Assessing Ectopic Fat Heterogeneity in Human Volunteers

A thesis submitted for the degree of
Doctor of Philosophy

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Declaration of Originality

The work or any other part thereof has not previously been presented in any form to the University for the purpose of assessment. The work is my own and any specific contributions or assistance are fully explained and appropriately referenced.

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He who finds a new path is a pathfinder, even if the trail has to be found again by others; and he who walks far ahead of his contemporaries is a leader, even though centuries pass before he is recognized as such.

Ibn Khaldun – 15th century historiographer & historian
ABSTRACT

Accumulation of ectopic fat in liver and pancreas are seen as key indicators of the metabolic syndrome affecting over a third of the population in the western world. Deposition of these ectopic fat depots is linked to insulin resistance, a major risk factor in the development of type 2 diabetes.

Quantifying ectopic fat deposition, without the use of biopsy, has always been a challenge. Magnetic resonance (MR) techniques have emerged as the gold-standard for estimating overall fat deposition. However, little is known on the regional variation of fat deposition in liver and pancreas in human subjects. The aim of this project was to determine, using an in vivo Multi-Echo MR imaging technique, regional variations in ectopic fat deposition in liver and pancreas and assess the potential impact of environmental factors, including BMI, age, and gender.

The overall ranges in liver and pancreas fat ranged from 0.69-32.27 % and 0.40-24.18% respectively. There was a strong correlation between liver fat, body weight, waist-to-hip ratio and waist circumferences. A significant correlation between pancreatic fat and waist-to-hip ratio, particularly in men, was also observed. The relationship between liver and pancreatic fat was surprisingly weak, suggesting different mechanism of accumulation.

Regional variations in liver and pancreatic fat were assessed by using surgical segmentations as anatomical markers, resulting in 8 anatomical segments for the liver and 3 for the pancreas.

Intra-abdominal adipose tissue and age had the largest effect on liver and pancreatic fat deposition.
Relative physical activity influenced the overall levels of liver ectopic fat content but did not significantly affect regional distribution in the pancreas. No gender differences in fat distribution in the liver were detected but a strong correlation with pancreas and men was detected.

Life-style interventions, such as weight loss programs, showed significant reduction in overall liver and pancreatic fat content. As for bariatric interventions, more significant reduction of overall ectopic fat in liver was seen with an increase in total and regional pancreas fat content.

In conclusion, I have shown, utilising an anatomical compartmentalisation of the liver and pancreas, that there are significant heterogeneity in fat deposition in the pancreas, and that different regions respond variably to lifestyle interventions. There is a more homogeneous fat deposition in the liver, which is more consistently modulated in response to lifestyle changes.
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Publications related to candidate

Abbreviations

¹H MRS Proton magnetic resonance spectroscopy

3D 3-dimensional

ALP Alkaline phosphatase

ALT Alanine Aminotransferase

ASAT Abdominal subcutaneous adipose tissue

AT Adipose tissue

ATP Adenosine triphosphate

BF% Body fat percentage

BMI Body Mass Index

cm centimetres

CoV Coefficient of variation

CoV Coefficient of variation

CT Computed Tomography

EUS Endoscopic ultrasound

FFA Free Fatty acid

FSE fast-spin-echo

FTO Fat mass and obesity-related gene

GCC Gulf Cooperation Council

GE Gradient-echo

GGT Gamma-glutamyl transferase

GL Glycaemic Load
GNPDA2 Glucosamine-6-phosphate deaminase 2
IAAT Intra-abdominal adipose tissue
IDEAL Iterative decomposition with echo asymmetry and least squares estimation
IHCL Intra-hepatocellular lipid
IMCL Intra-myocellular lipid
IPAQ International Physical Activity Questionnaire
IPCL Intra-pancreatic cellular lipid
IR Insulin resistance
KCTD15 Potassium channel tetramerisation domain containing 15
Kg Kilograms
L Litres
m metres
MC4R Melanocortin receptor 4
ME Multi-echo
MRI Magnetic resonance imaging
MRS Magnetic resonance spectroscopy
MTCH2 Mitochondrial carrier homolog 2
NAFLD Non-alcoholic fatty liver disease
NAIAT Non-abdominal internal adipose tissue
NASAT Non-abdominal subcutaneous adipose tissue
NASH Non-alcoholic steatohepatitis
NEGR1 Neuronal growth regulator 1
NF-κB Nuclear factor kappa light-chain enhancer of activated B cells
NHS National Health Service
PRESS Point-resolved-spectroscopy
SAAT Subcutaneous abdominal adipose tissue
SAT Subcutaneous adipose tissue
SH2B1 SH2B adapter protein 1
SNP Single nucleotide polymorphism
SSA Site Specific Assessment
T2DM Type 2 Diabetes
TAT Total adipose tissue
TE Echo time
TG Triglycerides
TIAT Internal adipose tissue
TMEM18 Transmembrane protein 18
TNF Tumor necrosis factor-alpha
TR Repetition time
TSAT Total subcutaneous adipose tissue
UAE United Arab Emirates
US Ultrasound
VLDL Very low density lipoprotein
WC Waist
WHO World health organisation
WHR Waist-to-hip ratio
WHtR Waist-to-height ratio
**Wt1** Wilms tumor protein

**ZDF** Zucker diabetic fatty

\( \beta \) Beta
Chapter I: Introduction

1.1 Obesity

The increased occurrence of obesity has focused attention on a worldwide crisis that is not one of famine or infection, but one of surplus. (Kahn, Hull et al. 2006) Obesity is defined as a multifactorial disease caused by abnormal or excessive adipose tissue accumulation. (Keys, Karvonen et al. 1972, WHO 2005, Shea, Diamandis et al. 2012)

Numerous reviews and academic books have been published on the causes, health consequences, and pathophysiological aspects of obesity. (Bray 2003, Haslam and James 2005, Kahn, Hull et al. 2006, Poirier, Giles et al. 2006, Van Gaal, Mertens et al. 2006) Unfortunately, the epidemic is increasing at an alarming rate and with it the occurrence of a number of comorbidities, including cardiovascular disease, type 2 diabetes and certain types of cancers. (Finucane, Stevens et al. 2011, Shea, Diamandis et al. 2012)

An individual's body weight and body composition are determined by interactions between the environment and genetics. (Loos 2012) The environment's influence on obesity relies on behaviours that increase the risk of positive energy balance, which in turn increases body mass in order to restore the energy balance. (Foreyt and Goodrick 1995, Hill and Peters 1998) Some individuals can avoid obesity in an unaccommodating environment by maintaining a pattern of healthy behaviours. (Klem, Wing et al. 1997, Hill and Peters 1998)
1.2 Obesity and Genetics

‘the genetic background loads the gun, but the environment pulls the trigger’. (Bray 2004)

Genetic makeup plays a part in regulating the strength of an individual's physiological resistance against gaining and maintaining body fat levels. (Sonnenberg, Krakower et al. 2004, Hebebrand, Bammann et al. 2010, Rhee, Phelan et al. 2012) There are a few theories that aim to explain this, such as the thrifty gene hypothesis, the fetal programming hypothesis, the sedentary lifestyle hypothesis and the ethnic shift hypothesis.(Walley, Asher et al. 2009, Heber 2010) All of these various theories offer a partial explanation regarding the impact of genes on body fat content, though none offer a full explanation.

Genetic contribution to obesity has been recognised through family, twin, and adoption studies. Twin studies have shown that genetic factors may be a factor of >40% of the variation in Body Mass Index (BMI) while lower heritability has been shown in families (>20%) and adoption (>20%) studies. (Stunkard, Foch et al. 1986, Stunkard, Sorensen et al. 1986, Turula, Kaprio et al. 1990, Hjelmborg, Fagnani et al. 2008)

The strongest obesity-related traits remains the association with variants within FTO (the fat-mass and obesity-related) gene.

FTO is expressed in many tissues, particularly in the hypothalamus and the liver. (Gerken, Girard et al. 2007, Fredriksson, Hagglund et al. 2008, Wang, Yang et al. 2011)
However, the molecular mechanism by which *FTO* variants influence obesity and thus lead to type 2 diabetes is still elusive.(Dina, Meyre *et al.* 2007, Frayling, Timpson *et al.* 2007, Scuteri, Sanna *et al.* 2007, Yeo and O'Rahilly 2012)

It has been recognised that obesity affects the endocrine function of adipose tissue by altering the secretion of adipokines, such as adiponectin and leptin, which have been related to ectopic fat accumulation, insulin sensitivity, and diabetes risk in epidemiological studies. (Soodini and Hamdy 2004, Perseghin, Lattuada *et al.* 2007)

Leptin is a multifunctional hormone produced mainly by the adipose tissue, and is involved in the regulation of food intake and energy homeostasis through its key actions.(Friedman and Halaas 1998) Although leptin receptors (LepR) are abundantly expressed in the brain, they are also present in peripheral tissues, indicating that leptin can exert peripheral actions.(Muoio and Dohm 2002, Paz-Filho, Mastronardi *et al.* 2012)

A more recent study from Bravard *et al.* (Bravard, Vial *et al.* 2014) have demonstrated a link between leptin receptor-signal transducers, activators of transcription 3 (LepR-STAT3) signalling pathway and the *FTO* gene, revealing a new role of *FTO* in the regulation of hepatic leptin action and glucose metabolism.

Other additional loci associated with obesity include *TMEM18, KCTD15, SH2B1, MTCH2, NERG1, GNPDA2* and *MC4R*. (Willer, Speliotes *et al.* 2009, Vimalaswaran and Loos 2010) Several reviews have assessed the implications of those loci to the development of human obesity.(Walley, Asher *et al.* 2009, McCarthy 2010, Vimalaswaran and Loos 2010)
Nevertheless, an acceptable consensus in the field is still lacking. It should be emphasized that whilst heritable factors are acknowledged in clarifying greatly the individual variation in risk of obesity, large genome-wide association studies identified so far <2% of common single nucleotide polymorphisms (SNPs) associated with BMI variability. (Bogardus 2009, Loos 2009, Hebebrand, Volckmar et al. 2010, Cheung and Mao 2012, Loos 2012)

1.3 Obesity Outbreak

The obesity outbreak seemed to begin almost simultaneously in most high-income countries in the 1970s and 1980s. (F Sassi 2009) Since then, coinciding growths in obesity in nearly all countries seem to be driven mainly by changes in the global food system.

This produces an unlimited supply of processed, highly palatable, energy-dense, affordable, and effectively marketed food. (Hill and Peters 1998, Swinburn, Sacks et al. 2011) These foods coupled with low levels of habitual physical activity have greatly contributed to the rise in global obesity.

1.3.1 Food System

The association between the global food system and shifts in obesity and related co-morbidities most notably diabetes, was first observed in high- and middle-income countries. (Popkin 2002)

This is now commonly seen in lower-income countries too, (Popkin and Slining 2013) although the relationship is not yet fully understood. (Monteiro, Moubarac et al. 2013) Perhaps this is
due to the underestimation of the full impact of industrialized food processing on dietary patterns and environments. (Monteiro, Moubarac et al. 2013)

Ever since the improvement of methods of preservation, such as smoking and fermentation, (Monteiro, Moubarac et al. 2013) the processing of food has enabled the evolution, adaptation and increase of humankind and of settled populations. (Ludwig 2011, Wrangham 2013) However, a more recent revolutionary development in the global food system and an acceleration in food science techniques can be dated as from the 1980s, (Monteiro, Moubarac et al. 2013) where transitional changes has been made from individual to mass provision thus “lowering the time price of food consumption”. (Cutler, Glaeser et al. 2003)

This ‘Food’ has been identified as ‘ultra-processed’; (Monteiro, Moubarac et al. 2013) It is ready-to-consume and is entirely or mostly made from industrial ingredients and additives such as sugar, fats, salt, and flavour enhancers, which makes it extremely profitable. (Godfray, Crute et al. 2010, Monteiro, Moubarac et al. 2013, Moodie, Stuckler et al. 2013) Food is now cheaper, hyper-palatable, processed and more readily available, than possibly at any time in human history. (Godfray, Crute et al. 2010, Monteiro and Cannon 2012, Stuckler, McKee et al. 2012, Monteiro, Moubarac et al. 2013, Moodie, Stuckler et al. 2013)

This increase in choice and abundance makes it difficult in reducing overconsumption. (Swinburn, Sacks et al. 2011) Additionally, marketing these processed food and beverages have been associated with increasing obesity rates. (Goris, Petersen et al. 2010) and is especially effective among children. (Gortmaker, Swinburn et al. 2012)
1.3.2 Active Living


The extent of the change in population levels of physical activity over time is largely driven by the role the built environment and sedentary behaviour play in decreased energy expenditure. (Day and Cardinal 2007, Gebel, Bauman et al. 2007, Sallis, Linton et al. 2009, Ding and Gebel 2012, Luke and Cooper 2013)

Data from cross-sectional studies and recent reviews have shown associations between environmental attributes, such as presence of sidewalks and proximity to parks in relation to outcomes such as physical activity and body mass. (McCormack, Giles-Corti et al. 2004, Kaczynski and Henderson 2008, Casagrande, Whitt-Glover et al. 2009) Furthermore, the existing environments within a country (the built environment, transport systems, active recreation opportunities, cuisines and food culture, and culture around body size) can have powerful effects and help to explain much of the differences in obesity prevalence between populations. (Swinburn, Sacks et al. 2011)
Undoubtedly, to consume a particular food or beverage or to exercise or not, is an individual decision. However, many of these decisions are automatic or subconscious. (Cohen 2008, Swinburn, Sacks et al. 2011) triggering a trend to consider influences on behaviour that are outside the person, such as the built environment.

