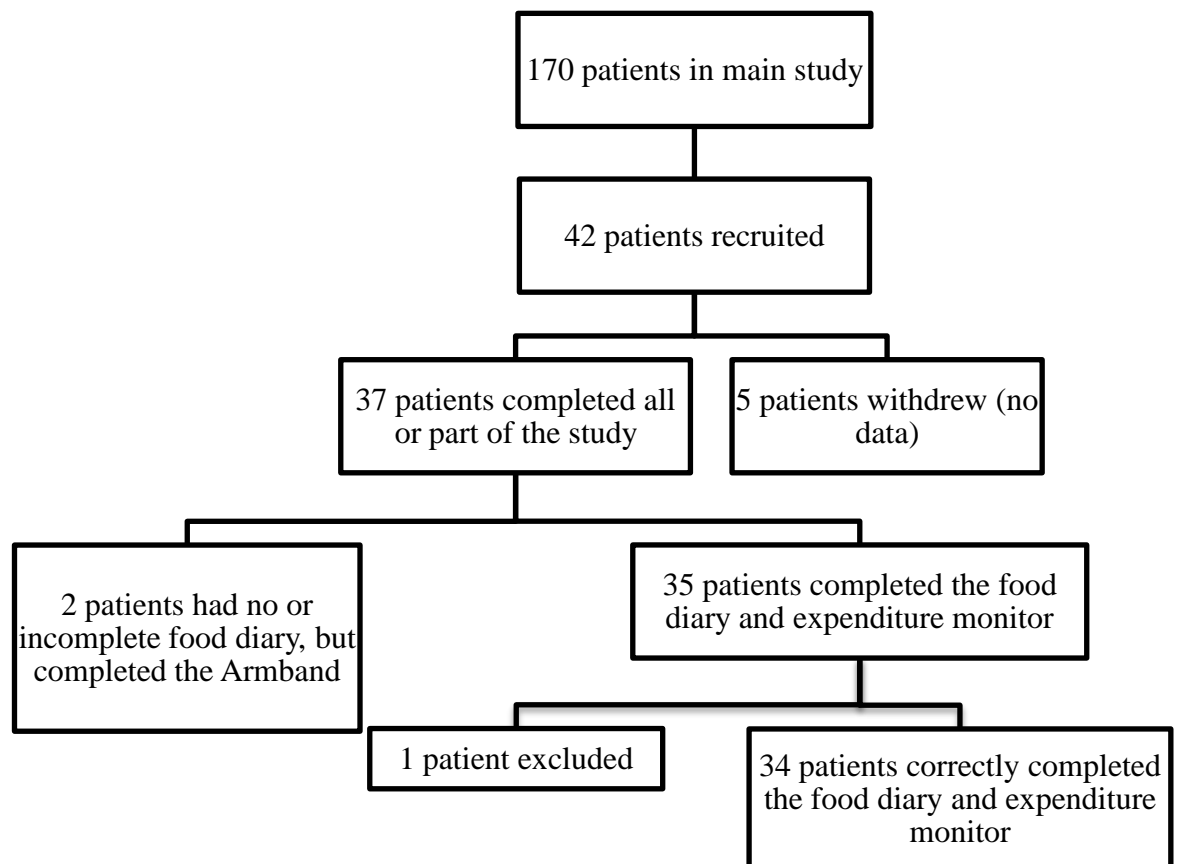


Figure 4.3: Dietary study patient recruitment

Results refer to the 36 patients who completed the Armband analysis with or without the dietary record. The median time from recruitment to study was  $2\pm 7.2$  days (range 1 to 33).

#### 4.6.2 Demographics

Median age was  $57.9\pm 12.2$  years (mean 55.8; range 32.3 to 72.9). Six (17%) patients were male. Thirty two (89%) were right-handed. Fourteen (39%) had dcSSc. Seven (19%) were anti-topoisomerase 1 and 9 (25%) were anti-centromere antibody positive. None required percutaneous enteral feeding or HPN.

The median interval from onset of Raynaud's phenomenon was  $143\pm 125$  months (range 0 to 608). The median interval from SSc onset was  $120\pm 88$  months (range 13 to 334).

#### 4.6.3 Nutritional status

This section describes the nutritional status of patients.

##### Body mass index and 'Malnutrition Universal Screening Tool'

The mean BMI was  $23.9\pm 3.9$  kg/m<sup>2</sup> (range 16.3 to 33.7). 'MUST' scores were calculated (Table 4.1).

<b>'MUST' component</b>	<b>Patients (%)</b>
<b>'MUST' BMI score</b>	
'MUST' = 0 (BMI >20 kg/m <sup>2</sup> )	30 (83%)
'MUST' = 1 (BMI = 18.5-20 kg/m <sup>2</sup> )	5 (14%)
'MUST' ≥ 2 (BMI <18.5 kg/m <sup>2</sup> )	1 (3%)
<b>Unintentional weight loss in past 3-6 months 'MUST' score</b>	
'MUST' = 0 (0-5% weight loss)	31 (86%)
'MUST' = 1 (5-10% weight loss)	4 (11%)
'MUST' ≥ 2 (>10% weight loss)	1 (3%)
<b>Total 'MUST' score</b>	
'MUST' = 0	28 (78%)
'MUST' = 1	4 (11%)
'MUST' ≥ 2	4 (11%)

Table 4.1: 'MUST' scores for included patients

### Mid-upper arm anthropometry

Few patients had MUAA  $\leq 5^{\text{th}}$  age-gender centiles (Table 4.2).

Non-dominant MUAA measurement	$\leq 5^{\text{th}}$ centile
MAC	3 (8%)
TSF	5 (14%)
MAMC	3 (8%)

Table 4.2: MUAAs below the 5<sup>th</sup> centile

### Bioelectrical impedance analysis

BIA results for the 36 patients are shown in Table 4.3.

Measurement	Mean	SD	Range
Percentage body fat (%)	33.9	7.7	12.1 – 45.4
Percentage body water (%)	52.0	6.3	41.9 – 67.7
Phase angle (50kHz)	4.5	0.82	2.7 – 6.3

Table 4.3: BIA measurements

#### 4.6.4 Gastrointestinal involvement

The mean inter-incisor distance was  $34.0 \pm 9.1$  mm (range 13 to 52). Twenty one (58%) patients had oesophageal and 10 (28%) had small bowel dysmotility. The spread of UCLA GI symptoms were tabulated (Table 4.4).

Symptom domain	Median	Mean	Range	IQR	No. scored minimum possible (%)	No. scored maximum possible (%)
Reflux	1.00	1.00	0.0 - 2.63	0.27 - 1.50	5 (14%)	0 (0%)
Distension / Bloating	1.75	1.65	0.0 - 3.0	1.00 - 2.25	1 (3%)	0 (0%)
Faecal soilage	0.00	0.69	0.0 - 3.0	0.00 - 1.00	21 (58%)	10 (6%)
Diarrhoea	0.50	0.53	0.0 - 2.0	0.00 - 1.00	16 (44%)	3 (8%)
Social functioning	0.58	0.52	0.0 - 1.8	0.00 - 0.67	10 (28%)	0 (0%)
Emotional well-being	0.44	0.67	0.0 - 2.0	0.03 - 1.11	9 (25%)	0 (0%)
Constipation	0.50	0.65	0.0 - 2.5	0.00 - 1.00	11 (31%)	3 (2%)
Total GI score	0.85	0.84	0.2 - 1.9	0.47 - 1.08	0 (0%)	0 (0%)

Table 4.4: UCLA scores

#### 4.6.5 Respiratory and cardiac involvement

Based on the Medsger severity scale, the majority of patients (69%) had normal cardiac function. The remaining patients (28%) had mild disease. Data was unavailable for 1 patient. None of the patients with moderate or severe cardiac disease were included in this study.

In contrast, based on the Medsger severity scale, most of the patients studied had respiratory involvement (39% = normal respiratory function; 22% = mild; 25% = moderate; 11% = severe; 3% = end-stage involvement).

#### 4.6.6 Functional status

The spread of patient-reported assessments of functional status (SHAQ) were calculated (Table 4.5). Patients displayed a wide range of responses across all domains.

	Mean	SD	Range
Total HAQ	1.43	0.76	0.0 - 3.0
SHAQ Pain	1.13	0.81	0.0 - 2.8
SHAQ GI	1.15	0.76	0.0 - 2.5
SHAQ lung	1.23	0.82	0.1 - 2.9
SHAQ global disability	1.48	0.79	0.0 - 3.0

Table 4.5: SHAQ scores

#### 4.6.7 Energy expenditure

In clinical practice, energy requirements are normally calculated using generic predictive equations, rather than being deduced from individual assessments of expenditure. This section describes and compares these predictions and measured expenditures.

##### Calculated energy expenditure

The mean predicted daily energy requirement was  $1930 \pm 265$  kcal (range 1453 to 2626). As expected, males (2376 kcal; range 2019 to 2626; n=6) had a higher predicted requirement than females (1840 kcal; range 1452 to 2173; n=30).

##### Recorded energy expenditure

The mean recording period was 2 days 23 hours and 8 minutes (range = 2 days 19 hours and 11 minutes to 3 days). During this period, the mean time the Armband was worn was 2 days 21 hours and 2 minutes (range = 2 days 16 hours and 41 minutes to 3 days). This represented 97% (range = 93 to 100%) of the recording period and 96% (range = 90 to 100%) of the full 3 days.

Most time was spent at rest or undertaking light exertion. Few patients completed any high intensity activity and those who did, only did so for a short period of time. There was a

wide variation in the amount of time spent by patients expending energy at a rate of >1.5 METs. Percentage daily time spent ranged from 5% (70/1440) to 40% (575/1440).

Times spent lying and sleeping were strongly correlated ( $r=0.92$ ;  $p<0.01$ ).

	<b>Mean</b>	<b>SD</b>	<b>Range</b>
Average total energy (kcal)	2027	476	1221 – 3400
Average corrected total energy (kcal)	2075	481	1283 – 3489
Average METs	1.4	0.3	0.9 – 1.9
Energy expended at (kcal):			
≤1.0 METs – rest	1019	341	399 – 1964
1.1 to 2.9 METs – light	675	262	160 – 1132
3.0 to 5.9 METs – moderate	359	298	11 – 1174
>6.0 METs – high	22	40	0 – 177
Time spent at (minutes):			
≤1.0 METs	986	235	472 – 1356
1.1 to 2.9 METs	365	194	78 – 826
3.0 to 5.9 METs	88	71	2 – 291
> 6.0 METs	3	5	0 – 19
Average number of steps per day	4035	3288	230 – 14148
Average time lying (minutes)	491	113	146 – 808
Average time sleeping (minutes)	397	110	128 – 672
Average time lying not asleep (minutes)	93	46	18 - 195

Table 4.6: Average daily energy expended

#### 4.6.8 Dietary assessment

The follow sections describe recorded dietary intake and, where applicable, compare intakes to RNIs, predicted requirements, expended energy and biochemical results.

##### Energy, macro- and micronutrient intake

The mean daily energy and macro- and micronutrient intakes were deduced (Tables 4.7 and 4.8). The mean daily combined weight of consumed food and fluid was  $2.6\pm 0.6$ kg (1.5 to 4.1kg). There was a substantial variation between patients' diets. Four patients took nutritional supplements (2 only Ensures, 1 only Complan and 1 Ensure, Vitajoule and Calogen).

Macronutrient or fluid intake	Mean	SD	Range
Water content in food and fluids (g)	2185	586	1113 - 3283
Number of drinks per day	6.9	1.7	2.7 – 10.3
Weight of water in fluids (g)	1615	586	382 - 2573
Energy (kcal)	1788	509	958 – 3498
Protein (g)	71.3	18.9	40.1 – 117.5
Nitrogen (g)	11.0	3.0	6.1 – 19.3
Total fat (g)	74.8	34.5	27.5 – 196.3
Saturated fat (g)	28.4	12.5	11.2 – 70.2
Carbohydrate (g)	212.1	53.4	92.3 – 354.9
Glucose (g)	13.4	8.3	1.9 – 41.4
Fibre (Southgate method) (g)	6.6	4.1	1.1 – 23.1
Non-starch polysaccharides (g)	9.8	4.0	4.9 – 21.1
Starch (g)	95.5	33.4	36.5 – 155.7

Table 4.7: Average daily macronutrient and fluid intake

g = grams; ml = millilitres; kcal (kilocalories)

The total energy intake did not correlate with age ( $r=0.284$ ;  $p=0.10$ ) or BMI ( $r=-0.01$ ;  $p=0.62$ ). Food substances provide differing amounts of energy per gram consumed. Fat is more energy dense (9kcal/g) than alcohol (7kcal/g), protein (4kcal/g) or carbohydrate (3.75kcal/g). Carbohydrates formed the bulk of most patients' diets (Figure 4.5).

However, the patients also acquired a significant proportion of their energy intake from fat. On average, fat provided 37% of their energy intake, but in one case this was as high

as 51%. The mean saturated fat intake was  $14.1 \pm 3.7\%$  (range 7 to 21). There were no significant correlations between the percentages of energy from fat and the risk of malnutrition ( $s=0.001$ ;  $p=0.99$ ) or BMI ( $r=-0.116$ ;  $p=0.51$ ).

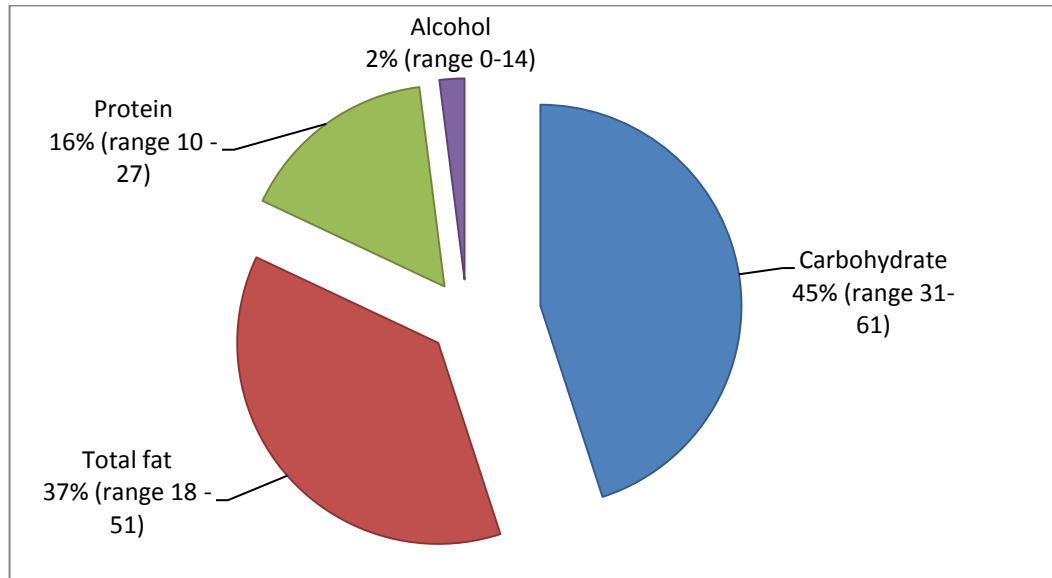


Figure 4.4: Pie chart of dietary energy sources

Micronutrient intakes were compared to RNIs, which are 2 SDs above Estimated Average Requirements (Table 4.8).

	Mean	SD	Range	Percent <RNI
Sodium (mg)	2504	898	968 – 5165	12
Potassium (mg)	2638	699	1641 – 4165	85
Calcium (mg)	819	300	396 – 1568	35
Phosphorus (mg)	1139	326	646 – 1978	0
Magnesium (mg)	244	80	145 – 489	65
Chloride (mg)	3702	1401	1487 – 7966	17
Iron (mg)	10.7	5.2	3.5 – 26.2	47
Copper (mg)	1.1	0.5	0.4 – 2.9	61
Zinc (mg)	8.6	4.1	3.9 - 25.9	44
Selenium (µg)	36.1	21.2	6.9 – 112.5	91
Folate (µg)	234	135	104 – 711	52
Vitamin D (µg)	3.1	3.2	0.0 - 15.5	N/A
Vitamin B12 (µg)	3.8	2.6	0.7 – 14.1	9

Table 4.8: Average daily micronutrient intake



Assuming that the average daily micronutrients recorded during the study are representative of participants' habitual intake, then >50% of patients are failing to meet the potassium, magnesium, copper, selenium or folate RNIs. Two patients whose oral magnesium intake was below the RNI were also receiving intermittent magnesium infusions. No one was prescribed oral magnesium. Of the 16 patients not meeting their iron RNI, 3 were prescribed oral iron. In addition, of the 18 patients meeting their 2 requirements, one was taking oral and one was taking intravenous iron.

No RNI exists for vitamin D. The main source of vitamin D is not dietary. In addition, 3 patients were taking a vitamin D preparation and 8 were taking a combined calcium and vitamin D preparation.

### Biochemical results

Biochemical results were only available for some patients (Table 4.9).

Biochemical test	Number of patients	Mean	SD	Range	below serum reference
Corrected calcium	28	2.40	0.09	2.25 – 2.60	0%
Phosphate	28	1.12	0.19	0.64 – 1.49	7%
Vitamin D	12	48.0	25.0	7.7 – 89.3	57%
Magnesium	21	0.82	0.1	0.5 – 1.0	5%
Iron	17	10.9	5.6	2.5 – 23.4	53%
Zinc	17	10.3	1.4	7.0 – 12.3	35%
Selenium	8	0.93	0.23	0.70 – 1.30	0%

Table 4.9: Biochemical results

None of the patients had a low serum calcium despite several being vitamin D deficient. 32% of all patients were prescribed a vitamin D supplement. Few patients were tested for vitamin D. However, of those tested, 57% were deficient or insufficient. 30% of those who were deficient were prescribed oral supplementation.

The one patient with low magnesium was prescribed oral supplementation. Very few patients were tested for selenium as this was only requested in a few cases (as described in Chapter 3).

### Comparison of micronutrient intake to biochemical results

Paired biochemical and dietary values were available for some patients (Table 4.10).

<b>Micronutrient</b>	<b>Number of patients</b>	<b>Number &lt;RNI (%)</b>	<b>Number with biochemical deficiency (%)</b>	<b>Number with intake &lt;RNI and biochemical deficiency (%)</b>
Corrected calcium	28	9 (32)	0 (0)	0 (0)
Phosphate	28	0 (0)	2 (7)	0 (0)
Magnesium	21	14 (66)	1 (5)	1 (5)
Iron	17	9 (47)	10 (53)	5 (26)
Zinc	17	9 (53)	6 (35)	4 (23)
Selenium	8	6 (57)	0 (0)	0 (0)

Table 4.10: Low dietary intakes and low biochemical results

There were no significant associations between patients not meeting their dietary RNI and those with biochemical deficiency (Chi-Squared). However, this comparison did not take into account dietary supplementation in excess of nutritional intake.

### Comparison of energy intake and nutritional status

There were no correlations between energy intake and 'MUST' ( $s=0.086$ ;  $p=0.62$ ) score or BIA percentage body fat ( $r=-0.326$ ;  $p=0.06$ ). After excluding those patients who reported recent weight loss, there were still no correlations between energy intake and 'MUST' ( $s=0.031$ ;  $p=0.87$ ) score or BIA percentage body fat ( $r=-0.344$ ;  $p=0.07$ ).

#### **4.6.9 Comparison of predicted, consumed and expended energies**

This study hypothesised that energy expenditures and intakes would be similar in weight stable patients and different in weight losing patients. Comparisons between weight stable/gaining patients and weight losing patients were not possible due to the low number ( $n=5$ ) of patients who reported weight loss within the preceding 3 to 6 months.

The following sections compare energy intakes and expenditures in all patients.

### Diary versus predicted energy

For all patients, there was no significant correlation between reported energy consumption and predicted ( $r=0.20$ ,  $p=0.25$ ) expenditure.

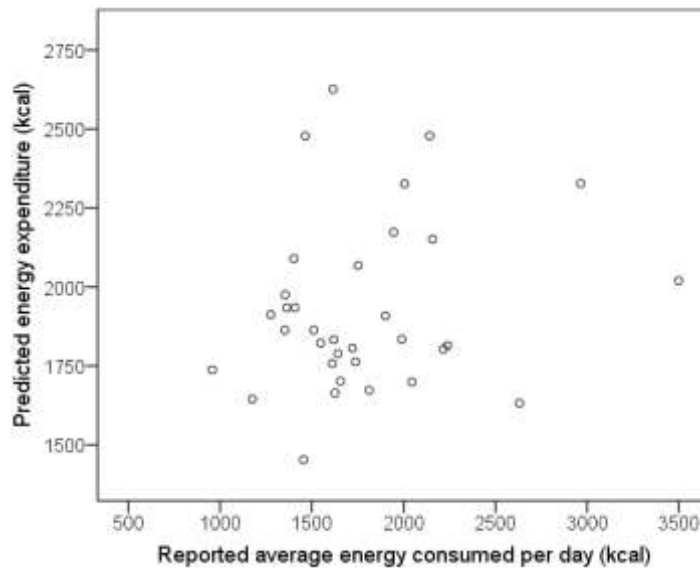


Figure 4.5: Predicted expenditure against consumed energy

### Diary versus measured energy

For all patients, there was no significant correlation between reported energy consumption and measured ( $r=-0.03$ ;  $p=0.89$ ) expenditure (Armband).

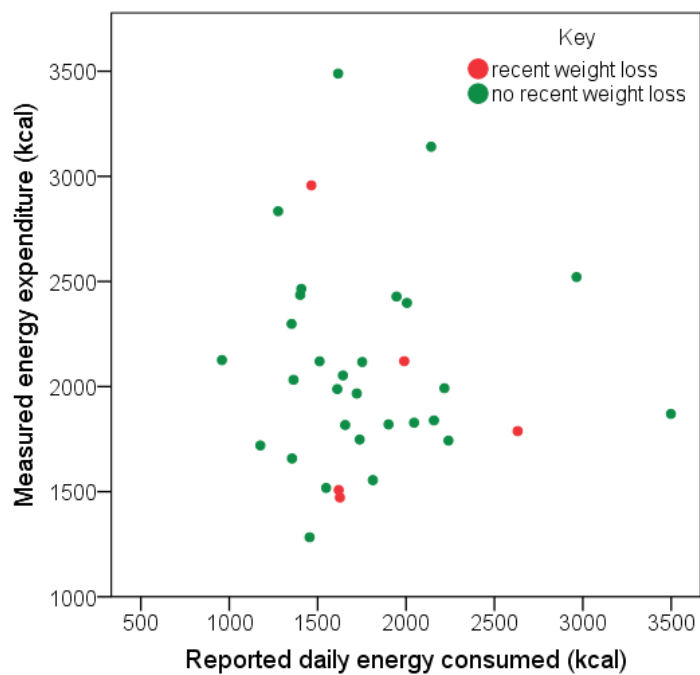


Figure 4.6: Energy expenditure against energy consumed

For all patients, the mean difference between recorded intake and expenditure was 241kcal. Fourteen patients reported having consumed more energy than they expended (mean  $400 \pm 416$  kcal; range 44 to 1654). Twenty patients expended more energy that they were reported to have consumed (mean  $689 \pm 491$  kcal; range 68 – 1784). Of the 5 patients who reported to have lost >5% weight over the preceding 3 to 6 months, only 2 had measured energy expenditures greater than their reported intakes (excess expenditures = 116kcal and 1438kcal). Weight losing patients were not asked whether they had modified their diet in an attempt to gain weight. However, of the 4 nutritional supplement taking patients, 3 were consuming in excess of their expenditure (range 230 to 1650kcal), while the fourth still had a 380kcal deficit.

### Measured versus predicted expenditure

A significant correlation was demonstrated between predicted and measured daily energy expenditures ( $r=0.81$ ,  $p<0.01$ ; Figure 4.7).

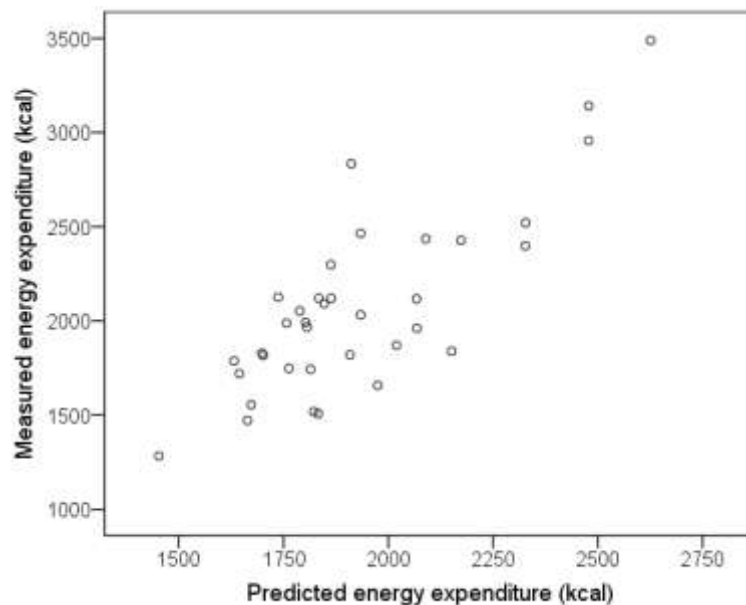


Figure 4.7: Measured against predicted energy expenditure

However, despite this, the actual values differed for individual patients (ICC = 0.62; 95% limits of agreement -459 to 751kcal; Figure 4.8). For those patients with higher mean energies, the expenditures often exceeded predicted requirements. In contrast, for patients with lower mean energies, predicted energies often exceeded measured expenditures.

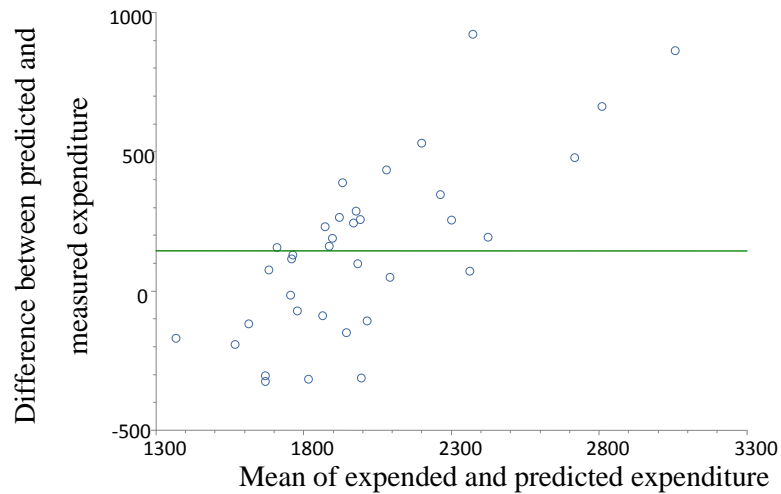


Figure 4.8: Agreement plot of expended and predicted energies

#### 4.6.10 Physical activity versus clinical manifestations

Different degrees of active energy expenditure (i.e. steps taken and time at >1.5METs) and time resting were recorded by the patients. There are many patient-specific characteristics and manifestations which may have influenced this activity. The influence of possible characteristics and manifestations recorded during this study are explored in this section.

Comparisons were not made to overall daily energy expenditure or energy expended at >1.5METS as these would have been subject to influences other than active expenditure (i.e. BMI and gender).

#### Demographics

##### Age

There was evidence of significant correlations between age and average daily number of steps ( $r=-0.57$ ;  $p<0.01$ ) and time at >1.5METs ( $r=-0.34$ ;  $p=0.04$ ). However, there were no associations between age and time spent lying down ( $r=-0.04$ ;  $p=0.80$ ) or asleep ( $r=0.06$ ;  $p=0.72$ ).

## Gender

Males and females did not differ significantly in their mean number of steps (females 4140±3470 vs. males 3514±2339; p=0.68) or time spent at >1.5METS (female 302±121 vs. males 286±72; p=0.75), lying (females 483±120 vs. males 533±67; p=0.33) or sleeping (females 390±107 vs. males 436±67; p=0.32).

## Disease-sub-type

On average, patients with lcSSc took more (5050±3490) daily steps than patients with dcSSc (2440±2227; p=0.02). However, there was no significant difference in the mean daily times spent at >1.5METs (lcSSc 317±106 minutes vs. dcSSc 272±122 minutes; p=0.26), lying down (lcSSc 490±84 minutes vs. dcSSc 492±153; p=0.96) or sleeping (lcSSc 396±75 minutes vs. dcSSc 400±137 minutes; p=0.93).

## **Nutritional status**

### Body mass index

Significant correlations were detected between BMI and time spent asleep (r=0.51; p<0.01) and lying down (r=0.43; p<0.01). However, there were no significant correlations between BMI and the number of steps (r=-0.04; p=0.83) or time at >1.5METS (r=-0.218; p=0.20).

### ‘Malnutrition Universal Screening Tool’

Significant correlations were identified between ‘MUST’ and time lying (s=-0.43; p<0.01) and sleeping (s=-0.49; p<0.01). However, there were no significant correlations identified between ‘MUST’ and time at >1.5METS (s=0.09; p=0.96) or daily steps (s=-0.10; p=0.57).

### Body composition

No significant correlations were detected between BIA percentage body fat and time at >1.5METS (r=-0.26; p=0.13), number of steps (r=-0.17; p=0.31) or time lying (r=0.09; p=0.62) or sleeping (r=0.20; p=0.23).

### Weight losing patients

There was a significant difference in the mean time lying down per day between weight losing and weight stable/increasing patients (weight losing  $393\pm 139$  minutes vs. weight stable  $507\pm 103$  minutes;  $p=0.04$ ) and time spent sleeping (weight losing  $300\pm 99$  minutes vs. weight stable  $413\pm 94$  minutes;  $p=0.02$ ). However, there was no significant difference in the mean daily time at  $>1.5$ METs (weight losing  $294\pm 135$  minutes vs. weight stable  $300\pm 112$  minutes;  $p=0.92$ ) or in the mean number of daily steps (weight losing  $4601\pm 4615$  vs. weight stable  $3944\pm 3117$ ;  $p=0.68$ ).

### **Functional ability**

#### Total HAQ

There was no evidence of any correlation between functional impairment (total HAQ score), number of steps ( $s=-0.260$ ;  $p=0.13$ ) or time spent at  $>1.5$ METs ( $s=-0.10$ ;  $p=0.57$ ) lying ( $s=0.17$ ;  $p=0.32$ ) or sleeping ( $s=0.26$ ;  $p=0.13$ ).

#### SHAQ - global disability

There was no evidence of any correlations between SHAQ global disability and number of steps ( $r=-0.27$ ;  $p=0.11$ ) or time spent at  $>1.5$ METs ( $r=-0.15$ ;  $p=0.38$ ), lying down ( $r=0.14$ ;  $p=0.43$ ) or asleep ( $r=0.15$ ;  $p=0.37$ ).

#### SHAQ - lung

There was no evidence of any correlations between SHAQ lung severity and number of steps ( $r=-0.30$ ;  $p=0.08$ ) or time spent at  $>1.5$ METs ( $r=-0.14$ ;  $p=0.43$ ), lying down ( $r=-0.07$ ;  $p=0.67$ ) or asleep ( $r=-0.10$ ;  $p=0.57$ ).

## **Cardiorespiratory involvement**

### Medsgger cardiac

There was no evidence of any correlations between severity of cardiac involvement and number of steps ( $s=-0.31$ ;  $p=0.07$ ) or time spent at  $>1.5$ METs ( $s=-0.20$ ;  $p=0.26$ ), lying down ( $s=0.11$ ;  $p=0.52$ ) or asleep ( $s=0.075$ ;  $p=0.69$ ).

### Medsgger lung

There was evidence of correlations between severity of respiratory involvement and number of steps ( $s=-0.35$ ;  $p=0.04$ ) and time lying down ( $s=0.33$ ;  $p=0.05$ ). However, there was no evidence of any associations between severity of respiratory involvement and time asleep ( $s=0.19$ ;  $p=0.28$ ) or at  $>1.5$ METs ( $s=-0.30$ ;  $p=0.08$ ).

### **4.6.11 Energy discrepancy versus clinical manifestations**

All patients had an energy discrepancy (expended minus consumed) between their intake and expenditure. The magnitude varied between individuals. Potential correlates were investigated.

### **Demographics**

A significant correlation ( $r=-0.50$ ;  $p<0.01$ ) was detected between age and the apparent energy discrepancy. Younger patients appeared to expend more energy than they reported consuming (Figure 4.9). In comparison, the energy discrepancies appeared to be less in older patients, and a few older patients appeared to consume more energy than they were expending.



