Investigating the mechanism of fatigue in subclinical hypothyroidism

A thesis submitted for the Doctorate in Medicine

Asgar Madathil

Student number 099201973

Institute of Cellular Medicine

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Abstract

Subclinical hypothyroidism (SCH) is a common medical condition affecting 4-10% of the population. Unlike overt hypothyroidism, clinical manifestations are unclear and treatment remains controversial. It is known that fatigue may improve with levothyroxine in these patients but the mechanisms linking symptoms with abnormal tissue function are poorly understood.

It was hypothesized that fatigue in patients with SCH is caused by peripheral tissue functional changes and that these changes are reversible with levothyroxine treatment. The objective of the study was to quantify the specific abnormalities in cerebral blood flow, cardiac function, cardiac and muscular energetic function, and autonomic function in patients with SCH, and to measure the changes in these abnormalities after levothyroxine therapy with any associated impact on fatigue. This was a pilot study as no previous studies looking into the mechanism of fatigue in patients with SCH have been reported.

Subjects with SCH (TSH 4.0 -10.0 mU/L, normal free T4) and fatigue were studied before and after levothyroxine therapy and were compared with age and gender-matched healthy controls (HC). Cerebral blood flow (CBF) was measured by MR arterial spin labelling. Cardiac function was measured using impedance cardiography. Cardiac and calf muscle energetic functions were measured by 31-Phosphorous Magnetic Resonance Spectroscopy. Autonomic function was assessed using heart rate variability.

At baseline, patients with SCH had increased CBF, impaired cardiac function, and lower cardiac and calf muscle energetic function, compared with HC. Autonomic function was equal to that of HC. After levothyroxine treatment, CBF decreased, cardiac function was unchanged, and cardiac energetic function improved. Calf muscle energetic function did not improve but autonomic function tests did. Although fatigue improved after levothyroxine treatment, these improvements were not correlated with peripheral tissue functional changes.

Novel physiological abnormalities in both CBF and cardiac and calf muscle energetic functions have been demonstrated by these studies. Improvements were seen in CBF,

cardiac energetic function and autonomic function after levothyroxine treatment. These parameters may play a role in the reduction of fatigue and warrant further investigation.

Dedication

I dedicate my MD thesis to my family; without their support I could not have undertaken this research project. My wife, Jeseem, has been extremely supportive and has stayed with me through “thick and thin”. She has shown exceptional patience at times of difficulty during my research work. My two darling daughters, Layyah and Ziyana, and our new addition, Zain, fill me with joy and happiness after a long day at work. Although they may never read this work, it is for them by all means!

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Abbreviations

Abbreviation	Meaning
AD	Autonomic Dysfunction
ADP	Adenosine Disphosphate
ANS	Autonomic Nervous System
ATP	Adenosine Triphosphate
BMI	Body Mass Index
BOLD	Blood-Oxygen Level Dependent
BRS	Baroreflex Sensitivity
CAD	Coronary Artery Disease
CBF	Cerebral Blood Flow
CO	Cardiac Output
CHD	Coronary Heart Disease
CI	Cardiac Index
DIO2	Type 2 deiodinase enzyme
DPG	2, 3-Diphosphoglycerate
EDI	End-diastolic Index
fMRI	Functional Magnetic Resonance Imaging
FIS	Fatigue Impact Scale
FT3	Free Tri-iodothyronine
FT4	Free Thyroxine
H^+	Hydrogen ion
HADS	Hospital Anxiety Depression Score
HC	Healthy control
HD	Hashimoto's Thyroid Disease
HF	High Frequency
HRV	Heart Rate Variability
HUT	Head-up Tilt
IC	Contractility Index
ICG	Impedance Cardiography
IHD	Ischaemic Heart Disease

Abbreviation	Meaning
LF	Low Frequency
LVET	Left Ventricular Ejection Time
LVWI	Left Ventricular Work Index
MRS	Magnetic Resonance Spectroscopy
MtTFA	Mitochondrial Transcription Factor-A
MVC	Maximum Voluntary Contraction
NMR	Nuclear Magnetic Resonance
Nu	Normalised Units
PBC	Primary Biliary Cirrhosis
PCr	Phosphocreatine/Creatine Phosphate
PDE	Phosphodiester
PET	Positron Emission Tomography
PGWI	Psychological General Well-Being Index
Pi	Inorganic Phosphate
PME	Phosphomonoester
^{31}P-MRS	^{31}Phosphorous Magnetic Resonance Spectroscopy
PSA	Power Spectral Analysis
PSD	Power Spectral Density
RCT	Randomised controlled trial
ROI	Region of Interest
RRI	R-R Interval
SCH	Subclinical Hypothyroidism
SDANN	Standard Deviation of all 5 minute Mean Normal to Normal R-R intervals
SDNN	Standard Deviation of Normal to Normal R-R Intervals
SF-36	Short Form-36
SPECT	Single-Photon Emission Computerized Tomography
TH	Thyroid Hormone
TPRI	Total Peripheral Resistance Index
TPO	Thyroid Peroxidase
TSH	Thyroid Stimulating Hormone

Abbreviation	Meaning
VLF	Very Low Frequency
VO2	Maximal Oxygen Uptake
WASI	Wechsler Abbreviated Scale of Intelligence
WMS	Wechsler Memory Scale
WTAR	Wechsler Test of Adult Reading

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Chapter 1 Subclinical hypothyroidism

1.1 Definition

Subclinical hypothyroidism (SCH) is a biochemical diagnosis defined as a state with raised serum thyroid stimulating hormone (TSH) levels and normal free thyroxine (FT4) and tri-iodothyronine (FT3) (Cooper and Biondi, 2012). SCH is a sign of early thyroid failure. It is often referred to as compensated thyroid failure with normal circulating thyroid hormones, despite disease affecting the thyroid gland.

SCH is divided into two categories: those with serum TSH between 4 and 10 mIU/L and those with serum TSH above 10 IU/L. Patients with serum TSH above 10 mIU/L have a higher chance of disease progression to overt hypothyroidism and a greater association with cardiovascular disease than those with serum TSH between 4-10 mIU/L (Rodondi et al., 2010, Diez et al., 2005). This classification helps to categorise patients with SCH who may potentially benefit from levothyroxine treatment. Younger patients (aged below 70 years) with serum TSH above 10 mIU/L are routinely treated with levothyroxine as recommended in the recent European Thyroid Association guidelines (Pearce et al., 2013). The guidelines also suggest treatment for a period of 3 months in those with serum TSH below 10 mIU/L if they have symptoms suggestive of hypothyroidism and to continue the treatment if significant improvement in symptoms are shown at the end of 3 months.

1.2 Prevalence

The prevalence of SCH is reported to be between 4-10% in various studies (Biondi and Cooper, 2008). The differences in prevalence between various studies are due to variations in serum TSH cut-offs for defining SCH, iodine status, age groups, gender and ethnicity. In the Whickham survey, the prevalence of SCH (serum TSH above 6 mIU/L) in women was 7.5% and in men was 2.8% (Vanderpump et al., 1995). In the NHANES III survey, SCH (serum TSH above 4.6 mIU/L) was prevalent in 4.3% of the reference population, which included multi-ethnic groups in the United States (Hollowell et al., 2002). The study has shown that SCH was more prevalent in Caucasians, and that the female gender, positive thyroid antibody status and age were

strongly associated with a higher prevalence of SCH. Iodine sufficient status was associated with a higher prevalence of overt and subclinical hypothyroidism than iodine deficient status in a Hungarian study (Szabolcs et al., 1997). In a given population several factors may determine the prevalence of SCH.

1.3 Diagnosis

Autoimmune thyroid disease accounts for 60-80% of SCH (Cooper and Biondi, 2012). Other less common causes include previous thyroid surgery, radioiodine treatment, drugs (Lithium and amiodarone) or a recent episode of thyroiditis. Ageing and obesity have been associated with a mild rise in serum TSH. Also, recovery from an acute illness has been associated with high serum TSH, which often resolves after 6-12 weeks. It is important to exclude transient elevations in serum TSH before making a diagnosis of SCH. A diagnosis of SCH should not be made on an isolated raised serum TSH and it is recommended that the serum TSH is repeated after a period of 3-6 months.

1.4 Impact of SCH

SCH by definition is a biochemical diagnosis without any overt clinical symptoms. However, a number of studies have shown associations of physical and cognitive symptoms with SCH. Classic symptoms of overt hypothyroidism have been shown in a large number of SCH patients in some studies, although the studies have shown conflicting results. The Colorado study showed that hypothyroid symptoms were more prevalent in SCH (n=2336) than in euthyroid subjects (13.7 vs. 12.1%,$p<0.05$) (Canaris et al., 2000). It was not a true population study as the subjects were from a health fair. This might have led to more subjects with symptoms and healthcare-seeking behaviour in the study and fewer subjects without symptoms. However, in an Australian population study involving women only, the mean Psychological General Well-Being Index (PGWI) and the Short Form-36 (SF-36) scores did not differ between SCH (n=80) and euthyroid controls (n=240) (Bell et al., 2007). The fifth Tromsø study was a large-scale population survey involving men and women that assessed the prevalence of hypothyroid symptoms in patients with SCH (n=89, mean serum TSH 5.57 mIU/L), using a pre-defined serum TSH upper limit of 10.0 mIU/L (Jorde et al., 2006). The study found no excess prevalence of hypothyroid symptoms in this cohort. Furthermore,

treatment of those subjects with SCH did not lead to improvement in symptoms typically associated with hypothyroidism.

A small (n=33) randomised double-blinded placebo controlled study (RCT) by Cooper et al. demonstrated improvement in hypothyroid symptoms with levothyroxine treatment in 8 out of 14 subjects with SCH (Cooper et al., 1984). The largest randomised placebo controlled trial of SCH (n=100, mean age 53.8 years, mean serum TSH 6.6 mIU/L) showed that fatigue improved with levothyroxine treatment in SCH (Razvi et al., 2007). Kong et al. showed that fatigue and weight gain were the most commonly-reported symptoms in SCH (Kong et al., 2002). However, no improvement in symptoms was seen with levothyroxine treatment in this study.

Overt hypothyroidism is known to cause various neuropsychiatric conditions, including reversible dementia and frank psychosis. Mild cognitive symptoms in SCH have been widely reported (Baldini et al., 1997, Samuels et al., 2007), (Haggerty et al., 1990, Bono et al., 2004). The study by Baldini et al. (mean age 52.9 years) showed reversible logical memory impairments in female SCH patients with goitre when compared to female euthyroid patients with goitre. The presence of goitre in both groups excluded perception of disease as the confounding factor in this study. Another study by Samuel et al. (age range 20-75 years) has shown that experimentally-induced SCH (randomized blinded fashion) was associated with working memory impairment (Samuels et al., 2007). These impairments were correlated with changes in FT4 or FT3 in these patients. However, the studies by Jorde et al. (mean age 62.5 years) and Park et al. (only above 65 years included) did not show any significant changes in cognitive function in patients with SCH (Jorde et al., 2006, Park et al., 2009).

As described above, the symptom reporting in SCH in various studies showed conflicting reports. There could be a number of reasons which might explain these variable results in different studies. This may be due to differing durations of disease, which is often not reported in many studies, and whether subjects were selected based on a single raised serum TSH or persistently raised serum TSH. Duration of disease can affect disease severity and hence severity of symptoms in SCH (Biondi and Cooper, 2008). If the subjects were included based on a single elevated serum TSH, then many of these patients may not have SCH upon repeat testing and hence may not show hypothyroid symptoms. Ageing is associated with decline in general health and subtle

abnormalities in cognitive function. It is also well known that in elderly patients, using symptoms to identify patients for thyroid hormone testing is less reliable (Eden et al., 1988). Ageing is associated with a mild rise in serum TSH and hence many of these patients may not have SCH and hence are unlikely to show any symptoms associated with a mild degree of hypothyroidism. The studies which showed no clear association between symptoms and SCH had higher numbers of elderly patients (Jorde et al., 2006, Lindeman et al., 1999, Gussekloo et al., 2004, Park et al., 2009, Roberts et al., 2006). The degree of serum TSH elevation may influence the presence of symptoms, which varied between studies assessing the symptoms in SCH. To summarise, there are several confounding factors which influence the outcome of symptom assessment in SCH. More studies are required to look into the mechanism of symptoms which correlate to disease severity in SCH.

SCH often progresses to overt hypothyroidism. According to the Whickham 20-year follow-up study, the annual risk of progression is 4.3% in women with positive thyroid peroxidase (TPO) antibodies and raised serum TSH; and 3% in those with negative antibodies and a raised serum TSH (Vanderpump et al., 1995). Patients with serum TSH above 10 mIU/L have a higher risk of progression to overt hypothyroidism than those with serum TSH below 10 mIU/L (Diez and Iglesias, 2004).

Cardiovascular morbidity and mortality have been studied in SCH recently. A recent meta-analysis has shown that in SCH, those with serum TSH above 10 mIU/L had higher coronary heart disease (CHD) events and CHD mortality than euthyroid controls (Rodondi et al., 2010). This was thought to be due to increased risk of hypercholesterolemia and atherosclerosis in SCH. These were observational studies and so far no RCTs have been performed to assess the true causal relationship between CHD and SCH.

1.5 Current treatment recommendations.

In the absence of long-term RCTs that assess the benefits and risks of treating SCH with levothyroxine, there have been a number of expert panel recommendations over the last 10 years (Surks et al., 2004, Cooper and Biondi, 2012, Garber et al., 2012, Pearce et al., 2013). Most recommend treating SCH with levothyroxine if serum TSH is above 10 mIU/L, based on a high rate of progression to overt hypothyroidism.

Controversy exists in treating patients with SCH if they have a serum TSH below 10 mIU/L. In this TSH range, some recommend treating patients less than 65 years of age with levothyroxine due to an increased risk of cardiovascular disease in this age group (Cooper and Biondi, 2012). Levothyroxine treatment also may be considered in this age group if they have any new onset hypothyroid symptoms, goitre or positive TPO antibodies (Pearce et al., 2013). Association of symptoms with thyroid status is less clear in this age and TSH range. Very few studies were done in younger patients with symptoms and serum TSH below 10 mIU/L. Therefore no recommendation exists for routine symptomatic treatment in this group. As mentioned previously, existing studies were mostly performed in older populations and did not show any convincing association with symptoms.

1.6 Summary

SCH is common in the general population. Autoimmune thyroid disease is the most common cause of SCH and diagnosis can only be confirmed after excluding a transient rise in serum TSH due to other conditions. Although it is a biochemical diagnosis, symptoms and cardiovascular disease have been attributed to SCH. Treatment of SCH is often controversial because of a lack of mechanistic studies linking SCH to a disease state and RCTs to prove a causal relationship between SCH and cardiovascular disease.

Chapter 2 Mechanism of fatigue in SCH

In this chapter, the mechanism of symptoms in SCH will be discussed. Potential targets for mechanistic studies and existing research will be explored. Finally, the research proposal and hypothesis for this project will be discussed.

Fatigue is defined as an extreme form of persistent and disabling tiredness, weakness or exhaustion, which could be physical or mental or both (Dittner et al., 2004). It is commonly seen in hypothyroid patients in varying degrees, as described previously. The mechanism of fatigue in hypothyroidism may be due to peripheral muscle, autonomic nervous system, or cardiac dysfunction, or alterations in cerebral blood flow. In the following sections, these subjects will be discussed in detail.

2.1 Fatigue due to muscle dysfunction

2.1.1 Muscle physiology- brief overview

Human skeletal muscle largely consists of striated muscle fibres. There are three types of muscle fibres:

Type I fibres: Appear red in colour due to high concentration of myoglobin. They are rich in capillaries and mitochondria. They solely use oxidative phosphorylation to generate ATP used for muscle contraction. These are often called slow twitch fibres because aerobic oxidation is slow to start and cannot sustain fast muscle contractions due to lack of anaerobic capacity. These fibres are fatigue resistant and can maintain prolonged aerobic activity due to a rich oxygen supply.

Type IIa: These are similar to type 1 fibres, but use both oxidative phosphorylation and glycolysis. Hence, these fibres can contract at a faster pace than type I fibres.

Type IIb: These fibres appear white because they are low in myoglobin concentration. They depend on glycolysis, and can contract at a faster rate than type I and type IIa fibres.

Human skeletal muscle often has mixtures of these fibres, although some muscle might have higher proportions of certain types of muscle fibres. For example, an *in vitro* study showed that the percentage of slow fibres in soleus and gastrocnemius was 60-100%

and 34-82% respectively (Gollnick et al., 1974). Muscle fibre types in individuals are determined mainly by genetic polymorphism, gender, hormonal factors and exercise training (Schiaffino and Reggiani, 2011). In animal studies, it has been shown that hypothyroidism causes fast (type II) to slow (type I) changes in fibre composition, i.e. a 99% reduction in the proportion of type II muscle fibres (Nwoye et al., 1982). Similar findings were demonstrated in muscle biopsies of hypothyroid patients (Khaleeli et al., 1983). The study showed a higher proportion of slow muscle fibres (type I) in hypothyroid patients, and after treatment with levothyroxine, a significant transformation to fast type muscle fibres was noted. These findings clearly reveal that thyroid hormones play a significant role in muscle fibre composition in addition to controlling muscle bioenergetics, as discussed later in the chapter.

2.1.2 Muscle energetic physiology.

Each muscle fibre contains several myofibrils, which are composed of actin and myosin filaments. Along the length of each myofibril are the repeats of sarcomeres, which are the functional unit of muscle contraction. Muscle mitochondria lie beneath the sarcolemmal membrane. Upon nerve stimulation, actin and myosin in each sarcomere slide over each other to create muscle shortening, which ultimately produces contraction in the whole muscle. The energy required for this process is derived from the hydrolysis of adenosine triphosphate (ATP) to adenosine disphosphate (ADP) and inorganic phosphate (Pi) by ATP-ases. During muscle exercise, ADP and Pi levels significantly increase and ATP levels are maintained by metabolic pathways, described below. A steady level of ATP concentration within the muscle is critical for optimal muscle function. ATP is synthesised in muscle under aerobic and anaerobic conditions via separate metabolic pathways.

2.1.3 Muscle bioenergetics under anaerobic conditions.

The anaerobic metabolism is the predominant type of energy source for fast muscle fibres during exercise, and during prolonged exercise in slow muscle fibre types. During the initial stages of exercise, the pre-existing ATP is used for muscle contraction. This ATP is depleted rapidly and the muscle phosphocreatine (PCr) serves as an intermediate source of high energy phosphate, which is used to make ATP for a brief period. The concentration of PCr in muscle fibre is 10 times that of ATP. The PCr combines with ADP and hydrogen ions (H^+) to produce ATP and creatine (Cr). This reaction is a

reversible process that occurs during recovery from exercise with re-synthesis of PCr. This is shown in the equation given below:

$$PCr + ADP + H^{+} \leftrightarrow Cr + ATP$$

The next source of ATP is via glycolysis in cytosol. Glucose is available either directly from blood circulation or via glycogenolysis. Glucose is metabolised down the glycolytic pathway to yield 2 ATP molecules and pyruvate. Under anaerobic conditions, pyruvate is converted into lactic acid and subsequently removed from the muscle cell. Although the glycolytic pathway produces only 2 ATP molecules per glucose, it is 2.5 times faster than oxidative ATP synthesis. Hence, it provides sufficient amounts of ATP quickly in fast type muscle fibres.

The figure 2.1 depicts the typical muscle spectra in a resting state from a phosphorus magnetic resonance spectroscopic study. The figure 2.2 shows the muscle spectra during exercising in which PCr undergoes depletion and replenished during recovery. The ATP level is maintained throughout the exercise to provide continuous supply of energy for muscle contraction during the exercise.

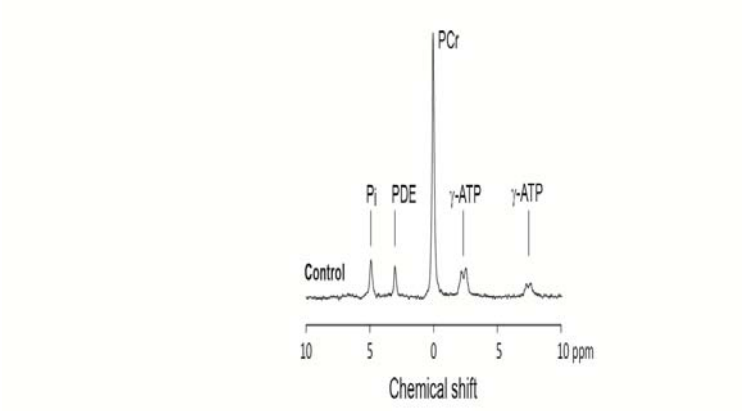

Figure 2-1 : Typical muscle spectra from a phosphorus magnetic resonance spectroscopic study.

It shows phosphocreatine (PCr), ATP, inorganic phosphate (Pi) and phosphodiester (PDE) peaks in parts per million (ppm).

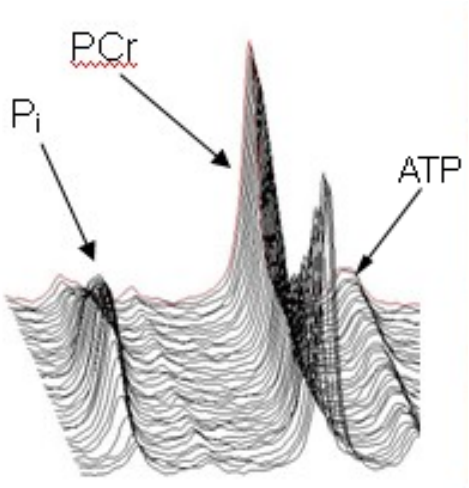

Figure 2-2 : Muscle spectra during exercise.

This figure shows that the phosphocreatine (PCr) level drops during the exercise and rise again during recovery after the exercise. The ATP level is maintained at a constant level during the exercise.

2.1.4 Muscle bioenergetics under aerobic conditions.

The major sources of fuel are glucose, lipids and amino acids in anaerobic conditions. This pathway occurs in the mitochondria using the electron chain transport system. In slow muscle fibres under aerobic conditions, the initial ATP synthesis is similar to ATP synthesis described under anaerobic conditions. The initial glycolysis is also common to both anaerobic and aerobic ATP synthesis. The pyruvate thus generated via the glycolytic pathway enters Kreb's cycle in the presence of oxygen and results in the generation of ATP molecules. This is a slow process, but yields 32 molecules of ATP from one glucose molecule, along with carbon dioxide and water. Thus, aerobic oxidation produces large amounts of ATP required for protracted periods of exercise in slow muscle fibres.

2.1.5 Thyroid hormone and mitochondrial function.

Muscle mitochondrial energetic functions are influenced by several factors, such as ageing, exercise and hormonal factors. Skeletal muscle mitochondrial membranes have triiodothyronine (T3) receptors, which suggests that thyroid hormones (TH) have a role in mitochondrial energetics (Sterling et al., 1978). The mitochondrial DNA contains promoter regions with response elements for thyroid hormone receptors. It is likely that TH affects mitochondrial protein expression by regulating the transcription of mitochondrial genes (Lanza and Sreekumaran Nair, 2010).

The effect of thyroid dysfunction on mitochondrial function has been extensively studied in animals. Mitochondria from hyperthyroid rats exhibited increased enzyme activities (Paradies et al., 1994). Exogenous thyroid hormone T4 increases mitochondrial volume density in both the liver and muscle in rats (Goldenthal et al., 2004, Wooten and Cascarano, 1980). It was found that with 14 days of liothyronine treatment in hypothyroid rats increased UCP2 and UCP3 expression as well as mitochondrial ATP synthesis rates in the soleus muscle, heart, and liver (Short et al., 2001). These data demonstrate that TH increases mitochondrial capacity for ATP synthesis in oxidative tissues, rather than simply increasing substrate oxidation to account for dissipation of the trans-membrane proton gradient (Lanza and Sreekumaran Nair, 2010).

To summarise, TH affects mitochondrial activity in several ways, including mitochondrial protein expression, volume density, enzyme activity and oxidative ATP synthesis. As a result, thyroid dysfunction often leads to alteration in muscle energetic function, resulting in clinically important symptoms like muscle fatigue or weakness.

2.1.6 Muscle fatigue in other diseases.

Fatigue due to muscle dysfunction has been shown in other diseases where fatigue is a prominent symptom. Our collaborators have investigated primary biliary cirrhosis (PBC) in which patients usually complain of disabling fatigue (Hollingsworth et al., 2008). Using ^{31}P-MRS studies of calf muscle, the study compared 15 patients with PBC to healthy controls. Subjects undertook calf muscle exercise at 25% and 35% of maximum voluntary contraction (MVC). Patients with PBC exhibited abnormal mitochondrial energetics, thought to be due to an autoimmune anti-pyruvate dehydrogenase complex. Greater fatigue severity assessed by the Fatigue Impact Scale (FIS) was reported in this group when muscle pH recovery time was prolonged. It was suggested that prolonged acidosis within the muscle might act as a stop signal, leading to perception of fatigue.

Fatigue in chronic fatigue syndrome has been investigated by our collaborator group (Hollingsworth et al., 2008). They studied patients with chronic fatigue syndrome, with ^{31}P-MRS in calf muscle, using plantar flexion at a fixed 35% load maximum voluntary contraction. These patients had delayed recovery of muscle pH following muscle

exercise. This was associated with abnormalities in proton efflux (the amount of acid pumped out of the myocyte following exercise, measured in mM/Minute) in the post-exercise recovery period. The maximum proton efflux was low and the time to maximum proton efflux was high in patients compared to healthy controls. These abnormalities can probably lead to muscle fatigue in patients. Whether those changes were due to physical deconditioning because of a lack of regular exercise or due to a primary muscle energetic defect is currently unknown. Further interventional studies in these patients may reveal the precise mechanism for these abnormal muscle bioenergetics.

The chronic fatigue syndrome was investigated by Wong et al. using ^{31}P MRS study in calf muscle (Wong et al., 1992) using a different protocol to the study mentioned previously by Hollingsworth et al. They measured muscle bioenergetics during dynamic graded exercise to the point of exhaustion. The patients reached muscle exhaustion earlier and had shown low ATP levels at the point of exhaustion when compared to healthy controls. They have suggested abnormal oxidative function as the mechanism leading to clinical fatigue in chronic fatigue syndrome.

These studies demonstrate, firstly, that fatigue in various diseases can be investigated using *in vivo* methods under physiological conditions, in real time, using exercise protocols. Secondly, that abnormal muscle bioenergetics have been consistently associated with fatigue. However, there are no interventional studies that show a causal relationship between fatigue and abnormal muscle bioenergetics. Our study aims to investigate muscle bioenergetics that might explain fatigue in patients with SCH and whether these changes, if any, will reverse with levothyroxine treatment, resulting in fatigue reduction.

2.1.7 Muscle dysfunction in SCH.

Skeletal muscle is one of the main targets for thyroid hormones and hence deficiency of thyroid hormones often leads to muscle symptom, such as pain, stiffness and/or weakness (Argov et al., 1988). These can result in patients complaining of fatigue or poor exercise tolerance in hypothyroidism.

^{31}P-MRS has been used to investigate the metabolic changes in hypothyroid muscle both in animals and humans (Argov et al., 1988, Kaminsky et al., 1991, Taylor et al.,

1992). Argov et al. demonstrated that the phosphocreatine/inorganic phosphate ratio (PCr/Pi) in hypothyroid muscle was low and this abnormality improved with levothyroxine treatment (Argov et al., 1988). Taylor et al. suggested that glycogen metabolic defect was the causative mechanism for the defective energy status in a hypothyroid state (Taylor et al., 1992), whereas two other studies suggested impaired mitochondrial function state for abnormal energetics in skeletal muscle (Argov et al., 1988, Kaminsky et al., 1991). Reduced levels of mitochondrial transcription factor A (mtTFA) were shown in muscle biopsy specimens of patients with Hashimoto's thyroid myopathy (Siciliano et al., 2002). The mtTFA has been shown to be one of the putative targets of thyroid hormones, and its lower levels may explain the molecular mechanism by which thyroid hormone deficiency leads to altered mitochondrial dysfunction in patients with hypothyroidism (Pillar and Seitz, 1997).