In addition, the literature implies that sedentary behaviours or sitting may be associated with obesity independent of physical activity. (Sugiyama, Healy et al. 2008, Dunton, Berrigan et al. 2009, van Uffelen, Wong et al. 2010, Mozaffarian, Hao et al. 2011, Proper, Singh et al. 2011, Thorp, Owen et al. 2011) Working adults spend about one half of their workday sitting down. (Mummery, Schofield et al. 2005, Jans, Proper et al. 2007, Ki, Pouliou et al. 2011) Adults also spend hours sitting in their leisure-time (e.g. TV viewing, using a computer, transports etc.) (Salmon, Owen et al. 2003, Dunstan, Salmon et al. 2005, Proper, Cerin et al. 2007, Ki, Pouliou et al. 2011) This has fairly significant public health implications, even though some adults could meet physical activity recommendations, they may sit for extended periods each day in different settings which could affect their health. (Chau, van der Ploeg et al. 2012)

1.4 Obesity and Economic Effects

By 2008, 502 million adults were classified as obese, a number that is expected to double by 2015. (Finucane, Stevens et al. 2011, Shea, Diamandis et al. 2012) One estimate, predicted that in 2030 an expected 2.16 billion adults worldwide will be overweight and 1.12 billion will be obese. (Kastorini, Milionis et al. 2011)
Consequently, the financial burden of obesity on healthcare worldwide is estimated to be about 3% or more of total healthcare expenditures. Bearing in mind that medical costs for obese individuals are extensively higher than for non-obese.(Finkelstein, Fiebelkorn et al. 2004, Shea, Diamandis et al. 2012)

Analysis of the patterns of the obesity epidemic in the past four decades is limited by the absence of representative data from different countries.(Wang and Lobstein 2006) Nevertheless, there is an evidence for a non-linear, stepwise increase of the prevalence over time. (Prentice and Jebb 1995, Olds and Harten 2001, Keith, Redden et al. 2006, Rokholm, Baker et al. 2010)

In low-income and middle-income countries such as Brazil, China, India and Mexico; groups of high socioeconomic status in urban areas tend to be the first to have high obesity prevalence, but the burden of obesity shifts to low socioeconomic status groups and rural areas as a country's gross domestic product (GDP) increases.(Monteiro, Moura et al. 2004, Mendez, Monteiro et al. 2005)

In addition, Popkin et al. (Popkin and Slining 2013) and Razak et al. (Razak, Corsi et al. 2013) support a potential rising shift in the BMI level among those who are already overweight and obese.
In recent years, interventions such as increasing the price of unhealthy food and beverages, (Mytton, Gray et al. 2007, Brownell and Frieden 2009, Sacks, Veerman et al. 2011) or decreasing the price of healthy foods, (Ni Mhurchu, Blakely et al. 2010) have been considered. However there has been very little research in the effect and resolution of sociocultural determinants such as food choices, physical activity, and body-size perception. (McCabe and Ricciardelli 2001) These determinants ought to be a priority for studies examining the high prevalence of obesity in specific ethnic groups, (Ogden, Carroll et al. 2006) given that prevalence of adverse body fat patterns associated with adverse effects are higher in populations from Asia, Latin America, the Middle East and Africa. (Barba, Cavalli-Sforza et al. 2004, Tuan, Adair et al. 2009)

1.4.1 Obesity in the Gulf Regions

Since the discovery of oil in the Arabian Gulf region in the 1960s, the countries that make up the Gulf Cooperation Council (GCC) – United Arab Emirates (UAE), Bahrain, Kuwait, Qatar, Oman and Saudi Arabia have experienced continued growth in population, per capita income and wealth. (Ng, Zaghloul et al. 2011)

Along with the rapid socio-economic growth of the GCC and change in lifestyle, (Malik and Razig 2008) there have been rises in nutritional health problems and related diseases. (Ng, Zaghloul et al. 2011) This is often referred to as a ‘nutrition transition’, which was first
perceived in developed countries, but has swiftly spread to growing economies and developing nations in the past two decades. (Popkin 2006)

Recent estimates on the prevalence of obesity in the GCC states are astonishing; particularly in Kuwait, Qatar, Saudi Arabia and Bahrain where between two-thirds to three-quarters of adults and 25–40% of children and adolescents are overweight or obese. These levels in some cases, may be exceeding, that of some developed countries. (Popkin 2006, Mabry, Reeves et al. 2010, Ng, Zaghloul et al. 2011)

Furthermore, there appears to be a gender difference where obesity is more prevalent among women than men (Women in Saudi Arabia ~79.2% vs. Men in Saudi Arabia ~74.5%). (Al-Mahroos and Al-Roomi 2001, Al-Riyami and Afifi 2003, Bener, Al-Suwaidi et al. 2004, Ministry of Health 2005)

Saudi Arabia seems to be the leading country globally in terms of diabetes prevalence worldwide, (Kamran, Bener et al. 2007, Al-Lawati, Mabry et al. 2008, Mabry, Reeves et al. 2010) with the highest recorded expenditure on diabetes consuming 21% of the country’s total health expenditure, with no data available on the health expenditure of obesity. (Farag and Gaballa 2011, Al-Rubeaan, Youssef et al. 2013)
1.5 Obesity and Measurement

In clinical practice, obesity has been commonly measured by expressing body weight as a function of height, the so called BMI, calculated as weight in kilograms divided by height in meters squared. (Keys, Karvonen et al. 1972, Consultation 2000, Barba, Cavalli-Sforza et al. 2004) This has long been used as an index of obesity, since it was originally described by Quetelet in 1869. (Quetelet 1869)

Population studies have consistently reported a J-shaped relationship between the BMI and morbidity/mortality risk. (Yusuf, Hawken et al. 2004, Prospective Studies, Whitlock et al. 2009, Berrington de Gonzalez, Hartge et al. 2010)

At present, several organizations use categories of BMI to define underweight, normal weight, overweight, and various classes of obesity, (Consultation 2000, Barba, Cavalli-Sforza et al. 2004) although it must be noted that these classifications by BMI do not apply to trained athletes. (Bray 2005) (Figure 1.0) Its limitations however, such as not distinguishing fatness from muscularity, and not taking into account the site where fat is deposited on the body, have left health professionals limited by the fact that obesity is a fairly a heterogeneous condition. (Keys, Karvonen et al. 1972, Despres, Lemieux et al. 2001, Tchernof and Despres 2013)
Figure 1.0 WHO (Consultation 2000) Classification

Accordingly, there has been a tendency for some time to adopt a one-size-fits-all approach to the management of obesity; interest has focused on detrimental characteristics of excess adipose tissue only considering the primary functions of this tissue i.e. storage and release of metabolic fuel. (Raz, Eldor et al. 2005)

However, adipose tissue is not simply a storage reservoir of excess energy in the form of fat but an active endocrine organ that plays multiple roles in the body. The metabolic role of adipocytes changes as they enlarge with increasing obesity and also according to their location. Indeed, they can be associated with a low-grade state of inflammation as a consequence of the
secretion of pro-inflammatory cytokines leading to metabolic complications. (Calabro, Golia et al. 2009, McQuaid, Hodson et al. 2011)

While it is acknowledged that obesity is the result of a positive imbalance between energy intake and output, the pathophysiology underlying the failure of energy homoeostasis remains unclear. (Tan and Vidal-Puig 2008)

1.6 Adipose Tissue Distribution

Epidemiological and metabolic studies conducted over the past several years have re-highlighted the concept introduced in the mid-forties by French physician Dr Jean Vague, (Vague 1947) who pointed out that the disease-causing complications that have been traditionally associated with obesity were more directly related to where the excess fat was rather than to excess weight per se. (Vague 1947, Krotkiewski, Bjorntorp et al. 1983, Kissebah, Freedman et al. 1989, Terry, Stefanick et al. 1991, Despres, Lemieux et al. 2001, Tchernof and Despres 2013)

Since then, recent studies have found that a high proportion of adipose tissue located in the abdominal part of the human body or a male type (upper body) “android obesity” has a harmful effect on a variety of important metabolic factors relative to peripheral or premenopausal female type (lower body) or “gynoid obesity”. (Kissebah, Vydelingum et al. 1982, Lapidus,


1.6.1 Adipose Tissue Depots

Whole body adipose tissue has commonly been subdivided in two main components: subcutaneous and internal adipose tissue. (Tchernof and Despres 2013) (Figure 1.1)

Subcutaneous adipose tissue is usually defined as the layer found just below the skin (hypodermis) and some evidence even suggests it might be protective, particularly in the gluteofemoral area. (Ross, Leger et al. 1992, Thomas, Saeed et al. 1998, Shen, Wang et al. 2003, Snijder, Visser et al. 2005, Porter, Massaro et al. 2009, Bays, Fox et al. 2010, Tchkonia, Thomou et al. 2013)
The main areas for subcutaneous fat deposition are the gluteal/leg regions, back, and anterior abdominal wall. In normal weight adults, ~ 80% of total body fat is found in the subcutaneous region. (Wajchenberg 2000, Ibrahim 2010, Tchoukalova, Koutsari et al. 2010, Tchkonia, Thomou et al. 2013) (Figure 1.2)

Internal adipose tissue on the other hand, comprises ~ 10% of total body fat, (Abate, Garg et al. 1995) and can be divided into intra-abdominal and non-abdominal sites. Visceral or intra-abdominal fat is mainly composed of omental, mesenteric and retroperitoneal depots. Non-
abdominal fat includes cardiac and inter-muscular depots. (Wajchenberg 2000, Ibrahim 2010, Item and Konrad 2012, Thomas, Fitzpatrick et al. 2013) (Figure 1.2)

Figure 1.2 Schematic Diagram showing Adipose Tissue Depots taken from (Thomas, Fitzpatrick et al. 2013)

Within the upper body, Scarpa’s fascia separates superficial and deep abdominal subcutaneous fat. Deep abdominal subcutaneous fat accumulation is interconnected with visceral fat accumulation, (Kelley, Thaete et al. 2000) showing that it may well present morphological and functional characteristic similar to visceral adipose tissue. (Walker, Verti et al. 2007) Although, Jensen et al. (Jensen, Sarr et al. 2003) observed that deep and superficial abdominal
subcutaneous fat behaved quite similarly with respect to meal fat storage, implying that these subdivisions may not be that functionally diverse. (Ebbert and Jensen 2013)

Fat accumulation and distribution varies extensively between sexes, among individuals and families, between different ethnicities, with aging and several disease conditions, as well as side effects to drugs and hormones. (Borkan, Gerzof et al. 1982, Enzi, Gasparo et al. 1986, Forbes and Brown 1989, Bouchard 1997, Wajchenberg 2000, Thomas, Parkinson et al. 2012, Tchernof and Despres 2013, Tchkonia, Thomou et al. 2013)

In young- to middle-aged subjects the upper limit of a normal and healthy body fat per cent (BF%) is ~25–30% in males and 35–40% in females. (WHO 1995)

As for determining the presence of abdominal obesity, Waist (WC) and Waist/Hip (WHR) ratio are used as markers where WC is >102 cm for men and >88 cm for women (Adult Treatment Panel III criteria). (Logue, Smucker et al. 1995, Expert Panel on Detection and Treatment of High Blood Cholesterol in 2001) This has allowed scientists to show that extra accumulation of fat in the visceral region gives rise to metabolic complications. (Lapidus, Bengtsson et al. 1984, Wajchenberg 2000, Lakka, Lakka et al. 2002, Despres and Lemieux 2006, Alligier, Gabert et al. 2013, Tchernof and Despres 2013, Tchkonia, Thomou et al. 2013) Though the explanation for this association is not fully understood. (Ebbert and Jensen 2013)
Intra-abdominal adipose tissue seems to play a major role in the pathogenesis of insulin resistance, diabetes, dyslipidemia, inflammation, hypertension and cardiovascular diseases. Whereas the metabolic consequences of subcutaneous adipose tissue are less well-defined. (Goldstone, Thomas et al. 2001, Despres 2006, Kuk, Katzmarzyk et al. 2006) Even though it had been linked to features of the metabolic syndrome independently of intra-abdominal fat. (Goodpaster, Thaete et al. 1997)

Whilst the distinct importance of visceral fat depot versus subcutaneous fat depot dysfunction has been challenged. (Frayn 2000) There is mounting evidence to suggest that visceral adipocytes are phenotypically dissimilar from subcutaneous adipocytes. (Perrini, Laviola et al. 2008, Tchkonia, Thomou et al. 2013) A recent study from Chau et al. (Chau, Bandiera et al. 2014) used genetically modified mice to confirm just that.