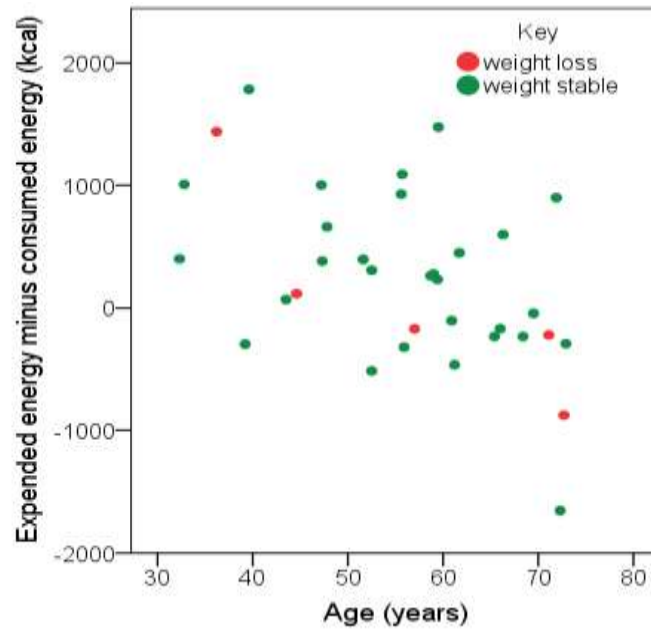


Figure 4.9: Expended energy discrepancy against age

No correlations were detected between the energy discrepancy and gender ( $s=0.11$ ;  $p=0.54$ ) or disease sub-type ( $s=-0.23$ ;  $p=-0.19$ ).

**Nutritional status**

There was a significant correlation between BMI and energy discrepancy ( $r=0.41$ ;  $p=0.02$ ). The discrepancy was greater in patients with higher BMIs (Figure 4.10).

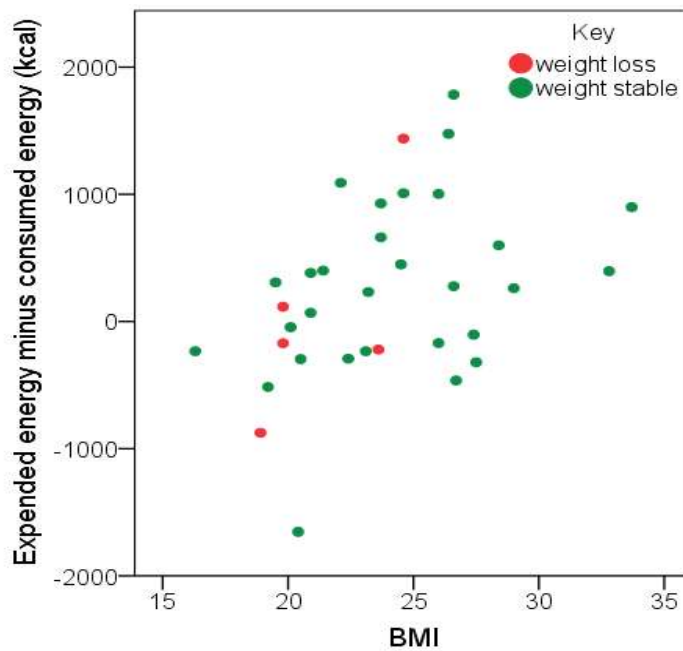


Figure 4.10: Energy discrepancy against BMI

There was no significant correlation between 'MUST' and energy discrepancy ( $s=-0.29$ ;  $p=1.0$ ). There was also no significant correlation between BIA percentage body fat and energy discrepancy ( $r=-0.64$ ;  $p=0.72$ ).

### **Functional status**

There were no significant correlations between energy discrepancy and either HAQ functional disability ( $r=0.05$ ;  $p=0.77$ ) or SHAQ global disability ( $r=0.07$ ;  $p=0.69$ ).

### **Clinical symptoms and manifestations**

There were no significant correlations between energy discrepancies and the UCLA reflux ( $s=0.03$ ;  $p=0.87$ ), bloating/distension ( $s=0.03$ ;  $p=0.91$ ) or total GI scores ( $s=0.07$ ;  $p=0.70$ ). There was no significant difference (t-test;  $p=0.85$ ) in the mean energy discrepancies of patients with (mean =  $260\pm 795$ kcal) and without (mean =  $214\pm 594$ kcal) oesophageal dysmotility. There was also no significant difference (t-test;  $p=0.12$ ) in the mean energy discrepancies of patients with (mean =  $-73\pm 766$ kcal) and without ( $354\pm 66$ kcal) small intestinal dysmotility. There were no significant correlations between energy discrepancies and the Medsger cardiac ( $s=-0.16$ ;  $p=0.36$ ;  $n=33$ ) or lung ( $s=-0.25$ ;  $p=0.15$ ;  $n=34$ ) severity scores.

#### **4.6.12 Macronutrient intake versus gastrointestinal manifestations**

In theory, dietary intake may be affected by GI manifestations. Possible relationships between GI symptoms and manifestations and oral aperture were explored.

### **Oral aperture**

There were no significant correlations between oral aperture and daily weights of total diet ( $r=-0.21$ ;  $p=0.24$ ), protein ( $r=-0.57$ ;  $p=0.75$ ), total fat ( $r=-0.13$ ;  $p=0.46$ ), carbohydrate ( $r=-0.17$ ;  $p=0.34$ ) or Southgate fibre ( $r=0.03$ ;  $p=0.89$ ). In addition, there were no significant correlations between oral aperture and percentages of energy from protein ( $r=0.1$ ;  $p=0.59$ ), total fat ( $r=-0.18$ ;  $p=0.32$ ) or carbohydrate ( $r=0.09$ ;  $p=0.63$ ).

## **Oesophagus**

There were no significant differences between patients with and without oesophageal dysmotility for daily weights of total diet (2.5kg vs. 2.8kg;  $p=0.25$ ), protein (69g vs. 74g;  $p=0.46$ ), total fat (72g vs. 78g;  $p=0.65$ ) or Southgate fibre (7.2g vs. 5.7;  $p=0.28$ ).

However, a significant correlation was detected between oesophageal dysmotility and carbohydrate (196g vs. 234g;  $p=0.04$ ). In addition, there were no significant differences between patients with and without oesophageal dysmotility for percentages of energy from protein (17% vs. 16%;  $p=0.50$ ), total fat (37% vs. 36%;  $p=0.67$ ) or carbohydrate (44% vs. 47%;  $p=0.20$ ).

Also, there were no significant correlations between UCLA reflux symptoms and daily weights of total diet ( $s=0.20$ ;  $p=0.25$ ), protein ( $s=0.24$ ;  $p=0.18$ ), fat ( $s=0.04$ ;  $p=0.83$ ), carbohydrate ( $s=-0.07$ ;  $p=0.71$ ) or Southgate fibre ( $s=0.16$ ;  $p=0.37$ ). In addition, there were no significant correlations between UCLA reflux symptoms and percentages of energy from protein ( $s=0.13$ ;  $p=0.48$ ), fat ( $s=0.10$ ;  $p=0.58$ ) or carbohydrate ( $s=-0.17$ ;  $p=0.34$ ).

## **Small intestine**

There were no significant differences between patients with and without small intestinal dysmotility for daily weights of total diet (2.7kg vs. 2.6kg;  $p=0.86$ ), protein (74g vs. 70g;  $p=0.60$ ), total fat (93g vs. 68g;  $p=0.06$ ), carbohydrate (s=232g vs. 205g;  $p=0.19$ ) or Southgate fibre (7.3g vs. 6.3g;  $p=0.53$ ). In addition, there were no significant differences between patients with and without small intestinal dysmotility for percentages of energy from protein (15% vs. 17%;  $p=0.21$ ), total fat (39% vs. 25%;  $p=0.18$ ) or carbohydrate (44% vs. 45%;  $p=0.62$ ).

There were no significant correlations between UCLA bloating/distension symptoms and daily weights of total diet ( $s=-0.14$ ;  $p=0.43$ ), protein ( $s=0.18$ ;  $p=0.31$ ), fat ( $s=-0.22$ ;  $p=0.21$ ), carbohydrate ( $s=-0.10$ ;  $p=0.58$ ) or Southgate fibre ( $s=-0.17$ ;  $p=0.92$ ). In addition, there were no associations between UCLA bloating/distension symptoms and percentages of energy from fat ( $s=-0.20$ ;  $p=0.26$ ) or carbohydrate ( $s=-0.16$ ;  $p=0.36$ ). However, an association was detected between bloating/distension and percentage of energy from protein ( $s=0.35$ ;  $p=0.04$ ).

## **4.7 Discussion**

This study is the first to compare measured energy intakes and expenditures in patients with SSc. However, despite measuring both, it failed to show any correlation. It also failed to find any significant correlations between energy intake and nutritional status, but the association with BIA body fat did approach significance. This study's failure to identify any correlations between intake and nutritional status is not unique [211]. In addition, with the exception of age and BMI, the present study failed to detect any correlations between the apparent energy discrepancies and other markers of nutritional status or SSc disease-related manifestations.

There are many potential reasons, other than the absence of any true association, to explain this study's lack of detection of any significant relationships. Limitations may have affected the assessment of either dietary intake or energy expenditure.

### **4.7.1 Dietary energy assessment**

Intakes of energy and macro- and micronutrients were assessed by 3-day estimated dietary records. Any errors incurred in this assessment may have masked any relationships with clinical manifestations.

Reporting biases may occur because the simple act of dietary documentation leads to an increased awareness of one's own diet and thus, in order to improve the appearance of their diet to the researcher, participants over or underreport their consumption. Reporting errors have been studied. By comparing 7 day weighed food reporting to predictions, a study involving healthy British adults showed a median under-reporting of 34% of predicted needs in men and 33% in women [387]. The scale of under-reporting differs between population sub-groups. There is a well established link between BMI and under-reporting, with frequent under-reporters tending to have greater BMIs [187]. This may help to explain why, in the present study, those patients with lower BMIs tended to report consuming more energy than they were expending and, in contrast, why patients with higher BMIs tended to report that they were consuming less energy than they were expending.

Furthermore, this difference in the energy discrepancies with BMI may also be linked to individuals' differing abilities to report portion sizes. It is well recognised that individuals'

abilities to estimate sizes is poor. However, it may be even worse with raised BMIs as was illustrated by a study in which obesity ( $\text{BMI} \geq 30 \text{ kg/m}^2$ ) was found to be associated with an 8% underestimation of portion size [183]. Therefore, this, in combination with intentional under-reporting intake by obese patients, may help to explain the tendency seen in the present study for heavier patients to appear to be consuming relatively less energy than their lighter counterparts.

In the present study, older patients appeared more likely to report consuming more energy than they were observed expending, whereas younger patients had a greater likelihood of reporting that they were consuming less energy than they were expending. This discrepancy may be, in part, attributable to differences in abilities to estimate portion sizes with age. Few studies have investigated this aspect of portion size estimation. However, an early study reported that older subjects tended to overestimate more than their younger counterparts [388]. In addition, another recent study, published only in abstract format, found older adults to be less able to estimate the portion sizes of certain food groups than younger adults (overestimation of small pieces and underestimation of spreads) [389].

It is unknown whether chronic GI diseases leading to altered dietary patterns, such as those seen with dysphagic or gastroparetic diets, have any effect on normal portion size perception.

#### **4.7.2 Dietary energy assessment and weight loss**

In the present study, no difference in the apparent energy discrepancy was noted in patients who reported recent weight loss than those without. However, this may be explained by limited numbers recruited with recent weight loss ( $n=5$ ).

#### **4.7.3 Dietary sources of energy**

UK recommendations are for daily energy intakes from total and saturated dietary fats to be up to 35% and 11% respectively [390]. Mean daily energies from total and saturated fat were 37% and 14% respectively which are slightly higher than UK recommendations. However, they were similar to the 37 to 39% total fat energy reported in a previous study of unselected patients with SSc [47].

#### **4.7.4 Dietary macronutrient intake**

High fibre diets may worsen GI symptoms in SSc [391]. Lower fibre intakes have been observed in patients compared to healthy volunteers [47]. In the present study, individuals' daily fibre intakes varied considerably (1.1g to 23.1g). However, no associations were found between fibre intake and GI symptoms/dysmotility or oral aperture. Equally, although the present study found an association between carbohydrate intake and oesophageal dysmotility, this was not the case for those with GI symptoms of reflux. There was, however, a significant positive correlation between the percentage of daily energy from protein and symptoms of distension/bloating.

#### **4.7.5 Dietary micronutrient intake**

Dietary micronutrient deficiencies appeared to be exceedingly common in the present study, with over 50% of patients failing to meet their RNIs for potassium, magnesium, copper, selenium or folate. However, despite this, few patients had biochemical deficiencies. While this may be readily explained by dietary under-reporting and inadequate reporting of supplement intake, the lack of associations may also relate to incomplete biochemical data and/or reduced total body stores not being reflected in serum measurements.

It is also worth highlighting that the chosen assessment method was the 3 day estimated diary. This was chosen to minimise patient burden. A 3 day assessment period is generally agreed to be acceptable for assessing macronutrient and energy intakes, but may fail to fully quantify normal micronutrient intakes which vary significantly on a day-to-day basis. Indeed, for some less commonly consumed micronutrients, studies in excess of 7 days may be needed to make an accurate assessment of some less commonly consumed dietary micronutrients [392-394]. Thus, over the 3 day assessment period of the present study, and in other studies involving patients with SSc, micronutrients may be underestimated, thereby giving rise to the false impression of patients with SSc failing to meet their RNIs.

#### **4.7.6 Total energy expenditure versus measured intake**

To-date, few nutritional studies involving patients with SSc have assessed or estimated energy expenditure. Previous studies comparing intakes to merely predictions have failed

to detect differences between patients with and without disease-related malnutrition [48]. In the present study, estimated expenditures positively correlated with actual expenditures. However, for individual patients, the values differed. In particular, for patients with higher mean energies, measured expenditures often exceeded the prediction, while for patients with lower mean energies the predictions often exceeded measured expenditures. This highlights the importance of measuring expenditures, rather than using predictions, in patients with diseases that may potentially influence their requirements and for whom an accurate assessment is essential

#### **4.7.7 Physical activity**

This study is only the second to use the SenseWear® Armband in patients with SSc. The first showed stable patients to have reduced daily physical activity in comparison to healthy controls [213]. The present study showed a wide variation in active expenditures between patients. In some, activity (steps, time at >3METs) was very low. Compared to the previous study, this study had a lower mean active expenditures (steps), but similar overall mean expenditures [213]. However, patients in the present study had a higher mean age and age was inversely correlated with activity.

There were no associations between physical activity (level or steps) and nutritional status (BMI, 'MUST' or percentage fat). However, in the previous study, negative correlations were noted, despite both studies including patients with identical mean BMIs [213].

Patients with worse lung involvement took significantly fewer steps, but did not spend significantly less time at >1.5METs, though this approached significance. No associations were detected between worsening lung-related functional disability (lung SHAQ) and less time at >1.5METs or number of steps, but the reduction in steps approached significance. This concurs with the finding of the previous study of reduced daily activity even with early lung involvement [213]. No similar correlations were detected between physical activity and cardiac involvement. However, none of the included patients had more than mild cardiac involvement.

The study was underpowered to assess differences in expenditure between patients with and without recent weight loss.

#### **4.7.8 Resting expenditure**

Inactivity (time lying and sleeping) was not reported in the previous SenseWear® study involving patients with SSc. Interestingly, some patients appeared to spend very little time resting. As expected, time spent resting did not differ with age, gender or disease sub-type. However, resting measures showed a positive correlation with BMI and negative correlation with nutritional risk. As with active expenditure, for inactivity correlations were seen with respiratory but not cardiac involvement. Thus, those patients with higher BMIs, less nutritional risk and worse respiratory disease spent more time at rest.

#### **4.7.9 Limitations and confounders**

A limitation to this study was the patient population who were recruited. This limitation should be considered when interpreting the results. Due to the method of recruitment, the patients who took part may not have been representative of all patients with SSc. Recruitment biases may have occurred due to a number of reasons.

The limited availability of the SenseWear® Armband led to the preferential recruitment of patients with early morning appointments. Though not formally assessed, those patients with more functional impairment may have been more likely to request late morning appointments. This may have occurred due patients with greater impairments requiring more time to get ready and to travel to the tertiary centre.

In addition, there may have been an element of patient self-selection. Patients with less disease burden and/or greater dietary interest may have been more likely to agree to participate.

Also, following recruitment, some of the more symptomatic patients were withdrawn due to the detection of biochemical deficiencies on baseline testing which required admission for replacement therapy. Thus, patients with significant deficiencies were excluded without assessment of their dietary intake.

#### **4.7.10 Summary**

This study highlighted the importance of measuring energy expenditure, rather than relying on predictive equations, when an accurate assessment of expenditure is needed



clinically. It also confirmed the association between SSc-related lung manifestations and inactivity, the understanding of which will aid the dietetic management of patients.

However, perhaps due to limitations in dietary assessment, it was unable to identify any correlations between the difference in energy intake and expenditure and clinical manifestations. In addition, it was underpowered to detect difference in weight stable and weight losing patients.

## **CHAPTER 5:**

### **ASSESSMENT OF AUTONOMIC**

### **DYSFUNCTION AND GASTRIC**

### **EMPTYING: A PRELIMINARY STUDY**

## **5 Assessment of autonomic dysfunction and gastric emptying**

### **5.1 Introduction**

Cardiovascular studies involving patients with SSc commonly identify autonomic dysfunction [252]. However, not all studies concur in their findings. Low HRV is reported to be common [251]. One study involving autonomic batteries using beat-to-beat HR, but not BP, have shown abnormal parasympathetic, sympathetic function or both in 79%, 55% and 41% of patients respectively [52]. However, another study, using similar methods, only detected abnormal HR and BP responses to standing [254]. In addition, another study, using continuous beat-to-beat BP monitoring, reported cardiovascular reflexes to be within normal limits, but failed to omit potentially confounding medications account [292].

Delayed gastric emptying is also common in patients with SSc. A radiolabelled breath test study using solid test meals reported delayed gastric emptying to affect 47% of all patients [51]. However, delayed emptying may be more common in patients with dcSSc than lcSSc [395].

Studies conducted to date have sought evidence of associations between autonomic dysfunction and GI involvement. One study investigating oesophageal involvement demonstrated a significant association between autonomic and oesophageal dysfunction [258]. Likewise, another study demonstrated an association between oesophageal dysfunction and cardiovascular autonomic neuropathy [259]. With regards to gastric involvement, one study showed delayed gastric emptying of solids to be associated with vomiting and postprandial bloating, but not nausea [50]. Similarly a study using functional ultrasonography showed delayed emptying of liquids to be linked to symptoms of fullness [52]. However, there was no association with appetite, satiety or cardiovascular autonomic dysfunction. In addition, another study, despite detecting cardiovascular autonomic dysfunction in 36% of patients, failed to show any association between liquid gastric emptying (functional ultrasonography) and cardiac autonomic dysfunction [261].

To-date, no studies have compared gastric emptying measured via radiolabelled breath tests to cardiovascular autonomic responses, autonomic questionnaires or postprandial cardiovascular responses. Thus, the following study was performed to investigate the link between gastric involvement (liquid gastric emptying and COMPASS 31) and autonomic

involvement using both beat-to-beat BP and HR in patients with SSc who have omitted their confounding medications. In addition, it is also the first to investigate for disturbances in postprandial cardiovascular responses in patients with SSc.

## **5.2 Hypotheses**

There are associations between:

- GI symptoms and the rate of gastric emptying
- GI symptoms and cardiovascular autonomic dysfunction
- The rate of gastric emptying and cardiovascular autonomic dysfunction

## **5.3 Aims**

This study thus aimed to identify the presence of any of associations between GI symptoms and rate of gastric emptying, between GI symptoms and cardiovascular autonomic dysfunction and between rate of gastric emptying and cardiovascular autonomic dysfunction.

### **5.3.1 Secondary purpose**

Due to technical and patient-specific problems encountered with the autonomic apparatus initially intended for use in this study, it became necessary after initiation to undertake nested developmental work on the methodology and approaches to be used. Therefore the results presented are to be considered as preliminary and as a platform for further work.

## **5.4 Ethical approval**

This study was granted ethical approval by the North West Ethics Committee (13/NW/0423).

## 5.5 Materials and methods

This section contains details of the participants studied, equipment used and the tests performed. An overview of the study sequence is shown below (Figure 5.1).

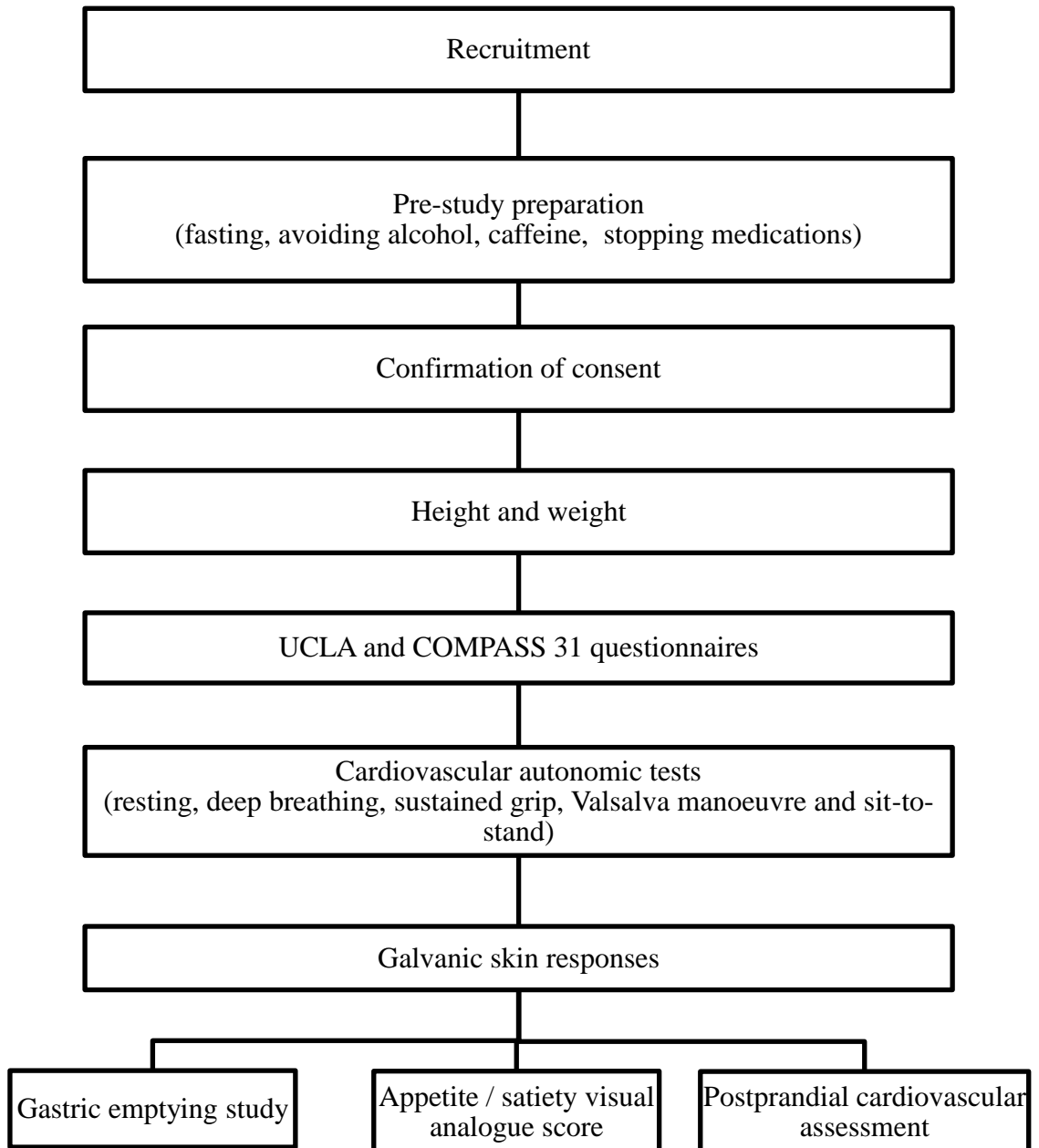


Figure 5.1: Flowchart showing the study sequence

### **5.5.1 Study participants**

Based on a sample size calculation, the intention was to recruit 20 participants with SSc, 20 age (+/- 5 years) and gender matched healthy participants and 15 non-age and gender matched healthy participants. The non-matched group was included to facilitate the identification of the potentially confounding effects of age on autonomic function due to the narrow age range of eligible patients with SSc.

All participants were required to be between 18 and 85 years old and able to give informed consent. All participants were required to have no history of diabetes mellitus, cardiac arrhythmias or current pregnancy.

#### **Participants with systemic sclerosis: criteria for inclusion and exclusion**

Due to the nature of the study participants with SSc were required to have no history of multiple digital amputations, severe finger contractions, severe breathing problems or sympathectomy. Due to the confounding effects of medications which could not be stopped, all participants had to have no history of medically-treated hypertension, renal disease, heart failure, mood disorder (i.e. depression or anxiety) or pain requiring opiate based analgesics. In addition, to the above requirements, participants taking medication for Raynaud's phenomenon had to be willing and able to stop their medication for the duration of the study. If possible, they were also requested to refrain from taking GI medications, such as proton pump inhibitors.

#### **Recruitment method**

Of the 170 patients with SSc recruited to the prospective nutrition study (Chapter Three), on account of the necessarily strict criteria only 46 patients were potentially eligible for this study. Potentially eligible patients were approached by means of invitation letter followed by either telephone contact or face-to-face discussion (at a scheduled hospital attendance).

### **Healthy participants: criteria for participation**

In addition to the requirements for all participants, healthy participants had to have no history of Raynaud's phenomenon, connective tissue diseases or respiratory, cardiac or neurological problems. They also had to not have any disease associated with gastroparesis or any symptoms suggestive of gastroparesis on initial questioning. Healthy participants could not be taking any potentially confounding medications (i.e. medications associated with autonomic side-effects or delayed gastric emptying).

### Recruitment method

The study was advertised via electronic and written advertising material. Only those healthy volunteers who fulfilled the above criteria were considered for inclusion in the study.

Age and gender matched healthy participants were matched to patients within +/- 5 years. The non-matched healthy participant group was intended to have a lower mean age and higher proportion of males.

### **5.5.2 Study environment**

Studies were conducted at Salford Royal Hospital in a quiet, softly-lit, temperature controlled room (23.5-25.5°C). A higher temperature was used than that which is normally recommended for autonomic studies, due the patients' sensitivity to the cold as a consequence of their secondary Raynaud's phenomenon.

Participants sat in a large, comfortable armchair (approximately 40° reclined) for the whole study, with the exception of the sit-to-stand manoeuvre. This position mimicked normal eating posture, whilst ensuring the participants' comfort when seated for a prolonged period of time. A nurse was present for studies involving patients with SSc.

### **5.5.3 Study preparation**

All participants were requested to attend between 8:30 and 9:30am. Prior to attendance, they were asked to fast overnight, to avoid caffeinated and alcoholic drinks for 12 hours and to avoid vigorous or unaccustomed exercise for 24 hours. Participants who smoked

were asked to refrain from smoking for 4 hours. Participants were asked not to apply skin creams.

### **Confounding medications**

Participants with SSc were asked to stop taking any potentially confounding medications for 5 half-lives prior to their attendance. Medications were for Raynaud's phenomenon (omitted 2-7 days), peripheral oedema (omitted on day of study) and GI symptoms (proton pump inhibitors 7 days; histamine H2 receptor antagonists 3 days; prokinetics 2 days; alginates 12 hours). Withheld medications were resumed after the study. Patients unable to stop medications for Raynaud's or oedema were not recruited. Participants unable to stop GI medications were still recruited due to the small effects of these medications on gastric emptying and the a priori need to include participants with GI involvement.

Due to the possible confounding effects of non-prescription medications, all participants were asked to notify the investigator if they required any medications in the 3 days prior to the study. If medications were potentially confounding, the study was re-scheduled. Potential confounding medications included anti-tussives, anti-histamines and analgesics.

#### **5.5.4 Clinical and demographic details**

Upon enrolment, date of birth, gender and handedness were recorded. Healthy participants confirmed their lack of any new GI or cardiovascular symptoms or diagnoses. As participants with SSc had already been recruited to the earlier study (Chapter Three), their disease demographics (e.g. sub-type, organ-involvement, disease duration) were known in detail.

Participants' heights and weights were recorded while wearing light clothing, without shoes. BMI was calculated.

#### **5.5.5 Gastrointestinal and autonomic questionnaires**

All participants completed the ULCA SCTC GIT 2.0 (Appendix 1) and COMPASS 31 (Appendix 11 and 12) questionnaires. The UCLA SCTC GIT 2.0 questionnaire assessed the frequency of any GI symptoms over the preceding 7 days. The COMPASS 31



questionnaire assessed the presence of symptoms suggestive of autonomic dysfunction over the preceding 1 to 5 years.

#### **5.5.6 Cardiovascular autonomic tests**

After the completion of the 2 symptom questionnaires, and whilst seated, participants' brachial BPs were recorded manually. Then participants were connected to the autonomic function analyser. The analyser and autonomic tests are described below.

As the study progressed and the technical difficulties in applying our in house apparatus and initial protocol to this challenging patient group became increasingly clear, it became necessary to adapt and develop as the study progressed as outlined below.

#### **Autonomic equipment**

The first 3 healthy participants and 8 patients with SSc were studied using the NeuroScope™ (MediFit Diagnostics Ltd, London, UK) autonomic function analyser. However, due to unanticipatedly large technical problems with this device in acquiring reliable data from SSc patients (on account of the underlying cutaneous pathology), this was then replaced by an analyser developed in-house by the Salford Medical Physics team, with a superior ECG acquisition system. It also recorded the raw ECG and beat-to-beat achieved strain pressure (sustained grip and VM) which facilitated later analysis and further stream-lined the analysis by automatically sending manually chosen, edited, R-R segments to the CMetX Cardiac Metric Software (available from: John J.B. Allen at [www.pyschofizz.org](http://www.pyschofizz.org)).

Both autonomic function analysers acquired BP from the ambulatory Portapres® system (Finapres Medical Systems, Amsterdam, The Netherlands).

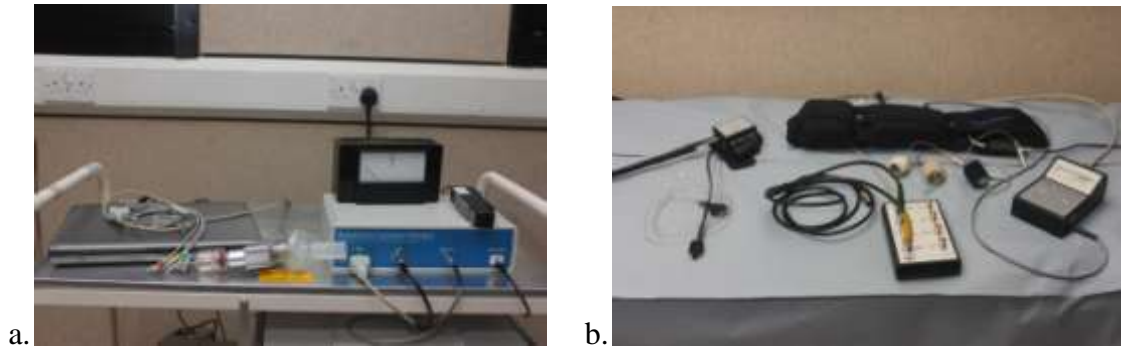


Figure 5.2: (a) In-house autonomic function analyser and (b) Portapres® system

Also, both autonomic function analysers acquired HR data from a 3-lead ECG. From this, they derived the R-R intervals, which were displayed in real-time. In order to optimize signal acquisition, the skin under the electrode was prepared using a mildly abrasive skin gel (Nuprep™, Weaver & Company, Colorado, USA) and Aqua-Wet gel backed Skintact® W-60 ECG electrodes (Leonhard Lang GmbH, Austria) were used.

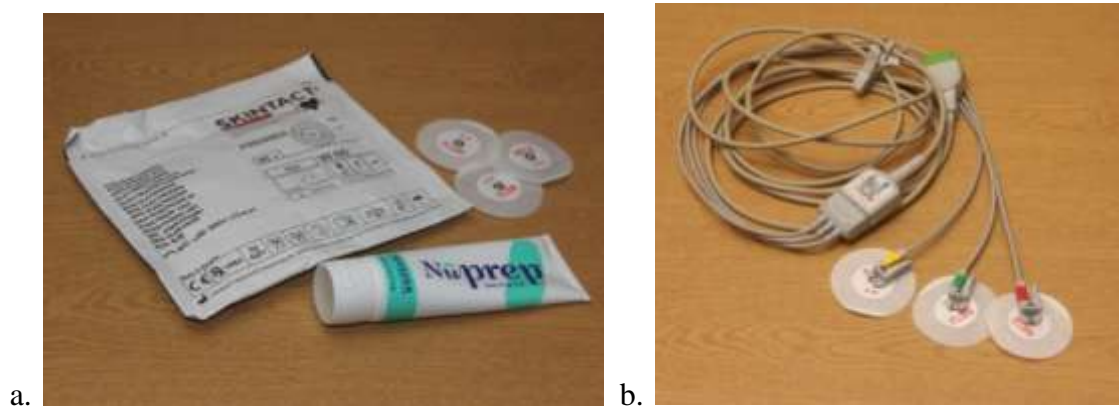


Figure 5.3: (a) Nuprep™ Skintact® electrodes and (b) ECG leads with electrodes

When using the NeuroScope™, the electrodes were placed in the standard locations (right and left subclavicular fossae and left 5<sup>th</sup> intercostal space in the mid-clavicular line). To reduce the effect of movement artefact, and thus improve signal acquisition, when using the in-house analyser different ECG electrode sites (right shoulder, left lower chest wall and left abdominal wall) were chosen. Due to its programming, the in-house analyser could interpret an ECG with this electrode configuration. This configuration generated a raw ECG with a R wave of greater relative amplitude to any artefact than the ECG from the NeuroScope™ and classical electrode position and was thus deemed technically superior.