In patients with SCH, muscular metabolic changes were found by Monzani et al. in 1997 (Monzani et al., 1997). They showed that mean lactate levels and mean lactate increments were higher in SCH patients than in healthy controls during exercise. This might suggest impaired mitochondrial oxidation in SCH. Another study showed reduced exercise tolerance with reduction in forearm muscle maximal power output and maximal oxygen uptake (VO2), with an increase in workload in SCH patients when compared to healthy controls (Caraccio et al., 2005). In SCH, a similar mechanism is possible given that overt hypothyroidism is associated with mitochondrial dysfunction, as described above.

In summary, muscular changes are common and have been found extensively in patients with overt hypothyroidism and SCH. To date, no study has correlated patient symptoms like fatigue with muscular metabolic changes in SCH. Thus, we propose to investigate using a ^{31}P-MRS method whether abnormal muscular metabolism exists in SCH, which may contribute to the pathogenesis of fatigue. We also aim to discover whether or not it will reverse with levothyroxine treatment.

2.2 Fatigue due to autonomic nervous system dysfunction

In this section, I will give a brief overview of the autonomic nervous system (ANS) followed by a description of the role of TH in the regulation of the ANS. Fatigue due to autonomic dysfunction will be discussed.

2.2.1 ANS physiology- A brief overview

The human autonomic nervous system is mainly composed of the sympathetic and parasympathetic nervous systems (Guyton, 2000). The sympathetic and parasympathetic systems often act together to maintain visceral functions and provide physiological adaptations to various stimuli, such as stress, food intake etc. Both systems primarily consist of two neuronal pathways. The pre-ganglionic neurons originate in the central nervous system and synapse with various peripheral autonomic ganglia. The postganglionic neurons arising from the autonomic ganglia innervate various tissues throughout the body. The sympathetic pre-ganglionic neurons are mainly located in lateral horns of thoracolumbar spinal segments, and postganglionic neurons arise from the paravertebral sympathetic chain in the corresponding spinal segments. The parasympathetic pre-ganglionic neurons originate from various cranial nerve nuclei within the brain stem, and sacral spinal segments 3 and 4. The parasympathetic post-ganglionic neurons arise from autonomic ganglia located within various cranial nerves, and on the walls of visceral organs located in the thoracic and abdominal cavities.

2.2.2 Autonomic nervous system and cardiovascular function

The vasomotor centre in the medulla and pons control cardiac functions via the sympathetic and parasympathetic nervous system. The sympathetic nerves for the heart originate from the thoracic spinal segments T3 to T6, whereas parasympathetic fibres mainly descend via the vagus nerve. Almost all of the sympathetic and parasympathetic preganglionic fibres secrete acetylcholine at their nerve terminals. In the heart, sympathetic and parasympathetic post-ganglionic nerve fibres secrete noradrenaline and acetyl choline respectively.

Sympathetic stimulation of the heart causes both inotropic and chronotropic effects (via beta-1 receptors) while parasympathetic stimulation results in opposing effects which help the heart to adapt to various physiological and pathological states. For example, during exercise, sympathetic stimulation increases cardiac muscle contraction so that

more blood is pumped out to target tissues, such as muscle, while parasympathetic stimulation decreases its pumping ability but allows the heart some degree of rest between bouts of strenuous exercise.

The ANS is also involved in cardiac autonomic reflexes, such as baroreceptor reflexes for maintenance of blood pressure. The baroreceptors are located in the walls of large arteries and send appropriate signals to the heart, based on arterial blood pressure. In response to standing from a supine or sitting position, the baroreceptors send immediate stimulatory sympathetic signals to increase heart rate and contractility, and increase peripheral vasoconstriction to augment the preload of the heart. At the same time, the parasympathetic inhibitory signals are deactivated and this further enhances cardiac output to raise the blood pressure. Thus, this reflex mechanism helps to prevent a decrease in blood pressure to the upper body and to maintain upright posture in human beings.

The peripheral vasculature is largely under sympathetic control in the resting state, under various physiological conditions. The sympathetic system innervates almost all blood vessels, including arterioles and veins (except capillaries, precapillary sphincters and most of the meta-arterioles). Noradrenaline is the main vasoconstrictor chemical substance at the sympathetic nerve terminals. The noradrenaline, and the adrenaline released from the adrenal medulla, act via alpha-adrenergic receptors to cause peripheral vascular smooth constriction in response to sympathetic activation. This mechanism helps to increase blood pressure in response to standing. The vasomotor centre in the medulla sends signals via the sympathetic nerves to keep the peripheral vasculature in a partially-constricted state in the resting state. This helps to maintain adequate tissue perfusion. In response to exercise, the sympathetic mediated vascular constriction in skin, splanchnic regions, kidney and inactive muscles helps to divert the blood to the active skeletal muscle. The sympathetic system facilitates adequate tissue perfusion in the resting state and rapidly provides adaptive vascular mechanisms during various physiological states.

In summary, the ANS regulates cardiovascular functions both directly and indirectly via several mechanisms. So, any pathological state that leads to autonomic dysfunction can lead to abnormal cardiovascular functions.

2.2.3 Role of thyroid hormones in regulation of the autonomic nervous system

It is well known that thyroid hormones play a key role in autonomic nervous system functions under physiological conditions and pathological states. It regulates physiological conditions, such as adaptation responses during physical activity or exposure to cold (Silva and Bianco, 2008). TH has major role in regulation of thermogenesis in human beings. In pathological states like thyrotoxicosis, patients exhibit a variety of autonomic nervous system manifestations, such as tachycardia and sweating, whereas in hypothyroidism, patients exhibit bradycardia and cold skin. In hypothyroidism, the lack of appropriate thermogenesis leads to intense cutaneous and subcutaneous vasoconstriction, leading to cold skin. The interaction between TH and ANS is bidirectional, as described below.

Effect of thyroid hormones on autonomic nervous system

TH has an inhibitory role on central sympathetic output, and at a peripheral level it enhances the sensitivity of catecholamine mediated by β-adrenergic receptors (Silva and Bianco, 2008). Hence, in hypothyroidism, despite increased levels of peripheral noradrenalin concentrations due to enhanced central sympathetic central output, there are depressed adrenergic responses (Manhem et al., 1992, Christensen, 1973). The increase in central sympathetic output is thought to be due to a compensatory response to reduced catecholamine receptor response, or due to reduced cardiac output (Braverman). Thus TH has a significant influence in modulating various physiological functions controlled by the ANS.

Effect of autonomic nervous system on thyroid hormones

Conversely, the ANS can affect TH secretion and function in many ways. It was suggested that sympathetic stimulation might have a direct stimulatory effect on the thyroid gland (Silva and Bianco, 2008). In animals, adrenergic stimulation leads to enhanced peripheral conversion of T4 to T3 in brown adipose tissue mediated by type II deiodinase (DIO2) enzyme. Human skeletal muscle has DIO2 enzyme (Salvatore et al., 1996). It has been shown in cultured human skeletal muscle cells that adrenergic stimulation leads to enhanced DIO2 activity (Hosoi et al., 1999). In hypothyroidism, DIO2 mediated peripheral skeletal muscle T3 production is increased (Maia et al., 2005) due to enhanced sympathetic activity.

This suggests that thyroid hormones and the ANS interact in various physiological and pathological conditions. Hence, abnormal thyroid function can lead to disturbances in the ANS, resulting in the clinical manifestations seen in thyroid disease.

2.2.4 Non-invasive in vivo human studies of autonomic dysfunction in hypothyroidism

There is substantial evidence to show that autonomic dysfunction (AD) leads to clinical manifestations in thyroid disease. Cardiac autonomic functional status can be studied by non-invasive methods in patients using power spectral analysis, as described by Bellavere et al. (Bellavere et al., 1992). The power spectral analysis (PSA) uses fast Fourier mathematical analysis to define underlying frequency bands in heart rate variability (HRV). This analysis will reveal low frequency (LF) bands (0.04-0.15Hz) and high frequency (HF) bands (0.15-0.4Hz) which are modulated by the sympathetic system (when expressed in normalised units (nu)) and parasympathetic system respectively. The very low frequency spectrum (VLF) is also revealed during the PSA and its physiological significance is thought to be related to thermoregulation and peripheral vasomotor systems. The total HRV represents all cyclical components of HRV during the testing period. Cardiac autonomic function can be also be studied by time domain parameters over a 24-hour period, which give rise to various parameters like standard deviation (SD) of normal to normal R-R intervals (SDNN) and SD of all 5-minute mean normal to normal R-R intervals (SDANN).

Cacciatori et al. studied cardiac autonomic functions in 7 overtly hypothyroid patients (mean serum TSH 55.5 mIU/L and Free T4 3.1 pmol/L) (Cacciatori et al., 2000). All patients had Hashimoto's thyroid disease (HD). PSA was carried out over a 10-minute period during rest and subsequently standing, deep breathing and Valsalva manoeuvre. In patients with HD, the high frequency component was low during rest and standing when compared to healthy controls, resulting in a high LF/HF ratio in patients with HD. The low frequency component in overt hypothyroid patients during rest was high during the standing position only. These changes were reversed after treatment with levothyroxine for 12-18 months, suggesting an enhanced sympathetic influence on the autonomic cardiovascular system, which was thought to be due to a secondary adaptation to an altered cardiovascular responsiveness.

In another study, PSA analysis was undertaken in 31 overtly hypothyroid patients (all due to HD, mean serum TSH 56 mIU/L and low free T4) over a 24-hour period (Galetta et al., 2008). The LF/HF ratio, which is a measure of sympatho-vagal balance, was significantly higher ($p < 0.05$) in hypothyroid patients. All these parameters (LF, HF and LF/HF ratio) improved significantly after treatment with levothyroxine for 6 months.

This study looked at PSA over a 24-hour period, unlike previous studies by Cacciatori et al. where they performed resting PSA for 10 minutes in a controlled environment. Both of the above studies included patients with severe hypothyroidism due to HD, but duration of disease was not specified. These studies have shown higher sympathetic function in hypothyroidism (raised LF/HF ratio), although absolute measurements in HF and LF were not consistent between the two studies, which might have contributed to differences in abnormal parameters of autonomic function. In the second study, the changes were reversible even with 6 months of treatment, which suggests that this period might be adequate to reverse autonomic functional abnormalities in hypothyroidism.

Unlike patients with HD, who are likely to have long-term hypothyroidism, Guasti et al. studied the effects of short-term overt hypothyroidism using the PSA method over a 5-minute period (Guasti et al., 2007). They performed the study in 42 patients with thyroid cancer after thyroid hormone withdrawal (mean serum TSH 87.4 mIU/L and low free T4) and after suppressive dose levothyroxine treatment. The LF/HF ratio was lower largely due to high HF in the hypothyroid state, and it improved after levothyroxine treatment. Heemstra et al. studied 11 post-thyroidectomy patients with short-term overt hypothyroidism demonstrating a low LF/HF ratio when compared to the post-treatment state, suggesting sympathetic withdrawal (Heemstra et al.). However, this study did not report any comparison of PSA analysis between healthy controls and the treatment group at baseline before thyroxine treatment. A significant improvement after levothyroxine treatment in LF/HF ratio suggests a reversible abnormality in autonomic function in short-term overt hypothyroidism.

Xing et al. studied 38 overt hypothyroid patients with mixed aetiology using 24-hour PSA analysis (Xing et al., 2001). They showed higher vagal tone demonstrated by raised HF in patients when compared to healthy controls, which improved significantly after treatment with levothyroxine. However, the LF was not significantly low in the

patients and did not alter after levothyroxine treatment. The duration of hypothyroidism after thyroidectomy and radioiodine treatment was not reported. It is likely these patients had short-term overt hypothyroidism following thyroidectomy and radioiodine treatment.

Some of these studies reported higher vagal tone in hypothyroid patients with short-term overt hypothyroidism (Guasti et al., 2007, Heemstra et al., Xing et al., 2001) while others describe increased sympathetic activity in patients with overt hypothyroidism (Cacciatori et al., 2000, Galetta et al., 2008). All these studies showed abnormalities in autonomic function in overt short-term and long-term hypothyroidism. However, the results were not consistent in that short-term overt hypothyroidism revealed higher parasympathetic tone and long-term overt hypothyroidism showed increased sympathetic activation. It is possible that short-term overt hypothyroidism induces impaired sympathetic function, resulting in relatively high parasympathetic tone. In long-term overt hypothyroidism, the compensatory mechanisms to increase sympathetic activation come into action and predominate over parasympathetic function. Differences in measuring PSA, age, and severity of hypothyroidism may also account for variation in autonomic dysfunction seen in all of these studies.

PSA was studied over a 24-hour period in patients with SCH by Galetta et al. (Galetta et al., 2006). They studied 42 patients with SCH (mean serum TSH 9.8 ± 1.7 mIU/L and normal free T4). Only patients with elevated serum TSH for 3 or more months were included in the study, which showed that even in patients with SCH, HF is low with a relative increase in the LF/HF ratio, i.e. sympatho-vagal imbalance. The LF was the same as in healthy controls. Moreover, serum TSH was positively correlated with LF/HF ratio ($r=0.42$, $p=0.006$). After 6 months of levothyroxine treatment, both HF and LF/HF ratio improved significantly. This implies a possible causal link between these changes and thyroid status. This study is inconsistent with the previous studies of overt hypothyroidism described earlier in this chapter. Sahin et al. did PSA analysis using a 24-hour method in 31 patients with SCH (mean serum TSH 10.55 mIU/L and normal free T4). The LF and HF showed no difference between patients and healthy controls. However, time domain parameters SDNN and SDANN showed a reduction in a subgroup of patients with serum TSH >10.0 mIU/L. The duration of SCH was not reported in this study, unlike in the previous study by Galetta et al. The functional

abnormalities in hypothyroidism partly depend on the duration of the disease. It may be that patients in the study did not have the disease long enough to reveal detailed functional abnormalities in the ANS.

In summary, non-invasive assessments of cardiac autonomic functions have been performed in several studies involving patients with varying degrees of hypothyroidism. They all consistently showed autonomic dysfunction in overt and subclinical hypothyroidism. There were inconsistencies in specific abnormalities of autonomic parameters between different studies. This could be due to differences in a variety of factors like age, methods of measurement of PSA, aetiology and severity of hypothyroidism. All studies revealed that autonomic dysfunction improved considerably after 6-12 months of thyroxine treatment. This suggests a possible causal link between autonomic dysfunction and hypothyroidism. These non-invasive clinical studies are in agreement with *in vitro* studies discussed earlier in the chapter, suggesting autonomic dysfunction is prevalent in hypothyroidism.

2.2.5 Functional abnormalities in the ANS and fatigue

Reversible autonomic dysfunctions are clearly evident in SCH. These abnormalities could contribute to clinical symptoms like fatigue. The studies discussed were not designed to correlate their findings with patient symptoms like fatigue; however, fatigue has been shown to be associated with autonomic dysfunction in other disease states where it is a prominent symptom. Our collaborator group studied patients with primary biliary cirrhosis (PBC) with the above methods to correlate fatigue and autonomic disturbances (Newton et al., 2007, Newton et al., 2006). It was shown that fatigue was correlated with reduced heart rate variability and abnormalities in other traditional autonomic tests like the Valsalva manoeuvre in patients with PBC. Fatigued patients with PBC (Fatigue Impact Scale score >80) had lower HRV than non-fatigued (FIS score <28) PBC patients. It was also found that baroreflex sensitivity (BRS), which is another marker of autonomic integrity, was abnormal in patients with PBC and this correlated with fatigue.

In summary, AD has been well researched previously in overt hypothyroidism and SCH using non-invasive methods. However, studies looking for a link between fatigue and SCH have not been performed before. Therefore we propose to investigate whether there is any link between fatigue and autonomic dysfunction in SCH. This might explain the mechanism of fatigue in these patients.

2.3 Fatigue due to cardiac dysfunction

2.3.1 Cardiac energetic physiology

The contractile unit of the cardiac myocyte is actin and myosin, similar to skeletal muscle, as described previously. Unlike skeletal muscle, it is almost entirely reliant on aerobic energy metabolism via oxidative phosphorylation. Cardiac myofibrils are highly enriched with abundant mitochondria which enables continuous energy supply by the creatine-kinase energy shuttle mechanism. Heart muscle is one of the biggest consumers of energy per tissue by weight within the human body. As explained previously, with skeletal muscle physiology, phosphocreatine (PCr) acts as an energy transporter and energy reserve molecule in cardiac energy metabolism (Holloway et al., 2011). ATP generated are used for cardiac myofibrillar contraction as well as calcium reuptake by the sarcoplasmic reticulum Calcium-ATPase pump (Holloway et al., 2011).

2.3.2 Control of myocardial energetics by thyroid hormones

Thyroid hormone affects cardiac function in many ways. At a molecular level, it positively regulates gene encoding for α-myosin heavy chain and beta-adrenergic receptors via genomic actions (Klein and Ojamaa, 2001). The role of thyroid hormones in cardiac mitochondrial functions has been studied in the recent past. These studies have shown that thyroid hormones stimulate cardiac mitochondrial biogenesis, increasing myocardial mitochondrial mass, mitochondrial respiration, oxidative phosphorylation, enzyme activities, mitochondrial protein synthesis cytochrome, phospholipid, and mitochondrial DNA content (Marin-Garcia). It is possible that thyroid hormone deficiency leads to impaired cardiac bioenergetic function, which might partly contribute towards abnormal cardiac haemodynamic functions.

2.3.3 Myocardial energetics as measured by ^{31}P-MRS

The cardiac PCr/ATP ratio, as measured by a ^{31}P-MRS technique, has been shown to be a good indicator of myocardial bioenergetic function, which in turn is a measure of mitochondrial activity (Radda, 1986). The PCr/ATP ratio reflects myocardial energy reserve (Hudsmith and Neubauer, 2009). It is low in various myocardial diseases, including coronary heart disease, valvular heart disease, cardiomyopathies and heart failure (Hudsmith and Neubauer, 2009). Even in subclinical stages of diseases affecting cardiac mitochondrial functions, it is found to be abnormal. For example, a study in Frederickson's cardiomyopathy showed a low cardiac PCR/ATP ratio in patients with normal left ventricular function (Lodi et al., 2001). In young patients with type 1 diabetes, with no history of coronary heart disease, the cardiac PCr/ATP ratio was found to be lower than in matched healthy controls (1.90 +/- 0.4 vs. 2.15 +/- 0.3, $p < 0.05$) (Metzler et al., 2002). These studies clearly show that cardiac PCr/ATP ratio as measured by ^{31}P-MRS is a reliable, sensitive and early indicator of various myocardial diseases.

The functional and clinical significance of cardiac PCr/ATP has been studied in the past. For example, it correlated with left ventricular ejection fraction in patients with dilated cardiomyopathy (Neubauer et al., 1995). It was found to be a better predictor of cardiac mortality in patients with dilated cardiomyopathy than the New York Heart Association functional class or left ventricular ejection fraction (Neubauer et al., 1997). However, Cardiac PCr/ATP ratio is not yet used in routine clinical practice because of the technical challenges involved in adapting the cardiac MRS techniques for routine clinical practice.

2.3.4 Cardiac dysfunction in SCH

Thyroid hormones have a significant effect on cardiovascular function. It is well known that patients with overt hypothyroidism have reduced cardiac output because of reduced stroke volume and heart rate (Klein and Ojamaa, 2001). Unlike patients with overt hypothyroidism, patients with SCH do not show overt cardiovascular disease or its symptoms.

Although SCH does not cause overt cardiovascular changes, mild disturbances in cardiac function have been described in previous studies involving patients with SCH,

using highly sensitive cardiac imaging studies. For example, a study using radionuclide angiography involving patients with SCH showed a reduction in the left ventricular ejection fraction during exercise, when compared to healthy controls (Forfar et al., 1985). Two other studies using Pulsed Wave Tissue Doppler Imaging showed right and left ventricular dysfunction in patients with SCH (Kosar et al., 2005) (Turhan et al., 2006). These changes improved with levothyroxine treatment.

It is possible that cardiac mitochondrial function is affected by mild thyroid deficiency in SCH. Hence, assessing PCr/ATP ratio may be a good non-invasive method to detect early changes in myocardial energetics in patients with SCH. This could possibly explain the mechanism of impaired cardiac function in SCH. No previous studies have been undertaken linking abnormal cardiac function in SCH and fatigue. Thus, we propose measuring the PCr/ATP ratio in patients with SCH using ^{31}P-MRS before and after levothyroxine treatment, and correlating these abnormalities to fatigue.

2.4 Cerebral dysfunction in SCH

Cognitive dysfunction has been investigated in SCH by many studies with conflicting results (Bono et al., 2004) (Davis et al., 2003). It is important to look at the physiological and pathological aspects of brain dysfunction in SCH. In this section, I will explore a brief physiology of brain and thyroid hormones, and potential mechanisms whereby abnormal brain physiology might lead to cognitive dysfunction in SCH.

2.4.1 Thyroid hormones and the brain

The foetal and neonatal human brain requires adequate levels of thyroxine for development (Timiras and Nzekwe, 1989). Severe impairment of brain growth can occur in the absence of adequate thyroxine during the critical period of brain development. The adult brain also requires thyroxine for normal functioning and its deficiency leads to various behavioural and cognitive changes (Bauer et al., 2002, Dugbartey, 1998).

The thyroid hormones exert their action via binding to nuclear thyroid responsive elements known as thyroid hormone receptors. Thyroid hormone receptors are present in neurons, oligodendrocytes and astrocytes (Anderson, 2001). The hippocampus and amygdala are rich in T3 receptors, with relatively low levels of T3 receptors present in

brainstem and cerebellum, as shown in animal studies (Ruel et al., 1985). Hippocampal neurogenesis was impaired in adult onset hypothyroidism in rats (Desouza et al., 2005). These parts of the brain have an important role in memory and behavioural functioning in adults. T3 is the key peripheral hormone which exerts its action through thyroid hormone receptors. As much as 50% of T3 in brain tissue is derived from T4 via local T3 production by type II deiodinase (Larsen, 1988). In hypothyroidism, there is a compensatory rise in local T3 production within the brain tissue (Dratman et al., 1982).

2.4.2 Mechanism of cerebral dysfunction in hypothyroidism

The exact underlying pathophysiology for cognitive and behavioural abnormalities in hypothyroidism is unknown. It was suggested that this may be part of a general hypometabolic state induced by tissue hypothyroidism (Braverman).

There have been several studies looking into cerebral metabolic activity and cerebral blood flow (CBF) in overt hypothyroidism of varying aetiology. In myxoedema, a decrease in CBF by 38% and a 2-fold increase in cerebral vascular resistance have been shown in 8 patients using the nitrous oxide method (Scheinberg et al., 1950). The study also showed a mean 27% decrease in mean cerebral glucose consumption. It was suggested that mental changes in myxoedema are due to decreased oxygen and glucose metabolism. Cerebral bioenergetic metabolism has been investigated using cerebral MRS in ten patients (aged 21-56 years) with severe acute overt hypothyroidism (Smith and Ain, 1995). The study showed decreased phosphocreatine/inorganic-phosphate ratio (PCr/Pi) in the frontal lobe area, which improved significantly after treatment with levothyroxine. This suggests that thyroid hormones control energetic functions in brain tissue similar to that which has been described in skeletal muscle previously. However, the exact functional significance of abnormal bioenergetics was not described and needs further mechanistic studies to see if these changes are related to cognitive or behavioural disturbances in hypothyroidism.

Over the last 10 years, several studies have looked at CBF in hypothyroidism using various methods. Positron emission tomography (PET) scans of the brain in patients with acute short-term severe hypothyroidism (post-thyroidectomy) showed global reduction in CBF and cerebral glucose metabolism (Constant et al., 2001). The CBF was measured when patients were euthyroid and after levothyroxine withdrawal. A

single-photon emission computerized tomography (SPECT) brain scan study was undertaken in patients with newly-diagnosed mild overt hypothyroidism due to autoimmune aetiology (mean age 45.9 years, mean serum TSH 14.6 mIU/L and low free T4) before and after levothyroxine treatment (mean duration 109 ± 44 days, range 63–215 days) (Krausz et al., 2004). The study showed significant reduction in regional CBF in areas affecting various cognitive functions in patients when compared with matched healthy controls. Regional CBF deficits in the right primary cortex may be related to psychomotor slowness found in hypothyroidism. There was no correlation between serum TSH and CBF, but the study involved only 10 patients. However, CBF did not improve with levothyroxine treatment. This may be due to shorter duration of treatment or persistent subtle abnormalities of cognitive function in treated hypothyroid patients (Krausz et al., 2004). Another SPECT study has shown improvement of CBF after restoration of euthyroidism in 56% of patients following a total thyroidectomy (Nagamachi et al., 2004). The affected areas were bilateral posterior parietal lobes and occipital lobes. The remaining patients did not show significant improvement in CBF with levothyroxine treatment in this study. This suggests a variable response in CBF to levothyroxine treatment. This is again consistent with varying responses of cognitive and emotional symptoms to levothyroxine treatment in hypothyroidism (Davis and Tremont, 2007).

In another study of patients with SCH, a functional MR brain study looked at the blood-oxygen level dependent (BOLD) signal changes before and after levothyroxine treatment (Zhu et al., 2006). The n-back working memory tasks (a computer-based executive memory test) were used to induce functional changes in various parts of brain. The study showed that in the pre-treatment patients with SCH the load effect of BOLD response was only found in the bilateral parietal areas and premotor areas. No activation was found in other frontal cortex regions of interest (ROIs) (bilateral middle/inferior frontal gyri, bilateral dorsolateral prefrontal cortex, the supplementary motor area/anterior cingulated cortex) which are relevant areas in working memory in patients with SCH. After 6 months of treatment with levothyroxine, the patients with SCH exhibited the same load effects in all ROIs as the euthyroid subjects along with an improvement of performance in n-back tasks. BOLD signal is a surrogate marker of neuronal activation and subsequent local blood flow changes in cerebral microcirculation (Logothetis, 2008). Hence, it was inferred from the above study that

hypothyroidism affects specific neuronal activity in certain parts of the brain which are relevant to working memory.

This was a preliminary study and no further similar functional MR brain studies have been published to show the reproducibility of these findings in a larger and different cohort of SCH patients. We also need to establish whether CBF is altered in SCH, as previous studies were done in overtly hypothyroid patients. Future studies aimed at correlating fatigue and abnormal cerebral physiologies with improvement after levothyroxine treatment are likely to yield answers as to whether SCH is truly associated with fatigue. This is especially relevant in the serum TSH range 4 to 10 mIU/L because these patients are not routinely treated with levothyroxine as per current recommendations. Also, as previously discussed, younger patients with SCH have shown more association with abnormal cognition than older patients. Older patients are more likely to have co-existing medical conditions, which make the interpretation of abnormal cerebral physiology more challenging. Hence, studies looking at younger age groups are more likely to show abnormal cerebral physiology in initial exploratory studies in SCH. None of the studies have looked at whether CBF is related to fatigue. Abnormal CBF could explain fatigue in SCH.