The research showed that the majority of visceral fat depots in the body can be traced back to a single type of cell in the developing embryo. These ‘bad’ fat originator cells, express a gene called Wt1 and are not found in the subcutaneous fat. (Chau, Bandiera et al. 2014) The highest number of these cells were found in the fat depots around the heart and stomach, which are known to be the most common places that give rise to metabolic complications.

response to overfeeding is due to an increase in cell number, not size, the opposite is true for abdominal fat gain. (DiGirolamo, Fine et al. 1998) Visceral fat mass changes principally through adjustments in adipocyte size, rather than number, regardless of sex and degree of adiposity. (Tchernof and Despres 2013) As well as a distinction in the expression of adipogenic genes and the secretion of adipocytokines. (Motoshima, Wu et al. 2002, Vohl, Sladek et al. 2004, Tchenna, Giorgadze et al. 2006, Kim, van de Wall et al. 2007, Yamamoto, Gesta et al. 2010)

Moreover, portal and systemic lipid kinetics in human subjects differ between these fat regions (Nielsen, Guo et al. 2004), suggesting that the release of lipids from subcutaneous fat depots are drained systemically. This indicates a less harmful effect than visceral (omental and mesenteric) fat depots, where lipids and pro-inflammatory cytokines are mostly drained by the portal vein, which is then transported directly to the liver leading to the impediments of ectopic fat. (Bjorntorp 1990, Bjorntorp 1991, Klein 2004, Nielsen, Guo et al. 2004, Jensen 2008, Item and Konrad 2012)

1.7 Ectopic fat

Human physiology needs to be well adapted to manage major disruptions in both the supply of and demand for energy. Adipose tissue is an important “energy bank” and requires an adaptability to have ‘a clear capacity to utilize lipid and carbohydrate fuels and to transition between them’. (Kelley, He et al. 2002, Sanyal 2005) Such ability exemplifies a healthy state and can be termed ‘metabolic flexibility’.
Furthermore, most tissues have a small intracellular reserve of lipids as a source for vital ‘housekeeping’ functions such as preservation of membrane structure, fluidity and intracellular signalling. (Rasouli, Molavi et al. 2007)

However, when adipose tissue stores, whether peripheral or central, become saturated either due to failure to develop sufficient adipose tissue mass (lipodystrophy), or to expand these stores suitably to contain increased energy intake (metabolic inflexibility), (Storlien, Oakes et al. 2004, Raz, Eldor et al. 2005, Tan and Vidal-Puig 2008, Virtue and Vidal-Puig 2010, Snel, Jonker et al. 2012) lipids may ‘overflow’ into other non-adipose tissues such as in the liver, pancreas, skeletal muscle, heart and kidneys. (Heilbronn, Smith et al. 2004, McQuaid, Hodson et al. 2011, Cohen, Syme et al. 2013) The storage of lipid droplets in these organs is known as an ‘ectopic fat’ storage. (Snel, Jonker et al. 2012) (Figure 1.3)
Ectopic fat accumulation appears to have clinical consequences; ectopic fats seem to disrupt metabolic processes and impair organ function and are closely related to the development of insulin resistance, type 2 diabetes mellitus, cardiovascular diseases and the metabolic syndrome. (Unger 2003, Montani, Carroll et al. 2004, Rasouli, Molavi et al. 2007, Snel, Jonker et al. 2012, Cohen, Syme et al. 2013)
These local fat availability and depositions are likely to be influenced by different genetic and environmental factors such as increased energy intake and physical inactivity, and appear to have varying metabolic implications. (Bachmann, Dahl et al. 2001, Stannard, Thompson et al. 2002, Kabir, Catalano et al. 2005, Schrauwen-Hinderling, Kooi et al. 2005, Bergman, Kim et al. 2006, Cohen, Syme et al. 2013) (Figure 1.3)

The effects of ectopic fat accumulation is dependant on the specific organ. However, the mechanisms leading to the disruption of organ function are quite similar at the cellular level. (Snel, Jonker et al. 2012) Lipids can diffuse intercellularly or accumulate intracellularly. Intercellular lipid accumulation could impair organ function via paracrine effects of through the release of adipokines. Though, it is mostly intracellular lipid accumulation that is associated with decreased insulin sensitivity. (Snel, Jonker et al. 2012)

Free fatty acids are usually taken up by the cell via specific transport proteins in the cell membrane along with passive diffusional uptake. (Glatz, Bonen et al. 2002) Inside the cell, Long-chain fatty acyl-CoA goes through β-oxidation and further degradation through the tricarboxylic acid cycle in the mitochondria. This may lead to an outcome of continuous supply of free fatty acids (FFAs) (caused by enhanced lipolysis and adipocyte dysfunction) together with an impairment in FFA oxidation in the mitochondria, resulting to intracellular lipid accumulation.
Metabolites such as long-chain acyl-CoA and ceramides are the ones that appear to be toxic to cells and not the FFAs themselves. These fatty acid metabolites induce a sustained activation of serine/threonine kinases such as protein kinase C isoforms which phosphorylate insulin-receptor substrates on serine residues. (Morino, Petersen et al. 2006) The subsequent defects in insulin signaling lead to a decrease in cellular function that depends on the cell type. (Morino, Petersen et al. 2006, Szendroedi and Roden 2009)

Correspondingly, it has been observed that that peripheral subcutaneous depots appear to play the role of a metabolic sink, buffering lipids to limit their deposition in other organs as well as maintaining the homeostasis of daily lipid fluxes. (Frayn 2002, Tan and Vidal-Puig 2008, Porter, Massaro et al. 2009) While visceral depot is more established as being part of the complex phenotype of adipose tissue storage dysfunction and ectopic lipid accumulation in several sites especially in the liver. (Despres 2011)

1.7.1 Ectopic fat in the Liver

The ectopic fat deposition in the liver, also known as intra-hepatocellular lipids (IHCL), in the absence of excessive alcohol ingestion leads to a spectrum of changes in the liver associated histologically with fat accumulation within the cytoplasm of hepatocytes. (Hamer, Aguirre et al. 2006, Oliva, Mortele et al. 2006) Common patterns of fat infiltration in the liver include diffuse fat accumulation, diffuse fat accumulation with focal sparing, and focal fat accumulation in an otherwise normal liver. (Hamer, Aguirre et al. 2006)
This spectrum encompasses non-alcoholic hepatic steatosis, also known as non-alcoholic fatty liver disease (NAFLD), a clinical condition predisposing to cirrhosis, liver failure and hepatocellular carcinoma. (Powell, Cooksley et al. 1990, Teli, James et al. 1995, Angulo, Keach et al. 1999, Matteoni, Younossi et al. 1999, Angulo 2002, Bugianesi, Leone et al. 2002, McCullough 2002, Dam-Larsen, Franzmann et al. 2004, Oliva, Mortele et al. 2006, Vauthey, Pawlik et al. 2006) (Figure 1.4)

![The Spectrum of NAFLD](http://www.hku.hk/press/news_detail_8345.html)

**Figure 1.4** Spectrum of NAFLD taken from http://www.hku.hk/press/news_detail_8345.html

Fatty infiltration of the liver is not a new occurrence, like civilization itself, it manifested in the agricultural revolution of the Fertile Crescent of Mesopotamia, where there was enough food being produced and less physical work to go around. (Charlton 2007)
In antiquity, the practice of fattening the liver of animals through overeating of figs derived from ancient Egypt, where Egyptians have noted that the liver of migrating geese and ducks, owing to the experience of overfeeding and inactivity these animals had before starting the migration, was delicious to eat. (K.F. Kiple 2000, Leuschner, James et al. 2001, Riva, Riva et al. 2011) Thus, on the grounds that both are inevitable in requiring overfeeding and inactivity, the concrete similarities between the French delicatessen ‘foie gras’ and human NAFLD are self-evident. (Leuschner, James et al. 2001)

However, until the last few decades, NAFLD was considered to be a somewhat benign condition. (Zelman 1952, Westwater and Fainer 1958, Mehta, Thomas et al. 2008) In 1980, Ludwig and colleagues introduced the term “non-alcoholic steatohepatitis” (NASH) to describe these histological findings in patients who did not consume alcohol. (Ludwig 1980) The mechanisms leading to hepatic fat accumulation remain, to this day, multifactorial and poorly understood. (Adams and Angulo 2005, Sanyal 2005)

The liver synthesizes circulating free fatty acids (FFA)s which are derived from lipolysis of triglycerides within adipose tissue, diet or de novo lipogenesis into triglycerides (TG)s. (Sanyal 2005, Ibrahim, Kohli et al. 2011) Once seized by the liver, FFAs can either be oxidised in the mitochondria to form adenosine triphosphate (ATP), or esterified into neutral TGs for storage, or combined into very low density lipoprotein (VLDL) particles. (Angulo 2002, Adams, Angulo et al. 2005, Mehta, Thomas et al. 2008)
Triglycerides accumulate in the liver when their synthesis surpasses their export via VLDL. (Angulo 2002, Adams, Angulo et al. 2005) Therefore they may serve as a probable biomarker for the flux of FFAs delivered to the liver. The total volume of circulating FFA is a critical index of the severity of the disease and degree of hepatocyte lipopoptosis. (Nehra, Angulo et al. 2001, Feldstein, Canbay et al. 2003, Ibrahim, Kohli et al. 2011)


Incidences of NAFLD are underreported and are highly variable with only a minority of patients ~3% progressing to cirrhosis. (Vernon, Baranova et al. 2011, Schuppan and Schattenberg 2013) Although it has been stated to affect over 20% to 30% of the general population in the western world. (Mehta, Thomas et al. 2008)

Numerous findings have now confirmed that both excessive intra-abdominal and hepatic fat accumulation are critical contributors to the development of the metabolic syndrome and type

However, the abnormalities of insulin secretion and insulin resistance that trigger type 2 diabetes have a convincing hypothesis of a single, common aetiology, i.e. excess lipid accumulation in the liver and pancreas. (Taylor 2008, Lim, Hollingsworth et al. 2011)

### 1.7.2 Ectopic fat in the Pancreas

Less is known about the human pathophysiological consequences of lipid accumulation in the pancreas, also known as intra-pancreatic cellular lipids (IPCL) with regards to the metabolic syndrome. (R. Paul Robertson 2004, Rossi, Fantin et al. 2011, Sepe, Ohri et al. 2011) Deposition of fat inside and around pancreas has been shown to be linked with a higher risk of diabetes. (Lee, Hirose et al. 1994, Nolan, Madiraju et al. 2006) It has also been suggested that it follows a similar process to NASH and is termed non-alcoholic steato-pancreatitis (NASP) or fatty pancreas. (N. S. Patel 2013)

β-cells are usually present with other endocrine cells in the Islets of Langerhans which are scattered throughout the exocrine pancreas. (R. Paul Robertson 2004) It has been suggested that excess ectopic pancreatic fat accumulation deteriorates β-cell function, exocrine

Pancreatic fat is normally present several years before type 2 diabetes is diagnosed and has been proposed as a potential marker to identify individuals at risk. (Kahn, Hull et al. 2006, van Herpen and Schrauwen-Hinderling 2008, Lingvay, Esser et al. 2009).

