



The effects of excess body weight on the heart and liver

Doctoral Thesis

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Preface

In 2007, Andrew Mitchell suggested that I contact his former colleague, Stefan Neubauer, in Oxford, about possible research into acquired heart and liver disease. Jersey, where I was then working with Andrew, had a high prevalence of cardiovascular and liver disease, stirring my interest. Four months later, Stefan and I met in the John Radcliffe Hospital with Oliver Rider to formulate a research plan to study the pathophysiology of obesity with promising new non-invasive magnetic resonance methods. Many of these methods were then in development, and had not been tested in vivo before. The work described here is born from that meeting, but has taken several tangents, which I hope the reader will understand and enjoy.

Obesity is a multi-system condition, with which we are all increasingly familiar. It is hoped that this thesis is accessible to anyone with an interest in obesity medicine, not just MR physicians and cardiologists.

This doctoral thesis is dedicated to all the volunteers and patients who gave their time so that we could try and better understand why we develop heart and liver disease, in the hope that this research would benefit us.

And you who want to demonstrate with words the form of man, put aside such an idea, because the more minutely you describe it, the more you will confound the mind of the reader and the further you will remove him from understanding of the thing described. Therefore it is necessary both to depict and to describe.

Translated from Leonardo Da Vinci, the first anatomist to dissect a human liver

Declaration

I declare that the work presented in this thesis is my own and has not been submitted for any other degree in this or any other University or Institute of Learning.

The studies described herein were designed by myself, with supervision from Professor Stefan Neubauer and Dr Paul Leeson. All volunteers and patients were recruited and scanned by me. Data for chapters 3 - 8 was derived from these studies. Chapter 3 was a follow-up study from a cohort of patients recruited by Dr Oliver Rider who were invited back from a longitudinal study. Chapter 5 includes 48 patients scanned as part of a collaboration with the Oxford Centre for Diabetes, Endocrinology and Metabolism (OCDEM) – all the liver MR data was acquired and analysed by myself.

The hypotheses tested, ethics applications and study administration were my work and responsibility.

Publications arising from this thesis

Gender-specific differences in left ventricular remodelling in obesity: insights from cardiovascular magnetic resonance imaging.

Oliver J. Rider, Adam Lewandowski, Richard Nethononda, Steffen E Petersen, Jane M Francis, Alex Pitcher, Cameron J. Holloway, Sairia Dass, **Rajarshi Banerjee**, James P Byrne, Paul Leeson and Stefan Neubauer.

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Myocardial Tissue Characterization using Magnetic Resonance Non-Contrast T1 Mapping in Hypertrophic and Dilated Cardiomyopathy

Sairia Dass, Joseph J. Suttie, Stefan K. Piechnik, Vanessa M. Ferreira, Cameron J. Holloway, **Rajarshi Banerjee**, Masliza Mahmood, Lowri Cochlin, Theodoros D. Karamitsos, Matthew D. Robson, Hugh Watkins and Stefan Neubauer

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Tackling Childhood Obesity as a Strategy in Cardiovascular Risk Reduction

Rajarshi Banerjee, Paul Leeson

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Waist circumference greater than 90cm is a risk factor for hepatic steatosis in otherwise healthy adults – a 3 Tesla magnetic resonance spectroscopy study

Rajarshi Banerjee, Belen Rial, Joseph Suttie, Adam Lewandowski, Matthew Robson, Jurgen Schneider, Barbara Fielding, Leanne Hodson, Jane Collier, Eleanor Barnes, Stefan Neubauer

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http://mobile.ilcapp.eu/EASL_161/poster_24643/program.aspx

Cardiac steatosis is associated with excess body weight in otherwise healthy adults

Rajarshi Banerjee, Belen Rial, Joseph J Suttie, Pete Cox, Adam J Lewandowski, Andrew Johnson, Oliver Rider, Cameron Holloway, Jane Francis, Matthew D Robson, Jurgen Schneider, Kieran Clarke, Paul Leeson, Stefan Neubauer

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Weight reduction surgery is associated with substantial long term reduction in left ventricular mass

Rajarshi Banerjee, Jane Francis, Cameron Holloway, Andrew Johnson and Stefan Neubauer

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Cardiac Steatosis Detected in Hypertrophic Cardiomyopathy, Dilated Cardiomyopathy and Aortic Stenosis

Joseph J Suttie; Sairia Dass; Sacha Bull; Cameron Holloway; **Rajarshi Banerjee**; Pete Cox; Alex Pitcher; Belen Rial Franco; Jane Francis; Jurgen Schneider; Matthew Robson; Hugh Watkins; Saul Myerson; Stefan Neubauer

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Abbreviations

BMI	=	body mass index
BP	=	blood pressure
CMR	=	cardiovascular magnetic resonance
CS	=	peak systolic mid-ventricular circumferential strain
D	=	dimensional
DNL	=	de novo lipogenesis
ECG	=	electrocardiogram
EDV	=	end-diastolic volume
EF	=	ejection Fraction
ESV	=	end-systolic volume
FA	=	fatty acid
IQR	=	interquartile range
¹ H	=	hydrogen, or proton
HDL	=	high density lipoprotein
HLC	=	hepatic lipid content
LDL	=	low density lipoprotein
LV	=	left ventricle/ventricular
LVM	=	left ventricular mass
MetS	=	metabolic syndrome
MR	=	magnetic resonance
MRCP	=	magnetic resonance cholangiopancreatography
MRI	=	magnetic resonance imaging
MRS	=	magnetic resonance spectroscopy
MTGC	=	myocardial triglyceride content
²³ Na	=	sodium
NAFLD	=	non-alcoholic fatty liver disease
NASH	=	non-alcoholic steatohepatitis

³¹ P	=	phosphorus
PSC	=	primary sclerosing cholangitis
RF	=	radiofrequency
RV	=	right ventricle/ventricular
SD	=	standard deviation
SEM	=	standard error of the mean
ShMOLLI	=	shortened modified look locker index
SSFP	=	steady-state free precession
STEAM	=	stimulated echo acquisition mode
SV	=	stroke volume
T	=	Tesla
TE	=	echo time
TR	=	repetition time
TSE	=	turbo spin echo
(c)T1	=	(iron-corrected) longitudinal, or spin-lattice, relaxation time constant
VLDL	=	very low density lipoprotein

Abstract

Obesity in adults and children is associated with increased cardiovascular mortality and morbidity. This is forecast to increase markedly in the next decade as childhood obesity is a burgeoning epidemic. Excess weight is clearly associated with insulin resistance, increased circulating triglycerides, and hypertension, all of these are related to progressive heart and liver disease. Ectopic fat deposition within organs is reported to cause lipotoxicity, which may lead to dysfunction and disease, but there have been few human studies to confirm this.

This doctoral thesis set out to study the early pathophysiology of obesity in adults and children using in vivo magnetic resonance (MR) imaging and spectroscopy to assess the composition and function of the heart and liver in lean and obese individuals. The central tenet of this project was to establish and validate a clinically viable method for measuring the fat content of viscera safely and accurately, and to determine a normal range for the triglyceride content of the heart and liver.

The initial study demonstrated that the heart remodels in response to weight loss, with over 20% reduction in LV mass, confirming that excess weight is genuinely a modifiable risk factor. Then, using spectroscopy, it was established that the healthy myocardium has a median triglyceride content of 0.37% (IQR 0.24% - 0.47%), which increases linearly in overweight and obese adults. Obesity, in the absence of any confounders, was also associated with a 10% reduction in cardiac contractile function. In comparison, healthy liver median lipid content was 0.67% (IQR 0.44% – 0.88%), which increased in obese adults to 2.9% (IQR 1.6% - 7.6%). There was a graded association between ectopic fat deposition in the liver and dyslipidaemia in adults, characterised by increased circulating triglycerides and reduced high-density lipoprotein. This dyslipidaemia may impair reverse cholesterol transport, and thus could be expected to exacerbate weight gain. Among obese and overweight subjects, there

were some with severe steatosis and evidence of coexistent hepatic inflammation and fibrosis.

To verify the accuracy of these spectroscopic measures for ectopic fat, a blinded, prospective comparison of non-invasive assessment of unselected liver disease in liver biopsy patients was completed. Liver disease presents with one or more of steatosis, fibrosis and haemosiderosis, all of which are associated with adverse cardiovascular outcomes. Fifty patients were recruited, and interobserver variability among pathologists was measured for histological reference standards for fat, fibrosis and iron deposition. MR measures of each of these metrics predicted the fibrosis, steatosis and haemosiderosis scores accurately. This enabled precise tissue characterisation of all forms of liver disease, including steatohepatitis, with one non-invasive test, to allow the diagnosis and monitoring of hepatic conditions.

Lastly, all these new biomarkers of early cardiac and liver disease associated with excess weight were applied to obese and lean children, to understand whether ectopic fat played a substantial role in early life. Obese children had increased ectopic fat in their hearts and livers, as well as impaired strain, evidence of dyslipidaemia, and in some cases evidence of active steatohepatitis, comparable to adults with severe disease.

The thesis therefore demonstrates that in vivo magnetic resonance techniques can be used for accurate measurement of visceral lipid content. Furthermore, there is evidence of significant ectopic fat deposition in both adults and children, with evidence of organ dysfunction, which raises the possibility that cardiovascular magnetic resonance may be of value to risk stratify obese individuals based on organ involvement. Finally, the developed methods may have broader applicability and offer a promising new method for the non-invasive diagnosis of chronic liver disease in other clinical settings.

Chapter 1: Introduction

1.1 Obesity and cardiovascular health in the 21st century

Obesity is a major worldwide health problem. Previously considered a disease of affluence, it is now apparent that many social, economic and political factors converge to create an ‘obesogenic’ environment which promotes behaviours resulting in excess calorific intake.^{1, 2} Increasing prevalence of obesity has been found in developing world societies as well as Western Europe and the USA. Much of this excess mortality is through cardiovascular disease, specifically coronary artery disease, heart failure and stroke.³

1.2 Definitions of obesity

Adults

Obesity in adults is conventionally defined by thresholds in the body mass index (BMI), which is calculated as $\text{Weight (kg)} / \text{Height (m)}^2$. The World Health Organisation (WHO) has defined underweight as $\text{BMI} < 18.5\text{kg/m}^2$, normal weight $18.5 - 24.9\text{kg/m}^2$, overweight $25 - 29.9\text{kg/m}^2$ and obese as $\geq 30\text{kg/m}^2$.⁴ Obesity can be further subclassified as severe ($\geq 35\text{kg/m}^2$) and morbid ($\geq 40\text{kg/m}^2$). There is a J-shaped curve between cardiovascular morbidity and BMI that corresponds to these categories.⁵ This has led to obesity becoming a risk factor for cardiovascular disease, and epidemiological surveys have confirmed excess body weight as a leading cause of preventable death.⁶ Importantly, it is seen as a preventable risk factor, like smoking. Excess body weight is also modifiable, although in clinical practice this is difficult to achieve, much like smoking cessation.

Childhood

At present there is no clear global consensus on the definition of childhood obesity. Children are routinely weighed and measured in infancy in most healthcare systems. Traditionally this was to ensure that there is no ‘failure to thrive’, although the scope of malnutrition has now come to encompass overfeeding. Growth charts for boys and girls are used as the basis for most assessments, with overweight usually being >85th centile, and obese >95th centile. The reference data may have come from a local, national or an international population.⁷ Use of data from the same population as a child has great inherent advantages at a local level in showing trends across time periods, but comparisons between regions or nations become difficult. In the UK, the National Child Measurement Programme has been set up in schools at reception (age 4-5) and at Year 6 (age 10-11), and uses a national reference chart from 1990, which is different from the growth charts used in the USA or the World Health Organisation. Moreover, growth curves and patterns of obesity vary between races, and in an increasingly multicultural society this is important.⁸ The WHO has addressed this in redefining BMI ranges for weight in different races in adults, but not yet in children.⁹

1.3 Obesity in medical history

Whilst obesity and cardiovascular disease have clear associations in the present day, this was not always the case. Corpulence was relatively rare in ancient times, and largely limited to the aristocracy, such that many praised it. Some, however, noted that it could lead to ill health. Hippocrates, in 400BC, was one of the first to state this:

‘It is injurious to health to take in more food than the constitution will bear, when at the same time one uses no exercise to carry off this excess...’

*Translated from Hippocrates, Greek physician circa 400BC*¹⁰

As prevalence increased, more writers and physicians became interested in larger bodies. The word ‘obesity’ was first used to advertise the healing properties of the water in Bath, Somerset:

‘...wherefore let those that feare obesity (that is, would not wax grosse), be careful to come often to our Baths; for by the use of them... keep their bodies from being unseemly corpulent.’

*Tobias Venner, English physician 1660*¹¹

Several famous physicians went on to catalogue the ill health associated by obesity, including the anatomist Joannes Morgagni, who was the first to describe ectopic fat from dissected post-mortem studies,¹² and William Osler, who listed it as a causative factor of angina pectoris.¹³ By the early 19th century, much of our modern understanding of ectopic fat was already accepted. In this description from 1811, Robert Thomas perfectly encapsulates our current understanding of diaphragmatic splinting from abdominal obesity:

‘Corpulency, when it arrives at a certain height, becomes an absolute disease. The increase of the omentum particularly, and the accumulation of fat about the kidneys and the mesentery, swell the abdomen, and obstruct the motions of the diaphragm; whence one reason of the difficulty of breathing, which is peculiar to corpulent people.’

*Excerpt from ‘The Modern Practice of Physic’ 1811*¹⁴

1.4 Pathophysiology of excess body weight

The pathophysiology of excess weight has been more difficult to determine in the two centuries since Dr Thomas' tome, and even today is a controversial area. Fat deposits have mechanical, endocrine, paracrine, psychiatric and metabolic effects on the body. Physical illness can be manifest directly (eg mechanical wear leading to accelerated osteoarthritis of the knee), or, as is often the case, through intermediate disease states brought on by obesity, such as diabetes mellitus, hypertension, dyslipidaemia, and chronic inflammation. All these processes promote atherosclerosis, and this has been the commonest explanation for the increased cardiovascular mortality seen in obese adults. This may be further augmented by behavioural change and psychiatric illness from obesity, which are much harder to quantify. For example, playground bullying of obese children can lead to low self-esteem, depression, 'comfort-eating' and an aversion to physical activity, leading to further weight gain. Moreover, depression, isolation and sedentary behaviour are all independently associated with increased cardiovascular mortality. Thus obesity presents a complex picture with several conventional, well-established risk factors alongside more qualitative markers.

The Metabolic Syndrome (MetS) was defined in appreciation of the clustering of the physical risk factors in obesity, and has replaced the vague 'Syndrome X'.¹⁵ Using the criteria established by the National Cholesterol Education Panel (NCEP III), the MetS is currently defined as three or more of the following:¹⁶

- 1) waist circumference for men greater than 102 cm and for women greater than 88 cm
- 2) fasting triglycerides ≥ 150 mg/dL, or ≥ 1.7 mmol/L
- 3) high density lipoprotein (HDL) cholesterol ≤ 40 mg/dL (≤ 1.0 mmol/L) for men and ≤ 50 mg/dL (≤ 1.3 mmol/L) for women
- 4) blood pressure greater than 130/85mm Hg
- 5) fasting plasma glucose of ≥ 110 mg/dL, or ≥ 6.1 mmol/L

All of these criteria are measured on continuous scales, and setting thresholds for normal or healthy ranges can be difficult. With HDL, for example, there is much published data to support the hypothesis that values greater than 1.5 mmol/l confer protection from cardiovascular disease.¹⁶ However, as the prevalence of MetS is between 34 and 42% in the USA, population surveys are biased towards lower values.¹⁷ Even if patients with a confirmed MetS diagnosis are excluded, the prevalence of overweight adults in the USA still brings down the mean HDL value for almost any general population. When HDL is measured in a 'healthy' population, such as 4014 men with normal lipid profiles from the EPIC cohort in Norfolk, the mean $\pm$ standard deviation obtained is 1.4 ± 0.3 mmol/l. Continuing on the spectrum of health, male endurance athletes have 40% higher HDL than sedentary, otherwise healthy men.¹⁸ Thus for a silent biomarker such as HDL we can have targets and arbitrary thresholds for elite health (or aspirational health), normal (meaning free from disease or heightened risk), and illness. Moreover, HDL is relatively easy to measure and reproduce using different assays; in other biomarkers, these added sources of error become very important.

The role of abdominal obesity and ectopic fat has been of great interest to the clinical and research communities in the past decade as the prevalence of MetS has increased markedly.¹⁹⁻

²³ For example, the prevalence of hypertriglyceridaemia in adolescence has increased from 4% of US adolescents to 17%, and there has been a doubling in the prevalence of adolescent hypertension.²⁴ Similar epidemics of obesity-related illness are reported in several epidemiological surveys of adults and children throughout Europe – table 1.1 shows an optimistic estimate of the burden of childhood disease in the EU currently.

Table 1.1. The burden of childhood obesity in Europe		
Obesity related cardiovascular risk factor	Lowest estimated prevalence in obese children (%)	Lowest estimated number of obese children in EU countries (2006)
Hypertension	21.8	1.11 million
Low HDL	18.7	950 000
High LDL	18.9	960 000
Raised triglycerides	21.5	1.09 million
Impaired glucose tolerance	8.4	420 000
Metabolic syndrome (≥ 3 of the above risk factors)	4.6	130 000
Hepatic steatosis	27.9	1.49 million
Type 2 diabetes	0.5	27 000

Even in the ‘best case scenario’, a fifth of obese children have significant acquired risk factors before adulthood, and **4.6%** have metabolic syndrome, with markedly increased risk of progression to heart disease.²⁵

Adapted from Lobstein T and Jackson-Leach RJ, Int J Paediatric Obes 2006; 1: 33-41

The clustering of these risk factors makes it difficult to study the early effects of obesity *per se* on cardiometabolic risk, and thus the attributable physiological manifestations of excess body weight and fat mass. There is a wide choice of possible surrogate end-points, representing either progression or regression of obesity or related disease. These include measures of weight (BMI), anthropometrics (waist circumference, hip circumference, waist:hip ratio, skinfold thickness), exercise capacity (peak oxygen consumption, maximal heart rate), gross anatomical change (cardiac dimensions, left ventricular mass, liver size, adipose tissue depot size), microscopic anatomical change (degree of steatosis on liver or muscle biopsy, size of adipocytes from adipose tissue biopsy), serum markers (glucose, insulin, lipid profile), metabolic flux (glucose tolerance test, lipoprotein synthesis rate), organ function (left ventricular ejection fraction, blood pressure, flow-mediated brachial artery dilatation, Epworth Sleep Score) and systemic well-being (SF-36 multi-dimensional health score). Any or all of these can be measured in combination.

As weight or BMI *per se* are poor measures of individual cardiovascular risk in adults and children, there has been increasing recent interest in the use of specific biomarkers of ‘cardiovascular health’. The most common pathological features of excess weight in adolescence and early adulthood are hepatic steatosis, hypertension and hypertriglyceridaemia. The mechanisms by which these three, in the absence of confounding factors, lead to disease are not entirely clear. LV mass is a well established surrogate endpoint for hypertensive heart disease, that is relatively easy to measure, and is known to increase with body size.²⁶ However, this hypertrophy may not occur in the early stages of obesity, and we do not know how modifiable it is, or what the ‘extra tissue’ is comprised of. Hepatic steatosis has only recently been amenable to accurate quantification using MR spectroscopy, first described in 1993.²⁷ Since then, it has become recognised as a key

cardiovascular risk factor, associated with a doubling in the prevalence of coronary artery disease.²⁰ Lastly, hypertriglyceridaemia is a classical risk factor, and can herald severe dyslipidaemia and atherogenesis, but we do not yet know how best to prevent or treat it.^{28, 29}

This thesis discusses the results of a series of proof-of-principle studies looking at heart and liver disease in patients. The aim is to refine our understanding of how obesity, through LV hypertrophy, hepatic steatosis and dyslipidaemia, leads to cardiovascular disease, and whether there is in fact a phenotypically distinct ‘obese heart’. The cardiac section was designed to study the early effects of excess weight in people with no known specific heart disease or other risk factors for atherosclerosis. It is hoped that this will shed light on the early pathophysiology of MetS, with minimal confounding. The liver section was designed to investigate the importance of weight in hepatic health, and validate new methods and biomarkers in the clinical assessment of liver disease. Finally, the children’s section describes a small study looking at the effects of adolescent obesity on cardiometabolic health.

1.5 The perils of excess weight – is it really so dangerous?

Popular print and televisual media frequently have updates on the threat to public health for the obesity epidemic, as well as individual stories of morbidity from extreme obesity.³⁰⁻³² In the medical community, it is well recognised that excess body weight is associated with poor health.³³ Although there are some inherited conditions associated with excess weight, such as congenital leptin deficiency or Prader-Willi syndrome, in over 90% of overweight patients the primary cause is an imbalance between calorific intake and energy expenditure, as described by Hippocrates earlier.^{34, 35} Most overweight and obese patients do not fall into the extreme phenotype of morbid obesity, with a body mass index greater than 40kg/m². Moreover, the accumulation of excess weight in an individual is a gradual process, so physiological and behavioural adaptations occur to accommodate it, such as wearing looser clothes or avoiding stairs. Each generation in the Western World seems to be heavier than their parents, a phenomenon of ‘passive obesity’, which leads to societal acceptance of larger phenotypes.¹ It is unfortunate that the MetS risk factors are mostly ‘silent’, in that hypertension, dyslipidaemia and gradual weight gain have no symptoms, in stark contrast to conditions like malaria, asthma or psoriasis, where early symptoms of systemic or organ dysfunction allow prompt treatment if healthcare is accessible. Thus the majority of overweight individuals do not perceive excess weight as a ‘health issue’, as there is no clear attributable disease.

Epidemiological surveys suggest otherwise – obesity is associated with increased asthma, gastro-oesophageal reflux disease, gallstones, osteoarthritis, early dementia, depression, certain cancers (including breast and endometrial) and chronic fatigue, as well as

cardiovascular disease, MetS and the complications of type 2 diabetes mellitus.^{17, 36, 37} The greatest economic cost from obesity is reportedly from increased lower back pain.³⁸

As with many acquired risk factors for disease, early prevention is seen by the medical community as being the most effective strategy for reducing morbidity.³⁹ This is further emphasised by our lack of treatment options – at present there is only one drug licensed for use for weight loss as an adjunct to lifestyle measures, and it has limited longterm efficacy.⁴⁰ Public health measures to reduce calorific imbalance are controversial. In a free-market society, potential constraints on consumption have many antagonists, including libertarians, the food production and retail industry, the motor industry, and other vested interests. A recurring argument is that excess weight in itself is not necessarily disadvantageous. In a variety of conditions, including heart failure and lung cancer, being overweight at the time of diagnosis seems to confer a survival advantage over being lean, the so-called ‘obesity paradox’.⁴¹ Furthermore, cardiovascular fitness, body fat content and distribution are of more importance than body weight *per se*, albeit harder to measure and stratify.^{42, 43} Thus we are left with a need to distinguish ‘healthy obese’ from ‘unhealthy obese’, and to determine why excess weight is harmful to some and not to others.³⁶ Nevertheless, the public health message should be clear – obesity is associated with significantly increased morbidity and mortality.³ Obese men and women are likely to have their life shortened by a median of 2-4 years, which rises to 8-10 years in the morbidly obese. Until we can identify who is at most risk, and separate them from individuals who can gain weight ‘safely’, all obesity should be treated with caution.

1.6 Early organ dysfunction

Much research has already been done, and it is interesting to note how scientists in genetics, epidemiology, early development, sociology, education and public policy have all separately approached the obesity epidemic.^{1, 35, 44-49} MetS is no longer seen as an arbitrary scoring system of risk factors for heart disease, but as a phenotype in itself with a high rate of liver disease, stroke, coronary artery disease and diabetic complications.^{21, 50} Biomarkers for MetS, such as waist circumference, serum lipid profile, glucose and insulin, are relatively easy to measure and compare, enabling research into different therapies that may reduce morbidity. However, we do not have many biomarkers for specific organ dysfunction resulting from MetS. Diabetic cardiomyopathy, severe steatohepatitis with fibrosis, obstructive sleep apnoea and transient cerebrovascular ischaemia are all sequelae from obesity with MetS, and a deeper understanding of the pathophysiology of early disease phenotypes is desirable. This would inform us about how and whom we should treat, and how public policy and health promotion strategies, coupled if necessary to screening programmes, might prevent obesity-related disease. Table 1.2 shows current diagnostic tests for these weight-related conditions.

Table 1.2. Diagnostic tests for conditions associated with excess weight and the metabolic syndrome				
Obesity-related condition	Serum markers	Clinical investigation for early disease	Clinical investigation for late disease	Research tools
Cerebrovascular ischaemia	C-reactive protein ⁵¹	Carotid artery ultrasound ^{52, 53} CT ⁵⁴	CT, MR	Functional and perfusion MR ⁵⁵
Diabetic cardiomyopathy (not including coronary artery disease)	Brain natriuretic peptide ⁵⁶	Echocardiography ⁵⁷	CMR PET Angiography	Collagen synthesis markers ⁵⁸ Matrix metalloproteinases CMR PET
Fatty liver disease ± fibrosis	AST and ALT ^{59, 60}	Ultrasound Liver biopsy	Liver biopsy, transient elastography	Collagen synthesis markers ^{61, 62} Matrix metalloproteinases
Obstructive sleep apnoea	ALT and AST ⁵⁹	Questionnaire ^{63, 64} Sleep study ^{65, 66}	Pulsoximetry, arterial blood gas analysis	Biosensors and portable monitoring ⁶⁷

Abbreviations: AST aspartate transaminase; ALT alanine transaminase; CT computed tomography; PET positron emission tomography

Serum markers have been investigated widely, and markers of inflammation, cell damage and collagen turnover are implicated in several diseases, as shown in Table 1.2. Unfortunately, they all lack specificity, and rarely provide a clear diagnosis that can dictate management in obesity-related diseases. There are a few clinical investigations that identify these conditions early. Of these, carotid ultrasound is used routinely in acute transient ischaemic attack patients, but neuroimaging for early cerebrovascular disease has not yet developed biomarkers for clinical application. Computed tomography can detect calcification of major cerebral arteries, but the dose of ionising radiation is too great, and the findings too non-specific, for this to be a useful screening tool.⁵⁴ Diabetic cardiomyopathy is defined as the presence of hypertrophy with either diastolic dysfunction or left atrial enlargement in the absence of significant coronary artery disease.^{57, 68} Most imaging studies of cardiovascular function use ultrasound-based techniques to measure a wide range of clinical end-points, such as ventricular volumes, systolic and diastolic function, carotid intima-media thickness, arterial waveforms, and cardiac output.^{26, 53, 69-76} Echocardiography is safe, repeatable, widely accessible, and can identify systolic and diastolic dysfunction, but lacks sensitivity, especially in large patients, and so there is a need for a better 'gold-standard'. Obese subjects are not suited to ultrasound examinations in general because of poor acoustic windows from thoracic wall and subcutaneous adipose tissue depots, and this disadvantage is even more marked in the largest patients. Magnetic resonance imaging, on the other hand, does not suffer from poor acoustic windows, although studies are limited to subjects who can fit into a conventional 60cm bore diameter. Over the past decade, there have been many MR studies into the effects of obesity, which have demonstrated the superior accuracy of this technique for the assessment of many clinical end-points previously measured with ultrasound.⁷⁷⁻⁸⁴ Magnetic resonance has become the optimal imaging modality for obesity research, and larger bore magnets are being constructed to facilitate this. Cardiac MR is very promising

here in that it allows assessment of function, anatomy, fibrosis, perfusion, metabolism and tissue oxygenation, thus enabling detailed phenotyping of the diabetic heart.⁸⁵

In fatty liver disease and steatohepatitis, ultrasound can detect excess fat by measuring the echogenicity of the organ, but lacks sensitivity, especially in the obese, and cannot assess fibrosis. Liver biopsy is routine practice, but costly and invasive. Moreover, there is considerable interobserver variation and sampling error from a needle biopsy.⁸⁶ Thus there is considerable research into new techniques to assess early liver inflammation and fibrosis, for chronic infective and inflammatory conditions as well as obesity-related illness.⁸⁷ With obstructive sleep apnoea (OSA), diagnosis of overnight hypoxia is easier to measure and verify, but remains time-consuming and costly. Importantly, as it is the only clearly symptomatic condition in Table 1.2, patient-based questionnaires allow reasonable diagnosis, such as the Epworth Sleepiness Scale, first developed in 1991.⁶³ This is a cost-effective and patient-centred form of diagnosis, with the added advantage of patient feedback and education, unlike serum markers and complex imaging.

Close inspection of Table 1.2 shows that in obese patients, the utility of diagnostic tools is blunted as they are ultrasound-based (echocardiography and carotid artery assessment), non-specific (CT, serum markers) or invasive (liver biopsy). Sleep studies are the exception, but they are costly and time-consuming to perform at present. Magnetic resonance methods, as well as physical and clinical associations with disease, are much more promising – the former does not use ionising radiation, and is therefore suitable for younger patients, whilst the latter is ideal for population screening, albeit difficult to standardise. Despite cost and accessibility issues, it may transpire that MR is the safest and most effective diagnostic modality in the assessment of the obese patient. With standardised acquisition and analysis software, MR may also provide the most cost-effective methods in comparison with other technologies.

1.7 Cardiovascular consequences of obesity

Obesity has been associated with ventricular hypertrophy, independently of age and blood pressure. This was first demonstrated by M-mode echocardiography in 3922 healthy subjects from the Framingham study in 1991.⁸⁸ LV mass, wall thickness and internal diameter were all positively associated with BMI. LV mass is conventionally indexed to body surface area to 'correct' for this. Similar changes in LV mass with concentric hypertrophy have been described in adolescent obesity and in overweight adults.^{72, 89, 90} This hypertrophy is accompanied by a reduction in contractile function, measured by echocardiographic strain. Some have made an argument for the existence of 'obesity cardiomyopathy' due to myocardial structural and functional alterations related to insulin resistance and to LV volume overload.⁷⁰ However, it is controversial to label a large proportion of the population as having a defined cardiomyopathy with preserved ejection fraction and, in many cases, no overt symptoms. Nevertheless, the 'obese heart' may also be a step on the continuum from the healthy/athletic heart to overt disease or diabetic cardiomyopathy, much like a HDL value of 1.1-1.3 mmol/l. The 'diabetic heart' has many features that suggest progression from obesity. Firstly, there is hypertrophy, with contractile dysfunction, despite normal coronary artery perfusion. Secondly, the diabetic heart has been found to have a higher myocardial triglyceride content compared to lean myocardium.⁹¹ This is thought to be due to altered metabolism in the diabetic heart, with increased fatty acid (FA) usage and reduced glucose and lactate oxidation.^{85, 92} It is likely that myocardial FA uptake exceeds oxidation rates, thereby resulting in lipid accumulation in the myocardium that may promote lipotoxicity. This has been reported by several groups, and occurs at an early stage, in the absence of any coronary ischaemia.⁹³ Importantly, obese patients are also reported to have measurable and significantly increased myocardial steatosis, and also to have altered myocardial energetics

during exercise, suggesting that they may also have altered metabolism.^{94, 95} Thirdly, there is impaired cardiac filling, especially in the supine position.⁹⁶ This may be due to haemodynamic factors and filling pressures of the ventricles, and/or because of higher myocardial wall thickness and fibrosis, with a resultant reduction in elasticity. Finally, although the definition of diabetic cardiomyopathy excludes coronary artery disease, many diabetic patients with contractile dysfunction will have some degree of myocardial hypoperfusion in stress, even with normal calibre arteries. In a recent adenosine perfusion single-photon emission computed tomography (SPECT) study from the Netherlands, of 120 asymptomatic diabetic patients, one third had significant abnormalities, and one quarter had clear myocardial perfusion defects.⁹⁷

Promisingly, weight loss has been shown to improve cardiac anatomy and function in the short term. Rider and colleagues showed that dietary weight loss and bariatric surgery could both reduce LV mass in patients over one year with no other cardiac risk factors.⁹⁸ Studies in the USA have yielded similar results, and the cardiac remodelling appears to persist for at least two years.^{77, 79} In these trial settings, excess weight does therefore appear to be a genuinely modifiable risk factor. However, early studies suggest that myocardial triglyceride content does not fall with weight loss, despite functional improvement of the heart.⁹⁹

There are many vascular consequences from obesity, with interlinked mechanisms. Fundamental to these are increased circulating volume, increased arterial pressure and increased endothelial shear stress. Consequently, obesity is associated with reduced aortic elasticity, and impaired endothelial function, both early markers of hypertensive disease.^{73, 81} These can then be further augmented by atherosclerosis, leading to reduced vascular compliance, further endothelial dysfunction, and end-organ hypoperfusion. The measurement of blood pressure in obese patients presents specific problems relating to the position of the

subject during measurement, the choice of cuff, and observer variation in auscultation – these can be overcome by adequate standardisation and appropriate sample size.

1.8 Hepatic consequences in obesity

One of the first sites of ectopic fat deposition with positive energy balance is the liver. Animal models of obesity have demonstrated clear associations between dietary weight gain and hepatic steatosis. In vivo human measurement has been available for almost two decades now, but is still not in routine clinical use. The first large cohort studies to study hepatic steatosis were based in Texas and Finland.^{100, 101} The Dallas Heart Study examined over 2000 patients, including 345 non-obese subjects with no cardiac risk factors, and determined the upper limit of normal liver fat to be 5.5% by ¹H MRS. From this, it was estimated that 34% of the Dallas general population had significant hepatic steatosis. The Finnish study examined 132 non-diabetic adults, and showed that liver fat correlated with intra-abdominal visceral fat accumulation and serum insulin. However, neither study determined the ‘healthy’ range for hepatic steatosis. Subsequently, a large cross-sectional study in London showed that in lean adults, the value for hepatic lipid content was much lower than in the Dallas or Finnish cohorts.¹⁰² Liver fat content has been shown to be as closely related to cardiometabolic risk factors as visceral fat content, and has even been labelled ‘the missing risk’. The arguments to back this are compelling. Firstly, hepatic insulin resistance can drive systemic insulin resistance, whilst simultaneously permitting high levels of gluconeogenesis. In type 2 diabetic patients, it is the hepatic lipid content that is the best predictor of insulin requirements.¹⁰³ Thus hepatic steatosis can lead directly to hyperinsulinaemic hyperglycaemia. Secondly, high liver fat states are associated with low-density-lipoprotein

(LDL) and very-low-density-lipoprotein (VLDL) overproduction, resulting in dyslipidaemia, and potentially driving the altered substrate utilisation seen in the myocardium.¹⁰⁴ In the words of Claude Bernard, the French 19th century physician who discovered glycogen and coined the term ‘homeostasis’:

“The liver is a factory that produces lipids and glucose in excess, both products being hallmarks in diabetes and hazards for vascular wall.”

Claude Bernard, first Chair of Physiology at the Sorbonne University, 1871.

The pathophysiology of hepatic steatosis is poorly understood, mainly because of the lack of clinical and experimental tools for in vivo studies discussed above. Previous studies have shown that simple steatosis can progress to low grade inflammation (steatohepatitis, or NASH), cirrhosis and finally to overt liver failure.^{105, 106} Between a quarter and a third of urban populations are overweight and have fatty liver disease, as suggested by Japanese and American studies.^{100, 107} Approximately one quarter of these will go on to develop steatohepatitis, and an as yet unknown fraction will go on to cirrhosis. These risk ratios will increase for any individual with alcohol misuse or exposure to hepatotoxic drugs and pathogens (Figure 1.1). All patients with fatty liver disease will have a high risk of cardiovascular morbidity, but whether this is due to accelerated MetS, or due to complications from progressive liver failure, such as disordered coagulation, is not known.²¹ In patients with liver disease from other causes than obesity, cardiovascular disease is still the commonest cause of death, suggesting that liver dysfunction exerts metabolic influence independently of body size.

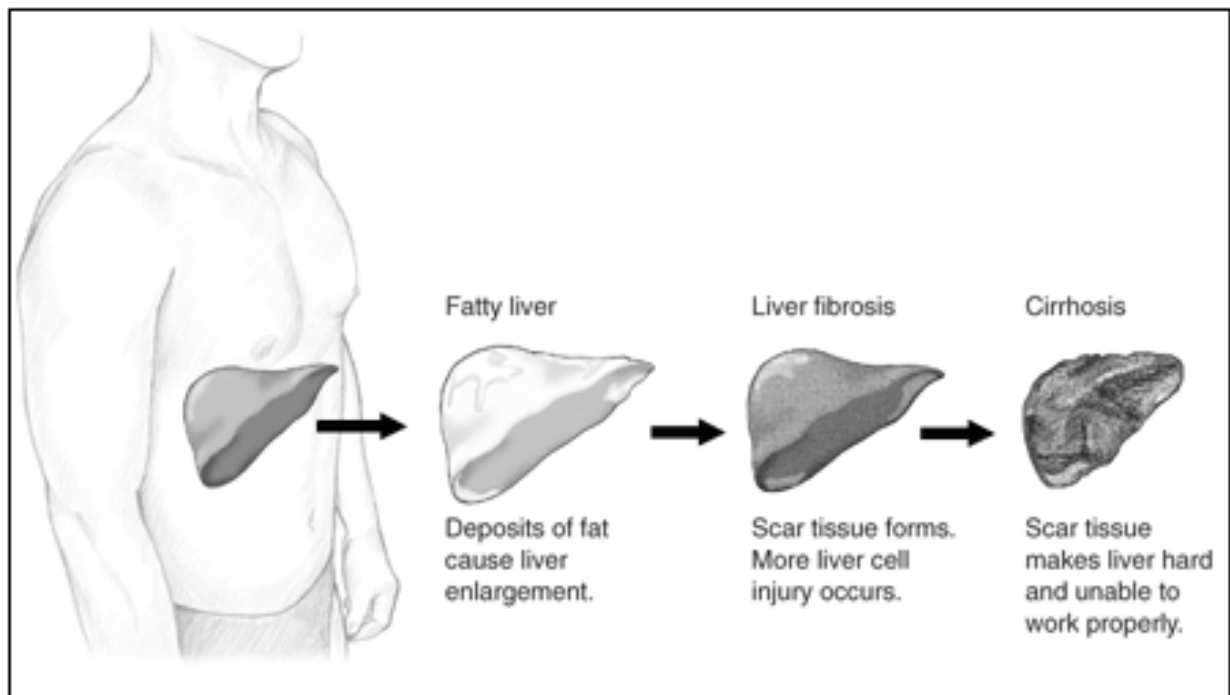


Figure 1.1. Progression of fatty liver disease

Approximately 25 – 35% of the general urban Western populations have fatty liver disease; ¼ of these will develop steatohepatitis, and of these some will develop cirrhosis.

Source: <http://protectyourliver.com/fatty-liver-disease/>

Of patients with fatty liver disease, it is currently very difficult to determine who has steatohepatitis, especially at an early stage. Much work has been done to try and resolve this, which will be discussed in further detail in Chapter 6. At present, the only clinical or research tool to establish a diagnosis of steatohepatitis as distinct from simple steatosis is a liver biopsy.

1.9 What interventions to use?

Many therapeutic strategies have been tried to combat the obesity epidemic. Health economists argue that we cannot wait for epidemiological data before making plans to address obesity, as the attributable burden of disease is growing in the meantime. Preventive measures are thought to be more effective than curative measures, especially in the absence of any non-surgical adult therapies with proven efficacy. Naturally, therefore, most resources are focussed on arresting the epidemic of childhood obesity.

Interventions to reduce childhood obesity range from individual behavioural treatment, group-work, school interventions, and public health campaigns. None of these have had unequivocal cost-effective success. Some school-based measures have been shown to be effective in reducing childhood weight in cluster-randomised trials, but no more so than in control groups (although crossover effect may have occurred).¹⁰⁸ This may be due to the fact that there are a variety of obese phenotypes. Some children are greater than the 95th centile for their age and gender matched weight because of ethnicity, variations in growth spurts and puberty, or athletic training – none of these confers any cardiometabolic risk, and some may indeed be protective. Others may be obese, unfit, and have marked insulin resistance, but remain below the 95th centile based on their BMI alone. Thus BMI charts are of limited value in children when used in isolation, and BMI reduction is a poor goal to aim for as a primary endpoint in intervention studies.

1.9.1 Diet

Several diets have been tried in different settings, but the principal message is not what children should eat, but what they should *not* eat – high calorie beverages, refined carbohydrates, excess dietary fat, and, most importantly, excess portion sizes. Sugar-sweetened beverages in particular have been studied extensively – a double-blinded randomised controlled trial in 641 healthy schoolchildren from the Netherlands suggested that reducing calorific intake from this source reduced weight gain over an 18 month period.¹⁰⁹ A similar study in obese American teenagers, however, found no appreciable weight loss from the elimination of sugar-sweetened beverages over two years.¹¹⁰ This is in keeping with previous studies which have shown that, up to the age of 14, dietary intervention alone is not effective at weight maintenance in overweight children. The wider debate includes the role of the food and advertising industries in addressing children's access to energy-dense foods (eg school-vending machines).

1.9.2 Exercise and sedentary behaviour

Current guidelines suggest a minimum of one hour's moderate intensity activity per day for children. Several exercise strategies in obese children have been shown to be beneficial, leading to a reduction in fat mass, lower insulin resistance and reduced blood pressure. However, formal exercise training depends entirely on the participation of the child and concordance, so the generalisability of trial data to our schools is questionable. In studies, overweight children are more likely to exercise when separated from normal, leaner, children. When playing with normal weight peers, overweight children expend more energy on a given locomotor activity, such as running, and also suffer increased biomechanical strain. This leads to early fatigue, and they are thus less likely to fully participate. Thus any prescribed

exercise programme for obese and overweight children must be tailored to them specifically. The type of exercise that best reduces obesity is still being debated. Strength training, aerobic exercise and high intensity interval training are all beneficial in different ways. Farpour-Lambert et al studied the effect of an additional 60 minutes of mixed intensity exercise on obese 6 – 11 year olds over 3 months and found quantifiable reduction in arterial stiffness, blood pressure (7mmHg systolic) and visceral adiposity, but BMI did not change as excess fat mass was replaced with muscle.¹¹¹

As well as formal exercise, the role of the physical environment in promoting or reducing activity has been studied. A recent study used GPS surveillance at 5 minutely intervals combined with accelerometry data in 100 children to determine where and when they were most active.¹¹² Their findings show that children tend to be active close to home, with 63% of strenuous physical activity occurring inside neighbourhoods. Amongst urban children, gardens (28%) and the local streets (20%) were the most commonly used environments for physical activity. Amongst rural children farmland (22%) and grassland (18%) were most frequently used. Technological developments, improved transport, urbanisation and changes in social play and interaction have created an ‘obesogenic environment’ – a child growing up in an inner city flat on a busy street with no garden is at risk from this environment, and it is no surprise to find a higher prevalence of childhood obesity in urban areas compared to rural areas. To counter this, simple measures such as walking to school, limiting television viewing and enabling active play (eg youth clubs) have been successfully promoted. Indeed, interventions to reduce sedentary activity have been more successful at arresting weight gain than dietary interventions, and the benefits are more sustainable in the longterm.¹¹³ As with diet, the message of what *not* to do in terms of staying sedentary is clearer than determining exactly what to do.

1.9.3 Family and Behavioural approaches

Treating obese children necessitates involvement of the family as, in practical terms, the diet of the child is dependent on the food provided by the parents or guardians. Family support also enhances and prolongs lifestyle changes that may lead to sustained weight maintenance. The child also acts as an agent of change in promoting healthier eating and more activity amongst other members of the family, fostering a positive cycle of health promotion.

1.9.4 Medical management and bariatric surgery

Numerous once-promising weight-loss drugs have been abandoned because of serious toxic effects: aminorex (which caused pulmonary hypertension), fenfluramine and dexfenfluramine (valvulopathy), phenylpropanolamine (stroke), rimonabant (suicidal ideation and behaviour), and most recently sibutramine (myocardial infarction and stroke). The removal of sibutramine from the market in 2010 left orlistat as the only prescription drug approved for the long-term treatment of obesity in adults and children.^{114, 115} A randomised controlled trial in 539 obese American adolescents showed only a 0.5kg weight gain over one year with the drug compared to a 3kg gain with placebo.¹¹⁶ Interestingly, both groups had dietary advice, behavioural therapy and a formal exercise programme, and yet the placebo arm was still not successful at weight maintenance. Orlistat is safe, but up to 50% of patients get gastrointestinal symptoms while on it, especially if they continue on a high fat diet.

To date, no randomized controlled trials have addressed the long-term safety and efficacy of bariatric surgery in severely obese adolescents.¹¹⁷ Long-term data are needed to show safety and efficacy of surgical treatments during the period of active growth and maturation, especially as these interventions can cause micronutrient depletion and malnutrition.

1.9.5 Multidisciplinary management

Obesity is a phenotype with many interacting factors, and the relative importance of each of these variables is difficult to assess, especially in children. As such, patient management lends itself to a combined approach involving diet, exercise, behavioural support, medical therapy and surgery when warranted. As a chronic condition, the model of care should be similar to modern adult heart failure management – referral to a multidisciplinary team for thorough initial assessment and investigation, an initial plan of treatment, regular follow-up via specialist nurses and clinics, with continued focus on patient education so that the child and family learn to manage the condition independently, with expert clinical support available when needed. This is similar to paediatric asthma and diabetes management as well. Unfortunately, no European health system has yet managed to implement such a system for weight management despite promising data from pilot studies.