2.5 Assessment of fatigue

The fatigue in clinical practice and research is commonly assessed by self-administered questionnaires. There are more than 30 types of fatigue-measuring questionnaires being used in clinical and research practice (Dittner et al., 2004). There are no fatigue-specific questionnaires available which are validated to measure fatigue in thyroid disease. Fatigue Impact Scale (FIS) is a widely-used questionnaire in fatigue research (Fisk et al., 1994a, Fisk et al., 1994b). This questionnaire has been used in investigating the mechanisms of fatigue in primary biliary cirrhosis and chronic fatigue syndrome. Therefore, FIS was used in our study to measure fatigue in patients and healthy controls.

2.6 Summary and hypothesis

SCH is a common medical problem affecting millions of people worldwide. It has been associated with fatigue. This is more controversial in patients with serum TSH ranging between 4.0-10.0 mIU/L. Cerebral, cardiac, autonomic and skeletal muscle dysfunction have been studied in SCH, as described previously. These are potential mechanisms which can cause fatigue in SCH. Hence, the following hypothesis was proposed:

Fatigue in SCH is due to functional abnormalities in peripheral tissues which are partly or wholly reversible with levothyroxine treatment.

As described in previous sections, the associations between symptoms and tissue dysfunction have not been studied in SCH. The aim of this research project was to study the cerebral blood flow, cardiac and skeletal muscle bioenergetics, and cardiac autonomics in patients with SCH before and after levothyroxine therapy. Measured parameters would be compared with an objective assessment of fatigue before and after levothyroxine therapy. This will help to explore whether functional abnormalities thus detected explain the mechanisms of physical symptoms in SCH.

Chapter 3 Methodology

3.1 Overall Study design

This was a pilot study investigating the mechanism of fatigue and cognitive dysfunction in patients with subclinical hypothyroidism (SCH). For the purpose of this MD thesis, the data was analysed for only the fatigue aspects of the study. The cognitive dysfunction and functional MR scan data were not included in this thesis. We planned to recruit 20 patients with SCH and their baseline data will be compared against 20 age and gender-matched euthyroid healthy controls. The patients were then treated with levothyroxine for 6 months to look for any improvement from the baseline results. The patients (n=20) were recruited from the secondary care endocrine clinics and various general practices in Gateshead. They were invited for a screening visit where they were assessed for inclusion and exclusion criteria. Those who met the criteria were entered into the study and underwent a functional MR scan of the brain, heart and leg, autonomic function tests and psychometric evaluation. They were then given levothyroxine at a dose of 1.6 mcg/kg once daily. During the subsequent 6 months, they had blood tests for FT4 and TSH every 6 weeks, and the levothyroxine doses were adjusted to keep TSH between 1-1.5 IU/L. At the end of 6 months, the tests were repeated i.e. MR scans, autonomic tests and psychometric evaluation. The patients then exited the study and were advised to discuss with their general practioners the need for continuing the thyroxine treatment. The healthy controls were recruited from the staff members of the research institute. The physical activity levels of both patients and healthy controls were not measured. The healthy controls underwent only baseline MR investigations. They did not undergo autonomic function tests and psychometric evaluation because of a lack of availability of rooms in the laboratory. The autonomic function data from age and gender-matched healthy controls in a previous study by our collaborators was used to compare with our patients cohort. There was no placebo arm in the study because it required a larger study group, which would have been very difficult to find and more funding resources would be required.

3.2 Rationale for the study design

This was a pilot study because a comprehensive research methodology looking into the mechanism of fatigue and cognitive dysfunction has not been undertaken previously in this specific group of patients. The data comparison between baseline pre-treatment

patients and age and gender-matched healthy controls will reveal whether there are abnormal results in patients. The data from patients between pre-treatment and post-treatment tests will be used identify changes related to treatment.

We have chosen to include patients between 18 years and 65 years because older patients might have more co-morbidities, which might be confounding factor underlying fatigue or cognitive dysfunction. Also, it is known that older patients might have raised serum TSH levels due to ageing itself. We selected patients with moderate-severe fatigue (FIS score >40) only, because of the pilot nature of the study. The patients had SCH for > 3 months, thus eliminating patients with transiently elevated TSH due to other illnesses or viral thyroiditis. Serum thyroid peroxidase (TPO) was measured in patients for identification of the aetiology of SCH.

We have excluded patients with co-morbidities that may be contributing factors to their fatigue, such as diabetes mellitus, anaemia, liver disease and renal disease. The patients with hypertension, hypercholestremia, heart disease and stroke disease were excluded because these patients may have reductions in blood flow due the underlying atherosclerosis and might interfere with cardiac spectroscopy and cerebral blood flow measurements. We have excluded patients with major psychiatric disease, which could contribute to abnormalities in cognitive tests. Subjects who have indwelling metals that might interfere with MRI scans were excluded because of safety reasons.

The symptoms of hypothyroidism are partly dependent on the duration of disease, as described in previous chapters. A longer duration of treatment is more likely to demonstrate changes with treatment rather than a shorter period of treatment. Hence, we chose 6 months for treatment duration so that functional changes due to treatment within various tissues studied may become more evident. This will also provide enough time to titrate the dose of levothyroxine to a target of serum TSH 1-1.5 mIU/L. The previous study by Razvi et al. showed that fatigue was significantly improved in the levothyroxine treated group (Razvi et al., 2007). The mean serum TSH in this group was 0.5 mIU/L. 10% of patients were over-replaced in that study. The serum TSH target of 1-1.5 mIU/L was chosen in our study because this might give the best chance of showing any functional changes in various tissues studied without exposing the patients to risk over-replacement. We chose to use a full replacement dose of thyroxine (1.6 mcg/kg per day) as recommended by several experts in the past for treating younger

SCH patients without heart disease. The dose titration of levothyroxine was done based on the results of thyroid function tests. If the serum TSH was out of target, then the levothyroxine dose was adjusted and re-checked at the next visit.

3.3 Patient recruitment

The patients with known SCH were identified in secondary care endocrine clinics and approached if they had raised TSH between 4.0 and 10.0 mIU/L, with normal FT4 on two occasions or more for at least 3 months apart, and had fatigue as one of their symptoms. In a primary care setting, after seeking permission from primary care physicians, the General Practice Database was searched for patients with SCH using the above biochemical criteria and whether fatigue was mentioned in the GP records. Any patients with major co-morbidities, such as vascular disease, diabetes mellitus and epilepsy, and those on drugs interfering with thyroid function tests, were excluded at this stage (pre-screening). The patient information letters were sent out via post to these pre-screened patients, and a reply to the research team was requested if they were interested in participating. Those who expressed an interest to participate in the study were invited to attend the screening visit after 12 hours of overnight fasting.

3.3.1 ***Screening Visit*** (Fasting)

Written informed consent was obtained after addressing any questions and explaining about the study in detail. Subjects were screened for inclusion and exclusion criteria.

3.3.2 Inclusion criteria:

1. Age 18 to 65 years

2. Subjects with confirmed SCH: Serum TSH between 4.1 and 10.0 mIU/L and normal FT4 for more than 3 months.

3. Fatigue Impact Scale > 40. Please refer to page 31 for a detailed explanation.

3.3.3 Exclusion Criteria

1. Subjects with previous thyroid disease or currently on thyroid hormone replacement, anti-thyroid drugs, amiodarone, Lithium, oral corticosteroids, hypotensive agents, aspirin, statins or ACE inhibitors/angiotensin receptor blockers.

2. Subjects with known diabetes mellitus/impaired glucose tolerance/impaired fasting glycaemia.

3. Known renal failure or a serum creatinine > 120 umol/l within the past 3 months.

4. Previous participation in a clinical trial within the past month.

5. Previous history of vascular disease (history and ECG).

6. Malignancy (any).

7. Active infections.

8. Major psychiatric disease (by history and Hospital Anxiety Depression score).

9. Drug abuse.

10. Previous major head injuries/epilepsy.

11. Pacemakers/cerebral aneurysm clips.

12. Pregnancy.

13. BMI >35 kg/m^2

Blood tests were taken for the following measurements: FT4, FT3, TSH, anti-thyroid peroxidise antibody, glucose, cholesterol profile, liver function tests, urea & electrolytes, bone profile and full blood count. During treatment phase, serum TSH and FT4 was measured at 6-weekly intervals. These were random (not fasting) samples and not timed in relation to the time of ingestion of levothyroxine tablets. The serum free T3 level was not measured during the treatment phase as the effect of levothyroxine treatment on serum free T3 level is unpredictable and its clinical significance is unknown (Jonklaas et al., 2014).

Medical history and clinical examination – cardiac auscultation to exclude any patients with likely structural heart disease, and height, weight and blood pressure were also measured.

Questionnaires

Fatigue impact Scale – those who scored less than 40 were excluded; this was to exclude patients without significant fatigue (Appendix A). This questionnaire evaluated overall impact of fatigue in a subject (Fisk et al., 1994b). This has not been validated for thyroid disease, but has been used for various diseases where fatigue is a clinical manifestation (Dittner et al., 2004). The FIS score was undertaken at the beginning of study in patients and healthy controls, and at the end of the study in patients.

Hospital Anxiety and Depression score: to exclude patients with significant active depression (Appendix B). Depression can cause fatigue, and SCH has been associated with low mood in previous studies (Samuels).

12-lead ECG: to exclude patients with ischaemic heart disease.

N-back Test: this is a computer-based working memory test. Subjects are shown series of alphabets on the computer screen. The task is to spot the repeats of the same alphabets in a particular order. When the same alphabet is repeated immediately afterwards, it is called 1-back; when the same alphabet is repeated 2 letters later, then it is 2-back; and when the same alphabet is repeated 3 letters later, it is 3-back.The subjects undergo this test during functional MR of the brain to activate the brain, and resulting BOLD signals are captured and analyzed. During the screening visit, the subjects undergo 2 practice sessions of 8 minutes each and a third practice session will be done immediately before the MR scan. This was done for analysis in conjunction with functional MR brain results. This was not analysed or described in this thesis.

The patients who met the inclusion criteria and did not have any exclusion criteria were entered into the study.

3.4 Biochemical Investigations:-

All screening blood samples were collected after 12 hours (from 9pm to 9am, water allowed) of overnight fasting. The samples were immediately analysed in the local clinical laboratory. The TSH, free T4 and free T3 were measured by Roche Cobas e601

and have coefficient of variation below 5%. The reference ranges for serum TSH, FT4 and FT3 were: 0.4 – 4.0 mIU/L, 9 – 25 pmol/L and 2.5 – 7.5 pmol/L, respectively.

3.5 Ethical approval:-

The North Tyneside Research Ethics Committee 2 and Gateshead Research and Development Committee approved the project.

3.6 Statistics:-

Power calculations were made using a 2-sample t-test to produce 80% power with 95% significance. From our collaborator's experience of variability in healthy subjects and patients in PBC and CFS, we estimate that by studying 20 subjects we can expect to detect an 8% difference in CBF, a 14% difference in the PCr/ATP in cardiac spectroscopy, and a 13% change in oxidative metabolism ($\tau_{1/2}$ PCr) of skeletal muscle. Depending upon the data distribution, paired Students t-tests or Mann Whitney U tests were used for comparison between before and after thyroxine treatment. Unpaired Student test or equivalent non-parametric tests were used to compare data between patients and healthy controls at baseline. Pearson analysis was undertaken for measuring association between 2 variables. The statistical analysis was performed using SPSS (version: 17).

3.7 Study visits

Summary of the study visits after the screening visit are given in Table-3

Visit	Setting	Preparation	Tests/intervention
Visit 1	Newcastle Magnetic Resonance Centre.	Nil	MR scans Heart, leg and brain.
Visit 2	Falls and Syncope Service, Royal Victoria Infirmary.	Avoid smoking, strenuous exercise and caffeinated drinks for 4 hours prior to tests	Autonomic Function Tests.
Visit 3	Bensham Research Centre	Nil	Neuropsychometric Assessment. Start thyroxine treatment.
Visit 4 6 weeks	Bensham Research Centre	Advised to bring any remaining tablets for compliance check.	Clinical assessment. Blood tests for free T4 and TSH. Thyroxine dose titration
Visit 5 12 weeks	Bensham Research Centre	Advised to bring any remaining tablets for compliance check.	Clinical assessment. Blood tests for free T4 and TSH. Thyroxine dose titration *TSH not on target-Visit 5a. TSH on target-Visit 6*
Visit 5a 18 weeks *(only if TSH not on target)*	Bensham Research centre	Advised to bring any remaining tablets for compliance check.	Clinical assessment. Blood tests for free T4 And TSH. Thyroxine dose titration.
Visit 6 24 weeks	Bensham Research centre	Advised to bring any remaining tablets for compliance check.	Neuropsychometric Assessment. Blood test for freeT4 and TSH.
Visit 7 24 weeks	Falls and Syncope Service, Royal Victoria Infirmary.	Avoid smoking, strenuous exercise and caffeinated drinks for 4 hours prior to tests	Autonomic Function Tests.
Visit 8 24 weeks	Newcastle Magnetic Resonance Centre.	Nil	MR scans Heart, leg and brain.

Visit 1

Newcastle Magnetic Resonance Centre, Newcastle General Hospital.

Preparation- Nil.

MRI screening questionnaire: Patients were asked to fill out a questionnaire to establish whether magnetic resonance scanning was contraindicated due to metallic components in their bodies and/or claustrophobia.

N-back practice: Third session is done prior to scans.

Scans were done in the following order.

1. MR 31P cardiac spectroscopy: Patients lie down on the scanner table in a prone position for 40 minutes.

2. MR 31Phosphurus Calf muscle spectroscopy: During rest, exercise and recovery after exercise (Section 4.4.2 and 4.4.3 for details of the protocol).

3. Resting functional scan of the brain, and BOLD response to n-back test.

Visit 2

Falls and Syncope service, Royal Victoria Infirmary, Newcastle upon Tyne.

Preparation – Patients were advised to refrain from smoking, caffeinated drinks and strenuous exercise (all of which can affect heart rate) for 4 hours prior to the visit. All patients had the tests at the same time of the day in a warm, quiet room.

Autonomic function tests – Heart rate and blood pressure were measured continuously for the duration of the test using surface ECG electrodes and phasic blood pressure, using digital photoplethysmography ('Portapres', Amsterdam, the Netherlands). The built-in software programme (TASKFORCE) calculated heart rate variability and baroreflex sensitivity during rest, and each of the manoeuvres is given below. Cardiac impedance electrodes were also applied to measure various cardiac indices during rest and different manoeuvres.

Rest - for 10 minutes.

Active standing for 2 minutes.

Valsalva manoeuvre.

Head-up tilt testing - 40 minutes.

Visit 3

Neuropsychometric assessment, Bensham Research Centre, Gateshead.

1. Preparation: Nil.

2. Setting: Single session lasting 90 minutes (with a 10-minute break after 60 minutes) was done in a warm, quiet and well-lit room. The session was supervised by myself or a research nurse for the study in the same order and methods of administration as below.

Wechsler Abbreviated Scale of Intelligence (WASI) – vocabulary, block design, word similarities and matrix reasoning.

Wechsler Test of Adult Reading (WTAR).

The Controlled Oral Word Association Test.

Wechsler Memory Scale-III abbreviated (WMS) - verbal story (immediate and delayed recall), family pictures (immediate and delayed recall), symbol search and digit span. Trail-Making.

3. Patients were started on levothyroxine at 1.6mcg/Kg body weight once daily after the above tests. They were advised to take thyroxine on an empty stomach before breakfast and were given 6 weeks of appointments for measuring TFTs and dose adjustment (if required).

Visit 4

Bensham Research Centre, Gateshead

1. Clinical assessment.

2. Compliance check.

3. Blood test - for FT4 and TSH.

4. Target TSH 1.0-1.5 IU/L and normal FT4.

5. If required, levothyroxine dose adjusted to keep TSH on target.

6. Next appointment in 6 weeks.

Visit 5

Bensham Research Centre, Gateshead.

1. Clinical assessment.

2. Compliance check.

3. Blood test - for FT4 and TSH.

4. Target TSH 1.0-1.5 IU/L and normal FT4.

5. If required, levothyroxine dose adjusted to keep TSH on target.

6. Next appointment in 12 weeks (Visit 6) if TSH on target.

If outside target, next appointment in 6 weeks (Visit 5a).

Visit 5a

Bensham Research Centre, Gateshead.

1. Clinical assessment.

2. Compliance check.

3. Blood test - for FT4 and TSH.

4. Target TSH 1.0-1.5 IU/L and normal FT4.

5. Levothyroxine dose adjusted to keep TSH on target.

6. Next appointment in 6 weeks (Visit 6).

Visit 6

Newcastle Magnetic Resonance Centre, Newcastle General Hospital.

Preparation - Nil.

MRI screening questionnaire: Patients were asked to fill out a questionnaire to establish whether magnetic resonance scanning was contraindicated due to metallic components in their body and/or claustrophobia.

N-back practice: Practice session prior to brain scan.

Scans were done in the following order:

1. MR 31P cardiac spectroscopy: Patients lie down on the scanner table in prone position for 40 minutes.

2. MR Calf muscle spectroscopy: During rest, exercise and recovery after exercise.

3. Functional scan of the brain and BOLD response to n-back test.

Visit 7

Falls and Syncope service, Royal Victoria Infirmary, Newcastle upon Tyne.

Preparation - Patients were advised to refrain from smoking, caffeinated drinks and strenuous exercise for 4 hours prior to the visit.

Autonomic function tests: Heart rate and blood pressure were measured continuously for the duration of the test using surface ECG electrodes and phasic blood pressure, using digital photoplethysmography ('Portapres', Amsterdam, the Netherlands). The built-in software programme (TASKFORCE) calculated heart rate variability and baroreflex sensitivity during rest, and each of the manoeuvres is given below. Cardiac impedance electrodes were also applied to measure various cardiac indices during rest and different manoeuvres.

Rest - for 10 minutes.

Active standing for 2 minutes

Valsalva manoeuvre - 2 attempts.

Tilt testing - 40 minutes.

Visit 8

Neuropsychometric assessment and blood tests for FT4 and TSH, Bensham Research Centre, Gateshead.

1. Preparation: Nil.

2. Setting: Single session lasting 90 minutes (with a 10-minute break after 60 minutes) was done in a warm, quiet and well-lit room. The session was supervised by myself or a research nurse for the study in the same order and method of administration.

Wechsler Abbreviated Scale of Intelligence (WASI) – vocabulary, block design, word similarities and matrix reasoning.

Wechsler Test of Adult Reading (WTAR).

The Controlled Oral Word Association Test.

Wechsler Memory Scale-III abbreviated (WMS) - verbal story (immediate and delayed recall), family pictures (immediate and delayed recall), symbol search, digit span, and Trail-Making. These were tests for various aspects of cognitive function. The data from cognitive studies are not being analysed or discussed in this thesis.

3. Blood tests for levothyroxine FT4 and TSH.

4. Patients exit from the study. Their general practioner was informed about the exit from the study and patients were advised to discuss with their doctor about continuing thyroxine in the long-term.

Chapter 4 Calf Muscle MR spectroscopy

4.1 Hypothesis:

Fatigue in SCH is (partly or wholly) due to abnormal energy metabolism in peripheral skeletal muscle and is reversible with levothyroxine treatment.

4.2 Primary Objectives:

To measure non-invasively the following energy metabolic variables using ^{31}P-MR dynamic spectroscopy and compare them with age and gender-matched euthyroid healthy controls:

(i) The baseline and end exercise metabolite concentrations

(ii) Maximal mitochondrial oxidative function inferred from phosphocreatine recovery from an exercise bout ($PCr_{t1/2}$). This was the key primary end point.

(iii) The time taken for muscle pH to normalise after cessation of exercise

(iv) The rate of removal of acid (the proton efflux) from the muscles in the post-exercise period.

4.3 Secondary objectives:

To examine the metabolic measurements before and after 6 months of levothyroxine treatment.

4.4 Use of Magnetic Resonance Spectroscopy:

Muscle energy metabolism can be studied non-invasively by using phosphorus-31 magnetic resonance spectroscopy (^{31}P-MRS). ^{31}P-MRS records signals from high-energy phosphate-rich compounds, which are central to muscle energy metabolism (Mattei et al., 2004). The signals are generated when a magnetic field is applied to phosphorus nuclei resulting in alteration of its spin properties. The signal intensity is proportional to the concentration of corresponding molecules. The spectrum typically consists of 7 signals corresponding to 7 metabolites i.e. Pi, PCr, γ-ATP, α-ATP, β-ATP, phosphomonoesters (PME) and phosphodiesters (PDEs) (Mattei et al., 2004). The concentration of adenosine diphosphate (ADP) at rest (10μM) produce insufficient

signals to measure directly, but can be indirectly estimated using the chemical equation based on the creatine-kinase equilibrium:

$$PCr + ADP + H^{+} \leftrightarrow Cr + ATP$$

Muscle pH can also be estimated indirectly using the relative chemical shift of Pi since the chemical shift of Pi is pH-dependent (Moon and Richards, 1973). Mitochondrial oxidative function is assessed by the rate of ATP synthesis during exercise. PCr recovery rate during recovery after exercise is a measure of mitochondrial capacity, because it reflects the rate of mitochondrial ATP production (Heerschap et al., 1999).

4.4.1 Advantages and disadvantages of ^{31}P-MRS-

Unlike *in vitro* studies, MRS is non-invasive and muscle metabolism can be studied under physiological conditions during varying degrees of exercise. It is important to mention that ^{31}P-MRS provides measurements of phosphate compounds at the tissue scale and not at a specific cellular or subcellular level (Heerschap et al., 1999). Only free and unbound phosphate compounds are measured using ^{31}P-MRS, unlike biopsy or freeze-clamping studies where the total cellular level of a compound is measured. But, ATP levels measured with *in vitro* methods and ^{31}P-MRS methods showed equivalent values, which suggests that the ATP pool is largely free and fully MR visible in muscle tissue (Gadian, 1995). PCr within muscle cells is also largely unbound and fully MR visible, but an *in vitro* study showed lower levels than *in vivo* studies because of rapid breakdown of PCr mediated by creatine-kinase reaction during the freeze-clamping procedure (Meyer et al., 1982).

4.4.2 Resting ^{31}P magnetic resonance spectroscopy

All MR scans for this study were undertaken using a Philips 3T Achieva scanner system. The part of the left calf with the greatest circumference was imaged using T1-weighted scans. With the patient lying supine, with their left calf at magnet isocentre, MRS data were acquired using a 14cm diameter ^{31}P surface coil for transmission/reception of signals, and the in-built body coil for anatomical imaging.

4.4.3 Exercise ^{31}P magnetic resonance spectroscopy

A purpose-built exercise apparatus has been designed to operate within the MRI scanner (Figure 4.1). The subjects lay down on the scanner table and were able to undertake

controlled plantar flexion exercise using this apparatus. The restraining straps helped to avoid the employment of other muscle groups (e.g. quadriceps). The exercise session involved two exercise bouts with the following sequence: rest, 3 minutes of exercise (plantar flexion at 0.5 Hz and 25% of Maximum Voluntary Contraction (MVC), which was quantified prior to spectroscopy), then 6 minutes' rest. The second exercise bout consisted of 3 minutes of the second exercise (plantar flexion at 0.5 Hz and 35% of MVC) and 6 minutes of rest, which allows the assessment of recovery from exercise. As lower intensity of exercise was used in the first exercise, which does not cause large decreases in pH, it permits the direct measurement of muscle oxidative metabolism. During the second exercise, a higher intensity exercise depresses pH and this allows the assessment of anaerobic metabolism and pH.

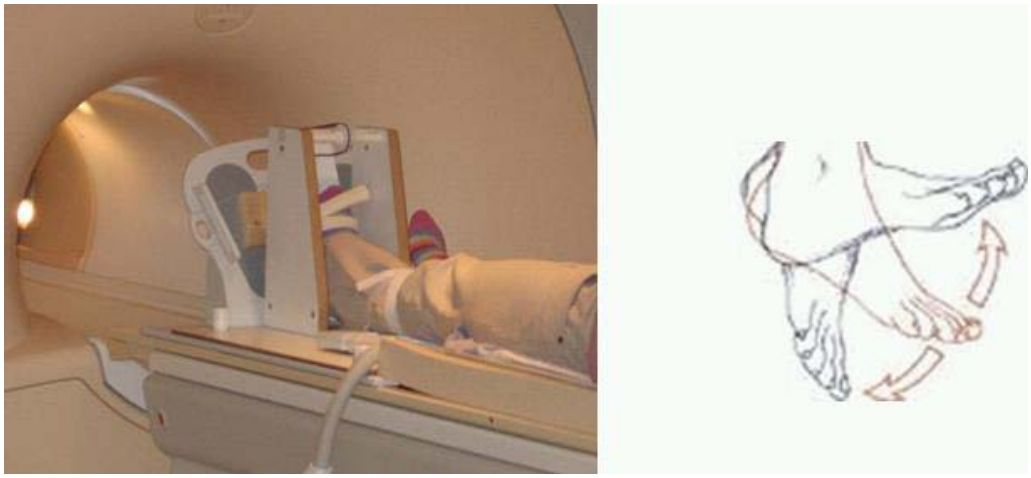

Figure 4-1: The apparatus used to permit exercise (plantar flexion) within the MR scanner (left) and the 0°-30° of plantar flexion involved (right).

4.4.4 Muscle metabolism during rest, exercise and recovery as observed by ^{31}P-MRS.

The various measurements made using ^{31}P-MRS muscle spectroscopy are shown in Table-4.1

Table 4.1: Metabolic parameters measured during rest and exercise with ^{31}P-MR Spectroscopy.

Leg exercise protocol stage	**Measurement**	**Unit**
Rest – before first exercise.	pH	
	PCr concentration	mM
	Pi concentration	mM
	ADP at rest	mM
During the first exercise (25%MVC)		
End of the first exercise	End Exercise ADP	μM
	PCr below basal	μM
	%PCr drop	
During recovery from the first exercise	V (PCr resynthesis)	mM/Min
	Qmax	mM/Min
	PCr $_{t1/2}$	Seconds
	ADP $_{t1/2}$	Seconds
Before the second exercise (35% MVC)	pH (resting)	
The end of the second exercise	End exercise pH	
During recovery from the second exercise	Minimum pH	
	pH recovery time	Seconds
	Initial Proton Efflux	
	Max Proton Efflux	

4.4.5 Interpretation of ^{31}P magnetic resonance spectroscopy:

A Java-based resonance interface (jMRUI version 2.0) was used to undertake the analysis of averaged (resting) and individual time series spectra (Vanhamme et al., 1999). As described by Kemp et al., at rest, an ATP level of 8.2 mM was assumed (Kemp et al., 1997). A non-linear least squares algorithm (AMARES) was used to quantify phosphocreatine (PCr), inorganic phosphate and pH. The chemical shift between PCr and Pi was used to assess pH (Kemp and Radda, 1994). To assess pH the following equation was used:

$pH = pkA+10 \log ([\Delta 1- \Delta 0]/[\Delta 0- \Delta 2])$

pkA = 6.75, Δ0=chemical shift (in ppm) between PCr and Pi, Δ1=3.27ppm, Δ2=5.63ppm.

The resting pH, end-exercise pH, and minimum pH following second-exercise pH, were determined. The end-exercise pH reflects the anaerobic glycolytic activity during an intense exercise. The pH recovery time was calculated by measuring the time from the cessation of exercise until pH returned to within 0.01 units of its pre-exercise value. This indicates the efficiency of proton efflux following exercise.

The PCr and pH values were utilised to calculate ADP levels (ATP value of 8.2 mM and PCr value of 42.5 mM was assumed) using the creatine-kinase equilibrium (Kemp and Radda, 1994, Kemp et al., 1997). The ADP concentration was estimated using the following equation:

$[ADP] = [ATP] [Cr] / [PCr] [H^+] K_{eq}$

K_{eq}= Creatine-kinase equilibrium constant =1.7 x 10^6 L.mM, [TCr] =Total creatine concentration =42.5mM, [ATP] = adenosine triphosphate concentration = 8.2mM.