This excess in accumulation may be achieved through a number of mechanisms including local free fatty acid release, triglyceride metabolite accumulation, oxidative stress, and release of pro-inflammatory factors and cytokine production, all of which stimulate β-cell injury. (R. Paul Robertson 2004)

In obese individuals, increased lipolysis contributes to high levels of circulating non esterified FAs, (McGarry 2002) subsequently, various mechanisms including the formation of reactive long-chain fatty acyl-CoAs and toxic metabolites, such as ceramide, may contribute to apoptosis and the decline of β-cell mass. (Shimabukuro, Zhou et al. 1998, Kharroubi, Ladriere et al. 2004, R. Paul Robertson 2004) Experimental and autopsy data have shown that fatty infiltration of the pancreas may add to a decrease in β-cell mass and function, by the local release of non esterified FAs and by adipocyte-derived proinflammatory and vasoactive factors. (R. Paul Robertson 2004, Newsholme, Keane et al. 2007)
However, the literature regarding the associations of pancreatic fat accumulation with β-cells function are scarce and less conclusive, with conflicting results, (Maarten E. Tushuizen 2007, Pinnick, Collins et al. 2008, Lingvay, Esser et al. 2009, Heni, Machann et al. 2010) leading to the fact that interaction of pancreatic fat with glucose regulation is somewhat complex. (N. S. Patel 2013)

In animal studies, adipocyte expansion and triglyceride accumulation increased in parallel in both exocrine and endocrine pancreatic regions. This leads to the eventual β-cell dysfunction, leading to lipoapoptosis and impaired insulin secretion as the animals develop pre-diabetes and diabetes. (Joseph L. Milburn 1995, Lingvay, Esser et al. 2009, Y Lee 2010)

Of late, studies have similarly demonstrated additional associations with fatty pancreas including hepatic steatosis, increased risk of pancreatic cancer, visceral fat accumulation and obesity. (Lee, Kim et al. 2009, Lingvay, Esser et al. 2009, Sijens, Edens et al. 2010)

1.7.3 Other ectopic fat stores

Whilst beyond the scope of this thesis there are other ectopic fat depots that need mention for the purpose of context. These other depot sites are the muscle, heart and kidneys.
1.7.3.i  Muscle

Accumulation of fat can be found stored within muscle cells – intra-myocellular lipids (IMCLs) and increased amounts of this ectopic depot have been linked to insulin resistance, type 2 diabetes and visceral adiposity. (Phillips, Caddy et al. 1996, Pan, Lillioja et al. 1997, Jacob, Machann et al. 1999, Snel, Jonker et al. 2012)

However, elevated IMCL has been reported in endurance-trained athletes, despite being highly insulin sensitive, an effect referred to as the ‘athlete’s paradox’. (Goodpaster, He et al. 2001, van Loon, Koopman et al. 2004)

IMCL is usually measured by skeletal muscle biopsies or using magnetic resonance spectroscopy (MRS). (Krssak, Falk Petersen et al. 1999, Perseghin, Scifo et al. 1999)

1.7.3.ii  Heart

Pericardial fat is the adipose tissue surrounding the heart. It consists of two layers: epicardial fat (i.e the true visceral fat depot of the heart) originating from mesothelial cells and surrounding the heart by 80% (Iacobellis, Corradi et al. 2005, Rabkin 2007, Sacks and Fain 2007, Snel, Jonker et al. 2012) and paracardial fat, originating from mesenchymal cells and comprising 20% of total heart weight. (Sacks and Fain 2007, Snel, Jonker et al. 2012)
Epicardial fat plays a dichotomous role, both unfavorable and protective and has been attributed to cardiovascular diseases. (Iacobellis and Barbaro 2008) This is due to its anatomic and functional proximity to the myocardium and the fact that it shares a common blood supply with the latter and encloses intense metabolic activity. (Iacobellis and Willens 2009, Ghosh 2014) Nevertheless, its physiology in animals and humans is not completely clear. (Iacobellis and Willens 2009)

1.7.3.iii Kidneys

Ectopic fat accumulation in the kidney especially around the renal sinus affects kidney function by compressing blood vessels as they exit the kidney, (Montani, Carroll et al. 2004) leading to hypertension and chronic renal disease. (Foster, Hwang et al. 2011, Ghosh 2014) Fatty kidneys have also been frequently seen in concurrence with abdominal obesity. (Ghosh 2014)

1.8 Body Composition Measurement

In order to fully understand the relationship between adiposity, ectopic fat and obesity related health risk, we must be able to accurately measure body fat content and distribution, as well as characterize regional fat distribution vis-à-vis ectopic fat without particularly relying on invasive histological and biochemical approaches. Consequently, there are two non-invasive methods to measure body fat content and ectopic fat: Indirect and Direct.
1.8.1 Indirect Methods

A number of technique have been developed to assess adiposity. (Bergman 2012) Indirect methods include: BMI, anthropometry, skinfold thickness, bioelectrical impedance, underwater weighing, and body water dilution. (Ayvaz, Kilinc et al. 2011, Bergman 2012, Thomas, Fitzpatrick et al. 2013)

While these methodologies do provide useful information, particularly at a population level, (Thomas, Fitzpatrick et al. 2013) they cannot be generalized among different ethnic groups. (Garrido-Chamorro, Sirvent-Belando et al. 2009, Rahman and Berenson 2010, Thomas, Fitzpatrick et al. 2013) and are often impossible to preform in individuals with extreme body types such as athletes or obese subjects. (Bergman 2012, Thomas, Fitzpatrick et al. 2013)

In addition, they give little information on different fat depots (van der Kooy and Seidell 1993, Pouliot, Despres et al. 1994, Kyle, Bosaeus et al. 2004, Shuster, Patlas et al. 2012) with no information concerning ectopic fat levels and distribution. (Thomas, Fitzpatrick et al. 2013)

1.8.2 Direct Methods

The fact that different fat depots and ectopic fats within the body appear to impact, to a varying extent, the risk of developing chronic diseases such as the metabolic syndrome, has made it clear that measuring total body fat content alone was not sufficient.
This lead to the development and implementation of new techniques that are continuously being perfected, and could accurately measure in-vivo adipose tissue content and distribution as well as fat depots in specific organs.

1.8.2. i Ultrasound

Ultrasound (US) is an important diagnostic tool that uses sound waves to create images of organs and structures inside the body. (Erikson, Fry et al. 1974) It is non-invasive, easy to use, safe, inexpensive and widely available. (Ricci, Longo et al. 1997, Strauss, Gavish et al. 2007, Roldan-Valadez, Favila et al. 2008)

It is also a suitable technique for estimating different adiposity depots. Although the time needed for a single measurement is very short, reproducibility and accuracy are poor. (Armellini, Zamboni et al. 1993, Suzuki, Watanabe et al. 1993) Moreover, care should be taken when interpreting ultrasound measurements as they are often subjective to the experience and abilities of the operator. (Mehta, Thomas et al. 2008, Shuster, Patlas et al. 2012)

Although a constant matter of debate, (Schwenzer, Springer et al. 2009) various studies have reported sensitivities and specificities for the detection of ectopic fat deposition with US, particularly in severe cases of fatty Liver. (Needleman, Kurtz et al. 1986, Joseph, Saverymuttu et al. 1991, Fishbein, Castro et al. 2005, Vernon, Baranova et al. 2011) It has been stated that
US had an acceptable level of sensitivity (60%–100%) (Angulo 2002, Saadeh, Younossi et al. 2002) but is is unable to provide precise determination and change of hepatic fat content. (Marchal, Verbeken et al. 1989, Mehta, Thomas et al. 2008, Sepe, Ohri et al. 2011)

Referring to a three point scoring system (‘mild’, ‘moderate’ and ‘severe steatosis’) based on hyperechogenic liver tissue, the increased discrepancy of echo amplitude between liver and kidney and the loss of echoes from the walls of the portal system, (Saverymuttu, Joseph et al. 1986, Hamaguchi, Kojima et al. 2007) grading of hepatic fat content using ultrasound, tend to be somewhat subjective.(Mehta, Thomas et al. 2008)

Ectopic fat in the pancreas, on the other hand, cannot be fully imaged with trans-abdominal US because of superimposing air in the stomach and small intestine (Sepe, Ohri et al. 2011), except when placing an endoscopic ultrasound (EUS) next to the pancreas parenchyma, where EUS can provide detailed image of fatty pancreas for analysis.(Al-Haddad, Khashab et al. 2009, Sepe, Ohri et al. 2011)

1.8.2. ii Computerized Tomography

Computed Tomography (CT) allows for the quantification and distribution of total adipose tissue content, as well as individual adipose tissue depots such as intra-abdominal and subcutaneous adipose tissue depot.(Borkan, Gerzof et al. 1982, Baumgartner, Heymsfield et
It can also be applied in inter-muscular depots, and other internal tissues and organs. (Baumgartner, Heymsfield et al. 1988, Kvist, Chowdhury et al. 1988, Gallagher, Kuznia et al. 2005, Thomas, Fitzpatrick et al. 2013) However the main drawback of CT scanning is the radiation dose it emits, which limits significantly its application in longitudinal studies and in paediatric populations. (Mehta, Thomas et al. 2008, Thomas, Fitzpatrick et al. 2013)

Contrast-unenhanced CT provides an accurate, reliable and more visible visualization of the whole liver in hepatic steatosis than contrast-enhanced CT. (Bydder, Kreeel et al. 1980, Park, Kim et al. 2006) Not only diffuse but also focal fatty infiltrations of the liver parenchyma can be observed. (Schwenzer, Springer et al. 2009) This is done by measuring the difference in liver and spleen attenuation values in Hounsfield units (HU). (Piekarski, Goldberg et al. 1980)

Normal healthy liver has an attenuation value of about 50–57 HU which is about 8–10 HU higher than the one of spleen. (Piekarski, Goldberg et al. 1980) Reduction in values of the liver which are less than 40 HU or 10 HU smaller than those of the spleen, can predict the occurrence of hepatic steatosis. (Hamer, Aguirre et al. 2005, Park, Kim et al. 2006) The image in the liver parenchyma appears darker than the spleen and even darker than the content of the hepatic vessels. (Schwenzer, Springer et al. 2009)
Lipid droplets in the pancreas are primarily in the interlobular septa. This results in a heterogeneous pattern on CT scans, making quantification of tissue density (fat) by using Hounsfield units unreliable and inaccurate. (Marks, Filly et al. 1980, Paivansalo 1984) Although, scans can be used to image the pancreas in its entirety.

1.8.2.iii Magnetic Resonance Imaging

Magnetic resonance imaging (MRI) is a medical imaging technique that uses strong magnetic fields and radiowaves to form images of the body. It allows measurement of fat and water proton signals and is considered one of the most accurate non-invasive technique for assessing adipose tissue content and distribution as well as assessing ectopic fat. (Siegelman and Rosen 2001, Cassidy, Yokoo et al. 2009) (Figure 1.5)

Image contrast in the MRI may be weighted to demonstrate different anatomical structures. This is measured by assessing how quickly tissues return to their equilibrium state after excitation by the independent processes of longitudinal T1 (spin-lattice) and transverse T2 (spin-spin) relaxation. (Haacke 1999) Signal intensities on T1 and T2 weighted images relate to specific tissue characteristics.
Figure 1.5 Methods in measuring Different Body Fat Content and Distribution

MRI has been validated against weighing of adipose tissue from human cadavers, with a reported difference of less than 5% (Abate, Burns et al. 1994)

The first studies utilising MRI to measure adipose tissue content and distribution in humans were published in the mid-1980s. (Foster, Hutchison et al. 1984, Kvist, Sjostrom et al. 1986, Seidell, Oosterlee et al. 1987, Baumgartner, Heymsfield et al. 1988) Since then, the technique has become the gold standard methodology for accurate and reproducible measurements of adipose tissue content and distribution in relation to health and disease. (Seidell, Bakker et al.)
Whole-body scanning provides a detailed quantitative map of adipose tissue content and distribution. However, this technique is very time-consuming, both in terms of MRI data acquisition (between 5 and 10 min) and image analysis (between 3 and 12 h) and is currently used by small groups world-wide. (Thomas, Fitzpatrick et al. 2013)

The standard protocol for full body scan, taken from the original publication by Ross et al. (Ross, Leger et al. 1992) involved the acquirement of a large dataset (>110 transverse slices) with small inter-slice gaps of 10 mm from head to toe, representing an excellent definition of the intra-abdominal fat depots and internal organs. (Thomas, Saeed et al. 1998, Machann, Thamer et al. 2005)

Moreover, whole-body imaging has other potential benefits; it can measure other tissues and determine organ volumes including skeletal muscle, liver, kidney, heart, pancreas and bone marrow. (Bosy-Westphal, Kossel et al. 2009, Lee, Kim et al. 2011, Watson, Pride et al. 2012)
Several alternatives to whole-body imaging have been suggested, either to overcome the technical short-comings of scanners and/or to shorten examination and analysis time while still giving full information on abdominal adiposity. (Thomas, Fitzpatrick et al. 2013)

CT has proven to be one technique that is widely available with lower cost and shorter examination time than MRI, its spatial resolution remains superior to the latter and is less susceptible to patient’s movements. (Earls 2000)

However, its ionizing radiation as stated previously limits significantly its application in longitudinal studies and in paediatric populations. Most significantly, studies have shown that patients with complicated disease and symptoms referable to hepatic, adrenal, and pancreatic disease may be best served by initial evaluation with MRI, which is more sensitive and specific in detecting and characterizing particular symptoms. (Earls 2000, Noone, Semelka et al. 2004, Mehta, Thomas et al. 2008, Thomas, Fitzpatrick et al. 2013)

US is another technique that is widely used for determining abdominal adiposity and may be the most cost-effective with a more common availability of the modality, level of optimization of the modality, staffing, and ease of patient handling. (Noone, Semelka et al. 2004) Although as mentioned previously reproducibility and accuracy are poor. (Armellini, Zamboni et al. 1993, Suzuki, Watanabe et al. 1993) Moreover, they are often subjective to the experience and abilities of the operator. (Mehta, Thomas et al. 2008, Shuster, Patlas et al. 2012)
Alternatively, this has led some groups to adopt a single-slice imaging technique with MRI for a compromise between accuracy and cost, where the anatomical landmark tends to be at the level of L4–L5. (Ross, Shaw et al. 1994, Abate, Garg et al. 1997, Shen, Wang et al. 2003, Thomas and Bell 2003, Kuk, Church et al. 2006, Kuk, Katzmarzyk et al. 2006, Maislin, Ahmed et al. 2012, So, Sasai et al. 2012) (Figure 1.5)

Similarly, acquisition of images from the abdominal region of the body, mainly visceral adipose tissue and hepatic ectopic fat, from multi-slice protocols has also been suggested. (Ross, Leger et al. 1992, Heymsfield, Wang et al. 1997, Thomas and Bell 2003, Gallagher, Kuznia et al. 2005, Yim, Heshka et al. 2007, So, Sasai et al. 2012)