1.9.6 Politics and Awareness

The HEALTHY study was a recent large, multi-component, school-based weight loss intervention in 4603 6th grade children (age 11 - 12) in the USA.¹⁰⁸ From 42 schools, 21 were randomised to have the intervention which consisted of improved dietetics, increased physical activity, behavioural training and social marketing. The remaining 21 schools acted as controls. After two years, there was no significant difference between the two groups, but both the controls and the intervention schools had a 4% reduction in overweight and obese pupils. As the schools were in the same areas, this would suggest that awareness by pupils, teachers and families of the study contributed hugely to the successful reduction in obesity. Far from being a negative study, this result has renewed political and academic will to raise awareness with local and national campaigns.

1.10 The role of ectopic fat deposition in early Metabolic Syndrome

A large body of work on obesity and cardiovascular disease has already established certain physical traits associated with poor outcomes, including left ventricular hypertrophy (LVH), increasing waist circumference, hypertension, fatty liver disease and ectopic fat deposition, chronic inflammation, abnormal lipid profiles and insulin resistance.^{91, 100, 118} To investigate early pathophysiology attributable to excess weight only, it was necessary to develop methods that could determine phenotypes associated with obesity, and use them in subjects with minimal confounding factors. With its excellent safety profile, reproducibility, flexibility to measure different biomarkers and precision, magnetic resonance was, and remains, the most useful investigative tool for obesity research. Chapter 2 will briefly review the history of magnetic resonance imaging and spectroscopy and introduce the conventional and new techniques used in the five studies described in this thesis.

Chapter 3 will focus on left ventricular mass, and how it changes with human weight loss in two small groups of obese adults over three years. LV hypertrophy is a sign of cardiac maladaptation in hypertension and valvular heart disease, and LV mass is a biomarker of the severity of disease. Drug trials for hypertension and surgical studies for aortic stenosis use LV mass regression as a key end-point to demonstrate efficacy.^{119, 120} Obesity is also associated with LV hypertrophy.^{80, 83, 84} It is hoped that correcting excess weight might also cause LV mass reduction. The effect of weight loss on blood pressure is also examined on this small cohort.

Chapter 4 will look at a new biomarker of heart disease, myocardial triglyceride content (MTGC). This was first described in vivo in 2003 in Texas, and has since been studied extensively in diabetes and obesity.⁹¹ Normal myocardium has low lipid content, and increasing MTGC in various disease states has been associated with poorer prognosis.^{121, 122}

However, it is not clear which variables determine cardiac steatosis. The literature to date is reviewed, and the role of gender, age and body size on this biomarker in one hundred adults with no known heart disease is examined.

Chapter 5 is a similar cross-sectional study of 148 adults, examining the associations between abdominal obesity, lipid profile and hepatic lipid content (HLC). HLC is easier to measure than MTGC as the liver is a large, static organ, unlike the interventricular septum of the heart. The normal value for HLC has been described as <7%, based on two epidemiological studies, and corroborated by a later study of 18 patients with liver biopsies.^{101, 123} The data here suggest that the established normal range is perhaps too generous, and that HLC levels in healthy individuals are typically <2%. Moreover, there is a clear correlation between waist circumference and HLC, suggesting that this may be a useful, currently under-utilised clinical measure.

Chapter 6 is a study of the diagnostic accuracy of multiparametric MR for all forms of liver disease. The data here validate the use of spectroscopy to determine liver fat by comparison with a reference standard of liver biopsy. There is currently a strong argument the MRS should be the gold-standard investigation for the assessment of steatosis, but as there are no standardised spectroscopic methods yet, each centre must establish their own reference data. Liver fibrosis and iron content can be measured by non-invasive methods – these are reviewed in detail. Moreover, we introduce novel MR mapping techniques that enable the rapid assessment of the iron and extracellular fluid content of parenchymal liver tissue, which allows a clinical diagnosis of the type and severity of liver disease to be made. The problems of liver biopsy acquisition and interpretation are also discussed.

Chapter 7 tests the various biomarkers and methods described before on children, to determine which, if any, are altered in the early stages of obesity. At present there are very

limited tools for the assessment of obesity-related disease in children, as invasive investigations and ionising radiation are inappropriate. Left ventricular mass, MTGC, cardiac strain, HLC and new biomarkers of liver inflammation can all be determined quickly, reproducibly and safely with a 3 Tesla magnetic resonance system. This study establishes the feasibility of this, and presents the results from 11 lean and 11 obese boys.

Finally, chapter 8 summarises and integrates the findings from this thesis, and suggests clinical applications for the methods developed herein. Future research questions and projects that may reveal yet more insights into heart and liver disease, in obese and non-obese patients, are discussed.

Chapter 2: General Methods of Magnetic Resonance Imaging & Spectroscopy

2.1 A brief history of magnetic resonance in diagnostic medicine

The main aims of this thesis require accurate non-invasive quantification of parenchymal tissue intracellular lipid content. This is best achieved with magnetic resonance spectroscopy (MRS). This technique provides information about the chemical constituents of a defined volume of interest within the bore of the magnet. It was first described for potential biomedical use in 1973 by Paul Lauterbur in New York, and then Peter Mansfield in Nottingham, as nuclear magnetic resonance imaging (NMR).¹²⁴⁻¹²⁶ Both used gradients within a magnetic field to localise water molecules within three orthogonal planes. However, anatomical imaging of the internal organs of the body was then, and is still now, the main developmental aim of the technology. In this respect magnetic resonance imaging (MRI) has been an unquestioned success, and the inventors were awarded the Nobel Prize for Medicine in 2003.¹²⁷ From the first clinical patient scanned in 1990, there were an estimated 20,000 MRI systems functioning around the world in 2002, producing 60 million patient scans per year. In 2011, there were 615 MR systems in the UK alone and an estimated 10000 in the USA.¹²⁸ Over 1000 MR systems are built by Siemens each year, and they are one of four major MR system manufacturers. From a clinical perspective there are many conditions for which MRI is now the first-choice diagnostic investigation, such as spinal cord compression.¹²⁹ There are even more circumstances where MR is the gold-standard investigation, but currently there is limited availability. Improved access to diagnostic MR is currently advocated for patients with acute stroke and suspected scaphoid bone injury.^{130, 131}

MRS utilises the magnetic property of atomic nuclei which resonate at different frequencies depending on the nature of the bonds surrounding a particular nucleus. The proton, or hydrogen atom, is the most commonly studied nucleus. A hydrogen atom bound to oxygen

in water will resonate at a certain frequency in a fixed magnetic field, whilst hydrogen bound to carbon will resonate at a different frequency. Moreover, the nature of the chemical bonds around the carbon will affect the resonating frequency, such that hydrogen in a methyl group ($-\text{CH}_2-$) can be distinguished from hydrogen in an unsaturated hydrocarbon ($=\text{CH}-$). MRS uses a strong magnetic field to polarise these protons within tissues, which then precess at their characteristic frequencies. A further field, oscillating at these resonant radiofrequencies, excites the protons. As they relax back to their equilibrium state they emit a weak radiofrequency signal which is detected and recorded by the scanner hardware and then analysed by computer software. The software plots a spectrum of the frequencies detected with peaks corresponding to the relative concentration of various chemicals. In addition, the specific chemicals that are present are identified, as they appear at different locations on a horizontal axis representing radiofrequency, measured in hertz. Proton (^1H) MRS detects approximately 15 distinct brain metabolites. These include lipids, lactate, N-acetylaspartate (NAA), glutamate/glutamine (Glx), creatine (Cr), choline (Cho) and myoinositol (mI).¹³²⁻¹³⁴ Brain and skeletal muscle are ideal organs for MRS because they lie still, so data can be acquired over a period of time. There is minimal motion that would add noise or lead to systematic errors in the received signal, unlike the heart or other pulsatile organs, such as the liver. The table below summarises the similarities and differences between MRI and MRS, and Figure 2.1 is an example of liver MRS used to measure fat content.

Table 2.1. A comparison of MRI and MRS

	MR imaging	MR spectroscopy
In vivo property examined	Magnetic resonance properties, including nuclear & ferromagnetic	Nuclear magnetic resonance only (although ferromagnetism changes line shapes)
Data presented as	Image brightness and density show <i>relative</i> differences within chosen scanning parameters. Two dimensional image (2D) presented – a series of these allows a 3D structure to be examined	<i>Relative</i> abundance of different resonances within chosen scanning parameters in chosen volume of interest. Each resonance corresponds to a specific chemical bond.
Scanning parameters	A wide variety, echo time, repetition time & field of view	A wide variety (eg PROBE ¹³⁵), echo time, repetition time & volume of interest
Prior knowledge required	Anatomical knowledge corresponding with image acquired in field of view	Resonances of pure chemical compounds (eg H ₂ O, purified alkanes, alkenes, amines, amides, organic acids & esthers) within a certain magnet field strength
Assumptions made	Minimal movement or ‘through-plane motion’ during image acquisition	Minimal movement during acquisition. Only the volume of interest provides signal, uncontaminated by noise from other parts of the body

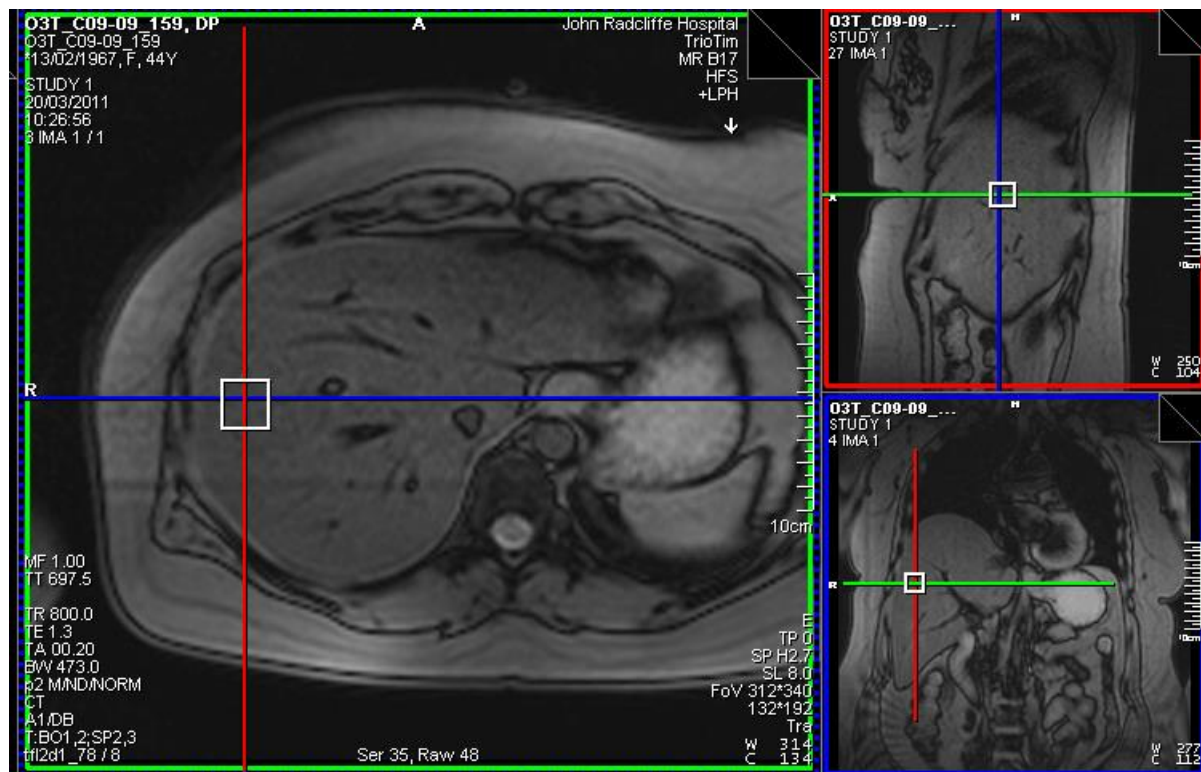
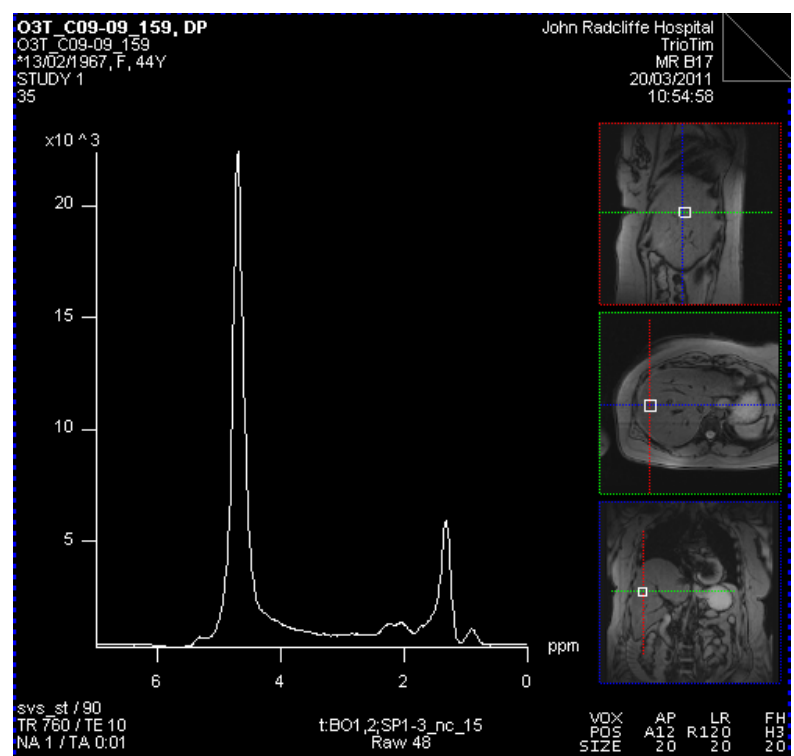


Figure 2.1. Liver ^1H MRS data acquisition

Liver MRI in a transverse plane, with coregistered sagittal and coronal images as insets, is shown in 1a (above); 1b (right) shows MRS of a voxel within the liver of the same patient, with a large water peak at 4.7ppm measuring 25 arbitrary units, and a smaller lipid peak at 1.3ppm measuring 6 units. Thus the hepatic lipid content in this voxel is $5.5/25 \times 100\% = 22\%$.



Unlike MRI, MRS has had much less clinical use or focus currently. This has been due to three main factors:

1. Technical expertise – only a few MRS sequences are automated, such as the PROBE (PROton Brain Examination) sequence.¹³⁵ Hence technical expertise and training are required to acquire the data, and these are usually provided by an MR physicist.
2. Post-processing – the spectra generated have to be analysed using computers to generate meaningful data, expressed as metabolite ratios to a given reference (eg creatine or water). Manufacturers have, thus far, not seen the need to include high-quality analysis software in their clinical systems, and there are no standardised software applications.
3. Clinical applications – there are many thousands of anatomical and medical imaging features that are reported as being clinically significant, in some cases with questionable evidence and reliability.¹³⁶⁻¹³⁹ However, imaging is quick and analysed in ‘real-time’, with endpoints that are recognisable and measurable (eg left ventricular end-diastolic volume). MRS and tissue characterisation have fewer defined end-points. Some have been shown to be of clinical use (eg PROBE), but widespread translation into practice has been slow.

Recent developments have tried to overcome these obstacles. With the rapid expansion in magnetic resonance diagnostic imaging there are more trained clinical and research MR physicists, leading to a greater availability of technical expertise. Since the approval of the PROBE by the Federal Drug Administration in 1995, clinical MRS has existed as an entity

solely for use in neurological evaluation, especially in brain tumour and epilepsy assessment. It has been used extensively in research, with several nuclei (eg ^1H , ^{31}P and ^{23}Na) in several organs (heart, liver, pancreas, skeletal muscle, as well as brain).¹⁴⁰ Several interesting and potentially useful studies have been performed, and techniques validated in different settings (eg ^{31}P heart and muscle to examine ATP usage, ^1H liver to determine fat content). A growing body of MR physicists and clinicians are becoming familiar with MRS, and it is hoped that technical expertise will grow further and lead to standardised protocols in different MR centres with different systems.

Post-processing of MRS data remains an obstacle to widespread use. However, different tools lead to comparable results and standardised methods are now possible for analysis. Java-based Magnetic Resonance User Interface (JMRUI) is a popular, commercially licensed software package for MRS data processing, with formalised training programmes. It is hoped that new user interfaces will be developed to tally with standardised acquisition methods, enabling simpler, more integrated methods.

There are many *potential* clinical applications for MRS. Higher magnetic field strength enables better signal-to-noise ratio (SNR) and more precise quantification of different metabolites, as well as the possibility of quicker MRS acquisitions. As 3 Tesla clinical scanners become more prevalent, more trials are being performed to evaluate MRS against imaging and histology for diagnostic and monitoring purposes. MRS endpoints and parameters are being investigated as markers of disease, such as MRS lipid:water ratio as a measure of fat content.¹⁰⁰ MRS has been shown to be non-inferior to, and safer than, histological quantification of steatosis in the liver and pancreas.⁸⁶ Standardised measures with internationally recognised normal ranges and definitions of disease and cost-effectiveness studies are now needed before clinical MRS outside the brain can begin in earnest.

2.2 Proton Magnetic Resonance Spectroscopy (^1H MRS)

Myocardial and hepatic lipids can be quantified using localised cardiac-triggered proton spectroscopy.¹⁴¹ At 3 Tesla, protons that are part of a methylene group ($-\text{CH}_2-$) resonate at 1.3ppm, while water resonates at 4.7ppm. Lipid content in a specified voxel of interest was measured by ^1H MRS in a 3 Tesla Siemens system (Tim Trio, Siemens Healthcare, Germany), using a stimulated echo (STEAM) sequence with water suppression. Subjects lay supine in the scanner, with a 24 channel spinal matrix underneath them and a 6 channel body matrix strapped in place over the anatomical region of interest. Breath-holding technique was explained and practised before data acquisition commenced, as variation in the depth of a subject's breath markedly altered the SNR. Subjects were advised to breathe in gently, breathe out, and then stop when they had reached the end of comfortable expiration. As normal expiration is a passive process driven by elastic recoil of the lungs, breath-holding and lung volumes are more consistent after expiration than after inspiration.

2.2.1 Cardiac data acquisition

Long and short axis cardiac localiser and FLASH cine images were obtained during end-expiratory breath-holds. Mid-diastole was determined from the cine images, and spectral acquisitions were all ECG-triggered to occur in this phase. A subject-dependent global calibration was used to minimise B_0 inhomogeneities. A single spectroscopic voxel of interest was placed in the interventricular septum at the level of the papillary muscles (23 x 8 x 36mm, see Figure 2.2). A further calibration pulse sequence was used to determine the optimum water suppression pulse scaling factor to better characterise the lipid peak at 1.3ppm. To minimise noise from septal movement, 5 acquisitions were obtained in mid-diastole during end-expiration, with a repetition time (TR) of 2 seconds to allow for

complete relaxation of the lipid signal between successive radiofrequency (RF) pulses. This required subjects to hold their breath and lie still for 12 - 14 seconds, which was comfortably managed by all but one of the participants. Spectroscopy parameters were set at an echo time (TE) of 10ms, a mixing time of 7ms, and 1024 points acquired at a bandwidth of 2000Hz. Spectra were obtained from 8 breath-holds – 7 with water suppression on to collect lipid data, and one with water suppression off (3 acquisitions, TR 4secs for complete relaxation of the water peak between RF pulses). To reduce chemical shift displacement, scan frequency was set at 1.3ppm during water-suppressed acquisitions and at 4.7ppm during the water-unsuppressed acquisition, corresponding to the lipid and water peaks respectively.

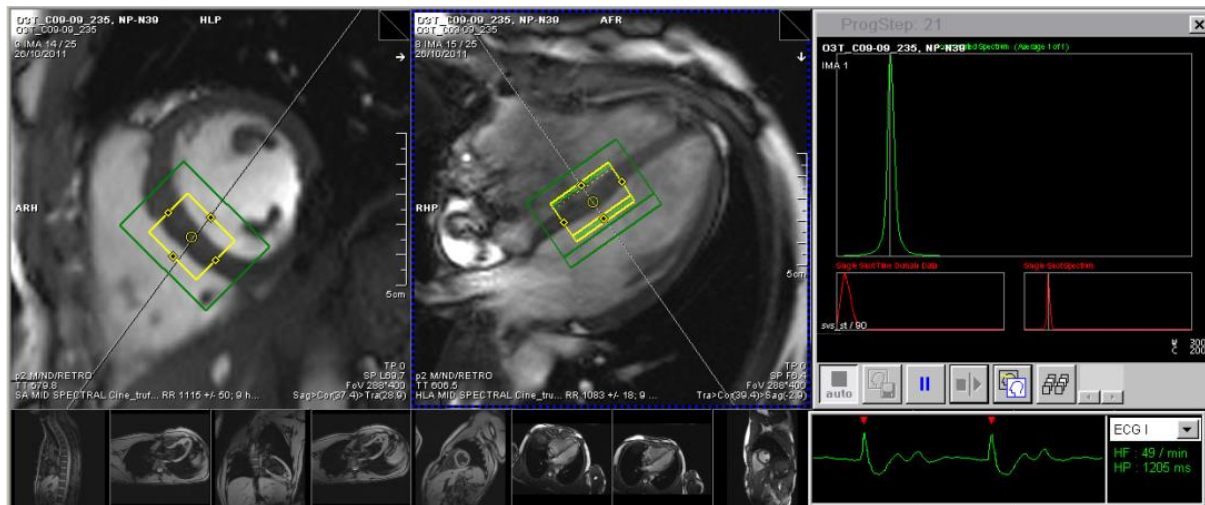
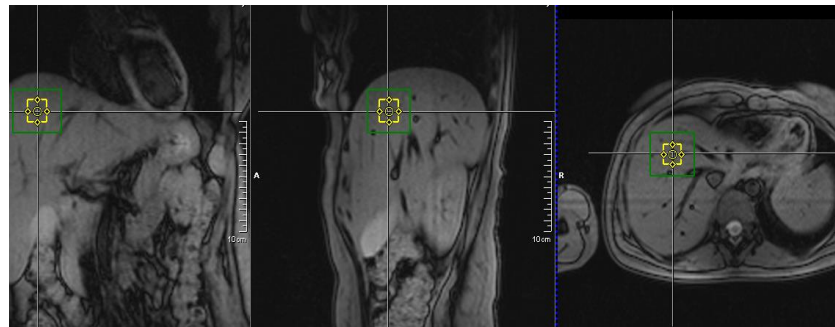


Figure 2.2. Cardiac ^1H MRS data acquisition

A 4.6ml (23 x 8x 25 mm) voxel was positioned in the interventricular septum on the four-chamber and short axis cine images at mid-diastole. Repetition time was of at least 2000ms (2R-R), and acquisition was ECG-triggered to commence in mid-diastole (in this case, 600ms after the QRS complex). Acquisition of water-unsuppressed spectrum is shown (top right window) – the water peak at 4.7ppm is the only visible peak.

2.2.2 *Hepatic data acquisition*

Localiser images were obtained during end-expiratory breath-holds in the transverse, coronal and sagittal planes (Figure 2.3). A first voxel of interest was placed in the lateral aspect of the right lobe of the liver, avoiding visible vessels and the biliary tree. As with the heart, a further calibration pulse sequence was used to determine the optimum water suppression pulse scaling factor to better characterise the lipid peak at 1.3ppm. Five acquisitions were obtained per breath-hold with ECG-triggering to minimise noise from variations in arterial blood flow in the liver. As with the heart, a TR of 2 seconds allowed for complete relaxation of the lipid signal between successive RF pulses. This required subjects to hold their breath and lie still for 12 - 14 seconds. Improved SNR in the liver due to less motion artefact compared to the heart meant that fewer datasets were required, so only five breathholds were used – 4 with water suppression on for lipid data, and one with water suppression off to determine the water signal. Spectroscopy parameters were otherwise similar to the cardiac setup described above (TE 10ms; mixing time 7ms; 1024 points acquired at a bandwidth of 2000Hz; scan frequency 1.3ppm for water-suppressed spectra and 4.7ppm for water-unsuppressed spectra; TR 2s for water-suppressed data and 4s for water-unsuppressed data).



MR images and STEAM proton MR spectra from a subject.

Voxel size: 2x2x2 cm (yellow box)

TR=2 s, TE=10 ms, avg = 21, spectral width 2000 Hz, 2048 points

Resonances:

- 1: triglyceride terminal methyls CH_3 (0.9 ppm)
- 2: methylene $(-\text{CH}_2-)_n$ (1.3 ppm)
- 3: $-\text{CH}_2-\text{CH}=\text{CH}-$ (2.0 ppm)
- 4: choline content components (3.2 ppm)
- 5: glycogen (3.6-4.0 ppm)
- 6: residual water (4.7 ppm)

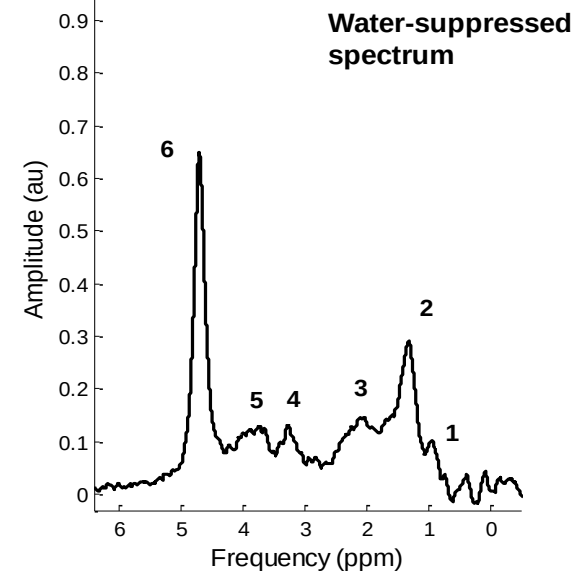
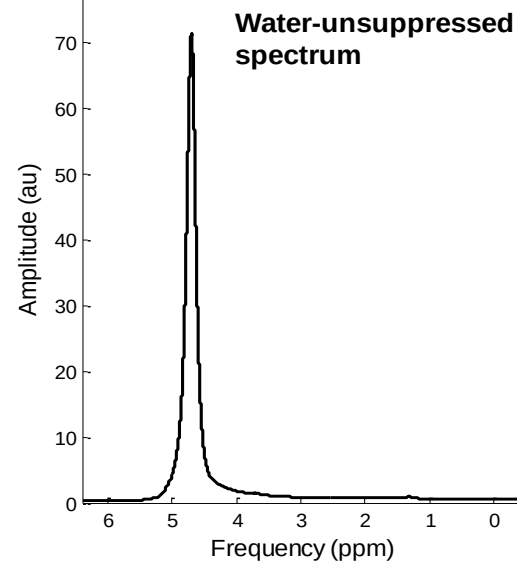


Figure 2.3. Hepatic ^1H MRS data acquisition and resonances of major liver metabolites

A larger voxel can be sampled (8cm^3) and there is less motion artefact, producing clearer spectra with less noise.

2.2.3 *¹H MRS data analysis*

Raw data was saved as rda files in an anonymised format and analysed offline by an investigator blinded to the anthropometric measurements and other data. Spectra were fitted using AMARES in the jMRUI package and MATLAB 2010b. Signals from different coil elements in each breath-hold were combined using a customised programme in MATLAB. Individual spectra were phase and frequency corrected prior to summation in MATLAB. Water-suppressed spectra were quantified using five identifiable peaks where possible, using prior knowledge and soft constraints. All peaks were fitted using Lorentzian lineshapes, and lipid content was expressed as a percentage of the water signal (Figure 2.4).

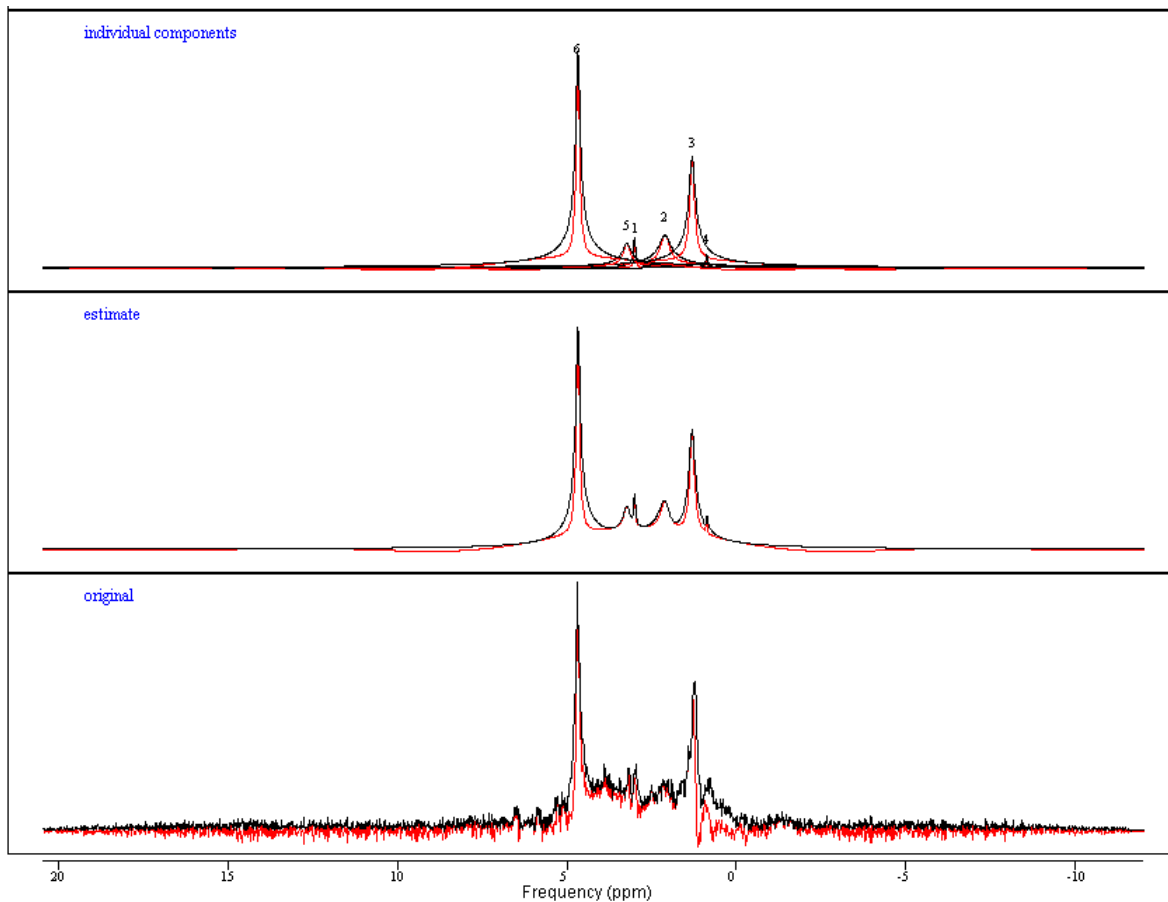


Figure 2.4. Cardiac water-suppressed MRS data processing in AMARES

Original data, from the same subject as above in Fig 2.2, is averaged from the 25 signals acquired (bottom panel). Individual peaks are identified (middle panel) and then fitted to metabolites with corresponding frequency (top panel). Peaks are 1) Creatine @ 3.0ppm, 2) Lipid CH unsaturated @ 2.14ppm, 3) Lipid CH₂ @ 1.33ppm, 4) Lipid methyl group @ 0.91ppm, 5) Choline @ 3.26ppm and 6) Residual water @ 4.7ppm. MTGC is calculated as the area under the lipid peak at 1.3ppm divided by the area under the water peak at 4.7ppm from the *unsuppressed* spectrum.

2.3 Magnetic Resonance Imaging

MRI has several advantages in clinical research. Firstly, imaging of internal organs is possible without any ionising radiation. Secondly, when compared to ultrasound-based techniques, image quality is much less dependent on the subject's body size. This was of great importance in designing studies for obese subjects. Thirdly, blinded analysis is possible by the same or other investigators at a later time-period. Although this is technically feasible with other imaging methods, it is much easier with MR, where all the data are automatically saved and coded. Lastly, MR image analysis has been widely tested and accepted as highly reproducible and less dependent on operator skill for the acquisition and the interpretation of data.¹⁴² MR assessment of cardiac volumes and mass is the gold-standard for these variables, and interobserver variability is less than 10%.

Strain is a dimensionless quantity that represents the percent change in dimension from a resting state to one achieved following application of a force, or stress. Thus, *negative strain* in the septum indicates compression or shortening, and is analogous to fractional shortening measured by 2D echocardiography. Peak circumferential strain can be measured in the heart using MR, and is more accurate at higher field strengths as measurement depends on SNR.¹⁴³ Thus strain data was acquired at 3 Tesla, along with cardiac spectroscopy data. To date, tagging has been the most validated method for assessing small changes in strain.¹⁴⁴ This method applies a grid of small volumes ('tags') to the myocardium at a given time-point, usually end-diastole. Myocardial deformation can then be measured by the movement of the tags through the cardiac cycle. An alternative method to assess strain, and also to assess diastolic function, is feature-tracking. In this technique, an operator draws epicardial and endocardial contours, as described above, and software tracks the deformation of the contours through the cardiac cycle. In both tagging and feature-tracking, circumferential

peak systolic strain is the most robust and reproducible metric. Radial and longitudinal strain can also be measured, but are less reproducible and more susceptible to operator bias. Feature tracking as a technique was in its infancy in 2009, and therefore the studies in this thesis used 2D tagging to measure peak systolic circumferential strain (CS).

There are some disadvantages with MR – temporal resolution is not as good as echocardiography for the assessment of diastolic function in the heart, or indeed in strain analysis. It is also costly – one hour's use of an MR system costs £400 - £1000, and the studies described in this thesis required over 400 hours of magnet time with subjects. This was in addition to time spent developing methods, learning and practising techniques, and determining reproducibility on normal volunteers and colleagues.

2.3.1 *Cardiac data acquisition*

Cardiac volumes were acquired using Steady State Free Precession (SSFP) imaging. This is possible at both 1.5T and 3T but, for work described in this thesis, data was acquired with a Siemens 1.5T Avanto system (Siemens, Erlangen). The methods used are standard guidelines for cardiac MR, with excellent reproducibility.^{145, 146}

Subjects lay supine in the scanner. The receiver coils were a posterior 24 channel spine array and an anterior 6 channel body coil. Subjects also wore an ECG monitor, so that cine images could be acquired with ECG-triggering. As with spectroscopy, data acquisition was during consistent breatholds to minimise motion artefact and anatomical changes due to different lung volumes.

Cardiac localisers were used to pilot long and short axis images. A series of ECG-triggered short axis cines was then acquired, covering the ventricles from base (at the level of the atrioventricular groove) to apex. Each of these slices covered the whole cardiac cycle and was 7mm thick, with a 3mm gap between slices (Figure 2.5).

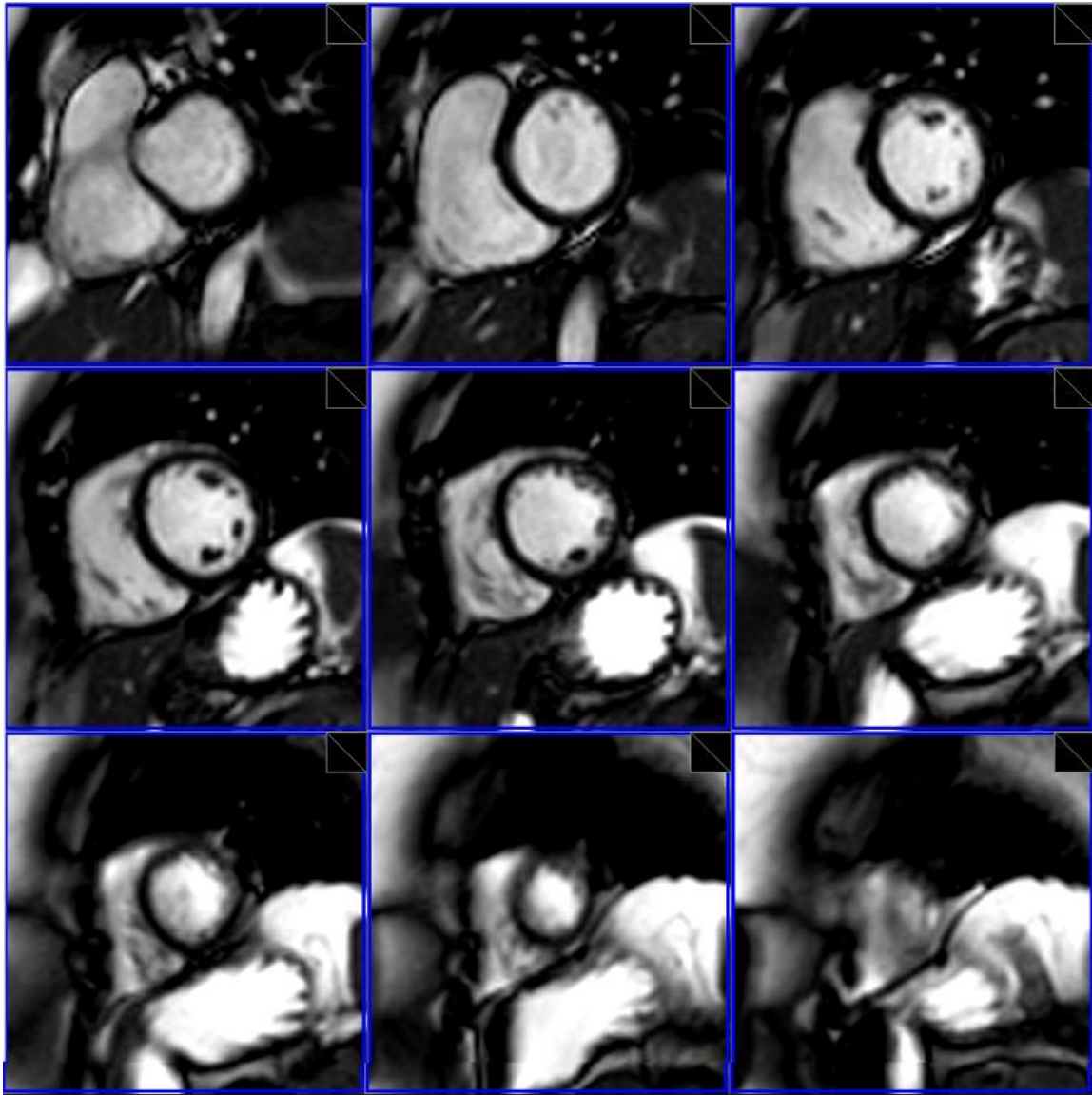


Figure 2.5. A stack of short axis images of the heart, acquired during end-expiration
Two images acquired per breathhold, from base (top left) to apex (bottom right). At each slice, the full cardiac cycle was captured – only the end-diastolic images are shown.

2.3.2 Cardiac data analysis

The short axis stack of cine images was loaded into ARGUS software package. End-diastole and end-systole were identified. Left ventricular epicardial and endocardial contours were drawn, enabling the quantification of left ventricular mass, end-diastolic and end-systolic volumes (Figure 2.6). Right ventricular endocardial contours were also drawn, enabling measurement of end-diastolic and end-systolic volumes. As all subjects in this thesis had no valvular disease, it was expected that left and right ventricular stroke volumes should be similar (<10mls difference). As a further test of precision, ten datasets were analysed by two independent cardiologists, and the coefficient of variation was 6%.

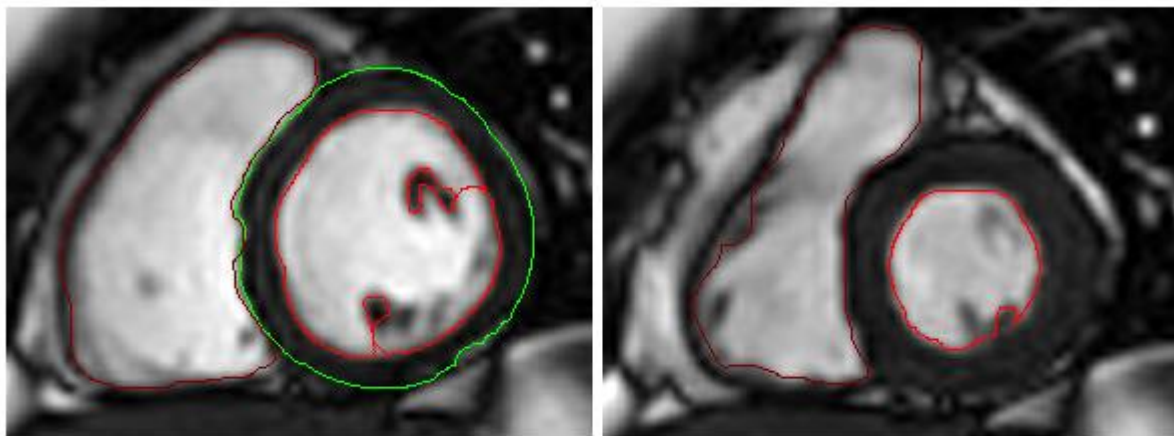


Figure 2.6. Determining LV volumes and mass

Short axis images at end-diastole (left) and end-systole (right) are shown, with endocardial (red) and LV epicardial contours (green). A stack of these images covering the heart from base to apex are analysed to calculate left and right ventricular end-diastolic and end-systolic volumes, and LV mass.

2.3.3 Strain data acquisition

A series of short axis images of the left ventricle were acquired, allowing a horizontal long-axis cine to be piloted. A gradient echo based high-resolution tagging pulse sequence was then performed at the basal, mid-ventricular and apical levels of the left ventricle. The mid-ventricular slice was chosen from the short-axis series, and the basal and apical slices were both equidistant from this mid-ventricular slice. All data was acquired during breatholds, and ECG-triggered to capture 90% of the R-R interval. Sequence parameters were TR 25ms, TE 7.4ms, flip angle 25° and heart phase interval < 40ms, giving 15 - 30 phases per cardiac cycle, depending on the heart rate. This acquired two sets of images with horizontally and vertically modulated stripes per breathold.

2.3.4 Strain data analysis

Tagged images were saved in an anonymised format and analysed by an experienced operator (JJS) blinded to the rest of the data using Cardiac Image Modeller software (CimTag2D v7 Auckland Medical Research, Auckland, New Zealand). Semi-automated analysis was performed by aligning a grid to the myocardial tagging planes in end-diastole. End-systole was determined visually, and tags adjusted at each frame through the cardiac cycle to derive peak systolic circumferential strain for the mid – ventricular slice, which was expressed as a percentage change from end-diastole. Normal circumferential strain has previously been described as -19 ± 2 ; impaired myocardial contractility is shown by a *more positive* value.¹⁴⁷

2.4 Abdominal adipose tissue imaging

Abdominal localisers were used to identify the 4th and 5th lumbar vertebrae. A transverse water-suppressed T1-weighted turbo-spin echo (TSE) sequence was acquired during a breath-hold to minimise motion artefact at the level of the 4th/5th lumbar intervertebral (L4/5) disc. The image acquired clearly showed adipose tissue as white, and visceral and subcutaneous depots were then contoured manually to determine cross-sectional area at this level (Figure 2.7). Sagittal abdominal diameter was also measured as the maximum antero-posterior distance at L4/5.

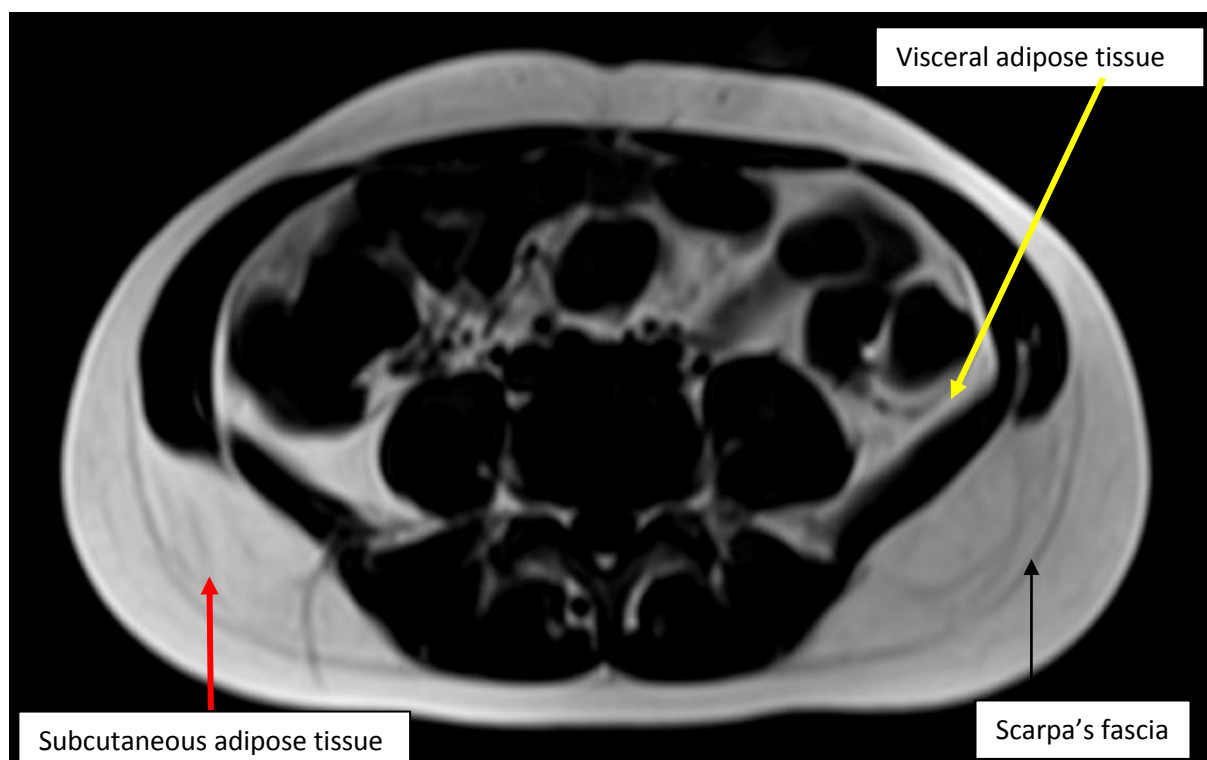


Figure 2.7. Measuring adipose tissue content.