The equation used for the calculation of the PCr re-synthesis following exercise is:

$V=\Delta[PCr]/\Delta t$

T=time

The percentage of PCr depletion at the end of the first exercise was calculated from the reduction of PCr level in the first exercise from the resting PCr. The measured initial

rate of [PCr] resynthesis (i.e. at cessation of exercise), V_{init}, and [ADP] concentration at end-exercise can be used to estimate the maximum possible oxidative ATP synthesis rate with unlimited ADP concentration. Assuming a hyperbolic relationship between ADP concentration and oxidative metabolism rate, the maximum possible oxidation rate, Q_{max} was estimated by:

$Q_{max} = V_{init}. (1 + (K_m/[ADP]_{end\text{-}exercise}))$ where $K_m = 90$ μM. K_m represents the concentration of ADP at which $V = Q_{max}/2$.

The Q_{max} measures the oxidative capacity of the muscle mitochondrial pool (Prompers et al., 2006). The rate of net proton efflux (E) from the acquired volume of muscle was estimated for every point after the cessation of exercise from the pH and the rate of phosphocreatine recovery. The rate at which protons are generated by net PCr resynthesis and an estimate of the titration of protons by cytosolic buffering, is taken into account (Kemp et al., 1993, Kemp and Radda, 1994).The rate of proton efflux was estimated from:

$E = \gamma V + \beta_T d(pH)/dt$

where β_T is the cytosolic buffer capacity, assumed to be 20 slykes (Kemp et al., 2001) and $-\gamma$ is a pH dependent empirical factor relating the rate of change of phosphocreatine to the free cytosolic protons produced, and is given by:

$\gamma = 1/(1+10^{(pH-6.75)}$

Mono-exponential fits to the recovery data were used to estimate the half-times for recovery to equilibrium of ADP ($\tau_{1/2}$ ADP) and PCr ($\tau_{1/2}$PCr). The $\tau_{1/2}$PCr is a surrogate measure of mitochondrial phosphorylation during the recovery from exercise. The proton efflux was calculated for every time point after cessation of exercise, and we noted the time point at which efflux reached its maximum, which should be the time point of cessation of exercise for healthy controls (Kemp et al., 1997). The proton efflux helps to recover cytosolic pH after exercise by pumping out hydrogen ions generated during exercise. Abnormalities in proton efflux have been associated with conditions such as chronic fatigue syndrome, and suggested as potential mechanism of fatigue in this condition (Jones et al., 2010) .

4.5 Results

After undergoing the screening process and taking informed consent as described in chapter 3, 25 patients and 20 healthy controls were recruited. The data from one patient had insufficient signal-to-noise to process. Another patient did not complete the study due to personal reasons after autonomic studies. Hence, 23 subjects in the patient group had a successful baseline MR spectroscopy assessment (see Appendix C for details). The baseline demographic data is shown in Table 4.2 for both patients and healthy controls. It shows that the 2 groups were comparable in terms of mean age, gender distribution, blood pressure and cholesterol. Patients had significantly higher BMI and fasting blood glucose than healthy controls. As expected, patients had significantly higher serum TSH (not normally distributed, hence given as median and IQR) and lower FT4 than healthy controls. Serum FT3 was also higher in patients than in healthy controls, although it was within the normal reference range in both groups. The TPO was positive in 13 out of 23 patients with SCH and only 1 out of 20 healthy controls (56% vs 5%). Both TPO positive and negative patients were comparable in their baseline data (data not shown). The mean HAD score was 9.6 (±5.2) for anxiety and 7.4 ((±4.1) for depression. m^2

Characteristic	SCH n=23 Mean(±SD)	HC n=20 Mean(±SD)	P value
Age (years)	41.6 (±12.4)	42.1 (±12.5)	0.888
No. of women (% of n)	19 (83)	17 (85)	0.832
BMI (kg/m^2)	29.0 (±6.0)	24.7 (±4.4)	0.012
Blood pressure (mmHg)	122/75 (±17/10)	121/77 (±19/11)	0.690
Blood glucose (mmol/L)	5.0 (±0.4)	4.7 (±0.5)	0.016
Serum Total Cholesterol (mmol/L)	5.6 (±1.0)	5.3 (±0.8)	0.447
Serum TSH (IU/L)	5.9 (IQR 5.25-8.2)*	2.1 (IQR 1.25-2.6) *	<0.001
Serum FT4 (pmol/L)	13.4 (±1.5)	14.7 (±1.4)	0.010
Serum FT3 (pmol/L)	5.1 (±0.7)	4.5 (±0.7)	0.035
FIS score	76.9 (±28.1)	4.3 (±5.0)	<0.001

Table 4.2: The baseline demographic data for patients and healthy controls are shown.

***The serum TSH is expressed as median (IQR-interquartile range)**

The obesity has been associated with raised TSH in previous studies (Pearce, 2012). Since the BMI was higher in SCH group when compared to HC group, it is possible that it can lead to high serum TSH in SCH group. The figure 4.2 shows that there was no correlation between serum TSH and BMI in the SCH group (r^2 =0.077, p=0.154). However, there was a positive correlation between serum TSH and BMI in the HC group (r^2 =0.247, p=0.007) as shown in Figure 4.3.

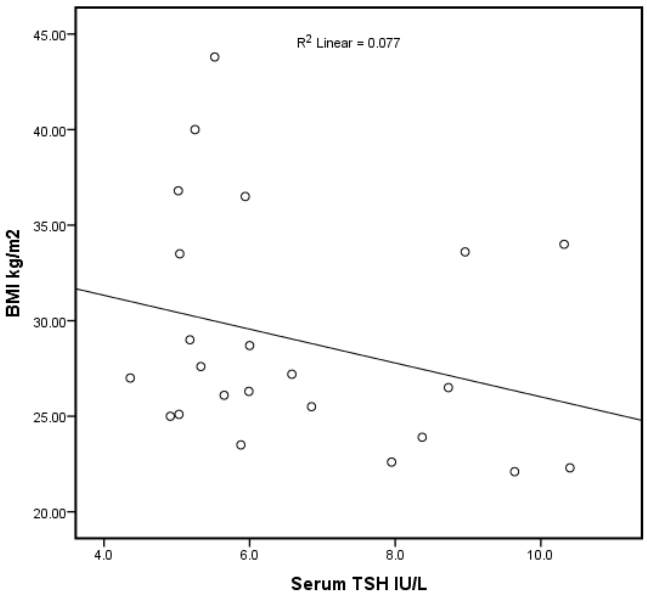

Figure 4-2: This shows the correlation between serum TSH and BMI in SCH group (p=0.154)

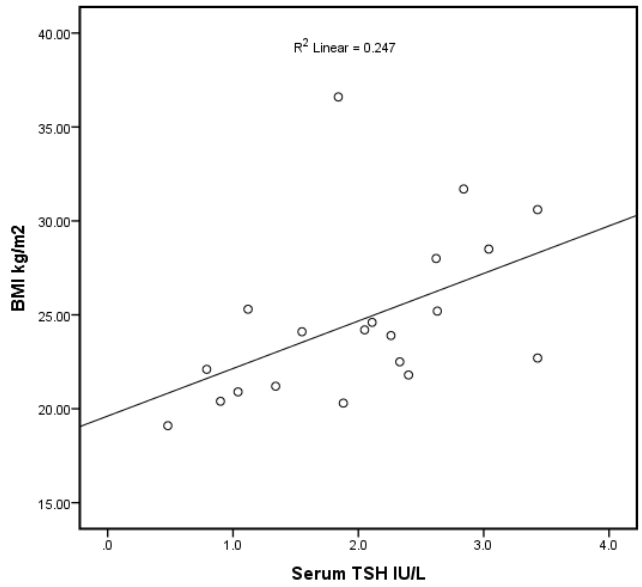

Figure 4-3: This shows the correlation between serum TSH and BMI in HC group (p=0.007)

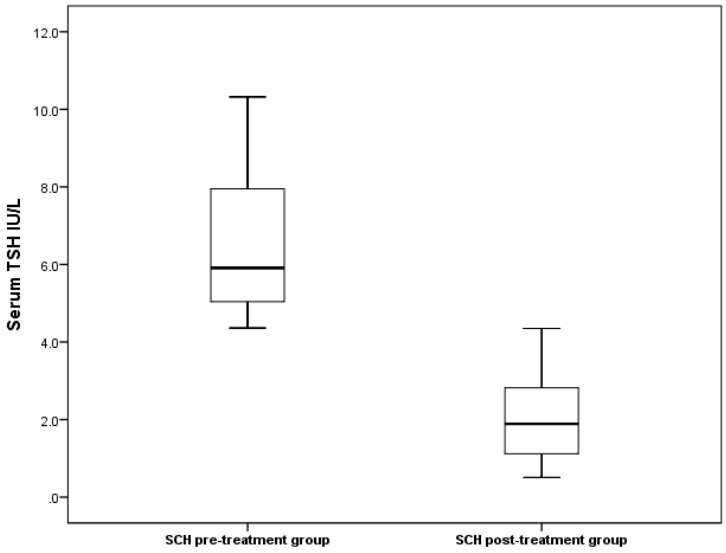

Figure 4-4: This shows the serum TSH distribution in pre- and post-treatment groups (n=18).

Three patients did not complete the treatment phase of the study (2 patients were unable to tolerate MR scans and 1 patient moved out of the area); a further 2 patients were poorly-compliant with levothyroxine treatment (had serum TSH outside normal range) and their data were not analysed. Hence, pre- and post-treatment data for 18 patients is presented. After treatment with levothyroxine (mean dose 105.3 ± 26.1 mcg/day) for 6 months, the mean serum TSH (note: normally distributed in post-treatment group), serum FT4 levels and FIS score were 2.1(±1.1) mIU/L, 19.1 (±2.3) pmol/L and 30.8 (±35.4) respectively. The distribution of serum TSH in pre- and post-treatment SCH groups is shown in Figure 4-4.

4.5.1 Resting calf muscle metabolism

The resting muscle spectroscopic data (Table 4.3) shows that there were no significant differences between patients and healthy controls except in resting PDE concentrations. The mean resting PDE was significantly higher in SCH group than in healthy controls ($p=0.002$) and this did not correlate with serum TSH, FT4, FT3 or FIS scores (all $p > 0.05$). Table 4.4 shows that there were no changes in any of the resting energy metabolites with levothyroxine treatment.

	SCH n=23 (mean±sd)	HC n=20 (mean±sd)	P value
Pi (mM)	3.9 (±0.8)	3.8 (±0.8)	0.686
PCr (mM)	33.9 (±3.8)	34.4 (±2.3)	0.669
ADP (μM)	9.7 (±0.6)	9.9 (±0.6)	0.486
pH	7.06 (±0.03)	7.06 (±0.02)	0.810
PDE (mM)	3.4 (±1.1)	2.4 (±0.8)	0.002

Table 4.3. The resting muscle spectroscopic data are shown for baseline patients and healthy controls. P value given for unpaired t-test

	SCH Pre-treatment N=18 (mean±sd)	SCH Post-treatment N=18 (mean±sd)	P value
Pi (mM)	3.8(±0.6)	3.7(±0.7)	0.881
PCr (mM)	33.8 (±4.2)	32.6 (±2.4)	0.239
ADP (μM)	9.7 (±0.4)	9.8 (±0.5)	0.390
pH	7.06 (±0.02)	7.06 (±0.02)	0.925
PDE (mM)	3.4 (±1.3)	3.1 (±1.3)	0.179

Table 4.4: The resting spectroscopic data are shown for patients before and after 6 months of levothyroxine treatment. P value give for paired t-test

4.5.2 Calf muscle metabolism during first exercise and recovery

Table 4.5 shows the muscle spectroscopic data during the first exercise and recovery for the baseline SCH group and healthy controls. The mean ADP concentration was significantly lower at the end of first exercise in patients when compared to healthy controls (p=0.037). The PCr concentration below basal level and the percentage drop in PCr at the end of first exercise was lower in patients than in healthy controls (p=0.036 and 0.029 respectively). The $\tau_{1/2}$PCr was the primary endpoint in the muscle spectroscopic data. The $\tau_{1/2}$PCr was similar between the 2 groups (p=0.976). The initial rate of PCr resynthesis (V) was lower in patients than in healthy controls (p=0.025). The

Pi concentrations were similar between the 2 groups at the end of the first exercise. In both patients and healthy controls, the abnormal metabolic parameters did not reveal any significant correlation with serum TSH, FT4, FT3 or FIS scores (all p >0.05). The TPO positive and negative patients showed no significant differences between them in any of the metabolic parameters measured in the muscle spectroscopy. The muscle spectroscopic data did not demonstrate any changes with levothyroxine treatment (Table 4.6).

	SCH n=23 (mean±sd)	HC n=20 (mean±sd)	P value
ADP conc at end of 1st exercise (μM)	24.4 (±10.2)	33.1 (±16.1)	0.037
PCr conc below basal at end of 1st exercise (mM)	6.2 (±3.2)	9.2 (±5.8)	0.036
% PCr drop at end of 1st exercise	18.1 (±8.4)	26.9 (±16.5)	0.029
V (initial PCr resynthesis) (mM/min)	6.7 (±3.6)	10.2 (±6.2)	0.025
Qmax (linear model) (mM/min)	42.3 (±10.7)	49.1 (±22.6)	0.209
$\tau_{1/2}$ PCr (sec)	35.2 (±8.4)	35.3 (±13.8)	0.976
$\tau_{1/2}$ ADP (sec)	27.3 (±6.8)	25.6 (±9.1)	0.476
Pi at end of 1st exercise (mM)	9.3 (±2.7)	10.5 (±5.0)	0.323
Pi Max during 1st exercise (mM)	10.8 (±3.3)	13.0 (±5.2)	0.111

Table 4.5: Muscle spectroscopic data during first exercise and recovery for baseline patients and healthy controls.

	SCH Pre-treatment n=18 (mean±sd)	SCH Post-treatment n=18 (mean±sd)	P value
ADP conc at end of 1st exercise (μM)	24.4 (±11.2)	22.1 (±6.5)	0.377
PCr conc below basal at end of 1st exercise (mM)	6.6 (±3.4)	5.8 (±2.3)	0.460
% PCr drop at end of 1st exercise	18.8% (±9.0%)	18.0% (±7.0%)	0.715
V (initial PCr resynthesis) (mM/min)	6.7 (±3.7)	8.2 (±5.2)	0.349
Qmax (linear model) (mM/min)	41.8(±10.1)	38.4 (±10.6)	0.112
$\tau_{1/2}$ PCr (sec)	35.6 (±8.0)	36.5 (±10.5)	0.516
$\tau_{1/2}$ ADP (sec)	26.7 (±5.3)	28.8 (±7.8)	0.105
Pi at end of 1st exercise (mM)	8.6 (±1.9)	8.6 (±2.5)	0.953
Pi Max during 1st exercise (mM)	10.1 (±2.9)	9.6 (±2.8)	0.627

Table 4.6 : Muscle spectroscopic data during first exercise and recovery for patients before and after levothyroxine treatment.

4.5.3 Calf muscle metabolism during second exercise and recovery

Table 4.7 shows the muscle spectroscopic data during the second exercise and recovery for both baseline patients and healthy controls. It reveals that the ADP concentration was significantly lower in patients (p=0.021) than in healthy controls, but pH changes were similar between the 2 groups. The initial proton efflux was similar between the 2 groups; however the maximum proton efflux was significantly lower in patients when compared to healthy controls (p=0.014). Interestingly, both maximum and end-exercise Pi concentrations were significantly lower in patients than in healthy controls (p=0.007 and 0.020 respectively).

	SCH N=23 (means±sd)	HC N=20 (means±sd)	P value
ADP conc at end of 2nd exercise (μM)	29.7 (±9.0)	42.9(±24.4)	0.021
pH at start of 2nd exercise	7.03 (±0.03)	7.04(±0.03)	0.685
pH at end of 2nd exercise	7.06 (±0.06)	7.05 (±0.05)	0.529
pH minimum during entire 2nd exercise and recovery	6.98 (±0.11)	6.97 (±0.10)	0.787
pH recovery time to within 0.01 of starting pH (sec)	119.6 (±111.7)	150.0 (±128.0)	0.410
Maximum proton efflux (2nd exercise) (mM/minute)	1.8 (±1.1)	3.7 (±3.4)	0.014
Initial proton efflux (2nd exercise) (mM/minute)	1.5(±1.1)	2.3(±2.0)	0.124
Pi at end of 2nd exercise (mM)	9.6 (±1.6)	12.3(±5.0)	0.020
Maximum Pi during 2nd exercise (mM)	10.4(±1.9)	13.7(±5.3)	0.007

Table 4.7: Muscle spectroscopic data during 2nd exercise and recovery at baseline for patients and healthy controls

However, the levothyroxine treatment did not modify any of these abnormal energetic parameters (all p>0.05) (Table 4.8). Abnormal metabolic parameters did not correlate with FIS, FT4, FT3 or TSH in patients and in healthy controls at baseline (all p>0.05).

	SCH Pre-treatment n=18 (mean±sd)	SCH Pre-treatment n=18 (mean±sd)	P value
pH at start of 2nd exercise	7.03 (±0.03)	7.04(±0.02)	0.295
pH at end of 2nd exercise	7.07(±0.03)	7.07(±0.02)	0.688
pH minimum during entire 2nd exercise and recovery	7.00(±0.04)	7.01(±0.02)	0.253
pH recovery time to within 0.01 of starting pH	127.8 (±115.3)	119.4 (±92.7)	0.835
Maximum efflux (2nd exercise)	1.8(±1.2)	1.6(±1.0)	0.634
Initial efflux (2nd exercise)	1.5(±1.2)	1.4 (±1.0)	0.823
Pi at end of 2nd exercise	9.4 (±1.6)	8.8 (±2.0)	0.245
Maximum Pi during 2nd exercise	10.2 (±1.9)	10.0 (±2.1)	0.649

Table 4.8: Muscle spectroscopic data during 2nd exercise and recovery for patients before and after levothyroxine treatment.

Correlation analysis between maximum proton efflux and minimum pH during entire second exercise and recovery showed an inverse relationship in HC (r^2= 0.598, p<0.001) (Figure 4.5), but not in patients (r^2=0.006, p=0.733) at baseline when all cases, including outliers, were included (Figure 4.6). However, there was significant correlation in patients (Figure 4.7) when 2 outliers were excluded in the correlation analysis (r^2=0.312, p=0.009).

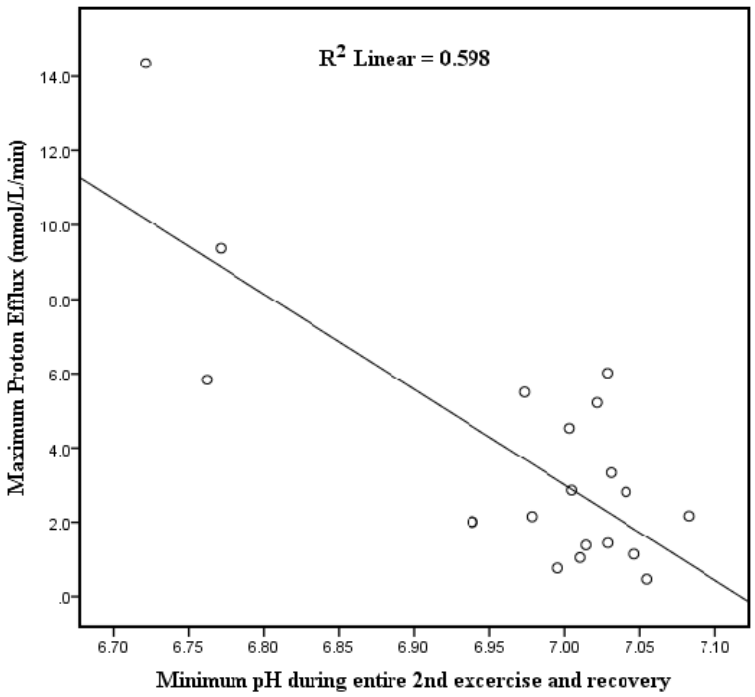

Figure 4-5: Correlation between maximum proton efflux and minimum pH during entire 2nd exercise and recovery in healthy controls ($p<0.001$).

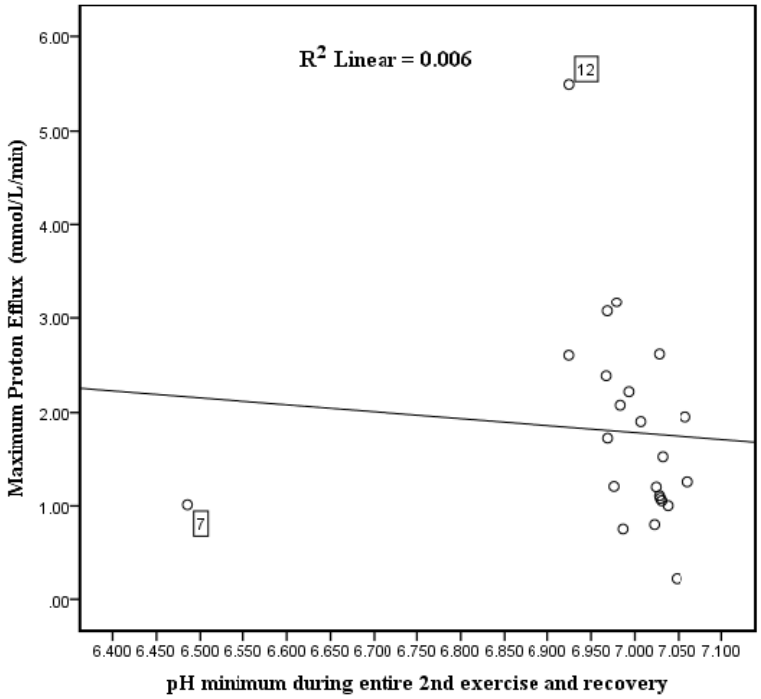

Figure 4-6: Correlation between maximum proton efflux and minimum pH during entire 2nd exercise and recovery in all patients at baseline ($p=0.733$). The outliers are labelled as cases 7 and 12.

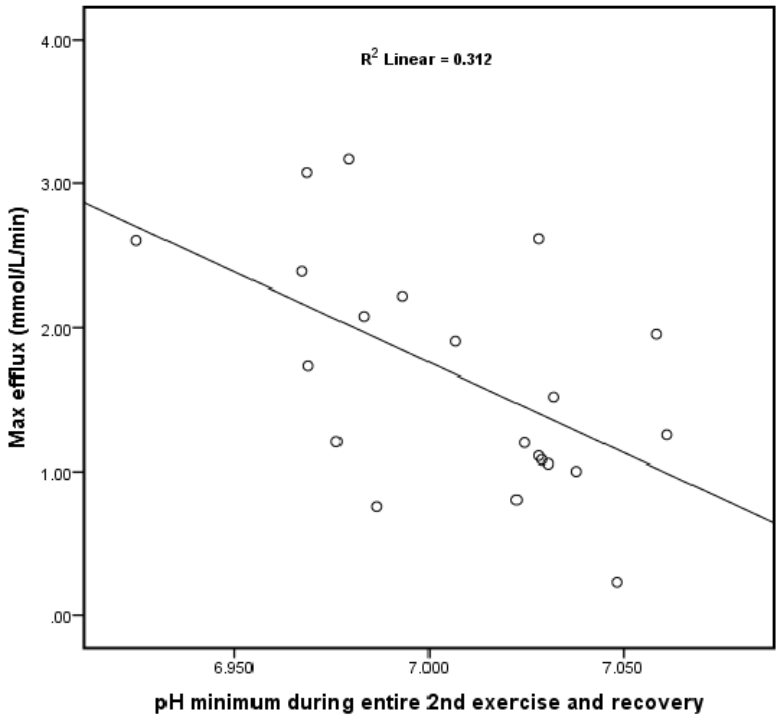

Figure 4-7 : Correlation between maximum proton efflux and minimum pH during entire 2nd exercise and recovery in patients after excluding the outliers (cases 7 and 12 on Figure 4-3)) at baseline (p=0.009).

Similarly, a strong positive relationship between maximum proton efflux and end-exercise ADP following second exercise was seen in HC (r^2 =0.686, p<0.001) (Figure 4.8) and not in patients at baseline (r^2 =0.026, p=0.461) (Figure 4.9). However, there was significant correlation in patients (Figure 4.10) when 1 outlier was excluded in the correlation analysis (r^2=0.347, p=0.005).

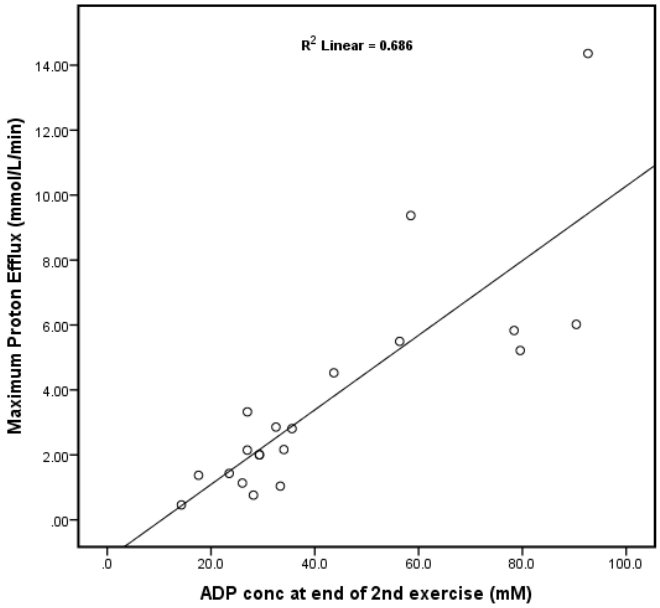

Figure 4-8: Correlation between maximum proton efflux and end-exercise ADP concentration following second exercise in healthy controls ($p<0.001$).

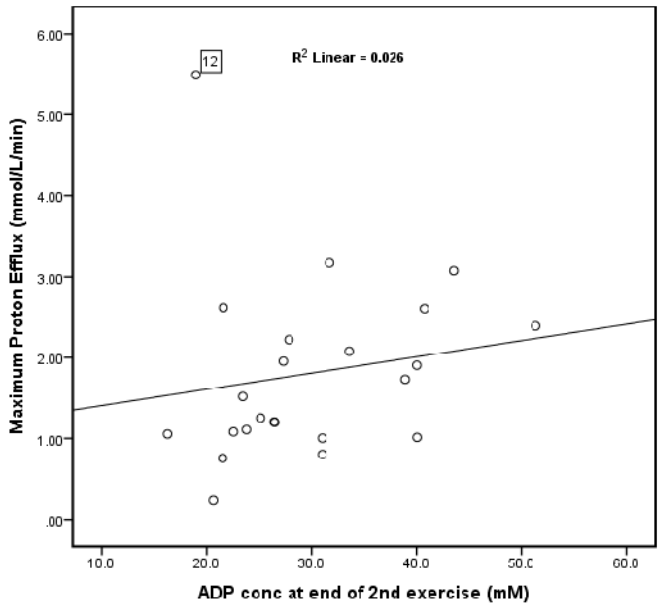

Figure 4-9: Correlation between maximum proton efflux and end-exercise ADP concentration following second exercise in all patients at baseline ($p=0.461$). The outlier labelled as case 12.