Despite the fact that whole body MRI is the gold standard in terms of accuracy, in practice, multiple-slice abdominal MRI is the favoured option in large population studies such as biobanks compared to whole body or single-slice MRI. (Shuster, Patlas et al. 2012, Thomas, Fitzpatrick et al. 2013)

Numerous studies have used MRI to measure adipose tissue, demonstrating differences in both content and distribution in addition to regional differences within the depots as an effect of age, BMI, gender, and ethnicity. (Misra, Garg et al. 1997, Thomas, Saeed et al. 1998, Toth, Tchernof et al. 2000, Smith, Lovejoy et al. 2001, Miyazaki, Mahankali et al. 2002, Ross, Aru et al. 2002, Shen, Punyanitya et al. 2004, Staiano and Katzmarzyk 2012, Thomas, Frost et al. 2012) As well as being used extensively to map unusual distributions of adipose tissue in various

MRI has also been employed in interventional studies, quantifying the impact of diet and exercise (Ross and Janssen 1999, Christiansen, Paulsen et al. 2009, Viljanen, Lautamaki et al. 2009, Ismail, Keating et al. 2012) as well the effect of various drugs (Marks, Moore et al. 1996, Miyazaki, Mahankali et al. 2002, Virtanen, Hallsten et al. 2003) and obesity surgery on adipose tissue content and distribution.(Johansson, Roos et al. 2008)

1.8.2.iv Proton Magnetic Resonance Spectroscopy (\(^1\)H MRS)

Proton magnetic resonance spectroscopy (\(^1\)H MRS) allows direct measurement of lipid content in different tissues. (Siegelman and Rosen 2001, Mehta, Thomas et al. 2008) It is a fast, safe, non-invasive method that has become an essential tool to accurately and reproducibly quantify metabolic data and is routinely used to detect fatty infiltration in skeletal muscle and liver. (Schick, Eismann et al. 1993, Boesch, Slotboom et al. 1997, Szczepaniak, Babcock et al. 1999, Szczepaniak, Nurenberg et al. 2005, Thomas, Hamilton et al. 2005, Thomas, Brynes et al. 2006, Mehta, Thomas et al. 2008, Thomas, Fitzpatrick et al. 2013)

It is also used to measure both cardiac (Szczepaniak, Dobbins et al. 2003) and pancreatic (Lingvay, Esser et al. 2009) fatty infiltrations. However, measurement of ectopic fat
In these organs is more technically arduous, due to motion when it comes to the heart, and size and location when it comes to the pancreas, leading to a predisposed contamination from surrounding adipose tissue signals. (Cohen, Syme et al. 2013, Thomas, Fitzpatrick et al. 2013)

In most studies, the frequently utilised localised technique is the point-resolved-spectroscopy sequence (PRESS) developed in the early 1980s, (Bottomley 1987) stimulating protons within the volume of interest with minimal stimulation outside of this volume.

An alternative sequence used, mainly in the liver, is Chemical Shift Imaging (CSI), which is a multi voxel technique that allows metabolite information to be measured in larger volumes of tissue that can be divided into smaller voxels of spectra asquisition covering the whole sample during the processing period. (Pykett and Rosen 1983, Brateman 1986) This allows spectra from across a 2D or 3D plane to be obtained in a single acquisition.(Thomas, Fitzpatrick et al. 2013)

1.8.2.v MRI-based methods for IHCL & IPCL

Recently, there have been an increasing number of publications, describing new MRI-based techniques that have emerged as useful modalities and allow for very high resolution assessment of regional variations of fat content. (Fishbein, Castro et al. 2005, O'Regan, Callaghan et al. 2008, Cassidy, Yokoo et al. 2009, Lee, Park et al. 2010, Hu, Nayak et al. 2011, Patel, Abeysekera et al. 2011, Reeder, Cruite et al. 2011, Thomas, Fitzpatrick et al. 2013)
These MRI-based techniques, mainly for evaluating ectopic fat, fall into two categories: those that are based on differences in the signal phase of fat and water, and those that utilize the chemical shift differences between fat and water resonances and isolate these two components. Thus, giving a quantitative measure of the signal fraction of both resonances per se. (Thomas, Fitzpatrick et al. 2013)

Those that are based on differences in the signal phase of fat and water are founded on the “DIXON” sequence. (Dixon 1984) The technique acquires two separate images with a modified spin echo pulse sequence. One is a conventional spin echo image with water and fat signals in-phase and the other is acquired with the readout gradient slightly shifted so that the water and fat signals are 180° out-of-phase.

The availability of both the water-only and fat-only images allows direct image-based water and fat quantitation which is now commonly used to incorporate all chemical-shift based sequences to attain separate water and fat images. (Thomas, Fitzpatrick et al. 2013)

A fine-tuning of the DIXON technique is the Iterative Decomposition with Echo Asymmetry and Least squares estimation (3D IDEAL) imaging and reconstruction method. (Reeder, Pineda et al. 2005) This technique combines asymmetrically acquired echoes with an iterative least-squares decomposition algorithm to maximize noise performance and be independent of the proportion of water and fat. (Reeder, Pineda et al. 2005) The advantage of this technique is that
the data is acquired as a volume, and is therefore a consistent and reproducible quantitative indicator of fat content in tissues. (Bydder, Yokoo et al. 2008) It has also been reported to have an improved outcome of fat-to-water ratios, by correcting the limiting factors for transverse relaxation (T1 bias and T (2)* decay) and overcoming field inhomogeneity difficulties. (Reeder, Cruite et al. 2011, N. S. Patel 2013, Thomas, Fitzpatrick et al. 2013)

More recently, the development and application of multi-echo (ME) MRI sequence has proven to be the most accurate in measuring ectopic fat. (O'Regan, Callaghan et al. 2008) ME MRI uses a series of echoes acquired as a train following after a single excitation pulse. Multiple symmetrical or asymmetrical echoes can be acquired, typically T2 weighted.

From the various images obtained with the ME sequence, decay curves are generated which present the change in signal intensity at each of the acquired echo times. This gives rise to oscillations in the decay curve depending on how high or low fat infiltration is enclosed in an organ. (Thomas, Fitzpatrick et al. 2013) From these data, ‘heat-maps’ can be made to visualise regional differences in fat deposition.

The ME-MRI has been regularly used in the liver and pancreas. (Figure 1.5) It has the advantage that regional differences in ectopic fat distribution can be measured. Furthermore, it is often possible to obtain a single slice containing the liver and pancreas, thus reducing
scanning time and allowing coinciding measurement of fat within these two separate organs. (Thomas, Fitzpatrick et al. 2013)

1.9 Factors that modulate fat distribution

Regulation of energy homeostasis is a complex process, and that fact conveys a significant challenge in trying to explain the pathogenesis of obesity. (Shea, Diamandis et al. 2012)

The intra-uterine environment and early post-natal life, genetic predisposition, environmental and behavioural factors certainly play a large role and are key determinants to the response of adipose tissue to excess energy intake over energy expenditure (Barker, Gluckman et al. 1993, Shea, Diamandis et al. 2012, Snel, Jonker et al. 2012)

1.9.1 Diet

Diets containing a great quantity of low-energy-dense foods and minimal amounts of high-energy-dense foods should hypothetically prevent obesity. (Baranowski, Cerin et al. 2009)

It has been known that the quality of a diet affects the specific location of the fat that is deposited. (Goss, Goree et al. 2013) Some studies have indicated that a relatively greater consumption of low glycaemic load (GL) diet is associated with a smaller waist
circumference. (Halkjaer, Sorensen et al. 2004) and induces reduction in intra-abdominal adipose tissue independent of weight change. (Goss, Goree et al. 2013)

Moreover, a number of cross-sectional studies have shown associations between dietary fatty acid composition and fat distribution patterns and accumulation. (Garaulet, Hernandez-Morante et al. 2006, Kishino, Watanabe et al. 2008, Tchernof and Despres 2013) However, research has not consistently supported this relationship. (Rampersaud, Pereira et al. 2005, Ledoux, Hingle et al. 2011) This may be due to failure in controlling for potential confounding variables such as physical activity. (Ledoux, Watson et al. 2011)

Short-term low energy dense diets can also mobilize ectopic fat stores in all organs. (Snel, Jonker et al. 2012), concluding that ectopic and intra-abdominal fat are very responsive to weight loss. A loss of 10% of the initial body weight in an obese person for example, can reduce visceral fat by ~30% (Borel AL 2012) and ~50% of ectopic fat in the liver. (Vitola, Deivanayagam et al. 2009)

Conversely, with high fat feeding, ectopic fat content in humans, especially IHCL, is easily modified, followed by visceral fat. (Bachmann, Dahl et al. 2001, Snel, Jonker et al. 2012)
1.9.2 Exercise

The health benefits of engaging in regular physical activity have been well established. (Morris, Everitt et al. 1980, Blair, Kohl et al. 1989) It has been regularly recommended as a method of improving weight management for its renowned ability to positively impact metabolic (Ryan 2005) and psychological health. (King, Hopkins et al. 2009, Messier, Rabasa-Lhorett et al. 2010)

Current physical activity recommendations suggest that ~250 min of weekly aerobic-type exercise is required for body weight management. (Donnelly, Hill et al. 2003, Haskell, Lee et al. 2007) Although, weight loss is often much less than anticipated, when exercise is used as the sole mean of intervention. (Miller, Koceja et al. 1997) The actual reduction of weight and body fat with the recommended amount of physical activity in overweight and obese individuals is often small but increases with exercise levels. (Franz, Vanwormer et al. 2007, Donnelly, Blair et al. 2009) However, there is an acknowledgement that even with intensive programmes, weight loss in excess of 3–4 kg is challenging to maintain. (Franz, Vanwormer et al. 2007)

In fact, diet-induced weight loss seemed to be more beneficial particularly in terms of ectopic fat depots and is associated with an improvement of the function of that organ. (Drenick, Simmons et al. 1970, Bugianesi, Marzocchi et al. 2004, Snel, Jonker et al. 2012, Schuppan and Schattenberg 2013)
Some studies reported that, in the short term, IHCL content is similarly reduced after diet only or exercise plus diet interventions. (Tamura, Tanaka et al. 2005, Albu, Heilbronn et al. 2010, Lazo, Solga et al. 2010)

The type of exercise also plays an important role; Johnson et al.(Johnson, Sachinwalla et al. 2009) has shown that aerobic exercise training programmes of lower energy expenditure than the recommended guidelines can induce a significant reduction in visceral and hepatic fat, even in the absence of weight loss. Conversely, resistance exercise training is known to positively affect insulin sensitivity and other developments associated with visceral fat accumulation. (Albright, Franz et al. 2000, Ismail, Keating et al. 2012)

1.9.3 Early Life programing

The intrauterine environment and early postnatal life are now recognized as key determinants of disease risk later in life. (Barker, Gluckman et al. 1993) The exposure to an unfavourable environment such as a sedentary lifestyle during post-natal life is hypothesized to results in compensatory developmental changes that become permanent.

Preterm infants have an altered body composition, demonstrating decreased lean body mass and significantly larger fat deposition in deep subcutaneous and internal abdominal compartments as well as a significantly greater ectopic fat deposition when compared to their
full-term born counterparts. (Uthaya, Thomas et al. 2005) Interestingly these differences appear to persist into adulthood with premature adults having increased visceral fat, liver fat, and muscle fat compared with control adults born at term. (Thomas, Al Saud et al. 2012)

In addition, low birth weight (LBW) arising from foetal growth restriction or preterm birth, is associated to an elevated risk of the metabolic syndrome. (Hales, Barker et al. 1991, Barker 1997) with an association with insulin resistance and Type 2 diabetes later in life.(Hales, Barker et al. 1991, Newsome, Shiell et al. 2003).

Over the last few years an increasing number of extremely preterm babies survived to adulthood, abnormalities related to insulin sensitivity, lipid metabolism, blood pressure and fat distribution appear to be recurring in these individuals. (Thomas, Al Saud et al. 2012) Moreover, unfavourable outcomes associated with preterm birth may extend to the next generation, a study by Mathai et al.(Mathai, Derraik et al. 2013) observed a similar increase in abdominal adiposity in children born at term of parents born preterm.
1.5 Hypothesis

Ectopic fat distribution in the liver and pancreas is heterogeneous and may be determined by physiological factors that predicts progressive liver disease and diabetes.

1.6 Aims of the thesis

Aims of the thesis are

- To evaluate Multi-echo (ME) imaging based techniques in measuring fat content and distribution in the liver and pancreas
- To determine variation in fat content and distribution in the liver and pancreas in lifestyle interventional studies using Multi-echo (ME) imaging based techniques
Chapter II: General Research Methods and Procedures

This chapter describes the experimental details of all the techniques and measurements used for each investigation in this Thesis.

**Personal contribution to research presented in thesis**

Owing to the fact that there have been collaborative projects published from aspects of the data included in this thesis, it is necessary to outline my personal involvement and contribution to the work presented.