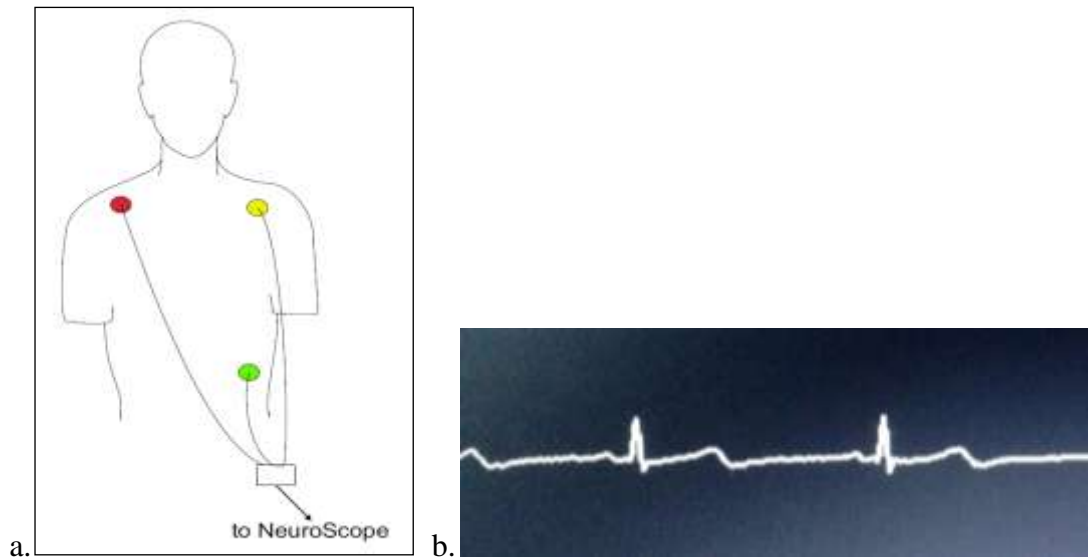


Figure 5.4: NeuroScope™ (a) ECG electrode configuration and (b) ECG signal

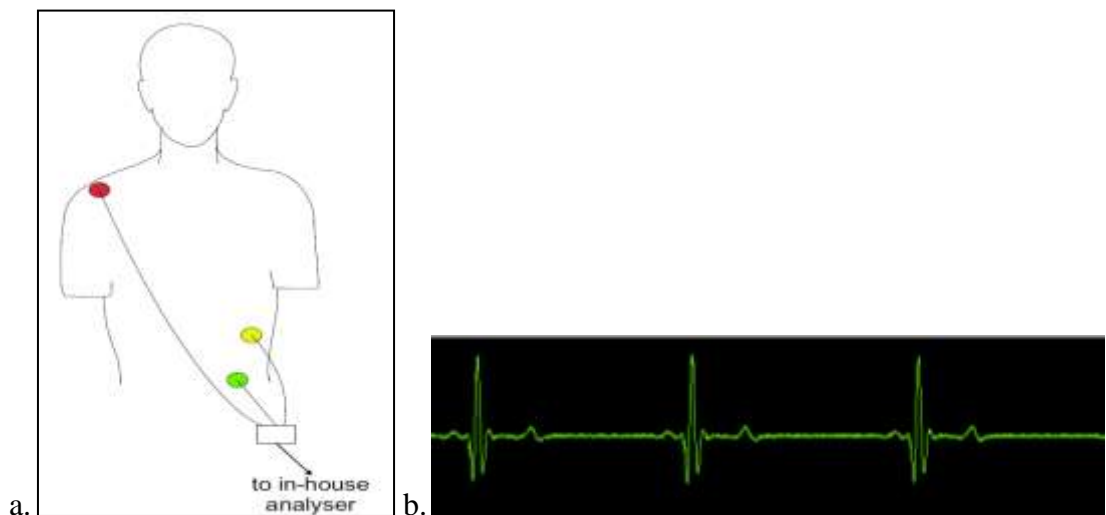


Figure 5.5: In-house (a) ECG electrode configuration and (b) larger ECG signal

Both analysers monitored beat-to-beat arterial BP via digital plethysmography using the non-invasive ambulatory Portapres® system. The raw SBP and DBP and generated MAP waveforms were displayed in real-time.

Prior to the attachment of the finger-cuff, hand temperature was felt and, if thought to be excessively cold, the participant's hands were warmed in water. In the first instance, the finger-cuff was fitted to the middle phalanx of the middle finger of the non-dominant hand. The non-dominant hand was chosen due to the use of the dominant hand in subsequent manoeuvres. If the finger-cuff failed to function, and the hand was not excessively cold, then alternative digits were tried (index, followed by ring, then thumb).

The attachment of the finger-cuff apparatus included the connection and ‘nulling’ of the height correction unit, which corrected for any hydrostatic effects.

If a reliable measurement could not be achieved (defined by an error message and/or PhysioCal interval <30sec) then, if appropriate, further attempts were made to warm the hand. Possible error messages included: ‘no plethysmogram’ (on starting), ‘contracted artery’ (during the study) and ‘no pulse’. Similarly, if the finger-cuff failed to function during the study, then further attempts were made to re-warm (heat pad) the hand and to keep it warm (covered by blanket).

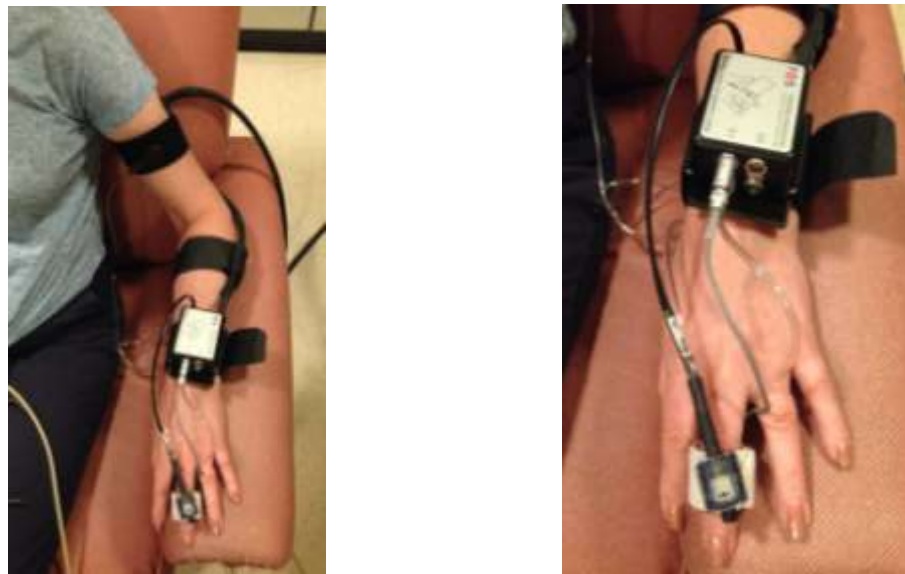


Figure 5.6: Finger-cuff attached to the middle finger

Prior to the start of any manoeuvre, participants were asked to rest to allow their cardiovascular measurements to stabilise. When recording, participants rested their hand with the finger-cuff attached on the armchair arm and kept it as still as possible, to reduce movement artefact and to minimise the chance of hydrostatic errors.

### **Resting heart rate and blood pressure variability**

The first cardiovascular autonomic assessment to be made was that of resting HRV and BP. For this, resting HR and BP were recorded over 5 minutes while the participant remained still and relaxed.

Following the study, the mean SBP, MAP and DBP were calculated. The R-R interval was manually inspected for errors such as missed, erroneous, or ectopic beats (Figure 5.7).

Any such abnormalities were manually corrected to enable analysis of the normal-to-normal R-R intervals on reliable recordings (Figure 5.8). For studies conducted using the in-house analyser, errors were confirmed using the recorded raw ECG.

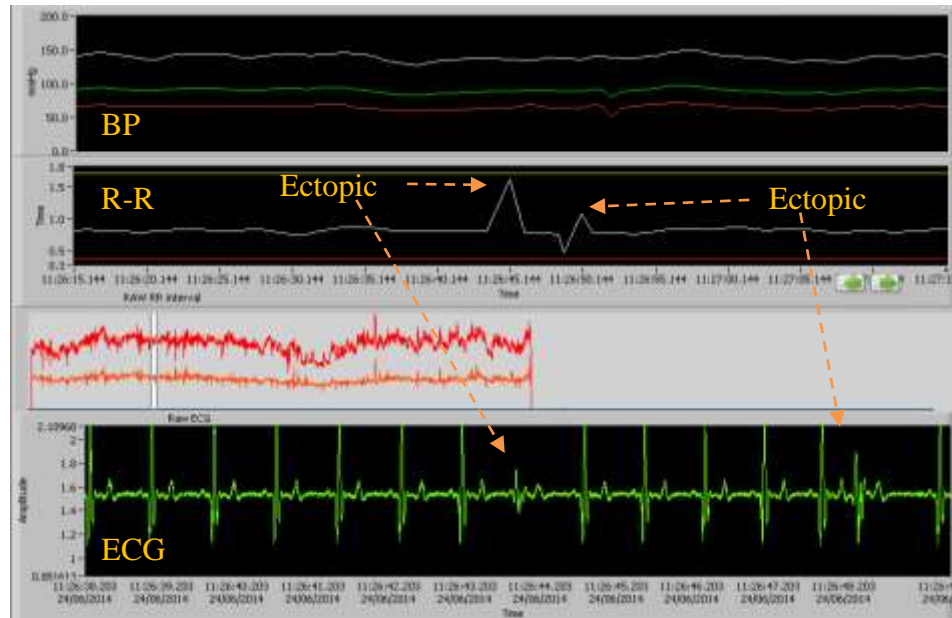


Figure 5.7: R-R and raw ECG traces showing 2 ectopic beats

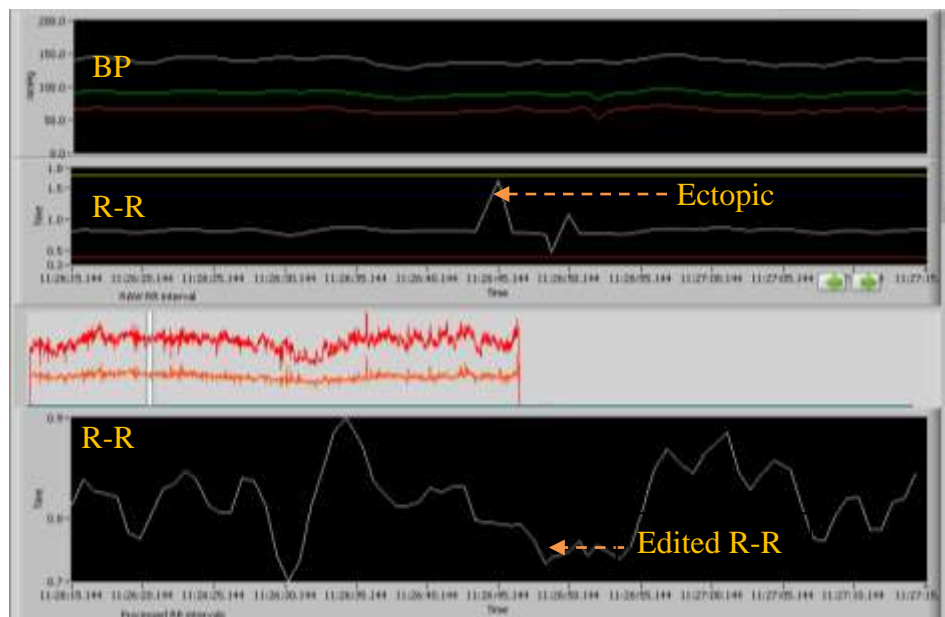


Figure 5.8: Raw R-R trace from Figure 5.7 with edited R-R segment

The corrected 5 minute R-R segment was analysed to determine the mean R-R interval and mean HR. IBIs were exported to the CMetX Cardiac Metric programme, a freely available command-line based computer programme, developed by Allen et al [278].

CMetX transforms unevenly spaced series of IBIs into a time series by interpolating data points at a fixed (10Hz) sampling rate. It then applies an optimal finite impulse response digital filter to the time-series representation of the IBI series to calculate various HRV metrics. The metrics reported in this study included SDNN (the recommended overall measure of HRV), Toichi's cardiac vagal index (CVI) and Toichi's cardiac sympathetic index (CSI). Allowances were made for the 12 seconds of data lost from the start and end of each 5 minute segment in order to retain the full assessment period.

### Heart rate response to deep breathing

The second autonomic assessment made was of change in HR during deep breathing. Participants were asked to breathe deeply, avoiding sudden gasps, at a standardised rate of 6 breaths per minute. Participants were verbally guided to inhale for 4 seconds and exhale for 6 seconds (repeated 6 times) whilst their HR was recorded. After an appropriate rest interval, the manoeuvre was repeated. Depth of breath was not recorded.

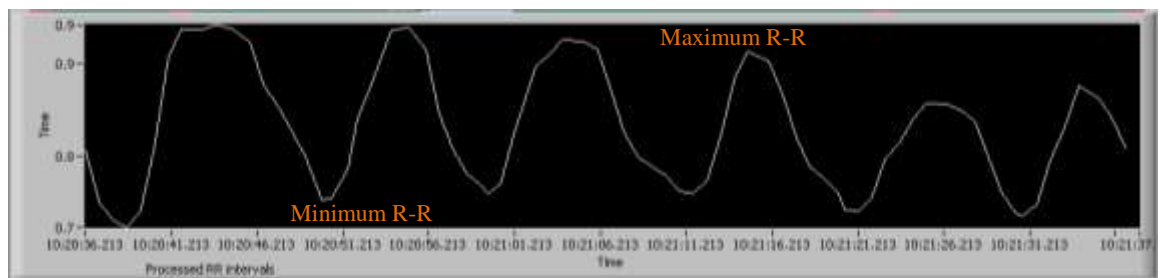


Figure 5.9: Variation in R-R interval during deep breathing

The difference between the maximum and minimum HRs for each of the 6 breaths was determined and used to calculate the average difference ( $HR_{DB}$ ). The mean of the 2 responses was calculated. A difference  $\geq 15$  beats/min is generally regarded as normal, while  $\leq 10$  beats/min is considered abnormal [268]. However, this does correct for age. The mean normal difference falls from 20 in healthy individuals aged 20-29 to only 9 in healthy individuals aged  $\geq 70$  [396]. Therefore, values were compared to age and gender minimum normal responses for use in the deduction of CASS scores.

In addition, the mean of the maximum R-R intervals and mean of the minimum R-R intervals over the 6 breaths was used to calculate the expiration/inspiration ratio (E:I ratio). The mean of the 2 manoeuvre was calculated.

### **Diastolic blood pressure response to sustained isometric handgrip**

The third autonomic assessment was of DBP response to sustained grip. Prior to starting, participants' unique voluntary maximum hand grip pressure (measured in Newtons) was determined by them squeezing the hand grip dynamometer as hard as possible using only their dominant hand muscles. The slave meter was automatically calibrated so that the maximum needle deflection represented this pressure.



Figure 5.10: Hand grip dynamometer and slave meter

Immediately prior to the assessment, in order to establish their baseline DBP, participants were asked to sit still holding the dynamometer. Baseline DBP was defined as the mean of beat-to-beat DBP measurements over a 20 second period. Then, using half their maximum force participants squeezed the hand grip dynamometer for 3 minutes. The slave meter provided visual feedback to help participants achieve and maintain a steady squeeze pressure. During analysis, the maximum DBP during the final 30 seconds was noted and compared to baseline (percentage change).



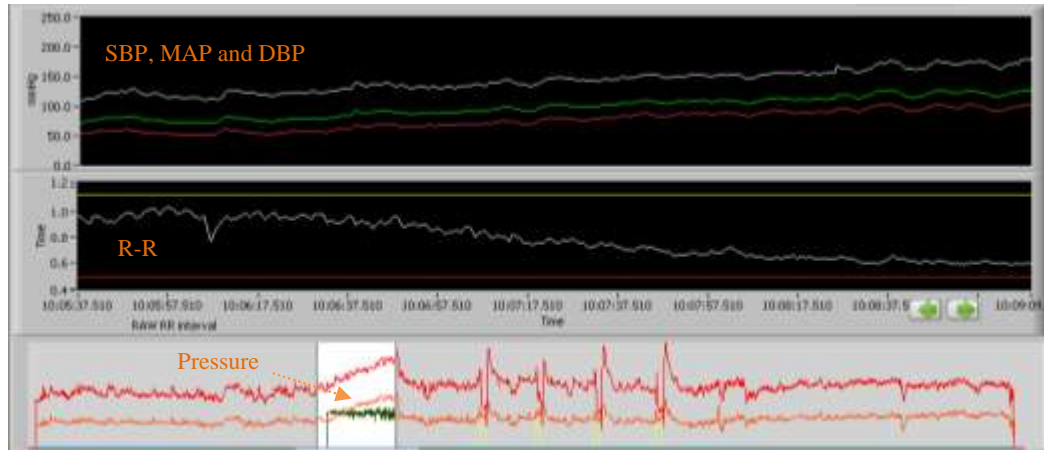


Figure 5.11: BP rises, R-R interval fall and dynamometer pressure during grip test

Achieved pressures were recorded, beat-to-beat, by the in-house analyser, but not the NeuroScope™. This is a difficult task and participants who struggled to maintain 40% of their maximum force were offered the opportunity to retry after resting. The average achieved pressures during the 3 minutes and the final minute were determined for in-house analyser studies.

### Heart rate and blood pressure response to Valsalva manoeuvre

The fourth autonomic assessment was of BP and HR changes during a VM. Participants started by sitting still holding the mouthpiece (Figure 5.12).



Figure 5.12: Valsalva mouthpiece and slave meter



To avoid data loss, the VM commenced after the completion of a PhysioCal. To indicate when to begin, the researcher counted down from 5 to 0. Towards the end of the countdown, participants were instructed to inhale but to avoid breath holding. When blowing, participants were instructed to maintain a constant pressure of 40mmHg for 15 seconds. Participants were given visual and verbal feedback about their achieved pressure and remaining time. Target pressure was indicated by the 50% mark on the slave meter (Figure 5.12). Upon completion, participants remained still during their HR and BP recovery. Participants performed at least 3 VMs.

No real-time assessment of VM adequacy was available. Beat-to-beat achieved pressures were displayed and recorded by the in-house analyser but, were only displayed by the NeuroScope. Therefore, VM adequacy assessment was based solely on researcher notes. Pressures recorded by the in-house analyser were subsequently analysed.

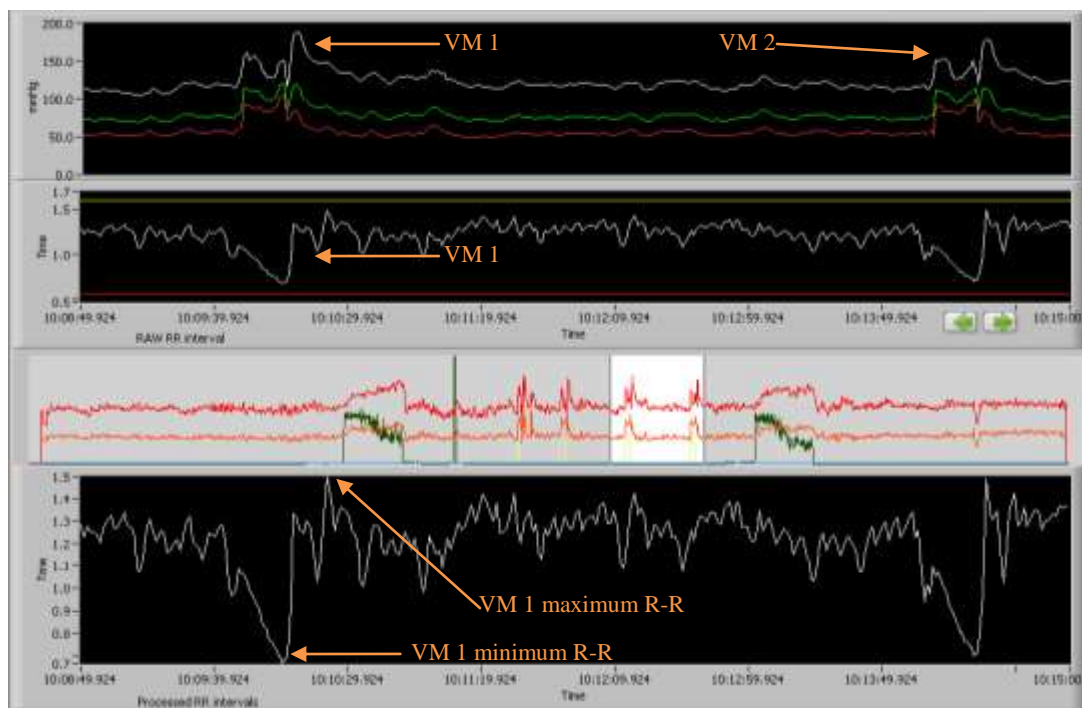


Figure 5.13: Changes in BP and R-R interval during VM

The VR was calculated for each VM. VR is the maximum HR generated by the VM strain, divided by the lowest HR recorded within 30 seconds of the peak HR. The mean VR was calculated from the 3 best VMs and compared to age and gender specific minimum normal values [275]. Baseline,  $II_E$  and  $II_L$  SBP, DBP and MAPs were extracted from the 3 chosen VMs. Pulse pressures (SBP minus DBP) were calculated.

### Blood pressure and heart rate response to sit-to-stand

The final part of the autonomic battery was the sit-to-stand test. For the first studies conducted, participants began seated in the low comfortable chair: this protocol was previously developed in diabetic gastroenteropathy patients.. However, due to difficulty for SSc patients instanding promptly, the protocol was modified to start from a higher firmer chair. Feet were however raised on a footstool to mimic the low-seated position. To avoid incurring hydrostatic errors on standing, participants' arms were supported in a sling.

Participants sat still for 5 minutes. Then, the researcher removed the footstool and participants stood up quickly (<3 seconds) without assistance. The standing timepoint was noted on the autonomic trace. Participants then stood, as still as possible, for 3 minutes.

Mean baseline SBP, MAP and DBP were derived by from measurements recorded over 20 seconds, 30 (+/-5) seconds before participants stood. Immediate post-stand changes were reported in terms of the SBP and DBP trough. In the absence of a clear trough, the lowest SBP and DBP within the first 10 seconds of standing were used. Baseline SBP and DBP were compared to lowest measurements recorded 1-3 min after standing.

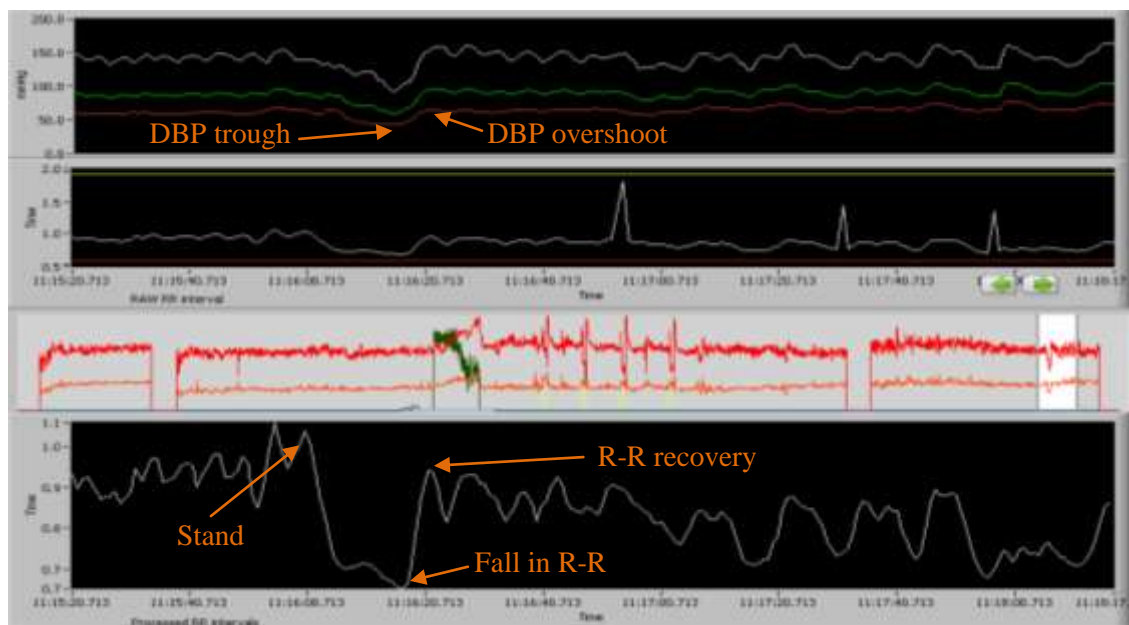


Figure 5.14: Changes in R-R interval and BP during sit-to-stand

To quantify the initial HR response to standing the secondary peak was used. Normally, this maximum HR is reached at approximately the 15<sup>th</sup> beat and the minimum at the 30<sup>th</sup>. However, as individuals differ considerably in their response times, use of the exact 15<sup>th</sup>

and 30<sup>th</sup> heart beats to calculate the 30/15 ratio may underestimate the true max/min ratio. Thus, the now accepted practice of using the highest and lowest HRs within the first 30sec of standing was used to calculate the HRmax/HRmin. As the HRmax/HRmin ratio decreases with age, the magnitude of HR change from baseline was also calculated.

### 5.5.7 Sympathetic skin response

Sympathetic sudomotor function was assessed by means of sympathetic skin response to a gasp stimulus. A sympathetic skin response is a momentary change in skin electrical potential, over the palm of the hand/fingers or the plantar surface of the foot/toes, in response to a range of internal or external stimuli. The sympathetic response reflects voltage changes due to the synchronised activation of palmer/plantar eccrine sweat glands. This method was chosen due to it being simple and fast to perform.

### Equipment

A PowerLab data acquisition system (ADInstruments Ltd, Oxfordshire, England), consisting of a digital analyser and 4 bipolar GSR) electrodes, was used in combination with a laptop running Chart™ 5 software (ADInstruments Ltd, Oxfordshire, England) (Figure 5.15).

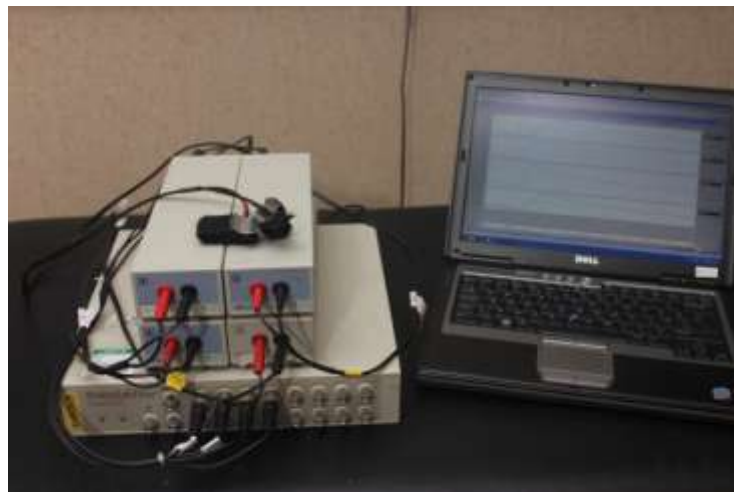


Figure 5.15: PowerLab analyser, 4 GSR and bipolar electrodes and Chart 5 laptop

GSR is an alternative term for skin conductance response. The GSR electrodes were polished stainless steel bipolar electrodes designed to be attached across 2 digits of the

same limb by Velcro. The 4 GSR system used allowed simultaneous recording of responses from all 4 limbs.

Each electrode passes a constant current between 2 skin contact points. Sympathetic sweat gland activation increases sweat secretion which reduces electrical resistance and increases conductance (Féré effect). The rise in conductivity occurs after a small lag period before decaying back to baseline. Changes in conductance are detectable between GSR electrodes. Responses generated are usually very small and the decay speed is participant dependent. Over time, due to increasing skin salt concentrations, baselines drift slowly upwards.

### Protocol

Participants were on request not wearing any skin cream. Any skin secretions were removed by wiping fingers and toes with warm water and drying thoroughly. As vasoconstriction negatively affects responses, excessively cold hands and feet were warmed with water. Electrodes were attached to limbs in sequence. Each GSR Amp was zeroed (open circuit), then attached using the Velcro and then re-zeroed (subject zero). Electrodes were attached to the plantar aspects of the distal phalanges of the second and fourth toes (Figure 5.16b) and the palmar aspects of the distal phalanges of the second and fourth fingers (Figure 5.16a).

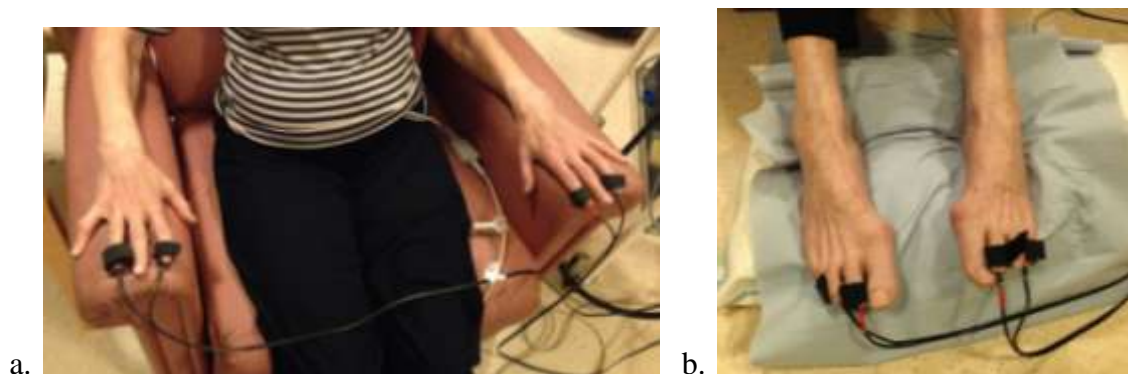


Figure 5.16: GSR leads connected to a participant's (a) hands and (b) feet

In order to avoid movement artefacts, it was imperative that participants kept their hands and feet stationary during recording since movement artefacts had larger amplitudes than responses. To assist with this, feet were rested on a soft pillow and hands on the chair arms. After the establishment of a stable baseline, participants were instructed to perform

a sudden inspiratory gasp. This was defined as a sudden deep inhalation, followed by a 2 second breath hold and then an exhalation. Then, they sat still and breathed normally while skin conductivities returned to a constant value. This gasp manoeuvre was repeated twice. Further repeats were impossible due to habituation.

The maximum amplitude reached (from baseline to peak) was recorded. Figure 5.17 shows a typical skin response. Normal responses may have low amplitudes in the range of 0.2 to 1.0 $\mu$ S [323]. Thus any responses >0.2 $\mu$ S were considered normal.

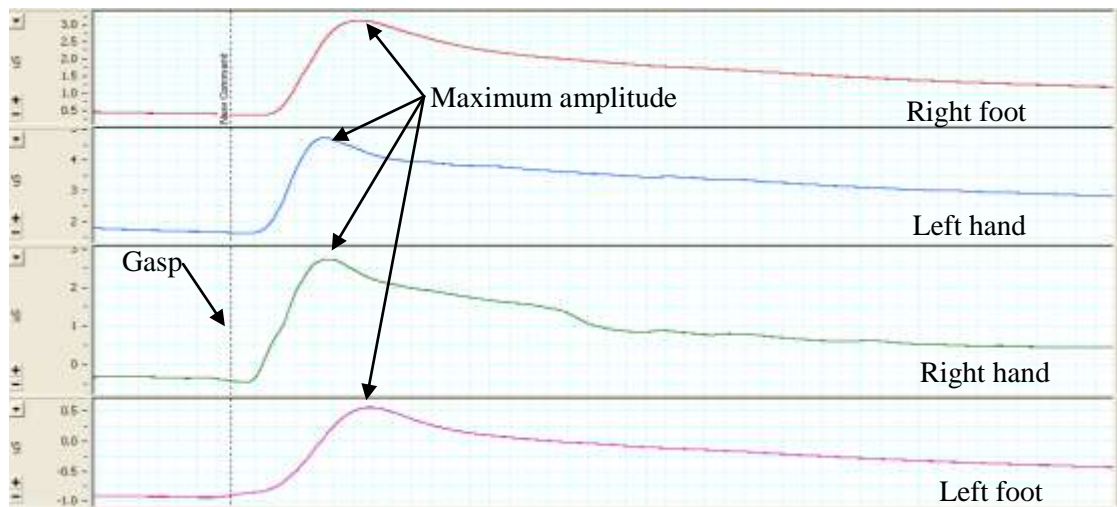


Figure 5.17: Chart 5 - typical normal GSR responses

### 5.5.8 Modified Composite Autonomic Score

For participants for whom sufficient data were available, individual CASS indexes and total scores were calculated. As the sudomotor function was assessed using the sympathetic skin responses, instead of the quantitative sudomotor axon reflex test or thermoregulatory sweat test, a modified sudomotor index was used (Table 5.1). Absence of a sympathetic skin response was defined as <0.2 $\mu$ S on first attempt.