Transverse water-suppressed turbo spin echo image at the level of the 4th/5th lumbar intervertebral (L4/5) disc. Manual contouring allows measurement of subcutaneous and visceral adipose tissue depots. Scarpa's fascia is also visible.

2.5 T1 mapping of the liver

The T1 relaxation time is an intrinsic electromagnetic property of tissue in a magnetic field. Contrast between tissue types in MR imaging is determined by T1, T2 and T2* relaxation times. The measurement of absolute T1 across an image is used to produce a T1 map. A recent development in cardiac imaging has been the shMOLLI sequence.¹⁴⁸ This enables cardiac T1 mapping for an 8mm slice in a 10-second breathhold.

This sequence samples the T1 recovery curve using single-shot steady state free precession (SSFP) acquisitions, and determines what the T1 value is per pixel by fitting the measured signal to the exponential relaxation curve. The same principles and approach were used to generate T1 maps of the liver in transverse and coronal planes. To minimise error, the acquisitions were cardiac-gated and acquired on end-expiration after a manual shim. There was also a quality assurance component – each acquisition generates an R^2 map for the fit of signal intensity to the exponential recovery curve.¹⁴⁹ For the studies described in chapters 6 and 7, an image was only considered for interpretation if the R^2 was $\geq 99\%$.

T1 values are lowest in regions where there is little unbound hydrogen, and highest where there is much unbound hydrogen (eg stomach acid = $\text{HCl} = \text{H}^+ + \text{Cl}^-$). The hypothesis tested in Chapter 6 tests the use of T1 to measure the volume of extracellular fluid (ECF) in a region of interest.

2.6 Anthropometric data

All subjects had height, weight, waist circumference and hip circumference measured while wearing light clothing only (eg hospital gown). Waist circumference was measured with the

subject standing and the measuring tape placed at the midpoint of the costal margin and the anterior superior iliac spine, as per WHO guidelines.¹⁵⁰ Hip circumference was measured at the maximum circumference of the buttocks.

2.7 Other clinical measurements

2.7.1 Blood sampling

Blood was taken from subjects while they were in a seated position to minimise variation in triglyceride level caused by lying supine. Subjects were instructed to fast for at least 6 hours prior to sampling, but to stay hydrated by drinking water. Once taken, an EDTA sample (purple tube) was centrifuged at 4°C for 12 minutes at 3000rpm. The supernatant plasma was stored at -80°C for later analysis. One sample with gel and clot activator (yellow tube) was sent for biochemical analysis immediately (eg total cholesterol, HDL, triglyceride, glucose, liver enzymes). Another was left to stand vertically for 30 minutes at room temperature before centrifugation at 3000 rpm for 12 minutes at 4°C, after which the supernatant serum was stored at -80°C for later analysis.

2.7.2 Electrocardiography

A 12-lead ECG was obtained with subjects lying supine during a 15 second breathhold to minimise excessive noise.

2.7.3 Blood pressure measurement

Systolic and diastolic blood pressures were measured during the screening appointment for each participant, and also during 1.5T scans using the MR-safe Veris vital signs monitor (Medrad Systems. Pennsylvania, USA).

Chapter 3: Cardiac remodelling after bariatric surgery

3.1 Introduction

Adult obesity in the absence of any other comorbidity is associated with left ventricular hypertrophy, which in itself is an independent risk factor for cardiovascular events.^{151, 152} Excess body weight has a linear relationship with vascular mortality.⁵ Surgical measures have been shown to be much more effective than dietary or pharmacological interventions in achieving weight reduction, although weight loss is greatest at one year, and many patients begin to resume weight gain after two years.¹⁵³ Previous studies have shown that weight reduction surgery is associated with left ventricular (LV) mass reduction one year later.⁹⁸ A more recent study using bariatric surgery in a different population of 15 hypertensive obese women showed progressive regression of LV hypertrophy at 2 years.⁷⁷

This study set out to see if surgical weight reduction could achieve longterm LV remodelling over three years without confounding hypertension. It also aimed to see if excess weight loss had a linear relationship with LV mass reduction, and to compare the cardiovascular consequences of dietary advice to surgical weight loss over 3 years.

3.1.1 Objective

To investigate the effects of dietary advice and weight reduction surgery on LV mass at 3 years in patients with obesity in the absence of other comorbidities.

3.2 Methods

We followed up 10 patients who had undergone weight reduction surgery (9 Roux-en-Y gastric bypass, 1 gastric band) for obesity in 2006-7, and 13 patients who had been given dietary advice for weight loss in 2007 (specifically, the ‘low glycaemic index’ diet). Height and weight were measured to determine body mass index (BMI).

Excess weight, defined as the proportion of an individual’s BMI greater than the ‘ideal’ of 23, was measured as:

$$(\text{Change in BMI}) \div (\text{baseline BMI} - 23)$$

or, expressed as a percentage change,

$$(\text{Change in BMI}) \div (\text{baseline BMI} - 23) \times 100\%$$

All subjects had no other identifiable cardiovascular risk factors (ie diabetes, hypertension, hypercholesterolaemia, smoking, obstructive sleep apnoea or known cardiac disease). The subjects had CMR scans (1.5 Tesla, Siemens, Erlangen, Germany) performed at baseline, 1 and 3 years. Cine imaging was used to determine LV mass (LVM), end-diastolic volume (EDV), end-systolic volume (ESV), stroke volume (SV) and ejection fraction (EF), calculated with ARGUS as described in Chapter 2.

3.2.1 Statistical analysis

Descriptive statistics were used to summarise subject characteristics in both groups. Data were presented as mean $\pm$ standard error of the mean (SEM). Analysis of variance (ANOVA) for repeated measures with Bonferroni correction for comparison of pairs of values was used for statistical evaluation. If there was a significant difference between mean values for repeatedly measured variables, paired t-test was used to compare time points.

The GraphPad Prism 5 software package was used for statistical analysis and data presentation.

Table 3.1. Baseline data for all participants (mean $\pm$ SEM)								
	BMI (kg/m ²)	LVM (g)	EDV (mls)	SV (mls)	LVEF (%)	LVM/EDV (g/mls)	SBP (mmHg)	DBP (mmHg)
Diet	35.3 $\pm$ 1.5	135 $\pm$ 9	148 $\pm$ 5	103 $\pm$ 3	69 $\pm$ 1	0.91 $\pm$ 0.04	115 $\pm$ 3	76 $\pm$ 2
Surgery	47.4 $\pm$ 1.6	137 $\pm$ 9	155 $\pm$ 6	106 $\pm$ 3	69 $\pm$ 2	0.91 $\pm$ 0.07	128 $\pm$ 3	78 $\pm$ 3

3.3 Results

Mean BMI in the diet group was $35.3 \pm 1.5 \text{ kg/m}^2$, compared to $47.4 \pm 1.6 \text{ kg/m}^2$ in the surgery group ($p < 0.001$), as shown in the baseline data in table 3.1. LV mass, cardiac volumes and LVEF were similar between the two groups. Systolic blood pressure was markedly higher in the surgery group ($p = 0.007$), as was pulse pressure ($p = 0.016$).

3.3.1 Weight loss

In the diet group there was a significant reduction of BMI by 7% from 35.3 ± 1.5 (mean $\pm$ SEM) kg/m^2 to $32.9 \pm 1.5 \text{ kg/m}^2$ at year 1 ($p = 0.016$; Figure 3.1a). This represents a reduction in excess weight of $19 \pm 7\%$. After three years BMI had risen to $34.8 \pm 1.6 \text{ kg/m}^2$, similar to baseline ($p = 0.07$). Importantly, following dietary advice, the group had not continued to gain weight, which is a positive result in the context of chronic adult weight gain. In excess weight terms, the dietary group had lost $4 \pm 7\%$ on average, although there was considerable variation.

In the surgery group there was a significant reduction in BMI at one year by from $47.4 \pm 1.6 \text{ kg/m}^2$ to $33.0 \pm 1.4 \text{ kg/m}^2$ ($p < 0.001$, Figure 3.1b). This represents a $59 \pm 5\%$ loss of excess weight. At three years, BMI was $33.7 \pm 1.5 \text{ kg/m}^2$, suggesting minimal change from Year 1. Significantly, even though the surgical group was much larger than the dietary group to begin with, they had achieved parity by the end of the first year, and a lower mean BMI by the end Year 3.

3.3.2 *Changes in LV mass*

Weight loss did not significantly affect LV end-diastolic volume or stroke volume in either group over the three years. In the diet group, there was a trend for a slow reduction of LV mass over time, which did not achieve statistical significance at Year 1. After three years, LV mass had decreased by 7% from $135 \pm 9\text{g}$ to $125 \pm 8\text{g}$ ($p = 0.045$, Figure 3.1c). There was no significant change in the LV mass / EDV ratio from a baseline of 0.91 ($p = 0.53$).

In the surgery group LV mass decreased by 15% from 137g to 117g ($p < 0.01$; Figure 3.1d) in the first year. At three years, there was a significant additional reduction in LV mass from 117g to 106g ($p = 0.04$), representing a further 8% reduction in LV mass from baseline (figure 3.2). Consequently, there was a significant difference in the LVM / EDV ratio from 0.91 to 0.74 at Year 3 ($p = 0.03$).

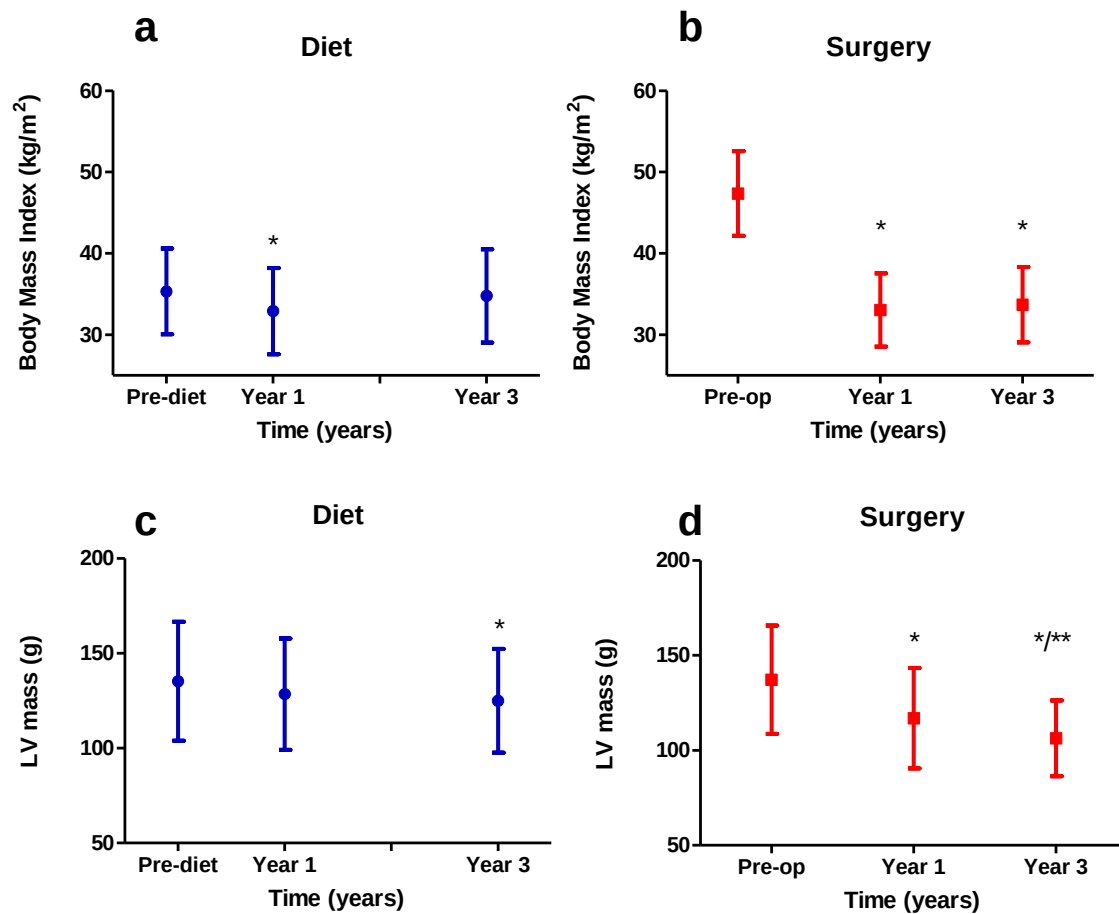
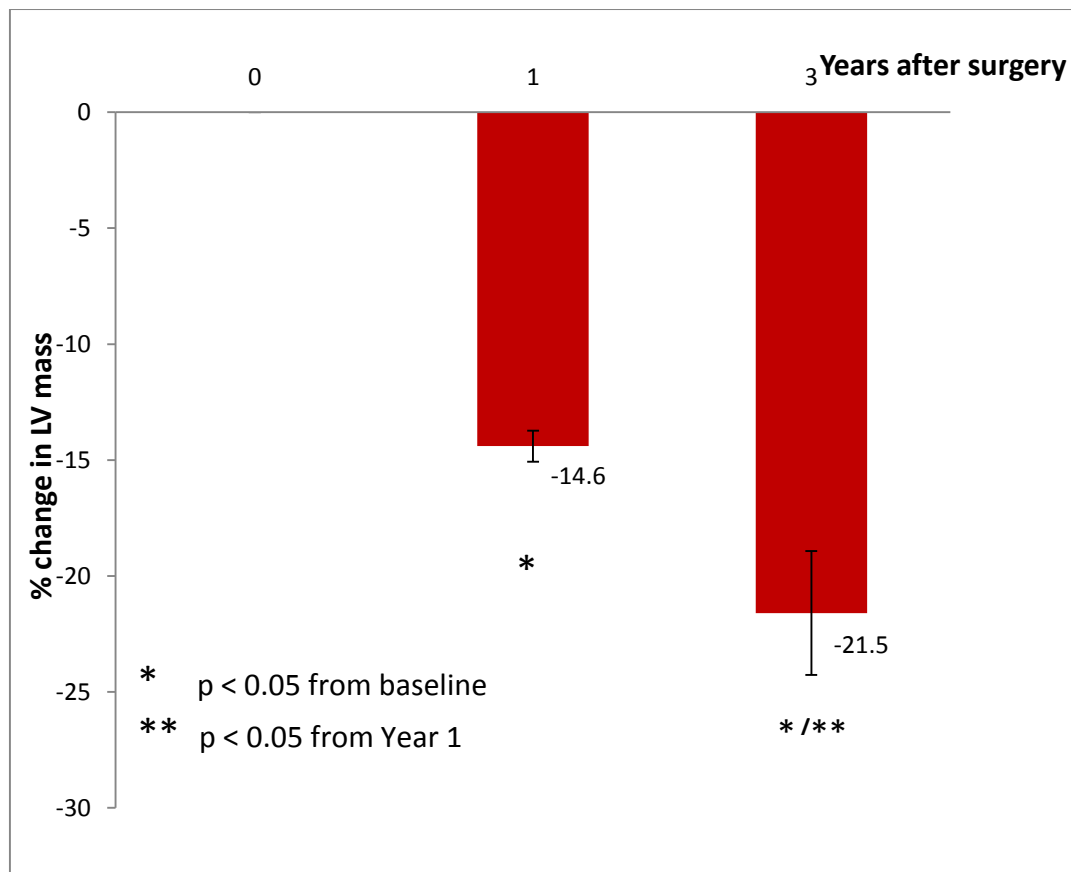


Figure 3.1 a-d. BMI and LV mass at baseline, 1 year and 3 years post-intervention

Data are shown as mean and standard deviation, to show the spread within each group; * denotes $p < 0.05$ for difference in mean value from baseline; ** denotes $p < 0.05$ for difference in mean value from Year 1 to Year 3.



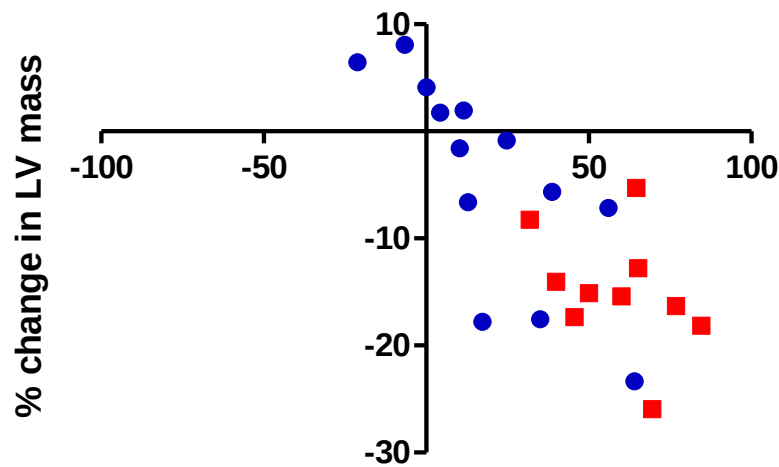
Figures 3.2. Percentage change in LV mass after bariatric surgery

Bar chart shows mean $\pm$ SEM. Although the BMI of the patients was essentially unchanged between year 1 and year 3, there was still significant further reduction in LV mass.

3.3.3 Excess weight lost and LV remodelling

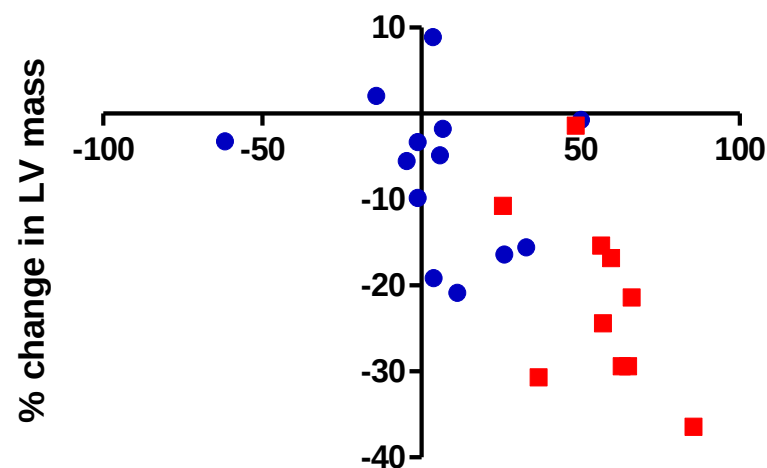
To examine the relationship between the excess weight loss and the change in LV mass, the data from the two groups of diet and surgery subjects were pooled. Loss of excess weight was strongly associated with LV mass reduction in a linear manner - $r_s = 0.72$ at 1 year and 0.63 at 3 years ($p < 0.001$ for both; Figure 3.3a and b). Although the amount of excess weight lost was much higher in the surgical group, the slope of the relationship was similar in both diet and surgery, suggesting that the degree of excess weight lost, not the method, was the key driver in LV remodeling.

Excess weight lost and LV remodelling at 1 year



$$r_s = 0.75^{***}$$

Excess weight lost and LV remodelling at 3 years



$$r_s = 0.66^{***}$$

● Diet
■ Surgery

Figure 3.3 a and b. Weight loss and LVM reduction

NB Excess weight change (%) is on x axes.

*** denotes $p < 0.001$.

Excess weight loss, regardless of method, was strongly associated with a reduction in LV mass at Year 1 and Year 3. Indeed, at Year 1, the greatest individual reduction in LV mass was achieved by diet. However, the surgical patients had much more success at keeping the excess weight off, and also had more regression of LV mass at 3 years.

3.3.4 Changes in blood pressure with weight loss

Even though patients with pre-existing hypertension were excluded from participating, and all subjects had values in the normal range for all time points, there was still a clear reduction in blood pressure in the surgical cohort, as seen in Table 3.2a. The most marked change was in systolic pressure (13mmHg reduction from baseline Year 1, maintained to Year 3). There were moderate reductions in diastolic and pulse pressure, but these did not attain statistical significance in this small sample. In the dietary group, which had less marked obesity, the systolic pressure was 115 ± 3 mmHg at baseline, and did not change over the three years (Table 3.2b). The diastolic pressure dropped gradually from 76 ± 3 mmHg to 70 ± 3 mmHg.

Table 3.2a. Changes in blood pressure in weight loss surgery patients				
Blood pressure	Year 0	Year 1	Year 3	ANOVA
Systolic (mmHg)	128 ± 3	115 ± 5	115 ± 3	$p = 0.01$
Diastolic (mmHg)	78 ± 3	73 ± 4	72 ± 2	$p = 0.15$
Pulse pressure (mmHg)	50 ± 3	42 ± 3	44 ± 2	$p = 0.06$

Table 3.2b. Changes in blood pressure following low GI diet				
Blood pressure	Year 0	Year 1	Year 3	ANOVA
Systolic (mmHg)	115 ± 3	116 ± 2	115 ± 3	$p = 0.98$
Diastolic (mmHg)	76 ± 2	74 ± 1	70 ± 3	$p = 0.03$
Pulse pressure (mmHg)	49 ± 2	42 ± 3	44 ± 2	$p = 0.40$

3.4 Discussion

Over 3 years, weight reduction surgery was associated with a significant progressive reduction in LV mass, which continued even after BMI had stabilised after 1 year. This may well represent long-term LV remodelling in response to marked weight loss. A small change in LV mass over 3 years may be due to variations in measurement between baseline and year 3 scans, inter-observer variability, or other confounders outside the study parameters (eg lifestyle changes during the study, such as exercise regime or change in employment). However, this degree of regression of LV hypertrophy is striking. For comparison, valve replacement for severe aortic stenosis, a clinical model for pathological LV hypertrophy in response to LV outflow tract obstruction and increased afterload which is then reversed, leads to a 30% regression in LV mass.¹²⁰ Aggressive pharmacological treatment of hypertension only produces a modest 10% reduction in LVM.¹¹⁹ In many patients where obesity and hypertension coexist as components of the Metabolic Syndrome, it may be that early surgical treatment of obesity may be more potent than medical treatment for the reversal of LV hypertrophy.

In the dietary patients, the trend in LVM reduction over three years was very similar to the drop in blood pressure. In the surgical group, the large blood pressure drop in the first post-operative year corresponded to a substantial drop in LVM. Although there was no further drop in pressure, it seems feasible that this marked drop in systolic pressure, and thence LV afterload, may have been a key factor in the regression in LV hypertrophy, which continued more modestly after the first year. Across all the obese patients here, weight loss and blood pressure reduction are closely associated, as previously shown.¹⁵⁴

LV mass is assumed to be entirely muscle, although we know from histology and post-mortem studies that the left ventricle is made of muscle, lipid and fibrous tissue.¹⁵⁵ It is conceivable that obesity is associated with excess fat deposition in the heart, and that weight loss reverses this, without affecting the muscular hypertrophy of the heart. Alternatively, weight loss may alter circulatory mechanics, including reduced LV afterload, leading to a reduction in LV muscle mass. Finally, it is also possible that myocardial fibrosis may occur in obesity, and lead to apparent LV hypertrophy with imaging, which may be reversible with weight loss. Post-mortem studies in diabetic cardiomyopathy have shown increased fibrosis.⁹⁶

Importantly, albeit in a small sample of 13 who were sufficiently motivated to take part in a clinical research study, dietary advice had a significant effect on LV mass and blood pressure, even though there was negligible weight loss. This is comparable in magnitude to the effect of antihypertensive therapy in patients with raised blood pressure, and reinforces the importance of dietary advice in the secondary prevention of heart disease in obese patients.

3.4.1 Potential Limitations

This was a three year non-randomised cohort study, where the subjects were all obese with no classical cardiovascular risk factors or evidence of heart failure. The study recruited by open advertisement, and is therefore susceptible to participants' self-selection bias. At the time of commencement, bariatric surgery was not widely available in the UK, and so the sample size is small. Participants in the weight reduction surgery group qualified on BMI criteria to undergo surgery, so there may well be confounding by indication. This also restricts more detailed analysis using multivariable regression. Obese men are seriously under-represented,

as only one took part in the surgical arm of the study. Weight management is very dependent on behaviour, and this study did not record dietary or exercise behaviour. However, dietary advice is routinely given in clinical practice for weight management, and so the diet group results are generalisable to a wider population of obese adults without other cardiovascular risk factors. Lastly, within the dietary group there was considerable variance in the subjects' weight gain and loss over the three years, which may distort some of the findings from this group.

3.5 Conclusions

Overall, changes in excess body weight correlate closely with changes in LV mass, and substantial weight loss results in dramatic LV remodeling, which is associated with reduced blood pressure. Other possible causes include a change in the endocrine profile acting on the heart, altered circulatory mechanics due to weight reduction, and changes in the fat and fibrous content of the heart. Moreover, these are not mutually exclusive. Anti-obesity surgery may prove useful in the treatment of LV hypertrophy and hypertension in Metabolic Syndrome, but further studies into the longterm effects need to be completed. The data further support the hypothesis that ventricular hypertrophy from excess body weight is modifiable. The consequent public health message is that losing excess weight can protect against heart disease.

Chapter 4: Excess body fat, myocardial steatosis and impaired myocardial strain

4.1 Introduction

Excess lipid accumulation, or steatosis, has long been associated with organ dysfunction in the liver, pancreas and skeletal muscle.^{22, 23, 156} Cardiac steatosis due to intramyocellular triglyceride deposition was first described in the post-mortem examination of obese patients in the 19th century.⁸² It is not known whether this can also lead to chronic low grade inflammation and eventual fibrosis, as occurs in hepatic steatohepatitis, but there is evidence from animal models that lipid and ceramide accumulation is associated with increased free radical mediated cellular injury.¹⁵⁷ Furthermore, altered myocardial metabolism in disease states can lead to increased fatty acid storage, ceramide synthesis and further lipotoxicity.⁴⁸

4.1.1 *Measuring myocardial triglyceride content in vivo*

Myocardial triglyceride content (MTGC) in humans was first measured in vivo by magnetic resonance spectroscopy (MRS) in 2003 by Szczepaniak and colleagues in Dallas.⁹¹ This group carried out the largest myocardial spectroscopy study to date of 177 adults in Texas, from which 134 datasets were obtained (78 diabetic, 15 lean, 21 obese and 20 with impaired glucose tolerance); the rest were excluded from analysis due to motion artefact.⁹⁵ Adults with diabetes mellitus had increased MTGC compared to lean and non-diabetic adults. The authors concluded that:

‘The data emphasize that in the presence of normal glucose tolerance, obesity per se is not sufficient to cause cardiac steatosis, because myocardial triglyceride content was dissociated from BMI.’

However, the study did not control for diabetic medication, dyslipidaemia or other cardiac risk factors, and was underpowered to assess the effects of obesity alone.

Subsequent studies in other cohorts have examined other variables affecting myocardial triglyceride content. Iozzo and colleagues compared 24 men to 17 women, and found that men had higher values (1.5% vs 0.9% of water signal).¹⁵⁸ However, this study was confounded as the cohort of 53 adults included lean, obese and diabetic subjects, some of whom were on statin and anti-hypertensive therapy. A recent French study comparing 30 obese to 33 lean subjects showed that MTGC correlated with epicardial fat volume, but did not then respond to surgical weight loss.⁹⁹ A Dutch study had previously described myocardial triglyceride content in 12 obese diabetic patients on insulin therapy falling in response to prolonged caloric restriction from $0.88 \pm 0.12\%$ to $0.64 \pm 0.14\%$ of the water signal.¹⁵⁹ This study also showed improvement in diastolic function with weight loss. A 12-week exercise training programme had a similar effect on cardiac steatosis in 14 healthy overweight men, reducing MTGC from 0.99 ± 0.15 to $0.54 \pm 0.04\%$.¹⁶⁰ The same group also described the functional consequence of cardiac steatosis in a later study of 42 diabetic men – MTGC was found to be an independent predictor of diastolic dysfunction and reduced biventricular strain measured by echocardiography.^{161, 162} Utz and colleagues later showed that insulin resistance was associated with increased MTGC content (0.83 ± 0.30 vs $0.61 \pm 0.23\%$) and reduced diastolic function in women with BMI $>27 \text{ kg/m}^2$, but with no change in systolic function.¹²¹ However, although they compared 29 patients with insulin resistance to 35 overweight or obese women with normal glucose homeostasis, there was no lean group for reference. Significantly, all the above studies were conducted on 1.5T systems.

Given the link between steatosis and impaired function, MTGC is an attractive biomarker for intervention studies in diabetes and obesity, although there is conflicting evidence as to whether it may be modified by weight loss and cardiorespiratory fitness. Obesity and insulin resistance are highly interlinked, both in their causes (eg sedentary lifestyle, social class, diet & age) and effects (hypertension, increased left ventricular mass, increased myocardial fatty acid uptake).

As such, excess body weight is a major confounder in the majority of previous studies investigating diabetic cardiomyopathy, Metabolic Syndrome (MetS) and MTGC changes. Moreover, as pharmacological treatment of diabetes may alter MTGC (eg insulin promotes lipogenesis), this adds further uncertainty. Despite this, no study has yet investigated the effect of excess body weight on MTGC in the absence of other identifiable cardiac risk factors to test if size alone is associated with increased cardiac steatosis.

4.1.2 Objectives

This study examined the effects of sex, age, excess body weight and body fat distribution on MTGC in lean, overweight and obese adults without any other potentially confounding classical cardiovascular risk factors. In addition, the relationship between excess body fat and myocardial peak circumferential strain was examined.

4.2 Methods

4.2.1 *Study design and power calculations*

The study protocol was approved by the National Research Ethics Service (09/H0505/97), and registered on the Thames Valley Comprehensive Local Research Network (CLRN ID 8632), as per the requirements of the British Heart Foundation and the UK National Health Service for clinical research. Participants were recruited by poster advertisement to take part in an observational magnetic resonance study to assess the effects of body composition on the heart and assigned to one of three BMI groups: lean (BMI 18.5 – 24.9 kg/m²), overweight (BMI 25 – 29.9 kg/m²) or obese (BMI > 30kg/m²).

Normal BMI adults are reported to have myocardial lipid content of 0.4% of the water signal. This rises to 0.8% in obese adults. The Office of National Statistics (ONS) analysed the proposal and recommended sample sizes of 20 in each category of lean men, lean women (to determine the effect of gender on MTGC), overweight and obese. To detect the predicted rise in myocardial lipids with 95% power, we would need at least 12 in each group, but the ONS suggested 20 as a normal distribution with the mean value for MTGC in the normal range could not be assumed.

Participants were excluded if they took any cardiovascular medication (including antihypertensive or lipid-lowering agents) or if they had a current or past smoking habit, diabetes mellitus (fasting serum glucose > 7.0mmol/l), hyperlipidaemia (cholesterol > 6.5mmol/l), hypertension (systolic > 140mmHg; diastolic BP > 90mmHg), obstructive sleep apnoea or an abnormal electrocardiograph (ECG). Participants with any history or clinical evidence of heart failure, valvular or congenital heart disease, or with any contraindication to MR scanning (eg pregnancy, known claustrophobia, metallic foreign body) or previous weight reduction surgery were also excluded. Recruitment began in October 2009 and ended in November 2011. Written

informed consent was obtained from all participants. Subjects were asked to eat a simple evening meal and then fast overnight prior to the study day.

4.2.2 Anthropometric and biochemical measurements

Height, weight, waist circumference and sagittal abdominal diameter (SAD) were measured, as described in Chapter 2. Venous blood was taken from subjects in the seated position and sent for biochemical analysis of total cholesterol, high density lipoprotein (HDL), serum triglyceride content, insulin and glucose.

4.2.3 Myocardial Magnetic Resonance Spectroscopy (^1H MRS)

Data acquisition

Myocardial lipids can be quantified using localised cardiac-triggered proton spectroscopy in a 3 Tesla system, as described in Chapter 2. Breath-holding technique was explained and practised before data acquisition commenced.

Data analysis

Raw data was saved as rda files in an anonymised format and analysed offline by investigators (RB & BR) blinded to the anthropometric measurements and other data.

4.2.4 Myocardial Strain Imaging (3T)

Data acquisition

Strain imaging using left ventricular tagging was used to determine global mid-ventricular systolic circumferential strain (CS), as described in Chapter 2. Gradient echo high-resolution

tagging pulse sequence was then performed at the basal, mid-ventricular and apical levels of the left ventricle. The mid-ventricular slice was chosen from the short-axis series, and the basal and apical slices were both equidistant from this mid-ventricular slice. All data was acquired during breatholds and ECG-triggering to capture 90% of the R-R interval.

Data analysis

Tagged images were saved in an anonymised format and analysed by an experienced operator (JJS) blinded to the rest of the data using Cardiac Image Modeller software (CimTag2D v7 Auckland Medical Research, Auckland, New Zealand), as described in Chapter 2.

4.2.6 Cardiac MR Imaging protocol (1.5T)

Subjects were studied in a 1.5T Siemens Avanto system to measure ventricular volumes, function and myocardial mass and function, using the method described in Chapter 2.¹⁴⁶ LV myocardial mass was calculated by subtracting the endocardial volume from the epicardial volume; LV mass estimation was based on prior knowledge of myocardial specific gravity (1.05 g/cm³). Interobserver variability was assessed by two operators (RB & JJS) reporting the data from the first ten patients. The coefficient of variation was 6%.

4.2.7 Assessment of adiposity and body fat distribution

Dual X-ray absorbitometry (DXA) studies were performed on a GE Lunar system to measure body composition from lean mass, bone mineral content and total fat mass. Body fat content was expressed as total body fat mass and body fat percentage.

A water-suppressed turbo-spin-echo (TSE) transverse axial 5mm slice at the level of the 4th/5th lumbar intervertebral (L4/5) disc was used to determine visceral and subcutaneous abdominal

adipose tissue (AT) content, which cannot be accomplished with DXA, by manual contouring of AT depots.

4.2.8 Statistical analysis

Descriptive statistics were used to summarise subject characteristics. Summary data was presented by BMI levels. The Kruskal-Wallis test was used to determine significant differences between BMI categories among continuous variables. Spearman correlation (r_s) was calculated to test associations between continuous variables.

Multivariable linear regression analysis was used to investigate cross-sectional associations between MTGC and CS as outcome variables and anthropometry, sex, age and insulin resistance as potential predictors. Separate regression models were considered for BMI and waist because of the high correlation between the two. As the MTGC distribution was positively skewed, it was log transformed (logarithm to the base e) prior to regression analysis. Statistical analyses were performed using Stata SE version 11.2 (StataCorp, College Station, TX, USA). For further description, please see Statistical Supplement at the end of this chapter.

4.3 Results

One hundred and two adults volunteered from the general public, of whom 100 subjects completed the study protocol and the remaining two were excluded on account of poor breath-holding and claustrophobia respectively.

In the 100 subjects included in the final analysis, 44 were lean (median BMI 21.9 (IQR 2.6) kg/m²), 28 overweight (26.8 (2.3) kg/m²) and 28 obese (39.4 (13.8) kg/m²). Spectroscopy data

was acquired and analysed in all subjects – examples are shown in Figure 4.1a and b. Strain data was available for 97 participants – examples are shown in Figure 4.2.

All variables showed significant differences between BMI levels except age, height, total cholesterol, LV end systolic volume (Table 4.1). There was no difference in LV ejection fraction between the lean, overweight and obese groups, but LV mass was increased in the overweight and obese groups, as expected.

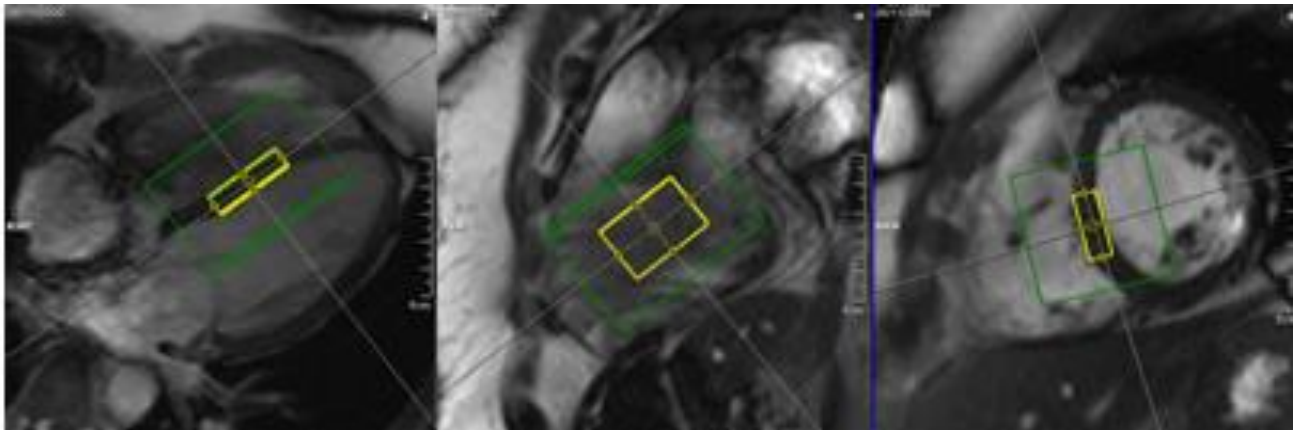
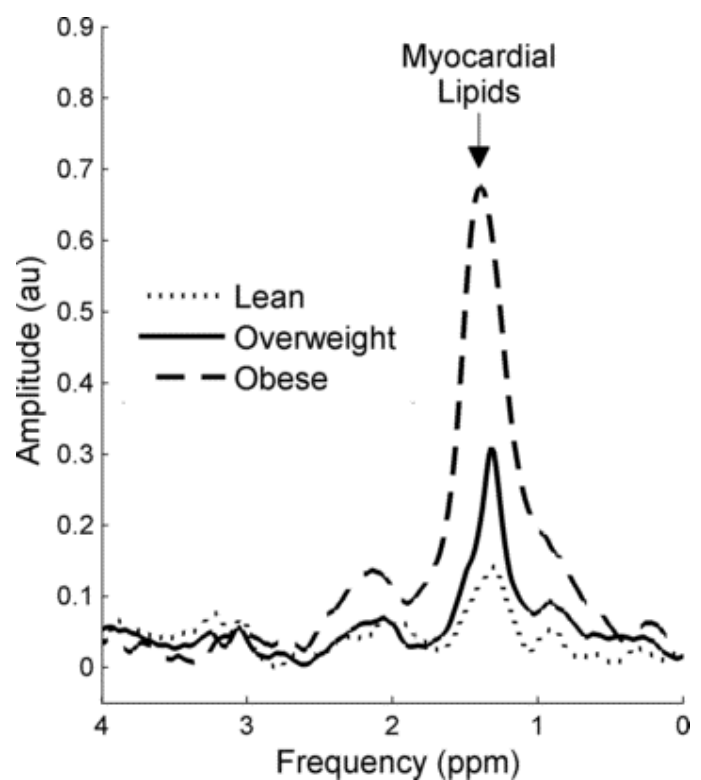


Figure 4.1a (above) and b (right). MTGC measurement with ^1H MRS

Figure 4.1a shows how a 4.6ml (23 x 8x 25 mm) voxel was positioned in the interventricular septum on the four-chamber and short axis cine images at mid-diastole. Figure 4.1b shows cardiac water-suppressed data for lean, overweight and obese subjects. MTGC is calculated from the area under the lipid peak at 1.3ppm.

In all three subjects, the unsuppressed water peak was 1.3 – 1.4 arbitrary units (au), so the water suppressed peaks shown here can be used for comparison.



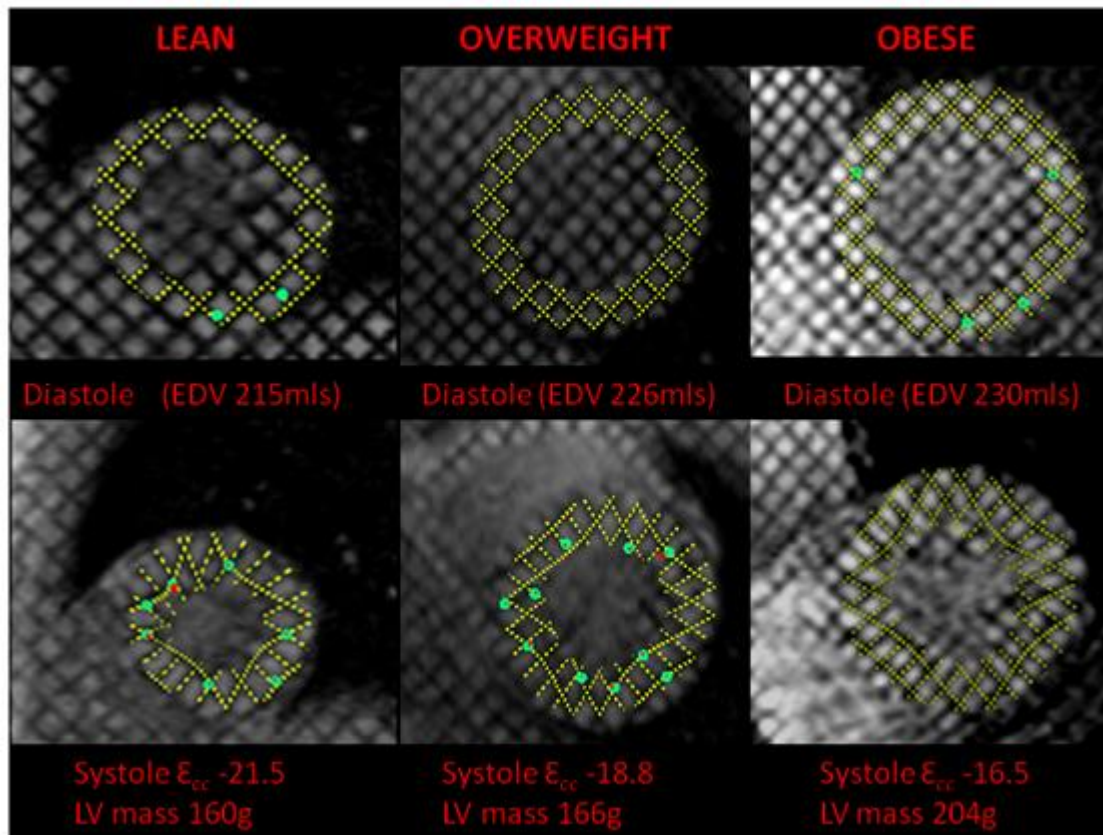


Figure 4.2. Myocardial strain imaging at 3T in obese and normal male subjects

Less deformation, and quantitatively more positive peak circumferential strain (ϵ_{cc}), can be seen in the overweight and obese individuals compared to the lean. They have similar ejection fractions (lean 66%, overweight 61%, obese 64%) and stroke volumes (142mls, 139mls and 147mls respectively), but the obese subject has markedly greater LV mass.

Table 4.1: Subject characteristics by BMI levels				
	Lean n=44 (BMI 18.5 – 24.9 kg/m ²)	Overweight n = 28 (BMI 25 – 29.9kg/m ²)	Obese n = 28 (BMI ≥ 30kg/m ²)	P value [‡]
Sex (No of women) [†]	26 (59%)	15 (54%)	18 (64%)	0.717 [†]
Age (years)	39.5 (26.50)	39.0 (12.5)	42.0 (14.5)	0.972
Anthropometric measurements				
Weight (kg)	64.9 (8.0)	80.0 (14.6)	112.5 (28.6)	<0.001
Body mass index (kg/m ²)	21.9 (2.6)	26.8 (2.3)	39.4 (13.8)	<0.001
Sagittal abdominal diameter (cm)	18.0 (2.3)	21.3 (2.3)	26.5 (5.3)	<0.001
Waist circumference (cm)	76.0 (10.0)	89.5 (13.0)	111.0 (18.0)	<0.001
Serum biochemistry				
Glucose (mmol/l)	4.9 (0.5)	5.2 (0.9)	5.2 (0.6)	0.015
Insulin (micromol)	11.8 (4.9)	12.1 (4.5)	17.8 (7.0)	<0.001
HOMA-IR	2.5 (1.3)	2.9 (1.4)	4.1 (1.6)	<0.001
Total cholesterol (mmol/l)	5.1 (1.1)	5.4 (1.0)	5.0 (1.3)	0.342
Measures of adiposity and ectopic fat deposition				
Total cross-sectional fat (cm ²)	168 (132)	378 (108)	744 (464)	<0.001
Subcutaneous fat (cm ²)	123 (108)	266 (106)	640 (376)	<0.001
Visceral fat (cm ²)	30.6 (31.6)	63.9 (33.6)	136 (84.8)	<0.001
Fat mass (kg)	14.7 (7.4)	26.3 (7.4)	47.5 (29.8)	<0.001
Fat (% of body mass)	24.9 (11.9)	33.1 (13.3)	46.7 (15.2)	<0.001
Myocardial parameters				
Left Ventricular Mass (g)	99 (33)	116 (63)	121 (30)	0.001
LV Ejection Fraction (%)	70.1 (7.5)	69.5 (6.4)	69.6 (8.5)	0.84
Data are a Median (Interquartile range). P value: Kruskal-Wallis rank test. For the variable sex [†] chi square test of independence was used.				