During my time as a Phd student I was directly involved in writing the complete ethics of the overfeeding study (11/LO/1097) (Appendix 1), as well as being present in the scanning procedures of all its participants and performing some of their anthropometric measurements.

I was not permitted to do the MRI analysis as data analysis was outsourced for reliability (see details in this chapter), but I was mentored by Dr Louise Thomas and radiographer
Julie A. Fitzpatrick to inform my work and understanding.

While MR spectroscopy required one trained analyst (for reliability) I performed all the Multi-echo analysis in this thesis.

During the course of the research, I personally recruited all the participants included in The overfeeding study (11/LO/1097) using emails, newspaper articles, flyers and posters. The remaining subjects from the cohort were from other studies in the group, to which I was granted access due to my contribution to performing all the ME data analysis.

2.1 Ethics

Ethical approval for this study was obtained from NRES Committee London - Queen Square (07/Q0411/19, 11/LO/1097, 12/LO/0139 and 10/H0711/91) (Appendix 1). The study was submitted for Site Specific Assessment (SSA) at each participating NHS Trust and was conducted in accordance with the recommendations for physicians involved in research on human subjects adopted by the 18th World Medical Assembly, Helsinki 1964 and later revisions.
All volunteers gave informed written consent for the study. Informed consent was obtained as follows: The study was discussed with each volunteer during a telephone screening checklist explaining the study procedures in detail, the potential benefits of participation, any potential risks and the rationale of the study itself.

After the call, the individual was sent a participant information sheet (Appendix 1) and was given the opportunity to ask questions about the study. If the volunteer wants to take part in the study, they are given a participant consent form during a formal screening visit. (Appendix 1) Any objection to participating was accepted, and the volunteers did not need to provide a reason for a refusal to take part. The volunteers were assured that they were not obliged to enrol in the study and it was also made clear that they could withdraw from the study at any time without giving a reason and without penalty.

2.2 Participants

Inclusion criteria required that participants be ambulatory with no medical conditions that could potentially affect any of the variables under investigation.

Participants were imaged at the Robert Steiner Magnetic Resonance Imaging (MRI) Unit, Hammersmith Hospital (London) according to a well-defined and well established protocol.
This MRI protocol has been used for over a decade to scan and determine body fat content and distribution. It also included a clinical health questionnaire and anthropometric measurements prior to the MR scan. (Appendix 1) All participants completed detailed screening to ensure suitability for inclusion. (Appendix 1)

2.2.1. Recruitment

All participants were recruited via public advertisements, email mailing lists and Imperial College Healthcare NHS Trust websites as well as hospital clinics for the bariatric surgery intervention and advertisement in newspapers and any existing research volunteer databases.

2.3. Assessment protocol

All participants were asked to attend the MRI Unit on the morning of their booked assessment and were asked to be fasted from 11pm the night before.

A complete check of medical history and general information documents were sent to all volunteers in advance before their first visit as well as witnessing the signed consent and metal check documents (Appendix 1). Participants were then asked to change into hospital issue
theatre garments (scrubs) which was provided for them, retaining only their underwear. All jewellery/metal objects were to be removed.

Baseline anthropometric measures were then performed. The MRI scan pre-check was completed to ensure that no metal was being taken into the magnet area. This included a verbal confirmation from the participant that their signed metal check was accurate.

### 2.4. Anthropometric measurements

Body mass (kg), height (cm), waist circumference (WC) (cm) and hip circumference (cm) were measured in each participant. Body mass was measured by means of a Seca scale (Vogel & Halke Hamburg, Germany), while height was recorded using a stadiometer (Invicta Plastics Ltd., Leicester, UK).

From these values, BMI (kg/m$^2$), waist-to-hip ratio (WHR) and waist-to-height ratio (WHtR) were calculated. To ensure accuracy and reliability of anthropometric assessment, all measurements used established, standardised protocols.
2.5 Whole Body Magnetic Resonance Imaging (MRI) Fat scan

On a single visit, participants underwent total body MRI scanning, \textit{in vivo} proton (\textsuperscript{1}H) magnetic resonance spectroscopy (MRS) and multi-echo MRI of their liver and pancreas.

2.5.1 Calibration

The MR equipment was calibrated each day according to manufacture guideline using a standardized phantom to detect drifts in measurements. Additionally, equipment servicing and upgrading was performed regularly.

2.5.2 Participant positioning and safety

Participants were instructed to lay still in a prone position with arms straight above the head (to minimise respiratory motion and to ensure that large subjects would fit in the bore of the magnet).
Sandbags and foam pads were used to support / brace limbs and ease muscle tension or unnecessary discomfort and ear defenders were worn at all times during scanning.

Participants were then scanned to up to 1 hour from their fingertips to their toes. Participants had constantly access to a buzzer to sound an alarm, and were able to hear and respond to instructions from the scanning console. The MR scanning environment is shown in (Figure 2.0).

Figure 2.0 Phillips Achieva 1.5T MRI Scanner at the Hammersmith Hospital, London used for all MRI/MRS/ME measurements.
2.6 MRI assessment of adiposity

2.6.1. MRI of Total Body and Regional Adipose tissue

Whole-body MRI was performed to determine a detailed quantitative map of adipose tissue content and distribution using a 1.5T multinuclear scanner (Phillips Achieva-TM, Philips Medical Systems, Best, Netherlands).

The images were acquired with a whole body axial T1-weighted fast-spin-echo (FSE) sequence using the Q-body coil, without respiratory gating. Imaging parameters: repetition time (TR) 560ms; echo time (TE) 18ms; slice thickness 10mm; inter-slice gap 10mm; flip angle 90 degrees; number of excitations 1. Transverse images were then acquired as nine equal stacks of 12 or 13 slices at the iso-centre of the magnet to avoid image distortion. Acquisition of images took between 5 and 10 min, depending on the length of the volunteer (Figure 2.1).
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2.6.2 MRI Adipose Tissue Quantification (Data Analysis Methods)

MR whole-body images were analysed using the commercially available software slice-O-matic™ (Tomovision, Montreal, Canada) that employs a threshold based system that relies on the image intensity differences between adipose and lean tissues. (Figure 2.2) From the
acquired images (120-140 images/subject) the area of adipose tissue depots in (cm²) was calculated as the product of pixel number and pixel area. The adipose tissue volumes (cm³) of each compartment were calculated by multiplying the adipose tissue depot area by the sum of the slice thickness (10 mm) and slice gap (10mm).

**Figure 2.2** a) T1-weighted Whole Body Coronal Image, for visual purposes this image has been segmented into (b) single slice black and white scale image initially processed to segment the subcutaneous and internal fat (c) these were labelled with specific colour codes for each depot: subcutaneous (green) and internal (red) fat.

Total and regional adipose tissue volumes were recorded in litres (l); comprising total adipose tissue (TAT), total subcutaneous adipose tissue (TSAT) and internal adipose tissue
These depots were then further refined to enable measurement of abdominal adipose tissue depots. (see Table 2.0)

Table 2.0 Classification of adipose tissue depots.

Thus, in summary:

Total Adipose tissue (TAT) = Subcutaneous AT + internal AT

Total Subcutaneous AT (TSAT) = Abdominal subcutaneous AT (ASAT) + non-abdominal (peripheral) subcutaneous AT (NASAT): TSAT = ASAT + NASAT
Total internal AT (TIAT) = intra-abdominal AT (IAAT) + non-abdominal (peripheral) internal AT (NAIAT): TIAT = IAAT + NAIAT.

Abdominal region: this was defined from the images covering the region from the top of the liver down to the top of the head of the femoral bone. For most subjects this corresponds to between 15–17 images (Thomas, Saeed et al. 1998), giving intra-abdominal adipose tissue (IAAT) and abdominal subcutaneous adipose tissue (ASAT).

Regular quality control duplicate datasets were also used to verify the accuracy of the image analysis. In these datasets adipose tissue areas were estimated by a single trained observer. The coefficient of Variation (CoV) of the analysis was very low, ranging from <1% for total fat to <5% for visceral fat. Adiposity data in this thesis is mainly expressed in absolute volume (adipose compartment volume in litres), though wherever necessary the total volume of an adipose compartment as a percentage of body weight (% body-fat) is also given.
2.6.3 \(^1\)H MR spectroscopy (MRS) of the Liver

During the same scanning session, a \(^1\)H MR spectrum was acquired using a flexible body coil. The golden standard \(^1\)H MR was used in this thesis as a correlation to the novel ME based technique of image analysis explained below.

Participants were positioned supine, arms resting by their side, and legs supported with the hips slightly flexed using a foam wedge (to prevent lumbar discomfort from flat surface of scanning table). Transverse images of the liver were used to ensure accurate positioning of a (2 × 2 × 2 cm\(^3\)) voxel in the liver, avoiding blood vessels, fatty tissue and the gall bladder.

\(^1\)H MR spectra was obtained from the right lobe of the liver using a MRS localisation technique known as the PRESS sequence (point-resolved-spectroscopy) (TR 1500ms, TE 135ms, 128 signal averages) without water suppression (Bottomley 1987, Thomas, Hamilton \textit{et al.} 2005). The right lobe was chosen owing to its larger size.

The coefficient variation of this method is <7\% for IHCL. (Thomas, Hamilton \textit{et al.} 2005). Acquisition of images took approx. 2 minutes. (Figure 2.3)
2.6.3.i $^1$H MR spectroscopy analysis of Liver Fat

$^1$H MR spectra were analysed to determine the levels of intra-hepatocellular lipid (IHCL) using the spectral fitting application jMRUI AMARES. (Naressi, Couturier et al. 2001) Quantification of the resonances was performed in the time-domain to fit water and fat, using established prior knowledge. (Vanhamme 1997, Thomas, Hamilton et al. 2005)

The resulting spectrum includes resonances arising from water at 4.7 ppm, and the -CH$_2$ and -CH$_3$ parts of the fatty acid chain at 1.3 and 0.9 ppm respectively. The raw spectra were manually phased and the highest point of the water peak set at 4.7 parts per million. (Thomas, Hamilton et al. 2005) (Figure 2.3)

IHCL was quantified in the spectrum as a percentage ratio of the -CH$_2$ lipid resonances with reference to water resonance, after correcting for T1 and T2. (Thomas, Hamilton et al. 2005) (Figure 2.3)
Figure 2.3 Transverse Image showing Location of a $2 \times 2 \times 2$ cm$^3$ PRESS voxel positioned within the liver and corresponding $^1$H MR Spectra from Volunteers with (a) low and (b) high levels of fat infiltration. Resonances can be seen arising from water, and the CH$_2$ and CH$_3$ parts of the triglyceride for hepatic fat. IHCL content is calculated as the ratio of CH$_2$ to water.

2.6.4 Multi-Echo (ME) Imaging of the Liver and Pancreas

During the same scanning session, multi-echo MRI sequence (ME-MRI) was also acquired (O'Regan, Callaghan et al. 2008). This method has the potential advantage over single voxel MRS in that regional difference in ectopic fat distribution can be assessed for IHCL and IPCL. (Thomas, Fitzpatrick et al. 2013) Imaging was performed by using a breath-hold
gradient-echo (GE) sequence with seven-echo readout, resulting in a total acquisition time of 4 seconds for a single section of the liver and/or pancreas.

A six-channel receiver coil was used, and images were acquired in held expiration. A single-section multi-echo sequence was performed in a transverse plane passing through the liver and spleen, superior to the main portal vein. Another single-section multi-echo sequence was performed in a transverse plane passing through the pancreas.

The multi-echo sequence parameters were as follows: flip angle, 20°; field of view, 530 mm; section thickness, 10 mm; acquired voxel size, 2.5 × 2.5 × 10 mm; repetition time, 17 msec; and number of signal average of 2. (O'Regan, Callaghan et al. 2008)

2.6.4.i ME-MRI analysis of IHCL and IPCL

In images acquired with the ME sequence, decay curves are formed which show the change in signal intensity at each of the acquired echo times. Signals are usually in-phase (higher signal) and out-of-phase (lower signal), at multiple echo times. This enables both simultaneous measurement of fat content and T2*.
Data obtained at each of the echo times corrects T2* effects, models the fat signal, and estimates fat and water proton densities from which the fat content can be calculated. Thus, an organ with little or no fat infiltration will generate a very smooth decay curve (without obvious oscillations in the decay), whereas one containing a higher level of fat will show significant oscillations throughout the decay. (Thomas, Fitzpatrick et al. 2013) From the single-section multi-echo sequence, the raw image data was exported from the scanner for off-line analysis using Matlab software with an in-house script (Matlab, version 7.0; Mathworks, Natick, Mass).

The program performed an automated pixel-by-pixel analysis, after the liver and pancreas were isolated manually, to obtain color-coded parametric maps (heat-maps) of the whole liver/pancreas fat. This helps visualise regional differences in fat deposition. For instance, (Figure 2.4) shows the heat maps from three individuals with varying levels of fat in their liver and pancreas.
Figure 2.4 A Series of Multi-echo Heat-map Images taken from our ‘Comparing Multi Echo MRI and MRS to Measure Pancreatic Fat’ poster at the MRC imaging group showing three volunteers with varying levels of ectopic fat in both the liver and pancreas. A scale reflecting fat content from blue (low) to red (high) is also shown. Image (a) shows low fatty infiltration in liver and pancreas. Image (b) shows high fatty infiltration in liver and pancreas. Image (c) shows high fatty infiltration in liver and low fatty infiltration in pancreas.