1 =	Absent sympathetic skin response in one limb
2 =	Absent sympathetic skin response in 2 limbs
3 =	Absent sympathetic skin response in >2 limbs

Table 5.1: CASS Sudomotor Index

The cardiovagal index compared HR<sub>DB</sub> or VR to published age and gender minimum normal responses [275]. The minimum normal value used was defined as the 5<sup>th</sup> centile and the 50% minimum normal value as the 2.5<sup>th</sup> centile.

1 =	HR <sub>DB</sub> or VR mildly decreased (above $\geq 50\%$ of minimum normal value)
2 =	HR <sub>DB</sub> or VR decreased to $< 50\%$ of minimum normal value
3 =	Both HR <sub>DB</sub> and VR decreased to $< 50\%$ of minimum normal value

Table 5.2: CASS Cardiovagal Index

The adrenergic index was not modified. Phase II<sub>E</sub> values were based upon one supportive result. Failure of phase II<sub>L</sub> to return to baseline was defined as one II<sub>L</sub> not returning to within 10mmHg of baseline or 2 II<sub>L</sub> within 10mmHg of baseline. Baseline MAP was the mean MAPs over 10 seconds prior to the VM. Participants were considered to have  $\leq 50\%$  change in pulse pressure if they met this criteria on 2 occasions.

1 =	Phase II <sub>E</sub> reduction $< 40\text{mmHg}$ but $> 20\text{mmHg}$ MAP below baseline <u>OR</u> Phase II <sub>L</sub> does not return to baseline <u>OR</u> Pulse pressure reduction to $\leq 50\%$ of baseline
2 =	Phase II <sub>E</sub> decrease of $< 40\text{mmHg}$ but $> 20\text{mmHg}$ MAP <u>AND</u> Phase II <sub>L</sub> or IV absent
3 =	Phase II <sub>E</sub> decrease of $> 40\text{mmHg}$ MAP <u>AND</u> Phase II <sub>L</sub> <u>OR</u> IV absent
4 =	Criteria for 3 <u>AND</u> Orthostatic hypotension (SBP decrease of $\geq 30\text{mmHg}$ ; MAP decrease of $\geq 20\text{mmHg}$ )

Table 5.3: CASS Adrenergic Index

Individual index scores were collated to derive the total CASS. CASSs were graded into mild (1-3), moderate (4-6) or severe (7-10) autonomic failure.

### 5.5.9 Postprandial assessment

The postprandial aspect of the study consisted of serial cardiovascular assessments, gastric emptying and VAS measures (Figures 5.18 and 5.19).

Baseline samples	<ul style="list-style-type: none"> <li>• Baseline gastric emptying sample</li> <li>• Baseline VAS questionnaire</li> </ul>
Autonomic preparation	<ul style="list-style-type: none"> <li>• Connection to autonomic analyser equipment and establishment of steady recording</li> </ul>
5min rest period pre-drink	<ul style="list-style-type: none"> <li>• Baseline 1min cardiovascular autonomic measures obtained (BP and HR)</li> </ul>
1min period pre-drink	<ul style="list-style-type: none"> <li>• Patients only - manual BP</li> </ul>
Drink	<ul style="list-style-type: none"> <li>• Participants asked to drink Ensure Plus</li> <li>• Stop clock started on completion of drink</li> </ul>
5min post drink	<ul style="list-style-type: none"> <li>• Gastric emptying sample</li> <li>• VAS questionnaire</li> </ul>
7 - 10min post drink	<ul style="list-style-type: none"> <li>• 1min analyser HR and BP</li> <li>• Patients only - manual BP</li> </ul>
10min post drink	<ul style="list-style-type: none"> <li>• Gastric emptying sample</li> <li>• VAS questionnaire</li> </ul>
12 - 13min post drink	<ul style="list-style-type: none"> <li>• Healthy volunteers only - Valsalva manoeuvre</li> </ul>
17 - 20min post drink	<ul style="list-style-type: none"> <li>• 1min analyser HR and BP</li> <li>• Patients only - manual BP</li> </ul>
20min post drink	<ul style="list-style-type: none"> <li>• Gastric emptying sample</li> <li>• VAS questionnaire</li> </ul>
22 - 23min post drink	<ul style="list-style-type: none"> <li>• Healthy volunteers only - Valsalva manoeuvre</li> </ul>
27 - 30min post drink	<ul style="list-style-type: none"> <li>• 1 min analyser BP and HR</li> <li>• Patients only - manual BP</li> </ul>
30min post drink	<ul style="list-style-type: none"> <li>• Gastric emptying sample</li> </ul>
37 - 40min post drink	<ul style="list-style-type: none"> <li>• 1 min analyser BP and HR</li> <li>• Patients only - manual BP</li> </ul>
40 min post drink	<ul style="list-style-type: none"> <li>• Gastric emptying sample</li> <li>• VAS questionnaire</li> </ul>

Figure 5.18: Postprandial assessment sequence (part 1)

42 - 43min post drink	<ul style="list-style-type: none"> <li>•Healthy volunteers only - Valsalva manoeuvre</li> </ul>
47 - 50min post drink	<ul style="list-style-type: none"> <li>•1 min analyser BP and HR</li> <li>•Patients only - manual BP</li> </ul>
57 - 60min post drink	<ul style="list-style-type: none"> <li>•1min analyser BP and HR</li> <li>•Patients only - manual BP</li> </ul>
60min post drink	<ul style="list-style-type: none"> <li>•Gastric emptying sample</li> <li>•VAS questionnaire</li> </ul>
64min post drink	<ul style="list-style-type: none"> <li>•Switch off BP finger cuff</li> </ul>
67 - 70min post drink	<ul style="list-style-type: none"> <li>•1min analyser HR</li> </ul>
77 - 80min post drink	<ul style="list-style-type: none"> <li>•1min analyser HR</li> </ul>
80min post drink	<ul style="list-style-type: none"> <li>•Gastric emptying sample</li> <li>•VAS questionnaire</li> </ul>
87 - 90min post drink	<ul style="list-style-type: none"> <li>•1min analyser HR</li> </ul>
97 - 100min post drink	<ul style="list-style-type: none"> <li>•1min analyser HR</li> </ul>
100min post drink	<ul style="list-style-type: none"> <li>•Gastric emptying sample</li> <li>•VAS questionnaire</li> </ul>
107- 110min post drink	<ul style="list-style-type: none"> <li>•1min analyser HR</li> </ul>
117 - 120min post drink	<ul style="list-style-type: none"> <li>•1min analyser HR</li> </ul>
120min post drink	<ul style="list-style-type: none"> <li>•Gastric emptying sample</li> <li>•VAS questionnaire</li> </ul>

Figure 5.19: Postprandial assessment sequence (part 2)

Postprandial measures were compared to pre-prandial assessments. Due to limitations encountered when conducting the study, some assessments specific to either healthy



participants (e.g. serial VM in participants) or patients (e.g. manual brachial BP) were introduced or removed.

### **Test drink**

A chilled, liquid, mixed nutrient, readily available drink (Ensure<sup>®</sup> Plus Milkshake, Abbott Nutrition, Berkshire, UK) was chosen as the ‘test meal’. Drinks are low residue, low volume (220mls) and nutritionally complete. Each portion consisted of a 220mls and contained approximately 330kcal, 11g fat, 49g carbohydrate and 13.8g protein. They were chilled to improve palatability. Ensures<sup>®</sup> are compatible with vegetarian, gluten-free, Halal and Kosher diets and thus met most participants’ dietary tolerances. Low volumes were preferable due to possible gastroparesis.

After mixing in the radiolabelled isotope, participants drank it relatively quickly (<5 minutes) via a straw. The time limit was specified to aid in the definition of a clear postprandial start time. Time spent drinking was noted. Any liquid remaining was measured.

### **Cardiovascular assessment in the postprandial phase**

The autonomic function analyser was re-attached and measurements were allowed to stabilize while participants sat still. The mean of measures over 1 minute were used as the baseline. A longer pre-prandial period was not used due to the need to limit the total recording period. Prolonged postprandial recording from a single digit may have caused discomfort to patients with Raynaud’s phenomenon.

Mean BP and HR measurements over pre-defined 1 minute intervals were recorded (Figures 5.18 and 5.19). It proved not to be tolerable for participants to remain still and quiet for the entire study. Therefore, participants only sat for at least 2 minutes prior to each interval with the exception of the first measurement due to the fifth minute VAS questionnaire. BP measures were stopped after one hour. A longer continuous recording period would have required more than one digit. Normal-to-normal R-R intervals were analysed using the CMetX Cardiac Metric Software. The percentage change in postprandial serial measures (HR, SBP, DBP, CVI, CSI) from baseline were calculated.

Due to concerns I developed over the reliability of digital BP measurements in patient studies, serial manual brachial assessments were introduced as an additional step after 5 patient studies. To prevent interference with the digital measures, the contra-lateral arm was used and measurements performed only in SSc patients after the paired autonomic assessment. To prevent observer bias, different researchers conducted the autonomic and manual measurements. Measurements were performed by research nurse present for patient safety rather than lone researcher.

It had been planned for all participants to perform serial postprandial VMs. However, due to difficulties experienced by initial patients, it was decided that postprandial VMs were to be omitted from patient studies. Only serial VRs were calculated.

### **Gastric Emptying**

The isotope used was the stable, water-soluble, non-radioactive  $^{13}\text{C}$  sodium acetate (100mg powder). Prior to each study, this was weighed on scales calibrated to 1mg and stored in a clean dry container. It was mixed with the Ensure<sup>®</sup> immediately prior to drinking. Pre-weighing prevent participants being left unattended during the study. Best efforts were made to ensure complete transfer of the powdered isotope.

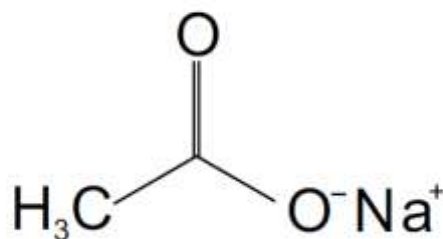


Figure 5.20: Sodium acetate chemical structure

The baseline end-expiratory breath sample was collected before consuming the labelled Ensure<sup>®</sup> test drink. Postprandial end-expiratory breath samples were collected at the 5, 10, 20, 30, 40, 60, 80, 100 and 120 minutes. Samples were collected using pre-labelled 100ml double-ended breath sample bags (Figure 5.20). Special one-way mouth pieces prevented air from escaping before placement of the stopper.



Figure 5.21: Sample bags, stoppers, mouth piece and sodium acetate

Samples were kept at room temperature until they could be analysed using the IRIS<sup>®</sup> <sup>13</sup>C-Acetate Breath Test System (Wagner Analysen Technik GmbH, Bremen, Germany). Samples were analysed within 1 week. Results were standardised for physiologic CO<sub>2</sub> production which is dependent upon body weight and height.

Reported indices for each time point included the volume of CO<sub>2</sub> (an indicator of test performance), measured percentage dose per hour and cumulative dose. The area under curve (AUC) was calculated for the percentage <sup>13</sup>C dose per hour.

### **Visual analogue scores**

Participants completed serial postprandial 100mm VAS questionnaires (Appendix 13) which scored various GI sensations and symptoms. Sensation scores of hunger, satisfaction, fullness and desire to eat more were adapted from the study by Flint et al [353]. Symptom scores of nausea, abdominal bloating and abdominal discomfort were devised for this study. For each question, individual scores, change in score from baseline and AUC were reported.

### **5.5.10 Statistical methods**

Statistical analysis was performed using SPSS (version 22). The differences between the participant groups' mean scores for the demographic, symptom (UCLA and COMPASS

31) and pre- and postprandial autonomic measures were assessed using Mann-Whitney U tests. Autonomic measures involving BP and HR were compared between paired groups (SSc vs. matched healthy; SSc vs. non-matched healthy; matched vs. non-matched healthy) rather than across all 3 groups due to the known confounding effect of age. Wilcoxon Signed Rank tests were used to compare pre-study mean manual brachial and digital DBP and SBP within groups.

As this was an exploratory rather than a mechanistic study correlations (Spearman's (s)) were sought between achieved pressure and percentage change in DBP during the sustained grip manoeuvre. For patients, correlations (Spearman's (s)) were also sought between GI symptoms and gastric emptying, between GI symptoms and autonomic measures, between GI symptoms and gastric emptying and between postprandial autonomic measures and CASS.

Differences between the 3 participant groups' gastric emptying results, postprandial VAS and postprandial cardiovascular responses were sought using Kruskal-Wallis and/or Mann-Whitney U tests. Similarities between serial postprandial VRs were sought using repeated Friedman's test. For all analyses, a significant difference was by convention accepted as a p value of  $\leq 0.05$ .

## **5.6 Results**

The study results are summarised in the following sub-sections. Given the complex and extensive nature of the analysis, the key positive and negative findings are later summarised in 5.5.17.

### **5.6.1 Participants recruited**

Recruitment took place between January and September 2014. Seventeen patients were recruited, exhausting the entire potential panel of Salford patients who might participate. The following figure describes their selection and response to study invitation.

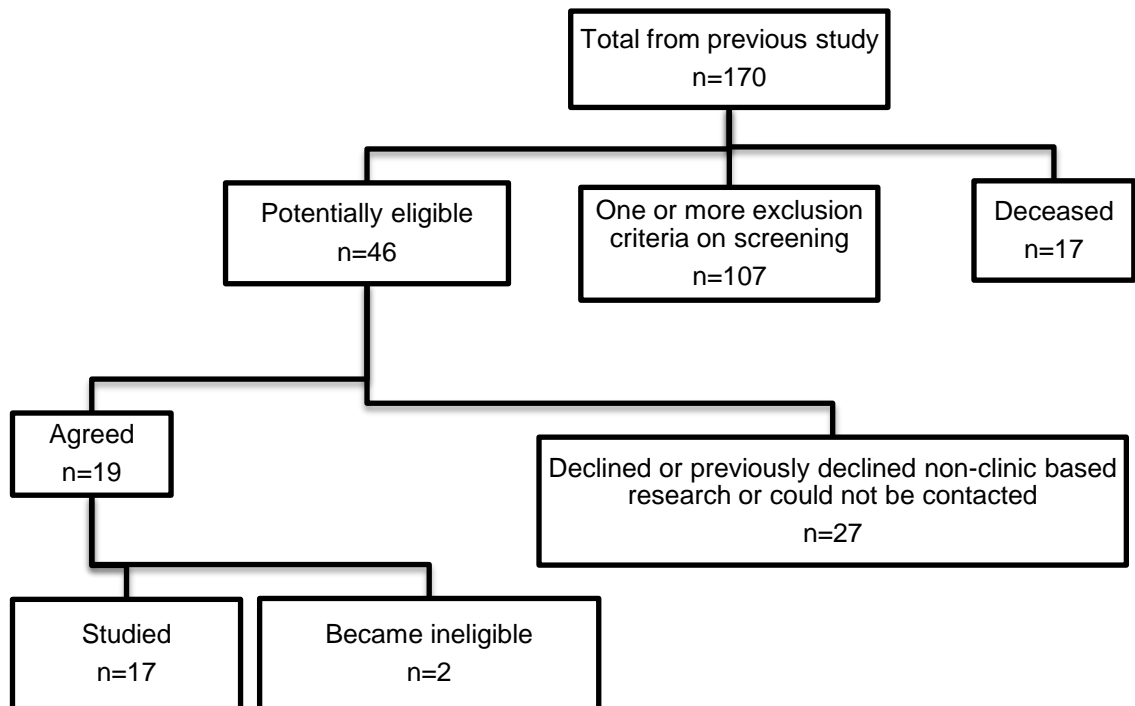


Figure 5.22: Recruitment of patients with SSc

Seventeen matched healthy participants were recruited. One study was abandoned after completion of the UCLA and COMPASS 31 questionnaires as she was found to be in atrial fibrillation. The GP was made aware and managed this. Fifteen non-matched healthy participants were recruited.

### 5.6.2 Demographics

Participant demographics are summarised below (Table 5.4).

	<b>SSc</b>	<b>Matched healthy controls</b>	<b>Non-matched healthy controls</b>
Number	17	17	15
Male:Female	1:16	1:16	4:11
Mean age (range)	63.2 (45.1 – 77.3)	62.2 (45.3 – 75.1)	36.3 (23.0 – 59.8)
Smokers: non-smokers	1:16	1:16	2:13
Mean BMI (range)	24.4 (19.0 – 33.3)	25.7 (19.0 – 32.7)	27.1 (18.4 – 45.8)

Table 5.4: Participant demographics

There was no significant (Mann-Whitney U) difference between the mean age of the patients and their matched healthy counterparts ( $p=0.66$ ). In addition, there was no significant (Mann-Whitney U) difference between the mean BMI of the patients and their matched healthy counterparts ( $p=0.27$ ). Similarly, there was no significant (Mann-Whitney U) difference between the mean BMI of the patients and their non-matched healthy counterparts ( $p=0.37$ ).

### **5.6.3 Clinical manifestations and potential confounders**

#### **Healthy participants**

In accordance with the exclusion criteria, none of the healthy participants was known to have a history of SSc, Raynaud's phenomenon, autonomic dysfunction, delayed gastric emptying or any other potentially confounding illness. However, one participant was found on attendance to have previously undiagnosed atrial fibrillation. No healthy participants were taking confounding medication.

#### **Patients with systemic sclerosis**

Sixteen (94%) patients had lcSSc. Eight (47%) were anti-centromere and 2 (12%) were anti-topoisomerase antibody positive. The median time from Raynauds' phenomenon onset was 196 (16 to 653) months and from SSc onset was 115 (12 to 348) months.

Six (35%) patients had normal Medsger lung severity scores, while 5 (29%) had mild, 5 (29%) had moderate and one (6%) had severe lung involvement. Due to the exclusion criteria no patients had cardiovascular involvement. Seven (41%) patients had normal Medsger GI severity scores, while 9 (53%) had mild involvement and one (6%) had moderate involvement.

Twelve patients were prescribed a proton pump inhibitor. Five of these were unable to stop it. In addition, 2 of these 5 patients required ranitidine and neither stopped it. No patients were prescribed prokinetics. Three patients took medication (2 nifedipine, 1 losartan) for Raynaud's phenomenon. All omitted it for the required time. Three patients were taking furosemide and all omitted it prior to the study.

#### 5.6.4 Gastrointestinal symptoms

This section summarises the results of the UCLA questionnaire. Significant differences (Mann-Whitney U) were identified in all the mean UCLA domain scores between the patients and both groups (matched and non-matched) of healthy participants. As above, there were no significant differences between the mean domain scores of the matched and non-matched healthy participant groups.

In addition, across all symptom domains, the absolute difference in mean scores between the patients and both of the healthy participant groups exceeded that of the minimally important difference required for there to be a clinical significance [88].

<b>Symptom domain</b>	<b>SSc mean (range) n=17</b>	<b>Matched healthy controls mean (range) n=17</b>	<b>Non-matched healthy controls mean (range) n=15</b>	<b>SSc vs. matched healthy controls</b>	<b>SSc vs. non matched healthy controls</b>	<b>Matched vs. non-matched controls</b>
Reflux	0.60 (0.0 – 2.0)	0.04 (0.0 – 0.4)	0.06 (0.0 – 0.5)	p<0.01	p<0.01	p=0.71
Distension / bloating	1.12 (0.6 – 3.0)	0.19 (0.0 – 1.0)	0.13 (0.0 – 1.0)	p<0.01	p<0.01	p=1.00
Faecal soilage	0.59 (0.0 – 3.0)	0.00 (0.0 – 0.0)	0.00 (0.0 – 0.0)	p=0.02	p=0.02	p=1.00
Diarrhoea	0.35 (0.0 – 1.5)	0.03 (0.0 – 0.5)	0.00 (0.0 – 0.0)	p=0.02	p=0.01	p=0.79
Social functioning	0.39 (0.0 – 1.0)	0.00 (0.0 – 0.0)	0.02 (0.0 – 0.3)	p<0.01	p<0.01	p=0.77
Emotional well-being	0.71 (0.0 – 2.1)	0.00 (0.0 – 0.0)	0.00 (0.0 – 0.0)	p<0.01	p<0.01	p=1.00
Constipation	0.63 (0.0 – 2.5)	0.07 (0.0 – 1.0)	0.00 (0.0 – 0.0)	p<0.01	p<0.01	p=0.58
Total GI score	0.63 (0.0 – 1.5)	0.04 (0.0 – 0.2)	0.04 (0.0 – 0.2)	p<0.01	p<0.01	p=0.79

Table 5.5: UCLA domain responses and comparison of means

### 5.6.5 Autonomic symptoms

This section summarises the results of the COMPASS 31 questionnaire (Table 5.6).

Symptom domain	SSc mean (range) n=17	Matched healthy controls mean (range) n=17	Non-matched healthy controls mean (range) n=15	SSc vs. matched healthy controls	SSc vs. non-matched healthy controls	Matched vs. non-matched controls
Orthostatic	9.88 (0.0 – 24.0)	2.82 (0.0 – 20.0)	2.4 (0.0 – 16.0)	p=0.05	p=0.05	p=1.00
Vasomotor	3.33 (0.0 – 4.2)	0.15 (0.0 – 2.5)	0.0 (0.0 – 0.0)	p<0.01	p<0.01	p=0.79
Secretomotor	5.17 (0.0 – 12.9)	0.50 (0.0 – 4.3)	0.0 (0.0 – 0.0)	p<0.01	p<0.01	p=0.41
GI	9.72 (0.0 – 25.0)	2.89 (0.0 – 7.1)	1.25 (0.0 – 4.5)	p<0.01	p<0.01	p=0.03
Bladder	2.22 (0.0 – 16.7)	0.26 (0.0 – 1.1)	0.0 (0.0 – 0.0)	p=0.01	p<0.01	p=0.26
Pupillomotor	1.96 (0.0 – 5.0)	0.82 (0.0 – 2.7)	0.60 (0.0 – 2.7)	p<0.01	p<0.01	p=0.48
Total	32.2 (0.0 – 54.9)	7.45 (0.0 – 24.9)	4.25 (0.0 – 22.1)	p<0.01	p<0.01	p=0.11

Table 5.6: COMPASS 31 domain scores and comparison of means

Significant differences (Mann-Whitney U) existed in all mean COMPASS 31 domain scores between patients and matched healthy participants. Significant differences were identified between all the mean COMPASS 31 domain scores of patients and non-matched healthy participants, in keeping with an ageing effect. With the exception of GI, scores did not differ significantly between matched and non-matched healthy participants. However, most healthy group participants had scored low.

### 5.6.6 Baseline cardiovascular battery

All participants completed the autonomic battery, with the exception of the excluded patient. However, for some subjects data were incomplete due to equipment failures or participant-related difficulties with certain manoeuvres. Technical recording failures were more common in the patient group.



In some patient studies an acceptable BP trace was simply unobtainable. Patient studies were more likely to use the index finger or thumb for BP. Seven patient studies used the thumb, 3 the index finger and one the ring finger, while only 6 (35%) used the middle finger. In comparison, no non-matched healthy participants and only 2 matched healthy (index finger and one thumb) did not use their middle finger. In some patient studies, BP traces deteriorated over time and hence measurements were only obtained for the initial manoeuvres. Missing data are reported in the relevant sections.

Patients were studied before their matched healthy counterparts. Therefore, the NeuroScope was used for 47% of patient, 12% of matched healthy and only 7% of non-matched healthy studies. The NeuroScope was more prone to movement artefact affecting R-R measurements. In addition, R-R trace loss resulted in simultaneous loss of BP recordings.

As a consequence of the above difficulties, patient studies often took longer than healthy participant studies. For patients, the mean time from successful finger-cuff application to completion was 73 minutes (range 48 to 107), while it was 64 minutes (range 52 to 87) in matched healthy participants and 61 minutes (range 50 to 75) in non-matched healthy participants. Furthermore, NeuroScope patient studies (mean 79 minutes; range 48 to 107) took longer on average than in-house analyser studies (mean 67 minutes; range 51 to 88).

### **Resting heart rate variability and blood pressure**

This section describes the baseline manual BPs and the mean HR, R-R and BP measurements during the 5 minute rest period.

#### Blood pressure

The patients' mean brachial BP was 120/74 (range 182/90 to 92/60). The matched healthy participants' mean brachial BP was 117/72 (range 172/108 to 82/48). The non-matched healthy participants' mean brachial BP was 116/72 (range 142/84 to 104/58). There were no significant differences (Mann-Whitney U) between the mean SBP and DBPs.

The analyser continuously measured BP over a 5 minute period. The patients' mean digital BP was 100/52 (range 141/74 to 60/33). The matched healthy participants' mean digital

BP was 122/63 (range 173/92 to 60/33). The non-matched healthy participants' mean digital BP was 122/63 (range 142/74 to 99/45). A significant difference was detected between the mean SBP ( $p=0.05$ ) and DBP ( $p=0.01$ ) of the patients and matched healthy participants. However, no significant differences (Mann-Whitney U) were identified between the mean SBPs and DBPs of the patients and non-matched healthy participant groups or between the healthy participant groups.

In addition, significant differences (Wilcoxon Signed Rank) were noted between the digital and brachial SBPs of the patients ( $p<0.01$ ), but not between the matched ( $p=0.17$ ) or non-matched ( $p=0.93$ ) healthy participants. All 3 groups were shown to have significant differences ( $p<0.01$ ) between their digital and brachial DBPs.

### Heart rate

ECG derived measurements were averaged over 5 minutes. Significant differences were sought between groups (Mann-Whitney U; Table 5.7).

	<b>SSc mean±SD (range) n=17</b>	<b>Matched healthy controls mean±SD (range) n=16</b>	<b>Non-matched healthy controls mean±SD (range) n=15</b>	<b>SSc vs. matched healthy controls</b>	<b>SSc vs. non-matched healthy controls</b>	<b>Matched vs. non-matched controls</b>
R-R interval (msec)	931±140 (728-1204)	908±127 (591-1150)	998±116 (827-1258)	$p=0.87$	$p=0.15$	$p=0.05$
HR (beats/min)	66±10 (50-82)	68±11 (52-102)	61±7 (48-73)	$p=0.85$	$p=0.17$	$p=0.06$
SDNN (HRV)	28.3±9.9 (16.7-53.5)	34.8±14.3 (11.8-68.7)	56.6±14.8 (26.6-86.3)	$p=0.13$	$p<0.01$	$p<0.01$
CVI	3.9±0.3 (3.5-4.4)	4.0±0.5 (2.7-4.7)	4.5±0.3 (3.8-4.9)	$p=0.23$	$p<0.01$	$p<0.01$
CSI	2.5±1.1 (1.1-4.9)	3.2±1.7 (1.7-8.1)	2.4±0.8 (1.3-4.2)	$p=0.17$	$p=1.00$	$p=0.20$

Table 5.7: Mean resting heart rate measures and comparison of means

## Deep breathing manoeuvre

HR measurements during deep breathing were obtained for all participants. Scores are reported in the following table.

	<b>SSc mean <math>\pm</math> SD (range) n=17</b>	<b>Matched healthy controls mean <math>\pm</math> SD (range) n=16</b>	<b>Non-matched healthy controls mean <math>\pm</math> SD (range) n=15</b>
E:I ratio	1.16 $\pm$ 0.07 (1.08 – 1.31)	1.12 $\pm$ 0.11 (1.08 – 1.42)	1.31 $\pm$ 0.14 (1.08 – 1.56)
Difference between maximum and minimum HR (DB <sub>HR</sub> )	10.1 $\pm$ 4.8 (5.0 – 18.7)	11.7 $\pm$ 6.0 (4.4 – 24.6)	17.3 $\pm$ 7.5 (5.3 – 31.8)

Table 5.8: Heart rate measures during deep breathing

No significant differences (Mann-Whitney U) were detected between the patients and matched healthy participants (E:I ratio  $p=0.40$ ; DB<sub>HR</sub>  $p=0.35$ ). However, there were significant ( $p<0.01$ ) differences between the results (E:I ratio and DB<sub>HR</sub>) of patients and non-matched healthy participants and between the non-matched and matched healthy participants (E:I ratio  $p<0.01$ ; DB<sub>HR</sub>  $p=0.02$ ). To explore whether this was linked to the different median age of the non-matched participants to that of the matched healthy participant and SSc groups, DB<sub>HR</sub> values were compared to normative values [275]. Participants aged above 69 were included in the 60 to 69 normative range.

	<b>SSc number (%) n=17</b>	<b>Matched healthy controls number (%) n=16</b>	<b>Non-matched healthy controls number (%) n=15</b>
$\leq 2.5^{\text{th}}$ centile	6 (35%)	5 (31%)	2 (13%)
2.5 <sup>th</sup> to $\leq 5^{\text{th}}$ centile	2 (12%)	1 (6%)	1 (7%)
$> 5^{\text{th}}$ centile	9 (53%)	10 (63%)	12 (80%)

Table 5.9: DB<sub>HR</sub> compared to age-matched centile lines

A greater percentage of non-matched healthy participants had a ‘normal’ ( $> 5^{\text{th}}$  centile) DB<sub>HR</sub>.

### Sustained grip manoeuvre

Not all sustained their target pressure for the full duration. Pressures achieved were only recorded by the in-house analyser. Patients and participants different resting BPs percentage, rather than absolute, DBP rise was used for statistical comparisons.

	<b>SSc</b>	<b>Matched healthy controls</b>	<b>Non-matched healthy controls</b>
Initial pressure (SD (number of patients; range))	123±55 (n=17; 47 - 248)	135±50 (n=16; 47 - 248)	211±80 (n=15; 122 - 364)
Percentage maximum mean pressure over 3 minutes (SD (number of patients; range))	48±2.8 (n= 9; 42 - 52)	47±5 (n=14;33 - 51 )	46±8 (n=14; 36 - 53)
Percentage of maximum mean pressure during final minute (SD (number of patients; range))	47.1±3.3 (n=9; 40 - 51)	47±5 (n=14; 33 - 52)	43±9 (n=14; 26 - 51)
Absolute DBP rise (SD (number of patients; range))	22.1±12.1 (n=17; 3 - 43)	29.0±13.9 (n=16; 11 - 65)	39.0±17.6 (n=14; 23 - 87)
Percentage DBP rise (SD (number of patients; range))	26.2±13.1 (n=17; 5 - 48)	29.7±9.7 (n=16; 16 - 49)	37.9±9.4 (n=14; 24 - 57)

Table 5.10: Sustained grip manoeuvre results

There was no significant difference (Mann-Whitney U) between the mean percentage rise in DBP between patients and the matched healthy participants (p=0.40). However, there were differences between the patients and non-matched healthy participants (p=0.02) and the matched and non-matched healthy participants (p=0.04). No correlations (Spearman's) were evident between either measure of achieved grip pressure and percentage DBP increase for any of the participant groups.

### Valsalva manoeuvre

All participants attempted VMs but, for some, data acquisition was incomplete. In addition, not all patients achieved the target pressure. During patient studies the mean pressure was 26±10mmHg (range 10 to 41; n=9), while during matched healthy participant studies it was 40±3mmHg (range 35 to 43; n=14) and during non-matched healthy participants it was 41±1mmHg (range 37 to 43; n=14). Pressures achieved by

patients were significantly lower (Mann-Whitney U) than pressures achieved by matched ( $p<0.01$ ) and non-matched ( $p<0.01$ ) healthy participants.

The patients mean VR was  $1.5\pm 0.2$  (range 1.2 to 1.9;  $n=15$ ), while the matched healthy participants was  $1.8\pm 0.4$  (range 1.3 to 2.5;  $n=15$ ) and the non-matched healthy participants was  $2.2\pm 0.3$  (range 1.6 to 2.6;  $n=15$ ). VR was unavailable for two patients and one matched participant. Significant differences were detected between the mean VRs of patients and matched healthy participants ( $p=0.02$ ), between patients and non-matched healthy participants ( $p<0.01$ ) and between matched and non-matched healthy participants ( $p<0.01$ ). To correct for the effects of age, the VR ratios were compared to age and gender normative values [275]. Participants over 69 years were included in the 60 to 69 normative range. All non-matched healthy participants had normal VRs ( $>5^{\text{th}}$  centile), but not all patients and matched healthy participants did (Table 5.11).