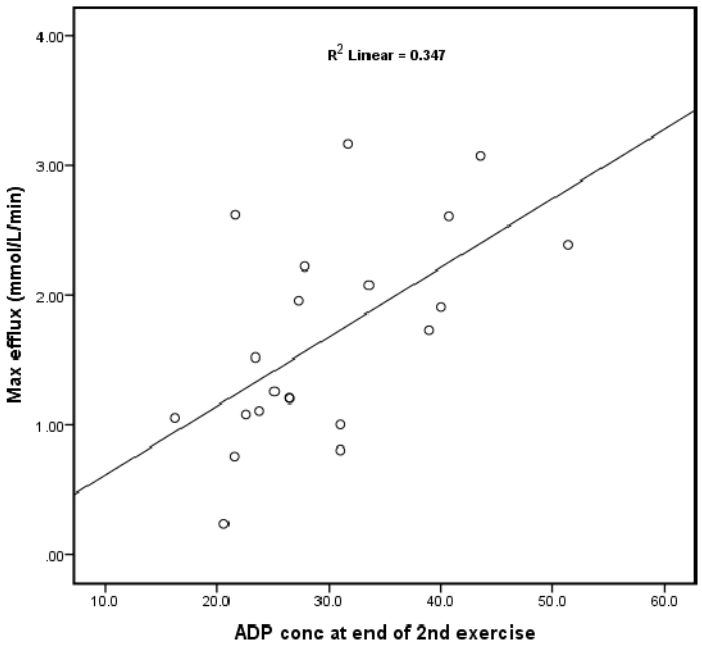

Figure 4-10 : Correlation between maximum proton efflux and end-exercise ADP concentration following 2nd exercise in patients after excluding the outlier (case 12 in Figure 4-9) at baseline (p=0.005).

4.6 Discussion

4.6.1 General discussion

To my knowledge, this is the first time that MR spectroscopic data has revealed impairments of skeletal muscle energy metabolism and proton handling in patients with SCH. These abnormalities were evident when participants underwent both the first and second exercise protocols, but not under resting conditions. However, there was no improvement in energy metabolism with 6 months of levothyroxine treatment in patients with SCH. The impaired metabolic abnormalities in patients did not correlate with thyroid function tests or FIS score. Hence, these metabolic changes do not explain the fatigue observed in patients with SCH.

The resting muscle energetic data did not demonstrate any significant abnormalities, apart from PDE concentrations being higher in patients at baseline when compared to healthy controls. A recent study in patients with SCH showed high PDE concentrations

and Pi levels in calf muscle using MR spectroscopy (Rana et al., 2012). MR spectroscopic studies on patients with overt hypothyroidism have also revealed resting high PDEs, Pi and low PCr (Khushu et al., Kaminsky et al., 1992b). Therefore, the high PDE levels in calf muscle seen in our study are consistent with previous similar studies of varying degrees of hypothyroidism. However, the normal levels of Pi seen in our study are not consistent with data by Rana et al. in patients with SCH. The precise reason for this result is unclear, but it is possibly due to the very mild nature of the disease in our cohort.

Glycerophosphorylcholine is a type of cell membrane phospholipid which contributes to PDE signals when measured by MR spectroscopy (Cerdan et al., 1986). A study by Burt et al. has shown that glycerophosphorylcholine levels were increased during fast-to-slow twitch muscle transformation via electric muscle stimulation in rabbits (Burt et al., 1982). In hypothyroidism, there is a change in muscle fibre composition from white type II (fast) to red type I (slow) fibres (Nwoye et al., 1982, Khaleeli et al., 1983). Hence, the muscle fibre transition has been proposed as a mechanism for high PDE levels seen in hypothyroidism.

During the first exercise, there is less depletion of PCr in patients than in healthy controls. During recovery from the first exercise, the PCr resynthesis (V) rate was significantly low ($p < 0.05$). However, the $\tau_{1/2}$ PCr and $\tau_{1/2}$ ADP were normal in patients. The skeletal muscle oxidative capacity during exercise recovery was normal in patients with SCH in a previous study (Rana et al., 2012). Our study findings are in accordance with those of the dynamic muscle exercise protocol by Kaminky et al. in patients with overt hypothyroidism, but they did not measure the oxidative capacity of skeletal muscle (Kaminsky et al., 1992b). However, the % depletion of PCr was similar between patients and healthy controls in that study, in contrast to our study findings of low percentage PCr depletion in patients with SCH. A low percentage PCr depletion might have led to a low PCr recovery rate (V_{init}) in our study.

The mechanism of abnormal muscle energetic metabolism in hypothyroidism has been well described previously. The human muscle mitochondria have T3 receptors (Sterling et al., 1978). Thyroid hormones directly affect mitochondrial protein expression by regulating the transcription of mitochondrial genes (Enriquez et al., 1999). A review in 2010 revealed multiple mechanisms whereby thyroid hormones influenced skeletal

muscle mitochondria (Lanza and Sreekumaran Nair, 2010). The thyroid hormones increase mitochondrial expression, mitochondrial volume density, mitochondrial enzyme activity and increased oxidative ATP synthesis. A review of specific effects of hypothyroidism on skeletal muscle function reveals a global inhibition of main oxidative pathways and of respiratory chain, which leads to clinical manifestations like muscle weakness, easy fatigability and muscle cramps. (Kaminsky et al., 1992a).

During the second exercise, there was reduced maximum proton efflux in patients when compared to healthy controls. The initial proton efflux was normal in patients. This indicates that the proton efflux started off normally in patients, but was subsequently impaired due to some unknown factors. In chronic fatigue syndrome, decreased maximum proton efflux has been reported previously and suggested as a potential mechanism of fatigue in chronic fatigue syndrome (Jones et al., 2010). However, FIS score was not correlated to maximum proton efflux in my study. Hence, abnormal proton efflux is unlikely to explain the mechanism of fatigue in SCH. The muscle pH during and recovery from exercise was normal in patients. Studies in overt hypothyroid patients have shown lower muscle pH towards the end of exercise (Khushu et al., Kaminsky et al., 1992b). The pH recovery following exercise was slow in overt hypothyroidism in another study (Taylor et al., 1992). Our findings are in contrast to these studies showing abnormal pH during exercise. But these were overtly hypothyroid patients, as opposed to SCH state, in our patients. No previous studies have reported proton efflux during exercise recovery in hypothyroidism. The study by Rana et al. did not mention any pH changes during resting state or exercise in patients with SCH (Rana et al., 2012). So, for the first time, my study has shown abnormal proton handling in SCH.

The correlation between maximum proton efflux and nadir pH during second exercise recovery did reveal a tight physiological correlation in both patients and healthy controls. The correlation data also reveals that the ADP is a stimulator of proton efflux in healthy controls and patients. These findings would suggest that the proton efflux is not influenced abnormally by intracellular nadir pH (i.e. acid production) or ADP levels in patients. There could be other factors which affect proton handling. Decreased muscle blood flow and inadequate Na^+/K^+ ATPase pump functioning can affect proton handling (Taylor et al., 1992). Autonomic dysfunction is well documented in SCH, as

discussed in the previous chapters. Disturbances in peripheral autonomic function can result in impaired muscle blood flow and this might lead to impaired proton removal, as proposed by Jones et.al (Jones et al., 2010). Thyroid hormones modulate the synthesis of Na^+/K^+ ATPase and hypothyroidism leads to reversible reduction in the number of ouabin binding sites in human skeletal muscle (Kjeldsen et al., 1984). This is another potential mechanism by which proton handling is affected in SCH.

The energetic metabolic and proton handling abnormalities did not change following levothyroxine treatment in our patients. Argov et al. reported improvement in resting PCr/Pi ratio and PCr recovery rate following treatment in 2 hypothyroid patients (after 4-6 weeks) and 8 male rats (Argov et al., 1988). Khushu et al. reported improvement in resting PCr/Pi ratio in 7 out of 9 patients after 12 weeks of levothyroxine treatment. However, the results of 2 patients and statistical significance were not given in the paper. Also, they did not report changes to PCr recovery rate and oxidative capacity following levothyroxine treatment. So, there is very little data in the literature of human studies to compare our treatment outcomes with previous studies.

Lack of response to levothyroxine in high energy phosphate metabolism and proton handling may be due to a number of factors. It may be due to a shorter duration (6 months) of levothyroxine treatment, although the study design precludes from drawing any definite conclusions. This might be also due to lack of stability of thyroid function during the 6 months of levothyroxine treatment. The study protocol involved continuation of the dose titration of levothyroxine during each visit if they did not achieve euthyroid status. Both studies previously mentioned have shown improvement in muscle energetic parameters within 3 months of levothyroxine treatment ((Argov et al., 1988, Khushu et al.). However, another study has shown that muscle energy substrates did not respond to one year of levothyroxine treatment in patients with SCH (Caraccio et al., 2005). Hence, the effects of a longer duration of levothyroxine treatment on skeletal muscle energy metabolism are unknown. It is obvious from the data that the patients were adequately replaced with levothyroxine because the mean serum TSH was well within the normal range. In addition, thyroid function tests did not correlate with any of the energetic metabolic abnormalities. Lack of correlation may be explained by a smaller sample size or lack of true association of abnormal metabolites and thyroid status. It is possible that metabolic changes observed in our study may not

be due to hypothyroidism and other factors may have influenced the energetic metabolic outcomes. The patients and healthy controls were well matched in terms of age, gender, blood pressure and cholesterol. Although the serum glucose was very slightly high in patients, it was well within the normal range. This may be due to higher BMI. It is unlikely that slightly higher glucose levels or higher BMI would have resulted in energetic metabolic changes in the muscle. It has been also shown that higher serum TSH in the SCH group was not due to higher BMI as there was no correlation between serum TSH and BMI in this group of patients. The positive correlation in HC group is an expected finding and is consistent with previous studies looking at the relationship between serum TSH and body weight (Pearce, 2012)

Despite lower FT4 levels, patients had slightly higher serum FT3 when compared to healthy controls. High BMI has been associated with higher FT3 (although within normal range) when compared to non-obese individuals (Pearce, 2012). In summary, apart from the duration of treatment, none of the other factors would have influenced significant muscle energetic abnormalities in patients with SCH in our study.

Patients are significantly fatigued compared to healthy controls. Fatigue can lead to exercise deconditioning and to impaired muscle metabolism. Chronic fatigue syndrome has been associated with abnormal proton handling, but causation was not proven (Jones et al.). It is possible that fatigue in our patients would have resulted in impaired skeletal muscle metabolism. However, FIS score did not correlate with abnormal metabolic parameters at baseline (both in patients and healthy controls) and improvement in fatigue with levothyroxine treatment did not lead to improvement in abnormal metabolic parameters. This would suggest a lack of effect of fatigue on skeletal muscle metabolism. Demonstrating this would require studying patients with SCH without fatigue and assessing their energy metabolism.

4.6.2 Strengths and limitations

The main strength of the study is that we selected patients with no interfering co-morbidities which might lead to fatigue. This helps to minimise the impact of any disease which can affect muscle metabolism. Also, patients with definite SCH were selected by ensuring there was raised serum TSH for 3 months or more prior to recruitment. This will exclude any patients with transient elevations in serum TSH

being labelled as having SCH. A previous muscle MRS study on SCH did not find any major energetic abnormalities, but duration of SCH was not reported in the study (Rana et al., 2012).

This study has important limitations. The small sample size in the study might have affected the results in terms of correlation analysis. There were no previous studies available to make power calculations for this study. We did not measure serum free T3 level during the treatment phase as the effect of levothyroxine treatment on serum free T3 level is unpredictable and the clinical significance is unknown (Jonklaas et al., 2014). The serum T3 was higher in healthy controls than patients at baseline, and it is unknown that the change in serum T3 during the treatment phase may have affected the metabolic parameters measured in the study. The FIS score calculates global impact of fatigue and not specifically for muscle fatigue. Hence, a muscle-specific questionnaire might have shown a significant correlation with muscle metabolic abnormalities. We did not have a group of patients without fatigue. This would have helped us to decide whether SCH without fatigue would have resulted in metabolic abnormalities in skeletal muscle and whether fatigue might have contributed to metabolic abnormalities in patients. The fitness level of healthy controls and patients were not measured in this study. The fitness level might have affected muscle energetic metabolism and I am unable to exclude this interfering factor in this study. The vitamin D status is known to affect skeletal muscle metabolism (Sinha et al., 2013). My study was undertaken throughout the year and the effect of vitamin D on skeletal energy metabolism during each season of the year is unknown in this study. The levothyroxine dose was adjusted during each study visit if the serum TSH levels were outside the target. Hence, this could have affected the measurements of metabolic parameters in the post-treatment group. This is a methodological limitation which need to be addressed in future similar studies.

4.6.3 Future directions

This study shows that the muscle ^{31}P-MRS can be used to study muscle metabolism in SCH. The muscle ^{31}P-MRS is sensitive enough to detect proton handling abnormalities in SCH which can used for measuring therapeutic efficacy in future studies with longer duration of levothyroxine treatment. The abnormal muscle metabolic results from this study may be used to do power calculations for future studies in SCH. Further studies

should be designed to include measurement of muscle fatigue with specific questionnaires which assess muscle function. Also, muscle strength and fitness level should be measured along with muscle metabolic abnormalities in the future studies.

Chapter 5 Cardiac Magnetic Resonance Spectroscopy

5.1 Hypothesis

Fatigue in patients with SCH is in part due to abnormal cardiac energetic function, leading to impaired cardiovascular haemodynamic function, and this is wholly or partly reversible with levothyroxine treatment.

5.2 Primary objectives

To measure cardiac Phosphocreatine/ATP ratio using non-invasive Cardiac Magnetic Resonance Spectroscopy and correlate with FIS score. These results will be compared with age and gender-matched euthyroid controls.

5.3 Secondary objectives

To compare baseline measurements in the aforementioned parameters with those following 6 months of levothyroxine therapy.

5.4 Method

We have already excluded subjects with clinical and electrocardiographic evidence of heart disease due to valvular pathology, hypertension with left ventricular hypertrophy, and ischaemic heart disease at the screening stage.

We used the same methodology as our collaborators in their study of cardiac function in patients with primary biliary cirrhosis using cardiac MR spectroscopy (Jones et al.). This has been described previously by Jones et al. The study used a 3T Intera Achieva scanner (Philips, Best, NL) with a 10cm diameter 31P surface coil (Pulseteq, UK) for signal transmission and reception. The study participants lay down in a prone position and their position was adjusted so that their heart was placed at the isocentre of the magnet. To verify location of the heart, an in-built body coil was used and images were localised. A cardiac-triggered, breath-held field map was carried out to undertake shimming. The liver and skeletal muscle interference was eliminated to avoid contamination of spectra. The first spectrum detected beyond the chest wall was chosen in the study analysis. The jMRUI processing software was employed to quantify the PCr, γ-ATP and 2, 3-DPG using the AMARES time domain routine fit (Vandamme). The blood has high levels of 2,3-DPG and can contaminate the ATP peak area. This contamination was corrected for by 1/6 of the amplitude of the combined 2, 3-DPG

peak (Conway et al., 1998). The T1 values of cardiac PCr and ATP were obtained from the literature and were used for correcting PCr/ATP ratio.

The typical cardiac spectrum from a healthy subject is shown below (Figure 5.1). It shows several peaks; however, in this study, the PCr/ATP ratio was analysed. This is widely studied and found to a marker of cardiac bioenergetic function (Radda, 1986).

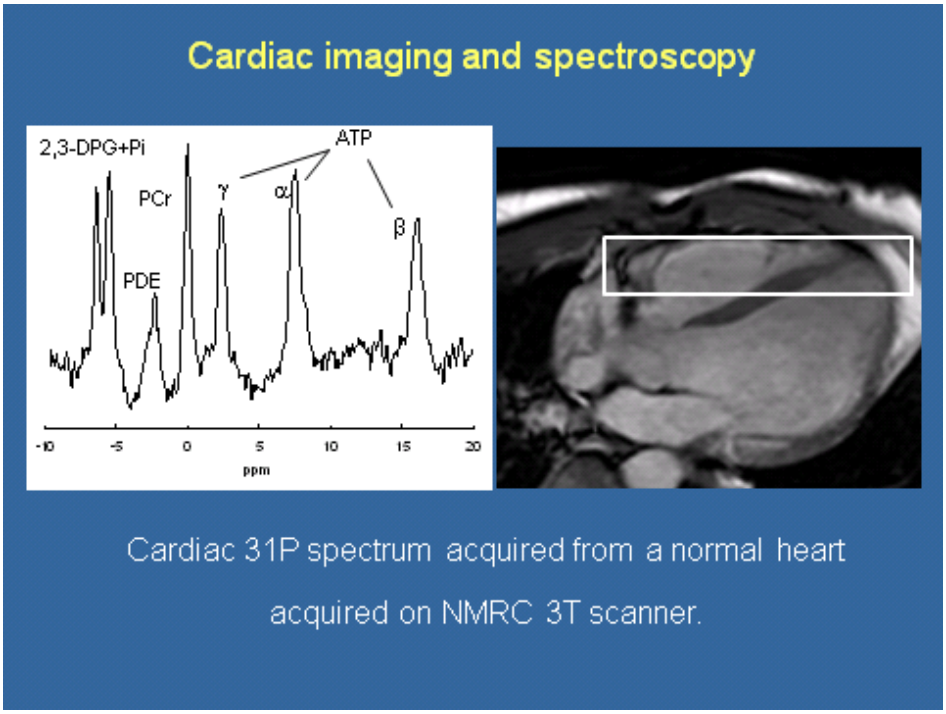

Figure 5-1: Typical cardiac 31P spectrum from a healthy subject.2, 3DPG, 2,3-disphosphoglycerate; PDE, phosphodiesters; PCr, phosphocreatine; ATP, adenosine triphosphate; ppm, parts per million

5.5 Results

Twenty-three patients with SCH had cardiac MR spectroscopy. Out of these, 2 had poor quality spectra and hence images were analysed for 21 patients (see Appendix C for details). Out of these 21 patients, 2 patients subsequently dropped out of the study (due to personal reasons), 1 had poor quality spectra on the post-treatment MRS scan and 2 patients were poorly compliant with levothyroxine. Therefore, data was successfully acquired for 16 patients with adequate levothyroxine replacement (the post-treatment SCH group). Out of 20 healthy controls (HC), 17 had good-quality spectra that were included in the final analysis, and the remaining 3 subjects had poor-quality spectra

which were excluded from the study. Rejection of spectra was based on inadequate signal-to-noise to fit any or all of PCr, the γ resonance of ATP or 2, 3-DPG.

The baseline demographic, clinical and biochemical data of the SCH and HC groups is provided in Table 5.1. At baseline, unlike serum TSH from the muscle spectroscopy cohort (patients and healthy controls, n=43), serum TSH is normally distributed in the cardiac spectroscopy cohort (patients and healthy controls, n=38) (Kolmogorov-Smirnov test, p=0.200). Both groups were well-matched for gender distribution, age, blood pressure and fasting blood cholesterol. The mean BMI was higher in the SCH group ($p<0.01$). The fasting blood glucose was slightly lower in HC than SCH ($p<0.05$). The mean starting and end of study doses of levothyroxine were 103.1(± 25.6) mcg and 102.3 (± 28.5) mcg respectively. The mean serum TSH and free T4 levels at the end of the study were 2.0 ±1.0 mIU/L and 19.0 ±2.4 pmol/L respectively. The serum TSH distribution in pre-and post-treatment groups is shown in Figure-5.2. The serum TSH was not normally distributed in the pre-treatment group (Kolmogorov-Smirnov test, p=0.016), but was normally distributed in the post-treatment group (Kolmogorov-Smirnov test, p=0.200).

Characteristic	SCH n=21 Mean(±SD)	HC n=17 Mean(±SD)
Age (years)	40.5 (±12.0)	43.3 (±13.2)
No. of women (% of n)	17 (81)	14 (82)
BMI (kg/m^2)	28.9 (±5.8)	24.7 (±4.8)*
Blood pressure (mmHg)	122/74 (±17/9)	121/75 (±20/11)
Blood glucose (mmol/L)	5.0 (±0.4)	4.7 (±0.5)*
Serum Total Cholesterol (mmol/L)	5.5 (±1.1)	5.4 (±0.8)
Serum TSH (IU/L)	6.5 (±1.7)	2.1 (±0.9)*
Serum free T4 (pmol/L)	13.6 (±1.3)	14.4 (±1.3)
Serum free T3 (pmol/L)	5.2 (±0.7)	4.4 (±0.8)*

Table 5.1: Baseline demographic, clinical and biochemical features of patients SCH compared to HC. * $p<0.05$.

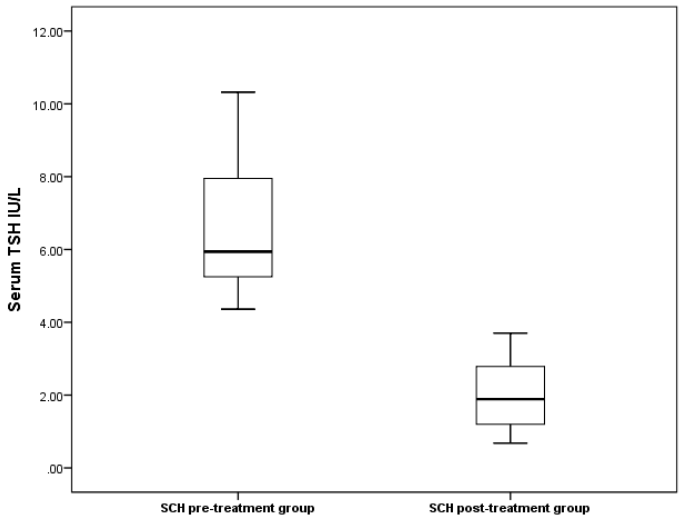

Figure 5-2: The figure shows distribution of serum TSH in both pre- and post-treatment SCH groups (n=16).

5.6 Results of cardiac PCr/ATP ratio

Figure 5.3 shows the typical cardiac MR spectra in a patient before and after treatment, and a healthy control. It clearly shows that resonance peak for PCr is shorter in SCH subject before levothyroxine treatment and became taller after levothyroxine treatment and this is comparable to that of healthy control.

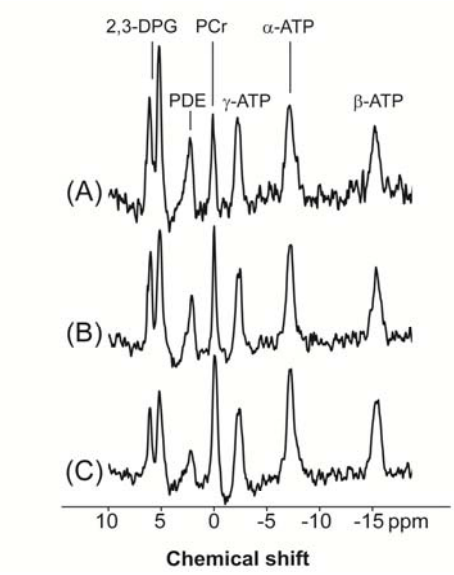

Figure 5-3: Cardiac ^{31}P spectra from SCH and healthy control subjects.

Sample cardiac ^{31}P spectra from a SCH patient (A) before and (B) after treatment with levothyroxine, demonstrating PCr/ATP ratios of 1.69 and 2.09 respectively. (C) shows a spectrum from a healthy control with PCr/ATP ratio 2.07 ppm (parts per million).

The cardiac PCr/ATP ratio in patients with SCH at baseline was significantly lower than HC (1.80 ± 0.26 vs. 2.07± 0.20, unpaired-t, p=0.001) (Figure 5.4).

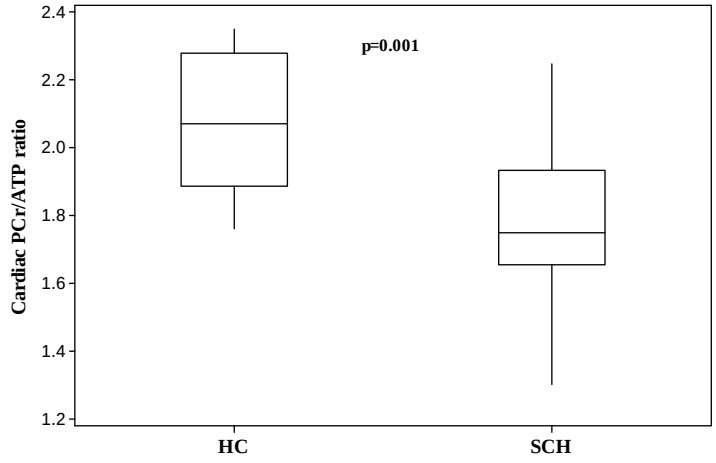

Figure 5-4: The baseline comparison of cardiac PCr/ATP ratio between SCH and HC groups. The cardiac PCr/ATP ratio was low in the SCH group at baseline (n=21) when compared with healthy controls (n=17); *unpaired-t, p=0.001.

Figure 5.5 shows the PCr/ATP ratio for each SCH subject before and after levothyroxine treatment. It shows that 13 patients had improvement and 3 patients had a decrease in cardiac PCr/ATP ratio with levothyroxine treatment.

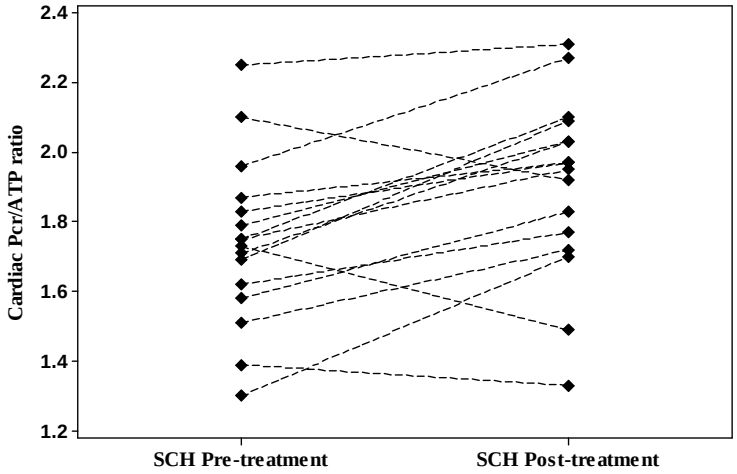

Figure 5-5: Cardiac PCr/ATP ratio of each SCH subject before and after levothyroxine treatment for 6 months

After treatment with levothyroxine for 6 months, mean cardiac PCr/ATP ratio improved significantly (1.74 ± 0.24 vs.1.91 ± 0.26, paired-t, p=0.004 (Figure 5.4). The mean cardiac PCr/ATP ratio in SCH after levothyroxine treatment is comparable to healthy controls (1.91 ±0.26 vs. 2.07 ± 0.20, p=0.051) (Figure 5.6)

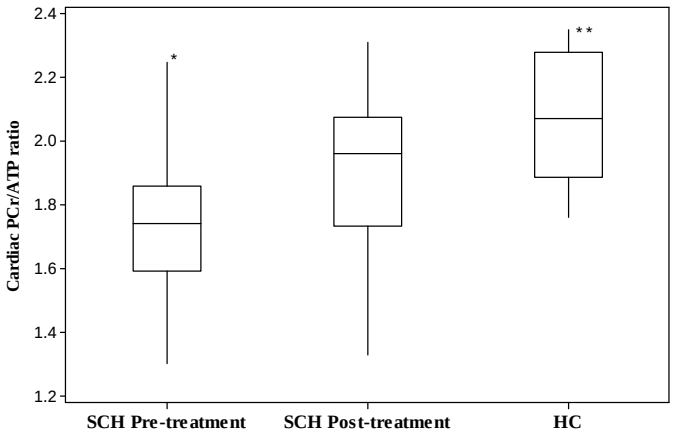

Figure 5-6: The comparison of cardiac PCr/ATP ratio between SCH pre- and post-treatment groups (* p=0.004), and HC and SCH post-treatment groups (**p=0.051)

In the SCH group, the cardiac PCr/ATP ratio was not correlated with baseline free T4, free T3, serum TSH, BMI, blood pressure, serum cholesterol or fasting blood glucose (data not shown). In the SCH post-treatment group, the change in serum TSH was not correlated to change in cardiac PCr/ATP ratio (data not shown) following treatment with levothyroxine.