2.7 Exercise Level Assessment

All participants filled a clinical health questionnaire prior to having the MR scan within the questionnaire aimed at determining relative weekly exercise activity (Appendix 1).

This was based on an abridged *International Physical Activity Questionnaires (IPAQ)* method that was previously used and validated by our group. The purpose of the questionnaires was to provide data on health–related physical activity from each volunteer.
The results were subdivided into: ‘Low’ (1-3 times per week) ‘Moderate’ (3-5 times per week) and ‘High’ (>5 times per week).

2.8 Interventional Studies

Subjects originally scanned for the basal phenotyping were recruited specifically to continue into interventional studies focused on either reducing or increasing calorie intake in a controlled manner. The aim of these two studies was to determine the effects of reduced/increased calorie intake on ectopic fat content and distribution.

Similar anthropometric measurements, MR imaging and analysis were obtained from all subjects at baseline and at follow ups.

2.8.1 Overfeeding intervention

Subjects were asked to take 600 kcal / day in the form of 2 x 125ml Fortisip Compact drinks (NutriDrinks, Perivale, UB6 7LQ, UK) after breakfast on top of their own standard diet for a period of 6 days.
All subjects were advised not to change their diet or physical activity during the duration of the intervention. The control group, which consisted of a gender/BMI matched individuals, were asked to drink a 0 kCal drink in the form of a 7UP soda (Dr Pepper Snapple Group Inc.; P.O. Box 86077; UK) for a period of 6 days (Appendix 1). Both groups were scanned immediately before and the morning after they finish their intervention.

2.8.2 Calorie Restriction intervention

Subjects were instructed to fill in a 7-day diet diary which was subsequently used as the basis for individualised dietary counselling as part of a weight-loss strategy. This required for each volunteer to record all food items and drinks that they consumed over a period of 7 consecutive days (including a weekend).

As part of the dietary assessment, volunteers were also asked to provide details regarding the method of food preparation and whether food was prepared at home or delivered/prepared, together with all available details of each brand, including packaging. Food diaries were analysed using DietPlan6 (Forestfield Software Ltd, West Sussex, UK) by a trained independent researcher competent in nutritional analysis.
The results from these analyses were subsequently used to advice each subject to reduce ~500 calorie intake to achieve a desired weight loss. On average it was aimed for subjects to achieve a 3.0 kg reduction in weight at the end of a 12 weeks intervention period.

2.8.3 Bariatric Surgery

Morbidly obese subjects were scanned before and after undergoing bariatric surgery to monitor the effect of the latter on ectopic fat content and distribution. Besides the normal exclusion criteria (Appendix 1), subjects were pre-selected based on their relative size due to magnet-bore size.

2.9 Statistical Analysis

Statistical calculations were performed using SPSS software (version 21.0, SPSS Inc,Chicago, IL, U.S.A) Microsoft Office Excel 2007 for Windows (Microsoft) and Unistat version 5.6.03, London, UK - throughout this thesis. Statistics appropriate to each study can be found in the relevant chapter.
Chapter III: Determination of Liver and Pancreatic Fat Content and Distribution

3.1 Introduction

Quantification of body and organ fat distribution is essential in physiology research since they are indispensably needed in our perception of the causes and effects of steatosis, obesity, the metabolic syndrome and their associated co-morbidities. (Hu and Kan 2013) Consequently, this has led to a significant increase in our understanding of the relationship between adipose tissue and various ectopic fat depots, along with the development of conditions such as type II diabetes, cardiovascular disorders and particular types of cancer. (Cassidy, Yokoo et al. 2009, Thomas, Fitzpatrick et al. 2013).

Up to the advent of imaging techniques, research into ectopic fat was, to a great degree, limited by the need to obtain tissue biopsies. Although muscle and occasionally liver biopsies could be attained for research purposes, study of ectopic fat in the heart and pancreas is clearly impractical and cannot be performed on living subjects. (N. S. Patel 2013, Thomas, Fitzpatrick et al. 2013) As a result, the use of non-invasive techniques for abdominal imaging and assessing ectopic fat content in vivo is experiencing exceptional growth in the last few decades. (Bartolozzi, Lencioni et al. 1999)
Ultrasound, computerized tomography (CT), magnetic resonance imaging (MRI) and proton magnetic resonance spectroscopy (¹H MRS) have all played a prominent role in this process, (Mehta, Thomas et al. 2008) with each technique having important advantages and limitations. (Lettner and Roden 2008, Cassidy, Yokoo et al. 2009, Thomas, Fitzpatrick et al. 2013) Reported sensitivities and specificities for the detection of ectopic fat deposition, in particular fatty liver deposition, have been widely documented, principally by the use of MRS. (Angulo 2007, Thomas, Parkinson et al. 2012, Angulo, Bugianesi et al. 2013).

In most cases, it has been stated that there is little or no variation in fat content across the liver. (Choji 1993, Angulo 2002, Saadeh, Younossi et al. 2002, Rinella, McCarthy et al. 2003) The most common and most frequently encountered MR imaging pattern in fatty liver is a diffuse and relatively homogeneous fat deposition across the organ. (Hamer, Aguirre et al. 2006) However, in a number of cases, fatty infiltration in the liver has been described as being patchy in distribution, its heterogeneous appearance is so pronounced that the fat is deposited in a constrained region (focal fatty infiltration). Alternatively, detached areas of the liver parenchyma remain uninvolved when the remainder of the liver is diffusely infiltrated with fat (focal fatty sparing). (Matsui, Kadoya et al. 1995, Hamer, Aguirre et al. 2006) The mechanism underlying these differences are yet unknown, nonetheless, these patterns may mimic inflammatory or vascular conditions, leading to confusion and to in some cases to unnecessary invasive diagnostic tests. (Hamer, Aguirre et al. 2006).

Conversely, fatty pancreas deposition, differs from the relatively general uniform steatosis of the liver in Non-Alcoholic Fatty Liver Disease (NAFLD). Furthermore, there are fewer
publications regarding the non-invasive measurements of ectopic fat in this organ, due to its size and location. (N. S. Patel 2013, Thomas, Fitzpatrick et al. 2013) Insufficient depiction of the whole organ and contamination by surrounding adipose tissue contribute to high variability and overestimation of lipid contents. (Szendroedi and Roden 2009) Some studies have described a focal accumulation of fat in the pancreas, particularly in the anterior aspect of the head. (Marchal, Verbeken et al. 1989, Matsumoto, Mori et al. 1995, Isserow, Siegelman et al. 1999, Kim, Byun et al. 2007) However, no consistent assessment of fat content and distribution within the pancreas has been undertaken so far.

This has in part occurred because established MRI techniques assessing steatosis are limited by T1 bias, T(2)* decay and multi-frequency signal-interference effects of protons in fat. (O'Regan, Callaghan et al. 2008) Furthermore, the gold standard technique, ¹H MRS, only measures ectopic fat content in a small single region of interest, requiring repeated measurement for any assessment of distribution to be made. (Thomas, Fitzpatrick et al. 2013).

In this chapter, I have used a Multi-echo (ME) imaging based technique to measure fat content and distribution in the liver and pancreas. The ability to accurately and noninvasively quantify fatty infiltration in organs such as the liver and the pancreas remains a critical component in understanding the link between obesity and its comorbidities such as type 2 diabetes and fatty liver disease. This technique overcomes some of the above limiting factors mentioned, and has therefore the potential to enable more accurate and effective measurement of tissue fat content and distribution in a single examination. (O'Regan, Callaghan et al. 2008, N. S. Patel 2013)
I will also be presenting a novel approach to assess ectopic fat distribution in the liver and pancreas, to further define potential changes across these organs. Initially, the technique will be tested and validated in a cohort of subjects to ascertain how age, gender, BMI, gestational age and life-style affect distribution of fat across the liver and pancreas and their relationship to total and abdominal adipose tissue.

3.2 Statistical Analysis

All Data are presented as mean ± standard deviation. Differences related to gender were examined using the Student’s t test and overall mean difference for BMI and Age groups using ANOVA and a post hoc Tukey test. The distributions of the obtained fat contents were not normal, thus non-parametric correlations were analysed using the Spearman rank test. Univariate and multivariable linear regression were used to assess the effect of age, gender and interaction between age x gender on all the variables. The estimate reported was coefficient ± standard error and p-values. Multi-echo imaging and spectroscopy methods were evaluated using Bland &Altman plot. Statistical significance was accepted at p<0.05
3.3 Results

3.3.1 Whole Cohort Anthropometry, Adiposity and Ectopic Fat measurements

A summary of the anthropometric and body fat measurements for the whole cohort are shown in Table 3.0. In total, 61 volunteers (37 male and 24 female) were studied. The mean age of all subjects was 45.25 ± 14.34 years (range 18-65 years).

The average BMI for this cohort was 31.4 ± 8.80 kg/m², with wide range of BMIs included in the group ranging from 18.7 to 50.9 kg/m². The average weight was 91.3 ± 22.8 kg (range 55.7-151.1 kg). The mean waist circumference was 101.8 ± 20.8 cm (range 65.0-145.5 cm) and waist-to-height ratio 0.64 ± 0.12 (range 0.32-0.93). Therefore the average measurements taken would make this cohort obese with a mean BMI >30 kg/m², and at increased risk of metabolic disease with mean waist circumference >100 cm.
**Table 3.0** Whole Cohort Anthropometry, Adiposity and Ectopic Fat measurements

<table>
<thead>
<tr>
<th></th>
<th>ALL COHORT (n=61)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± SD</td>
</tr>
<tr>
<td><strong>Anthropometric Variables</strong></td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>45.3 ± 14.3</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>91.3 ± 22.8</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>31.4 ± 6.1</td>
</tr>
<tr>
<td>WC (cm)</td>
<td>101.8 ± 20.8</td>
</tr>
<tr>
<td>Hip (cm)</td>
<td>109.3 ± 17.2</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>171.4 ± 10.3</td>
</tr>
<tr>
<td>WHR</td>
<td>0.93 ± 0.10</td>
</tr>
<tr>
<td>WHtR</td>
<td>0.64 ± 0.12</td>
</tr>
<tr>
<td><strong>Ectopic Liver Fat</strong></td>
<td></td>
</tr>
<tr>
<td>MRS IHCL</td>
<td>8.45 ± 13.47</td>
</tr>
<tr>
<td>ME IHCL</td>
<td>6.79 ± 7.53</td>
</tr>
<tr>
<td><strong>Ectopic Pancreas Fat</strong></td>
<td></td>
</tr>
<tr>
<td>ME IPCL</td>
<td>5.62 ± 6.03</td>
</tr>
<tr>
<td><strong>Adiposity Stores</strong></td>
<td></td>
</tr>
<tr>
<td>TAT (L)</td>
<td>37.20 ± 21.66</td>
</tr>
<tr>
<td>SAT (L)</td>
<td>30.20 ± 19.3</td>
</tr>
<tr>
<td>ASAT (L)</td>
<td>9.36 ± 7.08</td>
</tr>
<tr>
<td>NASAT (L)</td>
<td>20.84 ± 12.45</td>
</tr>
<tr>
<td>Internal (L)</td>
<td>6.96 ± 3.35</td>
</tr>
<tr>
<td>IAAT (L)</td>
<td>3.86 ± 2.21</td>
</tr>
<tr>
<td>NAIAT (L)</td>
<td>3.11 ± 1.29</td>
</tr>
<tr>
<td>IAAT/ASAT</td>
<td>0.52 ± 0.32</td>
</tr>
</tbody>
</table>

All data are presented as mean ± SD and range. WC: waist circumference; WHR: waist-to-hip ratio; WHtR: waist-to-height ratio; IHCL: intra-hepatocellular lipid; IPCL: intra-pancreatic cellular lipid, adipose tissue deposits are in litres (l); TAT: total adipose tissue; SAT: subcutaneous adipose tissue; ASAT: abdominal subcutaneous adipose tissue; NASAT: non-abdominal subcutaneous adipose tissue; Internal: total internal adipose tissue; IAAT: intra-abdominal adipose tissue; NAIAT: non-abdominal internal adipose tissue.
3.3.1.i Adipose Tissue Content and Distribution of Whole Cohort

Participants had mean TAT content of 37.2 ± 21.7 litres (range: 9.8 - 87.7 litres) with a mean SAT of 30.2 ± 19.3 litres (range 7.6 - 76.6 litres). Total internal adipose tissue was subdivided into abdominal (IAAT: mean 3.9 ± 2.2 litres, range 0.3 - 8.7 litres) and non-abdominal internal adipose tissue (NAIAT: 3.1 ± 1.3 litres (range 1.2 - 6.5 litres). The mean IAAT/ASAT ratio (0.52 ± 0.32) (range 0.10 - 1.57), see Table 3.0.