	<b>SSc number (%) n=15</b>	<b>Matched healthy controls number (%) n=15</b>	<b>Non-matched healthy controls number (%) n=15</b>
$\leq 2.5^{\text{th}}$ centile	4 (27%)	2 (13%)	0 (0%)
$2.5^{\text{th}}$ to $\leq 5^{\text{th}}$ centile	0 (0%)	0 (0%)	0 (0%)
$>5^{\text{th}}$ centile	11 (73%)	13 (87%)	15 (100%)

Table 5.11: VRs compared to normative centile

For some participants, BP recordings were of too poor quality. A phase II<sub>E</sub> MAP reduction of  $>20\text{mmHg}$  was not observed in any (0/13) of the patient studies but was seen in 20% (3/15) of the matched healthy and 6% (1/15) of the non-matched healthy participant studies. Phase II<sub>E</sub> pressure never fell by  $>40\text{mmHg}$ . In 7% (1/14) of patients phase II<sub>L</sub> MAP did not return to baseline. This compares to 40% (6/15) of the matched healthy participants and 26% (4/15) of the non-matched healthy participants. Phase II<sub>L</sub> was absent in 14% (2/14) of patients and 6% (1/15) of matched healthy participants, but it was not absent in any of the non-matched healthy participants (0/15). Phase IV was never absent. A fall in pulse pressure to  $\leq 50\%$  of baseline was seen in 62% (8/13) of patients, 40% (6/15) of matched healthy participants and 47% (7/15) of non-matched healthy participants.

### Sit-to-stand manoeuvre

The mean HRmax/HRmin ratio in patients was  $1.16 \pm 0.08$  (range 1.01 to 1.31; n=16), in matched healthy participants it was  $1.21 \pm 0.12$  (range 1.04 to 1.38; n=16) and in non-matched healthy participants it was  $1.40 \pm 0.23$  (range 1.14 to 2.07; n=15). The HR trace was unsuitable for analysis in 1 patient. No significant differences (Mann-Whitney U) were detected between the mean ratios of patients and matched healthy participants ( $p=0.18$ ). However, significant differences were detected between the patients and non-matched healthy participants ( $p<0.01$ ) and between matched and non-matched healthy participants ( $p<0.01$ ). The range of BP falls during the post-stand trough are displayed (Table 5.12).

	<b>SSc mean <math>\pm</math> SD (range) n=15</b>	<b>Matched healthy controls mean <math>\pm</math> SD (range) n=16</b>	<b>Non-matched healthy controls mean <math>\pm</math> SD (range) n=15</b>
SBP trough (mmHg)	27.6 $\pm$ 12.7 (3.9 – 48.1)	31.4 $\pm$ 10.8 (12.1 – 55.8)	25.0 $\pm$ 13.8 (1.6 – 51.7)
MAP trough (mmHg)	16.2 $\pm$ 6.6 (3.4 – 29.1)	22.1 $\pm$ 4.4 (13.4 – 30.6)	19.9 $\pm$ 8.0 (7.0 – 32.0)
DBP trough (mmHg)	10.8 $\pm$ 5.0 (2.6 – 21.7)	17.7 $\pm$ 3.7 (11.4 – 24.5)	17.4 $\pm$ 6.0 (5.6 – 27.3)

Table 5.12: Spread of BP sit-to-stand troughs

There were no significant differences (Mann-Whitney U) between the troughs of patients and either the matched or non-matched healthy participants. Likewise, there were no significant differences detected between the troughs of the healthy participant groups.

Between 1 and 3 minute post-stand, none of the patients (0/15) or non-matched healthy participants (0/15) had a SBP fall of  $\geq 30$ mmHg. In comparison, 6% (1/16) of matched healthy participants had a SBP fall of  $\geq 30$ mmHg. During the same period, 7% (1/15) of patients, 7% (1/16) of matched healthy participants and none (0/15) of non-matched healthy participants had a MBP fall of  $\geq 20$ mmHg. In addition, 7% (1/15) of patients, 13% (2/16) of matched healthy participants and 7% (1/15) of non-matched healthy participants had a DBP fall of  $\geq 10$ mmHg. Thus, matched healthy participants were more likely to have falls sufficiently great to meet CASS criteria for orthostatic responses.

### 5.6.7 Sympathetic sudomotor skin responses

All participants attempted this but, due to equipment errors, analysable data were not available for all. The range of responses is shown in Table 5.26.

	<b>SSc mean ± SD (n; range)</b>	<b>Matched healthy controls mean ± SD (n; range)</b>	<b>Non-matched healthy controls mean ± SD (n; range)</b>
Right foot	0.39±0.3 (n=12; 0.03 – 1.01)	1.07±1.07 (n=14; 0.07 – 3.95)	0.88±0.91 (n=14; 0.00 – 2.83)
Left foot	0.48±0.39 (n=12; 0.00 – 1.26)	1.09±1.12 (n=14; 0.02 – 4.01)	0.82±0.89 (n=14; 0.00 – 3.43)
Left hand	0.98±0.39 (n=12; 0.00 – 2.47)	1.44±0.97 (n=14; 0.15 – 3.19)	2.10±1.77 (n=14; 0.00 – 5.50)
Right hand	1.51±1.40 (n=11; 0.00 – 4.58)	1.41±0.74 (n=14; 0.19 – 2.67)	2.27±1.80 (n=13; 0.00 – 5.19)

Figure 5.23: Spread of SSR responses

The magnitude of positive responses varied substantially within and between individuals. Therefore, responses were only scored as either positive or negative. A negative response was defined as <0.2µS. Lower limb responses were more likely to be abnormal. Across all participants, 30% of all lower limb and 15% of all upper limb scores were abnormal

All 4 limb responses were normal in 67% (8/12) of patients, 71% (10/14) of matched healthy participants and 64% (9/14) of non-matched participants. One abnormal response was only noted in 7% (1/14) of non-matched healthy participants. Two abnormal scores were noted in 8% (1/12) of patients, 21% (3/14) of matched healthy participants and 14% (2/14) of non-matched healthy participants. Three abnormal responses were only generated by 8% (1/12) of patients. Four abnormal responses were produced by 17% (2/12) of patients, 7% (1/14) of matched healthy participants and 14% (2/14) of non-matched healthy participants. Responses were used to calculate the CASS sudomotor index (section 5.5.8). Two participants had data missing from one limb. Overall, when present, abnormal responses were more likely to affect all limbs or both feet.

### 5.6.8 Composite Autonomic Severity Score

The range of CASS index scores are described for each group (Table 5.13). Of the patients studied, only 12 had sudomotor scores and only 14 had adrenergic scores. Thus, total CASS scores could only be calculated for 11 patients with SSc.

	<b>SSc</b>	<b>Matched healthy controls</b>	<b>Non-matched healthy controls</b>
Mean sudomotor score $\pm$ SD (n; range)	0.92 $\pm$ 1.38 (12; 0-3)	0.64 $\pm$ 1.08 (14; 0-3)	0.79 $\pm$ 1.2 (14; 0-3)
Mean cardiovagal score $\pm$ SD (n; range)	1.06 $\pm$ 1.25 (17; 0-3)	0.87 $\pm$ 1.19 (15; 0-3)	0.33 $\pm$ 0.72 (15; 0-2)
Mean adrenergic score $\pm$ SD (n; range)	0.71 $\pm$ 0.61 (14; 0-2)	0.87 $\pm$ 0.52 (15; 0-2)	0.73 $\pm$ 0.46 (15; 0-1)

Table 5.13: Spread of CASS index scores for all groups of participants

Likewise, due to missing data, total CASS scores could only be calculated for 14 matched and 14 non-matched participants. CASS grades of autonomic failure are described (Table 5.14). No-one had severe autonomic failure. Surprisingly, many healthy participants appeared to have mild or moderate autonomic dysfunction. On further analysis, it was apparent that many healthy participants (22/30) had a low adrenergic index score.

<b>CASS</b>	<b>SSc n=11</b>	<b>Matched healthy n=14</b>	<b>Non-matched healthy n=14</b>
No autonomic failure	18%	14%	14%
Mild autonomic failure	46%	50%	64%
Moderate autonomic failure	36%	36%	22%
Severe autonomic failure	0%	0%	0%

Table 5.14: CASS autonomic failure

### 5.6.9 Gastric emptying rates

Only one patient was unable to drink the entire Ensure<sup>®</sup> (62/220ml remaining). Results were incomplete for 3 healthy participants (1 matched and 2 non-matched).

Mean % <sup>13</sup>C dose/hour values had wide standard deviations but, when plotted, mean values showed separation (Figure 5.24). For all participant groups, gastric emptying rates increased over the first hour, before peaking at approximately 100 minutes. The greatest separation in rates occurred during the latter part of this rise (approximately 80 to 100 minutes).



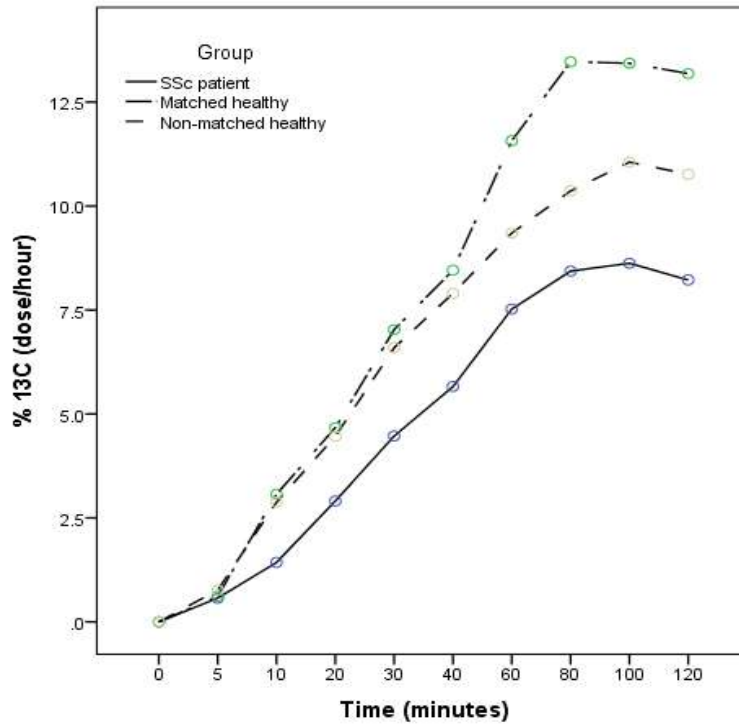


Figure 5.24: Mean gastric emptying rates (% <sup>13</sup>C dose/hour)

AUCs were not calculated for 3 healthy participants due to missing data points. The mean AUCs were  $43.8 \pm 21$  (range 8.5 to 94.0) for the patients,  $68.9 \pm 16.2$  (range 39.8 to 86.2) for the matched healthy participants and  $58.8 \pm 25.2$  (range 26.8 to 103.0) for the non-matched healthy participants.

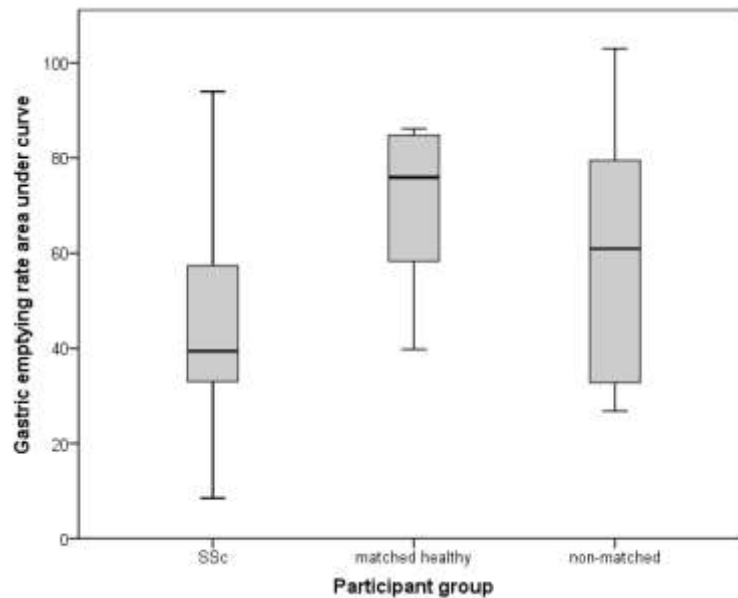


Figure 5.25: Box-and-whisker plot of gastric emptying AUC

On average, the patients had the slowest rate of gastric emptying. Consequently, at the end of the study, they would have had the largest residual gastric volume. However, the

patients also had the widest spread of gastric emptying rates due to the variation in individual results. In comparison, the matched healthy participants had the narrowest range and, on average, the fastest gastric emptying. The matched participants AUC differed significantly (Mann-Whitney U) from the patients ( $p < 0.01$ ) but not from the non-matched healthy participants ( $p = 0.11$ ). Similarly, the patients' AUC did not differ significantly from the non-matched healthy participants ( $p = 0.06$ ).

#### **5.6.10 Postprandial visual analogue scores**

Participants rated postprandial GI sensations (hunger, satisfaction, fullness and desire to eat) and GI symptoms (nausea, bloating and discomfort) at intervals using serial 100mm VAS scales. The following sections describe each sensation or symptom's mean baseline score and change from baseline. The potential range of postprandial change scores was +100 to -100mm.

##### **Hunger**

The mean baseline hunger scores were: SSc  $39.2 \pm 29.9$  (range 0 to 95), matched healthy participant  $44.3 \pm 31.2$  (range 4 to 100) and non-matched healthy participant  $48.9 \pm 24.6$  (range 3 to 84). No significant difference was detected between baseline scores of all participants (Kruskal-Wallis;  $p = 0.53$ ) or between the patients and the matched healthy participants (Mann-Whitney U;  $p = 0.63$ )

Mean changes in hunger appeared to differ between groups (Figure 5.26). The least relative mean hunger was reported earlier (5-10 minutes) by patients and matched participants than non-matched participants (40 minutes).

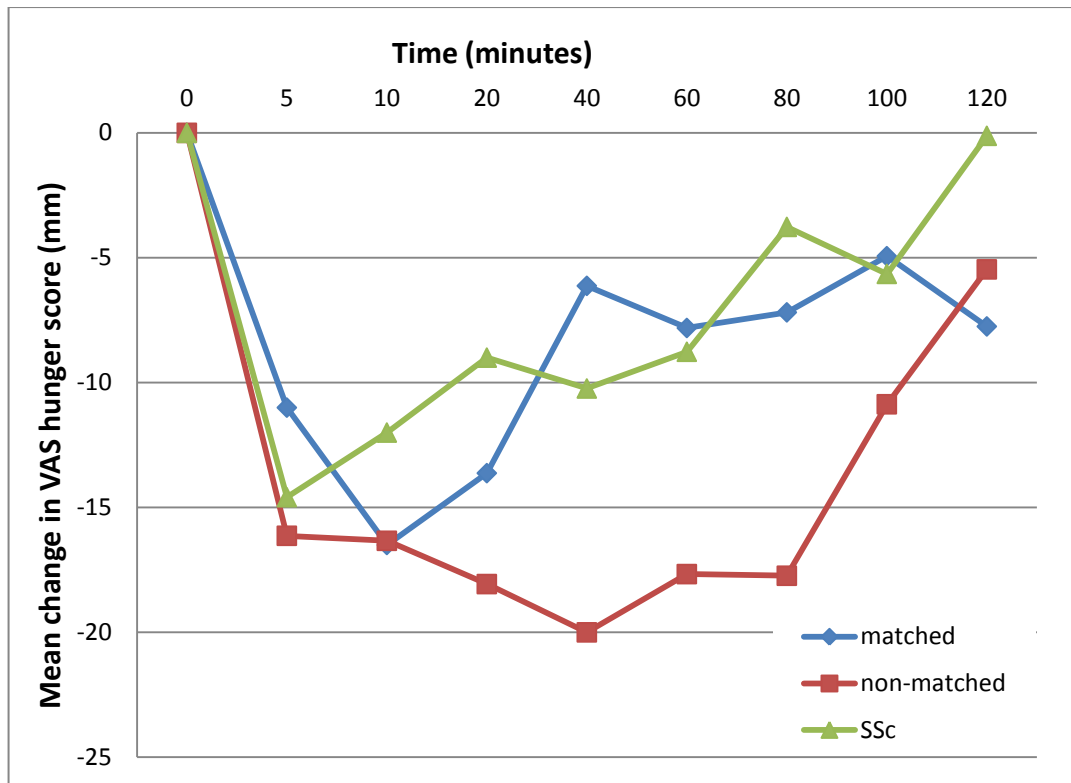


Figure 5.26: Change in postprandial VAS hunger score

AUCs were: SSc  $-64 \pm 224$  (range -588 to 231), matched healthy participant  $-71 \pm 241$  (range -613 to 435) and non-matched healthy participant  $-120 \pm 172$  (range -513 to 153). There was no significant difference between AUCs of all participants (Kruskal-Wallis;  $p=0.33$ ) or between the patients and the matched healthy participants (Mann-Whitney U;  $p=0.33$ ). AUC ranges crossed zero due to hunger exceeding baseline scores.

### Satisfaction

The mean baseline satisfaction scores were: patients with SSc  $22.4 \pm 29.9$  (range 0 to 95), matched healthy participants  $25.3 \pm 27.4$  (range 0 to 96) and non-matched healthy participants  $27.3 \pm 22.0$  (range 0 to 83). There was no significant difference between baseline scores of all participants (Kruskal-Wallis;  $p=0.54$ ) or between the patients and the matched healthy participants (Mann-Whitney U;  $p=0.71$ ).

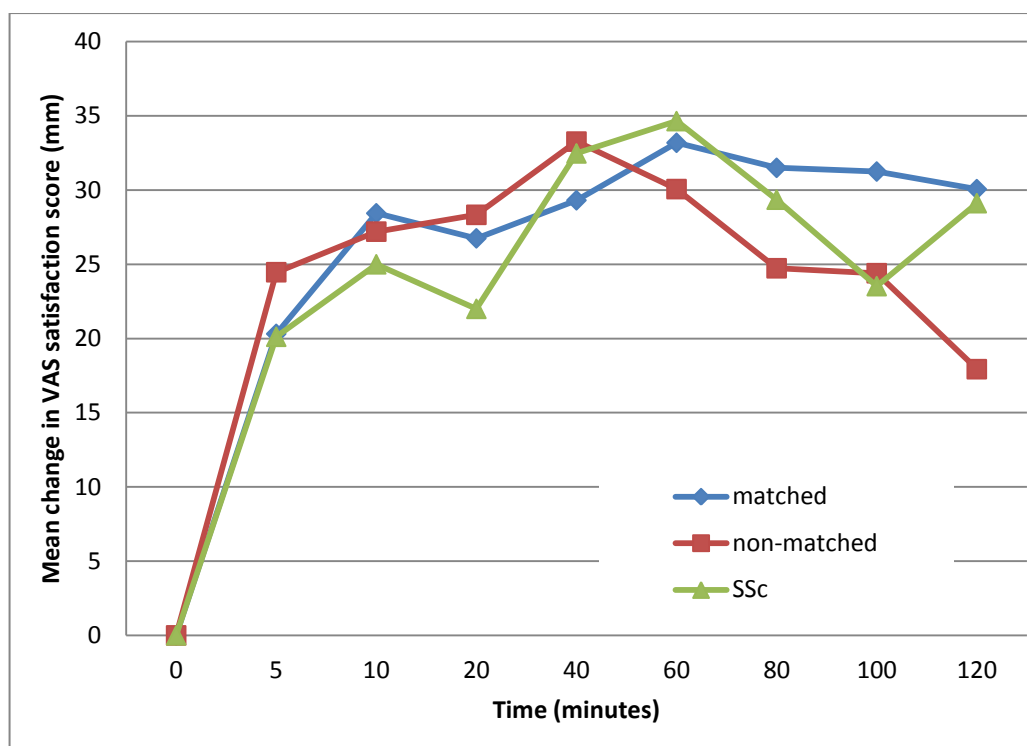


Figure 5.27: Change in postprandial VAS satisfaction score

The mean changes in scores were plotted (Figure 5.27). Mean satisfactions for all groups all peaked at 40 to 60 minutes. The mean AUCs were: SSc  $202 \pm 175$  (range -193 to 501), matched healthy participant  $215 \pm 241$  (range -67 to 685) and non-matched healthy participant  $-201 \pm 192$  (range -82 to 564). There were no significant differences between mean AUCs for all participants (Kruskal-Wallis;  $p=0.96$ ) or between the patients and the matched healthy participants (Mann-Whitney U;  $p=0.85$ ).

Despite, the mean serial scores being similar for all groups, individual responses differed widely. A few participants' overall satisfaction (AUC) was negative, indicating that for part of the study they were less satisfied than before the Ensure<sup>®</sup>. The greatest initial relative reduction was -50mm (5min), which was reported by a patient. Some, but not all of these participants, also reported an overall increase in hunger.

### Fullness

The mean baseline fullness scores were: SSc  $26.2 \pm 13.2$  (range 1 to 44), matched healthy participant  $17.1 \pm 24.4$  (range 0 to 96) and non-matched healthy participant  $19.7 \pm 16.9$  (range 0 to 50). There was no significant difference between baseline scores for all

participants (Kruskal-Wallis;  $p=0.52$ ) or between the patients and the matched healthy participants (Mann-Whitney U;  $p=0.40$ ).

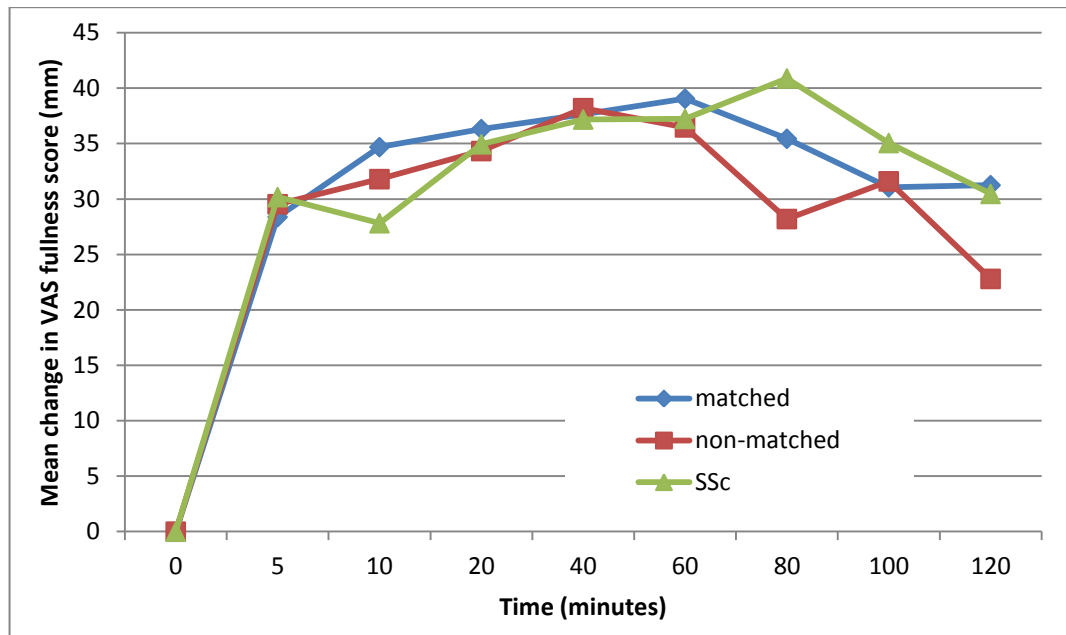


Figure 5.28: Change in postprandial VAS fullness score

All groups had similar mean changes in fullness, with scores increasing rapidly and then remaining constant until 80 minutes (Figure 5.28). This coincided with the fall in satisfaction and peak gastric emptying rate. Individuals in all groups showed a wide variation in scores. Within the first 10 minutes, 2 patients and 3 (2 matched) healthy participants reported a decrease ( $\geq 15$ mm) in fullness.

The AUCs were: SSc  $259 \pm 185$  (range -71 to 676), matched healthy participant  $-115 \pm 206$  (range -522 to 204) and non-matched healthy participant  $-194 \pm 165$  (range -562 to 123). There was no significant difference for all participants (Kruskal-Wallis;  $p=0.90$ ) or between the patients and the matched healthy participants (Mann-Whitney U;  $p=0.96$ ).

### Desire to eat

The mean baseline desire to eat scores were: SSc  $54.7 \pm 24.8$  (range 17 to 96), matched healthy participant  $56.1 \pm 25.9$  (range 13 to 100) and non-matched healthy participant  $69.2 \pm 22.5$  (range 23 to 100). There was no significant difference for all participants (Kruskal-Wallis;  $p=0.20$ ) or between the patients and the matched healthy participants (Mann-Whitney U;  $p=0.82$ ).

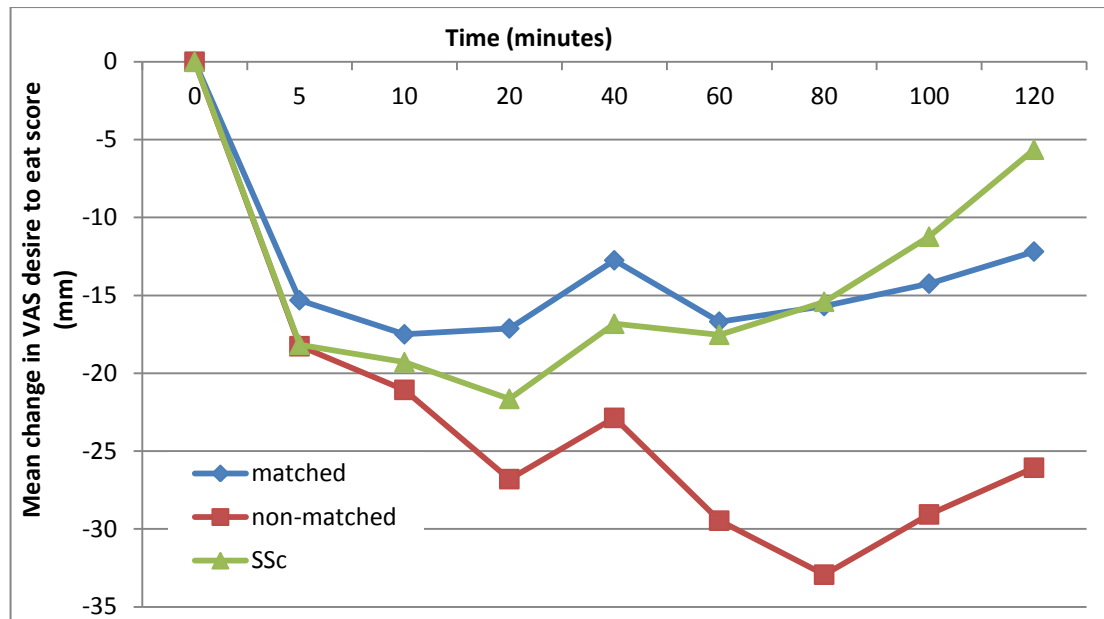


Figure 5.29: Change in postprandial VAS desire to eat score

All groups immediately reported a mean fall in their desire to eat. The greatest reduction in patients and the matched healthy participants occurred at 10-20 minutes, while in the non-matched participants, it was later at 80 minutes. The reduction was greatest in non-matched participants. Interestingly, 2 matched healthy participants and 2 patients reported an immediate increase in desire which persisted for over 1 hour.

The AUCs were: SSc  $-123 \pm 146$  (range -403 to 79), matched healthy participant  $-34 \pm 138$  (range -551 to 5) and non-matched healthy participant  $-5 \pm 17$  (range -67 to 0). There was no significant difference for all participants (Kruskal-Wallis;  $p=0.27$ ) or between the patients and the matched healthy participants (Mann-Whitney U;  $p=0.71$ ). Positive AUCs are due to some participants' increased desire.

## Nausea

The mean baseline nausea scores were: SSc  $5.06 \pm 16.4$  (range 0 to 67), matched healthy participant  $4.9 \pm 19.0$  (range 0 to 76) and non-matched healthy participant  $0.67 \pm 2.3$  (range 0 to 9). There was no significant difference for all participants (Kruskal-Wallis;  $p=0.41$ ) or between the patients and the matched healthy participants (Mann-Whitney U;  $p=0.47$ ). At baseline, very few participants reported having nausea. Two non-matched healthy participants reported mild nausea (<10mm). Two matched healthy participants reported nausea with one scoring it as 76mm and the other scoring it as only 2mm. In contrast, 5

patients reported nausea, with 3 scoring it as <10mm, 1 scoring it as 15mm and 1 scoring it as 67mm.

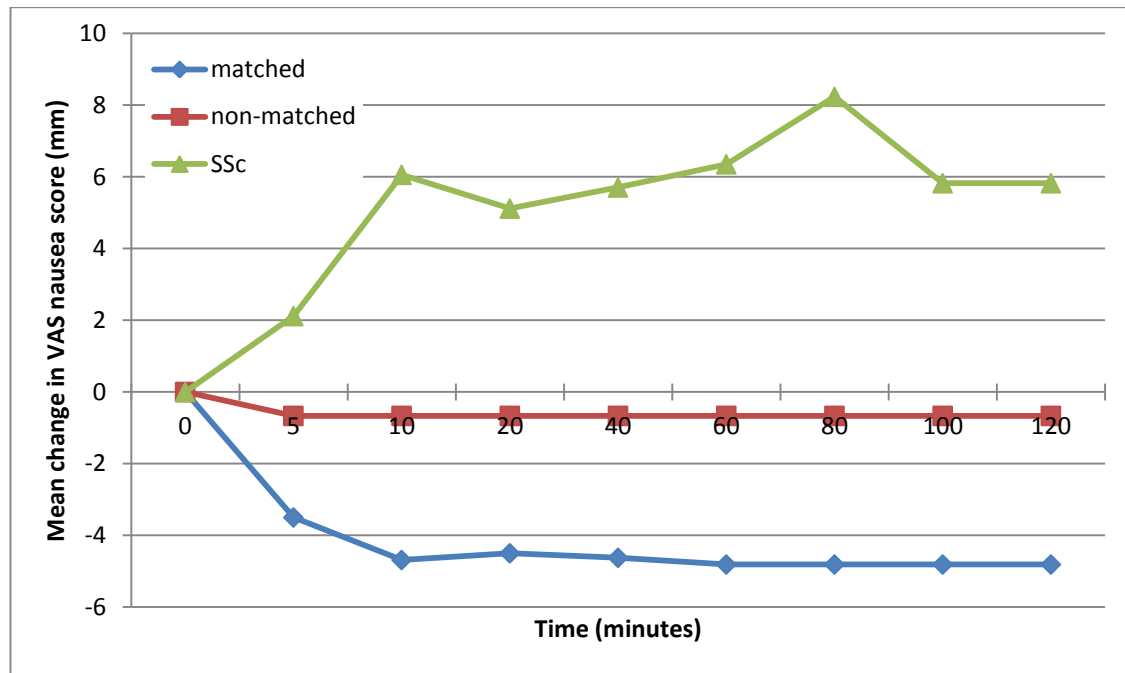


Figure 5.30: Change in postprandial VAS nausea score

Mean changes in the nausea scores of both the matched and non-matched participant groups fell, then remained low (Figure 5.30). This was due to the nausea reported by all healthy participants rapidly (<10minutes) resolving. In contrast, nausea scores of 5 patients increased. Only 1 patient's score fell within 10 minutes.

The AUCs were: SSc  $42 \pm 184$  (range -407 to 528), matched healthy participant  $-34 \pm 138$  (range -551 to 54) and non-matched healthy participant  $-5 \pm 17$  (range 0 to 68). There was a significant difference between the scores for all participants (Kruskal-Wallis;  $p=0.03$ ) but not just the patients and the matched healthy participants (Mann-Whitney U;  $p=0.15$ ).

### Abdominal bloating

The mean baseline bloating scores were: SSc  $11.8 \pm 21.8$  (range 0 to 72), matched healthy participant  $2.3 \pm 5.8$  (range 0 to 21) and non-matched healthy participant  $0.0 \pm 0.0$  (range 0 to 0). There was a significant difference for all participants (Kruskal-Wallis;  $p<0.01$ ) but not between the patients and the matched healthy participants (Mann-Whitney U;  $p=0.09$ ). None of the non-matched participants had any baseline abdominal bloating. Only 3

matched healthy participants reported mild baseline bloating. In comparison, 9 (53%) patients reported abdominal bloating and 2 had high baseline scores (>50mm).