4.3.1 *Myocardial Triglyceride Content (MTGC)*

Body size had a marked effect on cardiac steatosis, which was significant even after adjustment for age, sex and plasma insulin. The measured MTGC in the lean group was 0.37 (0.21), which is similar to that found in previous studies.^{91, 95} Increasing age was weakly, but significantly, associated with increasing MTGC ($r_s = 0.23$, $p < 0.05$), but gender was not a significant variable (Table 4.2). Compared to this lean group, overweight subjects had MTGC of 0.45 (0.27), and obese had 0.67(0.63), both significantly higher with $p < 0.001$ (Figure 4.3). All anthropometric and MR measures of adiposity were significantly associated with MTGC (r_s values 0.36 – 0.53, $p < 0.001$, supplemental data). Insulin concentration ($r_s = 0.39$, $p < 0.001$) and HOMA-IR ($r_s = 0.36$, $p < 0.001$) were also positively associated with MTGC. In multivariable regression analysis, a 1kg/m^2 increase in BMI was associated with a 4% cross sectional increase in MTGC. A 1cm increase in waist circumference was associated with a corresponding 2% increase in MTGC (Model II, Table 4.2). The addition of insulin did not change the results of this model, suggesting that adiposity and MTGC have an association independent of insulin sensitivity. The key determinants of MTGC in this cohort were measures of excess body weight, but not age, sex or insulin. The linear relationship between excess weight and MTGC is clearly shown in Figure 4.5A.

Table 4.2. Multivariable linear regression analysis for MTGC
Separate models for BMI and waist circumference

MTGC (% of water signal)[‡]

	(I)	(II)	(III) β (95% CI)
BMI model			
BMI (kg/m ²)	0.04 (0.03 , 0.06)***	0.04 (0.03 , 0.06)***	0.04 (0.02 , 0.06)***
Age (yrs)		0.01 (0.00 , 0.02)*	0.01 (0.00 , 0.02)*
Gender		0.03 (-0.19 , 0.25)	0.04 (-0.19 , 0.28)
Insulin (umol)			0.01 (-0.01 , 0.04)
R ²	33%	35%	35%
	(I)	(II)	(III) β (95% CI)
Waist circumference model			
Waist (cm)	0.02 (0.01 , 0.03)***	0.02 (0.02 , 0.03)***	0.02 (0.01, 0.03)***
Age (yrs)		0.01 (0.00 , 0.02)	0.01 (0.00 , 0.02)
Gender		-0.24 (-0.47 , -0.01)*	-0.22 (-0.46 , 0.02)
Insulin (umol)			0.01 (-0.02 , 0.04)
R ²	32%	36%	36%

[‡] Logarithm of MTGC was used as dependent variable.

Results can be transformed to % difference by using 100*[exp(β)-1].

*p<0.05, **p <0.01, ***p<0.001. CI: Confidence interval. R² (R squared)

I: Unadjusted only BMI or waist circumference was included in the model;

II: Adjusted for age and sex;

III: Adjusted for age, sex and insulin.

Results correspond to a 1 unit change 'increase' in each independent variable.

Figure 4.3: Distributions of Myocardial Triglyceride Content and Strain by BMI levels

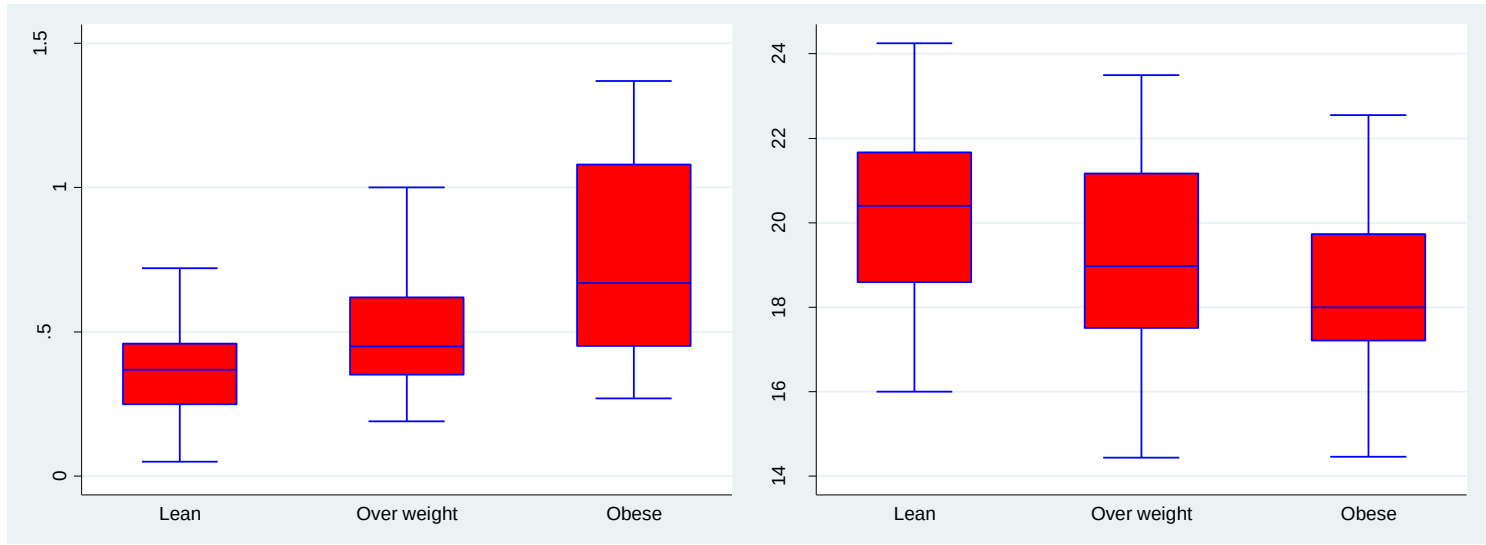


Figure 4.4: Distributions of Myocardial Triglyceride Content and Strain by gender

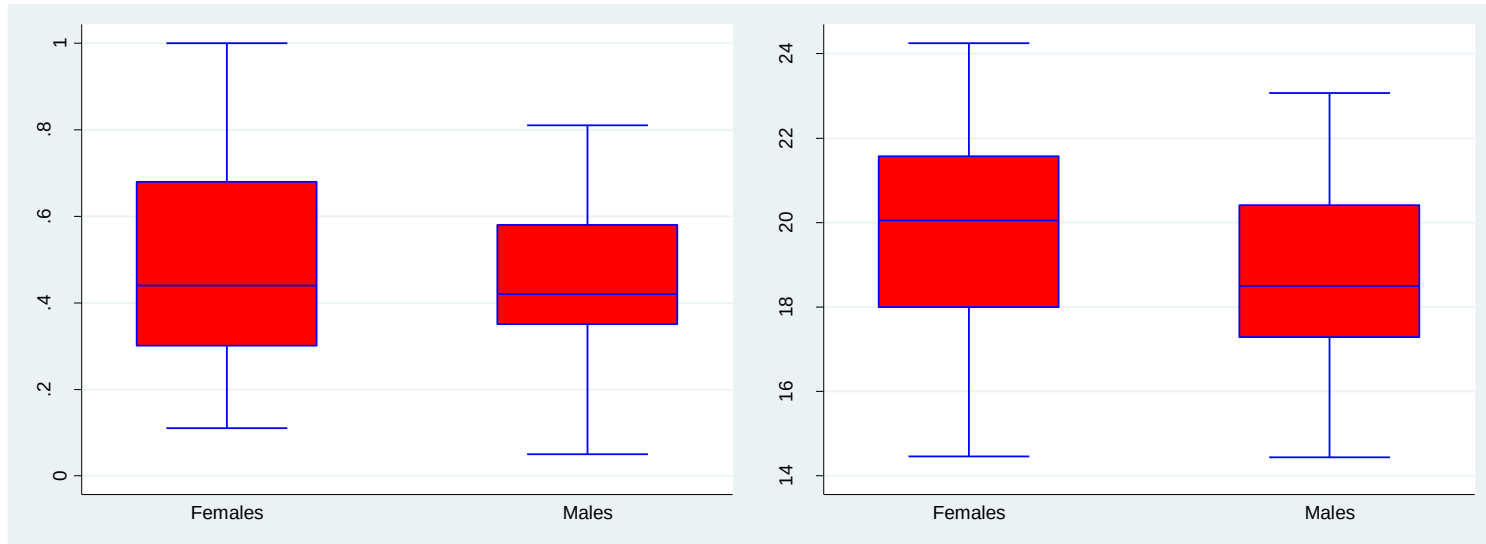
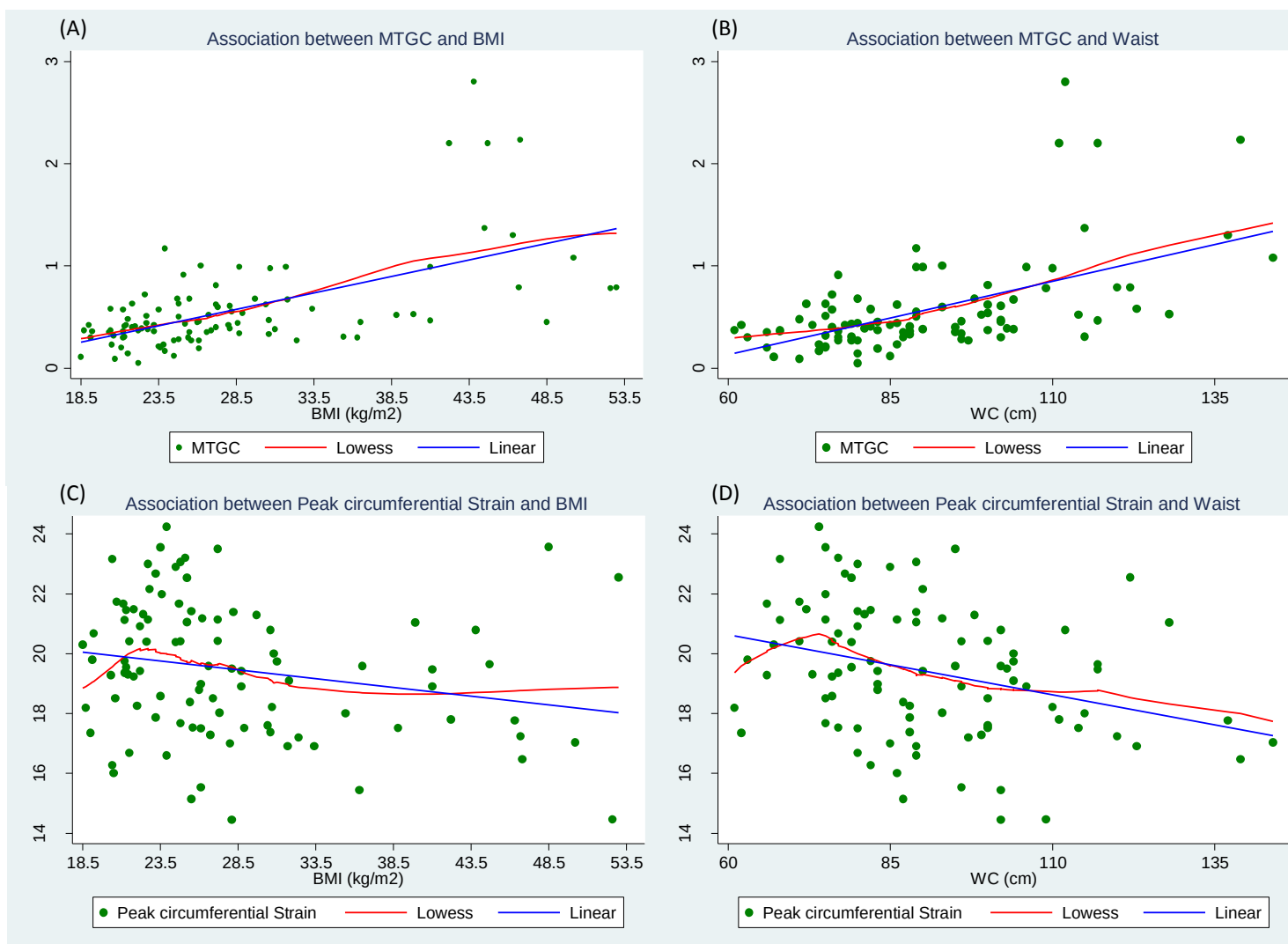


Figure 4.5. Adiposity and associations with Myocardial Triglyceride Content (A & B) and Peak Circumferential Strain (C & D)

Linear regression is plotted in blue; locally-weighted scatter plot smooth (LOWESS) curves to examine potential departure from nonlinearity are plotted in red.

For MTGC, there is a clear linear association with BMI ($r_s = 0.53$; $p < 0.01$) and with waist circumference ($r_s = 0.52$; $p < 0.01$). These are maintained even after adjustment for the effects of other variables (Table 4.2).

For CS, the associations are less marked with BMI ($r_s = 0.25$; $p = 0.04$) and waist ($r_s = 0.33$; $p = 0.01$). On multivariable analysis, they lose statistical significance, and are dominated by the effect of insulin (Table 4.3).

4.3.2 *Peak Circumferential Strain (CS)*

The overweight and obese groups were associated with more positive strain (Figure 4.3), although this did not reach statistical significance with the overweight group. CS was fairly normally distributed across the whole cohort (range -14.4% to -24.3%; see supplementary statistical data). Obese subjects had a median CS of -18.0 (2.5), which is 11.8% more positive than in their lean counterparts ($p < 0.007$). Women had significantly more negative CS compared to men ($p = 0.006$), as shown in Figure 4.4. BMI, waist, visceral fat content and insulin resistance were all associated with a more positive peak circumferential strain. Age did not have a significant association with strain.

In regression analysis, men had more positive strain compared to women, and higher fasting insulin levels was associated with a more positive strain, as shown in Table 4.3. Adjusting for age and gender did not change the associations between either BMI or waist circumference and CS. However, adding insulin rendered them statistically non-significant, whilst, in contrast, the effects of gender and insulin remained – a 1 micromol/l rise in insulin corresponded to a 0.7% more positive CS (-0.14, $p < 0.001$), whilst the male sex corresponded to a 5% more positive CS (-1.07, $p < 0.05$) compared to the female sex. Thus, insulin appears to be a key driver for myocardial strain, whereas it has no discernible effect on MTGC in this population. The R^2 for the strain models were lower than for the corresponding MTGC analyses: 20% in the BMI model and 21% in the waist circumference model. Bootstrap analysis confirmed the statistical significance found in Table 4.3 (see supplementary statistical data).

Table 4.3. Multivariable linear regression analysis for strain
Separate models for BMI and waist circumference

Peak circumferential strain

	(I)	(II)	(III) β (95% CI)
BMI model			
BMI (kg/m ²)	-0.06 (-0.11, -0.04)*	-0.07 (-0.012, -0.02)**	-0.03 (-0.09, 0.034)
Age (yrs)		0.02 (-0.02, 0.05)	0.01 (-0.03, 0.05)
Gender		-1.40 (-2.28, -0.53)**	-1.36 (-2.26, -0.47)**
Insulin (umol)			-0.13 (-0.23, 0.03)*
R ²	33%	35%	35%

	(I)	(II)	(III) β (95% CI)
Waist circumference model			
Waist (cm)	-0.04 (0.01, 0.03)**	-0.04 (-0.06, -0.01)**	-0.01 (-0.04, 0.02)
Age (yrs)		0.03 (-0.01, 0.06)	0.01 (-0.02, 0.05)
Gender		-0.84 (-1.73, 0.05)	-1.07 (-1.97, -0.17)*
Insulin (umol)			-0.14 (-0.25, -0.04)**
R ²	32%	36%	36%

Results can be transformed to % difference by using $100 \times [\exp(\beta) - 1]$.

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. CI: Confidence interval. R² (R squared)

I: Unadjusted only BMI or waist circumference was included in the model;

II: Adjusted for age and sex;

III: Adjusted for age, sex and insulin.

Results correspond to a 1 unit change 'increase' in each independent variable.

4.4 Discussion

These data represent the largest myocardial spectroscopy study to date in Europe, and show clear positive relationships between *all* measures of increasing excess body fat and MTGC. Moreover, it was conducted at 3 Tesla, with improved signal:noise ratio (SNR) compared to previous 1.5T studies. This was especially important in the assessment of lower MTGC values. Waist circumference and BMI predict MTGC independently of insulin in lean, overweight and obese adults, which has not thus far been reported. Cardiac steatosis has been previously described as a precursor to LV dysfunction in diabetic cardiomyopathy.⁹⁵ As obesity and type 2 diabetes mellitus are clearly co-linear, we sought to recruit only normoglycaemic obese adults with no hyperlipidaemia, the so-called ‘fit-fat’ phenotype, and have shown that cardiac steatosis occurs even in this group. The ranges of MTGC obtained for normal and obese subjects are similar to those previously reported for normal and diabetic patients.⁹⁵ Even in the subjects with the most steatosis in this study, the lipid fraction remained <3%, and does not account for the 20% increase in LV mass, supporting the hypothesis that increased stroke volumes required in larger people lead to a physiological hypertrophic response. In essence, obesity leads to substantial LV muscular hypertrophy, as seen in hypertensive disease, and also a linear rise in MTGC, but ectopic fat does not account for the increased LV mass.

Although the obese group had a significant reduction in strain as well as increased steatosis, there was no relationship between MTGC and peak circumferential strain. This suggests that functional impairment of cardiac contraction in these obese patients occurs independently of steatosis, not as a result of it. This is in contrast to the results from the 2010 study by Ng and colleagues, which found no association between glucose homeostasis and strain.¹⁶¹ The key predictor of strain in this non-diabetic cohort was insulin sensitivity, thus providing further

evidence in support of a 'metabolic syndrome cardiomyopathy'. Many studies have examined ventricular function in obesity, and have suggested impaired strain with multivariable logistic regression.^{26, 72, 163} However, the assessment of myocardial contractile function with echocardiographic techniques is inherently limited by poor acoustic windows, especially in severe obesity and in women. Other studies have shown that obesity is linked to heart failure, but have done so without controlling for diabetes, insulin resistance, hypertension and other weight-related cardiovascular conditions.¹⁶⁴

Diabetic cardiomyopathy, with marked steatosis, diastolic dysfunction and impaired contractility, may develop from 'benign' myocardial steatosis as a result of lipotoxicity and ceramide accumulation. This would mirror the pathology of liver fibrosis developing from fatty liver disease, which is also associated with visceral adiposity. Ceramides are small molecules consisting of a fatty acid, typically palmitate, bound to sphingosine.¹⁶⁵ Accumulation within cells in both normal and disease states leads to apoptosis, possibly through inhibition of mitochondrial function through interruption of the inner mitochondrial membrane. Ceramide is a known cardiotoxin which accumulates in mitochondria.¹⁶⁶ Unfortunately, ceramides and triglycerides have indistinguishable peaks in 3T human ¹H MRS, so we cannot measure them separately. Indeed, one of the key limitations of this study is that the measured MTGC represents a small ceramide fraction too, which may in fact be the salient, but indistinguishable, pathologic intermediate. If this is the case, it would be highly unlikely that this would diminish with any lifestyle intervention. Many drugs that alter metabolism or accumulate in the mitochondria, including amiodarone, are associated with ceramide formation and cardiotoxicity in animal models.^{166, 167}

As with the metabolic syndrome and atherogenesis, there are several interlinking mechanisms between obesity and impaired cardiac contractility. It may be that severe diabetic cardiomyopathy occurs with much higher MTGC and/or ceramide, just as steatohepatitis

follows from patients with markedly high liver fat, with the additional insult of ongoing inflammation and fibrosis. However, previous studies linking cardiac steatosis to impaired strain may have been confounded by the size of the diabetic subjects and the relative level of MTGC and ceramide.

In our multivariable regression models, both sex and insulin were significantly associated with impaired strain in a linear fashion, whereas BMI and waist circumference were not. The difference in strain between the sexes has been reported recently,¹⁴⁷ and may in part explain why the Framingham cohort showed the annual incidence of symptomatic systolic dysfunction to be higher in men than women (7.2/1000 v 4.7/1000).¹⁶⁸ Higher fasting insulin levels were also associated with a more positive strain, which is to be expected as insulin resistance is a marker of physical deconditioning and altered substrate utilisation. From the multivariable analysis, there was no statistically significant linear association between anthropometric indices and strain, due in part because the thinnest subjects in the lean category (measured by BMI and waist) had more positive strain (Figures 5C & D). Also, it may well be that adiposity and insulin resistance are entirely co-linear in determining strain, and further studies are needed to see if dietary and exercise interventions can improve CS by increasing insulin sensitivity. In populations with more advanced disease, further evaluation of pharmacological treatments to improve insulin sensitivity may alter myocardial strain, possibly at the expense of greater lipid accumulation in the heart. The longterm effects of this potential lipotoxicity will need to be monitored.

4.4.1 Potential Limitations

This was a cross-sectional observational single-time point study design, wherein all subjects had normal LV function with no classical cardiovascular risk factors, no symptoms or signs

of heart failure. Thus it cannot establish a causal link between excess bodyweight, measures of adiposity and insulin resistance on the one hand and MTGC or CS on the other hand. Moreover, many of the variables studied here are co-linear, and so there is potential confounding.

With regard to the spectroscopy method, one criticism is that the measured value is a ratio of lipid to water signal. In this largely healthy cohort, there is an assumption that the water content of myocardium is relatively constant. In disease states that involve fibrosis, with resultant expansion in interstitial space and higher tissue water content, this assumption may well be incorrect, and referencing to a fixed value may be required, especially when comparing different populations.

The study recruited by open advertisement, and is therefore susceptible to participants' self-selection bias. As a result, ethnic minorities and obese men are under-represented. Also, whilst diabetes was excluded, insulin and HOMA-IR are continuous variables that are influenced by excess weight, and there were predictable differences between BMI groups. Although it is highly likely that measures of exercise capacity and cardiopulmonary fitness may in part determine both CS and MTGC, these variables were not investigated. Each subject was at low risk for coronary artery disease, but they did not have formal stress perfusion or echocardiography examinations to exclude coronary ischaemia. Lastly, although each subject came for their study day after an overnight fast, we did not take into account participants' dietary, physical activity or sleep habits, which may also contribute to variation in MTGC and/or strain.

4.5 Conclusions

In summary, we have shown that excess body weight, measured by any parameter (BMI, waist circumference, total body fat) is linearly associated with increasing cardiac steatosis in the absence of any other cardiovascular risk factors and independently of insulin resistance. Both obesity and male sex are associated with more positive peak circumferential strain, which is evident despite a compensatory increase in LV mass in obese subjects. Obesity is associated with a 10% impairment of myocardial contractility, which is driven by insulin. From this population, it would seem that adiposity determines steatosis, whilst insulin and gender, but not MTGC, are key variables for strain.

4.6 Supplementary statistical data

Descriptive statistics were used to summarise subject characteristics including the distribution of age, MTGC and CS, measures of adiposity, anthropometrics and biochemical variables. Adiposity was assessed using cross-sectional fat, subcutaneous fat, visceral fat, total fat mass and body fat percentage (%Fat). Body size was measured using height, weight, body mass index (BMI), waist circumference (Waist) and SAD. Insulin resistance was assessed using insulin and HOMA-IR.

Data were presented as median and inter quartile range (IQR) for continuous variables. Summary data was presented by BMI levels lean ($\text{BMI} < 25 \text{ kg/m}^2$), overweight ($\text{BMI} \geq 25 \text{ kg/m}^2$ and $< 30 \text{ kg/m}^2$) and obese ($\text{BMI} \geq 30 \text{ kg/m}^2$). The Kruskal-Wallis test was used to determine significant differences between BMI categories among continuous variables, while the chi-square test of independence was used to test association between gender and BMI levels. Spearman correlation (r_s) was calculated to test associations between continuous variables and to detect putative confounding relationships between MTGC and/or CS and key predictor variables (shown in supplementary correlation matrix, below).

Table 4.4 Supplementary correlation matrix : Spearman measures

	Age (yrs)	BMI (kg/m ²)	Waist (cm)	Insulin	HOMA -IR	Viscera l fat (cm ²)	Total fat mass (kg)	Total body fat (%)	MTGC (% of water)	Peak Strain
Age (yrs)	1									
BMI (kg/m ²)	0.08	1								
Waist (cm)	0.12	0.89***	1							
Insulin (micromol)	0.02	0.55***	0.55***	1						
HOMA-IR	0.06	0.54***	0.57***	0.97***	1					
Visceral fat (cm ²)	0.29* *	0.76***	0.88***	0.51***	0.54***	1				
Total fat mass (kg)	0.19	0.93***	0.80***	0.54***	0.53***	0.75***	1			
Total body fat (%)	0.25*	0.77***	0.55***	0.42***	0.39***	0.55***	0.92***	1		
MTGC (% of water)	0.23*	0.53***	0.52***	0.39***	0.36***	0.51***	0.53***	0.46***	1	
Peak Strain	0.10	-0.25*	-0.33**	-0.32**	-0.33**	-0.30**	-0.22*	-0.09	-0.12	1

Regression analysis was used to investigate cross-sectional associations between MTGC and CS as dependent variables (outcome variables) and anthropometry, gender, age and insulin resistance variables as potential predictors. Separate regression models were considered for BMI and waist because of high correlation between the two.

Three regression models were considered:

I: unadjusted; only BMI or waist were included (separately) in the regression model.

II: adjusted for age and sex; these two variables were added to model I.

III: insulin was added to model II. BMI, waist, age and insulin were all included as continuous variables. For gender, the category “females” was included as a reference.

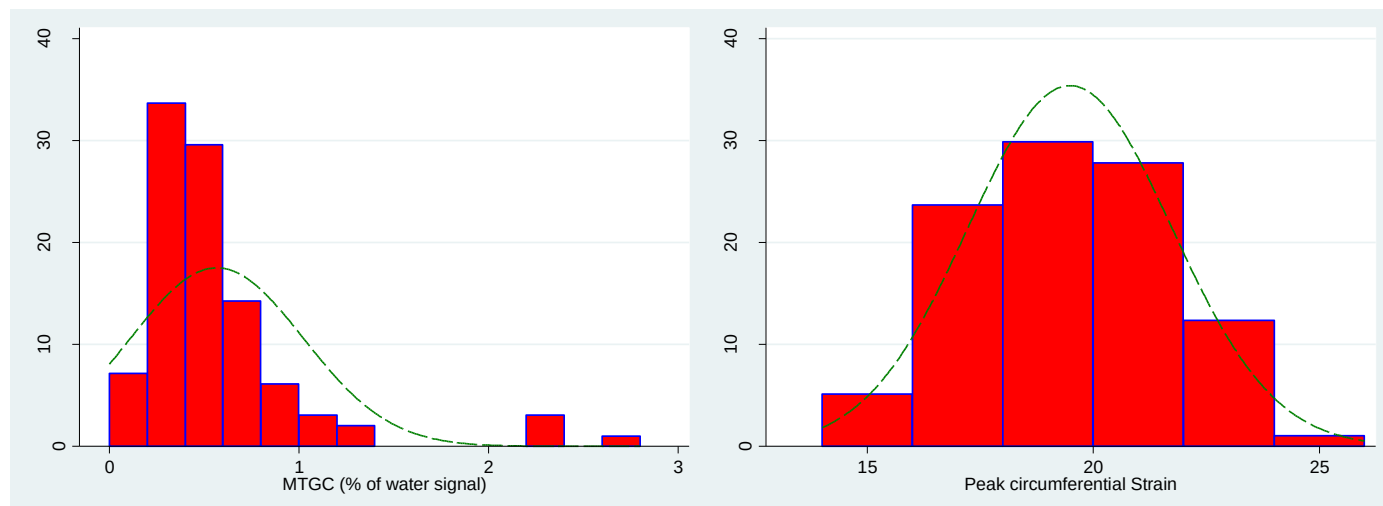
Because MTGC distribution was positively skewed (see supplementary data below), it was log transformed (logarithm to the base e) prior to regression analysis. In order to interpret the results as an average percentage difference in MTGC for a one unit increase in independent variables, the regression coefficients were then back transformed using the following equation:

$$100 * [\exp(\text{regression coefficient}) - 1]$$

CS was fairly normally distributed and therefore was included in the regression models without transformation (see supplementary data below). Although some predictors were positively skewed they were not log transformed in the regression models because there is no assumption that the predictor variables be normally distributed in linear regression.

Supplementary data

Distributions of Myocardial Triglyceride Content (% of water signal) and Peak Circumferential Strain



Locally-weighted scatter plot smooth (LOWESS) curves were investigated to examine potential departure from nonlinearity. The results show that there were no significant nonlinear trends between MTGC or CS and any of the variables studied.

In model III the variance inflation factor (VIF) was calculated to quantify the severity of any potential multicollinearity (predictors that are linearly related to each other). We also tested for heteroskedasticity of the residuals using the Breusch-Pagan/Cook-Weisberg test. Normality of the residuals was assessed graphically using kernel density estimate and also with the Shapiro-Wilk test for normality. To check the accuracy of confidence intervals based on the normality assumption of the residuals, bootstrap confidence intervals for the regression coefficients were calculated by creating 2000 resamples of the data through random sampling with replacement. Missing data was excluded from the analysis. Results from linear regression models were presented as beta coefficients (β) and 95% confidence

intervals (CI). Statistical analyses were performed using Stata SE version 11.2 (StataCorp, College Station, TX, USA).

Bootstrap analysis of linear regression models:

Bootstrap bias-corrected confidence intervals (Bbc CIs) were derived; this approach does not assume normality of the residuals which were considered to derive CIs in Table 3. For log(MTGC) models Bbc CIs were (0.02, 0.061) and (0.013,0.03) for BMI and waist respectively, while for insulin in the model with WCC (model III which includes (BMI or waist), age, sex and insulin) Bbc CIs were (-0.22,-0.05) and (-0.23,-0.06) for BMI and waist respectively. For gender, Bbc CIs were (-2.23,-0.55) and (-2.01,-0.19). These bootstrap results confirmed the statistical significance of the results in Table 3.

Chapter 5: The effect of excess body weight on hepatic lipid content

5.1 Introduction

5.1.1 *The liver and lipid metabolism*

The liver plays a key role in fat metabolism – it synthesises, stores, and distributes lipids around the body through lipoproteins, which it also produces. Several metabolic pathways are involved in lipid homeostasis, and variations in diet and energy expenditure are managed by all mammals, most graphically in those that hibernate. Adipose tissue is the preferred form for longterm storage of fuel because fat is the most energy-dense fuel store available to man, with 39 kilojoules per gram (kJ/g; Attwater system) of available fuel.¹⁶⁹ Glycogen, in comparison, has only 17kJ/g. The difference is attributable to two key features of fat. Firstly, fat is hydrophobic, and so there is no water within a gram of fat, whereas carbohydrates, being hydrophilic, exist as solvated complexes – 1 gram of glycogen will bind 2g of water, so the energy yield of 1g of ‘hydrated glycogen’, as it would exist in vivo, is only 5kJ/g. Secondly, the energy yield per molecule of fatty acid is three times greater than glucose, because β -oxidation is a highly efficient process compared to glycolysis.¹⁷⁰ However, because fat is insoluble, it is stored in adipose tissue depots separate from the bloodstream, and when required must be mobilised by hydrolysis to generate non-esterified fatty acids (NEFAs). These circulate in the blood, and can be directly taken up by skeletal and cardiac muscle, or by the liver where they can be incorporated into and then transported by lipoproteins to metabolically active tissues, all of which requires time and neurohumoral signalling. Thus we have evolved to store and utilise adipose tissue for longterm energy needs, while keeping an easily accessible pool of carbohydrate for more immediate requirements.

5.1.2 *Ectopic fat in the liver*

Persistent excess calorie consumption and insufficient expenditure through physical activity can lead to a net positive energy balance, which is very common in our ‘obesogenic’ environment. This calorie excess leads in turn to expansion of existing adipose tissue depots and ectopic fat deposition in organs and tissues not designed to store fat, such as the liver. This has been demonstrated in animal models, most famously in foie gras, first made by the ancient Egyptians as a delicacy. Overfed birds would develop marked ectopic fat in their livers, which would give them a soft and creamy texture when cooked. Excess liver fat develops in man as well, but there are a variety of causes for this. Firstly, the liver, when exposed to a high dietary sugar loads (eg sugar-sweetened beverages) or high circulating levels of insulin, increases production of fatty acids, by de novo lipogenesis (DNL). Alternatively there may be an increased flux of fatty acids from adipose tissue to the liver (portal vein hypothesis). This may increase the production of triglyceride, which can be incorporated into hepatocytes or utilised in VLDL production, and then transported in the bloodstream. Secondly, NAFLD can occur when the liver is unable to transport lipids to other organs, usually through a failure of lipoprotein synthesis or function.¹⁷¹ This occurs in protein malnutrition (kwashiorkor) and in abetalipoproteinemia (a hereditary condition with low circulating cholesterol, but high liver fat).¹⁷²⁻¹⁷⁴ Thirdly, disease processes and drug reactions within the liver can lead to a failure of homeostasis, and marked steatosis. This has been seen in patients receiving therapy with steroids, amiodarone and methotrexate.¹⁰⁶

5.1.3 *Alcohol and hepatic steatosis*

Alcohol is a special case in that it is very calorie-dense (29kJ/g), often comes associated with a sugar load, and is hepatotoxic. Unsurprisingly, this leads to marked steatosis and concomitant inflammation, giving rise to the term ‘alcoholic steatohepatitis’ (ASH), which is

seen quite clearly in liver biopsy specimens taken from patients after binge-drinking.¹⁷⁵ Following acute ingestion, most of the ethanol is converted to acetate, which is then used as a fuel in peripheral tissues instead of lipid or endogenous carbohydrate. Peripheral lipolysis and lipid oxidation are directly suppressed by increased circulating acetate. Moreover, ethanol promotes hepatic steatosis by inhibition of AMP kinase, and peroxisome-proliferator-activated receptor α (PPAR- α).^{176, 177} Hepatotoxicity is due in part to altered metabolism resulting in increased free radical production, and also because ethanol increases the permeability of the intestinal wall. Thus endotoxin from the gut lumen can translocate into the portal bloodstream and activate Kupffer cells in the liver, resulting in an acute cellular inflammatory response.¹⁷⁸ This is seen quite clearly in liver biopsy specimens taken from alcoholic patients, and alcoholic steatohepatitis (ASH) was pathognomic for alcoholic liver disease until 30 years ago.

5.1.4 Non-alcoholic fatty liver disease (NAFLD)

Fatty liver disease due to obesity alone as a common clinical entity was recognised in 1980 when a series of patients with little or no history of alcohol intake were found to have liver disease resembling fairly advanced alcoholic liver disease – liver biopsy samples showed extensive fatty deposition and early fibrosis.¹⁷⁹ Over the next 15 years, this was found in several patients, and the term ‘non-alcoholic fatty liver disease’ (NAFLD) became increasingly used to describe them.¹⁸⁰ NAFLD is now the commonest cause of abnormal liver blood tests in the community, and it is estimated that it has a prevalence of 6 - 20% in the UK and the USA, although this is likely to be an underestimate. In Dallas, it is thought to have 34% prevalence in the adult urban population.¹⁸¹

There has been increasing interest in NAFLD patients and potential therapies to see if they are cardioprotective. Moreover, hepatic lipid accumulation is a feature of the Metabolic Syndrome (MetS), for which abdominal obesity is a major determinant. Clearly there is likely to be a considerable overlap between obesity, NAFLD and other cardiac risk factors, but in the early pathogenesis of MetS, it is not known whether clinically significant hepatic steatosis occurs with abdominal adiposity in a 'normal' population, or whether it is a downstream feature of MetS. MRS is an ideal tool for measuring hepatic lipid content (HLC), and has been shown in previous studies to perform better than liver biopsy because it is less susceptible to sampling error and interobserver reliability.¹²³

5.1.5 Objectives

The aim of this cross-sectional study was to determine the relationship between hepatic steatosis and waist circumference in normal, overweight and obese adults with no other MetS risk factors.

5.2 Methods

Adults with no evidence of diabetes, hypertension, hyperlipidaemia, smoking, known heart or liver disease were recruited. After an overnight fast and abstinence from alcohol for 24 hours, waist circumference, height and weight were measured, and blood was collected for biochemical analysis.

Hepatic lipid content (HLC) was measured with proton magnetic resonance spectroscopy (^1H MRS) at 3 Tesla, as described earlier in Chapter 2. Spectra were acquired from an 8cm^3 voxel positioned in the right lobe of the liver, avoiding blood vessels & the biliary tract. Peaks for water and lipid resonances were quantified from the spectra using AMARES (jMRUI software), and HLC expressed as a percentage of the hepatic water content.

A turbo-spin-echo transverse axial 5mm slice at the level of the 4th /5th lumbar vertebrae disc was used to determine visceral and subcutaneous adipose tissue content.

5.3 Results

148 adults were recruited - subject characteristics are shown in Table 5.1.

Table 5.1. Anthropometric and biochemical subject characteristics			
	Men	Women	All
n =	41 (28%)	107 (72%)	148
Age (years)	38.0 (17.5)	44 (7.0)	42.0 (21.0)
Anthropometric measurements			
Height (m) ^a	1.80 ± 0.06	1.65 ± 0.06	1.69 ± 0.12
Weight (kg)	80.5 (25.5)	68.0 (17.2)	70.0 (23.0)
Body mass index (kg/m ²)	25.5 (7.9)	25.1 (6.4)	25.3 (6.6)
Sagittal abdominal diameter (cm)	21.3 (5.8)	19.2 (3.7)	19.7 (3.8)
Waist circumference (cm)	90.0 (19.5)	81.0 (18.0)	83.5 (20.0)
Measures of adiposity and ectopic fat deposition			
Total cross-sectional fat (cm ²)	306 (255)	281 (213)	290 (224)
Subcutaneous fat (cm ²)	215 (168)	236 (164)	234 (180)
Visceral fat (cm ²)	71.6 (93.6)	45.0 (42.2)	50.7 (53.2)
Serum biochemistry			
Glucose (mmol/l)	5.25 (0.81)	4.80 (0.71)	4.90 (0.65)
Insulin (micromol)	13.63 (7.51)	11.48 (4.11)	11.91 (5.20)
Total cholesterol (mmol/l)	5.10 (0.95)	5.20 (1.20)	5.20 (1.10)
Triglycerides (mmol/l)	1.02 (0.86)	0.82 (0.41)	0.86 (0.52)
HDL cholesterol (mmol/l) ^a	1.35 ± 0.37	1.69 ± 0.41	1.60 ± 0.42
Cholesterol:HDL ratio	4.2 (1.75)	3.14 (1.35)	3.33 (1.66)

^aAll data presented as median (interquartile range), except height and HDL cholesterol (in grey), which are presented as mean ± standard deviation, as these data are distributed normally. Normality was determined by the D'Agostino & Pearson omnibus normality test.

5.3.1 *Abdominal obesity and hepatic steatosis*

In normal weight adults (BMI 18.5 – 24.9 kg/m²), hepatic lipid content was 0.9% (IQR 0.6). In overweight adults, this was significantly greater at 2.5% (2.2), and the trend extended to obese adults, with 8.1% (11.2). In both sexes, HLC was most strongly associated with visceral fat content (Women $r_s = 0.74$, men $r_s = 0.78$, both $p < 0.0001$) and waist circumference (Women $r_s = 0.65$, men $r_s = 0.70$, both $p < 0.0001$), more so even than BMI (Women $r_s = 0.57$, men $r_s = 0.68$, both $p < 0.0001$) or total cross-sectional fat (Women $r_s = 0.60$, men $r_s = 0.77$, both $p < 0.0001$). Thus it would appear that abdominal obesity is a better predictor of hepatic steatosis than BMI.

Increased waist circumference seemed to be a prerequisite for hepatic steatosis in this population – a waist circumference ≤ 90 cm excluded HLC $> 5\%$ in all but one subject (Figure 5.1). The sensitivity of this threshold was 95%, with a negative predictive value (NPV) of 99% (Table 5.2). Lean individuals with low waist circumference in both sexes were associated with progressive fall in hepatic lipid content to $< 1\%$; this was best seen when HLC was log transformed (Figure 5.2).

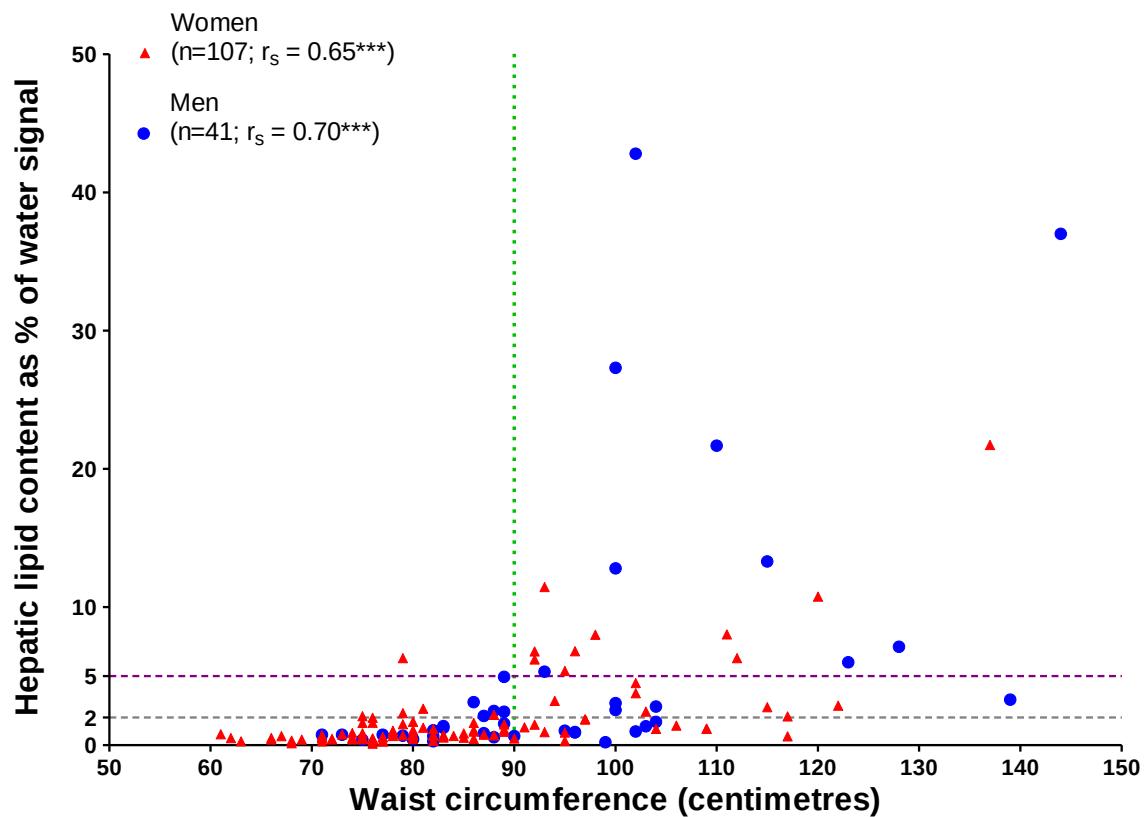


Figure 5.1. Waist circumference and hepatic steatosis

Increasing waist circumference is associated with increasing hepatic lipid content, most clearly seen when waist circumference > 90cm in both sexes.

Table 5.2. Sensitivity and specificity of waist circumference for hepatic steatosis

Comparison of waist circumference > 90cm and gold standard ^1H MRS for the diagnosis of clinically significant hepatic steatosis.

	HLC > 5%	HLC ≤ 5%	
Waist > 90cm	19	29	PPV = 19/48 = 40%
Waist ≤ 90cm	1	99	NPV = 99/100 = 99%
	Sensitivity = 19/20 = 95%	Specificity = 99/128 = 77%	

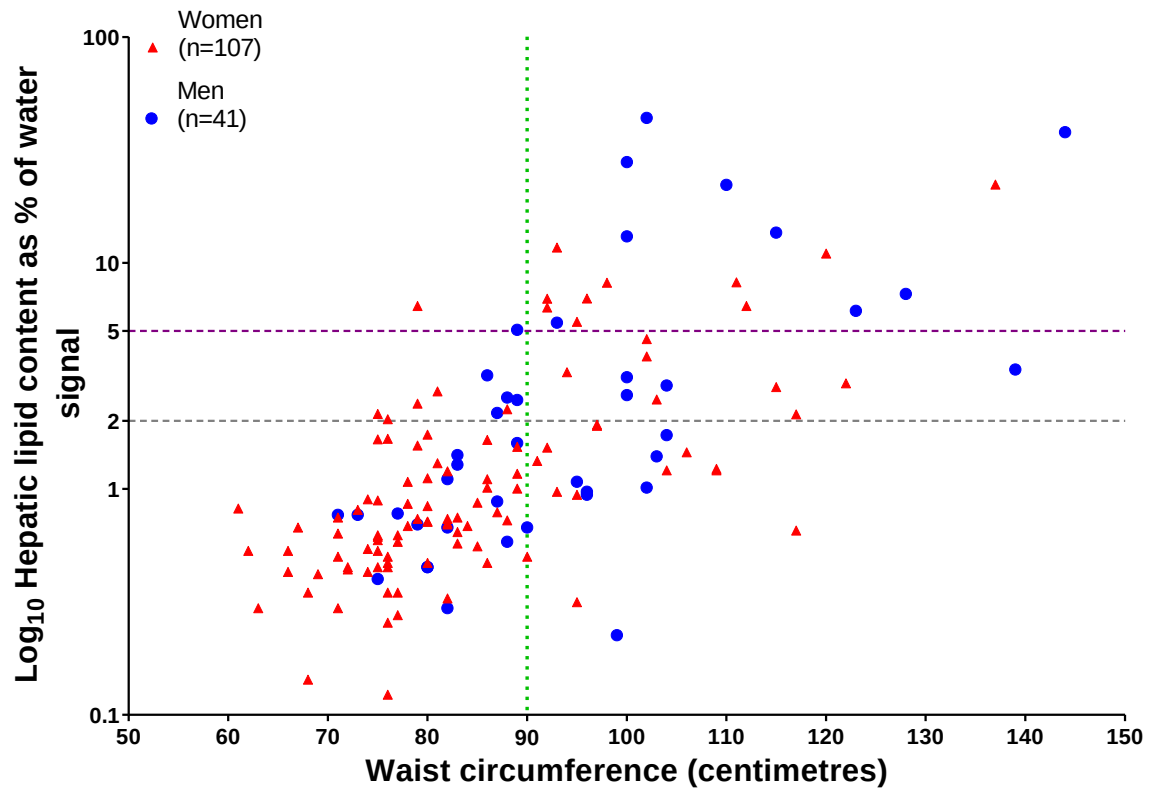


Figure 5.2. Log₁₀ HLC and waist circumference

Log₁₀ HLC and waist circumference have a clear linear relationship – ‘healthy’ HLC is <2%, and is clearly associated with lower waist circumference.