Further correlation analysis between serum TSH (log transformed to attain linearity) and Cardiac PCr/ATP ratio was performed after combining both HC and SCH groups. This was done to look for a linear relationship between the two variables across a broader range of serum TSH. A significant inverse correlation was found between serum TSH and cardiac PCr/ATP ratio (Pearson's r= -0.37, p=0.026) (Figure 5.7).

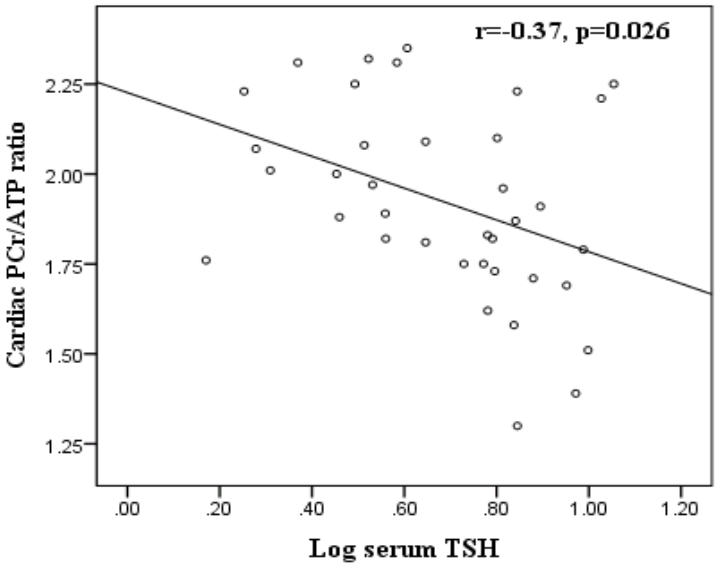

Figure 5-7: Correlation between PCr/ATP and serum TSH (Pearson r=-0.37, p=0.026)

The cardiac PCr/ATP ratio can be influenced by various factors other than thyroid status. A linear regression analysis was undertaken with cardiac PCr/ATP ratio as the outcome variable and gender, age, BMI, thyroid status (SCH and HC), cholesterol, glucose, blood pressure and FIS as the predictor variables. The results are shown in Table 5.2. It revealed that SCH status was a negative predictor for cardiac PCr/ATP ratio, independent of other variables (odds ratio (OR) -0.30, 95% confidence interval (CI) -0.13 to -0.47, p=0.001). Age was also a negative predictor of cardiac PCr/ATP ratio (OR -0.01, CI -0.01 to 0.00, p=0.04). BMI and FIS were not significant predictors of cardiac PCR/ATP ratio in this analysis.

Predictor variables	B value	Confidence interval for B value		P value
		Upper Bound	Lower Bound	
Gender	0.07	-0.12	0.27	0.46
Age	-0.01	-0.01	0.00	0.04
Thyroid status	-0.49	-0.82	-0.16	0.01
BMI	-0.01	-0.02	0.00	0.18
Cholesterol	0.01	-0.09	0.11	0.79
Glucose	0.19	0.00	0.38	0.05
Systolic BP	0.00	-0.01	0.00	0.59
Diastolic BP	0.00	-0.01	0.01	0.51
FIS (pre-treatment)	0.00	0.00	0.01	0.18

Table 5.2: Results of linear regression analysis using cardiac PCr/ATO ratio as the outcome variable and confounding variables listed above as the predictors.

5.7 Discussion

5.7.1 General discussion

Our results show that the cardiac PCr/ATP ratio was reduced in patients with SCH but improved significantly after treatment with levothyroxine for 6 months. It did not show any correlation between fatigue and abnormal cardiac PCr/ATP ratio. Serum TSH was inversely correlated with cardiac PCr/ATP ratio and SCH status was a negative predictor of cardiac PCr/ATP ratio in this cohort.

Our findings strengthen the results of previous studies assessing cardiac function in SCH and may further our understanding of the bioenergetic basis of myocardial dysfunction. A recent meta-analysis of 14 cross-sectional studies in patients with SCH using echocardiography have consistently revealed cardiac systolic and diastolic dysfunction (Chen et al., 2013). Various mechanisms for cardiac dysfunction have been proposed. A study in SCH using cardiac MR showed that the combination of decreased

cardiac preload and increased afterload as the possible mechanism of cardiac dysfunction, as opposed to a direct cardiac ionotropic impairment (Ripoli et al., 2005). However, direct effect of thyroid hormones on cardiac contractility has been suggested in another study by Monzani et al. (Monzani et al., 2001). Whether the low PCr/ATP ratio seen in our study is the effect of peripheral circulatory disturbances in SCH on myocardial function or a direct effect of thyroid hormones on myocardial function remains unknown.

It is possible that thyroid hormone directly affects myocardial energetic function. At a molecular level, it positively regulates gene encoding for α-myosin heavy chain and beta-adrenergic receptors via genomic actions (Klein and Ojamaa, 2001). Work on the role of thyroid hormones in cardiac mitochondrial functions has shown that thyroid hormones stimulate cardiac mitochondrial biogenesis, increasing myocardial mitochondrial mass, mitochondrial respiration, oxidative phosphorylation, enzyme activities, mitochondrial protein synthesis, cytochrome, phospholipid, and mitochondrial DNA content (Marin-Garcia). Direct non-genomic action of triiodothyronine (T3) in hypothyroid sheep has been studied in the past. The study by Portman etc. al. showed that intravenous infusion of T3 in thyroidectomised sheep resulted in an increase of cardiac PCr/ATP ratio, as measured by 31P MRS (Portman et al., 2005). Therefore, it is possible that thyroid hormone deficiency directly leads to impaired cardiac bioenergetic function, i.e. a low cardiac PCr/ATP ratio, which might contribute towards abnormal cardiac haemodynamic functions.

It is well known that patients with ischaemic heart disease have a low cardiac PCr/ATP ratio (Sardanelli and Quarenghi, 2006). Studies have shown reversible coronary micro-circulation impairment in SCH (Baycan et al., 2007, Oflaz et al., 2007, Traub-Weidinger et al., 2012). Subtle lipid alterations have been demonstrated in SCH, which might explain the mechanism of impaired coronary micro-circulation (Althaus et al., 1988, Bindels et al., 1999, Razvi et al., 2007). It is possible that impaired coronary micro-circulation might explain the low cardiac PCR/ATP ratio seen in our cohort.

Abnormal cardiac PCr/ATP ratio has been found in asymptomatic patients with diabetes and obesity (Shivu et al., 2010, Perseghin et al., 2007). These studies have also shown that an abnormal cardiac PCr/ATP ratio can be seen without evidence of coronary circulatory impairment. Another study has shown that high levels of free fatty-acids are

associated with a reduced cardiac PCr/ATP ratio in diabetic patients (Scheuermann-Freestone et al., 2003). We have not measured circulating free fatty acids in our study. SCH has been associated with several metabolic changes seen in an insulin resistance state similar to diabetes (Pucci et al., 2000). Therefore, a direct circulatory metabolic effect may cause an abnormal cardiac PCr/ATP ratio in SCH, in addition to the mechanisms described previously.

To summarise, multiple mechanisms are possible for a low cardiac PCr/ATP ratio in SCH, but the most plausible explanation would be a direct effect of myocardial tissue hypothyroidism. The serum free T3 levels were higher (but within the reference range) in patients than in healthy controls, possibly due to higher BMI. The intra-cellular concentration of T3 is largely determined by genetic polymorphisms in deiodinase enzymes (Jonklaas et al., 2014). Hence, the significance of raised serum T3 and its effects on cardiac myocytes is unknown and needs further studies to clarify this confounding factor. Clinical studies are unlikely to provide definitive answers because of overlapping factors like simultaneous effect of thyroid hormones on both vascular system and cardiac inotropic state. We need further *in vitro* studies to delineate the mechanism of low PCr/ATP in SCH.

Patients with SCH had higher fasting blood glucose and free T3 levels than HC, but these were well within normal ranges. It is intriguing to find slightly higher free T3 levels in SCH than HC. Previous studies have shown that SCH patients have mid to low-normal range free T3 levels (Pacchiarotti et al., 1986). Although the SCH subjects had higher BMI than the healthy controls, previous work has shown no effect of this degree of BMI difference on PCr/ATP ratio (Rider et al., 2012a, Rider et al., 2012b). There was no significant correlation between BMI, glucose, serum free T3 and PCr/ATP ratio in this study.

Serum TSH was not correlated to cardiac PCr/ATP ratio in the SCH cohort. This could be due to small number of subjects in our SCH cohort and the narrow range of serum TSH (4-10 IU/L) for this study. However, when we combined both SCH and HC groups, resulting in a larger number of subjects and wider serum TSH range, cardiac PCr/ATP ratio inversely correlated to serum TSH. Serum TSH is widely accepted as a surrogate marker of tissue hypothyroidism. Hence, an inverse correlation between serum TSH and cardiac PCr/ATP ratio suggests an association between tissue thyroid

status and cardiac function. Similar findings have been demonstrated in previous studies in SCH. The serum TSH was inversely correlated to stroke volume in a study by Ripoli et al. using Cardiac MR in patients with SCH (Ripoli et al., 2005). Another study by Monzani et al. using Doppler echocardiography and videodensitometric analysis have shown an inverse correlation between serum TSH and cardiac cycle variation index (a measure of intrinsic myocardial contractility) (Monzani et al., 2001). However, these studies did not report the effects of potential confounding variables affecting cardiac function. In our study, the linear regression analysis showed that SCH status is a negative predictor of cardiac PCr/ATP ratio, even after adjusting for age and other potential confounding variables like BMI and FIS score. BMI and FIS score did not predict cardiac PCr/ATP ratio on this regression model. These findings strengthen the case for the association between tissue thyroid status and cardiac dysfunction. Normalisation of serum TSH in the SCH group with levothyroxine treatment led to significant improvement of cardiac PCr/ATP ratio and this was non-significantly different from HC. This suggests a possible causal relationship between serum TSH and cardiac PCr/ATP ratio in this study. The reproducibility of these findings needs to be shown in further studies involving a larger cohort of subjects to reveal the true causal relationship between thyroid function and cardiac bioenergetics.

5.7.2 Clinical implications

Fatigue Impact Scale scores were not correlated to an abnormal cardiac PCr/ATP ratio in our cohort. This data does not support our hypothesis that fatigue might be due to abnormal cardiac PCr/ATP ratio in SCH. This may be a true lack of effect on fatigue by cardiac dysfunction in patients with SCH. But, this could also be explained by mild reductions (as opposed to severe reductions) in cardiac PCr/ATP ratio which do not lead to overt clinical symptoms like fatigue. Although the FIS questionnaire is a good general-purpose tool for measuring impact of fatigue (Frith and Newton) , it has not been validated in SCH or thyroid disease. Also, it does not measure the fatigue severity, but assesses the impact of fatigue in different areas of individual's functioning i.e. physical, cognitive and psychosocial domains (Dittner et al., 2004). Cardiac dysfunction leads mainly to physical fatigue and does not directly affect cognitive function. Hence, a total FIS score may not be a good tool to reflect upon, especially since the physical

aspect of fatigue affected by cardiac dysfunction. This could be another reason for not showing any correlation between FIS score and cardiac PCr/ATP ratio.

SCH has been found to be associated with ischaemic heart disease (IHD) in large-scale epidemiological studies (Razvi et al., 2010, Walsh et al., 2005). A recent retrospective study has shown that levothyroxine treatment reduces the incidence of IHD in SCH patients (Razvi et al., 2012). Patients with IHD have low cardiac PCR/ATP ratio with or without overt occlusive coronary artery disease (Weiss et al., 1990, Buchthal et al., 2000). After adjusting for coronary artery disease (CAD) and cardiac risk factors, a phosphocreatine-adenosine triphosphate ratio reduction of 1% increased the risk of a cardiovascular event by 4% (P=0.02) (Johnson et al., 2004).

The treatment of SCH is controversial and many experts recommend treatment with levothyroxine if serum TSH is more than 10 IU/L because of the high rate of progression to overt hypothyroidism (Biondi and Cooper, 2008, Surks et al., 2004). For patients with serum TSH between 4 and 10 IU/L, the treatment benefits are uncertain (Surks et al., 2004), but surrogate markers of cardiac dysfunction have been shown to improve with levothyroxine treatment in this group (Monzani et al., 2001). Our study adds to the evidence that even with serum TSH below 10 IU/L, reversible cardiac dysfunction as manifested by abnormal PCr/ATP ratio can be demonstrated. Our study supports the treatment of SCH with levothyroxine for cardiovascular benefits, although large randomised controlled studies are required to prove clinical benefits in SCH.

5.7.3 Strengths and Limitations

Only patients with stable SCH were selected i.e. patients with raised TSH for more than 3 months, so that tissue level bioenergetic changes were measurable with a cardiac MRS study. The disease manifestations in SCH depend on the duration and severity of the disease and peripheral sensitivity of the target tissues to thyroxine hormones (Biondi and Cooper, 2008). Patients with cardiovascular diseases or major risk factors for cardiovascular diseases were excluded to limit the effects of these confounding factors on the results of cardiac PCR/ATP ratio.

The limitations of the study include lack of matching for BMI, which might have contributed to a lower cardiac PCr/ATP ratio, although correlation analysis did not confirm this potential confounder. Obesity is associated with raised serum TSH (Pearce,

2012). Also, exercise levels were not measured in SCH and HC, which might have caused the lowering of cardiac PCr/ATP ratio in SCH due to cardiac deconditioning in people with fatigue. The serum T3 was not measured during the treatment phase. However, it is unknown whether this has affected the cardiac PCr/ATP ratio. Abnormal cardiac mass (which was not measured morphologically) might have contributed to an abnormal cardiac PCr/ATP ratio in SCH. However, levothyroxine treatment improved the cardiac PCr/ATP ratio, which suggests that exercise and abnormal ventricular mass were not contributing significantly to a low cardiac PCr/ATP ratio in this SCH cohort.

5.7.4 Future directions

This study has shown that the cardiac PCr/ATP ratio is low in SCH and increases with levothyroxine treatment. The results from this study could form the basis for future large-scale randomised controlled trials in subclinical hypothyroidism. These trials may demonstrate the true reversibility of cardiac effects in this common medical condition. Cardiac MR has been used in patients with SCH to detect ventricular dysfunction (Ripoli et al., 2005). Future studies should be designed to include cardiac MR ventricular functional assessment to detect subtle changes in the systolic and diastolic function along with cardiac PCr/ATP measurement. This may be able to assess whether impaired cardiac PCr/ATP ratio could contribute to ventricular dysfunction.

5.7.5 Summary

In summary, this study has shown that the cardiac PCr/ATP ratio is low in SCH and increases with levothyroxine treatment. This might be due to the direct effect of mild thyroid hormone deficiency and micro-vascular coronary ischemia in SCH. However, it did not prove our hypothesis that fatigue could be related to abnormal cardiac PCr/ATP ratio in SCH. The results from this study could form the basis for future large-scale randomised controlled trials in subclinical hypothyroidism. These trials may demonstrate the true reversibility of cardiac effects of this common medical condition.

Chapter 6 Cardiac autonomics and impedance

6.1 Hypothesis

Fatigue in subjects with SCH is in part due to abnormal cardiac autonomic nervous system functions and is reversible (partly or wholly) with levothyroxine therapy.

6.2 Primary Objective

To measure cardiac autonomic function parameters non-invasively and compare with age and gender-matched healthy controls, and correlate with fatigue.

6.3 Secondary Objective

To measure the above parameters after 6 months of levothyroxine treatment in patients with SCH.

6.4 Method of cardiac autonomic function assessment

Participant preparation: The tests were done in a non-fasting state and participants were advised to refrain from smoking and consuming caffeinated beverages for 4 hours prior to tests.

Setting: The tests were done at the Falls and Syncope Service at the Royal Victoria Infirmary, Newcastle upon Tyne. They were performed in a warm, quiet room.

Method: The subject was asked to lie down on a couch. The standard ECG electrodes were applied using limb leads I or II and digital blood pressure was recorded using Portapress® digital plethysmography, applied on the left forefinger or middle finger. This recorded continuous ECG and blood pressure throughout both the rest period and various manoeuvres (given below). A computerised system (TASKFORCE®) calculated heart rate variability (HRV) and baroreflex sensitivity (BRS) using autoregressive mathematical method (Bellavere et al., 1992) from the ECG RR intervals and continuous digital blood pressure recording. The absolute value of digital blood pressure was corrected automatically using a standard oscillometric blood pressure cuff applied to the right arm. The cardiac impedance electrodes were applied on the lower anterior chest and over the posterior aspect of the neck for measuring cardiac indices. The impedance cardiography (ICG) works by measuring changes in thoracic electrical bio-impedance over changes in time in relation to the cardiac cycle. The electrical and

impedance signal changes due to thoracic volume variations with each heartbeat were captured by these electrodes and were utilised with the help of algorithms to derive various cardiac haemodynamic parameters during rest and various manoeuvres. (Reference-http://www.impedancecardiography.com)

1. Resting for 10 minutes: The subject was instructed to lie down on the couch for 10 minutes without speaking or reading. They were advised to keep awake.

2. Active standing: The subject was instructed to stand up from lying down position and remain in standing position for 2 minutes.

3. Valsalva manoeuvre: In a sitting position, the subject was requested to blow into a 10 ml syringe which was connected to sphygmomanometer. The subject sustained continuous gentle blowing to keep the pressure at 40mmHg for 15 seconds. This was repeated after 2 minutes.

4. Passive Head-up Tilt (Passive HUT): The subject was instructed to lie down on the tilt-table with safety straps around waist and knees. The table was raised at the head-end and kept at a 70-degree angle for 40 minutes. The subject was advised not to speak unless symptomatic and refrain from reading and sleeping.

The following parameters were measured during rest and above manoeuvres:

Mean RR interval (milliseconds): The period from R wave to R wave derived from ECG signal. The mean calculated from all the RR intervals during each testing period. Non-beating sinus beats are removed semi-automatically and corrected using interpolation of preceding beats.

Heart rate variability using power spectral analysis: The mean calculated from all the RR intervals during each testing period. The values were also given after correcting for body surface area (nu). Non-beating sinus beats are removed semi-automatically and corrected using interpolation of preceding beats. Using fast Fourier transform based techniques, various spectral analysis (analysis of heart rate variability in time and frequency domains) are performed by the computer-based TASKFORCE software as per international guidelines (1996).

Total HRV (ms^2): This reflects all cyclical components of heart rate variability.

High Frequency (HF) spectra (0.15-0.40 Hz): This is synchronised with breathing phases and associated with parasympathetic activity.

Low Frequency spectra (LF) (0.04-0.15 Hz): This largely represents sympathetic activity.

Very Low Frequency (VLF): This component accounts for long-term regulatory mechanisms related to humoral factors and thermoregulation.

LF/HF ratio: This is a measure of sympatho-vagal balance.

Cardiac indices:

Cardiac Output (L/minute): This measures the amount of blood the left ventricle ejects each minute and broadly represents overall cardiac function.

Left Ventricular Ejection Time (milliseconds): The time from opening to closure of the aortic valve.

Total Peripheral Resistance Index (mmHg.min.m^2/L): It is an indicator of cardiac afterload.

End-Diastolic Index: This correlate with cardiac preload.

6.5 Validation

The TASKFORCE monitor is a commercial kit validated for clinical use and is widely used in in both clinical and research settings. No formal validating studies have been done in our research unit for this particular monitoring kit. Previous research studies undertaken using this kit have revealed consistent results comparable to other studies published in literature (Newton et al., 2006, Newton et al., 2007).

6.6 Results of cardiac autonomic function measurements

At baseline, there were 24 subjects with SCH who had autonomic tests. We analysed data from those who had completed a full 40 minutes of passive HUT protocol. There were 4 patients who did not complete the full 40 minutes of passive HUT and 1 patient's data was not recorded correctly (see Appendix C for details). The data from 1 SCH subject was omitted to match the exact number of healthy control data (and nearest age and gender matching) from the historical cohort in the baseline data analysis (but

this subject's data was included in the pre-and post-comparison analysis). So, there were 18 SCH subjects in the baseline group and we selected 18 age and gender-matched healthy controls from the historical cohort. The historical cohort was part of the previously published studies by our collaborative team (Hollingsworth et al., 2010). The mean age of SCH at baseline and HC were similar and gender distribution was equal in both groups. The serum TSH results were not available for HC, but they were free of any known thyroid and cardiovascular diseases.

Among the 19 SCH subjects who had ANS tests undertaken in the post-treatment group, 14 patients' data were included in the analysis. Five subjects were excluded (from the baseline cohort of 19) from the data analysis of the post-treatment group due to the following reasons: 1 did not complete the full 40 minutes for passive HUT; 2 left the study due to personal reasons; and 2 patients had serum TSH out of target (poorly compliant). The mean serum TSH was 6.7 (±1.8) IU/L and 2.2 (±1.1) IU/L in pre- and post-treatment SCH groups respectively. The serum TSH distribution is shown in the Figure-6.1 in both groups. The serum TSH is not normally distributed in the pre-treatment SCH group (Kolmogorov-Smirnov, p=0.042), but it was normally distributed in the post-treatment SCH group (Kolmogorov-Smirnov, p=0.200).

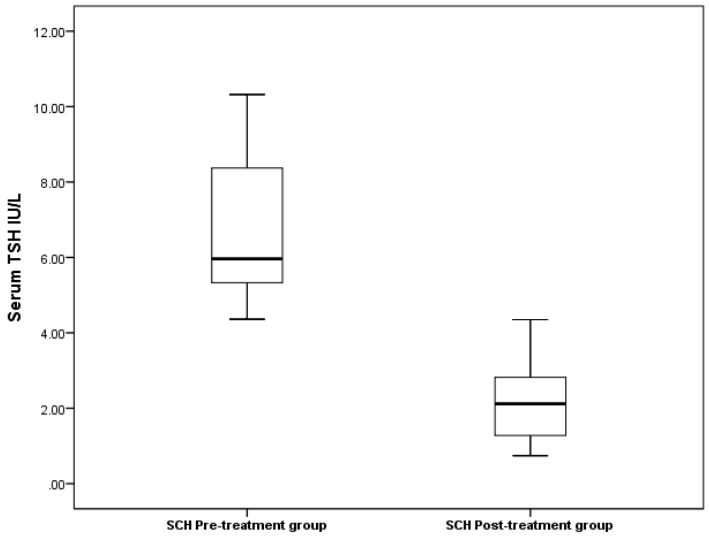

Figure 6-1: The distribution of serum TSH in pre- and post-treatment SCH groups is shown in this figure (n=14)

Table 6.1 shows the resting cardiac autonomic data for SCH at baseline and HC groups. It reveals that there were no significant differences between the 2 groups in any of the variables measured.

Variables	SCH n=18	HC n=18	P value
LFnu-RRI (%)	55.4±16.9	60.0±16.3	0.372
HFnu-RRI (%)	44.6±16.9	40.0±16.3	0.372
VLF-RRI (ms^2)	289.6±292.0	638.2±993.6	0.176
LF-RRI (ms^2)	726.9±817.9	479.3±256.6	0.225
HF-RRI (ms^2)	793.2±1490.0	376.2±341.9	0.218
PSD-RRI (ms^2)	1809.7±2507.3	1493.8±1256.0	0.586
LF/HF-RRI	1.8±1.3	2.8±2.5	0.187
LF/HF	1.5±0.9	1.9±1.4	0.362

Table 6.1: Comparison of resting cardiac autonomic parameters between baseline SCH and HC. Values are mean (SD)

Table 6.2 shows the cardiac autonomic data during passive HUT protocol for baseline SCH and HC groups. It does not reveal any significant differences between the 2 groups.

Variables	SCH n=18	HC n=18	P value
LFnu-RRI (%)	76.0±9.3	79.6±11.0	0.218
HFnu-RRI (%)	24.0±9.3	20.4±11.0	0.218
VLF-RRI (ms^2)	939.1±1226.0	707.2±982.1	0.499
LF-RRI (ms^2)	625.3±678.9	547.9±396.9	0.618
HF-RRI (ms^2)	135.5±100.1	101.1±79.9	0.203
PSD-RRI (ms^2)	2798.1±4827.8	1356.2±1139.0	0.194
LF/HF-RRI	6.7±5.8	10.5±8.0	0.133
LF/HF	5.2±4.9	8.4±7.0	0.148

Table 6.2: Comparison of cardiac autonomic parameters during head-up tilt (HUT) between baseline SCH and HC. Values are mean (SD)

Table 6.3 shows the mean change in cardiac autonomic data during passive HUT from the resting value for baseline SCH and HC groups. It did not reveal any significant changes between the 2 groups.

Variables	SCH n=18	HC n=18	P value
LFnu-RRI (%)	20.6±13.7	19.7±11.5	0.858
HFnu-RRI (%)	20.6±13.7	19.7±11.5	0.858
VLF-RRI (ms^2)	661.8±1280.1	68.9±1495.7	0.206
LF-RRI (ms^2)	101.6±935.5	68.6±288.3	0.497
HF-RRI (ms^2)	657.8±1457.4	275.1±272.6	0.246
PSD-RRI (ms^2)	988.4±4777.8	137.6±1702.9	0.329
LF/HF-RRI	4.9±5.4	7.7±6.7	0.190
LF/HF	3.7±4.7	6.5±6.1	0.160

Table 6.3: Comparison of change in cardiac autonomic parameters during head-up tilt (HUT) between baseline SCH and HC. Values are mean (SD)

Table 6.4 shows the resting cardiac autonomic data for pre- and post-treatment SCH groups. It reveals that there were no significant differences between the 2 groups in any of the variables measured.

Variables	SCH Pre-treatment n=14	SCH Post-treatment n=14	P value
LFnu-RRI (%)	54.6±16.6	57.4±14.0	0.374
HFnu-RRI (%)	45.4±16.6	42.6±14.0	0.374
VLF-RRI (ms^2)	291.0±334.0	494.1±616.1	0.334
LF-RRI (ms^2)	735.2±905.6	409.4±374.0	0.205
HF-RRI (ms^2)	878.3±1688.8	347.4±413.7	0.259
PSD-RRI (ms^2)	1904.6±2840.3	1250.9 ±968.5	0.429
LF/HF-RRI	1.8±1.4	2.1±1.7	0.178
LF/HF	1.5±1.0	1.6±0.8	0.442

Table 6.4: Comparison of resting cardiac autonomic parameters between pre- and post -SCH groups. Values are mean (SD)

Table 6.5 shows the cardiac autonomic data during passive HUT protocol for pre- and post-treatment SCH groups. It shows that the pre-treatment SCH group had lower LFnu-RRI than the post-treatment group (77.1±8.4 vs. 80.2±7.0 %, p=0.016).It also shows that the pre-treatment SCH group had higher HFnu-RRI than the post-treatment group (22.9±8.4 vs.19.8±7.0 %,p= 0.016). A unit change in HFnu-RRI and LFnu-RRI during HUT with treatment did not correlate significantly with a unit change in TFTs, FIS score and Cardiac PCr/ATP ratio.

Variables	SCH Pre-treatment n=14	SCH Post-treatment n=14	P value
LFnu-RRI (%)	77.1±8.4	80.2±7.0	0.016
HFnu-RRI (%)	22.9±8.4	19.8±7.0	0.016
VLF-RRI (ms^2)	2417.1±5426.2	307.6±161.9	0.173
LF-RRI (ms^2)	581.9±718.3	937.0±1583.8	0.172
HF-RRI (ms^2)	108.2±81.2	111.1±81.1	0.891
PSD-RRI (ms^2)	3107.2±5467.8	1355.6±1679.2	0.277
LF/HF-RRI	7.4±6.4	9.5±9.0	0.218
LF/HF	5.7±5.4	7.2±7.3	0.279

Table 6.5: Comparison of cardiac autonomic parameters during head-up tilt (HUT) between pre- and post-SCH groups. Values are mean (SD)

Table 6.6 shows the mean change in cardiac autonomic data during passive HUT from the resting value for the pre- and post-treatment SCH groups. It did not reveal any significant changes between the 2 groups.