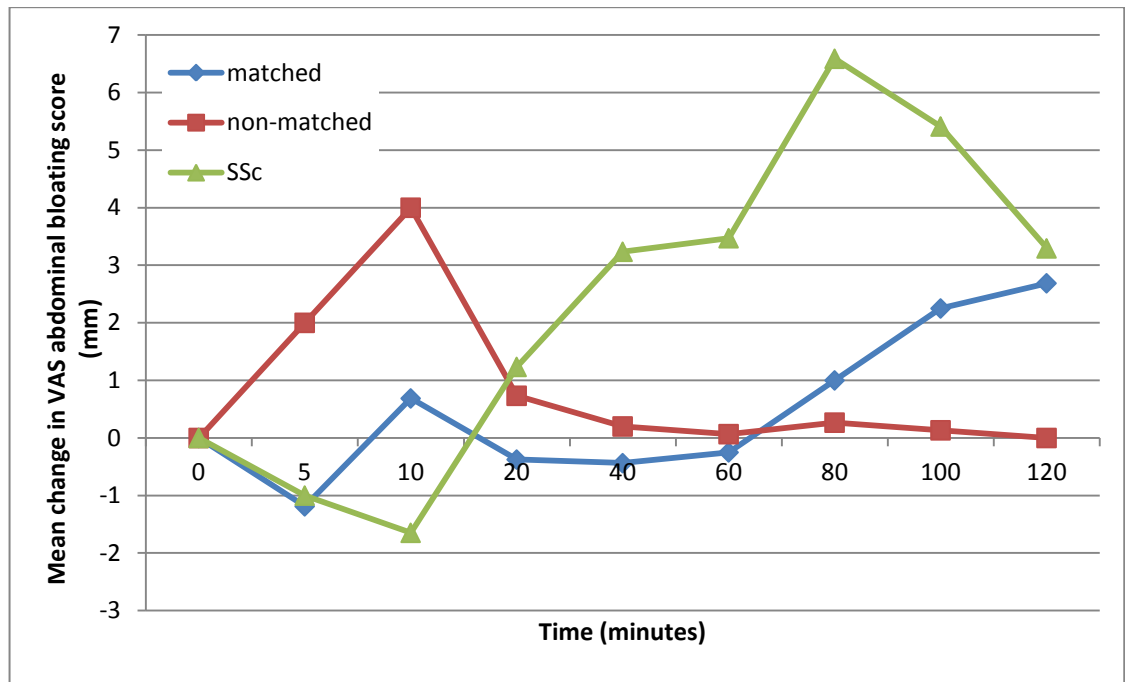


Figure 5.31: Change in postprandial VAS abdominal bloating score

Two non-matched participants developed transient abdominal bloating which subsequently resolved. Three matched participants either developed new abdominal bloating or had an increase in pre-existent bloating. In all but one case, this fully resolved. In comparison, almost 60% of patients reported persistent abdominal bloating. The AUCs were: SSc  $19 \pm 110$  (range -296 to 260), matched healthy participant  $3 \pm 30$  (range -90 to 55) and non-matched healthy participant  $7 \pm 21$  (range 0 to 80). There was no significant difference for all participants (Kruskal-Wallis;  $p=0.42$ ) or between the patients and the matched healthy participants (Mann-Whitney U;  $p=0.35$ ).

### Abdominal discomfort

The mean baseline abdominal discomfort scores were: SSc  $6.7 \pm 11.1$  (range 0 to 38), matched healthy participant  $0.1 \pm 0.5$  (range 0 to 2) and non-matched healthy participant  $4.9 \pm 15.6$  (range 0 to 60). There was a significant difference for all participants (Kruskal-Wallis;  $p=0.02$ ) and for patients and the matched healthy participants (Mann-Whitney U;  $p=0.03$ ).



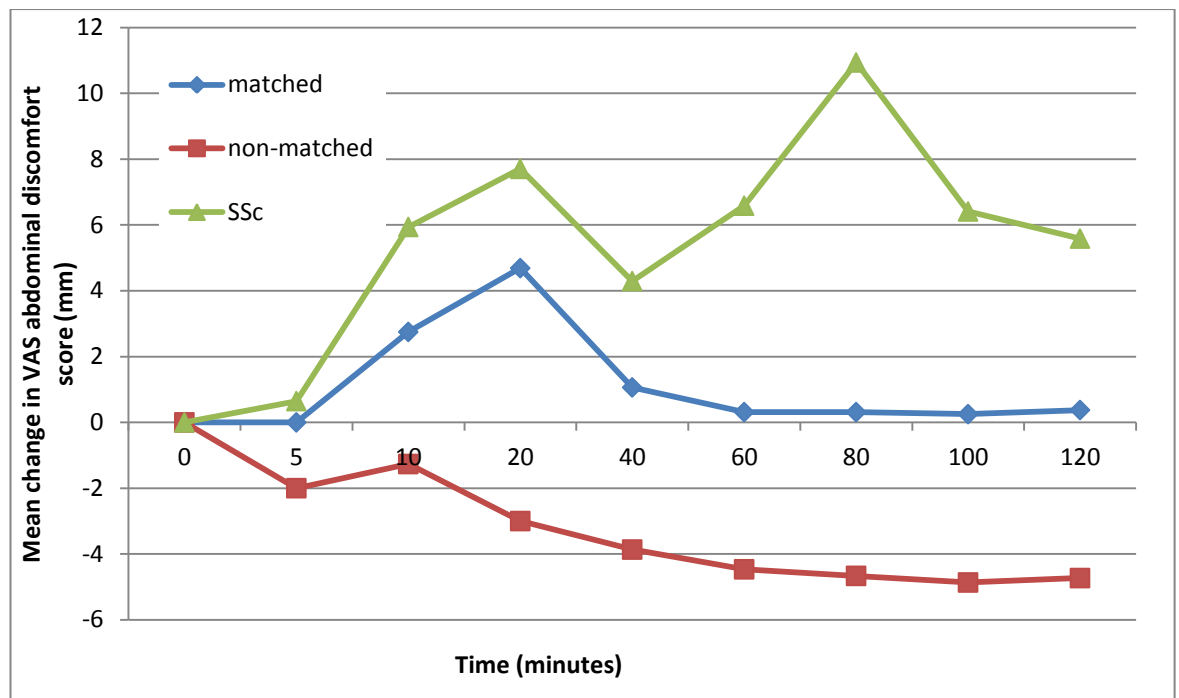


Figure 5.32: Change in postprandial VAS abdominal discomfort score

Only one non-matched participant reported baseline abdominal discomfort, which persisted. In addition, 2 non-matched participants developed (10 to 20 minutes) mild, but transient, abdominal discomfort. Two matched healthy participants reported baseline abdominal discomfort (mean 37mm), which resolved within 60 minutes. Another 2 matched participants reported mild (mean 17mm) transient discomfort which rapidly peaked (10min) before resolving. In comparison, 8 (47%) patients described baseline abdominal discomfort. All also had abdominal bloating. Over the course of the study, only 4 patients reported no abdominal discomfort. Discomfort peaked at 80 minutes, and most had some persistent discomfort.

AUCs were: SSc  $45 \pm 105$  (range -180 to 274), matched healthy participant  $10 \pm 26$  (range 0 to 99) and non-matched healthy participant  $-26 \pm 96$  (range -357 to 42). There was a significant difference between AUCs for all participants (Kruskal-Wallis;  $p=0.03$ ), but not between the patients and the matched healthy participants (Mann-Whitney U;  $p=0.11$ ).

### 5.6.11 Postprandial cardiovascular response

Serial postprandial BP (60 minutes) and HR (120 minutes) are described.

#### Blood pressure

Continuous digital and intermittent manual brachial (introduced after fifth study) BPs were measured in patients. Digital measures were hampered by difficulties maintaining reliable measurements. Patients affected often reported a cold hand. Problems increased over time, but on one occasion began before the Ensure<sup>®</sup>.

Patients' mean baseline manual BP was  $118 \pm 23 / 75 \pm 9$  (range 168/86 to 90/60; n=12) and digital BP was  $115 \pm 24 / 59 \pm 11$  (range 162/88 to 82/43; n=16). Wilcoxon Signed Rank tests showed no significant difference between SBPs (p=0.61) but did between DBPs (p=0.05). Percentage BP changes from baseline were compared.

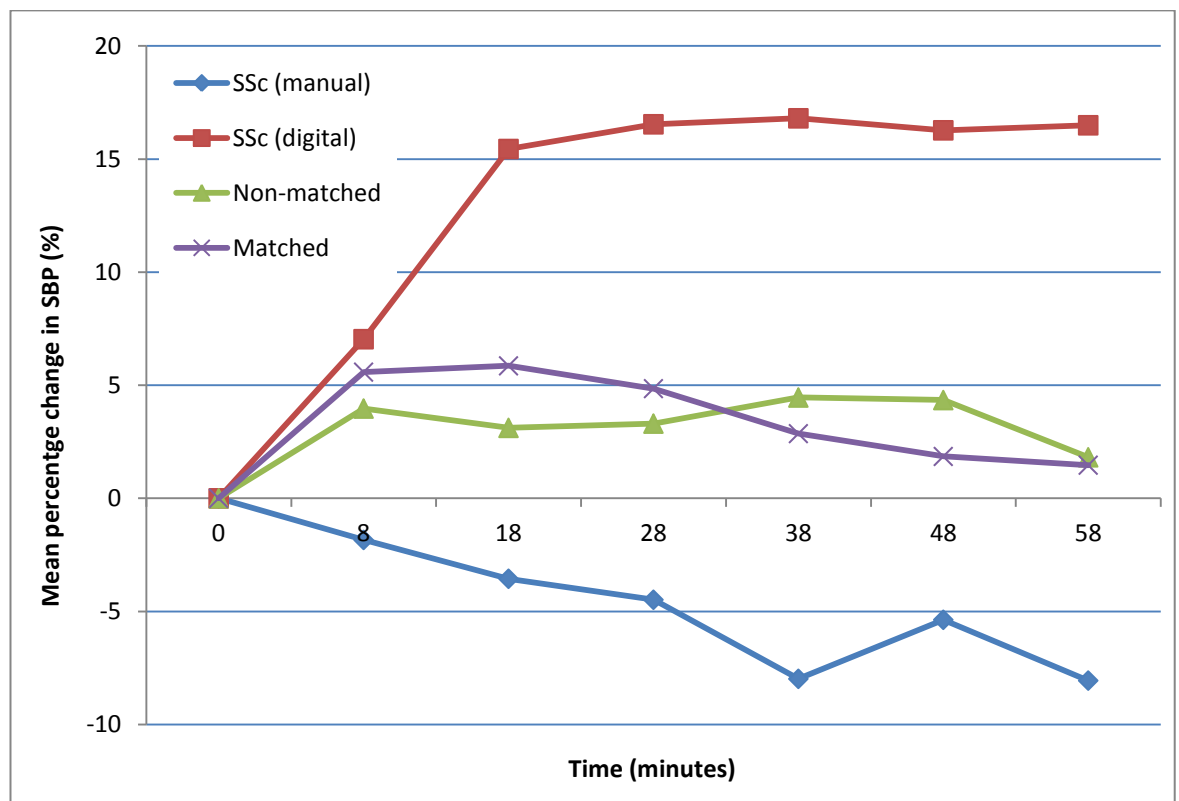


Figure 5.33: Postprandial percentage change in mean SBP

Continuous digital SBP increased in all groups (Figure 5.33) with the greatest relative increase in the patients. In contrast, the patients' manual SBP fell. Digital SBP was only recorded at all time points for 10 patients. The others had 1 to 5 postprandial SBPs. BPs

failed to record after 8 minutes in 3 patients, and 2 of these had SBP falls of >10% at 8 minutes.

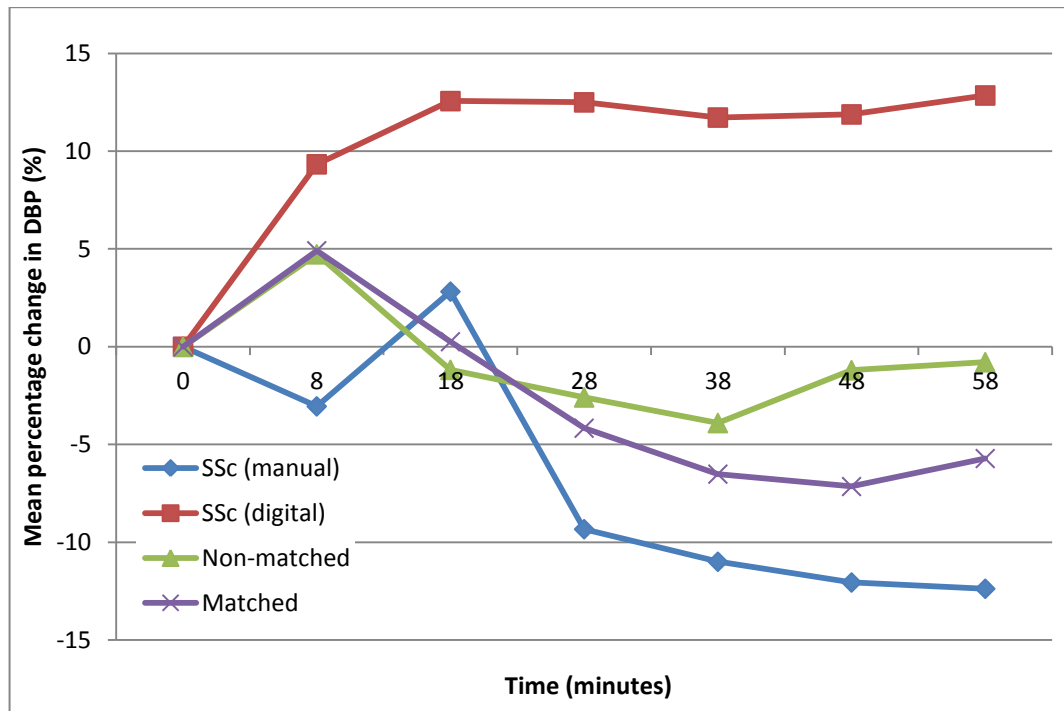


Figure 5.34: Postprandial percentage change in mean DBP

DBPs were smaller than SBPs. Changes of <5% were only 2 to 3mmHg. However, patients had a relative digital DBP increase. DBPs were only complete for 12 patients. Three patients had 1 to 5 DBPs. Two patients had no data.

The mean change in SBP AUC was  $86 \pm 56$  (range 3 to 154,  $n=11$ ) for patients (digital), (manual),  $-27 \pm 43$  (-101 to 31) for patients (manual),  $17 \pm 33$  (range -45 to 65;  $n=16$ ) for matched healthy participants and  $25 \pm 21$  (range -18 to 59;  $n=15$ ) for non-matched healthy participants. The mean change in DBP AUC was  $61 \pm 78$  (range -67 to 221;  $n=12$ ) for patients (digital),  $-39 \pm 20$  (-71 to -3) for patients (manual);  $-2 \pm 37$  (range -50 to 102;  $n=16$ ) for matched healthy participants and  $-19 \pm 26$  (range -65 to 14;  $n=15$ ) for non-matched healthy participants. Mann-Whitney U showed a significant difference between the mean changes in SBP ( $p < 0.01$ ) and DBP ( $p < 0.01$ ) AUCs between patients (manual) and matched participants.

## Heart rate

Mean baseline HRs were  $66.3 \pm 11$  (range 51 to 85) for the patients,  $67 \pm 11$  (range 52 to 101) for the matched healthy participants and  $58 \pm 6$  (range 48 to 67) for the non-matched healthy participants. Significant differences (Mann-Whitney U) were found between patients and non-matched healthy participants ( $p=0.03$ ) and between patients and matched healthy participants ( $p=0.01$ ), but not between patients and matched healthy participants ( $p=0.93$ ).

Percentage HR changes from baseline were compared. The groups followed similar paths. Non-matched participants showed an earlier HR rise (8 minutes).

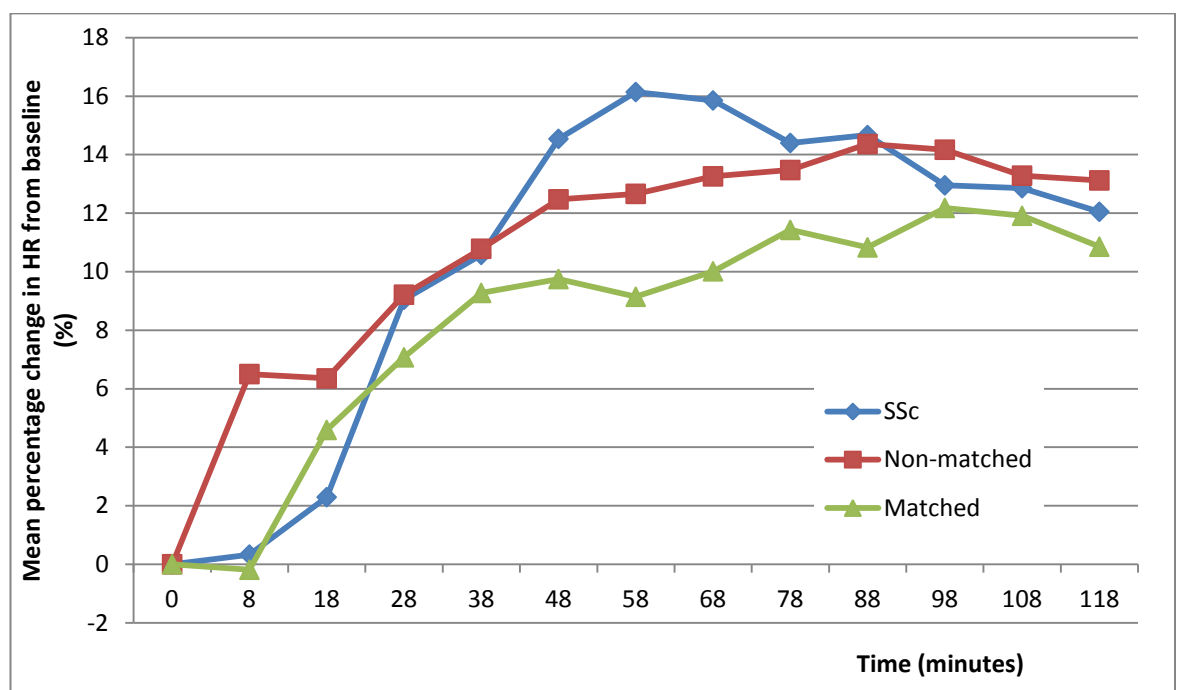


Figure 5.35: Postprandial percentage change in mean HR

The mean AUCs for HR change were  $130 \pm 72$  (range 24 to 344;  $n=17$ ) for patients,  $123 \pm 95$  (range 12 to 395;  $n=16$ ) for matched healthy participants and  $109 \pm 72$  (range -19 to 202;  $n=16$ ) for non-matched healthy participants. Kruskal-Wallis showed no significant difference for all participants ( $p=0.86$ ). Mann-Whitney U showed no significant difference for patients and matched healthy participants ( $p=0.53$ ).

### Cardiovagal index

The mean baseline CVIs were  $4.0 \pm 0.2$  (range 3.4 to 4.4) for the patients,  $4.0 \pm 0.5$  (range 2.8 to 4.9) for the matched healthy participants and  $4.5 \pm 0.3$  (range 4.0 to 5.0) for the non-matched healthy participants. Significant differences (Mann-Whitney U) were found between patients and non-matched healthy participants ( $p < 0.01$ ) and between patients and matched healthy participants ( $p < 0.01$ ), but not between patients and matched healthy participants ( $p = 0.79$ ).

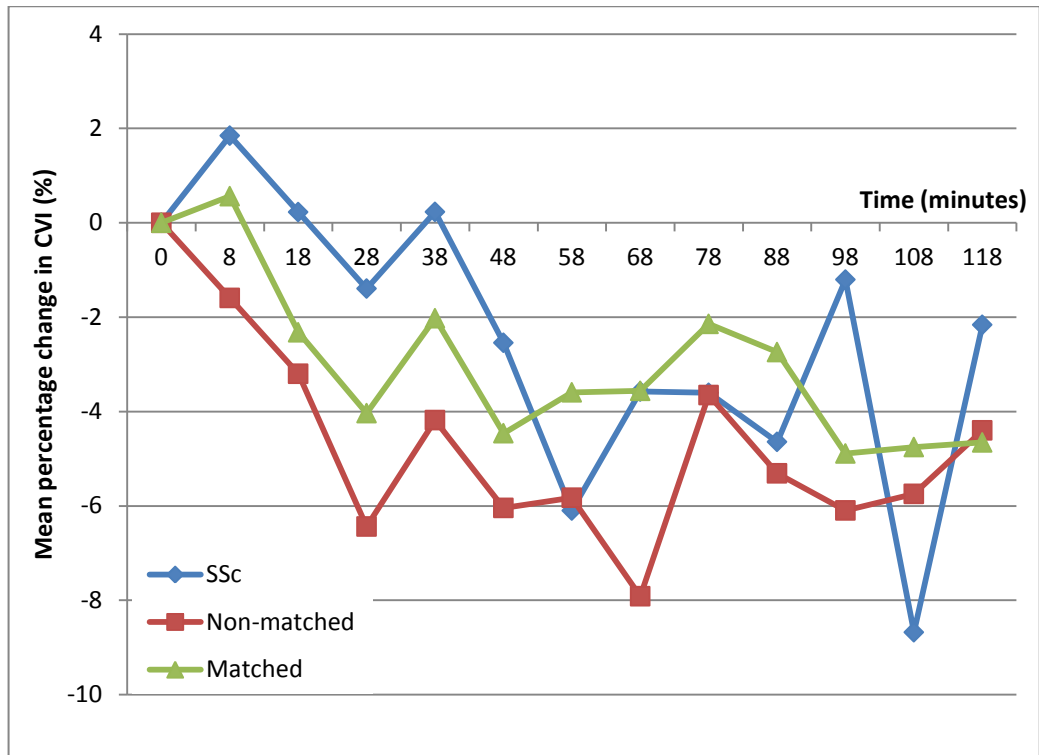


Figure 5.36: Postprandial percentage change in mean CVI

At all postprandial time points mean CVI was lower than baseline. The only exception was an early CVI rise in patients. In contrast, the non-matched participants showed a rapid CVI fall. Mean AUCs for change in CVI were  $-31 \pm 54$  (range -160 to 71;  $n = 17$ ) for patients,  $-49 \pm 50$  (range -164 to 11;  $n = 16$ ) for matched healthy participants and  $-44 \pm 46$  (range -106 to 35;  $n = 15$ ) for non-matched healthy participants. Kruskal-Wallis indicated no significant difference for all participants ( $p = 0.50$ ). Mann-Whitney U showed no significant difference for patients and matched healthy participants ( $p = 0.35$ ).

### Cardiosympathetic index

Mean baseline CSIs were  $2.6 \pm 1.1$  (range 1.1 to 5.2) for the patients,  $2.6 \pm 1.1$  (range 1.2 to 4.9) for the matched healthy participants and  $1.9 \pm 0.7$  (range 1.1 to 3.5) for the non-matched healthy participants. Significant differences (Mann-Whitney U) were found between patients and non-matched healthy participants ( $p=0.04$ ) and between patients and matched healthy participants ( $p=0.03$ ), but not between patients and matched healthy participants ( $p=0.90$ ).

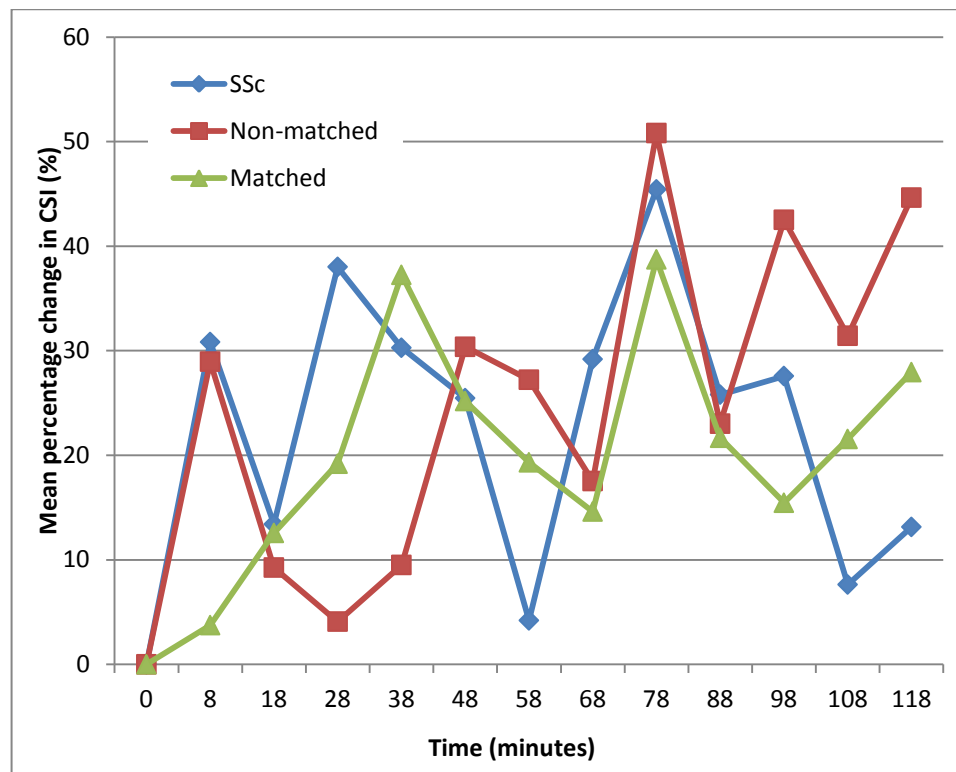


Figure 5.37: Postprandial percentage change in mean CSI

Mean CSI increased in all groups and remained elevated. Individual groups were not distinct. Individuals' CSIs fluctuated widely. As short 1 minute assessment intervals were used, measurements would have been strongly influenced by any small IBI change (e.g. altered breathing or movements). Mean AUC for change in CSI was  $284 \pm 442$  (range -388 to 1256,  $n=17$ ) for patients with SSc,  $191 \pm 422$  (range -627 to 970;  $n=16$ ) for matched healthy participants and  $350 \pm 445$  (range -230 to 1594;  $n=15$ ) for non-matched healthy participants. Kruskal-Wallis showed no significant difference for all participants ( $p=0.71$ ). Mann-Whitney U showed no significant difference for patients and matched healthy participants ( $p=0.66$ ).

### Serial Valsalva Ratios

Twenty eight healthy participants completed 3 VMs within the first hour VRs were calculated. The mean VR at 12 minutes was  $1.8 \pm 0.4$  (range 1.8 to 2.7). The mean VR at 22 minutes was  $1.9 \pm 0.4$  (range 1.2 to 2.9). The mean VR at 42 minutes was  $1.9 \pm 0.4$  (range 1.1 to 2.6). Friedman's test showed no significant difference in individuals' VRs ( $p=0.80$ ).

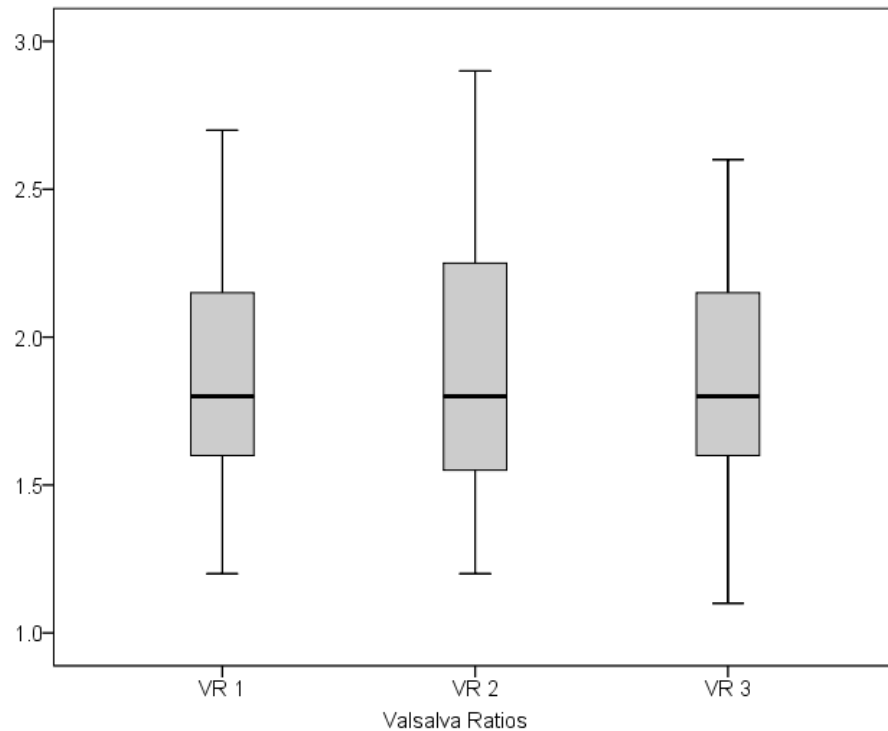


Figure 5.38: Box-and-whiskers plot showing serial postprandial VRs

Fourteen were matched and 14 were unmatched healthy participants. The matched group's mean VRs were  $1.7 \pm 0.4$  (range 1.2 to 2.7) at 12 minutes,  $1.7 \pm 0.5$  (range 1.2 to 2.9) at 22 minutes and  $1.7 \pm 0.3$  (range 1.1 to 2.3) at 42 minutes. The non-matched group's mean VRs were  $2.0 \pm 0.4$  (range 1.4 to 2.5) at 12 minutes,  $2.0 \pm 0.4$  (range 1.5 to 2.7) at 22 minutes and  $2.0 \pm 0.3$  (range 1.6 to 2.6) at 42 minutes. For both groups, Friedman's test showed no significant difference between the individuals' VR (matched  $p=0.92$ ; non-matched;  $p=0.55$ ). As expected, given the difference between autonomic battery VRs, there were significant differences (Mann-Whitney U) between matched and non-matched healthy participants' VRs (VR1  $p=0.04$ ; VR2  $p=0.01$ ; VR3  $p<0.01$ ).

### 5.6.12 Gastrointestinal symptoms versus gastric emptying rate

The following section compares GI symptoms and sensations to gastric emptying rate (AUC of the percentage  $^{13}\text{C}$  dose/hour) with the aim of detecting any correlations between these measures for patients with SSc.

#### UCLA SCTC GIT 2.0

Total UCLA scores were compared to gastric emptying (Figure 5.39).

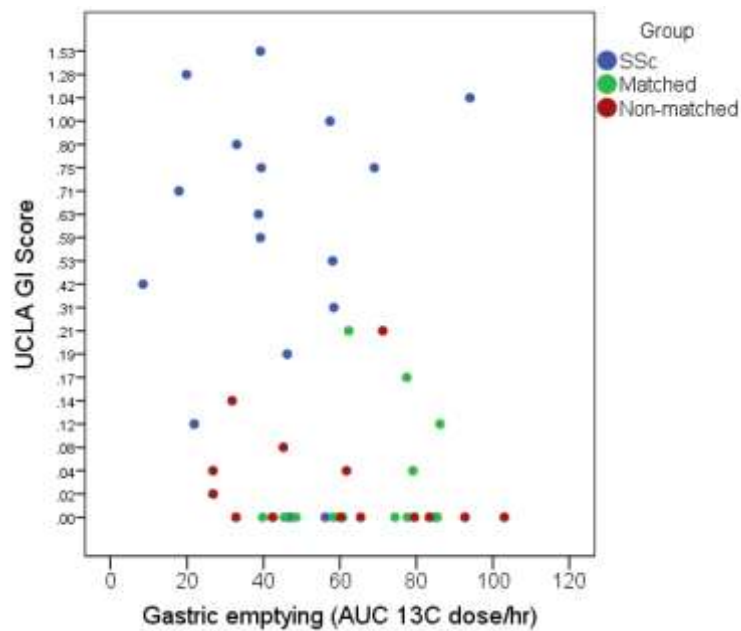


Figure 5.39: Total UCLA against gastric emptying

There was no association between patients' UCLA and gastric emptying scores ( $s=-0.039$ ;  $p=0.88$ ). There was also no significant association between patients' distension/bloating UCLA domain and gastric emptying scores ( $s=0.230$ ;  $p=0.38$ ).



## COMPASS 31

Total COMPASS 31 scores were compared to gastric emptying rates.

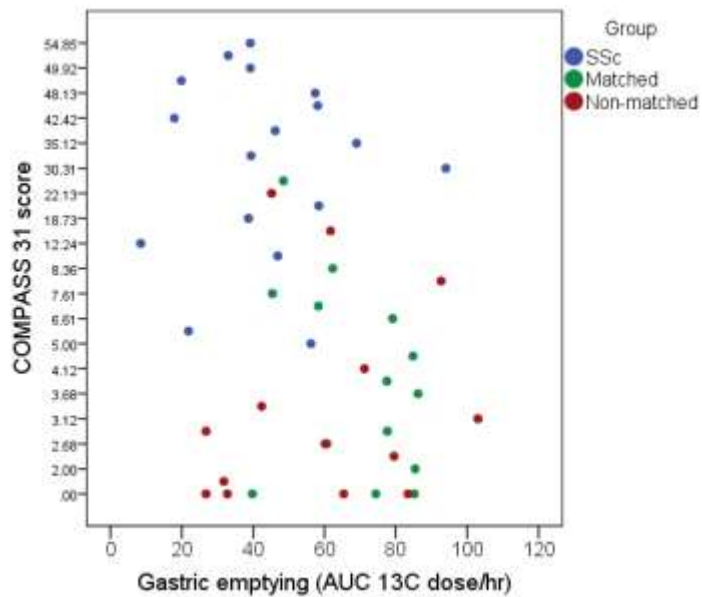


Figure 5.40: COMPASS 31 against gastric emptying

There was no association between patients' COMPASS 31 scores and gastric emptying rates ( $s=-0.108$ ;  $p=0.68$ ).