5.3.2 *Hepatic lipid content and dyslipidaemia*

Increasing HLC was strongly associated with increasing serum triglycerides (Women $r_s = 0.44$, men = 0.60, both $p < 0.0001$), increasing insulin (Women $r_s = 0.43$, men = 0.69, both $p < 0.0001$) and reduced HDL cholesterol (Women $r_s = -0.48$, men $r_s = -0.66$, both $p < 0.0001$). Increasing HLC quartiles correlated with the developing dyslipidaemia of MetS, namely increasing serum triglyceridaemia and reduced serum HDL (Figure 5.3).

In multivariable linear regression analysis for the determinants of HDL, gender, triglyceride and $\log_{10}$ HLC were all significant, and the resultant model predicted HDL well ($R^2 = 0.35$, Table 5.3). Insulin and HOMA-IR were not significant predictors of HDL. The addition of waist circumference as a variable improved the model ($R^2 = 0.39$), but $\log_{10}$ HLC was no longer a statistically significant predictor. This confirms that $\log$ HLC and waist circumference are highly colinear, suggesting that one may be an intermediate to the other for the fall in HDL seen in early MetS.

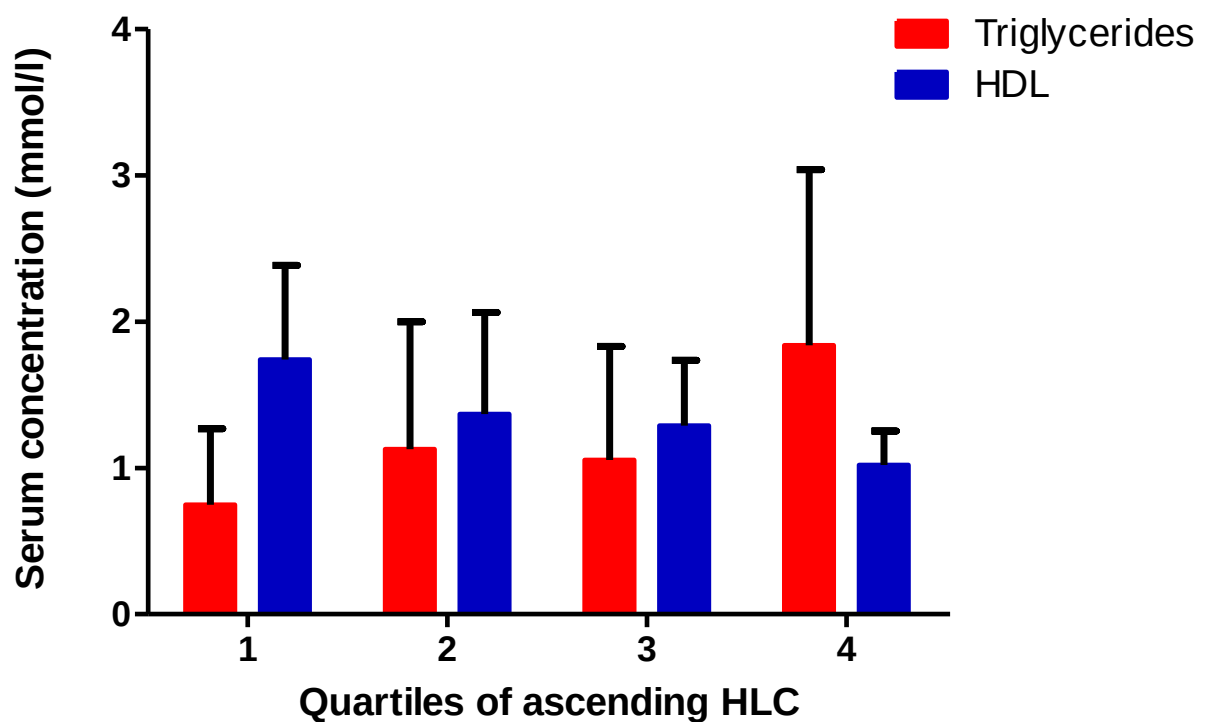


Figure 5.3. Increasing hepatic lipid content is associated with dyslipidaemia

Increasing HLC, shown in quartiles, is closely associated with the dyslipidaemia seen in Metabolic Syndrome of hypertriglyceridaemia and falling HDL. Inset table (below) shows HLC mean and standard deviation(SD) values for each quartile.

HLC	Mean $\pm$ SD
Quartile 1	0.51 $\pm$ 0.34
Quartile 2	1.00 $\pm$ 0.39
Quartile 3	2.40 $\pm$ 1.24
Quartile 4	17.8 $\pm$ 26.2

**Table 5.3. Multivariable linear regression analysis for determinants of HDL-cholesterol
Separate models for BMI and waist circumference**

HDL-cholesterol (mmol/l) [‡]	β (95% CI)
Model 1: Gender, serum triglycerides, and log HLC	
Gender	-0.21 (-0.34 , -0.08)**
Triglycerides (mmol/l)	-0.17 (-0.29 , 0.04)**
Log HLC (% of water signal)	-0.13 (-0.19, -0.07)***
R ²	35%
Model 2: Waist circumference, gender, serum triglycerides and log HLC	
Waist (cm)	-0.007 (-0.011, -0.002)**
Gender	-0.19 (-0.32, -0.06)**
Triglycerides (mmol/l)	-0.16 (-0.29 , -0.04)**
Log HLC (% of water signal)	-0.067 (-0.14 , 0.008)
R ²	39%
[‡] HDL-cholesterol was used as dependent variable. For gender, women were considered as normal, and men as the variant * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. CI: Confidence interval. R ² (R squared)	
Results correspond to a 1 unit change 'increase' in each independent variable.	

Model 1 (above) shows that gender, triglyceride and logHLC are all significant. Being male is associated with an approximately 0.2 mmol/l reduction in HDL across this population (CI - 0.34, -0.08, $p = 0.002$ in model 1, -0.32, -0.06 in model 2, $p = 0.005$). Model 2 (below) shows that the statistically significant impact of logHLC is lost with the addition of waist circumference, suggesting a high degree of colinearity between HLC and waist circumference. Gender and triglyceride remain significantly associated with HDL.

5.3.3 *Hepatic lipid content and serum liver function tests (LFTs)*

83 subjects in the cohort had serum transaminases, alkaline phosphatase, bilirubin and albumin measured. HLC did not correlate with gamma-glutamyl transaminase, bilirubin, alkaline phosphatase or albumin. Alanine transaminase (ALT) and aspartate transaminase (AST) did correlate with HLC – increasing HLC was associated with higher ALT ($r_s = 0.41$, $p = 0.001$; Figure 5.4) and AST ($r_s = 0.32$, $p = 0.006$). Elevated transaminase levels signify inflammation and hepatocyte damage rather than steatosis per se, and so this association is probably driven by the occurrence of steatohepatitis in subjects with steatosis. However, LFTs are not specific to liver disease, and not sensitive either – many patients with liver cirrhosis have ‘normal’ transaminase levels. Thus these associations, although statistically significant, cannot be interpreted further, especially as this study did not control for alcohol consumption.

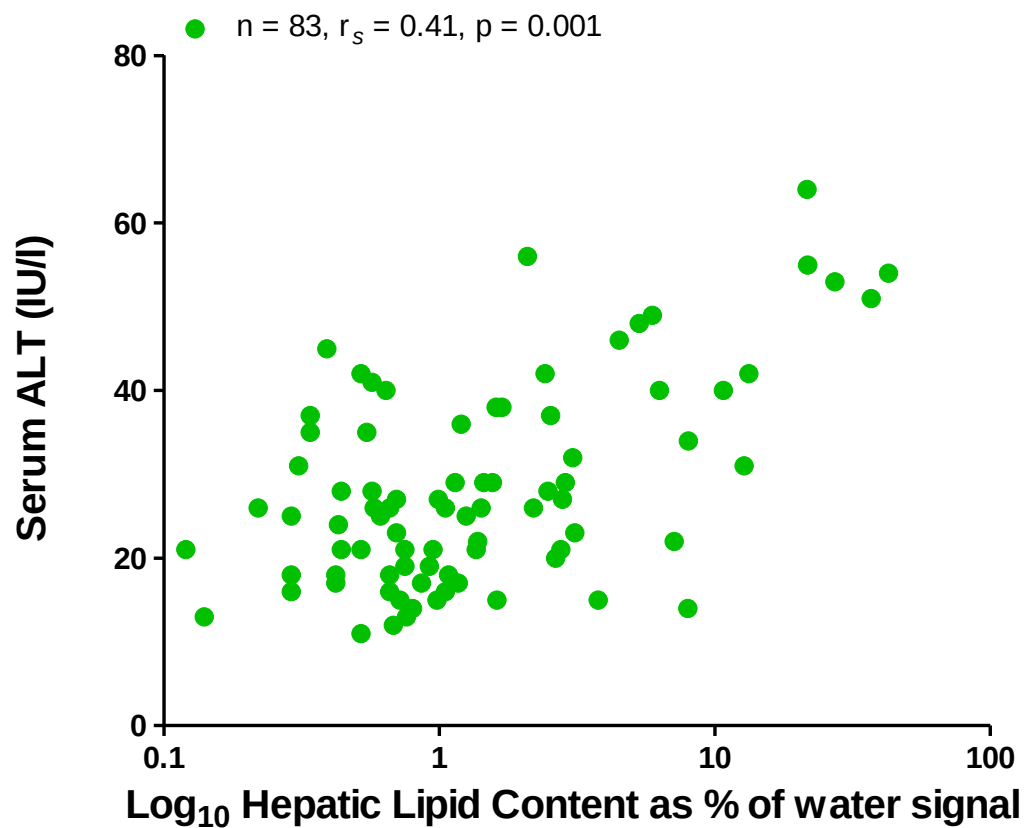


Figure 5.4. Hepatic steatosis and serum ALT

Subjects with increasing HLC are associated with higher ALT, especially when $\text{HLC} > 10\%$, suggesting some degree of inflammation and hepatocyte damage.

5.4 Discussion

This study set out to determine the effects of increasing body size on hepatic lipid content in the absence of any other classical cardiovascular risk factors. Patients with fatty liver disease are twice as likely to get early coronary artery disease compared to the healthy population, and heart disease is the main cause of death in these patients.²⁰ Previous studies in European and North American populations have reported that normal HLC is <6%.^{100, 101} In the cohort described here, HLC in lean adults with no cardiovascular risk factors was <2%. This is a lower ‘normal range’ than previously reported. However, as the majority of the general population is either overweight or obese, one can argue that the normal distribution of size in the population should be reflected in the normal distribution of liver fat content. This dilemma is found in many continuous variables, such as blood pressure and HDL. One solution is to describe HLC <2% as the ‘healthy’ range, but this requires further studies in different populations.

Hepatic steatosis in this cohort of subjects with no classical cardiovascular risk factors is associated with increasing abdominal obesity (waist circumference and visceral fat content), dyslipidaemia, and increased serum transaminase levels. HLC, fasting insulin, visceral fat content and waist circumference are highly colinear, suggesting that they confound each other. It is possible that increased abdominal obesity leads to altered fat metabolism and neuroendocrine signalling, resulting in increased liver fat. This may be mediated by increased leptin and insulin production upregulating hepatic lipogenesis. Alternatively, it is also plausible that dietary excess, especially in carbohydrate intake, leads to de novo lipogenesis in the liver and ‘acute hepatic steatosis’, which over time predisposes to visceral and ectopic fat formation when subcutaneous adipose tissue depots are saturated. – the ‘overflow hypothesis’.¹⁸² These two hypotheses are not mutually exclusive, and it may well be that

hepatic steatosis and visceral adiposity form part of a ‘vicious cycle’, leading to worsening dyslipidaemia and more aggressive MetS. It is interesting to note that these relationships were apparent in this low risk group of mainly Caucasian adults – previous studies have shown a high prevalence of fatty liver in obese adults with abnormal LFTs and/or coexistent hypertension and other risk factors.^{19, 20, 50, 183} As hepatic steatosis and abdominal adiposity seem to predict the changes of MetS even with unremarkable LFTs and in the absence of diabetes, this would suggest that they occur early in the pathogenesis of the syndrome. Since the liver produces HDL, it is feasible that early liver dysfunction manifests as dyslipidaemia. Subsequent reduction in reverse cholesterol transport would lead to adipose tissue accumulation and fit the ‘vicious cycle’ hypothesis, wherein weight gain accelerates as liver synthetic function falls.

Differences in hepatic metabolism, DNL and lipid storage may account for higher cardiovascular risk occurring in ethnic groups with higher visceral adiposity (eg South Asians), and may be related to carbohydrate consumption (eg rice based diets).¹⁸⁴ Waist circumference and NAFLD are partly reversible with dietary modification and weight loss in overweight adults, and the former is very easy to measure. Thus it is an ideal ‘public health biomarker’ – maintaining a waist circumference below 90cm (or 35”) seems to prevent fatty liver and MetS, although this threshold must be tested in different populations. This is important because there are no current generalisable or tractable therapies for the huge numbers of patients with NAFLD and NASH – the most effective management strategy will be a population-based effort to encourage people to reduce their waist size, and this will require clear and simple targets that can be measured in primary care or by individual patients themselves. This also assumes that reversal of weight gain will result in reversal of hepatic steatosis. Current guidelines suggest a variety of targets for waist circumference based on gender and ethnicity, based on population surveys from several organisations in different

regions, which may partly account for why waist circumference is not routinely measured in clinical practice despite being a key predictor of MetS.¹⁷ Recent American Heart Association guidelines set thresholds of 104cm for men and 88cm for women, but with lower thresholds for high risk ethnicities.¹⁸⁵

Future studies could examine the relationship between HLC and HDL metabolism to determine why HDL concentrations are lower in fatty liver disease, and whether this is reflected in a reduction in reverse cholesterol transport from adipose tissue to the liver, the primary role of HDL. Dynamic studies of liver metabolism in normal subjects and in NAFLD patients may reveal more about why NAFLD is so closely linked to atherosclerosis, and offer novel therapeutic options for the modification of liver fat. Previous studies have shown that insulin fails to suppress hepatic VLDL secretion in patients with high liver fat, compared to subjects without NAFLD, leading to overproduction of VLDL.^{104, 186} It is conceivable that insulin sensitisers, such as glucagon-like peptide agonists (GLP1-agonists), may correct this imbalance, and early animal and in vitro human data have been promising.¹⁸⁷⁻¹⁸⁹

5.4.1 Study limitations

Subjects were recruited by poster advertisement, and were mainly Caucasian, and thus ethnic differences were not adequately studied. Most subjects were female, but there were enough men (n = 41) to allow multivariable analysis. All subjects fasted for a minimum of eight hours, and abstained from alcohol for 24 hours before their study visit. However, alcohol intake, dietary habits and exercise capacity were not assessed – all of these three variables are known to impact on hepatic metabolism. Alcohol excess in particular can cause marked acute hepatic steatosis, which may take weeks to recover. Lastly, C-reactive protein and other

blood biomarkers of hepatic inflammation were not measured, although they are not specific to the liver.

5.5 Conclusions

Increasing waist circumference is strongly associated with increasing hepatic steatosis, even in the absence of other cardiovascular and MetS risk factors. In this cohort, hepatic steatosis was closely linked to the developing dyslipidaemia of MetS, suggesting that it may be an intermediate stage in the development of MetS in overweight and obese adults.

Chapter 6: Multiparametric magnetic resonance and the diagnosis of liver disease

6.1 Introduction

Over 10% of adults in Western populations have some degree of recognisable liver disease.¹⁹⁰⁻¹⁹² Viral hepatitis affects over 450 million worldwide, and the increasing prevalence of obesity and alcohol-related liver disease has lead to an epidemic of steatohepatitis and progression to cirrhosis.¹⁹³⁻¹⁹⁵

There is a pressing need for a reliable diagnostic tool for liver disease to identify early stages and to target therapies to those patients that may benefit (eg antiviral therapy in progressive hepatitis C, weight reduction surgery in fatty liver disease).¹⁹⁶ Liver biopsy is the current gold-standard for diagnosing liver disease, but it is invasive and costly. Most importantly, it carries a significant risk of serious bleeding complications, which is further amplified as patients with severe liver disease have abnormal coagulation. Furthermore, with a biopsy only 0.002% of the liver is examined, and there is great intra- and inter-observer variability in histological interpretation. This has led to several different grading and staging systems for liver fibrosis, such as Knodell, Ishak, METAVIR, Scheuer, Brunt & the NAFLD Activity Score.¹⁹⁷⁻²⁰² Agreement between these classifications is limited, even when the same slides are assessed in comparative studies.²⁰³ Histological interpretation is especially complicated in patients with mixed or coexisting disease, as different conditions have specific scoring systems that require prior knowledge of the aetiology. Clinical trials use different scoring systems, some of them further modified from those described above, and thus comparison and meta-analysis are rendered almost impossible.

A percutaneous liver biopsy gives information on tissue architecture, steatosis, iron content, fibrosis and cellular damage. No other individual test can exactly match this level and breadth of detail. However, considerable progress has been made in non-invasive methods to assess individual elements traditionally examined by histology, as shown in Table 6.1.

Table 6.1. Developments in non-invasive markers of liver disease				
Histology	Laboratory reference gold-standard	Non-invasive technique	Diagnostic performance: Area under receiver operator curve (AUROC)	Limitations
Steatosis	Chemical lipid analysis	Ultrasound	0.74	Obesity, acoustic windows, poor sensitivity Susceptible to iron and fibrosis Availability
		MRI	0.91	
		MRS	0.97	
Haemosiderosis	Dry weight hepatic iron content (HIC; 2 nd biopsy)	MRI	0.99 vs HIC (liver fibrosis not assessed)	Routine practice for known iron overload, but cannot distinguish low levels of haemosiderosis
Fibrosis	Explanted liver collagen proportionate area	Serum markers	0.80 vs Scheuer ≥ 3	Not specific, generalisability
		Transient Elastography	0.83 vs METAVIR ≥ 2 0.89 vs METAVIR ≥ 3	Not specific, cannot be used in obese, requires a prior ultrasound
		MR Elastography	0.94 vs METAVIR ≥ 2	Cost, availability, generalisability

6.1.1 Steatosis

Moderate and severe steatosis are clearly diagnosed on liver biopsy, as the lipid-rich vesicles are highly visible (see Figure 6.1). However, there is considerable inter-observer variation in absolute quantification of histological steatosis. This is partly due to sampling error – two pathologists may look at different sections of the same slide and come to different conclusions. Clinically, this is not a problem as, for diagnostic purposes, steatosis is essentially a binary variable – if present it suggests fatty liver disease (FLD), alcoholic or non-alcoholic steatohepatitis (ASH or NASH). There are no medical therapies for any of these conditions at present, so quantitative assessment has never been performed outside of a few clinical trials. In 1968, a serial percutaneous biopsy study showed that even moderate alcohol intake caused acute hepatic steatosis in healthy volunteers.¹⁷⁵ A 5 year cohort study of 42 patients with NASH suggested that disease progression was slower than in ASH, but significantly the severity or type of hepatic change did not correlate with the degree of obesity, hyperlipidemia or hyperglycemia.²⁰⁴ As with many numerical scales, the highest degree of variation is in the mid-range of severity, between 20% and 70%.

¹H MR spectroscopy (MRS) has been compared to liver biopsy for the assessment of steatosis in many recent studies. The hepatic lipid content (HLC) correlates very well with histological steatosis, although different relationships are seen in fibrotic and non-fibrotic patients.^{205, 206} Indeed, because of the reduced sampling error, and better interobserver reproducibility, MRS is now considered to be the reference standard for the measurement of hepatic steatosis.^{86, 206}

MR imaging, rather than spectroscopy, can be used to measure fat, with gradient-echo chemical shift imaging, also known as the Dixon method.^{207, 208} Developments in this method enable hepatic fat fraction (FF) to be quantified with comparable accuracy to ¹H MRS, but a universal sequence has not been standardised, and the method is susceptible to variations in liver iron content and fibrosis.²⁰⁹

Ultrasonography is not specific, is not sensitive in early disease, and is of limited efficacy in obese patients.^{139, 210, 211} Histological analysis, by eye or by more complex semi-quantitative methods, determines the percentage of hepatocytes with visible lipid vesicles. Steatosis is graded as 0 (0-5%), 1 (5-33%), 2 (34-65%) and 3 (>66%), with a value >5% considered significant.²⁰⁶ Ultrasonic echogenicity only identifies significant steatosis with a sensitivity of 53-62%, a specificity of 81-93%, and a reported area under the receiver-operator characteristic curve (AUROC) of 0.74.^{206, 211, 212}

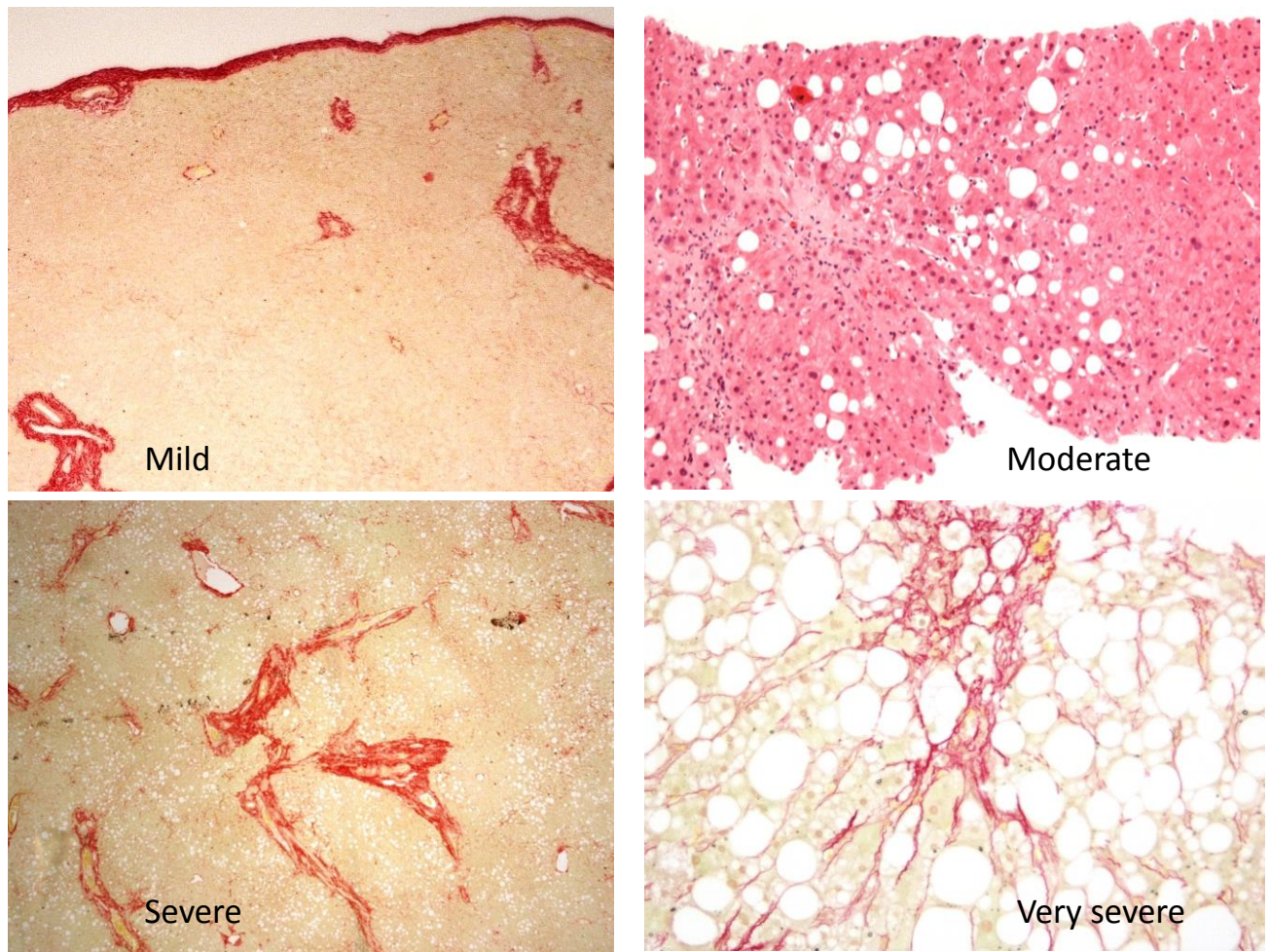


Figure 6.1. Mild, moderate, severe and very severe steatosis

Lipid-rich vesicles do not stain with haematoxylin and eosin, and so appear white. Of note is the potential sampling variation in the patient with moderate steatosis – some areas have many lipid-rich hepatocytes, whereas others are relatively normal. The patients with severe and very severe steatosis both have pericellular fibrosis, seen as strands of collagen (red) around each cell.

Images are from slides taken from patients in the Rapid Imaging Assessment of the Liver study, with permission.

6.1.2 *Iron overload (haemosiderosis)*

Iron overload causes hepatic and cardiac failure, and can occur at a young age in patients with inherited or acquired disease. Inherited iron overload occurs most commonly in haemochromatosis, an autosomal recessive disorder, with an estimated prevalence in the population of 1 in 500, and several subtypes, defined by the affected iron-transport protein (eg HFE, ferroportin, transferrin, haemojuvelin).²¹³ Furthermore, any condition, inherited or acquired, that requires multiple blood transfusions can lead to haemosiderosis, as humans cannot excrete iron unless they bleed. Thus, patients with thalassaemia, sickle cell disease, bone marrow failure and myelodysplastic syndromes are all at risk. Historically, these groups were offered iron chelation therapy when they became symptomatic with heart or liver disease. Diagnosis of haemosiderosis was made either by a conventional liver biopsy, semi-quantitatively analysed with Perls' stain, or more accurately by a second biopsy for the assay of dry weight hepatic iron content (HIC).²¹⁴ This was costly and risky, especially as it was performed serially in young patients. The development of MR techniques to measure cardiac and liver iron has changed practice – patients at risk of iron overload are screened by MR with defined biomarkers (T2* and R2) and thresholds have been defined for treatment with chelation to prevent organ damage.^{215, 216} Thus, preventive therapy is targeted at those most likely to benefit, and consequently mortality in this group of patients has reduced greatly.²¹⁷ Standardised sequences are available for use on different MR systems. However, there are patients who have mild-moderate, hitherto undiagnosed haemosiderosis in conjunction with fibrotic liver disease. One series reported that 33% of cirrhotic patients had haemosiderosis.²¹⁸ In this group, conventional biopsy is poor at determining the degree and relevance of iron overload, and there is considerable interobserver variability in the interpretation of Perls' stained slides. MR is a known powerful diagnostic tool for HIC – no other non-invasive modality has been shown to work comparably. Nevertheless, it is not known how HIC affects liver fibrosis progression. It would be ideal to measure HIC in an

unselected population and compare it to a biomarker for fibrosis, thus removing the need for paired dry weight and conventional liver biopsies.

6.1.3 Fibrosis

Individual serum markers of fibrosis are sensitive in many cases, but not specific. It was hoped that combining a series of serum markers of abnormal extracellular matrix turnover would improve diagnostic accuracy. The Extended Liver Fibrosis panel (ELF™) examines and forms a composite fibrosis score from three biomarkers: hyaluronic acid (HA), procollagen III N-terminal propeptide (PIIINP), and the tissue inhibitor of metalloproteinase 1 (TIMP-1). First described in 2004, it reported a sensitivity of 90%, but had a specificity of only 41%, with an AUROC of 0.80 for the detection of severe fibrosis, defined as Scheuer score ≥ 3 .⁶¹ In a separate study of 196 NAFLD patients, the ELF score predicted moderate or severe fibrosis with an AUROC of 0.82.²¹⁹ A subsequent 7 year follow-up study suggested that an ELF score predicted mortality.²²⁰ Patients were excluded from the study if they took aspirin or had cardiovascular disease, cancer, advanced cirrhosis with evidence of decompensation, suspicion of drug-induced liver disease, or any disorder associated with extrahepatic fibrosis, including rheumatic, renal, or lung disease. This limits the generalisability of this study – in an unselected population this panel of markers may overestimate liver fibrosis since they may reflect extrahepatic abnormal extracellular matrix turnover.²²¹ ELF™ has nevertheless been approved for use in the UK and Europe, and is available from Siemens Health Diagnostics. It has not been licensed for use in the United States. Other similar panels of liver fibrosis biomarkers are available, but are of limited clinical use – for example, FibroTest™ has an AUROC of 0.74 for the detection of METAVIR F2-4 fibrosis.²²²

Transcutaneous elastography (TE) can aid in quantifying fibrosis. In vivo elastography was developed from technology used by the French food industry to determine the maturity of large blocks of cheese by measuring internal stiffness, and first described in 2002.^{223, 224} A probe is placed on an intercostal space over the lateral lobe of the liver. A mechanical shear wave is emitted, which travels faster through stiff ($\approx$ fibrotic) parenchyma, and more slowly through soft ($\approx$ healthy) tissue. The speed of this shear wave is measured by successive ultrasonic waves, and from this the stiffness of the liver is derived mathematically.²²⁵ Several studies have demonstrated use in various liver conditions, but always in preselected populations, and liver stiffness does not discriminate between early and moderate stages of fibrosis. A large meta-analysis of 38 studies gave a mean AUROC of 0.83 for the diagnosis of significant fibrosis (METAVIR ≥ 2) in different liver diseases.²²⁶ Moreover, TE is subject to operator error from variance in force applied, probe positioning and angulation to the skin. Use in obese patients is severely impaired due to poor acoustic windows and the larger distances through which the shear wave has to travel before entering the liver.^{227, 228} However, it is a continuous variable, and is reported to give valuable prognostic information in certain conditions.²²⁹ FibroScan™ is an elastography device licensed for use in Europe since 2006, but not in the USA.

A similar, but more accurate, ultrasound-based technology is Acoustic Radiation Force Impulse (ARFI) shear wave imaging. This uses brief, high energy, focussed acoustic pulses to generate radiation force in tissue, and conventional ultrasound to track the resulting tissue displacements in order to determine mechanical properties, such as elasticity. The use of ARFI in liver assessment was pioneered in Duke University in collaboration with Siemens, and has been evaluated in a prospective study of 172 NAFLD patients due to undergo biopsy.²³⁰ Data was obtained from 135 patients (78%). The utility of ARFI was limited in patients with BMI $> 30\text{kg/m}^2$ – 26% of these patients (31 of 118) could not be assessed. In patients who did have successful data acquisition and analysis, the shear stiffness threshold of

4.24 kPa distinguished low (fibrosis stage 0–2) from high (fibrosis stage 3–4) fibrosis stages with a sensitivity of 90% and a specificity of 90% (AUC of 0.90). Thus, like FibroScan™, this technology is very promising in thinner patients, but is of limited use in obese patients.

Magnetic resonance elastography (MRE) uses the same shear wave principles as TE in an MR system. It was developed in the Mayo clinic, and first described in healthy volunteers in 2006.²³¹ The operator error is reduced because the shear wave is delivered by an automated acoustic driver, and MR, rather than hand-held ultrasound, is used to measure its speed and propagation through the whole liver. Thus it is superior to TE, but expensive, and limited in obese patients because the shear wave still has to travel through adipose tissue where it is mollified.^{232–234} AUROC for the diagnosis of significant liver fibrosis has been reported as 0.94 in different studies.^{235, 236} MRE is approved for use in the USA, but not widely available yet as it can only be performed on GE systems, and requires the purchase of the dedicated acoustic driver and the MRE software.

Importantly, although these methods can distinguish cirrhotic livers from normal, none can identify early liver disease with any specificity, and they do not give the breadth of clinical information obtained from liver biopsy with regard to fibrosis, inflammation, steatosis, haemosiderosis and tissue architecture. Moreover, ultrasonic methods, including all elastography, cannot be used if there is any fat or fluid between the chest wall and the liver, thus excluding all severely obese patients and all patients with ascites. Thus a combination of the lack of good non-invasive investigations coupled to the human and economic costs of liver biopsy leaves many patients with suspected early liver disease under-investigated, with no definite diagnosis.

Magnetic resonance techniques are well established at assessing anatomical detail. The principle of MR tissue characterisation has been applied before for cardiac tumours, where pre-operative biopsy is ill-advised, and for cardiomyopathies.²³⁷ Recent advances have

enabled MR systems to examine and quantify the constituent properties of organs by their lipid and iron content.^{205, 216, 238, 239} Measurement of fat allows accurate assessment of hepatic steatosis, and assessment of liver iron by MR is already widely used in thalassaemia and sickle cell disease.^{239, 240} The assessment of parenchymal tissue water content has been described as a potential method to identify inflammation and/or fibrosis, as both involve expansion of the interstitial space.²⁴¹ The hypothesis was that a combination of all three of these metrics in a rapid MR protocol would enable diagnosis of liver conditions accurately, non-invasively, and safely, in adults and children.

6.1.4 Objective

The diagnostic accuracy, discrimination and clinical usefulness of multiparametric MR for the assessment of liver disease was evaluated in a prospective, blinded study of an unselected patient population referred for liver biopsy.

6.2 Methods

6.2.1 Study population

From March 2011 to July 2012, we invited all patients referred for liver biopsy at two UK study centres – the John Radcliffe Hospital in Oxford and the Royal Berkshire Hospital in Reading, to take part. Exclusion criteria were any contraindication to 3 Tesla MR imaging (eg severe claustrophobia, pacemaker, metallic implant, pregnancy). Patients attended for their non-invasive assessment prior to their liver biopsies, and all data analysis by the research team was performed with no knowledge of the reason for referral. Histological results for steatosis, fibrosis and haemosiderosis were then compared to non-invasive MR results.

6.2.2 Study Oversight

The study was approved by the institutional research departments and the National Research Ethics Service. The trial was registered with clinicaltrials.gov (NCT 01543646) as a prospective study of diagnostic accuracy. All patients and volunteers gave written informed consent.

6.2.3 *Histological interpretation of liver biopsy samples*

As there is considerable interobserver variation in reporting of liver biopsy, all patient samples were independently assessed by three separate histopathologists (LMW, DWD & KAF), all of whom specialise in hepatobiliary disease. Hepatic lipid content was measured by determining the percentage of hepatocytes with visible lipid vesicles. This was graded as 0 (0-5%), 1 (5-33%), 2 (34-65%) and 3 (>66%), as per the conventional score suggested by Brunt.²⁰¹ All samples were assessed for fibrosis by Ishak stage and by collagen proportionate area (%).¹⁹⁸ Stainable iron was estimated using a Perls' histochemical stain and semi-quantified using a four tier grading system – separate dry weight iron analysis was not routinely performed as this would have required a second biopsy for research purposes. Interobserver variability between the three for steatosis, fibrosis and haemosiderosis was determined using weighted kappa statistics. The final histology report for all patients was determined by consensus agreement without knowledge of the non-invasive data for each of these metrics.

6.2.3 *Magnetic resonance protocol*

All examinations were performed with the patient lying supine in a 3 Tesla Siemens system (Tim Trio, Siemens Healthcare, Germany). To minimise motion artefacts from respiration and the cardiac cycle, data was acquired during expiratory breath-hold and with ECG-gating. This required subjects to repeatedly hold their breath and lie still for 12 - 14 seconds, which was comfortably managed by all of the participants. The full MR protocol for this study is shown in Figure 6.2. For the determination of normal ranges for the three metrics, 50 healthy subjects with $\text{BMI} \leq 25\text{kg/m}^2$, normal liver enzymes, and no known liver disease were studied. These volunteers were recruited by poster advertisement.

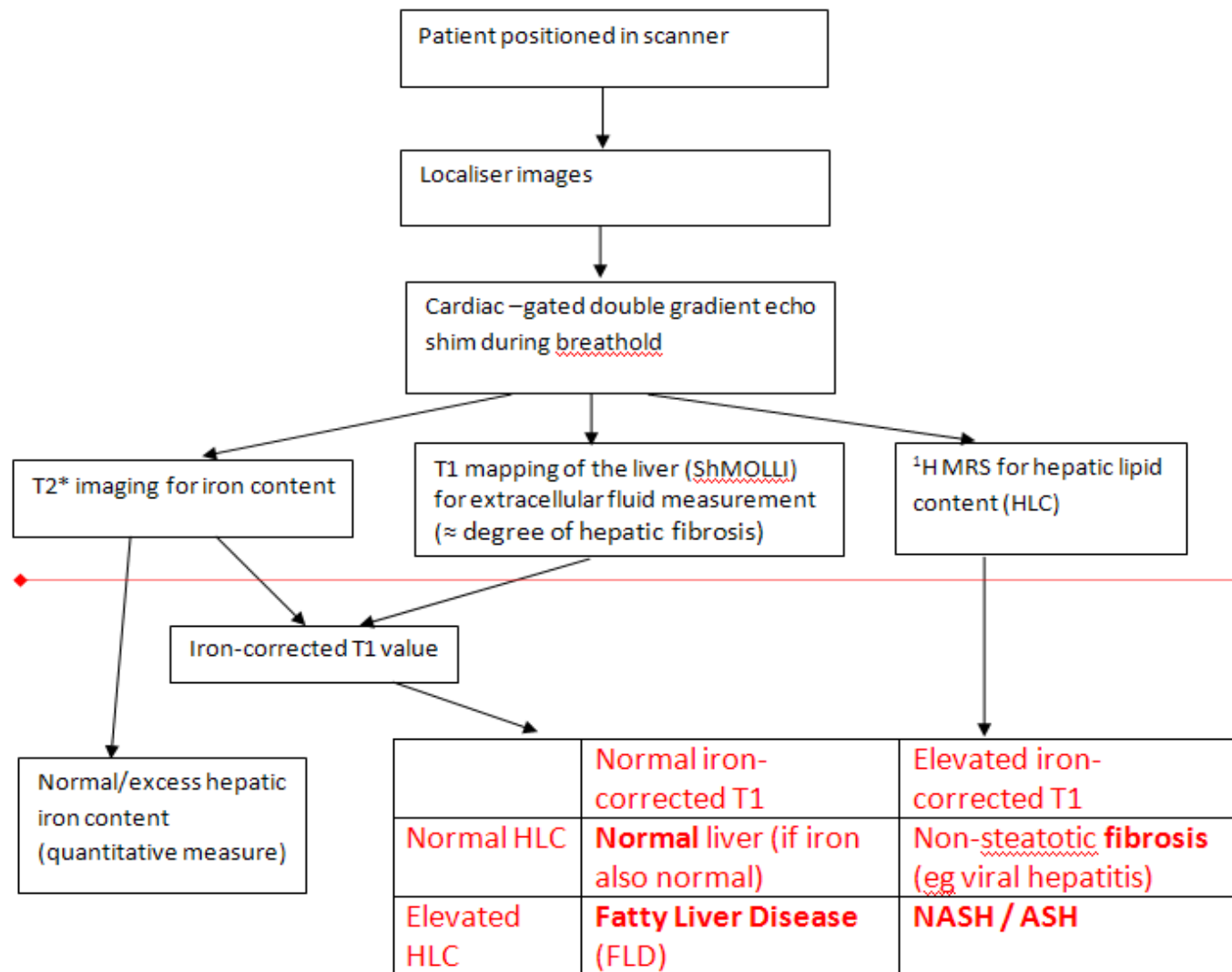


Figure 6.2 RIAL study MR protocol
 Data acquisition (above the red line) required 20 minutes lying supine in the scanner. Iron correction was applied to T1 values if the T2* was <15milliseconds.

6.2.5 *Hepatic steatosis measurement with ^1H MRS*

Hepatic lipid content can be quantified using localised cardiac-triggered proton spectroscopy, as described in Chapter 2. Hepatic lipid content (HLC) as a percentage of the liver water content using ^1H MRS was measured with water suppression in an 8cm^3 voxel in the lateral right lobe of the liver, from where most percutaneous biopsy sampling occurs. If the patient was comfortable at the end of their MR scan, and time allowed, the measurement was repeated in a voxel in the left lobe of the liver, corresponding to the region of interest in transjugular liver biopsy samples, and enabling comparison of HLC across both lobes.

6.2.6 *Iron content imaging*

A multi-gradient-echo acquisition was used to calculate a $T2^*$ map of the liver with a slice thickness of 3mm. The iron content of the liver has marked effects on $T1$ and spectroscopy measurements: $T1$ is very sensitive to any reduction in $T2^*$, and in ^1H MRS the water peak is broader in patients with low $T2^*$. Thus this protocol quantified HIC with different techniques, to increase its reliability.

6.2.7 *Extracellular water imaging with $T1$ mapping*

A $T1$ relaxation time map was acquired using the ShMOLLI sequence in a transverse plane.¹⁴⁸ This sequence samples the $T1$ recovery curve using single-shot steady state free precession (SSFP) acquisitions. There is a quality assurance component – each acquisition generates an R^2 map for the fit of signal intensity to the exponential recovery curve.¹⁴⁹ For this study, an image was only considered for interpretation if the R^2 was $\geq 99\%$. The effect of iron on the measured $T1$ was assessed by developing a two-compartment model for the liver, consisting of liver parenchyma and a variable proportion of extracellular fluid, with higher fractions of ECF corresponding to a more fibrotic liver. The dependence of $R1$, $R2$ and $R2^*$

of liver parenchyma on iron concentration at 3T was based on data from previous studies.²⁴²⁻
²⁴⁵ The R1 value for ECF was $0.44/\text{s}$ ²⁴⁶. R2 and R2* values for ECF were the same as those for the liver parenchyma, based on the large distance (relative to the length scale of the hepatic iron distribution) traversed by water molecules during T2 relaxation.²⁴⁷ Bloch simulations were used to calculate the combined signal from the two compartments during the ShMOLLI readouts and then the measured T1 calculated using the ShMOLLI algorithm. A look-up table was used to correct the measured T1 for a given HIC to the T1 which would have been measured in the case of normal HIC.

6.2.8 Image analysis

MRI – Data were analysed in real-time while the patient was leaving the scanner. Regions of interest that excluded vessels and the biliary tree were selected on the transverse T1 and T2* maps, corresponding to the lateral right lobe of the liver, from where most biopsies are taken. Mean T1 and T2* were recorded.

MRS – Data were analysed offline by a researcher blinded to the anthropometric measurements and imaging data using AMARES in the jMRUI package and customised software running within MATLAB 2010b. Signals from different coil elements in each breath-hold and were combined, and individual spectra phase and frequency corrected prior to summation. HLC was expressed as % of water signal.

6.3 Results

54 patients and 50 normal volunteers with no known liver disease were recruited to the study. Of these, 3 did not undergo liver biopsy (1 liver-related death before biopsy, one refused, one improved and clinical decision to biopsy reversed), and one patient did not undergo MR imaging (claustrophobia). There were 50 complete patient datasets included in the final analysis – characteristics are shown in Table 2.

Table 6.2. Patient characteristics	
Male : Female	36 : 14
Age (years)	52 ± 14
BMI (kg/m ²)	27.8 ± 5.7
Waist circumference (cm)	97 ± 14
<i>Serum lipid profile</i>	
Total cholesterol (mmol/l)	4.67 (1.65)
Triglycerides (mmol/l)	1.90 (0.82)
HDL-c (mmol/l)	1.23 ± 0.35
LDL-c (mmol/l)	2.67 (0.90)
<i>Liver enzymes</i>	
Bilirubin (IU/l)	11 (6.6)
ALT (IU/l)	64 (73)
AST (IU/l)	53 (55)
GGT (IU/l)	80 (189)
Alkaline phosphatase (IU/l)	202 (131)
Albumin (g/l)	44 (3.8)
Values shown as mean ± SD for normally distributed variables, and median (IQR) for non-normally distributed variables	

6.3.1 *Assessment of steatosis*

^1H MRS predicted the degree of steatosis with great accuracy when compared to the Brunt scoring system ($r_s = 0.88$, $p < 0.001$). Figure 6.3 shows how each pathologist graded steatosis in each of the 50 biopsies, and shows how there is good agreement in grades 0 and 3, but more variation in 1 and 2. There was modest agreement in the assessment histological steatosis, with the trivariate weighted kappa statistic for Brunt scoring of 0.79. Figure 6.4 shows the mean and range in the histological assessment of the percentage of hepatocytes with visible macrovesicular lipid inclusions by the three pathologists. Variance was more marked when scoring was on a continuous % scale, and the trivariate weighted kappa statistic fell to 0.75. Overall correlation between HLC and the mean % steatosis from the three pathologists was excellent ($r_s = 0.88$, $p < 0.001$). Interestingly, ^1H MRS correlated very well with each of pathologists individually (Figure 6.4).

Hepatic lipid content measured in both the right and left lobes of the liver was very similar ($r_s = 0.97$, $p < 0.001$), as shown in Figure 6.5, suggesting that the distribution of fat in the liver is uniform.