Variables	SCH Pre-treatment n=14	SCH Post-treatment n=14	P value
LFnu-RRI (%)	22.5±14.2	22.8±12.5	0.923
HFnu-RRI (%)	-22.5±14.2	-22.8±12.5	0.923
VLF-RRI (ms^2)	2126.1±5385.5	-186.5±590.6	0.131
LF-RRI (ms^2)	-153.3±990.0	527.6±1525.1	0.061
HF-RRI (ms^2)	-770.2±1637.3	-236.3±381.6	0.248
PSD-RRI (ms^2)	1202.6±5426.6	104.7±1303.5	0.489
LF/HF-RRI	5.6±6.0	7.4±8.5	0.254
LF/HF	4.3±5.2	5.6±6.9	0.281

Table 6.6: Comparison of change in cardiac autonomic parameters during head-up tilt (HUT) between pre- and post-SCH groups. Values are mean (SD)

6.6.1 General discussion

The results showed that cardiac autonomics did not differ significantly between SCH patients and HC at baseline during rest and HUT. The pre-treatment SCH group had significantly lower LFnu RRI and higher HFnu RRI than post-treatment SCH during HUT. These results suggest lower sympathetic and higher parasympathetic activity during HUT in SCH patients, which improved significantly with levothyroxine treatment. Lack of correlation between HRV variables (HFnu-RRI and LFnu-RRI) and FIS suggests that fatigue is not related to HRV abnormalities in SCH patients.

Previous studies have shown inconsistent results in hypothyroid patients when assessed for cardiac autonomic function. Short-term overt hypothyroidism was associated with lower sympathetic and higher parasympathetic responses in a study conducted by Heesmstra et al. in 11 post-thyroidectomy patients (Heemstra et al.). Overt hypothyroidism due to Hashimoto's thyroiditis was associated with lower sympathetic activity in a study by Inukai et al.(Inukai et al., 1998). A higher parasympathetic tone in overt hypothyroidism due to varied aetiology has been shown by Xing et al. (Xing et al., 2001). Our results are consistent with these studies. In contrast to these studies, a reduction in parasympathetic and increased sympathetic activity has been shown in overt hypothyroidism (Cacciatori et al., 2000). The conflicting results may be due to different methods of measurement of HRV, and varying aetiology, severity and duration of hypothyroidism.

In SCH patients (n=42, mean serum TSH 9.8±1.7 mIU/L), Galetta et al. reported a reduction in parasympathetic tone, which improved with levothyroxine treatment. Sympathetic tone was not different between patients and HC, and did not alter after thyroxine treatment. However, a study by Sahin et al. has shown no significant changes in cardiac autonomic function in SCH patients with serum TSH below 10 mIU/L (n=18), but revealed decreased sympathetic tone in SCH patients with serum TSH above 10 mIU/L (n=13) (Sahin et al., 2005). Our results have shown similar results in SCH patients. This study did not report a response to levothyroxine treatment and measured 24-hour HRV rather than specific dynamic stress tests (i.e. HUT) for autonomic function tests, as measured in this study. The abnormalities in HRV in our study became significant during HUT following treatment with levothyroxine. This indicates that changes in HRV exist in SCH patients with serum TSH below 10 mIU/L.

But these changes are very mild and not obvious during rest, and only dynamic tests may show significant abnormalities in HRV in SCH.

In this arm of the study, multiple measurements of various HRV frequency domains have been made. If we were to correct for multiple testing with the Bonferroni method, then the results will not be significant. However, the results are consistent with previously reported similar studies and are physiologically plausible in hypothyroidism, as explained in the following paragraph.

The mechanisms of abnormal HRV in hypothyroidism have been described previously. In hypothyroidism, central sympathetic output is increased in response to reduced peripheral vascular and cardiac sensitivity to catecholamines (Coulombe et al., 1977). Diminished catecholamine responsiveness in hypothyroidism is explained by the reduced number of catecholamines receptors and post-receptor defects in hypothyroidism (Silva and Bianco, 2008).

6.6.2 Clinical implications

Fatigue in our SCH group is not explained by abnormal HRV measurements. The hypothesis is not proven by our results. This might be due to the smaller number of subjects or true lack of correlation between fatigue and thyroid status in SCH. No previous studies have been done to show any relationship between symptoms of hypothyroidism and autonomic dysfunction, although many of the clinical features of hypothyroidism can be explained physiologically by diminished adrenergic activity. Fatigue in primary biliary cirrhosis and chronic fatigue syndrome has been associated with autonomic dysfunction (Pagani and Lucini, 1999, Newton et al., 2006). But these were association studies only and no causal link has been established.

Cardiac autonomic dysfunction has been associated with increased cardiac morbidity and mortality. These has been shown in a number of diseases, including diabetes (Maser and Lenhard, 2005, Chico et al., 2005). SCH has been associated with increased cardiovascular disease, as previously described (Rodondi et al., 2010). Recently it has been shown that mortality is increased in patients with chronic heart failure and SCH (Rhee et al., 2013). So, it is possible that cardiac autonomic dysfunctions seen in SCH may be linked to these cardiac morbidities and mortality.

6.6.3 Strengths and limitations

Overt heart disease or hypertension can alter the results of cardiac autonomic evaluation (1996). We have excluded patients with overt cardiovascular disease or risk factors for cardiovascular diseases. This minimised the chance of underlying diseases affecting HRV measurements in this study.

In this study, HRV and other dynamic testing were performed under supervised testing conditions. This is in contrast to many other studies where SCH patients were assessed using 24-hr Holter monitoring for cardiac autonomic evaluation. 24-hour monitoring can be influenced significantly by very low-frequency oscillations (1996). It is not a supervised testing condition, hence subject factors such as emotional stress situations or bouts of exercise can lead to abnormal HRV. Hence, short-term HRV carried out under testing conditions are more accurate and reproducible when compared to long-term 24-hour monitoring.

Some of the studies in the past did not show any improvement in HRV with levothyroxine treatment in hypothyroidism (Celik et al., 2011, Heemstra et al.). This may be due to shorter duration of treatment, inadequate dosing, or irreversible damage to ANS by hypothyroidism. The study by Heemstra et al. (Heemstra et al.) treated hypothyroid patients with levothyroxine for 2 months, which may not be long enough to demonstrate significant changes in ANS after treatment. In the study by Celik et al., although 6 months of levothyroxine treatment was given, serum TSH after treatment was 4.1 (normal range 2.6-5.6 μIU/mL) (Celik et al., 2011). This is a higher serum TSH than that achieved by many other studies like Galetta et al. and in this study. This shows that achieving adequate therapeutic targets for longer periods, as in the FIS study, is important to demonstrate tissue level changes in SCH.

However, the study has important limitations. One of the inherent limitations of the HRV frequency domain measurements is that the normal range is not established in healthy controls in the literature. This is because of significant differences in testing conditions, variation in duration of measurements and significant random errors in HRV frequency domain measurements. Hence, it is recommended to have validation and test-retest reproducibility studies in each centre for HRV measurements, and to obtain normal ranges based on age (Tannus et al., 2013). For LFnu during the tilt test, the coefficient of variation (CV) for HC was 8.7%. There was a 4.0% increase in LFnu

during tilt test in the SCH group after levothyroxine treatment. Although, it is statistically significant, it is still within the CV of tilt test for HC. So, it is important to design future studies so that more sensitive methodologies are used to assess serial changes in HRV measurements. We have not repeated HRV measurements in HC, because these were historical controls. The effects of repeat testing in the SCH group could not be verified because the SCH group did not have a placebo arm. This was done as a pilot study, hence future studies need to be designed so that the effects of repeat testing in the SCH group could be ascertained.

The small sample of the study group is a potential reason for not showing any significant results. Previous studies have shown significant changes in HRV with larger study sample sizes in SCH (Galetta et al., 2006, Akcakoyun et al., 2009, Celik et al., 2011). There were no power calculations made for this study with regards to the cardiac autonomics arm of the study. The data from this study might serve for future studies for power calculations so that adequate sample size can be calculated.

The healthy control data was obtained from the historical controls who had undergone the same methodology as in the FIS study. Thyroid function data were not available for the HC in the historical cohort, although participants with a history of thyroid disease were excluded. Previous studies have shown that the prevalence of SCH in the general adult population is between 4-10%, more prevalent in the older population than the younger population (Biondi and Cooper, 2008). Hence, it is possible that 1 or 2 participants in the HC group might have SCH. However, this is unlikely to affect the final outcome of the study findings.

It has not been possible to match for body mass index (or body surface area) and blood pressure. This might have affected the results at baseline comparison between SCH and HC. Also, the presence of subclinical heart disease is not apparent on history, examination, and on a 12-lead ECG, and cannot be ruled out in participants in this study. Subclinical heart disease can be ruled out to a large extent using cardiac stress testing and echocardiography. Future studies should be designed so that full pre-study evaluations are conducted for potential interfering factors with HRV measurements.

6.6.4 Future directions

Future studies should be designed so that full pre-study evaluations are conducted for potential interfering factors with HRV measurements. A larger study group with and without fatigue would help to differentiate between fatigue-associated changes and changes due to SCH. Long-term significance of abnormal HRV in SCH is yet be determined with large prospective observational studies.

6.7 Results of cardiac impedance measurements

Table 6.7 shows the resting cardiac impedance data for SCH at baseline and HC groups. It reveals that the systolic BP was lower in SCH than HC ($p<0.05$). The measurements of cardiac pumping i.e. stroke volume, stroke volume index, cardiac output and cardiac index were all lower in SCH than HC ($p<0.05$). The afterload (TPRI) was higher in SCH than HC ($p<0.05$). The preload (EDI) was lower in SCH than HC ($p<0.05$). The cardiac contractility and LVWI were lower in SCH than in HC ($p<0.05$).

Variables	SCH n=18	HC n=18	P value
Heart rate (beats/min)	66.9±6.9	67.9±10.9	0.727
Systolic blood pressure (mmHg)	113.2±14.2	126.1±18.1	0.024
Diastolic blood pressure (mmHg)	72.1±13.3	79.1±11.3	0.115
Stroke volume (mls)	64.9±12.1	81.4±21.6	0.013
Stroke volume index [ml/meter^2]	36.0±10.0	46.3±11.8	0.012
Cardiac output (litres/min)	4.3±1.1	5.4±1.4	0.018
Cardiac index (litres/minute/meter^2]	2.4±0.8	3.1±0.8	0.022
Total peripheral resistance (dyne second /centimetre^5)	1672.0±445.8	1391.8±420.0	0.069
Total peripheral resistance index (dyne second meter^2/centimetre^5)	3144.7±1076.6	2460.4±806.9	0.036
End-diastolic index (ml/meter^2)	59.2±15.3	74.2±17.7	0.020
Left ventricular ejection time (milliseconds)	315.2±14.8	318.2±18.5	0.521
Left ventricular work index (mmHg x litres/min/meter^2)	2.8±0.8	3.8±1.1	0.010
Contractility index (1000/seconds)	36.7±19.2	53.6±19.9	0.022

Table 6.7: Comparison of resting cardiac impedance parameters between baseline SCH and HC. Values are mean (SD)

In patients during resting state, the free T4 was positively correlated to systolic BP ($r=0.580$, $p=0.012$) (Figure-6.1) and there was an inverse association between serum free T3 and LVET ($r=-0.556$, $p=0.025$) (Figure-6.2).

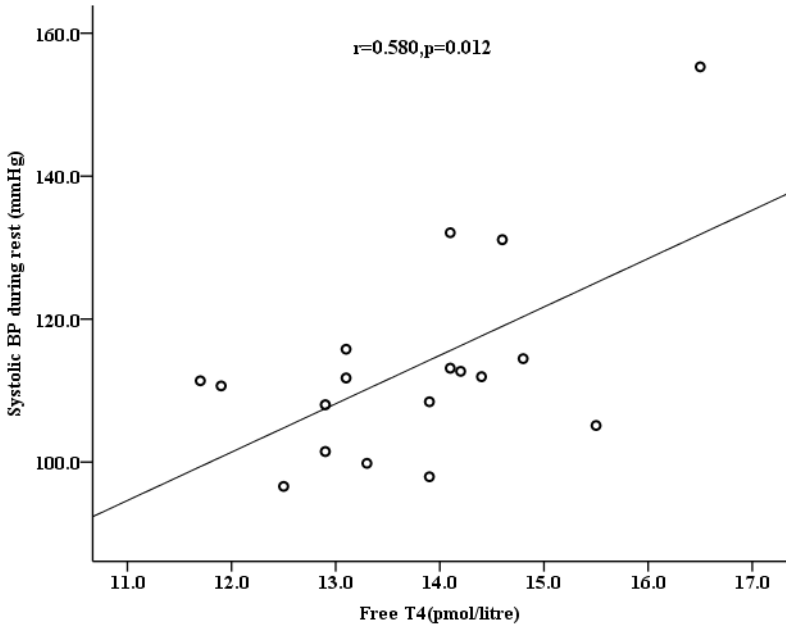

Figure 6-2: Relationship between systolic blood pressure and free T4 (pmol/litre) during rest in SCH

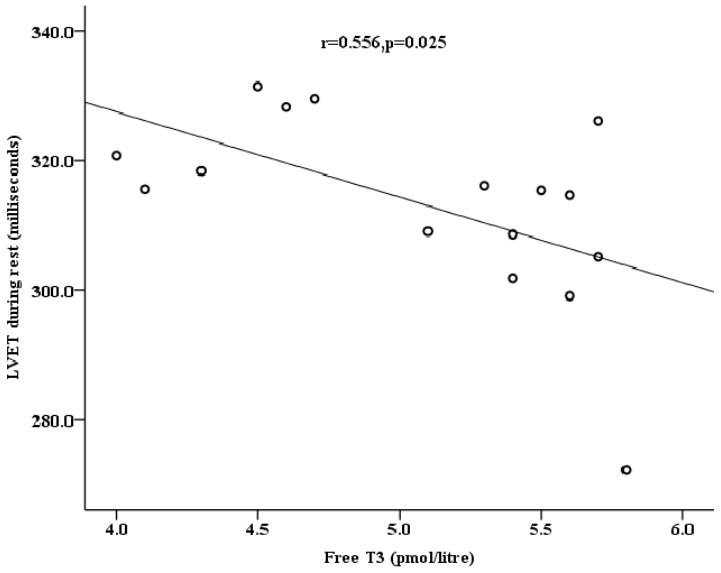

Figure 6-3: Relationship Left ventricular Ejection Time (LVET) (milliseconds) and free T3 (pmol/litre) during rest in SCH

To explore the mechanism of low CI in SCH patients, further correlation analysis was undertaken between CI and key variables affecting cardiac index i.e. EDI, TPRI and IC.

It showed positive correlations between CI and EDI (Figure-6.4), CI and IC (Figure-6.5); and an inverse correlation between CI and TPRI (Figure-6.6) (p value for all <0.001).

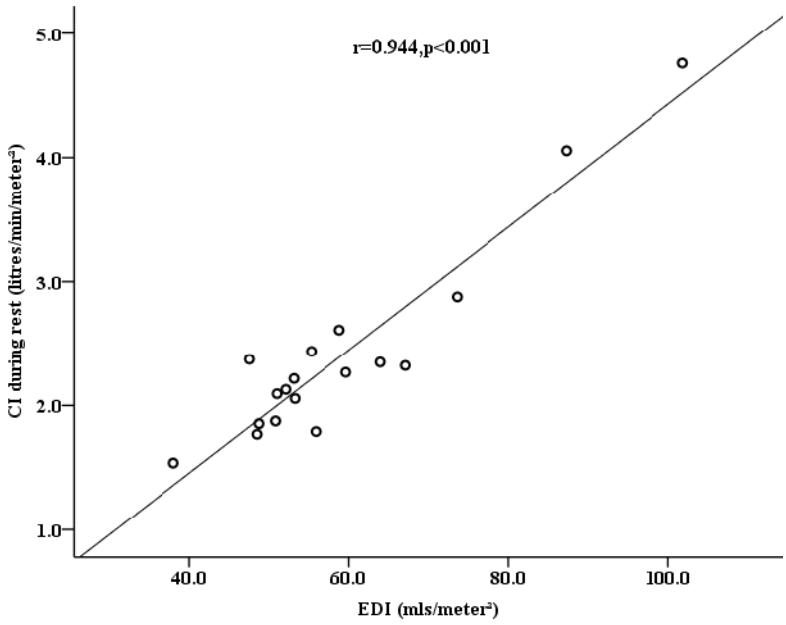

Figure 6-4: Relationship between cardiac index (CI) (litres/min/meter^2) and end-diastolic index (mls/meter^2) during rest

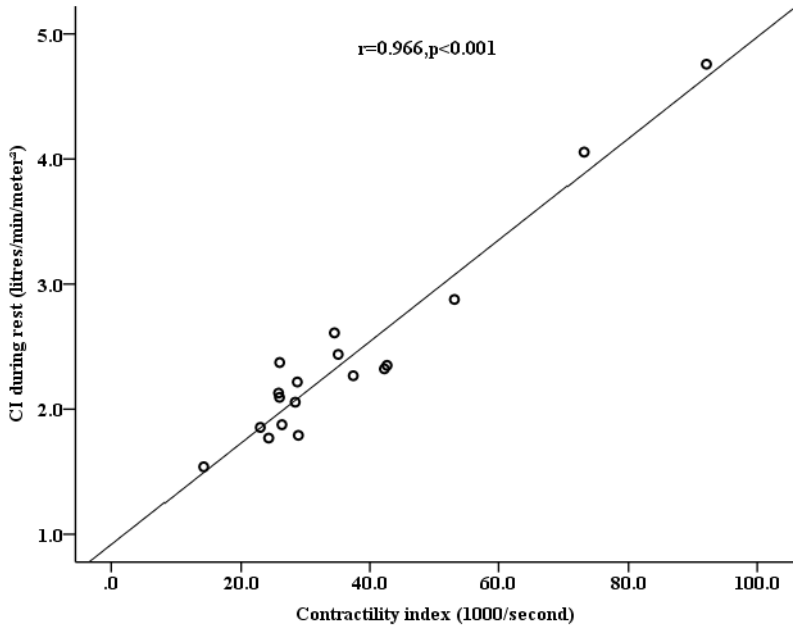

Figure 6-5: Relationship between cardiac index (CI) (litres/min/meter2) and contractility index (1000/second) during rest

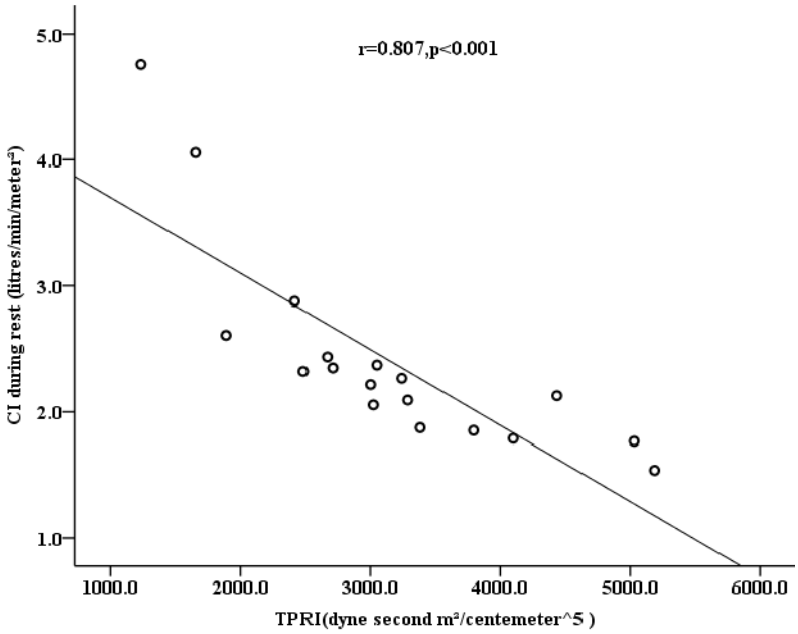

Figure 6-6: Relationship between cardiac index (CI) (litres/min/meter^2) and total peripheral resistance index (TPRI) (dyne second meter^2/centimetre^5) during rest

Table 6.8 shows the cardiac impedance data during passive HUT protocol for baseline SCH and HC groups. The mean heart rate was similar between both groups, but both systolic and diastolic BP were not different between the 2 groups (p values were 0.212 and 0.088 respectively). Both stroke volume index and cardiac index were significantly lower in SCH than HC ($p < 0.05$)). Afterload (TPRI) was similar between both groups, but preload (EDI) was lower in SCH than HC ($p<0.05$). The amount of work (LVWI) and force of work (contractility index) was significantly lower in SCH than HC ($p<0.05$).

Variables	SCH n=18	HC n=18	P value
Heart rate (beats/min)	80.2±6.7	82.5±13.7	0.572
Systolic blood pressure (mmHg)	122.7±16.4	129.6±19.1	0.212
Diastolic blood pressure (mmHg)	83.4±11.2	90.4±13.6	0.088
Stroke volume (mls)	52.5±7.3	58.1±11.0	0.140
Stroke volume index [ml/meter^2]	28.6±4.3	33.0±6.0	0.030
Cardiac output (litres/min)	4.2±0.6	4.7±0.9	0.107
Cardiac index (litres/minute/meter^2]	2.3±0.4	2.7±0.6	0.023
Total peripheral resistance (dyne second /centimetre^5)	1872.3±313.4	1734.9±517.7	0.468
Total peripheral resistance index (dyne second meter^2/centimetre^5)	3475.2±787.6	3068.6±999.1	0.225
End-diastolic index (ml/meter^2)	51.5±7.7	59.9±11.3	0.028
Left ventricular ejection time (milliseconds)	283.8±13.6	279.1±	0.559
Left ventricular work index (mmHg x litres/min/meter^2)	3.0±0.5	3.6±0.6	0.012
Contractility index (1000/seconds)	26.8±8.1	36.1±12.3	0.024

Table 6.8: Comparison of cardiac impedance parameters during head-up tilt (HUT) between baseline SCH and HC. Values are mean (SD)

Table 6.9 shows the mean change in cardiac impedance data during passive HUT from the resting data for both SCH and HC groups. The change in stroke volume and stroke index were lower in SCH than HC ($p<0.05$).

Variables	SCH n=18	HC n=18	P value
Heart rate (beats/min)	13.3±5.9	14.6±7.3	0.668
Systolic blood pressure (mmHg)	9.4±11.8	3.4±11.9	0.208
Diastolic blood pressure (mmHg)	11.3±9.1	11.3±8.1	0.851
Stroke volume (mls)	-12.4±13.2	-23.4±13.9	0.024
Stroke volume index [ml/meter^2]	-7.3±8.2	-13.3±7.7	0.036
Cardiac output (litres/min)	-0.1±1.1	-0.7±0.9	0.103
Cardiac index (litres/minute/meter^2]	-0.1±0.6	-0.4±0.5	0.170
Total peripheral resistance (dyne second/centimetre^5)	200.3±412.4	343.1±333.4	0.191
Total peripheral resistance index (dyne second meter^2/centimetre^5)	330.5±792.7	608.2±587.9	0.178
End-diastolic index (ml/meter^2)	-7.7±12.2	-14.3±10.0	0.106
Left ventricular ejection time (milliseconds)	-31.4±15.1	-39.1±19.8	0.209
Left ventricular work index (mmHg x l/min/meter^2)	0.2±0.8	-0.2±0.7	0.139
Contractility index (1000/seconds)	-10.0±15.3	-17.5±11.6	0.116

Table 6.9: Comparison of change in cardiac impedance parameters during head-up tilt (HUT) between baseline SCH and HC. Values are mean (SD)

The change in TPRI during HUT was inversely correlated to free T4 (r=-0.522, p=0.026) (Figure-6.6).

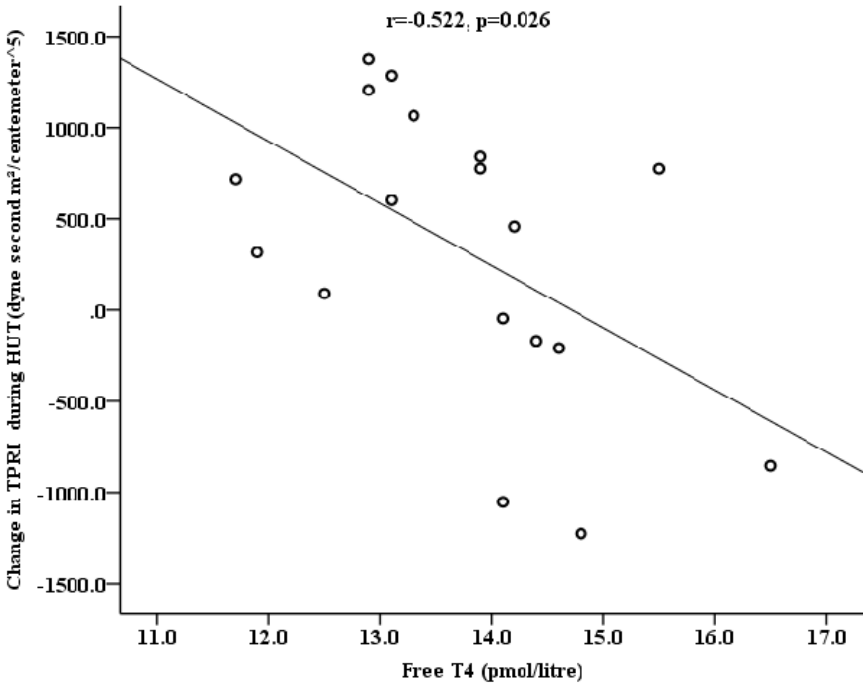

Figure 6-7: Relationship between change in total peripheral resistance Index (TPRI) (dyne second m^2/centimetre^5) and free T4 (pmol/litre) during Head-up tilt (HUT) in SCH.

Table 6.10 shows the resting cardiac impedance data for pre- and post-treatment SCH groups. It reveals that there were no significant differences between the 2 groups in any of the variables measured.

Variables	SCH Pre-treatment n=14	SCH Post-treatment n=14	P value
Heart rate (beats/min)	67.1±7.6	68.3±5.3	0.504
Systolic blood pressure (mmHg)	115.1±15.7	115.9±12.2	0.815
Diastolic blood pressure (mmHg)	72.6±12.1	74.3±11.5	0.474
Stroke volume (mls)	64.7±11.7	63.1±9.4	0.375
Stroke volume index [ml/meter^2]	35.6±10.0	34.7±8.7	0.375
Cardiac output (litres/min)	4.3±1.0	4.3±0.7	0.848
Cardiac index (litres/minute/meter^2]	2.4±0.8	2.4±0.6	0.804
Total peripheral resistance (dyne second/centimetre^5)	1682.1±351.0	1692.8±344.8	0.885
Total peripheral resistance index (dyne second meter^2/centimetre^5)	3191.3±974.5	3171.6±843.6	0.895
End-diastolic index (ml/meter^2)	58.3±15.3	57.5±14.0	0.559
Left ventricular ejection time (milliseconds)	313.4±16.0	311.5±12.9	0.633
Left ventricular work index (mmHg x l/min/meter^2)	2.9±0.8	2.9±0.6	0.977
Contractility index (1000/seconds)	36.07±19.0	34.6±15.6	0.409

Table 6.10: Comparison of resting cardiac impedance parameters between pre-and post-CH groups. Values are mean (SD).