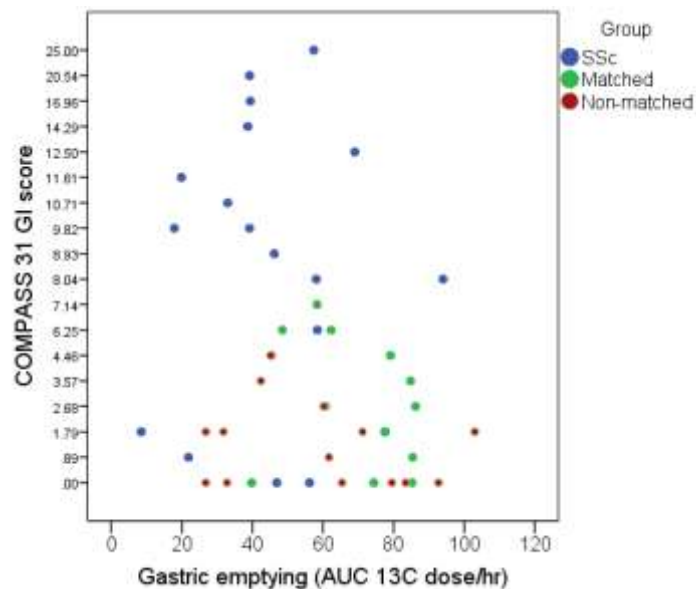


Figure 5.41: COMPASS 31 GI against gastric emptying

The COMPASS 31 GI domain score was also compared to gastric emptying. There was no association between the patients' COMPASS 31 GI domain scores and gastric emptying rates ( $s=-0.051$ ;  $p=0.85$ ).

### Gastrointestinal symptoms and sensations

As part of this exploratory study, correlations were sought between patients' change in GI symptoms or sensations AUCs and gastric emptying rate (AUC percentage  $^{13}\text{C}$  dose/hour). However, there were no correlations evident.

	<b>Gastric emptying (AUC percentage <math>^{13}\text{C}</math> dose/hour)</b>	
Hunger	s=-0.023	p=0.93
Satisfaction	s=-0.426	p=0.09
Fullness	s=-0.226	p=0.38
Desire to eat	s=-0.050	p=0.85
Nausea	s=-0.188	p=0.47
Abdominal bloating	s=-0.117	p=0.66
Abdominal discomfort	s=-0.065	p=0.81

Table 5.15: Individual GI symptoms/sensations versus gastric emptying

### 5.6.13 Gastrointestinal symptoms versus autonomic measures

This section compares GI symptoms and sensations to measures of autonomic dysfunction for patients only.

#### UCLA SCTC GIT 2.0 and COMPASS 31

UCLA and COMPASS 31 scores were compared to autonomic functioning.

	UCLA		COMPASS 31	
	Total	Distension / bloating	Total	GI domain
HRV	s=-0.115 p=0.66	s=-0.065 p=0.81	s=-0.115 p=0.66	s=0.112 p=0.64
VR	s=-0.130 p=0.64	s=-0.061 p=0.83	s=-0.086 p=0.76	s=-0.007 p=0.98
Percentage increase in DBP with sustained grip	s=-0.150 p=0.57	s=-0.232 p=0.37	s=-0.282 p=0.27	s=-0.379 p=0.13
E:I ratio	s=-0.244 p=0.35	s=-0.217 p=0.40	s=-0.337 p=0.19	s=-0.361 p=0.16
HRmax / HRmin	s=-0.202 p=0.45	s=-0.176 p=0.52	s=-0.309 p=0.29	s=-0.476 p=0.06
CASS score	s=0.217 p=0.52	s=-0.335 p=0.31	s=0.490 p=0.13	s=0.431 p=0.19
CASS category	s=0.265 p=0.43	s=-0.318 p=0.34	s=0.554 p=0.07	s=0.548 p=0.08

Table 5.16: UCLA and COMPASS 31 scores versus autonomic functioning

### Gastrointestinal symptoms and sensations

Few correlations were found between patients' GI symptoms/sensations and autonomic measures; with the multiple analyses undertaken these minor associations are unlikely to be clinically important.

AUC for change in score	HRV (SDNN)	VR	% DBP increase with grip	E:I ratio	HRmax / HR min ratio	CASS score
Hunger	s=0.164 p=0.53	s=0.032 p=0.91	s=0.088 p=0.74	s=0.209 p=0.42	s=-0.432 p=0.10	s=-0.018 p=0.96
Satisfaction	s=0.502 p=0.04	s=0.004 p=0.99	s=0.065 p=0.80	s=-0.135 p=0.61	s=0.150 p=0.58	s=-0.014 p=0.97
Fullness	s=0.480 p=0.05	s=-0.339 p=0.22	s=-0.266 p=0.32	s=-0.336 p=0.19	s=-0.015 p=0.96	s=0.476 p=0.139
Desire to eat	s=-0.064 p=0.81	s=-0.382 p=0.16	s=0.028 p=0.94	s=-0.064 p=0.81	s=-0.009 p=0.97	s=0.559 p=0.07
Nausea	s=-0.258 p=0.32	s=0.213 p=0.45	s=0.014 p=0.96	s=-0.289 p=0.26	s=-0.137 p=0.61	s=-0.068 p=0.84
Abdominal bloating	s=-0.124 p=0.64	s=-0.346 p=0.21	s=-0.459 p=0.06	s=-0.689 p<0.01	s=-0.402 p=0.12	s=0.280 p=0.41
Abdominal discomfort	s=0.462 p=0.06	s=-0.055 p=0.85	s=-0.416 p=0.10	s=-0.266 p=0.30	s=-0.355 p=0.18	s=0.275 p=0.41

Table 5.17: GI sensations/symptoms versus automatic functioning

### 5.6.14 Autonomic measures versus gastric emptying

Autonomic functioning was compared to gastric emptying for patients. No relationships existed with the exception of HRmax/HRmin.

	<b>Gastric emptying (AUC percentage <sup>13</sup>C dose/hour)</b>	
HRV	s=-0.310	p=0.23
VR	s=0.054	p=0.85
Sustained grip percentage increase in DBP	s=-0.280	p=0.28
E:I ratio	s=-0.268	p=0.30
HRmax / HRmin	s=-0.546	p=0.03
CASS score	s=-0.213	p=0.53
CASS category	s=-0.108	p=0.75

Table 5.18: Gastric emptying versus autonomic functioning

### 5.6.15 Postprandial autonomic response versus autonomic battery

Individual autonomic scores were compared to the postprandial autonomic measures for just the patients. As manual BPs were used only 12 patients were included.

<b>Post meal change (AUC)</b>	<b>HRV (SDNN)</b>	<b>VR</b>	<b>% DBP increase with grip</b>	<b>E:I ratio</b>	<b>HRmax / HR min ratio</b>
SBP	s=0.622 p=0.03	s=0.378 p=0.26	s=0.515 p=0.09	s=0.343 p=0.28	s=0.316 p=0.32
DBP	s=-0.287 p=0.37	s=0.042 p=0.90	s=0.242 p=0.50	s=0.028 p=0.93	s=0.228 p=0.48
HR	s=0.255 p=0.32	s=-0.232 p=0.41	s=-0.060 p=0.82	s=-0.221 p=0.39	s=-0.248 p=0.36
CVI	s=-0.14- p=0.59	s=0.179 p=0.52	s=0.390 p=0.12	s=0.276 p=0.28	s=0.402 p=0.12
CSI	s=-0.123 p=0.64	s=-0.114 p=0.69	s=0.017 p=0.95	s=-0.178 p=0.50	s=0.052 p=0.51

Table 5.19: Postprandial versus battery autonomic results

### 5.6.16 Postprandial autonomic response versus gastric emptying

Patients' autonomic scores were compared to their postprandial autonomic measures. As manual BPs were used only 12 patients were included

Post meal change (AUC)	Gastric emptying (AUC)	
SBP	s=-0.511	p=0.09
DBP	s=-0.550	p=0.06
HR	s=-0.110	p=0.67
CVI	s=-0.412	p=0.10
CSI	s=-0.325	p=0.20

Table 5.20: Postprandial autonomic results versus gastric emptying

### 5.6.17 Summary of key findings

This section summarises key findings for patients and matched healthy participants.

The key positive findings in this study were:

- Patients had worse autonomic and GI symptoms
- Patients had smaller (non-significant) post-stand DBP and MAP troughs
- Patients had lower VR
  - But mean VM pressures <30mmHg by patients
- Patients had slower mean gastric emptying
- Patients had a greater postprandial falls in SBP and DBP

The key negative findings in this study were:

- No difference in HRV, CVI, CSI, E:I ratio, HR<sub>DB</sub>, HR<sub>max</sub>/HR<sub>min</sub> or DBP to grip
- No difference in postprandial GI sensations
- No difference in postprandial GI symptoms
- No difference in postprandial HR, CVI or CSI
- No associations between postprandial GI symptoms/sensations and gastric emptying
- No associations between GI or autonomic symptoms and slower gastric emptying
- No associations between GI or autonomic symptoms and autonomic measures

- No associations between postprandial GI sensations and autonomic measures
  - Except SDNN and hunger /satisfaction
- No associations between postprandial GI symptoms and autonomic measures
  - Except E:I ratio and abdominal bloating
- No associations between gastric emptying and autonomic measures
  - Except HRmax/HRmin
- No associations between postprandial autonomic measures and gastric emptying
- No associations between postprandial and battery autonomic measures
  - Except postprandial SBP and SDNN

## **5.7 Discussion**

This pilot study was the first in patients with SSc, who had stopped their confounding medications, to involve beat-to-beat BP and HR and postprandial cardiovascular indices together with detailed autonomic questionnaire profiling. As a pilot study, it was designed not only to investigate the study hypotheses, but also to investigate the feasibility of this research technique in patients with SSc. However, the older age than anticipated or planned of the recruited patients profoundly limited the study's ability to detect any pathological autonomic changes between patients and healthy controls, thus preventing the study from adequately addressing the hypotheses. Age transpired to be a larger factor.

### **5.7.1 Participants**

As a consequence of the inclusion criteria and study burden, the patients recruited to this study were not representative of those recruited to the prospective study (Chapter 3). They were older and less likely to have dcSSc or be male. Age has a well documented detrimental effect on autonomic functioning [253]. This age-related dampening of responses lessened the study's ability to detect disease-related changes. In addition, participants were excluded if known to have cardiac disease, and thus perhaps autonomic dysfunction. Thus, conclusions drawn from the autonomic components of this study are not representative of all patients with SSc.

The positive findings observed between controls of different ages do however support a potential utility of the protocol developed, if applied to patient groups less technically challenging than SSc.

### **5.7.2 GI and autonomic symptoms**

This is the first study to use the COMPASS 31 autonomic questionnaire in patients with SSc [263]. Patients scored significantly higher across all categories. Thus, based on the questionnaire, patients were more likely to have autonomic dysfunction, albeit not generalised. However, both patients and healthy participants reported GI symptoms. Matched participants also scored significantly higher than non-matched for GI symptoms. In healthy participants, the highest scoring questions related to occasional bloating, diarrhoea and/or constipation. Thus, the GI autonomic question domains appeared less discerning than those of the other symptom domains.

Patients also reported significantly worse GI symptoms (UCLA) than healthy participants, despite some continuing to take GI medications. Unlike COMPASS 31 scores, due to their shorter assessment period, GI medications may have confounded UCLA scores. Thus, both the UCLA and COMPASS 31 differentiate between patients and matched participants. Scores indicate that patients have more GI and autonomic symptoms.

### **5.7.3 Resting blood pressure**

There were no significant differences between the groups' manual BPs. However, there were between the patients and matched healthy participants' mean finger BPs, but not between healthy participant groups. Only the patients had a significant difference between their brachial and finger SBPs. Thus, differences appear disease-related and are almost certainly technical. Disease-related limitations which may have impacted upon the accuracy of the finger cuff in patients with SSc include secondary Raynaud's phenomenon, cutaneous manifestations and alternative finger choice.

In response to cooling, Raynaud's phenomenon results in vasospasm. Full arteriole constriction leads to finger cuff failure. In addition, finger cooling is linked to higher SBPs and lower DBPs [291]. Following vasoconstriction, re-warming is needed to re-establish measurements. However, re-warming in patients with secondary Raynaud's phenomenon



triggers a decrease in finger SBP [297]. These limitations were not reported in a previous SSc study; however, confounding medications for Raynaud's phenomenon, which may have limited vasospasm, were continued [292].

Cutaneous involvement may have affected the light emitting diode or generated increased resistances to inflation. Healthy participants' studies report small BP differences between fingers [291]. Thus, the need to use different fingers in patients may have impaired inter-individual comparisons. Cuff dysfunction was more common postprandially, perhaps because the finger had not fully recovered from the baseline study. Thus, the interpretation of finger BPs in patients was subject to many errors, which particularly affected the longer components of the study, such as the postprandial period.

#### **5.7.4 Resting heart rate variability**

Reduced HRV is linked to increased cardiac risk. Studies have shown significantly lower HRV in patients with SSc [397]. In the present study, although the mean HRV (SDNN) was lower in patients than matched healthy participants, this did not achieve significance. However, the younger non-matched healthy participants had a significantly higher mean HRV than the matched healthy participants.

During patient NeuroScope™ studies technical limitations which affected ECG capture led to an increased frequency artefacts requiring manual correction. Editing risks R-R over-correction, as corrected points are assumed to be halfway between neighbouring points. Despite the limitations, it is likely that the older age of patients masked any effect of SSc on HRV. However, differences between healthy participants demonstrated the functionality of the in-house analyser.

#### **5.7.5 Resting cardiac vagal index and cardiac sympathetic index**

Using frequency domain methods, studies excluding patients aged over 60 have shown increased sympathetic activity and parasympathetic dysfunction [251, 397]. This is the first SSc study to report CVI and CSI from time domain methods by comparing adjacent R-R intervals. There was no significant difference in CSI between the patients and matched healthy participants or matched and non-matched healthy participants. Likewise,

there was no significant difference in CVI between the patients and matched healthy participants, but there was between the matched and non-matched healthy participants.

Cardiac sympathetic regulation occurs at a lower frequency than parasympathetic regulation. Thus, CVI was able to detect a difference with age, but not disease, when assessed over 5 minutes. In contrast, 5 minutes offers fewer opportunities to measure sympathetic influences, which may explain its failure to detect a difference with age.

### **5.7.6 Deep breathing**

Despite most patients having some degree of respiratory involvement, this manoeuvre was completed with ease.  $HR_{DB}$  responses and E:I ratios did not differ significantly between patients and non-matched healthy participants, but they did between matched and non-matched healthy participants. Compared to age centiles, a similar proportion of patients and matched healthy participants had abnormal results [275]. Thus, the absence of any differences between patients and matched participants was likely due to the confounding effect of age. Had the study included a younger patient cohort, disease-related effects may have been detected but this remains speculative.

### **5.7.7 Sustained grip**

There was no significant difference in the percentage DBP rise of patients and matched healthy participants, but there was between matched and non-matched healthy participants. Thus, the absence of any difference between patients and matched participants was likely due to age's confounding effect. Musculoskeletal involvement did not appear to hinder responses. Patients and matched participants achieved similar maximum pressures and similarly achieved their 50% targets. If this manoeuvre had been performed by younger patients, results might have been affected by autonomic dysfunction. Reduced autonomic responses in younger patients compared to controls have been shown by previously [252]. Younger participants achieved higher target pressures than older participants. Higher pressures may be more difficult in selected patients with SSc. Thus, due to SSc-related musculoskeletal manifestations this may not be the first choice autonomic test for every patient with SSc.

### **5.7.8 Valsalva manoeuvre**

Patients and matched healthy participants had significantly different mean VRs. Likewise, matched and non-matched healthy participants had significantly different mean VRs. However, comparable frequencies of patients with abnormal BP responses were seen in patients and matched participants. Thus, VR but not BP responses suggest the influence of age-related and disease-related effects. Disease-related effects have been reported previously [253, 398]. However, disease-related influences may not have been solely due to autonomic dysfunction.

BP data, especially when using the NeuroScope™, were prone to interference. Most patients had respiratory involvement which hindered pressure generation and reduced oral apertures, which compromised their ability to form a seal with the original mouthpiece. Their resultant lower pressures may have been insufficient to reliably generate VM responses. Thus, due to the confounding effects of disease on performance ability, VM responses may not be solely reflective of autonomic dysfunction in patients with SSc.

### **5.7.9 Sit-to-stand**

Patients had non-significantly lower HRmax/HRmin ratios than the matched healthy participants and significantly lower ratios than non-matched healthy participants. In addition, matched participants had significantly lower HRmax/HRmin ratios than non-matched participants. Thus, effects of age and disease were evident. Disease-related effects on HRmax/HRmin have been noted previously [398, 399]. Disease effects may have been from autonomic dysfunction or confounding influences.

Patients had non-significantly smaller MAP and DBP troughs than matched participants. During the 1 to 3 minute post-stand interval, fewer patients than healthy participants had significant BP drops. BP measurements may have been confounded by the previously discussed limitations. In addition, being the final battery manoeuvre, patients' finger cuffs were more likely to dysfunction. Also, CASS criteria were based on brachial measures from lying-to-standing, rather than sitting-to-standing. Cut-offs may not be transferable.

Thus, the patients' abnormal responses to standing may have been due to autonomic involvement or confounders. Studies involving younger patients and completion at the start of the autonomic battery may help to define this further.

### **5.7.10 Sympathetic skin scores**

The mean amplitude of responses was smaller in the lower than the upper limbs. Thus, 'abnormal' responses were more common in the lower limbs. Both patients and healthy participants generated 'abnormal' responses. A very low baseline skin conductance was observed in some participants in association with abnormal responses. Responses may have been due to autonomic dysfunction, age or study limitations. Previously abnormal sympathetic skin responses have been shown in patients with SSc [327].

Due to technical problems, results were unavailable for many participants. With age, latency increases and amplitude decreases, especially in the lower limbs [326]. Some participants struggled to perform a sudden inspiratory gasp to command. If not performed, a repeat attempt may have been subject to habituation.

Thus, assessment was subject to many technical difficulties and limitations (including age) which hindered its ability to assess sympathetic skin manifestations.

### **5.7.11 Modified composite autonomic severity score**

Using this score, as many of the healthy participants as patients had autonomic failure while few patients had severe autonomic failure. However, this is not unexpected given that every element of the score had limitations and patients with severe cardiac involvement were excluded.

Healthy participants scored highly on the sudomotor and adrenergic scores. Sudomotor scores were entirely based upon sympathetic skin responses. Their limitations were described in 5.6.10. Adrenergic scores were based upon VM BP changes. No one scored high enough for consideration of CASS orthostatic criteria. VM BP changes were affected by many limitations (Section 5.6.8). In contrast, the cardiovagal scores which were calculated from  $HR_{DB}$  and VR were more discriminating between participant groups, but were still affected by confounding disease-related effects. In summary, CASS grades were affected by limitations in the autonomic assessment process. Limitations were related to age, non-autonomic disease manifestations and technical problems.

### **5.7.12 Gastric emptying**

Liquid gastric emptying rates increased rapidly over the first 80 to 100 minutes before declining slowly. Patients had the slowest gastric emptying followed by the non-matched and finally the matched healthy participants. All groups showed wide variation in individual results, with the greatest being in the patients. Thus, the patient group included people with delayed and normal emptying. Delayed emptying is common in SSc [51, 348]. Patients' AUC differed significantly to that of the matched healthy but not the non-matched healthy participants, though it approached significance. There was no significant difference between healthy groups.

Compared to autonomic function, gastric emptying results are less heavily influenced by age. Age is included in IRIS<sup>®</sup> machine calculations. However other limitations were present. Based upon carbon dioxide concentrations in the 'end-expiratory' breath samples, it would appear that some individuals had difficulty providing samples. This may have led to under-reporting of gastric emptying. Also, radiolabelled studies falsely assume no, or consistent, radioisotope retention [341]. However, despite possible limitations, gastric emptying studies appeared able to differentiate between patients and also between patients and matched participants.

### **5.7.13 Postprandial sensations and symptoms**

Participants temporal ratings showed similar profiles, but individuals' measures varied substantially, as is known to occur with VAS studies [353]. There were no significant differences in patients' and matched participants' baseline GI sensations or symptoms with the exception abdominal discomfort which was higher in patients. The groups showed no significant differences in the AUC for the change in sensations. Plots for GI symptoms appeared to show divergent courses. However amplitudes were low and analysis showed no significant difference in AUC. This differs from a previous study which reported a significant difference in epigastric fullness [52]. However, it found no difference in appetite, satiety, nausea or epigastric pain. Thus, postprandial scores failed to differentiate between groups, and thus offer little diagnostic benefit.

#### **5.7.14 Gastric emptying and autonomic dysfunction**

Despite delayed gastric emptying and autonomic dysfunction both being common in patients with SSc, previous studies have failed to identify any significant relationships [52, 261]. This study also failed to identify any clear relationships. However, correlations between postprandial BP responses and gastric emptying approached significance. The absence of significant correlations may be due to the patients' older age and study limitations. Thus, based on this study, a link cannot be supported.

#### **5.7.15 Gastric emptying and GI and autonomic symptoms**

In its validation studies, UCLA distension/bloating domain scores correlated with gastroparesis and SIBO [89]. Despite this, the present study failed to detect any associations between patients' scores and gastric emptying rates. Perhaps, in the present study, other influences to patients' UCLA scores may have precluded the detection of correlations with gastric emptying.

COMPASS 31 has not previously been assessed alongside gastric emptying. Despite some patients generating higher scores than healthy participants, implying that some may have an element of generalised autonomic dysfunction, there were no significant relationships between COMPASS 31 scores and gastric emptying. As COMPASS 31 was validated for autonomic dysfunction, the absence of any associations would appear to refute the hypothesised link between gastric emptying and autonomic dysfunction. Alternatively, this failure may simply be a reflection of inconsistencies in symptom reporting between individuals. In summary, the COMPASS 31 questionnaire appears unable to assist in the identification of patients with delayed gastric emptying, which may suggest the absence of any association between autonomic dysfunction and gastric emptying. However, to confirm this would require further studies.

#### **5.7.16 Gastric emptying and postprandial sensations and symptoms**

It was hypothesised that postprandial symptoms and sensations would be related to gastric emptying, as it was thought that those patients with the slowest gastric emptying would have greater fullness, reduced desire to eat and greater symptoms of bloating and discomfort. However, no significant relationships were detected. This lack of any

association was despite patients having a range of gastric emptying rates and sensation/symptom scores. Thus, it may reflect the known difficulties in VAS based inter-individual comparisons. Hence, VAS tools do not appear useful for clinical assessments of the effects of gastric involvement.

#### **5.7.17 Autonomic dysfunction and GI and autonomic symptoms**

The UCLA questionnaire was designed for patients with SSc, whose GI manifestations may be due to autonomic dysfunction. Despite this, there were no significant correlations between UCLA scores and any measures of autonomic dysfunction. However, as the UCLA score was not validated for autonomic dysfunction, conclusions cannot be drawn from the absence of any associations about the pathogenesis of gastric emptying. In addition, despite the COMPASS 31 questionnaire being an autonomic questionnaire, its results did not correlate with measures of autonomic dysfunction. Thus, the UCLA and COMPASS 31 questionnaires may be unable to distinguish between disease-related and age-related autonomic dysfunction.

#### **5.7.18 Autonomic dysfunction and postprandial sensations and symptoms**

There were no significant associations between changes in postprandial GI sensations and autonomic measures, with the exception of between HRV and both satisfaction and fullness. With regard to postprandial GI symptoms and the autonomic measures, with the exception of the E:I ratio and abdominal bloating, there were no significant associations. These autonomic measures all distinguished the patients from younger non-matched, but not older age-matched, healthy participants. Confounding disease-related effects unrelated to autonomic functioning (e.g. inability to perform breathing components) could not be excluded in patients with blunted responses secondary to age. Thus, firm conclusions about the presence or absence of any relationships cannot be made without repeating the study in younger patients and adjusting the test protocol to better accommodate patients with SSc.

### **5.7.19 Postprandial autonomic measures**

Postprandially patients mean manual and brachial SBP and DBP diverged, with digital finger pressures increasing and manual brachial pressures decreasing. This confirms the observed unreliable nature of postprandial finger cuff measures in this group, so the postprandial finger data was disregarded for further analyses in this study. Raised pressures were likely due to finger cooling and vasospasm. However, postprandial hypotension, evident on manual testing, may also have compromised peripheral circulation. Due to this, only manual pressures were used for analyses despite this reducing the power.

Postprandial, patients' SBP and DBP fell and differed significantly from that of matched participants. These changes would be compatible with postprandial autonomic dysfunction [237]. Both patients and matched healthy participants had statistically similar rises in mean HR. Thus, BP appeared to be a more sensitive marker of autonomic dysfunction than mean HR. A link between postprandial hypotension and autonomic dysfunction is supported by the association between postprandial SBPs and the battery HRV. This is in spite of the previously discussed limitations to autonomic testing in this study. In addition, the correlations between gastric emptying and DBPs approached significance ( $p=0.06$ ), thereby potentially suggesting a link between postprandial hypotension and delayed gastric emptying. Further studies would be needed to explore this further.

There were no significant differences in postprandial CSI or CVI responses. Serial measurements in individuals showed wide fluctuation. The problem with a short 5 minute assessment period has already been discussed. This was likely worsened with the even shorter assessment period used postprandially. VRs are another measure of vagal function. Serial postprandial VRs performed by healthy participants showed no difference over time, suggesting no significant change in vagal functioning at the times studied.

### **5.7.20 Summary**

This study was initially intended to investigate for associations between GI symptoms and rate of gastric emptying, between GI symptoms and cardiovascular autonomic dysfunction and between rate of gastric emptying and cardiovascular autonomic dysfunction. However, due to technical and patient-related difficulties encountered during its undertaking, which required modification to the study apparatus and protocol, it struggled



to adequately address its primary aims. However, the functionality of the modified apparatus and protocol has been established and validated, in particular by demonstrating healthy participants' age-related decline in autonomic function, and could now be used confidently in future studies. Also, directions for future studies in a younger SSc patient cohort have been highlighted.

## **CHAPTER 6:**

## **GENERAL DISCUSSION**

## **6 General discussion**

### **6.1 Introduction**

In the past, GI involvement was listed as a significant cause of death (>10%) in patients with SSc [400]. However, in recent years, the proportion of patients dying from SSc-related GI disease has halved [201, 400]. As GI manifestations are as yet irreversible, this decline in mortality is presumably the result of better awareness of GI involvement, improved symptom management and, as involvement of the GI tract may have nutritional implications, the availability and provision of superior nutritional support.

The severest cases of GI involvement may culminate in the development of IF which, without nutritional intervention, would result in death through malnutrition.

Unfortunately, until it is possible to modify GI disease progression, identification and support is the best that can be offered.

This thesis includes the longest and largest SSc-specific HPN series conducted to-date. It shows that, in patients with SSc and IF, HPN is safe and nutritionally effective. However, the desired goal would be for patients not to progress to IF requiring HPN. Instead, it would be to be able to identify early, during routine clinic attendances, those selected patients with SSc who are at risk of developing malnutrition, either as a consequence of GI or other SSc-related manifestations. To do this would require the use of a rapid, easily completed screening method which reliably detects those at risk patients and then allows for close monitoring of the nutritional course.

One such screening tool, which is widely used in UK clinical practice, is 'MUST' which incorporates assessments of BMI, recent weight loss and 'acute disease' effect. However, like all such tools it is not perfect in its detection of patients at nutritional risk, and thus cannot be relied upon in isolation. Indeed, its strict BMI cut-offs and reliance on patient awareness and recall of recent weight change, lead to its failure to identify some patients who may benefit from early nutritional intervention. Thus, there is a need to use more than one assessment modality and re-assess frequently.

Other assessment modalities which may prove useful in the detection and monitoring of patients' nutritional status include BMI, MAC, 4-site anthropometry and oral aperture. BMI is easily measurable, but in selected patients with SSc may prove difficult to perform or interpret. Measurements may be complicated by peripheral oedema and

reduced mobility. MAC, on the other hand, correlates well with change in weight, can be performed when seated and, as it requires only a tape measure to perform, can be performed at the bedside. Though not normally recognised as a nutritional assessment modality, the present study suggests the same characteristics appear to be true for oral apertures. Four-site anthropometry is an additional low-cost, rapid to perform nutritional assessment modality, which requires little expertise and has been shown to be reliable in many patients with SSc for serial measures. As an additional measure, which may augment more regular BMI and/or MAC assessments, it also provides details regarding body composition. Thus, rather than just assessing weight, its inclusion would also allow evaluation for changing body composition, which would highlight muscle loss as opposed to adipose tissue gain to the managing clinician.

Once identified, the hope would be that any 'at risk', weight and/or muscle losing patients could receive tailored nutritional support which would negate their decline. The provision of this would normally include an assessment of not only nutritional status, but also their intakes, requirements and any contributing SSc-related manifestations. However, this study showed that routine dietary records are prone to significant confounding, and thus their routine completion may only be of little clinical benefit. However, they may still have a role in some highly motivated patients, as part of a focused dietitian-led dietetic assessment. This study also showed the discrepancy between predicted energy requirements, based on standard equations, and actual expenditures measured using of kinematic monitors such as the SenseWear® Armband in patients with SSc. Thus, in selected patients who continue to decline nutritionally, despite outwardly appearing to be meeting their nutritional requirements, there may be a role for assessment of expenditure rather than simply relying upon a predictive equation.

The goal would also be to be able to predict those patients likely to develop nutritional compromise based on their disease characteristics. Unfortunately, the present study, perhaps due to its relatively short follow-up period, failed to identify predictors of subsequent decline. However, when assessed at baseline, there were clear associations with small intestinal and respiratory involvement. This is in spite of patients with respiratory involvement being less physically active. Based on the study findings, clinicians should be additionally vigilant for the development of nutritional decline following the development of small intestinal involvement or worsening respiratory disease. Whilst respiratory involvement may be easy to detect, given the normal practice

of annual or biennial respiratory assessments, small intestinal involvement may be more covert. Investigations for small intestinal involvement are not normally conducted at scheduled intervals, instead patients are normally only referred for abdominal imaging or breath tests for SIBO following the development of clinical manifestations or nutritional compromise. Thus, there is a need for a more rapid, non-invasive, safe test to enable the earlier detection and hence management to prevent decline.

Looking forward, it is not sufficient to merely aim to detect nutritional decline and to manage GI symptoms. Instead, the hope for the future must be to be able to cure SSc-related GI manifestations by targeting the underlying disease processes. However, before this can be accomplished, the underlying pathogenesis must be understood. With this in mind, and knowing about the autonomic nervous systems' involvement in normal GI motility and that GI dysmotility is the hallmark of GI involvement in patients with SSc, this study sought to determine the presence of any associations between delayed gastric emptying and autonomic dysfunction. However, this task was hindered by the older age of patients who were recruited to the study and the patient and technology-related limitations experienced, which made it difficult to draw any firm conclusions. However, the data would suggest that autonomic problems are not the principle driver of gut dysfunction. The study also showed that relying on symptoms alone is unreliable.

## **6.2 Future directions**

Despite that which was already known, and that which has been discovered through the completion of these studies, there remains many unanswered questions with regard to the assessment and monitoring of patients' nutritional status and GI manifestation.

### **6.2.1 Development of a composite nutritional assessment tool**

This study has investigated various possible clinically applicable modalities which could be used for the routine monitoring of patients with SSc at their attendance at their routine clinical attendance. Several have been shown to be clinically applicable. However, due to clinic time constraints, not all measures can be performed at every routine attendance. Therefore, there exists the need for the development of a composite screening tool which incorporates aspects of the questionnaire and clinical measures. For instance, the

questionnaire could contain relevant aspects of the UCLA questionnaire and subjective assessments of recent unintentional weight change and recent dietary difficulty. Meanwhile, the routine assessment could include measurement of BMI (and hence weight) and MAC. At index assessment, these measurements could be compared to a pre-defined cut-off, while at subsequent attendances, absolute and/or percentage changes could be compared to the previous measure. Comparison to previous measurements would ensure the detection of weight losing obese patients, who may not otherwise score if compared to a pre-defined lower weight threshold. Further assessments of body composition, such as 4-site anthropometry could be performed annually in all patients and more frequently in those patients triggering action on the composite scoring tool.