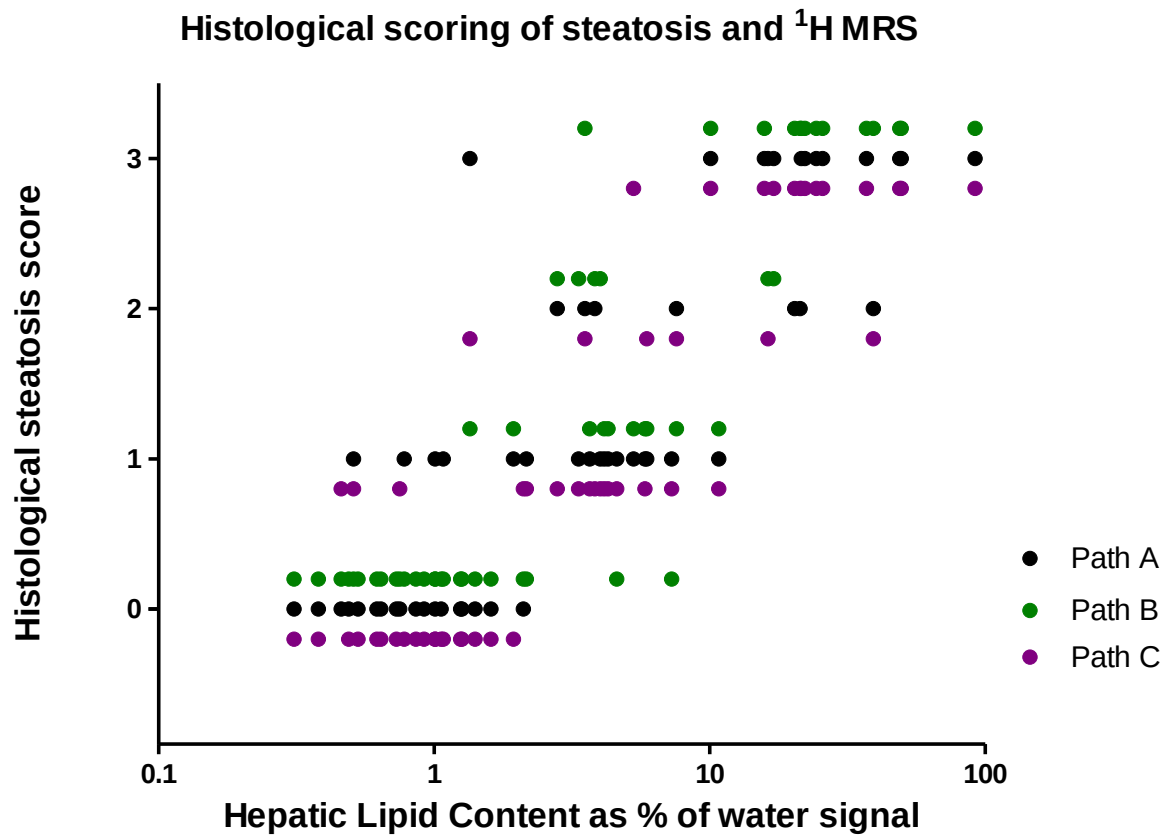


Figure 6.3. ^1H MRS accurately predicts histological steatosis

Non-invasive HLC measurement correlated well with semiquantitative histological assessment of steatosis using the Brunt scoring system ($r_s = 0.88$, $p < 0.001$).

This graph shows how each pathologist rated each of the 50 biopsies for steatosis, using the HLC measures by MRS as a reference. For stage 0 and stage 3 there was good agreement between the pathologists, but in stages 1 and 2 there was more variation in assessment. Overall, the trivariate weighted kappa statistic from pathologists A, B and C was 0.79.

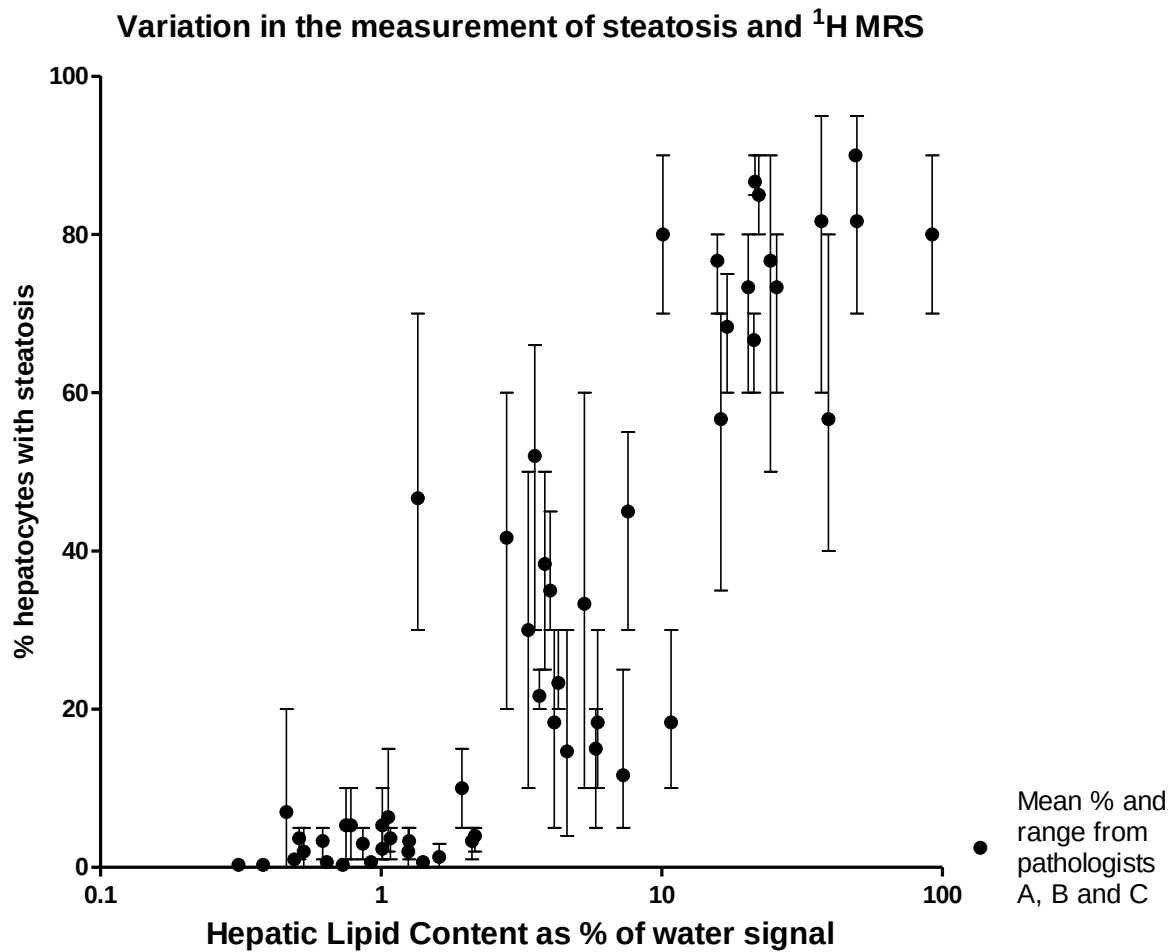


Figure 6.4. Variance in the assessment of steatosis, using ^1H MRS as a reference

Each point represents the mean from the 3 pathologists for the percentage of hepatocytes with visible lipid inclusions from a single biopsy, with the range shown by the error bars. As with the Brunt scoring system, there was good agreement for low and high percentages, but in the middle there was considerable variance, especially for values of hepatic lipid content between 2 and 12%. The trivariate weighted kappa statistic from pathologists A, B and C was 0.75. Spectroscopy predicted the mean % steatosis from the three pathologists very well ($r_s = 0.88$, $p < 0.001$). Correlation with individual pathologists was also good:

Path A $r_s = 0.83$, Path B $r_s = 0.86$, Path C $r_s = 0.85$, all $p < 0.001$.

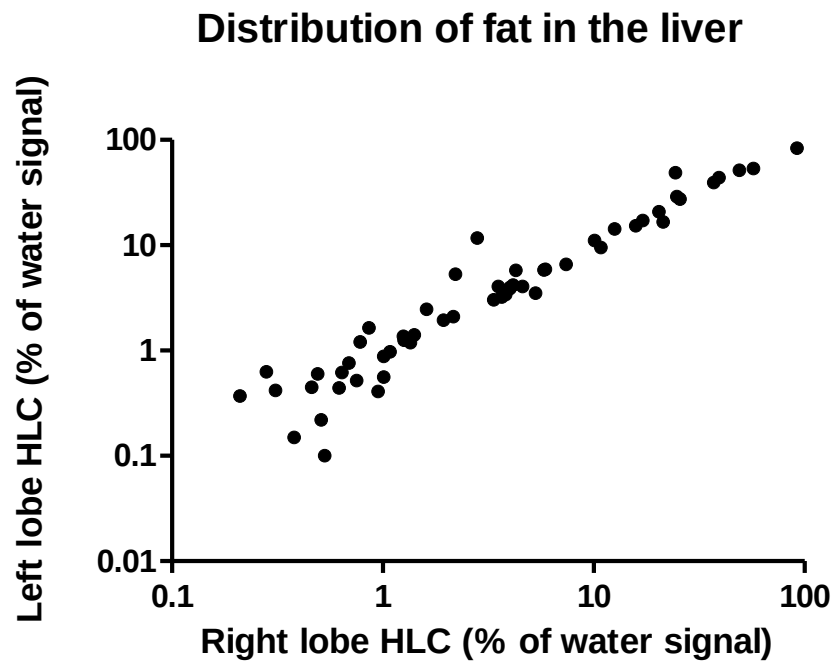


Figure 6.5. HLC in both lobes of the liver is very similar

Correlation between HLC measured in the right and the left lobe was excellent ($r_s = 0.97$, $p < 0.001$). At very low values ($<0.7\%$), the variation may well be due to measurement error, inclusion of small bile ducts in one of the voxels, or other artefacts. This suggests that fat is distributed in most patients equally in the right and left lobes of the liver.

6.3.2 *Quantifying hepatic iron*

The optimal method for the assessment of haemosiderosis is dry weight iron measurement, but this requires a dedicated second biopsy, and thus is not acquired unless there is clinical suspicion beforehand (eg a known haemochromatosis patient). No patient in this series had a dry weight iron assessment, but all samples were assayed with Perls' stain. However there is considerable interobserver variability in the semiquantitative staging of haemosiderosis. In 50 patients, the trivariate weighted kappa statistic was 0.61.

T2* measurement correlated with the mean haemosiderosis score of the three pathologists fairly well ($r_s = -0.73$, $p < 0.001$), as shown in Figure 6.6. A threshold of T2* = 15ms was a useful measure in excluding significant iron overload, as agreed by all three pathologists, and had a negative predictive value of 97%, as shown in Table 6.3. Intra-observer variance in the assessment of haemosiderosis was not determined (ie the pathologists were not asked to reanalyse the data again to determine reproducibility).

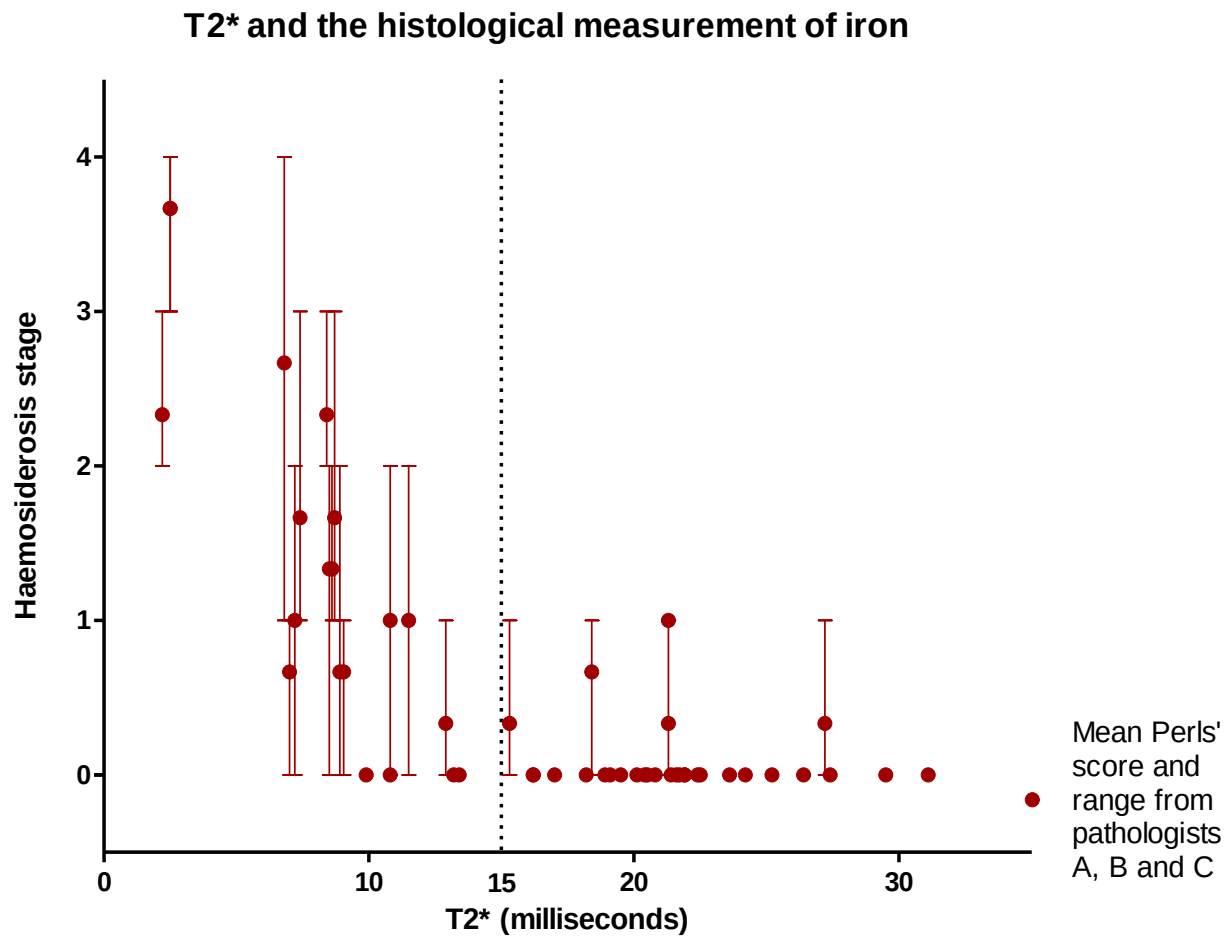


Figure 6.6. T2* and semiquantitative assessment of haemosiderosis

There was only modest agreement in the histological assessment of iron overload, with a trivariate weighted kappa statistic of 0.61. A threshold of $T2^* = 15$ separated 8 of the 9 patients with definite iron overload, agreed by all three pathologists, leaving only one ‘false negative’.

Table 6.3. Sensitivity and specificity of T2* for the detection of haemosiderosis

	Pathological haemosiderosis reported by all 3 pathologists	No haemosiderosis, or lack of agreement between 3 pathologists	
$T2^* \leq 15\text{ms}$	8	12	PPV = $8/20 = 40\%$
$T2^* > 15\text{ms}$	1	29	NPV = $29/30 = 97\%$
	Sensitivity = $8/9 = 89\%$	Specificity = $29/41 = 71\%$	

6.3.3 *Measuring liver fibrosis*

Iron corrected T1 (cT1) predicted the degree of fibrosis in patients, regardless of the aetiology of disease. As Figure 6.7 shows, there was a close correlation between the cT1 value for a region of interest and the Ishak fibrosis stage ($r_s = 0.71$, $p < 0.001$). When looking at the 19 patients with viral hepatitis, this increased to $r_s = 0.88$ ($p < 0.001$), as shown in Figures 6.8 and 6.9. There was marked variance in the assessment of fibrosis when the pathologists were blinded to the clinical data – the trivariate weighted kappa statistic was 0.70. Figure 6.10 shows the mean and range of ISHAK scores from the three pathologists for each of the fifty biopsies, using cT1 as a reference. As with the assessment of steatosis earlier, the greatest variance was in the mid-range values for fibrosis. cT1 correlated with each of the pathologists' findings – $r_s = 0.54$ for pathologist A, 0.63 for B, and 0.67 for C (all $p < 0.001$), and $r_s = 0.71$ with the clinic-pathological consensus report ($p < 0.001$).

Corrected T1 and the histological measurement of fibrosis

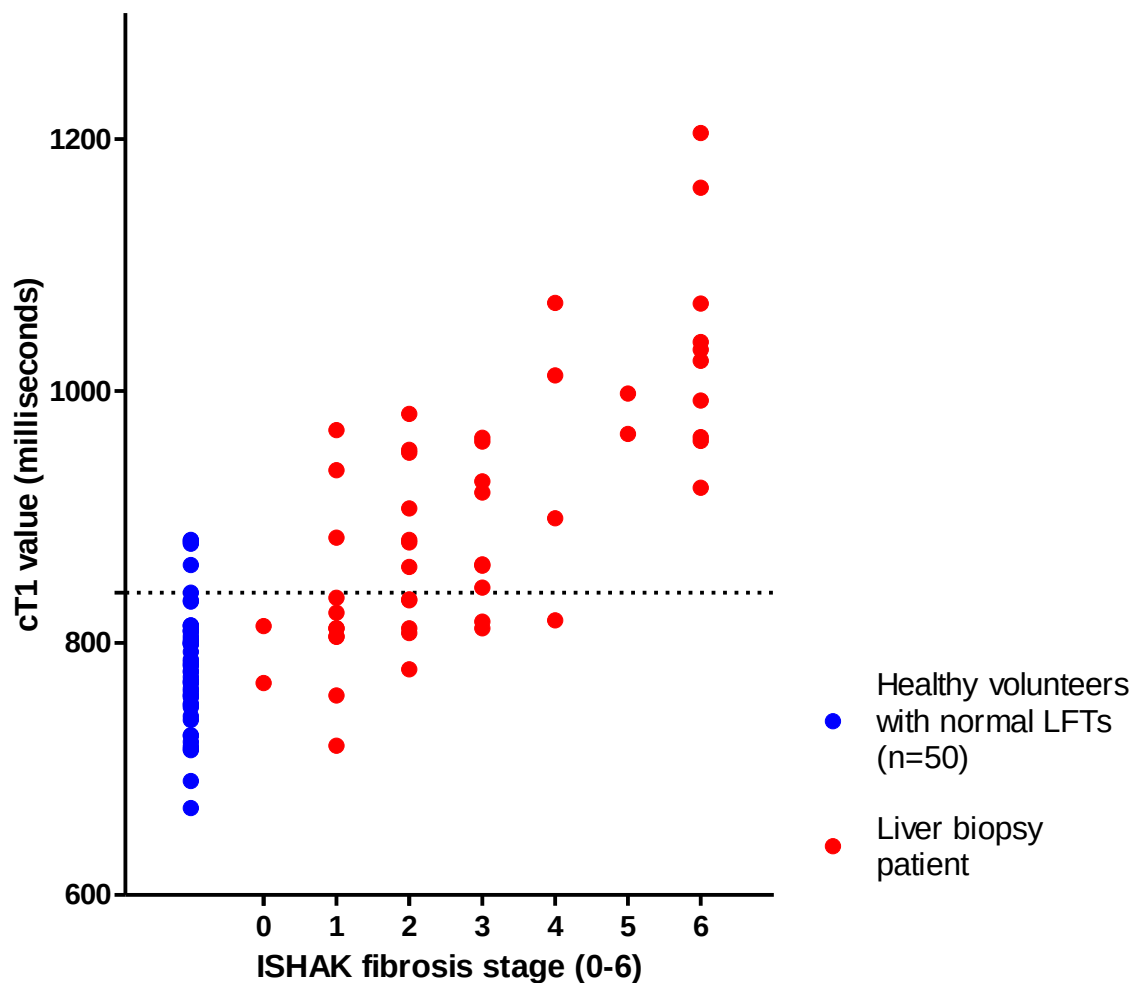


Figure 6.7. Corrected T1 (cT1) discriminates between degrees of fibrosis

cT1 values from 50 volunteers with presumed healthy livers (blue) and from 50 liver biopsy patients (red), acquired with no knowledge of any clinical data or the indication for biopsy. The Ishak fibrosis score shown is the consensus score from 3 histopathologists with knowledge of the clinical data. The dotted line at cT1 = 840ms has a sensitivity of 90% and a specificity of 88% for the detection of Ishak > 2.

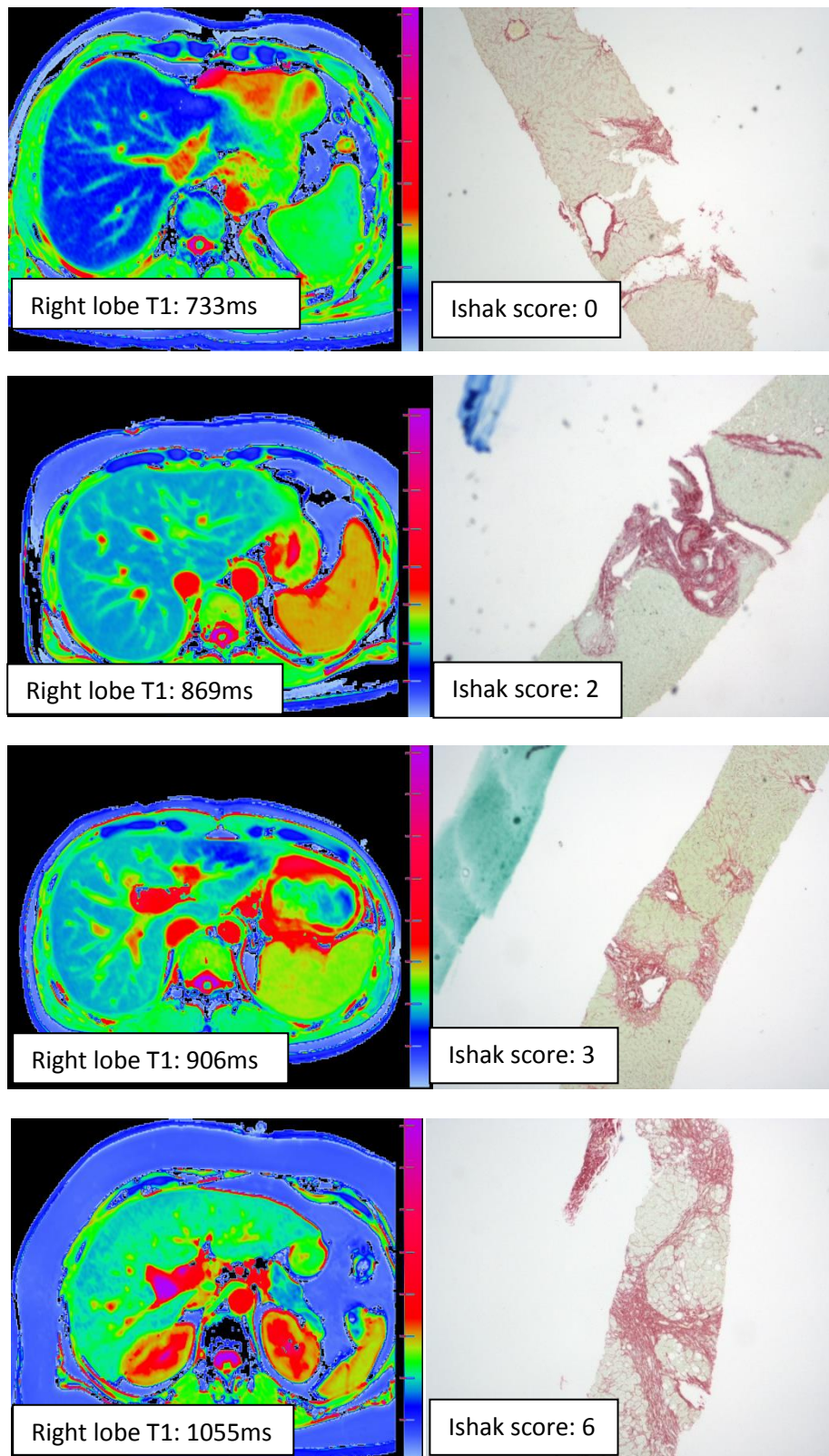


Figure 6.8. Viral hepatitis fibrosis staging. Examples of transverse liver cT1 maps taken at the level of the spleen from 4 patients with corresponding Sirius Red stained liver biopsies for fibrosis (Ishak 0 – 6). The appearance of the map, and the quantitative measure of ECF, clearly correlate with the degree of fibrosis as assessed by the Ishak score.

Predicting liver fibrosis stage

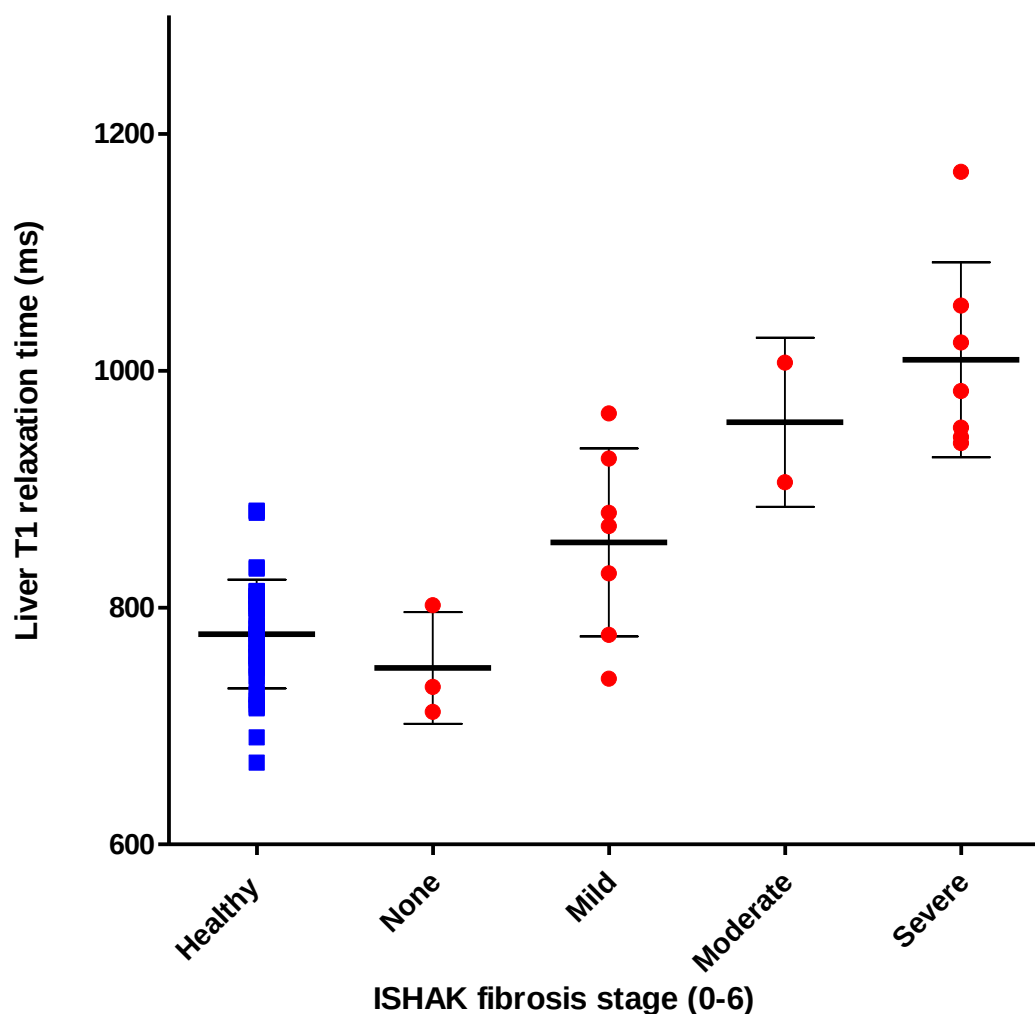


Figure 6.9. MR cT1 predicts fibrosis even more accurately in viral hepatitis

Mean $\pm$ SD cT1 values for levels of fibrosis from the clinic-pathological consensus in 19 patients with viral hepatitis (red), and in 50 presumed healthy volunteers (blue). ANOVA (right) suggests significant differences in the means between healthy volunteers and any mild, moderate or severe disease. Moderate disease was not clearly distinguished from either mild or severe disease, but there were only 2 patients with Ishak stage 3-4.

Bonferroni's Multiple Comparison Test	Mean Diff.	P < 0.05?	Summary
Healthy vs None	28.6	No	ns
Healthy vs Mild	-77.4	Yes	*
Healthy vs Moderate	-179	Yes	***
Healthy vs Severe	-232	Yes	***
Mild vs Moderate	-102	No	ns
Mild vs Severe	-154	Yes	***
Moderate vs Severe	-52.8	No	ns

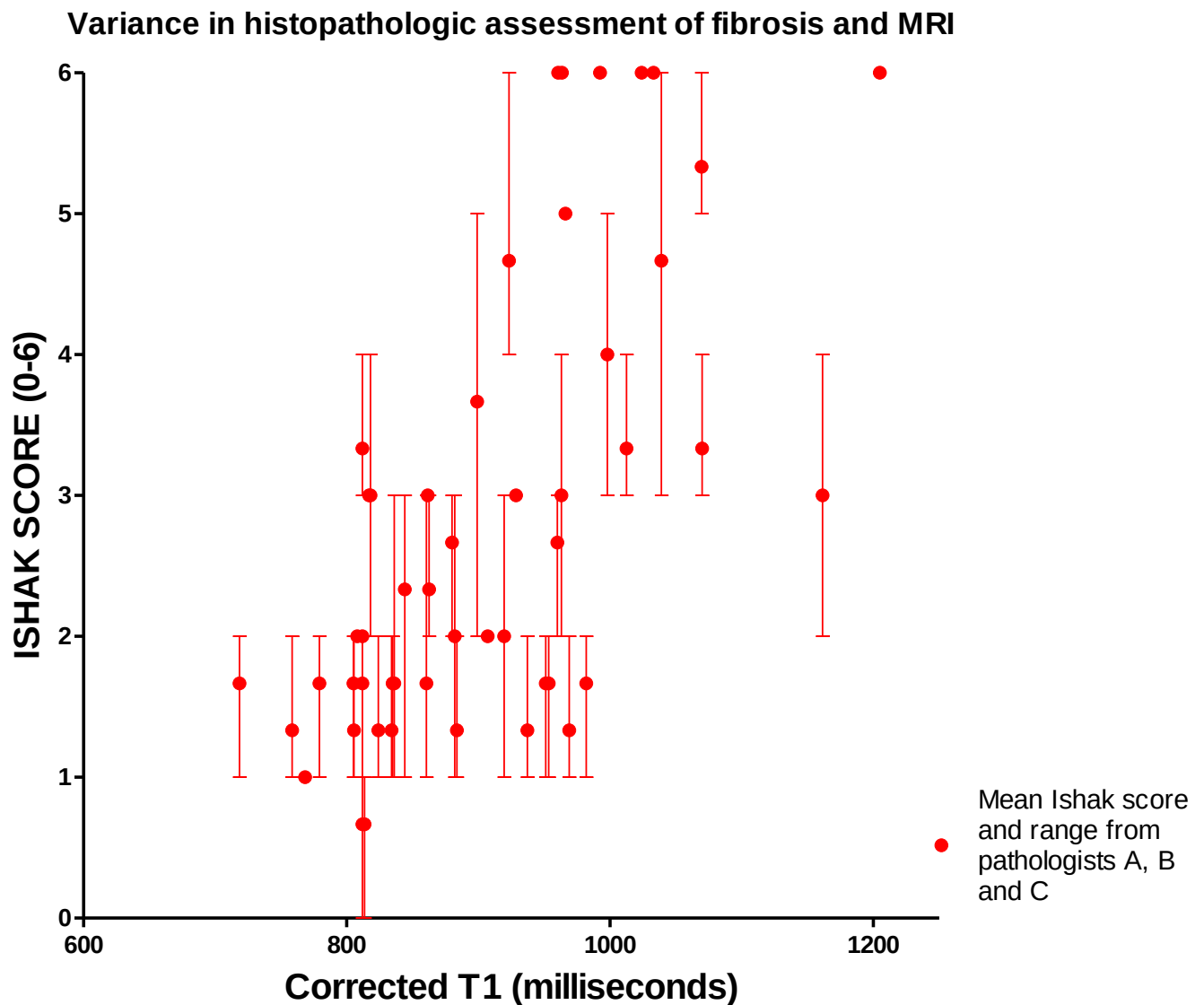


Figure 6.10. Variance in histological assessment of fibrosis, using MR as reference

There was noticeable variance between pathologists in the assessment of the ISHAK score from each sample. Corrected T1 predicted individual pathologist's assessments – $r_s = 0.54$ for pathologist A, 0.63 for B, and 0.67 for C (all $p < 0.001$). Corrected T1 predicted the consensus Ishak score even more accurately, with $r_s = 0.71$ ($p < 0.001$).

6.4 Discussion

This new method of liver assessment by multiparametric MR imaging and spectroscopy provides detailed tissue characterisation of a large sample of liver parenchyma, and can measure fibrosis, steatosis and iron content quantitatively, quickly and safely. It has proved challenging to develop non-invasive measures of liver disease historically because of the known interobserver variance – we have addressed this by comparing multiparametric MR in a prospective study to the results of three expert pathologists and their variance, with blinded analysis of tissue from the outset, and further comparison with the clinical consensus report.

For each of steatosis, haemosiderosis and fibrosis, there was notable interobserver variance, In each of these metrics MR offered an equivalent biomarker which matched the values agreed by the pathologists, both individually and by consensus. Fibrosis, as measured by corrected T1, has not been reported before, and steatosis and haemosiderosis have not been judged in prospective, blinded, unselected patient studies with MR.

The detailed microarchitecture of the liver at the level of the portal tract is not seen, but macroarchitecture and the distribution of fibrosis through the liver can be determined, which is of particular interest in conditions with patchy fibrosis, such as primary sclerosing cholangitis and hepatic infarcts. Most importantly, because this is the first quantitative non-invasive technique for the assessment of liver extracellular fluid, it can be used for the safe longitudinal assessment of disease progression and/or response to therapy. This has not been possible in the past, and opens up many possibilities for research and clinical use into new therapeutic strategies for hepatic and metabolic conditions. For example, the degree of liver inflammation caused by the ingestion of alcohol, paracetamol and other drugs may be quantified over time, without the need for serial biopsies.¹⁷⁵ Corrected T1 is a marker of ECF, which can rise with oedema in the acute setting or fibrosis in chronic illness, or both if there

is acute insult to a fibrotic liver. In this regard, it is not a pure biomarker for fibrosis. However, interpretation based on the clinical picture, history, and simple biochemistry from the patient can guide the observer towards the correct choice between acute and/or chronic liver disease.

This method may prove of particular use in patients with coexisting pathology, such as viral hepatitis and NASH. Currently, there are separate scoring systems for these pathologies, and interpretation can be difficult. An overweight patient with hepatitis C may have a first biopsy reported on the METAVIR score for fibrosis and the Brunt score for steatosis, and a second biopsy reported on the Ishak and NAS scores. All four systems are in common usage, and comparison is thus difficult, even before sampling variation and inter-observer variance are considered. In contrast, this method correctly identified patients with both steatosis and fibrosis, regardless of aetiology.

These results suggest that many patients have a significant element of haemosiderosis, but that hepatic iron content is not properly measured, as a dry weight iron biopsy is not taken at the time. Risk stratification and resource management are possible with MR: patients with $T2^* < 15\text{ms}$, may need a dry weight biopsy for HIC, an patients with a $cT1 > 840\text{ms}$ are more likely to benefit from liver biopsy. The image quality and detail far surpasses those obtained by any ultrasonic method, although of course this requires access to an MR system. However, as MR cholangiopancreatography is a standard investigation for suspected biliary disease, this is not a major hurdle. The multiparametric nature of this method also enables reinforced diagnosis. Iron overload is determined by three features – a reduced $T2^*$, a markedly reduced $T1$, and a broadened water peak on spectroscopy. This type of pattern recognition is common in medicine, and using different methods to determine a tissue characteristic gives the interpreter more confidence in the diagnosis by reducing the likelihood of false positive

results. Further improvements in the assessment of iron metabolism may be possible in the near future as hepcidin assays become more affordable.

6.4.1 Study limitations

This is a relatively small study, and all scans were performed in single centre. However, this MR protocol can be implemented on other 3T systems. Patients were recruited from two UK hospitals, and all the biopsies was analysed by three blinded pathologists. The patient group was heterogenous, and no category was excluded from participation, so these results are generalisable to an adult population with suspected liver disease. None of these patients required liver transplantation, and so comparison of liver macroarchitecture by pathology and non-invasive MR was not possible. Percutaneous liver biopsy is not an ideal reference standard for fibrosis, steatosis or haemosiderosis, but we have addressed this by measuring interobserver variance.

6.5 Conclusions

We have developed a new, accurate and completely safe method to characterise the severity and type of liver disease, thus aiding diagnosis and staging. The non-invasive data generated is equivalent to liver biopsy measurements of steatosis, haemosiderosis and fibrosis, as demonstrated in this prospective, blinded study in an unselected liver biopsy population. MR is increasingly accessible, and it is hoped that this technique can be validated in clinical multicentre studies to determine if it can be used for all liver disease in adults and children, and how cost-effective it is when compared to other diagnostic algorithms. For the assessment of fatty liver disease, especially in younger and high-risk patients, these techniques offer a promising tool for the differentiation of simple steatosis from steatohepatitis. Further work to evaluate the technology, ideally in a prospective multicentre study using liver biopsy as an immediate reference and patient outcomes as a key endpoint, is necessary, and should adhere to the STARD criteria (STAndards for the Reporting of Diagnostic accuracy studies).²⁴⁸

Chapter 7: The effects of obesity on cardiometabolic risk in children

7.1 Introduction

Over the past 20 years there has been an alarming increase in childhood obesity in the developed and developing world.²⁴⁹ This is due to a variety of well-publicised factors, including increased television viewing, reduced access to open play, more sedentary lifestyles and commuting habits, changes in dietary composition and eating habits, and increasing acceptance as overweight being part of the ‘normal distribution’ within the population. In the US, 18% of all teenagers are obese, compared to 5% in 1980, and a further 25% are overweight.²⁵⁰ In the UK in 2010, the prevalence of childhood obesity in children under 10 years old was 15%, compared to 10% in 1995 (Figure 7.1). In secondary school children, the prevalence was higher still at 18%, up from 15% in 1995.

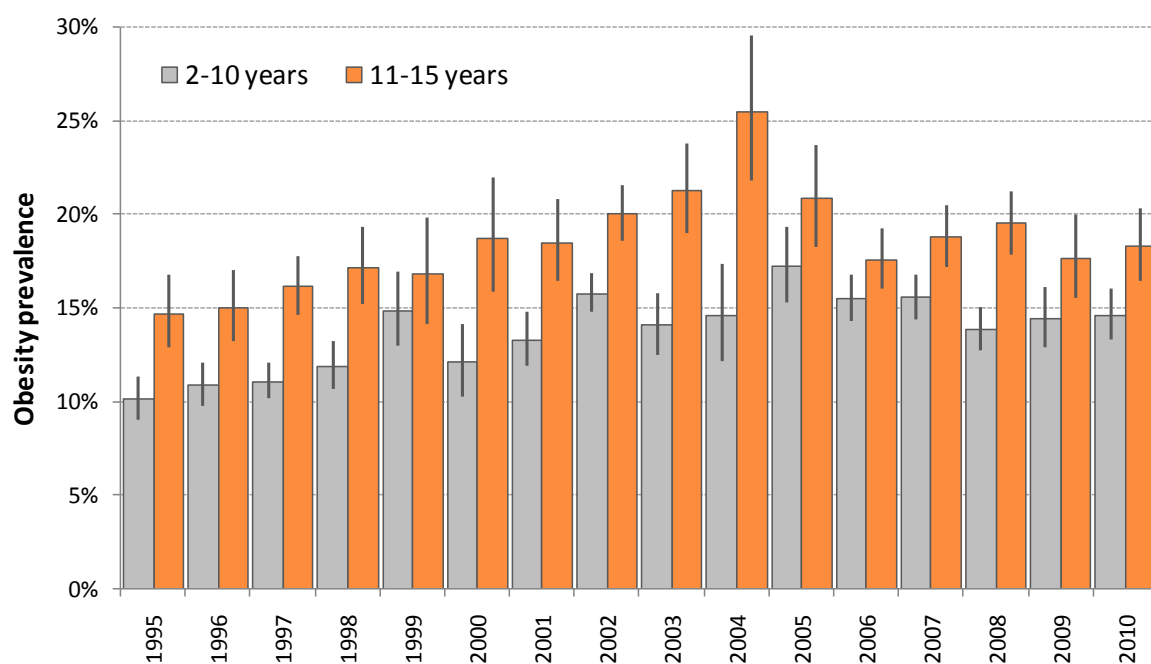


Figure 7.1. Prevalence of obesity among children aged 2-15 years

Child obesity is defined as BMI $\geq$ 95th centile of the UK90 growth reference chart

Source: Health Survey for England 1995-2010

7.1.1 *Childhood obesity and disease in childhood*

Even during childhood, obesity leads to increases in risk factors such as high blood pressure, diabetes mellitus, hypercholesterolaemia and the metabolic syndrome as well as non-cardiac sequelae that include musculoskeletal defects, NASH, gallstones, increased risk from certain cancers, bullying and depression.^{251, 252} There is considerable evidence that increased body weight has direct effects on vascular development, cardiac size and function, even in the absence of the above additional cardiovascular risks.²⁵³ Excess abdominal fat mass is associated with reduced aortic distensibility and increased pulse wave velocity. This, coupled to the increased circulating volume that is found in obese children may well underlie the increased blood pressure seen in over 20% of obese teenagers.²⁵⁴ Independently of hypertension, childhood obesity is also associated with increased left ventricular mass and eccentric left ventricular hypertrophy.²⁵⁵ Furthermore, autopsy studies in obese children reveal increased atherosclerotic plaque burden.²⁵⁶

7.1.2 *Childhood obesity and adult disease*

Adolescent obesity also predicts who will be obese as adults with levels of BMI tracking in to adulthood, where it is associated with atrial dilatation, reduced distal aortic distensibility, increased circulating volumes and ‘obesity cardiomyopathy’. The odds of childhood obesity persisting into adulthood are smaller in the early years compared with adolescence, but the long term impacts of overweight & obesity in the first decade of life have recently been described in large prospective studies incorporating North American and European populations.²⁵⁷ The Bogalusa Heart Study, commenced in 1972, is one of the first studies to provide prospective data through to adulthood on the impact of obesity and, based on 14000 children, has demonstrated the manifestation of the risks of excess body weight in terms of atherosclerosis, dyslipidaemia and early onset type 2 diabetes.^{256, 258-261}

However, not all obese children become obese adults. Those that normalise through late adolescence carry no extra cardiometabolic risk.²⁵⁶ Thus it is very difficult to target weight management measures to high-risk children as at present it is difficult to distinguish them from the ‘benign adolescent obese’ phenotype. It is desirable to identify biomarkers of early vascular and metabolic disease which would allow better risk-stratification.

7.1.3 Novel biomarkers for the assessment of childhood obesity

Results from Chapter 4 suggest that body size is a key determinant of cardiac steatosis, which is currently being investigated as a possible marker of altered myocardial metabolism. Moreover, impaired peak circumferential strain is a more sensitive indicator of early systolic dysfunction than LV ejection fraction in progressive inherited cardiomyopathies (eg Becker’s muscular dystrophy), so it may be similar in progressive acquired heart disease.²⁶² Strain has been shown to be more positive in paediatric renal transplant patients compared to normal controls.²⁶³ If ‘obesity cardiomyopathy’ exists in children, strain may be a useful early indicator of disease. Elevated hepatic lipid content may be a key intermediate step between abdominal obesity and the dyslipidaemia of MetS, as discussed in Chapter 5, and has been investigated in obese children recently.²⁶⁴ Finally, if hepatic steatosis leads to steatohepatitis, then perhaps multiparametric MR, as described in Chapter 6, could identify this in a paediatric population. The diagnosis of NASH is particularly difficult in children as liver biopsy is rarely performed. However, it is the paediatric NASH population which theoretically carries the highest cardiometabolic risk.^{265, 266}

7.1.3 Objectives

1. To study the impact of childhood obesity on cardiac and hepatic steatosis using novel MR techniques.
2. To determine if multiparametric MR is feasible in children to characterise early obesity-related cardiovascular and liver disease in a single non-invasive investigation.

7.2 Methods

7.2.1 Study design and oversight

The study protocol was approved by the National Research Ethics Service (09/H0505/125). The Office of National Statistics recommended sample sizes of 12 to show a 50% difference in myocardial and hepatic triglyceride content between groups. Eleven boys, aged 10 -15, and defined as obese by being >95th centile on the UK growth reference chart, were recruited from local community paediatrics' services. Obese children are identified by the National Child Measurement Programme in Year 6 at school, corresponding to 10-11 years of age, and routinely referred to local medical services. Eleven lean boys were recruited by poster advertisement in local schools and sport clubs. Exclusion criteria were any contraindication to MR imaging, known diabetes mellitus, hypertension, known cardiovascular disease, any previous hospitalisation >5 days, and hyperlipidaemia. All children denied smoking.

All boys were invited for a 3 hour assessment of cardiovascular function and metabolic risk. Cardiac volumes, LV mass and contractile function, myocardial triglyceride content (MTGC), peak circumferential strain (CS), hepatic lipid content (HLC), liver T1, abdominal fat distribution and blood pressure were all measured, as described in Chapters 2 and 6, in a 90 minute 3 Tesla scan. Boys came after an overnight fast, but were encouraged to stay

hydrated with water. There was an option for the boys to donate venous blood as part of the study after topical local anaesthetic application to measure lipid profile, liver enzymes and glucose.