Table 6.11 shows the cardiac impedance data during passive HUT protocol for pre- and post-treatment SCH groups. It does not reveal any significant changes between the 2 groups.

Variables	SCH Pre-treatment n=14	SCH Post-treatment n=14	P value
Heart rate (beats/min)	79.7±6.4	80.5±3.6	0.677
Systolic blood pressure (mmHg)	125.1±17.7	127.7±11.4	0.566
Diastolic blood pressure (mmHg)	85.0±10.2	86.0±6.9	0.650
Stroke volume (mls)	52.8±8.7	51.4±6.3	0.326
Stroke volume index [ml/meter^2]	28.5±4.9	27.8±3.4	0.358
Cardiac output (litres/min)	4.2±0.7	4.1±0.6	0.681
Cardiac index (litres/minute/meter^2]	2.3±4.1	2.2±0.3	0.723
Total peripheral resistance (dyne second/centimetre^5)	1925.6±376.3	1979.7±377.8	0.456
Total peripheral resistance index (dyne second meter^2/centimetre^5)	3600.5±847.3	3660.5±705.2	0.656
End-diastolic index (ml/meter^2)	51.1±8.7	49.8±6.5	0.308
Left ventricular ejection time (milliseconds)	284.7±12.7	283.4±7.2	0.712
Left ventricular work index (mmHg x l/min/meter^2)	3.0±0.5	3.0±0.3	1.000
Contractility index (1000/seconds)	26.2±9.0	25.1±6.6	0.348

Table 6.11: Comparison of cardiac impedance parameters during head-up tilt (HUT) between pre- and post-SCH groups. Values are mean (SD).

Table 6.12 shows the mean change in cardiac impedance during HUT from the resting values for pre- and post-treatment SCH groups. It did not reveal any significant changes between the 2 groups.

Variables	SCH Pre-treatment n=14	SCH Post-treatment n=14	P value
Heart rate (beats/min)	12.6±6.5	12.2±5.0	0.681
Systolic blood pressure (mmHg)	10.1±12.5	11.8±10.8	0.521
Diastolic blood pressure (mmHg)	12.5±8.2	11.7±12.5	0.761
Stroke volume (mls)	-11.9±13.4	-11.7±10.9	0.883
Stroke volume index [ml/meter^2]	-7.1±8.3	-6.9±7.1	0.810
Cardiac output (litres/min)	-0.1±1.2	-0.2±0.9	0.896
Cardiac index (litres/minute/meter^2]	-0.1±0.7	-0.1±0.5	0.979
Total peripheral resistance (dyne second/centimetre^5)	243.5±465.5	286.9±446.3	0.531
Total peripheral resistance index (dyne second meter^2/centimetre^5)	409.2±867.3	488.9±784.7	0.518
End-diastolic index (ml/meter^2)	-7.2±12.6	-7.7±10.9	0.656
Left ventricular ejection time (milliseconds)	-28.8±13.8	-28.0±11.4	0.839
Left ventricular work index (mmHg x l/min/meter^2)	0.1±0.8	0.1±0.6	0.977
Contractility index (1000/seconds)	-9.9±16.0	-9.6±12.6	0.850

Table 6.12: Comparison of cardiac impedance parameters during head-up tilt (HUT) between pre- and post-SCH groups. Values are mean (SD).

6.7.1 General Discussion

At rest, the cardiac impedance data shows impaired cardiac index in SCH when compared to HC. These changes did not occur with levothyroxine treatment. The heart rate was similar between both groups. Low cardiac index was related to reduced contractility (CI) and preload (EDI), and increased afterload (TPRI) in SCH. This suggests multiple impairments in cardiovascular physiology in SCH during resting conditions. The serum FT4 is positively related to systolic blood pressure, but the significance of this is unclear. The serum FT3 in inversely related to LVET, meaning

higher FT3 is associated with lower left ventricular ejection time. FIS and cardiac PCr/ATP ratio were not correlated to cardiac indices during rest. These findings indicate that the cardiac PCr/ATP ratio is not contributing to low cardiac output, and fatigue is not caused by low cardiac output during the resting stage.

During passive HUT, SI, CI, EDI, LVWI and IC were low in SCH when compared to HC. This shows that the patients were unable to respond sufficiently to physiological strain of HUT. However, the TPRI were not significantly different between SCH and HC. This suggests that peripheral response was appropriate in SCH during HUT. So, the low CI was due to low EDI and IC. Levothyroxine treatment did not improve any abnormalities in SCH.

The findings from this study are in accordance with some of the studies that reported cardiac dysfunctions previously. A cardiac MR study in SCH showed lower end diastolic volume, SV,CI; and a higher peripheral resistance in SCH than healthy controls (Ripoli et al., 2005). These findings were similar to my study. They inferred that the reduction in afterload and preload were responsible for reduction in CO in SCH. This study has shown that cardiac contractility is also low, which is contributing to lower CO in SCH. They found significant improvement in cardiac function with levothyroxine for a median duration of 86 days. Unlike this study, my study did not find any improvement in cardiac function following levothyroxine treatment for 6 months. Cardiac impedance was measured in patients with SCH before and after levothyroxine treatment in another study (Faber et al., 2002). This study did not compare patients with healthy controls, but shows improvement in CO and a reduction in peripheral resistance with levothyroxine treatment after a mean duration of 157 days in patients. However, another study using cardiac impedance in patients with SCH did not show any improvement in cardiac function with levothyroxine treatment (La Viola et al., 2003). In summary, my study did corroborate some of the previous studies, but this needs further studies in bigger cohorts to draw definitive conclusions.

The lack of response to levothyroxine in cardiac function in SCH is unexplained. In addition to the discussions in the previous sections, general factors like obesity may have caused subtle cardiac dysfunction in SCH. Obesity is associated with diastolic dysfunction, which improves with weight loss (Rider et al., 2012b). The patients in my cohort did not have very high BMIs and this degree of raised BMI was not associated

with diastolic dysfunction. The cardiac impedance data may not be sensitive enough to identify subtle improvements in cardiac function in SCH with levothyroxine treatment. This could be another reason for not showing any improvement with levothyroxine treatment, unlike previously-mentioned studies in this section.

6.7.2 Strengths and limitations

The general strengths and weakness of the study were described in previous sections. These are applicable to this particular arm of the study as well, especially since small sample size might have limited the power of the study in the pre- and post-treatment arm of the study.

Chapter 7 Cerebral Blood Flow using Arterial Spin Method

7.1 Hypothesis

Fatigue in subjects with SCH is mediated through (wholly or partly) altered CBF and is reversible with levothyroxine treatment.

7.2 Primary endpoints

To quantify whole-brain grey matter blood flow using MR arterial spin labelling (ASL) in SCH and compare with age and gender-matched euthyroid healthy controls.

7.3 Secondary endpoints

To quantify whole-brain grey matter blood flow after 6 months of levothyroxine treatment in patients with SCH.

7.4 Method

To examine this hypothesis, the ASL technique was used to measure CBF in SCH patients both at the baseline and after 6 months of levothyroxine treatment, as well as in age and gender-matched controls (HC).

MRI was performed on a 3T whole body scanner (Philips Medical Systems, Best, Netherlands) using the integrated body coil for transmission and signal detection through an 8 channel SENSE head coil. A T_1 weighted anatomical volume with 1 mm isotropic resolution was collected using a standard clinical protocol (3D MPRAGE sequence, FOV 240 x 240 x 180mm^3, TE/TR =4.6/9.6ms, SENSE factor 2). Cerebral blood flow (CBF) was measured using a FAIR arterial spin labelling (ASL) sequence (Kim and Tsekos, 1997, Kim, 1995) in the same manner as previously described (Tryambake et al., 2013). The ASL images were processed as previously described (Tryambake et al., 2013) to derive whole-brain grey matter CBF.

7.5 Results

Out of 25 of those recruited, 3 patients with SCH did not have complete brain MR scans (unable to tolerate or attend brain MRI scans) and 1 subject had a poor-quality image. Hence, we had 21 SCH subjects with completed data, with the data from 1 SCH subject not analysed, to match with 20 healthy controls (see Appendix C for details). The

patient demographic information is shown in Table 7.1 for SCH at baseline (n=20) and HC (n=20). It shows that the subjects in each group were well matched, except for BMI. As expected, the mean serum TSH (serum TSH was normally distributed as per Kolmogorov-Smirnov test, p=0.083), FT4 and FIS score were significantly different in each group. The whole-brain grey matter CBF for SCH at baseline and HC were 49.3(±6.6) vs. 46.9(±5.8) ml/100g/min (p=0.225) respectively.

Parameters	SCH n=20	HC n=20	Unpaired t-test (p value)
Age (years)	40.6(±12.2)	42.2 (±12.5)	0.685
Gender (no. of females)	18	17	0.229
Body Mass Index (kg/m2)	29.5 (±6.3)	24.7 (±4.4)	0.008
Blood Pressure (mmHg)	123.2/76.6 (±17.8/10.1)	121.2/77.1 (±19.2/11.5)	0.728
Total Cholesterol (mmol/L)	5.5 (±1.0)	5.3 (±0.8)	0.500
Glucose (mmol/L)	4.9 (±0.4)	4.7 (±0.5)	0.121
TSH (mIU/L)	6.7 (±1.9)	2.0 (±0.9)	<0.001
Free T4 (pmol/L)	13.5 (±1.3)	14.7 (±1.4)	0.012
Fatigue Index Score	77.6 (±22.7)	4.3(±5.0)	<0.001

Table 7.1: **Characteristics of subclinical hypothyroid patients at baseline were compared to healthy controls. Values are mean (±SD).**

Out of 20 subjects in the baseline SCH cohort, 2 subjects were poorly compliant with levothyroxine and 1 had head rotation during the repeat imaging. Hence we had 17 subjects in the post-treatment SCH group (see Appendix C for details). Table 7.2 shows the comparison of SCH patient groups before and after levothyroxine treatment. Figure-7.1 shows the distribution of serum TSH in pre- and post-treatment groups. In the pre-treatment SCH group, the serum TSH was not normally distributed (Kolmogorov-Smirnov test, p=0.028), but serum TSH in the post-treatment SCH group was normally distributed (Kolmogorov-Smirnov test, p=0.200). The CBF in pre- and post-treatment SCH groups were 50.6 (±8.5) vs. 46.7 (±8.5) (p=0.013) respectively. The CBF in SCH post-treatment group was not non-significantly different from HC (46.9±5.8 vs.46.7 (±8.5) ml/100g/min, p=0.947). The whole grey matter CBF for HC, SCH pre-levothyroxine treatment and SCH post-levothyroxine treatment is shown in Figure 7.2, and the corresponding CBF in SCH (pre- and post-levothyroxine treatment) is indicated by a line.

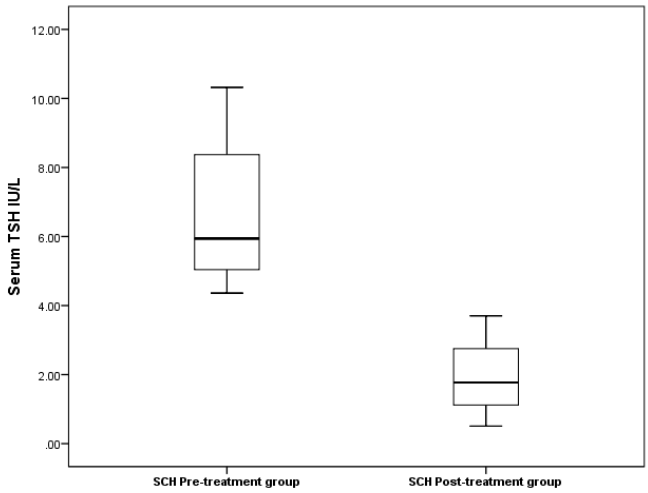

Figure 7-1: This graph shows the distribution of serum TSH in pre- and post-treatment SCH groups (n=17).

Parameters	SCH pre T4T (N=17)	SCH post T4T (N=17)	Paired t-test (p value)	Magnitude change
TSH	6.6 (±1.8)	1.9 (±1.0)	<0.001	-4.7 (±2.1)
Free T4	13.5 (±1.4)	19.2 (±2.4)	<0.001	+5.6 (±2.2)
FIS	79.9 (±23.9)	34.2 (± 35.7)	<0.001	-45.7 (±31.0)
CBF	50.6 (±8.5)	46.7 (±8.5)	0.013	-3.9 (±5.7)

Table 7.2: Changes in TSH, free T4, fatigue index score and cerebral blood flow after 6 months of levothyroxine treatment in SCH group. The last column is the result of a t-test for difference between groups. Values were mean (SD).

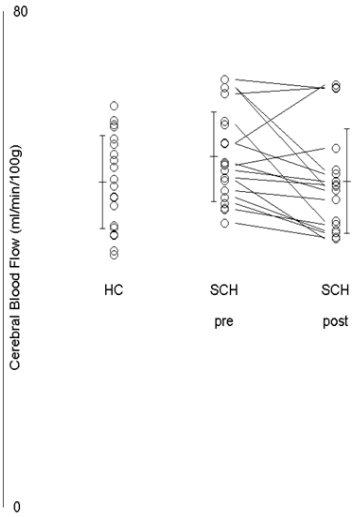

Figure 7-2: Cerebral blood flow of each individual. Cerebral blood flow significantly decreased in the SCH post-thyroxin treatment compared to the baseline. The levothyroxine treatment appears to have a normalisation effect on CBF (HC vs. SCH post-treatment, p=0.947)

7.6 Discussion

7.6.1 General discussion

In this study, we have shown that the CBF in SCH was significantly reduced by levothyroxine treatment to the level seen in HC. FIS is significantly higher in SCH baseline compared to HC, and levothyroxine treatment significantly relieves the fatigue in SCH.

The results suggest that increased CBF was secondary to the SCH state where the main effects of SCH are on the cellular level. At cellular level, thyroxine hormone (T4) is converted to triiodothyronine (T3) by deiodinase enzymes in brain. In hypothyroidism, there is upregulation of type II deiodinase enzyme (DIO2) activity in brain, and animal studies have shown enhanced local production of T3 within brain tissue in hypothyroidism (Dratman et al., 1983). T3 has known vasodilatory properties by acting on vascular smooth muscles (Ojamaa et al., 1993). Hence, it was postulated that a slight increase in CBF in SCH may be an over-compensatory response to mild tissue hypothyroidism. Treatment of the hypothyroid state by levothyroxine led to a decrease

in DIO2 activity (Burmeister et al., 1997) and tissue T3 levels, which can lead to normalisation of tissue blood flow.

Normalisation of increased cerebral blood flow velocities after levothyroxine treatment in overt hypothyroidism was found in a study by Utku et al. using transcranial Doppler sonography (Utku et al., 2011). They found a similar increase of mean blood flow velocities of bilateral middle cerebral arteries in both over hypothyroidism (n=30,mean age 37.4 years, men/women ratio 3/27, mean serum TSH 19.8 IU/L) and SCH (n=30 , mean age 34.4 years, men/women ratio 4/26, mean serum TSH 6.3 IU/L) groups. The SCH group had similar demographic characteristics, as in our study. They reported normalisation of blood flow after levothyroxine treatment in overt hypothyroidism, but did not report the response to levothyroxine treatment in the SCH group. My study has shown a reduction of CBF in the SCH group with levothyroxine treatment. The proposed mechanism for altered cerebral blood flow was of mild atherosclerosis and consequent narrowing of major cerebral arteries, leading to increased blood velocity or due to systemic hypertension (Utku et al., 2011). The precise cerebral auto-regulatory mechanism in hypothyroidism remains unknown, as is the case with various diseases affecting cerebral circulation (Paulson et al., 1990). Further studies are required to reveal the true pathophysiological mechanisms of cerebral circulation in SCH.

7.6.2 Clinical implications

The observed fatigue was not associated with CBF in SCH, suggesting that CBF is not a marker of fatigue in SCH. So, the data does not support the hypothesis that fatigue may be due to altered CBF. This may be due to the fact that very early tissue hypothyroidism may not be severe enough to cause altered neuronal or direct cerebral circulatory dysfunctions leading to fatigue in SCH. This could also be due to small sample size of the SCH group. Fatigue in SCH could be related to peripheral muscular dysfunction (Beyer et al., 1998, Caraccio et al., 2005) rather than due to a central cause. Patients with a greater severity of SCH (i.e. serum TSH >10 IU/L) might have shown more pronounced changes in CBF. We studied patients with serum TSH below 10 IU/L, as those with serum TSH above 10 IU/L are routinely treated because of the high rate of progression to overt hypothyroidism (Surks et al., 2004). Hence, my study aim was to explore for any reversible pathophysiological changes in SCH with borderline elevated serum TSH who are not routinely treated with levothyroxine at present (Surks et al.,

2004). FIS measures composite of three domains *viz.* cognitive, physical and social (Fisk et al., 1994b). CBF may not affect physical and social factors directly; hence, the total FIS score may not correlate with CBF.

7.6.3 Strengths and weaknesses

The major strength of this study is that we selected only subjects with no overt vascular disease and without major risk factors for vascular disease. No subjects were on any drugs which could affect vascular function. The disease manifestations in SCH depend on the duration and severity of the disease and peripheral sensitivity of the target tissues to thyroxine hormones (Biondi and Cooper, 2008). Hence, only patients with stable SCH (i.e. those with raised serum TSH for more than 3 months) were selected, and patients were started on a full replacement dose of levothyroxine to maximise the chance of detecting any tissue level changes on CBF. The limitation of the study is that the small size of the sample might have reduced the power of the study, but this was a preliminary exploratory study and we were unable to carry out the precise power calculations for the study. We did not repeat scans for HC to see whether changes in CBF in SCH were not due to random variations on repeat measurements.

7.6.4 Future directions

The findings from this study would need to be reproduced in a larger and separate SCH cohort because our study was the first one to look at this specific group of patients with SCH i.e. younger patients with serum TSH below 10 IU/L. Also, data from this study could be used for future power calculations for similar studies in SCH. The effect of combination therapy of hypothyroidism with levothyroxine and triiodothyronine (known as "T4+T3 therapy") on CBF has not been studied before. The clinical benefits of T4+T3 therapy has been shown to be inconsistent in previous studies (Chakera et al., 2012). It was suggested that a specific subgroup of hypothyroid patients with common variation in the DIO2 gene might benefit from T4+T3 combination therapy (Panicker et al., 2009). The effect of T3 therapy on tissue levels of T3 has been demonstrated in the past in animal models (Short et al., 2001). MR methods like ASL may be used to study tissue level changes in hypothyroidism in this subset of hypothyroid patients and to correlate tissue levels changes with genetic variations in DIO2 enzyme. This might help

many thousands of patients who are not gaining significant improvement on levothyroxine alone.

7.7 Summary

To conclude, we found a reduction of CBF in SCH patients after levothyroxine treatment, and its physiological significance is unknown. The CBF did not correlate with fatigue in our cohort. Hence, CBF is not a marker of fatigue in SCH. Future studies are warranted to look at CBF in SCH patients.

Chapter 8 Overall Discussion

The hypothesis of the study was that fatigue in SCH is due to functional abnormalities in peripheral tissues which are partly or wholly reversible with levothyroxine treatment. The hypothesis is not proven in that none of the target tissues showed any abnormalities which will explain the mechanism of fatigue in SCH.

The muscle MRS data showed abnormal PCr recovery and maximum proton efflux. These findings were consistent with existing literature. However, in patients these abnormalities did not improve with levothyroxine treatment. These abnormalities did not correlate with thyroid function tests at baseline or fatigue. The functional significance of this impaired skeletal muscle metabolism is unknown in our study. But, as explained previously, other studies have linked fatigue to these abnormalities in primary biliary cirrhosis and chronic fatigue syndrome. In our study, these were not linked to fatigue, which might be due to true lack of association or methodological limitations, as described previously.

The cardiac MRS data showed a low cardiac PCr/ATP ratio which improved with levothyroxine treatment. However, it did not correlate with FIS score and hence indicates that fatigue is not contributed by impaired myocardial energetics. After combining both patients at baseline and healthy controls, serum TSH had an inverse correlation with cardiac PCr/ATP ratio. Thyroid status was a strong independent predictor of cardiac PCr/ATP ratio. Although thyroid status seems to modulate cardiac bioenergetics, it may not be severe enough to lower cardiac output significantly. The cardiac autonomics data showed improvement in sympathetic and parasympathetic tone in patients with levothyroxine treatment. The impedance data showed impaired cardiac indices at baseline, which did not improve with levothyroxine treatment. There were no significant correlations between FIS and any of these abnormal parameters, which show that fatigue is not caused by abnormal cardiac function. The CBF data showed surprise results of increased CBF which normalised with levothyroxine treatment. These results were consistent with another study, but it did not correlate with FIS score.

There were several potential reasons for hypothesis not being proven in this study. SCH may not have led to severe tissue dysfunction, which can cause symptoms. FIS score measured global fatigue rather than specific organ dysfunction and associated

symptoms. Also, the small sample size might have affected the power of the study. However, it is possible that combination of functional changes in peripheral tissues might have contributed to the fatigue. These need further investigation in future studies. Although the hypothesis is not proven, the study has revealed interesting functional abnormalities in SCH. This shows that even with serum TSH between 4 and 10 mIU/L, tissue level abnormalities are seen in myocardium, peripheral skeletal muscle and autonomic nervous system. The true clinical significance of these findings needs to be explored in future studies.

Appendix

Appendix A

Fatigue Impact Scale Questionnaire

Please complete

Do you agree with these statements?

Because of fatigue (low energy)...

If the question is not relevant to you, please tick "no problem" (eg. See question 28).

	no problem	small problem	moderate problem	big problem	extreme problem	
1. I feel less alert						
2. I feel that I am more isolated from social contact						
3. I have to reduce my workload or responsibilities						
4. I am more moody.						
5. I have difficulty paying attention for a long period						
6. I feel I cannot think clearly.						
7. I work less effectively (this applies to work inside or outside the home)						
8. I have to rely more on others to help me or do things for me.						
9. I have difficulty planning activities ahead of time						
10 I am more clumsy and uncoordinated						
11 I find that I am more forgetful						

	no problem	small problem	moderate problem	big problem	extreme problem	
12 I am more irritable and more easily angered						
13 I have to be careful about pacing my physical activities						
14 I am less motivated to do anything that requires physical effort						
15 I am less motivated to engage in social activities						
16 My ability to travel outside my home is limited						
17 I have trouble maintaining physical effort for long periods						
18 I find it difficult to make decisions						
19 I have few social contacts outside my own home						
20 Normal day to day events are stressful for me						
21 I am less motivated to do anything which requires thinking						
22 I avoid situations that are stressful for me						
23 My muscles feel much weaker than they should do						
24 My physical discomfort is increased						
25 I have difficulty dealing with anything new						
26 I am less able to finish tasks that require thinking						
27 I feel unable to meet the demands that people place on me						
28 I am less able to provide financial support for myself and my family						
29 I engage in less sexual activity						
30 I find it difficult to organise my thoughts when I am doing things at home or at work						
31 I am less able to complete tasks that require physical effort						
32 I worry about how I look to other people						
33 I am less able to deal with emotional issues						
34 I feel slowed down in my thinking						

	no problem	small problem	moderate problem	big problem	extreme problem	
35 I feel it hard to concentrate						
36 I have difficulty participating fully in family activities						
37 I have to limit my physical activities						
38 I require more frequent or longer periods of rest						
39 I am not able to provide as much emotional support to my family as I should.						
40 Minor difficulties seem like major difficulties						

Appendix B

Hospital Anxiety and Depression Scale (HADS)

GL assessment
the measure of potential

Name: _____ Date:_____

FOLD HERE

Clinicians are aware that emotions play an important part in most illnesses. If your clinician knows about these feelings he or she will be able to help you more.

This questionnaire is designed to help your clinician to know how you feel. Read each item below and **underline the reply** which comes closest to how you have been feeling in the past week. Ignore the numbers printed at the edge of the questionnaire.

Don't take too long over your replies, your immediate reaction to each item will probably be more accurate than a long, thought-out response.

FOLD HERE

A	D		A	D
		I feel as if I am slowed down		
3		Nearly all the time		3
2		A lot of the time		2
1		From time to time, occasionally		1
0		Not at all		0
		I still enjoy the things I used to enjoy		
	0	Definitely as much		
	1	Not quite so much		
	2	Only a little		
	3	Hardly at all		
		I get a sort of frightened feeling as if something awful is about to happen		
3		Very definitely and quite badly		
2		Yes, but not too badly		
1		A little, but it doesn't worry me		
0		Not at all		
		I can laugh and see the funny side of things		
	0	As much as I always could		
	1	Not quite so much now		
	2	Definitely not so much now		
	3	Not at all		
		Worrying thoughts go through my mind		
3		A great deal of the time		
2		A lot of the time		
1		Not too often		
0		Very little		
		I feel cheerful		
	3	Never		
	2	Not often		
	1	Sometimes		
	0	Most of the time		
		I can sit at ease and feel relaxed		
0		Definitely		
1		Usually		
2		Not often		
3		Not at all		
		I get a sort of frightened feeling like 'butterflies' in the stomach		
		Not at all	0	
		Occasionally	1	
		Quite often	2	
		Very often	3	
		I have lost interest in my appearance		
		Definitely		3
		I don't take as much care as I should		2
		I may not take quite as much care		1
		I take just as much care as ever		0
		I feel restless as if I have to be on the move		
		Very much indeed	3	
		Quite a lot	2	
		Not very much	1	
		Not at all	0	
		I look forward with enjoyment to things		
		As much as I ever did		0
		Rather less than I used to		1
		Definitely less than I used to		2
		Hardly at all		3
		I get sudden feelings of panic		
		Very often indeed	3	
		Quite often	2	
		Not very often	1	
		Not at all	0	
		I can enjoy a good book or radio or television programme		
		Often		0
		Sometimes		1
		Not often		2
		Very seldom		3

Now check that you have answered all the questions

	A	D
TOTAL		

Appendix C

The table below shows the individual subjects with SCH who had each test at pre- and post-treatment visits. The explanation of the alphabets in the table is given below, which state the reason why certain individuals did not have tests or why their data not included at each pre- and post-treatment visit.

a=poor scan quality, b=intolerant of MR scan, c=drop out, d=omitted to match the data e=poorly compliant, f=incomplete recording, g=did not complete the tilt test

Recruited	MUSLCE		HEART		ANS		CBF	
Subject No.	PRE	POST	PRE	POST	PRE	POST	PRE	POST
1	1	1	1	1	1	1	1	1
3	3	3	3	a	3	3	a	a
10	10	10	10	10	10	10	10	10
11	a	a	a	a	g	g	11	11
12	12	c	b	b	g	c	b	b
13	13	c	13	c	13	c	d	d
15	15	c	15	c	c	c	b	b
17	17	e	17	e	17	e	17	e
18	18	18	18	18	18	18	18	18
21	21	21	21	21	21	21	21	21
22	22	e	22	e	22	e	22	e
23	23	23	a	a	f	f	23	23
24	24	24	24	24	24	g	24	24
30	30	30	30	30	d	30	30	30
31	31	31	31	31	31	31	31	a
33	33	33	33	33	33	33	33	33
36	36	36	36	36	g	g	36	36
37	c	c	c	c	37	c	c	c
41	41	41	41	41	41	41	41	41
44	44	44	44	44	44	44	44	44
46	46	46	46	46	46	46	46	46
47	47	47	47	47	47	47	47	47
48	48	48	48	48	48	48	48	48
49	49	49	49	49	g	g	49	49
50	50	50	50	50	50	50	50	50
Total								
25	23	18	21	16	18	14	20	17

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