Patients scoring on the composite tool could be referred for dietetic assessment and if appropriate tailored nutritional input. In addition, it would highlight to the clinician that careful consideration was required in order to identify and address any potential causes for their nutritional deterioration.

The development and testing of such a composite nutritional tool would require the completion of longer-term, multi-centred studies including more patients with a range of clinical manifestations. However, despite this initial heavy investment, if successfully developed, the end product which could be used in non-specialist centres with little financial investment may lead to a substantial improvement in the nutritional assessment of patients with SSc.

### **6.2.2 The need to assess muscle mass**

When assessing nutritional status, it is important to not only measure total weight and adiposity, but also muscle (lean) mass. Muscle may be lost through malnutrition, chronic disease and/or sarcopenia. Sarcopenia, which is the loss of muscle mass and function with age, is known to be aggravated by various disease processes including inflammation. It is an increasing problem globally as the population ages. Preserving muscle mass is important not only because of the links to nutrition, but also because its loss leads to a decline in function and thus autonomy.

As explored in this thesis, clinical assessments of body composition in patients with SSc are troublesome, due to the confounding effects of disease on caliper and BIA

measurements. In theory, assessment could also be confounded by co-existent myositis [401]. Other potential assessment methods, more suited to intermittent research applications than repeatable clinical measures, include dual energy absorptiometry and cross-sectional imaging.

A recent dual energy absorptiometry study found patients with SSc to have significantly lower relative skeletal mass index than healthy controls [211]. Lower skeletal mass had many associations including dcSSc, longer disease durations, lower energy intakes and BMIs, more severe skin involvement and raised inflammatory markers. However, correlations were not sought with functional impairment and any confounding inflammatory effects of myositis were not defined. With individually tailored nutritional interventions, another small study showed a 15% (2/13) reduction in the number of patients classed as sarcopenic after 6 weeks, but longer follow-up data was not included [222]. Furthermore, the positive effects of graded exercise, including resistance exercise, have yet to be explored.

Thus, as yet, many key questions remain which, if answered, may help to address the problems of low muscle mass in patients with SSc and which may not only improve patients' nutritional status, but also their functional ability. These could, perhaps, be addressed through the completion of longitudinal interventional studies involving serial assessments of body composition, function (e.g. walking test and grip strength) and myositis and nutritional and physical interventions.

### **6.2.3 The need for earlier detection of small intestinal involvement**

This study has highlighted the need for early identification of small intestinal involvement with a view to instigating earlier management in the hope of preventing nutritional decline. However, unlike screening for respiratory involvement, due to the associated radiation risk, costs, time commitments and low yield, screening cannot simply be annual or biannual imaging studies or breath tests. Thus, there exists the need for a dedicated, cost effective, simple, cheap, low burden screening tool.

The UCLA questionnaire is a validated questionnaire for patients with SSc. Higher UCLA scores, though not specific, have been reported in patients with SIBO [402]. The present study was unable to confirm or refute this, as the status of patients with SIBO (i.e. treated

or untreated) was not considered. Thus, higher or increasing scores may prompt the need to consider SIBO. However, further studies are needed to confirm this. Also further studies are required to explore whether, in order to detect developing small intestinal involvement, clinicians should consider all components of the UCLA score or merely those elements directed towards small intestinal symptoms (i.e. diarrhoea, bloating and flatulence).

Alternatively, though not investigated by this study, could the COMPASS 31 have any role to play in the detection of small intestinal disease? Links have previously been shown between autonomic dysfunction and oesophageal, but not gastric, involvement [258]. Links to small intestinal involvement have not been sought. However, if small intestinal involvement was linked to generalised autonomic dysfunction then an association may be expected with the total COMPASS 31 score. Thus, in theory, increasing COMPASS 31 scores could perhaps form part of a screening tool, which would alert the clinician to the possibility of risk of worsening GI and thus gastric and small intestinal involvement. To consider this possibility would require further autonomic studies involving patients below the age of 60, to determine the presence of any associated autonomic dysfunction, and a wider range of GI manifestations (none to severe), perhaps with more 'real life' inclusion criteria. However, other mechanisms for dysfunction, including fibrosclerosis of the gut itself, also need to be addressed. Future advances in matrix biology and therapeutics may prove pivotal for this disease.

Faecal calprotectin is a widely available, simple, non-invasive, reproducible biomarker test for the detection of intestinal inflammation. Recent interest has been directed towards a possible role for faecal calprotectin in patients with SSc. Increased concentrations have been reported in patients in the presence of GI involvement, with levels elevated especially in the presence of SIBO [403]. Furthermore, levels have been shown to fall with eradication. Further studies are still needed to confirm this association in light of previous less supportive studies not involving patients with SSc [404, 405]. However, if proven, it together with the UCLA questionnaire and/or COMPASS 31 questionnaire might form the basis of a screening pathway to identify patients to refer for further confirmatory studies. Should this be possible, longitudinal nutritional studies could be conducted to delineate the nutritional and clinical benefits of earlier detection of small intestinal involvement.



### **6.3 Conclusions**

Malnutrition and GI involvement continue to pose significant problems for both patients with SSc and the clinicians caring for them. The studies undertaken as a part of this work sought to better understand the development, assessment and management of nutritional problems and to identify any relationships between gastric involvement and autonomic dysfunction. However, despite this study's positive and negative findings, there still remain many unanswered questions relating to patients' nutritional and GI manifestations. These areas will form the basis for future research.

# APPENDICES

# Appendix 1

## UCLA SCTC GIT 2.0 Questionnaire [86]

Name: \_\_\_\_\_ Date: \_\_\_\_\_

The following questions ask about your gastrointestinal (gut, GI) symptoms and how they affected your life over the last 7 days. 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 <u>past 1 week</u> , how often did you....	Check one response for each question			
		No Days	1 – 2 Days	3 – 4 Days	5 – 7 Days
REFLUX	... have difficulty swallowing solid food?	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
	... have an unpleasant stinging or burning sensation in your chest (heartburn)?	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
	... have a sensation of bitter or sour fluid coming up from your stomach into your mouth (acid reflux)?	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
	... have heartburn on eating 'acidic' foods such as tomatoes and oranges?	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
	... regurgitate (throw up or bring up small amounts of previously eaten food)?	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
	... sleep in a 'raised' or 'L-shaped position'?	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
	... feel like vomiting or throwing up?	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
	... vomit or throw up?	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

DISTENSION	... feel bloated (a sensation of gas or air in the stomach)?	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
	... notice an increase in your belly, sometimes requiring you to open your belt, pants or shirt?	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
	... feel full after eating a small meal?	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
	... pass excessive gas or flatulence?	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

SOILAGE	... accidentally soil (dirty) your underwear before being able to get a bathroom?	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
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DIARRHOEA	In the <u>past 1 week</u> , how often did you...	Check one response for each question			
		No Days	1 – 2 Days	3 – 4 Days	5 – 7 Days
	... have loose stools (diarrhoea)?	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
	In the <u>past 1 week</u> , have you noticed your stools becoming...	Check one response for each question			
		Yes		No	
	... watery?	<input type="checkbox"/>	<input type="checkbox"/>		

SOCIAL FUNCTION	In the <u>past 1 week</u> , how often did the following interfere with <u>social activities</u> (such as visiting friends or relatives)?	Check one response for each question			
		No Days	1 – 2 Days	3 – 4 Days	5 – 7 Days
	... Nausea	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
	... Vomiting	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
	... Stomach ache or pain	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
	... Diarrhoea	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
	... Worry you would accidentally soil your underwear	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
... Bloating sensation	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	

EMOTIONAL WELL-BEING	In the <u>past 1 week</u> , how often did you....	Check one response for each question			
		No Days	1 – 2 Days	3 – 4 Days	5 – 7 Days
	... feel worried or anxious about your bowel problems?	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
	... feel embarrassed because of your bowel symptoms?	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
	... have problems with sexual relations because of your bowel symptoms?	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
	... fear not finding a bathroom?	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
	... feel depressed or discouraged due to your bowel symptoms?	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
	... avoid or delay travelling because of your bowel symptoms?	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
	... feel angry or frustrated as a result of your bowel symptoms?	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
	... have problems with your sleep as a result of your bowel symptoms?	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
... feel 'stress' or an upset mood worsens your bowel symptoms?	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	

	In the <u>past 1 week</u> , have you noticed your stools becoming....	Check one response for each question			
		Yes	No		
	... harder?	<input type="checkbox"/>	<input type="checkbox"/>		
CONSTIPATION	In the <u>past 1 week</u> , how often...	Check one response for each question			
		No Days	1 – 2 Days	3 – 4 Days	5 – 7 Days
	... were you constipated or unable to empty your bowels?	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
	... did you have hard stools?	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
... did you have pain while passing your stools?	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	

## Appendix 2

UCLA SCTC GIT 2.0 Interpretation scales [86]

Scales	None to mild	Moderate	Severe to very severe
Reflux	0.00-0.49	0.50-1.00	1.01-3.00
Distension/ /Bloating	0.00-1.00	1.01-1.60	1.61-3.00
Faecal soilage	0.00-1.00	1.01-2.00	2.01-2.50
Diarrhoea	0.00-0.49	0.50-1.00	1.01-2.00
Emotional well-being	0.00-0.49	0.50-1.00	1.01-3.00
Social functioning	0.00-0.49	0.50-1.00	1.01-3.00
Constipation	0.00-0.49	0.50-1.00	1.01-3.00
Total GI score	0.00-0.49	0.50-1.00	1.01-3.00

### Appendix 3

UCLA SCTC GIT 2.0 minimally important difference estimates [88]

Domain	Qualitative Change	Minimally important difference estimates
Reflux	Somewhat better	-0.26
	Somewhat worse	0.19
Distension/ /Bloating	Somewhat better	-0.14
	Somewhat worse	0.12
Faecal soilage	Somewhat better	-0.19
	Somewhat worse	0.06
Diarrhoea	Somewhat better	-0.19
	Somewhat worse	0.07
Emotional well-being	Somewhat better	-0.36
	Somewhat worse	0.16
Social functioning	Somewhat better	-0.07
	Somewhat worse	0.21
Constipation	Somewhat better	-0.17
	Somewhat worse	0.13
Total GI score	Somewhat better	-0.20
	Somewhat worse	0.12

## Appendix 4

Health Assessment Questionnaire Disability Index used in clinic [406]

**USA Scleroderma HAQ  
HEALTH ASSESSMENT QUESTIONNAIRE**

Name \_\_\_\_\_ Hospital No. \_\_\_\_\_ Date \_\_\_\_\_

In this section we are interested in learning how your illness affects your ability to function in daily life. Please feel free to add comments.

Please tick the one response that best describes your usual abilities  
**IN THE PAST SEVEN DAYS:**

	Without <b>ANY</b> difficulty	With <b>SOME</b> difficulty	With <b>MUCH</b> difficulty	<b>UNABLE</b> to do	
<b>DRESSING &amp; GROOMING</b> Are you able to:					<b>DRESSING</b> _____
• Dress yourself, including tying shoelaces and doing buttons?	_____	_____	_____	_____	
• Shampoo your hair?	_____	_____	_____	_____	
<b>ARISING</b> Are you able to:					<b>RISE</b> _____
• Stand up from an armless straight chair?	_____	_____	_____	_____	
• Get in and out of bed?	_____	_____	_____	_____	
<b>EATING</b> Are you able to:					<b>EATING</b> _____
• Cut your meat?	_____	_____	_____	_____	
• Lift a full glass to your mouth?	_____	_____	_____	_____	
• Open a new milk carton?	_____	_____	_____	_____	
<b>WALKING</b> Are you able to:					<b>WALKING</b> _____
• Walk outdoors on flat ground?	_____	_____	_____	_____	
• Climb up five stairs?	_____	_____	_____	_____	

FOR OFFICE USE  
KEY \_\_\_\_\_  
VISIT TYPE  
\_\_\_\_ (4) clinic  
\_\_\_\_ (1) hospital  
\_\_\_\_ (2) follow-on call  
\_\_\_\_ (8) mail

Please tick any **AIDS or DEVICES** that you usually use for any of these activities:

_____ Cane	_____ Devices for dressing (button hook, zipper pull, long-handled shoe horn, etc.)
_____ Walker	_____ Built up chair
_____ Crutches	_____ Special or built-up utensils
_____ Wheelchair	_____ Other (specify: _____)

Please tick any categories for which you usually need **ASSISTANCE FROM ANOTHER PERSON:**

_____ Dressing & Grooming	_____ Eating
_____ Arising	_____ Walking



**USA Scleroderma HAQ  
HEALTH ASSESSMENT QUESTIONNAIRE**

Name \_\_\_\_\_ Hospital No. \_\_\_\_\_ Date \_\_\_\_\_

Please tick the one response which best describes your usual abilities **IN THE PAST SEVEN DAYS:** FOR OFFICE USE

	Without <b>ANY</b> difficulty	With <b>SOME</b> difficulty	With <b>MUCH</b> difficulty	<b>UNABLE</b> to do	
<b>HYGIENE</b>					<b>HYGIENE</b> ____
Are you able to:					
• Wash and dry your entire body?	_____	_____	_____	_____	
• Take a tub bath?	_____	_____	_____	_____	
• Get on and off the toilet?	_____	_____	_____	_____	
<b>REACH</b>					<b>REACH</b> ____
Are you able to:					
• Reach and get down a 2 kilo object (such as a bag of sugar) from just over your head?	_____	_____	_____	_____	
• Bend down and pick up clothing off the floor?	_____	_____	_____	_____	
<b>GRIP</b>					<b>GRIP</b> ____
Are you able to:					
• Open car doors?	_____	_____	_____	_____	
• Open jars that have been previously opened?	_____	_____	_____	_____	
• Turn taps on and off?	_____	_____	_____	_____	
<b>ACTIVITIES</b>					<b>ACTIVITY</b> ____
Are you able to:					
• Run errands and shop?	_____	_____	_____	_____	
• Get in and out of a car?	_____	_____	_____	_____	
• Do everyday household cleaning?	_____	_____	_____	_____	

Please tick any **AIDS or DEVICES** that you usually use for any of these activities:

- |   |   |
|---|---|
| _____ Raised Toilet Seats                 | _____ Bathtub Seat                            |
| _____ Long-handled appliances for reach   | _____ Jar Opener (for jars previously opened) |
| _____ Long-handled appliances in bathroom | _____ Other (specify: _____)                  |

Please tick any categories for which you usually need **ASSISTANCE FROM ANOTHER PERSON:**

- |               |                                   |
|---------------|-----------------------------------|
| _____ Hygiene | _____ Gripping and opening things |
| _____ Reach   | _____ Errands and chores          |

## Appendix 5

UK Functional Questionnaire used in clinic [107]

NAME:

HOSPITAL NUMBER:

DATE:

### 11 ITEM FUNCTIONAL QUESTIONNAIRE

#### SCORING SYSTEM

0= Able to perform in normal manner

1= Can manage with some alteration of style

2= Can only manage with some difficulty

3= Impossible to achieve

PLEASE CIRCLE

- |   |         |
|---|---------|
| 1) Can you lift and pour water (about 1.5 litres) from a saucepan ?                   | 0 1 2 3 |
| 2) Can you unscrew a lid from a jam jar already opened before ?                       | 0 1 2 3 |
| 3) Can you take money ( 20p and £1) out of a purse with the thumb and second digits ? | 0 1 2 3 |
| 4) Can you hold a pen and write your name ?   | 0 1 2 3 |
| 5) Can you hold a pen and write half a sheet of A4 typing paper ?                     | 0 1 2 3 |
| 6) Can you do and undo shirt buttons ?  | 0 1 2 3 |
| 7) Can you tuck your shirt or blouse into the waistband ?                             | 0 1 2 3 |
| 8) Can you comb the back of your hair ?   | 0 1 2 3 |
| 9) Can you wash your hair ?   | 0 1 2 3 |
| 10) Can you get up from the toilet without using your hands ?                         | 0 1 2 3 |
| 11) Can you walk 20 steps without using a banister rail ?                             | 0 1 2 3 |

## Appendix 6

Norms for Upper Arm Anthropometry for American Men and Women [155]

### a. Men - MAC

Age	Percentile - Men - MAC						
	5 <sup>th</sup>	10 <sup>th</sup>	25 <sup>th</sup>	50 <sup>th</sup>	75 <sup>th</sup>	90 <sup>th</sup>	95 <sup>th</sup>
18-24	25.7	27.1	28.7	30.7	32.9	35.5	37.4
25-34	27.0	28.2	30.0	32.0	34.4	36.5	37.6
35-44	27.8	28.7	30.7	32.7	34.8	36.3	37.1
45-54	26.7	27.8	30.0	32.0	34.2	36.2	37.6
55-64	25.6	27.3	29.6	31.7	33.4	35.2	36.6
65-74	25.3	26.5	28.5	30.7	32.4	34.4	35.5

### b. Women - MAC

Age	Percentile - Women - MAC						
	5 <sup>th</sup>	10 <sup>th</sup>	25 <sup>th</sup>	50 <sup>th</sup>	75 <sup>th</sup>	90 <sup>th</sup>	95 <sup>th</sup>
18-24	22.1	23.0	24.5	26.4	28.8	31.7	34.3
25-34	23.3	24.2	25.7	27.8	30.4	34.1	37.2
35-44	24.1	25.2	26.8	29.2	32.2	36.2	38.5
45-54	24.3	25.7	27.5	30.3	32.9	36.8	39.3
55-64	23.9	25.1	27.7	30.2	33.3	36.3	38.2
65-74	23.8	25.2	27.4	29.9	32.5	35.3	37.2

### c. Men - TSF

Age	Percentile – Men - TSF						
	5 <sup>th</sup>	10 <sup>th</sup>	25 <sup>th</sup>	50 <sup>th</sup>	75 <sup>th</sup>	90 <sup>th</sup>	95 <sup>th</sup>
18-24	4.0	5.0	7.0	9.5	14.0	20.0	23.0
25-34	4.5	5.5	8.0	12.0	16.0	21.5	24.0
35-44	5.0	6.0	8.5	12.0	15.5	20.0	23.0
45-54	5.0	6.0	8.0	11.0	15.0	20.0	25.5
55-64	5.0	6.0	8.0	11.0	14.0	18.0	21.5
65-74	4.5	5.5	8.0	11.0	15.0	19.0	22.0

d. Women - TSF

Age	Percentile – Women - TSF						
	5 <sup>th</sup>	10 <sup>th</sup>	25 <sup>th</sup>	50 <sup>th</sup>	75 <sup>th</sup>	90 <sup>th</sup>	95 <sup>th</sup>
18-24	9.4	11.0	14.0	18.0	24.0	30.0	34.0
25-34	10.5	12.0	16.0	21.0	26.5	33.5	37.0
35-44	12.0	14.0	18.0	23.0	29.5	35.5	39.0
45-54	13.0	15.0	20.0	25.0	30.0	36.0	40.0
55-64	11.0	14.0	19.0	25.0	30.5	35.0	39.0
65-74	11.5	14.0	18.0	23.0	28.0	33.0	36.0

e. Men - MAMC

Age	Percentile – Men - MAMC						
	5 <sup>th</sup>	10 <sup>th</sup>	25 <sup>th</sup>	50 <sup>th</sup>	75 <sup>th</sup>	90 <sup>th</sup>	95 <sup>th</sup>
18-24	23.5	24.4	25.8	27.2	28.9	30.8	32.3
25-34	24.2	25.3	26.5	28.0	30	31.7	32.9
35-44	25.0	25.6	27.1	28.7	30.3	32.1	33.0
45-54	24.0	24.9	26.5	28.1	29.8	31.5	32.6
55-64	22.8	24.4	26.2	27.9	29.6	31.0	30.7
65-74	22.5	23.7	25.3	26.9	28.5	29.9	30.7

f. Women - MAMC

Age	Percentile – Women - MAMC						
	5 <sup>th</sup>	10 <sup>th</sup>	25 <sup>th</sup>	50 <sup>th</sup>	75 <sup>th</sup>	90 <sup>th</sup>	95 <sup>th</sup>
18-24	17.7	18.5	19.4	20.6	22.1	23.6	24.9
25-34	18.3	18.9	20.0	21.4	22.9	24.9	26.6
35-44	18.5	19.2	20.6	22.0	24.0	26.1	27.4
45-54	18.8	19.5	20.7	22.2	24.3	26.6	27.8
55-64	18.6	19.5	20.8	22.6	24.4	26.3	28.1
65-74	18.6	19.5	20.8	22.5	24.4	26.5	28.1

## Appendix 7

Age and gender specific co-efficients [162]

Male	17-19yrs	20-29yrs	30-39yrs	40-49yrs	>50yrs
C	1.1620	1.1631	1.1422	1.1620	1.1715
M	0.0630	0.0632	0.0544	0.0700	0.077

Female	16-19yrs	20-29yrs	30-39yrs	40-49yrs	>50yrs
C	1.1549	1.1599	1.1423	1.1333	1.1339
M	0.0678	0.0717	0.0632	0.0612	0.0645

## Appendix 8

Schofield equation: age and gender specific co-efficients [175]

Male	10-17yrs	18-29yrs	30-59yrs	60-74yrs	$\geq 75$ yrs
A	17.7	15.1	11.5	11.9	8.4
B	657	692	873	700	821

Female	10-17yrs	18-29yrs	30-59yrs	60-74yrs	$\geq 75$ yrs
A	13.4	14.8	8.3	9.2	9.8
B	692	487	846	687	624

## Appendix 9

### Food portion images used in study

#### Picture examples to help you assess the quantity of food you have consumed

























Use the pictures on pages 2 to 6 to help you indicate the size of portion you have eaten.  
The pictures are much smaller than life size.

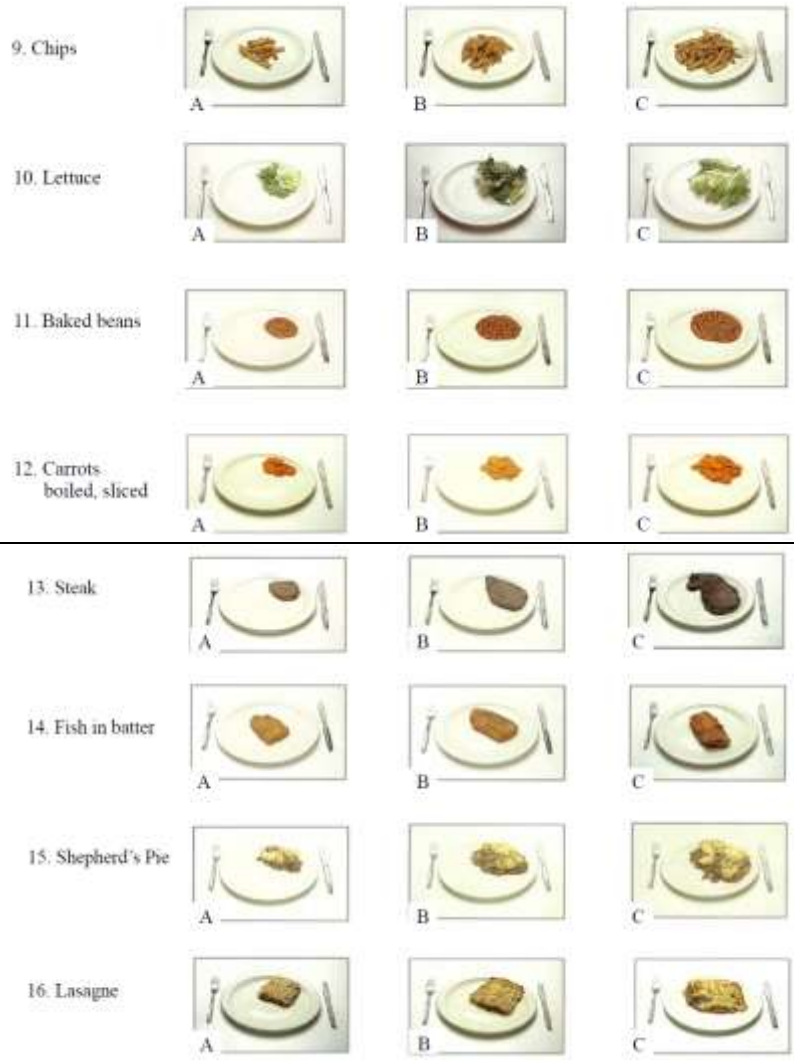
The actual size of the dinner plate, shown with a knife and fork, is 10inches (25cm), the side plate, with either a knife or a fork, is 7inches (18cm) and the bowl, with a spoon, is 6.3inches (16cm).

The food in the picture does not have to be exactly the same as that which you ate.  
For example, you may have eaten chocolate cake but the picture shows sponge cake.

On the food record, write the food you actually ate, the picture number and size (A, B, or C) nearest to your own helping, for example, bran flakes - picture 1 - C

The photographs used in this document are taken from: 'A Photographic Atlas of Food Portion Sizes' by Michael Odean, Mary Adams and Susan Murray.  
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1. Breakfast cereal	 A _____	 B _____	 C _____
2. Ginger cake	 A _____	 B _____	 C _____
3. Sponge cake	 A _____	 B _____	 C _____
4. Quiche	 A _____	 B _____	 C _____
<hr/>			
5. Cheddar Cheese	 A _____	 B _____	 C _____
6. Soup	 A _____	 B _____	 C _____
7. Pasta	 A _____	 B _____	 C _____
8. Jacket potato	 A _____	 B _____	 C _____





## Appendix 10

### UK Reference Nutrient Intakes [177]

	<b>Male RNI</b>	<b>Female RNI</b>
Sodium (mg)	1,600mg	1,600mg
Potassium (mg)	3,500mg	3,500mg
Calcium (mg)	700mg	700mg
Phosphorus (mg)	550mg	550mg
Magnesium (mg)	300mg	270mg
Chloride (mg)	2,500mg	2,500mg
Iron (mg)	8.7mg	8.7mg >50yr 14.8mg <50yr
Copper (mg)	1.2mg	1.2mg
Zinc (mg)	9.5mg	7.0mg
Selenium (µg)	75µg	60µg
Folate (µg)	200µg	200µg
Vitamin D (µg)	N/A	N/A
Vitamin B12 (µg)	1.5µg	1.5µg

## Appendix 11

### COMPASS 31 questionnaire and scoring [permission to reproduce granted] [263]

Please complete this questionnaire by ticking the appropriate boxes, or by writing in the spaces provided.

Please answer all the questions as best as you can.

1. In the past year, have you ever felt faint, dizzy, 'goofy', or had difficulty thinking soon after standing up from a sitting or lying position?

Yes

No (if you marked No, please skip to question 5)

2. When standing up, how frequently do you get these feelings or symptoms?

Rarely

Occasionally

Frequently

Almost always

3. How would you rate the severity of these feelings or symptoms?

Mild

Moderate

Severe

4. In the past year, have these feelings or symptoms that you have experienced:

Gotten much worse

Gotten somewhat worse

Stayed about the same

Gotten somewhat better

Gotten much better

Completely gone

---

5. In the past year, have you ever noticed colour changes in your skin, such as red, white or purple?

Yes

No (if you marked No, please skip to question 8)

6. Which parts of your body are affected by these colour changes? (Tick all that apply)

Hands

Feet

7. Are these changes in your skin colour:

Getting much worse

Getting somewhat worse

Staying about the same

Getting somewhat better

Getting much better

Completely gone

8. In the past 5 years, what changes, if any, have occurred in your general body sweating?

I sweat much more than I used to

I sweat somewhat more than I used to

I haven't noticed any changes in my sweating

I sweat somewhat less than I used to

I sweat much less than I used to

---

9. Do your eyes feel excessively dry?

Yes

No

10. Does your mouth feel excessively dry?

Yes

No

11. For the symptom of dry eyes or dry mouth that you have had for the longest period of time, is this symptom:

I have not had any of these symptoms

Getting much worse

Getting somewhat worse

Staying about the same

Getting somewhat better

Getting much better

Completely gone

12. In the past year, have you noticed any changes in how quickly you get full when eating a meal?

I get full a lot more quickly now than I used to

I get full more quickly now than I used to

I haven't noticed any change

I get full less quickly now than I used to

I get full a lot less quickly now than I used to

---

13. In the past year, have you felt excessively full or persistently full (bloating feeling) after a meal?

- Never
- Sometimes
- A lot of the time

14. In the past year, have you vomited after a meal?

- Never
- Sometimes
- A lot of the time

15. In the past year, have you had a cramping or colicky abdominal pain?

- Never
- Sometimes
- A lot of the time

16. In the past year, have you had any bouts of diarrhoea?

- Yes
- No (if you marked No, please skip to question 20)

17. How frequently does this occur?

- Rarely
  - Occasionally
  - Frequently \_\_\_\_\_ times per month
  - Constantly
-

18. How severe are these bouts of diarrhoea?

Mild

Moderate

Severe

19. Are your bouts of diarrhoea getting:

Much worse

Somewhat worse

Staying the same

Somewhat better

Much better

Completely gone

20. In the past year, have you been constipated?

Yes

No (if you marked No, please skip to question 24)

21. How frequently are you constipated?

Rarely

Occasionally

Frequently \_\_\_\_\_ times per month

Constantly

---

22. How severe are these episodes of constipation?

Mild

Moderate

Severe

23. Is your constipation getting:

Much worse

Somewhat worse

Staying the same

Somewhat better

Much better

Completely gone

24. In the past year, have you ever lost control of your bladder function?

Never

Occasionally

Frequently \_\_\_\_\_ times per month

Constantly

25. In the past year, have you had difficulty passing urine?

Never

Occasionally

Frequently \_\_\_\_\_ times per month

Constantly

26. In the past year, have you had trouble completely emptying your bladder?

- Never
- Occasionally
- Frequently \_\_\_\_\_ times per month
- Constantly

27. In the past year, without sunglasses or tinted glasses, has bright light bothered your eyes?

- Never (if you marked Never, please skip to question 29)
- Occasionally
- Frequently
- Constantly

28. How severe is this sensitivity to bright light?

- Mild
- Moderate
- Severe

29. In the past year, have you had trouble focusing your eyes?

- Never (if you marked Never, please skip to question 31)
  - Occasionally
  - Frequently
  - Constantly
-



30. How severe is this focusing problem?

Mild

Moderate

Severe

31. Is the most troublesome symptom with your eyes (i.e. sensitivity to bright light or trouble focusing) getting:

I have not had any of these symptoms

Much worse

Somewhat worse

Staying about the same

Somewhat better

Much better

Completely gone

---

## Appendix 12

COMPASS 31 scoring [263]

Domain	Item	Answer	Points	Domain	Item	Answer	Points	
Orthostatic intolerance	1	1	1	GI	12	1	2	
	2	2	1			2	1	
		3	2		13	2	1	
		4	3			3	2	
		3	1		1	14	2	1
	2		2		3		2	
	3		3		15	2	1	
	4	1	3			3	2	
		2	2		16	1	1	
		3	1			17	2	1
					3		2	
					4		3	
					18		1	1
Vasomotor	5	1	1			2	2	
	6	1	1			3	3	
		2	1		19	1	3	
	7	1	3			2	2	
		2	2			3	1	
		3	1		20	1	1	
						21	2	1
					3		2	
					4		3	
Secretomotor	8	1	1		22	1	1	
		4	1	2		2		
		5	2	23	3	3		
	9	1	1		1	3		
	10	1	1		2	2		
	11	2	3	3	1			
		3	2	2	2			
		4	1	3	1			
						3	1	

Domain	Item	Answer	Points	Domain	Item	Answer	Points	
Bladder	24	2	1	Pupillomotor	27	2	1	
		3	2			3	2	
		4	3			4	3	
	25	2	1		28	1	1	
		3	2			2	2	
		4	3			3	3	
	26	2	1		29	2	1	
		3	2			3	2	
		4	3			4	3	
		30	1		1	31	2	3
			2		2		3	2
			3		3		4	1

Raw domains are derived by adding the points for the question comprising each domain. Where an answer is not assigned a point, the score of that answer is zero. The final domain scores are generated by multiplying the raw score with a weight index. The total score is the sum of all domain scores.

## Appendix 13

VAS for appetite, satiation and abdominal symptoms used in the autonomic study  
(Chapter 5)

Subject number: \_\_\_\_\_ Date: \_\_\_\_\_

Time point: baseline / 5min / 10min / 20min / 40min / 60min / 80min / 100min / 120min

Please place a vertical line on the lines below to indicate the magnitude of your feeling.

I am not at all hungry | How hungry do you feel? | I have never been more hungry

I am completely empty | How satisfied do you feel? | I cannot eat another bite

Not at all full | How full do you feel? | Totally full

Nothing at all | How much do you think you can eat? | A lot

I am not at all nauseous | How nauseous do you feel? | I have never been more nauseous

None at all | How much abdominal bloating do you have? | Very bloated

None at all | How much abdominal discomfort do you have? | A lot

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