7.2.2 Statistical analysis

Descriptive statistics were used to summarise subject characteristics in both groups, and unpaired t-test was used to determine the significance of any difference in mean values. Spearman correlation (r_s) was calculated to test associations between continuous variables. GraphPad Prism 5 software was used for statistical analysis and data presentation.

7.3 Results

Cardiac volumes, blood pressure, abdominal fat content and MTGC were determined in all subjects. Peak circumferential strain was measured in 19/22 boys, HLC in 20/22 boys, and liver T1 in 18/22. Three lean boys were unable to complete the MR protocol as they became uncomfortable and fidgety, and so accurate shimming became impossible to measure HLC, and breath-holding was poor in one lean boy, so strain imaging was not analysable; T1 measurement was not performed in three lean and one obese boy. Five of the lean boys and eight of the obese boys agreed to give a blood sample. Anthropometric, biochemical, cardiac and blood pressure data are shown in Table 7.1. The average age of the lean boys was slightly higher than that of the obese (13.1 v 11.9 years, $p = 0.054$). There was no significant difference between the groups in fasting glucose or total cholesterol.

Table 7.1. Anthropometric and biochemical subject characteristics			
	Lean	Obese	P value
N	11	11	
Mean age (years)	13	11	0.05
<i>Anthropometric measurements</i>			
Height (m)	1.65 ± 0.17	1.63 ± 0.06	0.74
Weight (kg)	50.8 ± 13.7	79.2 ± 27.8	0.008
Body mass index (kg/m ²)	18.4 ± 2.1	28.9 ± 6.5	<0.001
Sagittal abdominal diameter (cm)	15.3 ± 1.8	22.1 ± 4.0	<0.001
Waist circumference (cm)	68.9 ± 6.5	97.5 ± 17.0	<0.001
<i>Measures of adiposity and ectopic fat deposition</i>			
Total cross-sectional fat (cm ²)	62 (46)	395 (314)	<0.001
Subcutaneous fat (cm ²)	48 (43)	372 (263)	<0.001
Visceral fat (cm ²)	12 ± 4	47 ± 28	<0.001
<i>Serum biochemistry</i>			
Glucose (mmol/l)	4.50 (0.37)	4.80 (0.45)	0.26
Total cholesterol (mmol/l)	3.7 ± 0.6	4.0 ± 0.5	0.42
Triglycerides (mmol/l)	0.53 ± 0.10	1.04 ± 0.57	0.04
HDL cholesterol (mmol/l)	1.40 ± 0.25	1.03 ± 0.18	0.03
Cholesterol:HDL ratio	2.75 ± 0.45	4.04 ± 0.91	0.008
<i>Cardiac size and function</i>			
LV ejection fraction (%)	72 ± 5	71 ± 6	0.86
LV end-diastolic volume (mls)	121 ± 31	129 ± 34	0.56
LV mass (g)	92 ± 26	97 ± 22	0.56
RV ejection fraction (%)	64 ± 6	62 ± 4	0.49
RV end-diastolic volume (mls)	132 ± 33	147 ± 36	0.36
<i>Blood pressure</i>			
Systolic BP (mmHg)	104 ± 8	123 ± 13	<0.001
Diastolic BP (mmHg)	61 ± 10	73 ± 10	0.01
Pulse Pressure (mmHg)	44 ± 9	50 ± 10	0.14

7.3.1 Cardiac size and function

There were no significant differences between the two groups in ventricular systolic function or end-diastolic volume. However, the obese children did have markedly higher systolic and diastolic blood pressures. Four of the obese boys had systolic BP > 130mmHg, the threshold for the diagnosis of prehypertension, and one of the criterion for MetS. The mean pulse pressure was not significantly different between the two groups. Left ventricular mass (LVM) across all boys increased with higher pulse pressure ($r_s = 0.64$, $p=0.001$; Figure 7.2). However, neither systolic nor diastolic pressures were correlated with LVM.

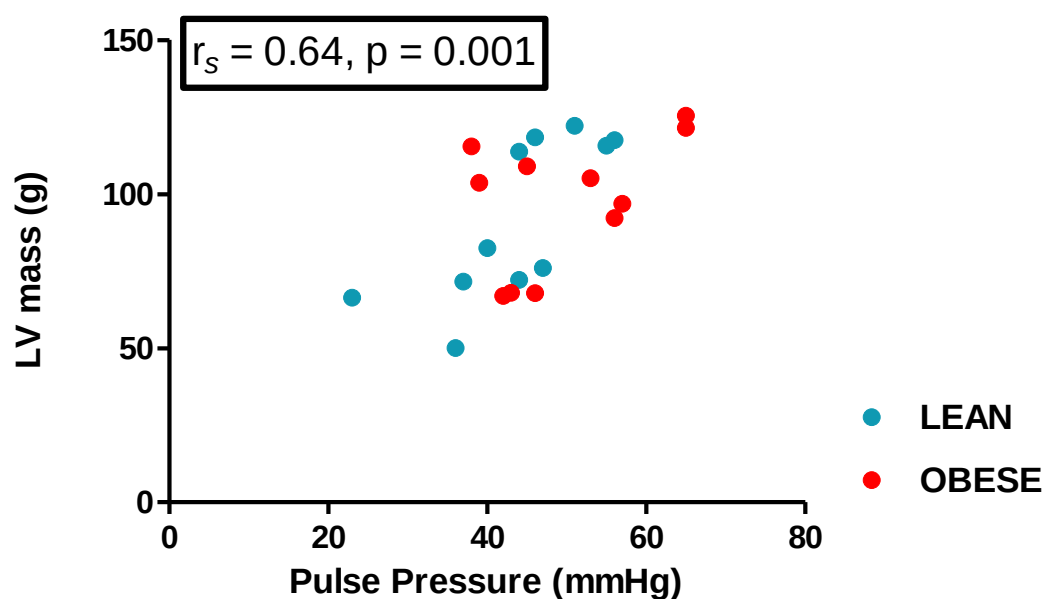


Figure 7.2. Increasing pulse pressure in all boys is associated with increased LV mass

7.3.2 Myocardial triglyceride content

Lean boys had very low MTGC, with a range of 0.06 – 0.38% of the water signal, a median of 0.15% and a mean of 0.16%. Indeed, this may be an overestimate, as below 0.1% the assessment of MTGC spectra, compared to the baseline noise in the spectra, is difficult, and the three boys with MTGC <0.1% may be better described as having undetectable MTGC. The obese boys had much higher MTGC ($p = 0.04$), with a range of 0.07 – 0.46% of the water signal, a median of 0.29% and a mean of 0.28% (Figure 7.3, below). Examples of spectra are shown in Figure 7.4.

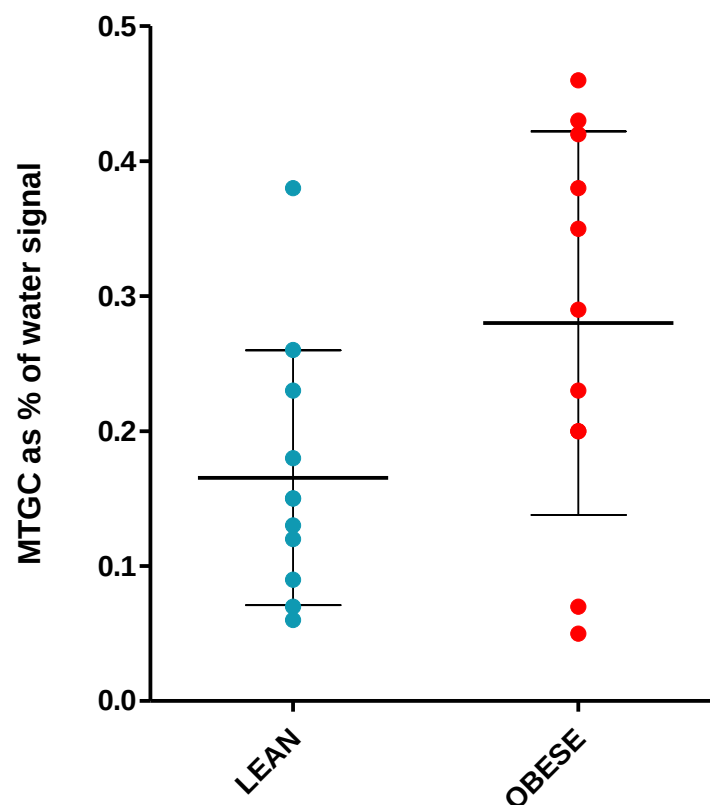


Figure 7.3. Myocardial triglyceride content (MTGC) in lean and obese boys

Values for all 22 subjects are shown, with mean $\pm$ SD in each group. Obese boys had a significantly higher mean MTGC ($0.28 \pm 0.14\%$ versus lean $0.16 \pm 0.09\%$, $p = 0.04$).

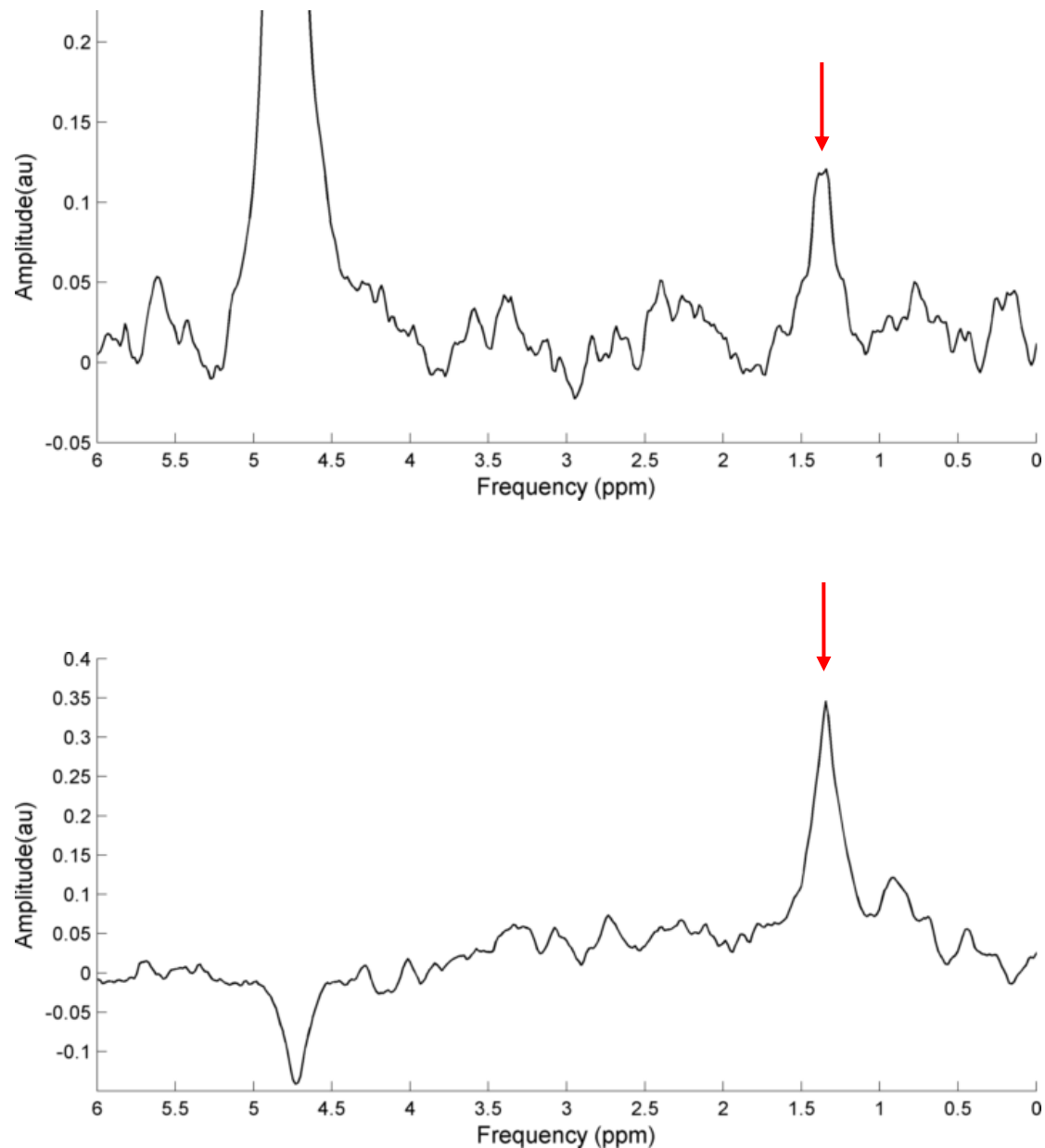


Figure 7.4. Examples of ^1H MRS water suppressed spectra from a lean (above) and an obese (below) boy

The methyl peak ($-\text{CH}_2-$), representing lipids, is at 1.3ppm (shown with red arrow). Note that the y-axes are on different scales.

7.3.3 Myocardial peak circumferential strain

Lean boys had CS values of -17.5 to -21.6, with a mean of -20.2 and a median of -21.3. These are comparable to lean adults, and to previously published normal values. Obese boys had CS values of -14.2 to -19.8, with a mean of -17.6 and a median of -17.7. This was significantly more positive than the lean group ($p = 0.006$), suggesting a clear association of obesity and impaired strain at this early age (Figure 7.5).

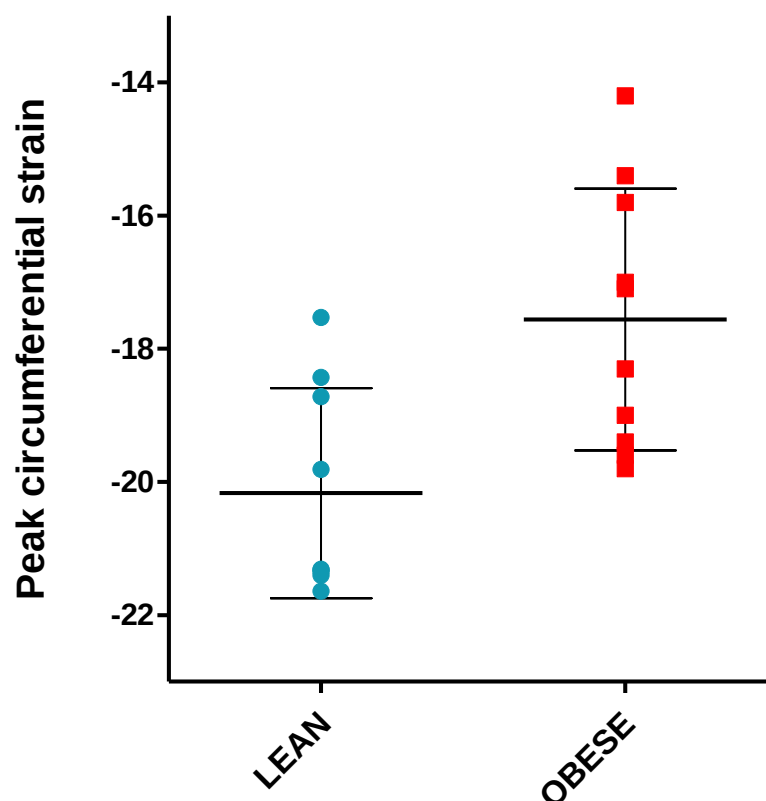


Figure 7.5. Peak circumferential strain (CS) in obese and in lean boys

Values for 19 boys are shown (9/11 lean; 10/11 obese), with mean $\pm$ SD in each group. Obese boys had a significantly more positive CS (-17.6 ± 2.0 versus lean -20.2 ± 1.6 ; $p = 0.006$).

7.3.4 *Hepatic lipid content*

Median HLC was 0.46% (IQR 0.04-0.79) in the lean group. The obese boys had markedly elevated HLC ($p = 0.04$), with a median of 2.79% (IQR 1.53 - 5.79). Seven obese boys had HLC > 2%, compared to none in the lean group (Figure 7.6). Two obese boys had HLC > 20%, suggesting very severe steatosis. Across both groups, there were strong positive correlations between HLC and all measures of abdominal adiposity, most clearly seen with waist circumference ($r_s = 0.80$, $p < 0.001$; Figure 7.7a) and visceral fat content ($r_s = 0.82$, $p < 0.001$, Figure 7.7b).

The obese boys had significantly reduced HDL and elevated triglyceride concentrations compared to the lean boys (Table 7.1). There was a strong negative correlation between HLC and HDL ($r_s = -0.65$, $p = 0.03$), and the pattern of dyslipidaemia associated with MetS was also evident (Fig 7.8a-c), if not statistically significant due to the paucity of data from the lean group.

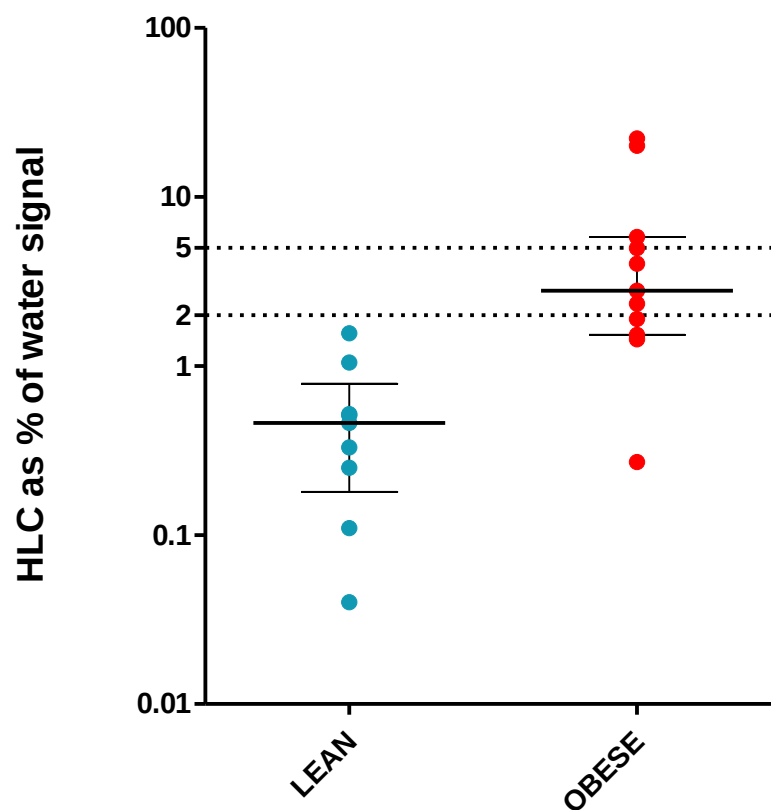


Figure 7.6. Hepatic lipid content in lean and obese boys

HLC is <2% in all lean boys, and markedly higher in the obese group. Median HLC and interquartile range is shown for both groups (lean 0.46, obese 2.79, $p = 0.04$), as the data is not normally distributed. HLC is shown on a logarithmic scale.

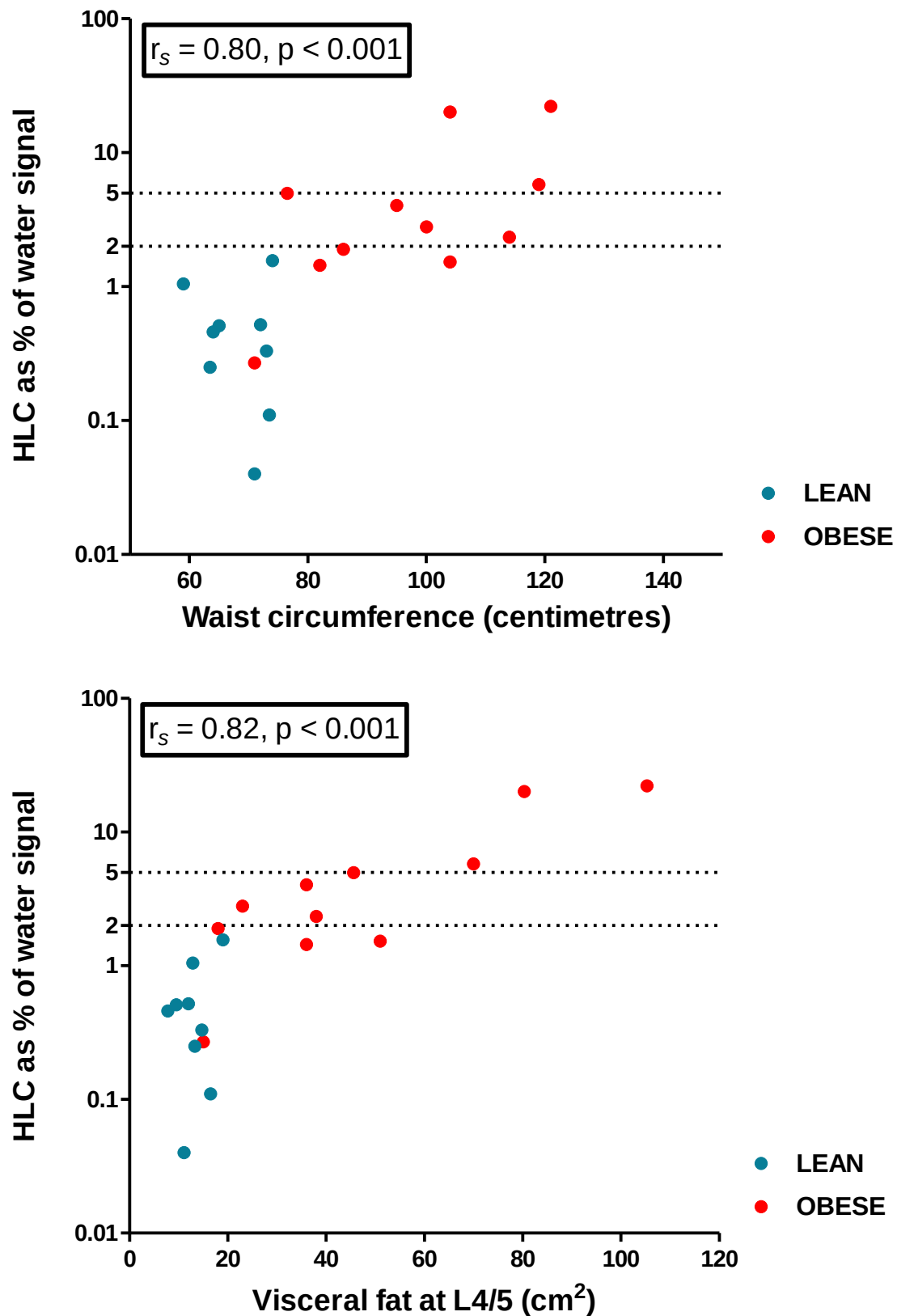
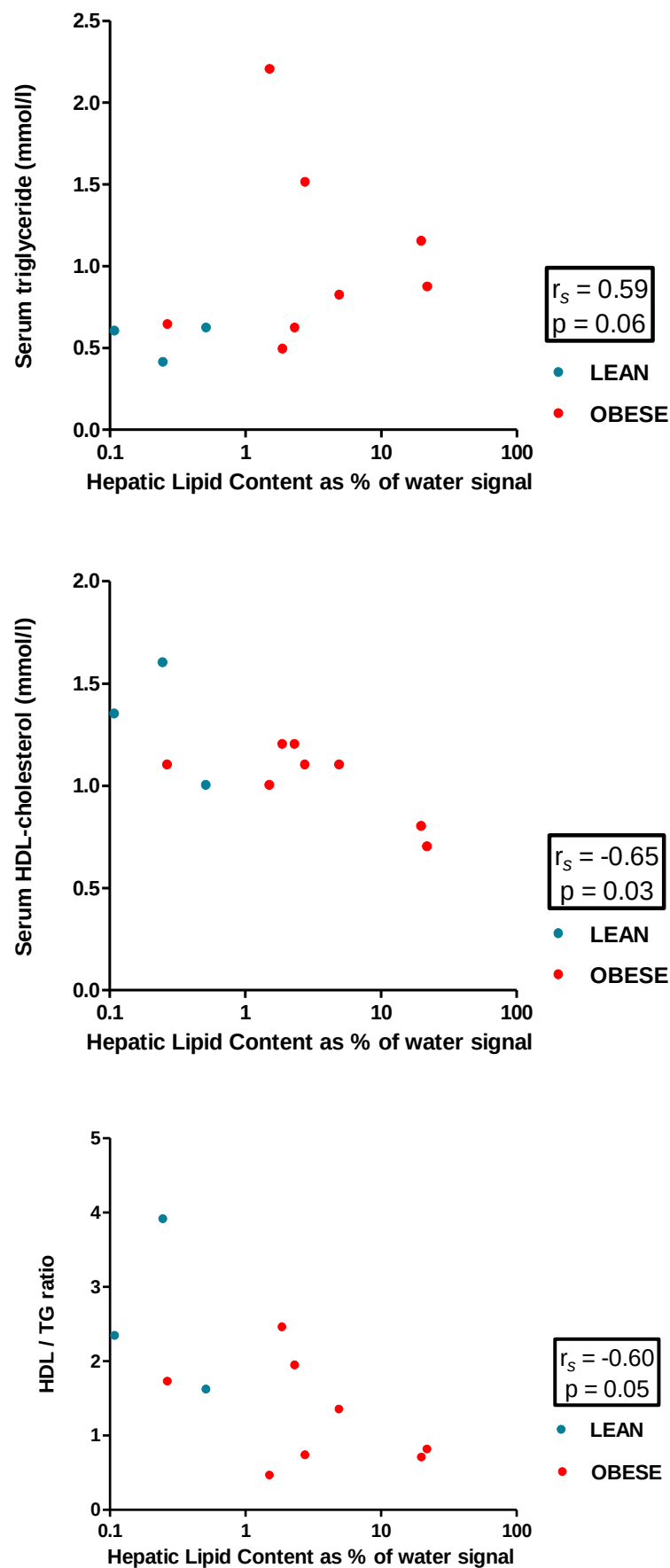


Figure 7.7 a (above) and b (below). Hepatic lipid content and abdominal adiposity

HLC has a strong positive association with waist circumference and visceral fat content across all 22 boys. HLC on the y-axis is shown on a logarithmic scale.



7.3.5 Liver T1 values as a measure of fibrosis

The lean group had a normal distribution of T1 values, with a mean of 783 ± 9 ms (range 749 – 820; median 780ms). This was similar to the normal adult value for T1 found in Chapter 6. The obese boys had a wider range of T1 values, and a markedly higher mean of 858 ± 30 ms (range 764 – 997; median 829; unpaired t test $p = 0.04$). Three obese boys had $T1 \geq 970$ ms, indicating markedly more extracellular fluid in their hepatic parenchyma (Figure 7.9). Most significantly, these three boys also had the highest HLC, suggesting steatohepatitis (Figure 7.10). Furthermore, all three of these boys had the same distinctive pattern of increased T1 seen throughout their livers, similar to the pattern described for pericellular fibrosis (Figure 7.11). None of the children had elevated iron on T2* mapping, which might have altered their T1 (ie no iron correction was required for any subject).

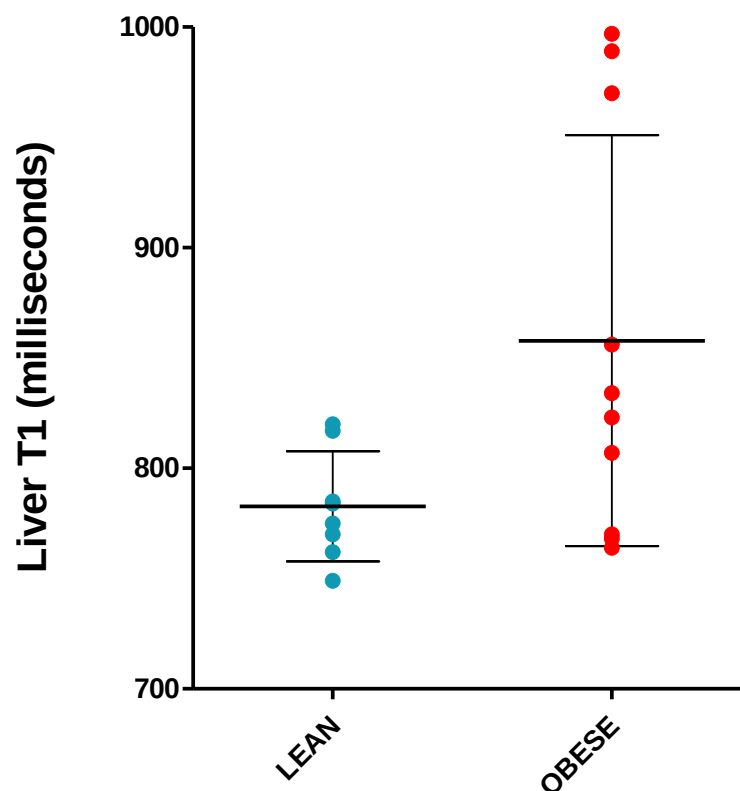


Figure 7.9. Liver T1 values in lean and obese boys

Mean liver T1 is markedly higher in the obese boys (858 ± 93 ms) compared to the lean group (783 ± 25 ms). Mean $\pm$ standard deviation of T1 (SD) are shown for both groups.

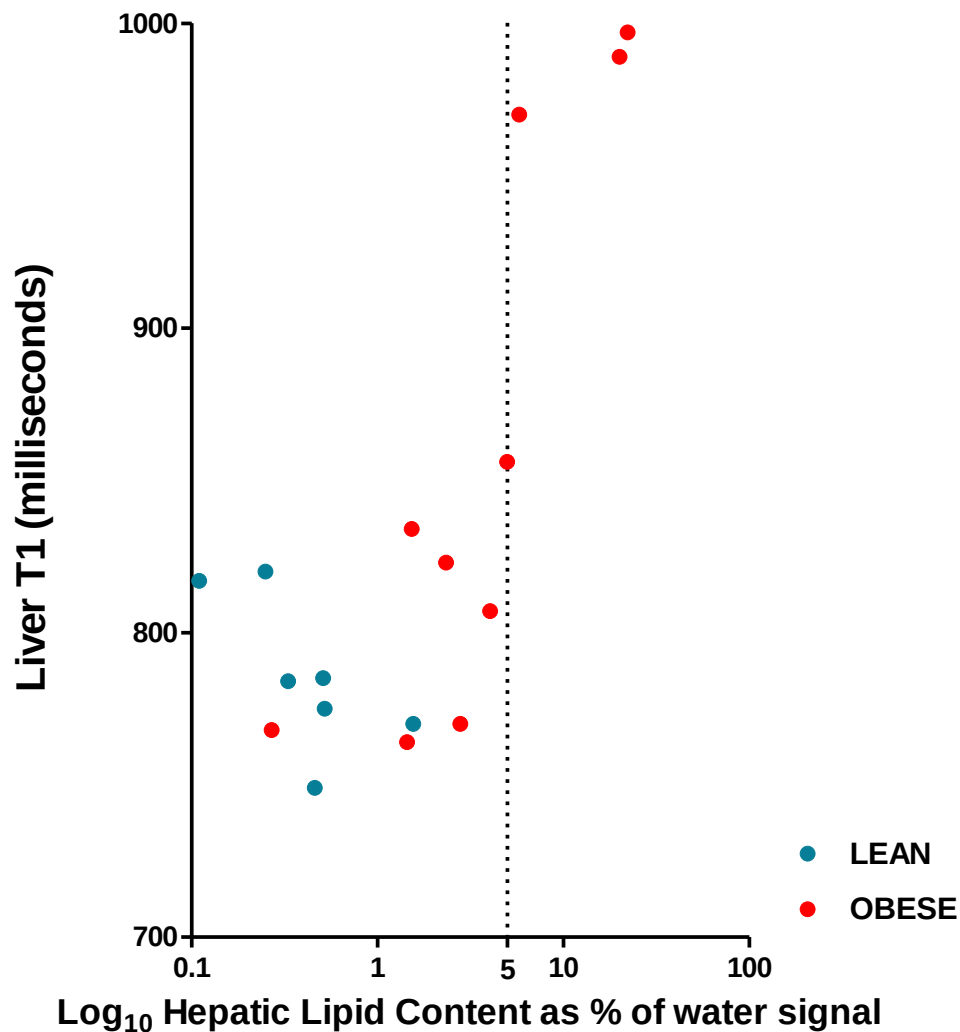


Figure 7.10. Non-invasive diagnosis of steatohepatitis in children

The three boys with high T1, suggesting increased ECF, and high HLC (data not shown here), suggesting steatosis, are likely to have NASH. With current clinical diagnostics, the only way to confirm this would be with liver biopsy. For all 22 boys, hepatic lipid content correlated with liver T1 ($r_s = 0.57$, $p = 0.016$), providing further evidence that progressive steatosis is associated with increased hepatic extracellular fluid.

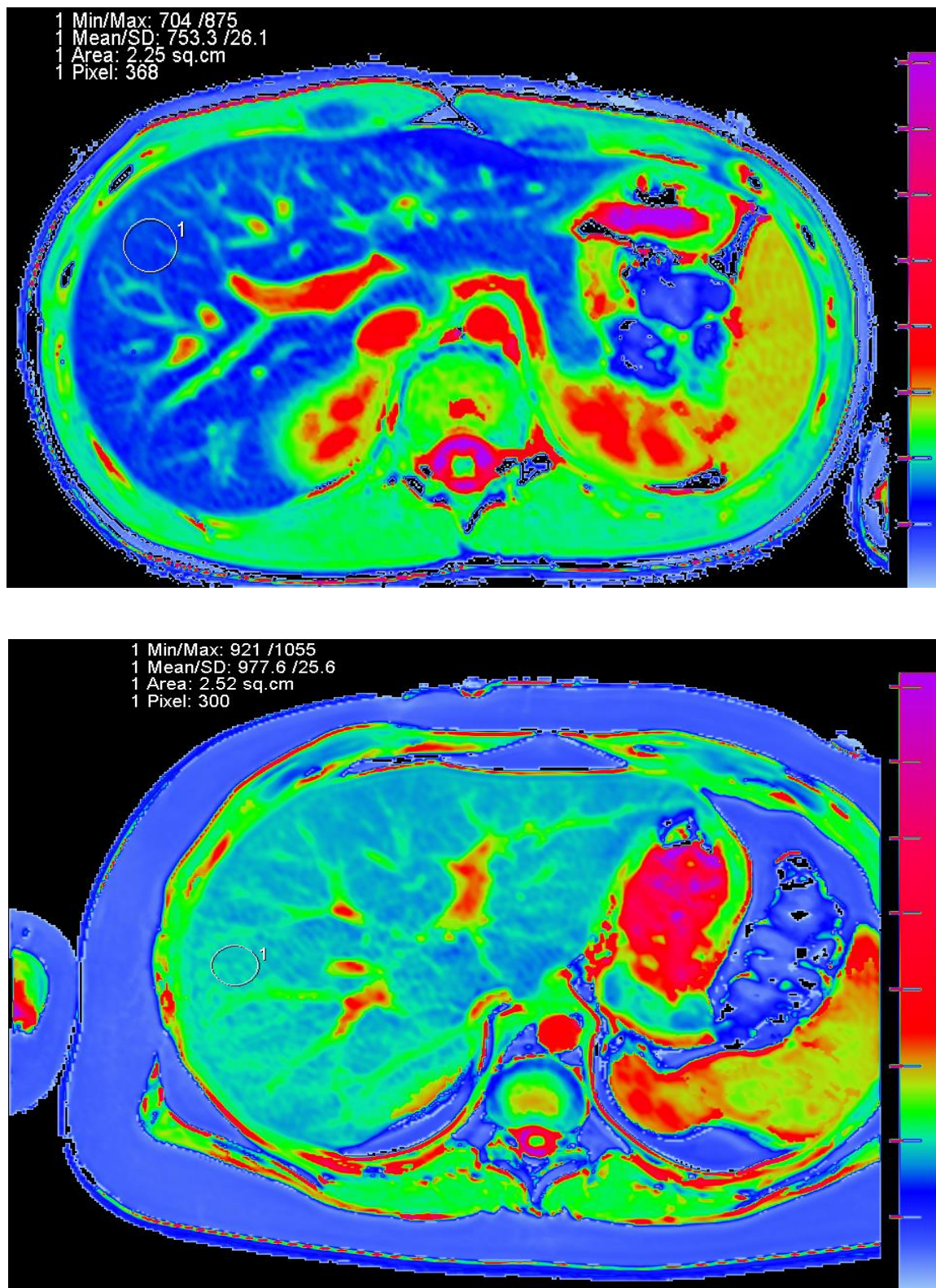


Figure 11. A normal boy's liver (above), and an obese boy's liver with MR evidence of NASH (below). The lean boy has a T1 of 753 ± 26 ms, and HLC of 0.5%. The obese boy has a T1 of 978 ± 26 ms, and the characteristic texture of steatohepatitis. His BMI is 30.8 kg/m^2 with a waist circumference of 104 cm, and HLC of 20.1%.

7.4 Discussion

This small pilot study aimed to see if new biomarkers could help determine the impact of childhood obesity on cardiometabolic health. There were notable differences in blood pressure and serum triglycerides, both established ‘classical’ risk factors for heart disease. More interestingly, the data suggest that multiparametric MR could identify significant hepatic steatosis even at this early age, and the dyslipidaemia of MetS that is more expected in adulthood. The epidemiology of paediatric NASH is poorly defined. An autopsy study of 742 children aged 2-19 from 1993 to 2003 in California showed that 13% had histologically defined NAFLD and 3% had NASH, but the authors noted that this may have been biased by alcohol-induced motor vehicle deaths.²⁶⁷ This corroborates data derived from national surveys in the USA and Asia in the same decade in which approximately 3% of adolescents had elevated serum ALT > 30IU/l.^{268, 269} Boys and older children are more likely to have NAFLD and NASH, which is understandable as disease severity is exacerbated by hepatic lipid accumulation and the ‘vicious cycle’ discussed in Chapter 5. Ultrasound studies using echogenicity as a marker of steatosis under-report the NAFLD prevalence at 2-4% as a consequence of the technique’s lower sensitivity. With the increasing prevalence of obesity, as well as teenage alcohol misuse, all these estimates of prevalence are likely to be outdated.

The current MRS thresholds for HLC are >5% for moderate steatosis and >9% for severe steatosis.²⁶⁴ This small cohort of 22 boys suggests that these thresholds are not sufficiently sensitive. HLC $\approx$ 5% is equivalent to 30-60% of hepatocytes containing significant lipid inclusions. Conventionally, severe histological steatosis is > 33%. Thus, a threshold of MRS HLC > 5% may misclassify many patients as moderate rather than severe. Furthermore, liver biopsy is prone to sampling error, as previously discussed. The clear association in childhood

between central obesity and hepatic steatosis is a useful public health message in itself – overfed, obese children have liver disease, unlike their lean peers.

The dyslipidaemia observed in the obese boys is similar to that observed in the adult study in Chapter 5. According to AHA recommendations, the ‘acceptable’ upper limit for serum triglyceride in boys aged 10-14 is 0.74 mg/dl.²⁷⁰ Although only three samples from lean boys were studied, their serum triglyceride of 0.53 ± 0.1 mg/dl was well within this range, whereas the obese boys had a much higher 1.04 ± 0.57 mg/dl ($p = 0.04$). The obese boys had much lower HDL as well, and it is likely that this lipid profile predisposes to early atheroma formation, especially when coupled to the increased blood pressure seen here also. Early atherosclerotic plaque development is a very poor prognostic marker, and has been described before.^{250, 256, 271, 272}

Elevated T1 in the three boys with the highest HLC, suggesting steatohepatitis, was seen with the same characteristic pattern seen in biopsy-proven NASH patients in Chapter 6. Although there is no biopsy confirmation of NASH from these children, these data suggest that the technique is feasible, and further studies are necessary in a larger cohort to determine the utility of this method for clinical use and targeted screening in adolescent obesity for liver disease. Furthermore, cohort studies are warranted to determine how children with NASH fare in early adulthood. Data from the Mayo clinic suggests that paediatric NASH patients have a standardised mortality ratio of 13 compared to normal children over a 20 year follow-up.²⁶⁵ Non-invasive longitudinal studies would be much more tractable than biopsy studies.

The obese boys had significantly higher MTGC, although not as high as lean or obese adults. Myocardial triglyceride accumulation seems to be a slow, gradual process, unlike hepatic lipid accumulation. The reversibility of MTGC has not been studied as yet. An alternative explanation might be that the higher MTGC may be a measure of myocardial ceramide

concentration – the ceramide peak is indistinguishable from the methyl (-CH₂-) peak. Importantly, there was no difference between lean and obese in stroke volume or left ventricular mass. This contrasts with previous CMR studies, which suggested that LV hypertrophy was an early cardiac adaptation to obesity.^{89, 273} Friburg et al studied 19 obese children and 20 lean controls and reported a 20% increase in LV mass. However, their obese group was significantly older and had more boys (10/19 compared to 9/20). Male gender and increasing age both contribute to increased LV mass, and may have contributed to the observed difference.

The difference in CS between lean and obese boys here is interesting, in that it mirrors the trend seen in adults studied in Chapter 4. It is notable that, although the obese boys had significantly higher systolic and diastolic blood pressures, it was the pulse pressure which correlated with LVM. This suggests that whilst obesity may be more strongly associated with increased blood pressures, cardiac adaptation is driven by increased afterload in early life, and this may account for the difference in strain. This process would also explain the changes in strain seen in children with severe renal disease. Unfortunately, the role of insulin resistance cannot be determined from our study as blood sampling was optional. Larger studies are warranted to enable multivariate analysis in children, accounting for variables not investigated here such as gender, exercise capacity and ethnicity.

7.4.1 Study limitations

This was a small, slightly underpowered, study, but nevertheless there were striking differences between groups. Subjects were recruited by poster advertisement for lean boys and from local paediatric clinics for obese boys – the different recruitment strategy for each group may have introduced a selection bias. Ethnic differences in body fat distribution,

although they are known to affect insulin resistance, were not studied. All subjects fasted for a minimum of eight hours, before their study visit, but dietary habits and exercise capacity were not assessed. All of the children denied smoking or alcohol use, but this was not verified biochemically. Only three of the lean children opted to give a blood sample, and so this pilot study is underpowered in itself to demonstrate clear differences in lipid profile between groups. Moreover, serum insulin and other pertinent biomarkers (eg leptin, C-reactive protein) were not measured. However, the obese boys' lipid results are different from published reference ranges for lean children.²⁷⁰

7.5 Conclusions

In summary, this study shows that childhood obesity is associated with increased blood pressure, altered myocardial strain, dyslipidaemia and hepatic lipid accumulation, and that some obese boys have increased hepatic extracellular fluid content. This is the first demonstration that non-invasive imaging can identify NASH in children, although the techniques are equally applicable to other liver conditions. Importantly, the degree of hepatic steatosis seen in the obese children compared to the more modest changes in blood pressure and the lack of difference in LV mass suggest that lipid accumulation in the liver is a more upstream event in the development of metabolic syndrome.

Chapter 8: Conclusions and suggested further work

8.1 Introduction

There is a strong argument that obesity is not a medical problem, but a societal one.^{274, 275} Nevertheless, there are medical consequences from excess body weight which cannot be ignored. Moreover, fear of obesity-related illness is a powerful motivator both for the individual and for the policy-makers who shape our economic and physical environment. These studies have sought to characterise some of the obesity-related conditions in adults and children with no known heart or liver disease. We have shown that sustained weight loss can lead to a regression in LV mass, and that obesity is associated with both impaired myocardial strain and increased intramyocardial lipid content. In the liver, we have shown that obesity is strongly associated with steatosis and the dyslipidaemia of Metabolic Syndrome. The MR methods used to quantify steatosis have been verified against the current ‘gold-standard’ of liver biopsy, and we have developed new ways of assessing other types of liver disease non-invasively. Finally, we have looked at the feasibility of assessing children with MR to diagnose early cardiometabolic disease, and have identified clear differences between obese and lean boys for several biomarkers of early disease.

8.2 Ventricular hypertrophy and cardiac steatosis

It is well established that obese patients have LV hypertrophy. However, the cause of this hypertrophy has not yet been fully elucidated. Compensatory hypertrophy in response to hypertension and/or increased stroke volumes, myocardial fibrosis, endocrine adaptation and cardiac steatosis have all been suggested as putative mechanisms, and are not mutually exclusive. Previous studies have shown that this hypertrophy is reversible with weight loss surgery, and the effect is preserved for two years.^{77, 79, 80, 84} The small follow-up study in Chapter 3 confirms these findings at three years following surgery. The extent of cardiac steatosis as a component of the increased LV mass in obesity is small – the highest value seen in 100 subjects, of whom 27 were obese, is only 2.8%, or less than 5g of myocardium. Thus the reduction seen with weight loss, which was over 20%, equivalent to 30g of muscle, cannot be due to loss of intramyocardial fat alone. Regression of hypertrophy must therefore be driven by another process, such as muscular adaptation to a reduced afterload.

One possible mechanism is through the change in cardiac axis that occurs with weight loss. Many obese patients have left axis deviation due to diaphragmatic splinting, pushing the cardiac apex up towards the axilla and producing a curved aortic root leading up to the aortic arch. This is most marked with central obesity. Consequently, blood from the left ventricular outflow tract passes through 90° in the first few centimetres of the ascending aorta, producing marked shear stress on the aortic wall. This has recently been suggested as a causal link between obesity and aneurysm formation in animal models.²⁷⁶ As the diaphragmatic splinting is resolved with abdominal weight loss, the cardiac apex normalises, and the angle between the LV outflow tract and the aortic arch decreases. Further studies in bariatric patients are necessary to see if this hypothesis is true, ideally with 4-dimensional flow mapping to determine the flow patterns in the aorta associated with obesity.

Another mechanism would be through direct compression of the abdominal aorta by excess adipose tissue. In the latter weeks of pregnancy, the mother carries approximately 8kg of extra tissue on her lower abdomen, and this is known to cause compression of the inferior vena cava in the supine position.²⁷⁷ Although the arterial wall of the aorta is thicker, more muscular, and less likely to collapse under this extra burden, it is not inconceivable that excess abdominal weight does lead to increased transmural aortic pressure, and thus a higher pulse pressure is required. Moreover, some obese patients have deconditioned paraspinal and abdominal core muscles, predisposing them to tracheal obstruction in sleep apnoea and obesity hypoventilation syndromes.²⁷⁸ This process would make them especially vulnerable to abdominal weight pressing down on their descending aorta at night, when their muscles are relaxed. Indeed, loss of diurnal variation of blood pressure is one of the earliest signs of hypertension in obese teenagers, and predicts the Metabolic Syndrome in all age groups.²⁷⁹ To investigate this further, a study on abdominal aortic distensibility in lean patients could determine if the addition of extrinsic abdominal weight (eg 10kg) alters distal aortic flow in the supine position. Moreover, this should not occur in the prone position, as the spine would prevent extrinsic aortic compression. To test the hypothesis fully would require invasive arterial pressure studies to measure distal aortic pressure waveforms while supine before and after the addition of an extrinsic 10kg weight over the abdomen. This may be tractable in patients due for diagnostic coronary angiography who would be happy to take part in research into the mechanisms of hypertensive disease.

Left ventricular hypertrophy in obesity may also be due to endocrine and/or paracrine signalling. Adiponectin, leptin and ghrelin have recently been implicated as causes of LVH, each being shown to have independent associations with hypertrophy.²⁸⁰⁻²⁸² However, the signalling pathways involved have not been fully elucidated, and the hormones themselves are very difficult to measure as serum levels vary in response to stress, temperature, appetite,

insulin resistance, feeding and satiety. It is hoped that further work may determine the interaction of these highly conserved satiety hormones, and establish how they affect cardiovascular function and structural adaptation to increased size.

Myocardial fibrosis and disarray is a final possible explanation for the observed LV hypertrophy seen in obesity. In the studies described here, it is assumed that all the tissue between the endocardial and epicardial borders of the left ventricle was myocardium. In certain inherited and acquired heart diseases, such as hypertrophic cardiomyopathy and aortic stenosis, MR imaging has demonstrated that pathological hypertrophy consists of disarrayed myocardial fibres and collagen fibrosis, in addition to normal myocardium.^{283, 284} These features have not yet been shown in obesity, but contrast and fibrosis imaging are promising tools for use in this field.²⁸⁵ The role of anti-fibrotic pharmacotherapy in obesity-related liver disease is already under investigation with ongoing clinical trials using losartan – it may transpire that cardiac remodelling is also possible in patients with myocardial fibrosis as a consequence of chronic obesity.²⁸⁶

8.3 Weight loss to treat heart disease

Maintaining normal weight and preventing obesity is seen as a keystone in cardiovascular risk reduction, both at the individual and the population level. However, when overweight and obese patients are found to have heart disease, management of their weight is often neglected. Over the next few decades, the number of obese patients with heart disease will rise markedly. Physicians and surgeons often suggest weight loss to improve cardiovascular fitness, and this often occurs during cardiac rehabilitation following myocardial infarction. From a clinical perspective, cardiovascular improvement after weight loss in these patients is

a promising area for further research, as obesity is increasingly seen as a modifiable risk factor. Increased availability of weight reduction surgery and longterm data on efficacy and cost-effectiveness has been published in the last few years, and it is now standard practice for the treatment of diabetes mellitus in morbid obesity.¹⁵³ If weight loss does indeed improve afterload, then perhaps it would benefit obese and MetS patients with moderate aortic stenosis? The perioperative risk of laparoscopic sleeve gastrectomy would be much lower in this group than the risk of valve replacement, and a clinical trial would be justified. Similarly, in overweight and obese patients with coronary artery disease, stable angina and LV hypertrophy, surgical weight loss may be beneficial. In theory, weight loss will lead to LV remodelling will result in a lower muscle mass, which will be better perfused, resulting in less symptomatic angina. Moreover, this strategy would also improve insulin sensitivity, thus reducing the rate of atherosclerotic disease progression.

It is unfortunate that there has not been much progress in the development of pharmaceutical therapies for obesity, as these would be easier to evaluate on patients with co-existing heart disease. Sibutramine and rimonabant have been withdrawn from the market because of safety concerns.²⁸⁷⁻²⁸⁹ Metformin and orlistat are used for weight reduction, and may offer some benefit in obese patients awaiting elective surgery, but there is no existing evidence for this. Liraglutide and growth hormone analogues are currently under investigation as possible weight loss therapies in diabetic and lipodystrophic populations. Non-invasive MR biomarkers of cardiometabolic disease may be more useful surrogate endpoints in these patients than weight, waist circumference or BMI, especially as the recent history of drug development in this field has had many false dawns.²⁸⁷

8.4 Liver disease in the assessment of cardiovascular risk

Atherosclerosis is increasingly seen as a pathological process determined by multisystem deregulation. A 'Western' diet, coupled to abnormal cholesterol transport, leads to ectopic fat deposition, organ dysfunction, and inflammation. In the arterial tree, these processes, along with hypertension and reduced endothelial function combine so that areas of endothelium become foci for lipid-rich macrophage and platelet deposition. The liver's role in these processes has been extensively studied, but the paucity of non-invasive biomarkers of hepatic disease has been a major hurdle. Although NASH was described 30 years ago, only since 2004 have spectroscopy studies shown that abdominal obesity is closely linked to hepatic lipid content, and that this can be altered by weight loss.^{100, 101, 290} Alcohol is the biggest cause of mortality in Russia, where excess usage is endemic, and accounts for over half of all adult deaths.²⁹¹ Could this be due partly to severe liver disease resulting in accelerated atherosclerosis? A further possibility is that alcohol overuse leads to myocardial steatosis, and that the resultant depression in contractile function seen in 'alcoholic cardiomyopathy' may be similar in aetiology to diabetic heart disease. The epidemiology supports these theories of causation, but mechanistic studies are needed to show how alcohol affects lipoprotein metabolism, which patient groups are most susceptible, and what can be done to treat them. We cannot accurately determine the burden of alcohol - related disease in most countries because of the lack of consumption data and the lack of liver data. In the healthy volunteer group in Chapter 6, with presumed normal liver function, three subjects had markedly increased cT1 values, suggesting either inflammation or fibrosis. This may have been due to recent alcohol use, or to chronic asymptomatic, hitherto undiagnosed liver disease. As with any health survey, incidental findings of abnormal function are expected. However, in liver disease, we have not had sufficiently sensitive and acceptable research

tools in the past to allow these surveys, unlike other branches of medicine (eg blood pressure measurement, echocardiography or electrocardiography in cardiovascular science, pulse oximetry and lung function assessment in respiratory medicine). Multiparametric MR may offer many opportunities to assess heart and liver disease in adults and children, thus giving insights into the role of metabolism in atherosclerosis, and in other forms of heart disease, such as alcoholic cardiomyopathy.

8.5 Assessing liver disease – changes over time

Multiparametric MR holds many promises in the scope of addressing the huge forecasted burden of hepatobiliary disease. The study described in Chapter 6 was conceived to validate spectroscopy as the optimal method for the assessment of hepatic steatosis. Although further work is required in a multicentre study, it may transpire that MR is the fastest, most accurate, and cost-effective method to assess a patient presenting with suspected liver disease. Much of the information gathered from a biopsy can be obtained, with none of the attendant risk or sampling error, and the quantitative measurements reduce inter-observer variance. The technique is suitable for longitudinal studies to assess disease progression, or response to therapy (eg bocepravir for hepatitis C).

Figure 8.1 shows a patient who took part in the liver biopsy study, and subsequently had weight reduction surgery. Figure 8.2 shows her transverse liver T1 map and a corresponding biopsy, taken during her gastric band insertion. Her T2* was normal, so no iron-correction was required. The T1 and histology match, and suggest inflammation, with an Ishak score of 3 by consensus, and 3, 4 and 5 by blinded individual pathologists. Table 8.1 shows her pre and post-operative serum biochemistry. There was little or no change in her bilirubin, ALT, glucose and total cholesterol, but marked differences in HDL, triglyceride, GGT, and CRP. Her pre-operative hepatic lipid content, measured by ^1H MRS, was 20.7%. Figure 8.3 shows changes in her hepatic lipid content and her T1 – according to her MR parameters, she had severe NASH, and has now been cured by this operative measure. A second biopsy to confirm this histologically could not be justified ethically.

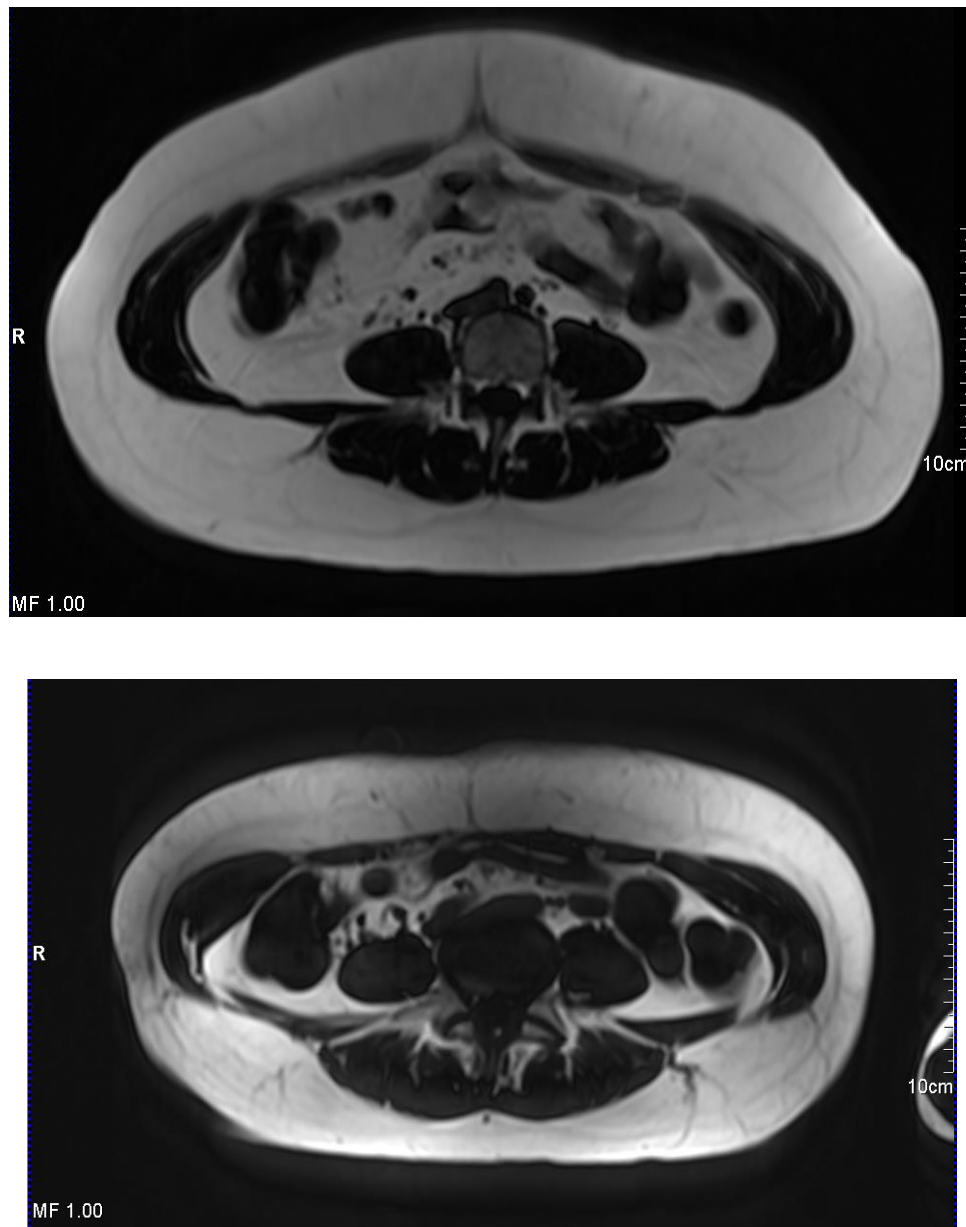
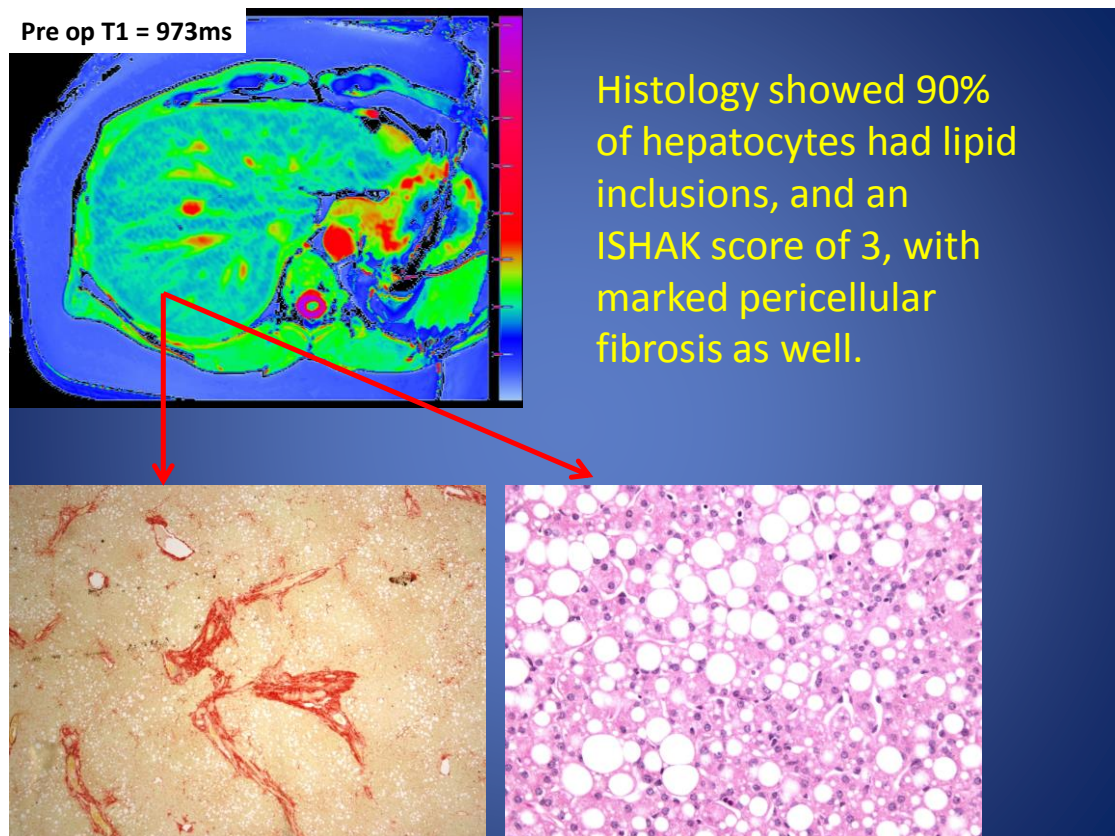


Figure 8.1. Weight loss following gastric band insertion

Transverse TSE images at the L4/5 intervertebral disc, with the same scale, from a 44 yr old lady, pre (above) and one year after (below) gastric bypass surgery. Her pre-op BMI was 34.3 kg/m², with a visceral fat content of 131cm²measured at L4/5. Her post-op BMI was 24.4 kg/m²,with a visceral fat content of 28 cm².

Figure 8.2. Pre-operative fibrosis on MR and histology**Table 8.1. Markers of cardiometabolic risk before and after surgery**

Serum biochemistry	Pre	Post
Fasting glucose mmol/l	5.5	4.5
Total cholesterol mmol/l	5.6	5.6
Triglyceride mmol/l	1.89	1.31
HDL-c mmol/l	1.1	1.7
Calc LDL-c mmol/l	3.6	3.3
Bili micromol/l	10	12
ALT IU/l	68	59
Alk phos IU/l	158	153
GGT IU/l	53	20
CRP mg/l	2.2	<0.5

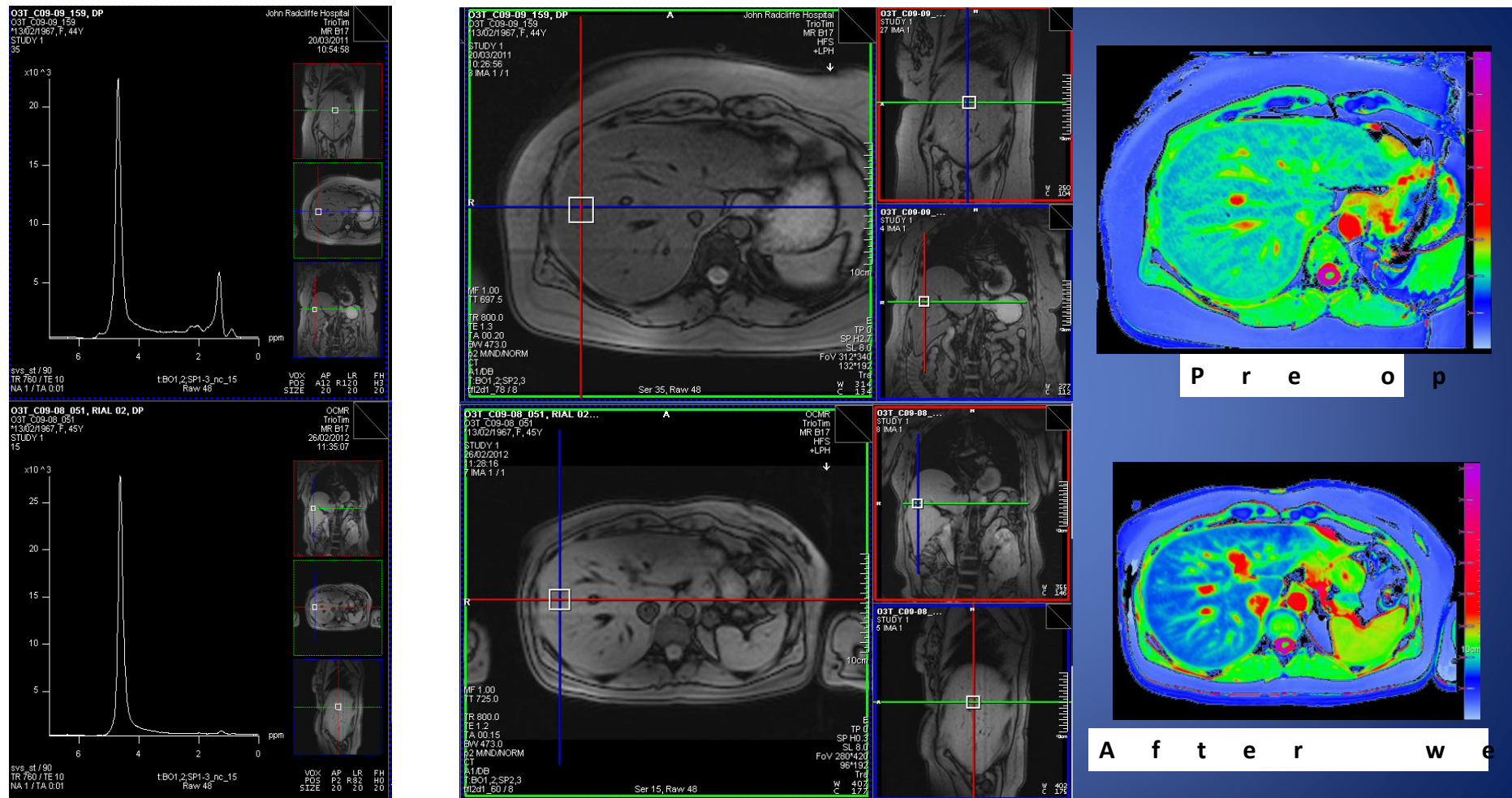


Figure 8.3. Changes in steatosis and fibrosis over one year. ¹H MRS results are shown in the first column – her hepatic lipid content fell from 21.7% to 2.1% of her water signal. The voxel position is shown in the middle panel, and was identical on both visits, although little variation in HLC is expected. Her T1 dropped from 973ms to 817ms, suggesting a resolution of liver inflammation and/or fibrosis.

Previous studies examining the effects of therapies in liver disease have been fraught with controversies arising from interobserver variance. A study by Olivieri et al in 1998, published in the New England Journal of Medicine, claimed that the iron-chelating agent deferiprone was associated with increased hepatic fibrosis.²⁹² This led to one of the most protracted medico-legal battles in history between the authors, their institute (the Toronto Sick Kids Hospital), and Apotex Inc, the manufacturer of deferiprone, centering on the interpretation of 72 liver biopsies taken from 39 patients.²⁹³ Of these, 19 had received deferiprone therapy, and 20 had received standard care with desferoxamine. To this day, deferiprone is not licensed in North America, although it is widely used in thalassaemia patients throughout Europe. The acrimonious litigation surrounding the ‘Olivieri affair’ inspired the author John le Carré to write ‘The Constant Gardener’.^{294, 295} Fifteen years after the original study, we may have the tools to assess the best iron chelation agents for use in children and their effects on hepatic fibrosis, without the risks of biopsy, and with far less controversy. Furthermore, multiparametric MR biomarkers as surrogate endpoints for liver therapies are very promising because they cover a broad spectrum of liver pathology. A treatment strategy that in the past may have improved steatosis but possibly aggravated fibrosis would have needed complex study design and large numbers to power, as the measurement of both metrics was flawed. This partly explains why 90% of experimental drugs are not licensed because of concerns regarding hepatic metabolism, usually a non-specific elevation in liver enzymes. By measuring steatosis, iron and fibrosis simultaneously and safely, these studies are now tractable. The damage to an individual from hepatotoxic drugs, such as methotrexate and amiodarone, or alcohol, can now be better assessed. Critics may suggest that biopsy data are still essential for this, but the counterargument is that the sampling and observer renders this method ineffective. A reductionist approach to the largest internal organ is not logical.

8.6 Assessing liver disease – regional variation in tissue damage

Certain conditions are known to present with heterogenous damage across the liver, unlike isolated NASH. Primary sclerosing cholangitis (PSC) is an autoimmune liver disease that currently has no cure other than liver transplant. PSC is rare, but accounts for 8% of all orthotopic liver transplants.²⁹⁶ The condition was first described in 1867, and presents with obliterative inflammatory fibrosis of the biliary tree, affecting small, medium and large biliary ducts.^{297, 298} Patients can present in childhood or early adulthood – mean age at presentation in an Italian retrospective cohort was 35.²⁹⁹ Early non-specific symptoms of lethargy and right upper quadrant discomfort are followed by deepening cholestatic jaundice. Diagnosis is difficult to achieve – the patchy nature of PSC can lead to marked sampling error in percutaneous biopsy. Cholangiography, which became available in the 1970s, has markedly improved radiological assessment of the biliary tree. In one series, hepatic histology was diagnostic in only (36%), whereas cholangiography was diagnostic in 18/22 (82%).²⁹⁷ Intrahepatic ducts are difficult to visualise with cholangiography, and so small duct disease is hard to identify. This is a major problem clinically, because definitive early diagnosis of the disease is not possible. The increasing availability of MR cholangiopancreatography (MRCP) will hopefully address this, especially in young patients where diagnostic investigations with ionising radiation for non-specific symptoms and jaundice are often withheld until clinical disease progression has occurred. In a series of 46 patients followed over a median of 6 years by the Mayo clinic, 76% with asymptomatic PSC had progressive disease, and 24% had liver failure, resulting in death or liver transplantation.³⁰⁰ To date, the most striking examples of the heterogeneity of liver damage have been in explanted liver tissue from patients with primary sclerosing cholangitis.³⁰¹⁻³⁰³

Figure 8.4 shows transverse and coronal images of a 21 year old man who took part in the study described in Chapter 6. He had first presented to his general practitioner aged 17 with non-specific abdominal pain and lethargy. Two years later, he was found to have small gallstones, in itself a highly unusual finding in a lean young man, and had a laparoscopic cholecystectomy. He was then admitted with severe abdominal pain, jaundice and fatigue. His liver enzymes and full blood count were highly suggestive of a severe hepatitis – ALT 3930 IU/l, AST 3546 IU/l, bilirubin 88umol/l, alkaline phosphatase 461 IU/l, prothrombin time 16.8s and activated partial thromboplastin time 34.8s. His subsequent percutaneous biopsy of the right lobe showed severe inflammation (Figure 8.5). However, there was noticeable sparing of his left lobe on his MR imaging.

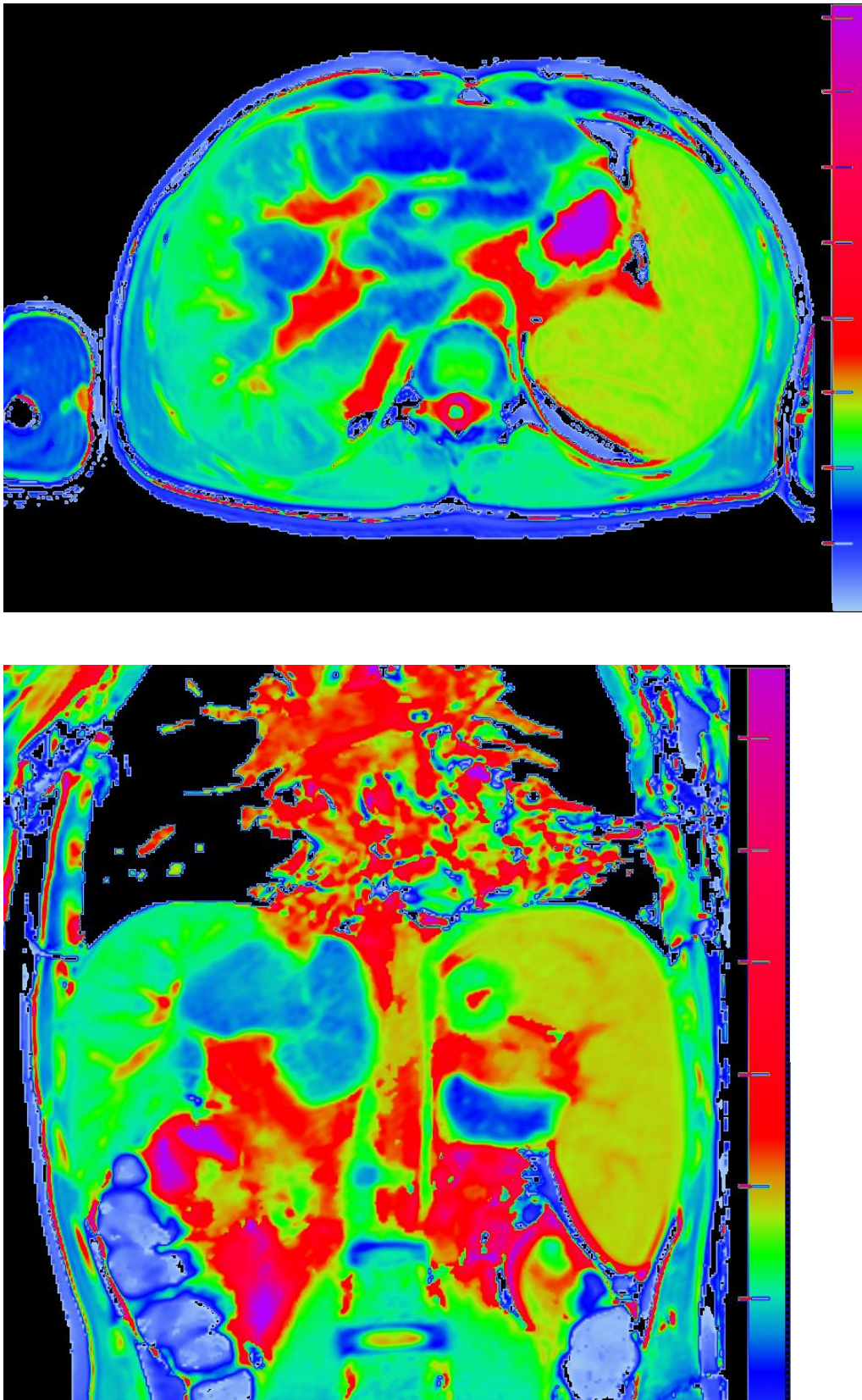


Figure 8.4. Non-invasive assessment of patchy liver disease. The transverse (above) and coronal (below) T1 maps show severe inflammation of the right lobe of the liver, with relative sparing of the left lobe. Also of note, the spleen is enlarged.

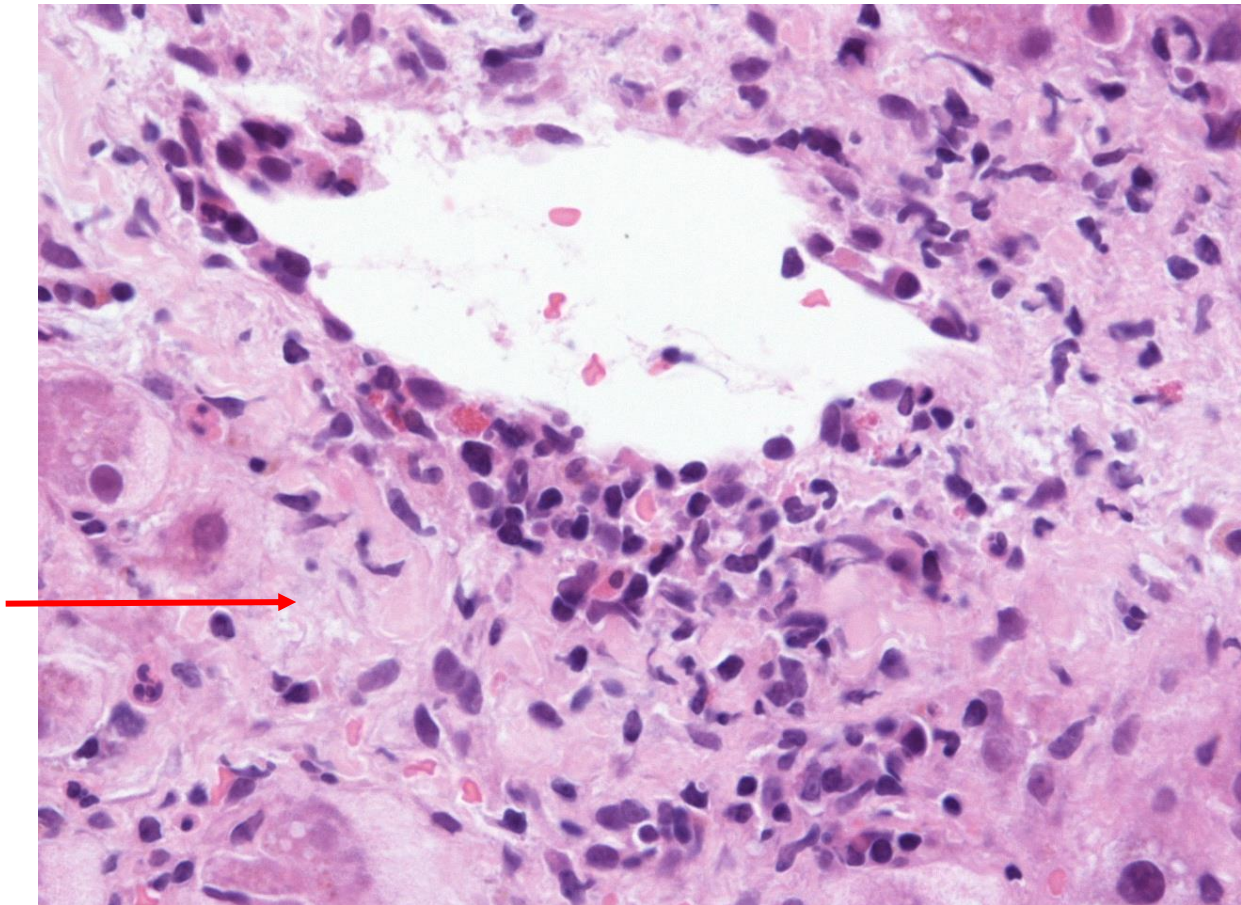


Figure 8.5. Histology of acute liver inflammation

This is a hematoxylin and eosin stained slide of the liver biopsy taken from the patient in Figure 8.4 from the right lobe, corresponding to an area of markedly high T1 (1064 ms). The patient had normal hepatic iron content, so no Fe-correction is required. There is considerable perivenular cellular dropout, with interstitial expansion (red arrow). This will become scar. Note also some multinucleate cells, and large number of plasma cells infiltrating liver parenchyma (pink nuclei), suggesting chronic inflammation. The consensus amongst three pathologists was that this biopsy showed severe inflammation and 6/6 fibrosis (although note that the Ishak score was not designed for acute disease or non-viral hepatitis).

The patient's clinical course is of great interest – with deepening jaundice and worsening coagulopathy, he was transferred to a quaternary centre for liver failure and put on the liver transplant waiting list. He slowly recovered, and was discharged 3 weeks later. One hypothesis is that he had severe biliary obstruction and cellular damage in his right lobe, resulting in the marked transaminase levels, but that his liver synthetic function was maintained from his left lobe. As he did not have cholangiography or a transjugular biopsy, this cannot be proved. Four months later, he had returned to full employment, and is no longer on the emergency transplant waiting list. He is on steroid therapy, but will almost certainly need liver transplantation in the next few years.

Multiparametric MR, in conjunction with MRCP, may well be the ideal technique for the diagnosis and monitoring of PSC – small duct disease should show on cT1 maps, as will hepatic fibrosis and acute inflammation. Conventional MRCP at 3 Tesla would give complementary data on the anatomy and duct size of the biliary tree. A small pilot study on patients with confirmed or suspected PSC would be appropriate to determine feasibility. If successful, this would naturally translate into a longitudinal cohort study to see if MR biomarkers predicted disease progression, and which patients were most at risk of needing transplantation within one year.

Moreover, we do not know if other liver conditions have this degree of regional heterogeneity. Just as cardiac imaging can predict prognosis by determining the burden of disease in the myocardium, it may be that the burden of hepatic disease, if quantified, may identify high-risk patients.

8.7 Childhood obesity and cardiometabolic risk

We do not yet have longterm epidemiological data on the effects of excess weight at a young age on atherosclerosis progression, partly because adolescent obesity is a relatively recent epidemic. Adolescent body mass index and waist circumference are known to predict adult obesity, type 2 diabetes mellitus, dyslipidaemia and MetS, and so it is logical that there will be more cardiovascular morbidity for obese children.^{253, 304, 305} We have shown that non-invasive tissue characterisation and strain assessment may be able to detect children with incipient NASH and at high cardiometabolic risk, defined by more positive circumferential strain, LV hypertrophy, hepatic and/or cardiac steatosis, insulin resistance and dyslipidaemia. If verified by further trials, the use of these biomarkers to determine those at greatest risk in adolescence may be of great importance in resource management and targeting future therapies to those children most likely to benefit. Importantly, and somewhat unexpectedly, it was no more difficult to study children than adults. The completion rate, even with a very detailed MR protocol requiring 90 minutes of scan time, was very high (19/22). Quantifying myocardial triglyceride content took the longest time, and thus for future studies this may not be the most useful metric to acquire first. Streamlining and standardising data acquisition and analysis will be essential to allow large, multicentre interventional trials on different MR systems, but this is not an impossible hurdle. Established therapies for dyslipidaemia and insulin resistance, such as fibrates and metformin, may be the most obvious choices as safe, accessible pharmacologic therapies for childhood obesity with high cardiometabolic risk. Newer therapies, such as GLP-1 agonists, may also be appropriate. Importantly, non-pharmacologic therapies can also be similarly evaluated, such as exercise regimes and family therapy. In the long run, this will enable proper meta-analysis of the most effective strategies to reduce the health burden of childhood obesity. In itself, 3 Tesla MR will not fix the

problem, but it can deliver better diagnosis and monitoring of adolescent obesity, and it is hoped that these new biomarkers may be useful surrogate endpoints for efficacy. This will help determine a concerted effort, backed by science and public policy, which is the only way forward to tackle the issue.

8.8 Conclusions

This thesis set out to determine the effects of excess body weight on the heart and liver, based on the premise that ectopic fat deposition had adverse consequences and associations. The studies here suggest that, with increasing body size, ectopic fat is deposited in the heart, as shown in Figure 8.6. In adults, gender had no impact. This establishes a clear reference range for further studies in which MTGC may be a study variable (lean adults had mean 0.38%, of water signal, with median 0.37% and IQR 0.24% - 0.47%). In obese adults, there was increased lipid deposition in a linear, predictable relationship, and also evidence of a 10% impairment of circumferential strain. The two are not obviously linked in a causal fashion – multivariable regression suggests that the impaired strain is due mostly to insulin resistance, rather than cardiac steatosis. The impaired strain is a significant finding, as it is evidence of contractile dysfunction due to a modifiable physical parameter, which can be corrected.

Obesity is associated with dramatic hepatic steatosis and evidence of liver oedema in the absence of any other risk factors, as shown in Figure 8.7. Many obese adults had increased AST, and some obese children had markedly elevated T1 values, pointing to active steatohepatitis. Novel MRI techniques suggest that it is possible to identify simple hepatic steatosis from steatohepatitis non-invasively and quantitatively – this will allow much earlier and better stratification of liver disease in the future. Patients with steatosis and inflammation have evidence of marked dyslipidamia (low HDL, high triglycerides), which may exacerbate weight gain and does accelerate atherosclerosis.

The ability to identify and quantify obesity-related heart and liver disease early offers promise in the future for the development of strategies to combat the obesity epidemic.

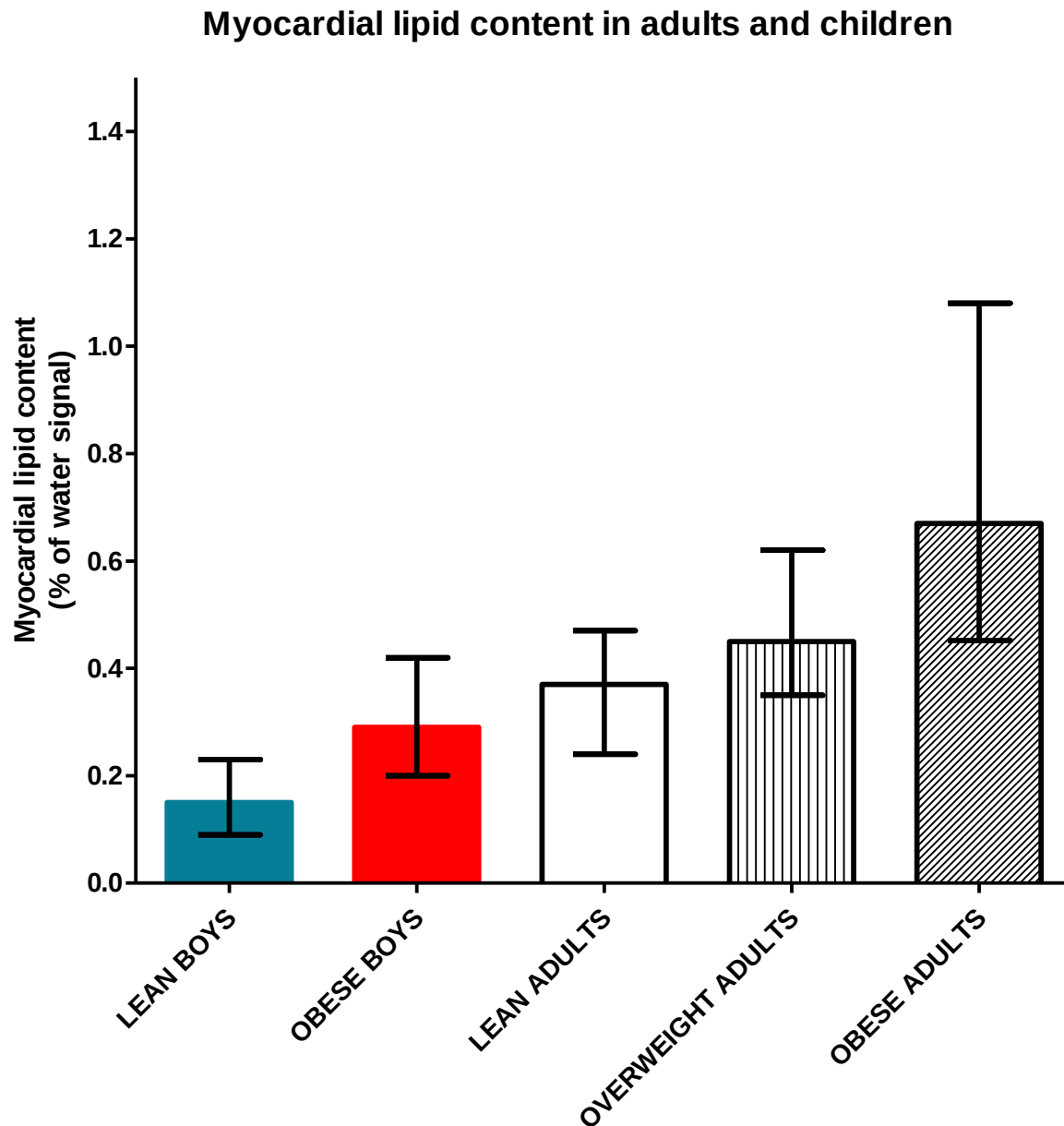


Figure 8.6. Comparison of MTGC in all adults (100) and children

Bars show median, whiskers denote interquartile range (adult data not normally distributed). Myocardial triglyceride content was lowest in lean boys, and highest in obese adults. Lean adults had significantly higher MTGC than lean boys – puberty may be the cause of this increase.

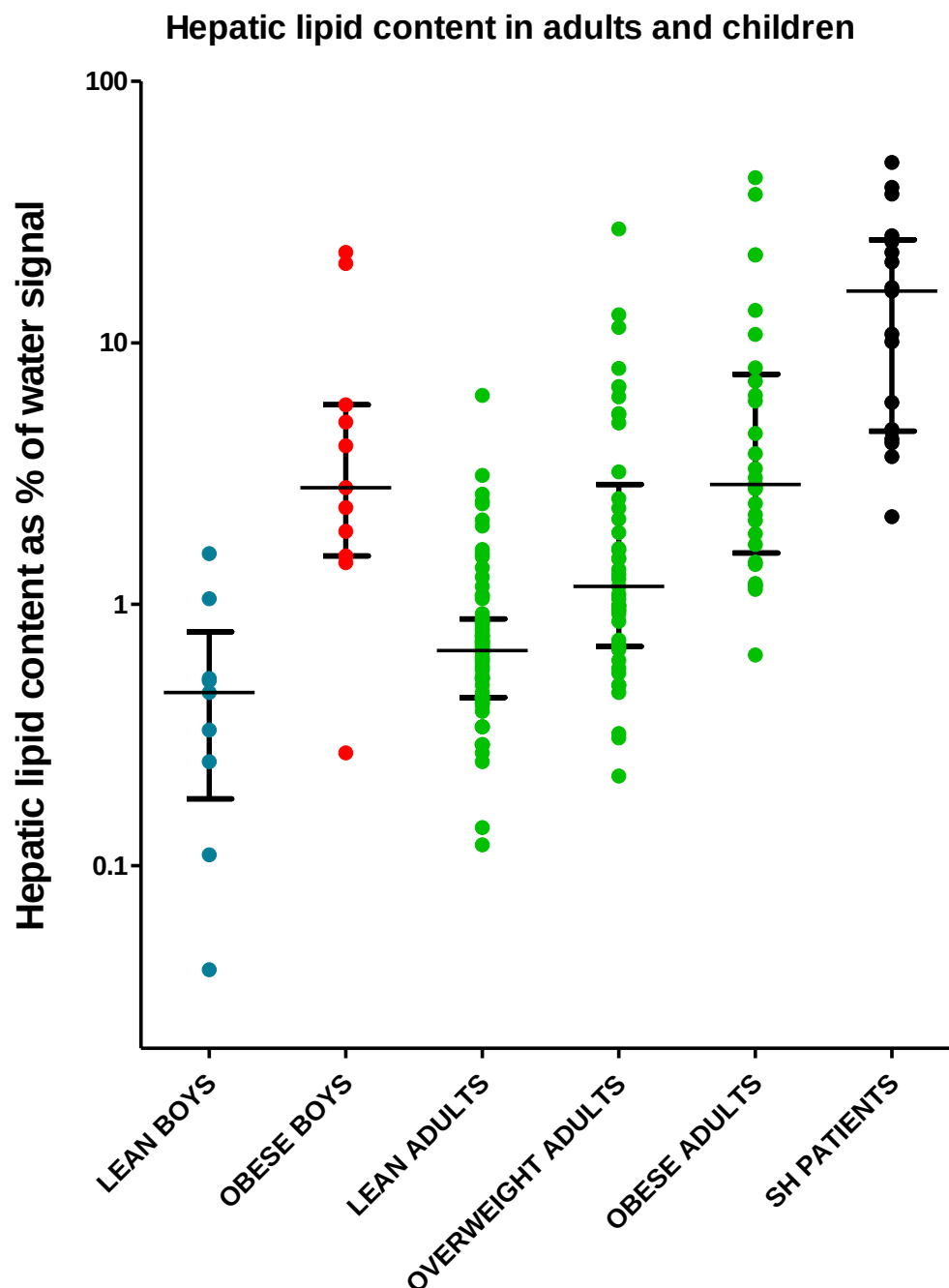


Figure 8.7. Comparison of hepatic steatosis in all adults (148) and children and 20 patients with biopsy-proven steatohepatitis

Median $\pm$ interquartile range are shown for each category, as data is not normally distributed. Obesity in adults and children is associated with marked hepatic steatosis. Hepatic lipid content is shown on a logarithmic scale. Of note, all the steatohepatitis patients (SH) had histologically significant steatosis, but there were many obese adults and children who had similar levels of steatosis.

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