3.3.1.ii Liver Fat Content for the Whole Cohort

IHCL was assessed by two different methods; MRS and ME (see Table 3.0). The mean IHCL value using $^1$H MRS was 8.45 ± 13.47 (range 0.0-60.54) as for ME was 6.79 ± 7.53 (range 0.69-32.27). The measurements of IHCL obtained using MRS were stratified as being in the healthy, normal or elevated range, see Table 3.1.
Table 3.1 Classification of IHCL measured by $^1$H MRS in the Study Population

<table>
<thead>
<tr>
<th>IHCL class</th>
<th>Number</th>
<th>proportion of cohort</th>
</tr>
</thead>
<tbody>
<tr>
<td>‘Healthy’ range &lt;2% IHCL</td>
<td>29</td>
<td>47.5%</td>
</tr>
<tr>
<td>Normal range &lt;5%</td>
<td>37</td>
<td>60.7%</td>
</tr>
<tr>
<td>Elevated IHCL &gt;5%</td>
<td>24</td>
<td>39.3%</td>
</tr>
<tr>
<td>High IHCL &gt;16%</td>
<td>11</td>
<td>18.03%</td>
</tr>
</tbody>
</table>

Values of healthy, normal and elevated levels of IHCL as established by our group. NB Normal values also include values from ‘healthy’ group, similarly subjects with liver fat >16% are also included in the group >5%. Clinical governance within the MRI unit at Hammersmith Hospital states that volunteers with a measured IHCL by $^1$H MRS of >16% should have this reported to their GP, with the recommendation that they have follow-up clinical investigation.

3.3.1.iii Pancreatic Fat Content for the Whole Cohort

Pancreatic fat content was obtained only using ME imaging, since the small size of the pancreas makes measurement by MRS unreliable due to contamination from surrounding adipose tissue signals. The mean pancreatic fat content was $5.62 \pm 6.03$, range 0.40-24.18 (see Table 3.0). The measurements of pancreatic fat obtained were also stratified as to being in the healthy, normal or elevated range, using the normative values for ectopic fat content previously derived in the liver see Table 3.2.
Table 3.2 Classification of Pancreatic Fat measured by ME Imaging in the Study Population

<table>
<thead>
<tr>
<th>Class</th>
<th>Number</th>
<th>Proportion of Cohort</th>
</tr>
</thead>
<tbody>
<tr>
<td>‘Healthy’ range &lt;2%</td>
<td>18</td>
<td>29.5%</td>
</tr>
<tr>
<td>Normal range &lt;5%</td>
<td>41</td>
<td>67.2%</td>
</tr>
<tr>
<td>Elevated &gt;5%</td>
<td>20</td>
<td>32.8%</td>
</tr>
<tr>
<td>High &gt;16%</td>
<td>6</td>
<td>9.8%</td>
</tr>
</tbody>
</table>

There is limited published data of normal ranges of pancreatic fat content available; therefore values of healthy, normal and elevated levels of ectopic fat found in the liver were used as a general guide. NB Normal values also include values from ‘healthy’ group, similarly subjects with pancreas fat >16% are also included in the group >5%.

3.3.2 Correlation Analysis for Whole Cohort Variable

Whole cohort correlation analysis for all anthropometric variables, adipose tissue (in litres) and ectopic fat stores (arbitrary units) are shown in Table 3.3.

3.3.2.1 Relationship between Anthropometric Measurements and Adipose Tissue Depots

Most of the adiposity store variables correlated with anthropometric variables to a significant degree (p<0.01). BMI was the variable, which correlated to the greatest degree with all adiposity stores (TAT: r=0.91, SAT: r= 0.89, Internal : r= 0.80, ASAT : r= 0.90, IAAT : r= 0.75, p<0.01, Table 3.1) followed by WHtR (TAT: r=0.92, SAT: r= 0.93, Internal: r= 0.66,
ASAT: \( r = 0.92 \), IAAT: \( r = 0.60 \), \( p < 0.01 \), Table 3.1) and Hip circumference (TAT: \( r = 0.91 \), SAT: \( r = 0.91 \), \( p < 0.01 \), Table 3.3).
Table 3.3 Linear Correlation Analysis between Anthropometric Measurements, Lipid Stores and Body Fat Stores in Whole Cohort.

<table>
<thead>
<tr>
<th>ALLCOHORT</th>
<th>ANTHROPOMETRIC VARIABLES</th>
<th>ECTOPICT FAT STORES</th>
<th>ADIPOSITY STORES in LITRES</th>
</tr>
</thead>
<tbody>
<tr>
<td>n=61</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AGE</td>
<td>-0.025</td>
<td></td>
<td></td>
</tr>
<tr>
<td>WEIGHT</td>
<td>-0.440**</td>
<td>0.078</td>
<td></td>
</tr>
<tr>
<td>HEIGHT</td>
<td>0.221</td>
<td>0.855**</td>
<td>-0.156</td>
</tr>
<tr>
<td>WAIST</td>
<td>0.207</td>
<td>0.813**</td>
<td>-0.298*</td>
</tr>
<tr>
<td>HIP</td>
<td>0.228</td>
<td>0.550**</td>
<td>0.120</td>
</tr>
<tr>
<td>WHR</td>
<td>0.348**</td>
<td>0.612**</td>
<td>-0.617**</td>
</tr>
<tr>
<td>BMI</td>
<td>0.247</td>
<td>0.854**</td>
<td>-0.368**</td>
</tr>
<tr>
<td>MRS Liver</td>
<td>0.224</td>
<td>0.660**</td>
<td>-0.021</td>
</tr>
<tr>
<td>ME total IHCL</td>
<td>0.225</td>
<td>0.662**</td>
<td>0.001</td>
</tr>
<tr>
<td>ME total IPCL</td>
<td>0.513**</td>
<td>0.358**</td>
<td>-0.176</td>
</tr>
<tr>
<td>TAT</td>
<td>0.274*</td>
<td>0.764**</td>
<td>-0.434**</td>
</tr>
<tr>
<td>SAT</td>
<td>0.222</td>
<td>0.724**</td>
<td>-0.471**</td>
</tr>
<tr>
<td>Internal</td>
<td>0.395**</td>
<td>0.767**</td>
<td>-0.146</td>
</tr>
<tr>
<td>SAAT</td>
<td>0.254*</td>
<td>0.747**</td>
<td>-0.451**</td>
</tr>
<tr>
<td>NASAT</td>
<td>0.226</td>
<td>0.701**</td>
<td>-0.488**</td>
</tr>
<tr>
<td>IAAT</td>
<td>0.432**</td>
<td>0.721**</td>
<td>-0.113</td>
</tr>
<tr>
<td>NAIAT</td>
<td>0.302*</td>
<td>0.769**</td>
<td>-0.210</td>
</tr>
<tr>
<td>IAAT/ASAT</td>
<td>0.232</td>
<td>-0.164</td>
<td>0.441**</td>
</tr>
</tbody>
</table>

All data are presented as Spearman rank test correlation. *p<0.05, **p<0.01. Abbreviations: WC: waist circumference; WHR: waist-to-hip ratio; WHR: waist-to-height ratio; IHCL: intra-hepatocellular lipid; IPCL: intra-pancreatic cellular lipid, TAT: total adipose tissue; SAT: subcutaneous adipose tissue; ASAT: abdominal subcutaneous adipose tissue; NASAT: non abdominal subcutaneous adipose tissue; Internal: total internal adipose tissue; IAAT: intra-abdominal adipose tissue; NAIAT: non-abdominal internal adipose tissue.
3.3.2.ii Relationship between Liver Fat, Adipose Tissue and Anthropometry

There were significant correlations between IHCL and measures of anthropometry and adiposity, regardless of which method, (MRS or ME imaging), was used to measure IHCL content (see Table 3.3). For simplicity from here on, correlations from both methods will be presented in the tables, whilst only data obtain by ME will be given in the text.

There was a significant correlation between IHCL and body weight ($r=0.66$, $p<0.01$) and waist circumference ($r=0.71$, $p<0.01$). Internal adipose tissue, particularly IAAT was the variable which correlated most strongly with IHCL content ($r=0.74$, $p<0.01$), see figure 3.1 followed by the Internal adiposity store ($r=0.74$, $p<0.01$).

There were significant, but weaker correlations between IHCL and subcutaneous adipose tissue depots ($r=0.49$, $p<0.01$).
3.3.2.iii Relationship between Pancreatic Fat, Adipose Tissue and Anthropometry

There were significant correlations between IPCL, anthropometry and adiposity; however, these were in general weaker than those observed in the liver (Table 3.3).

There was a strong correlation between age and IPCL (r= 0.51, p<0.01), indeed the correlation between IPCL and age, was stronger than the correlation with age and any of the other variables measured.
As with IHCL, the most significant correlations were between IPCL and internal adipose tissue (internal: $r=0.59$, $p<0.01$, IAAT: $r=0.61$, $p<0.01$). Interestingly, the relationship between IPCL and IHCL was weaker (see Figure 3.2), although still significant ($r=0.40$, $p<0.01$).

**Figure 3.2** Relationship between IHCL and IPCL both measured by ME Imaging.
3.3.3 Comparison between MRS and ME to measure IHCL

There was a very strong correlation (r=0.96, p<0.01) between the MRS and ME imaging technique in assessing IHCL in the whole cohort. To further assess the validity of the techniques, Bland-Altman plot (Bland and Altman 1986) was utilised. MRS was analysed against ME to determine the 95% limits of individual agreement, see Figure 3.3.

Agreement between MRS and ME in measuring IHCL showed a mean difference of 1.66±6.75. In general there was good agreement between the two methods. However, substantial differences were observed between the two techniques at very high levels of IHCL. This arises from the differences in the formula used to calculate IHCL content in the two methods (MRS: fat/water ratio and ME: (fat/water + fat) ratio) rather than a differentiation in absolute levels of IHCL detected by the two techniques.
Figure 3.3 Bland Altman Plot showing agreement between MRS and ME in measuring IHCL. Central Line is group mean difference and the outer lines represent ± 2 standard deviations.
3.3.4 Gender Differences in Anthropometry, Adiposity and Ectopic Fat Measurements

There were significant gender differences within this cohort, see Table 3.4. Females were significantly older (50.2 ± 11.1 vs. 42.1 ± 15.4 years, p=0.02) with a much greater BMI than their male counterparts (36.5 ± 10.0 vs. 28.1 ± 6.1 kg/m², p<0.01). Similarly female subjects had significantly higher hip circumference (120.5 ± 17.4 vs. 102.1 ± 12.7 cm, p<0.01) compared with the male subjects. Waist circumference was also greater in the female group, though this did not reach significant (107.8 ± 23.6 vs. 97.8 ± 17.9 cm, p=0.08).
Table 3.4 Gender Differences in Anthropometry, Adiposity and Ectopic Fat Measurements

<table>
<thead>
<tr>
<th></th>
<th>Male Subjects (n=37)</th>
<th>Female Subjects (n=24)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± SD</td>
<td>Range</td>
<td>Mean ± SD</td>
</tr>
<tr>
<td><strong>Anthropometric Variables</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>42.1 ± 15.4</td>
<td>19-65</td>
<td>50.2 ± 11.1</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>88.7 ± 20.2</td>
<td>63.2-151.1</td>
<td>95.4 ± 26.3</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>28.1 ± 6.1</td>
<td>18.7-48.1</td>
<td>36.5 ± 10.0</td>
</tr>
<tr>
<td>WC (cm)</td>
<td>97.8 ± 17.9</td>
<td>65-139</td>
<td>107.8 ± 23.6</td>
</tr>
<tr>
<td>Hip (cm)</td>
<td>102.1 ± 12.7</td>
<td>55-133.5</td>
<td>120.5 ± 17.4</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>177.7 ± 6.7</td>
<td>165-192</td>
<td>161.6 ± 6.60</td>
</tr>
<tr>
<td>WHR</td>
<td>0.96 ± 0.09</td>
<td>0.80-1.18</td>
<td>0.89 ± 0.10</td>
</tr>
<tr>
<td>WHtR</td>
<td>0.57 ± 0.10</td>
<td>0.32-0.75</td>
<td>0.75 ± 0.11</td>
</tr>
<tr>
<td><strong>Liver Fat</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MRS IHCL</td>
<td>8.16 ± 13.78</td>
<td>0.00-59.57</td>
<td>8.91 ± 13.25</td>
</tr>
<tr>
<td>ME IHCL</td>
<td>6.80 ± 7.95</td>
<td>0.70-32.27</td>
<td>6.78 ± 7.00</td>
</tr>
<tr>
<td><strong>Pancreas Fat</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ME IPCL</td>
<td>5.18 ± 6.12</td>
<td>0.40-24.18</td>
<td>6.29± 5.96</td>
</tr>
<tr>
<td><strong>Adiposity Stores</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TAT (L)</td>
<td>27.18 ± 15.08</td>
<td>9.79-70.33</td>
<td>52.54 ± 21.48</td>
</tr>
<tr>
<td>SAT (L)</td>
<td>20.68 ± 12.2</td>
<td>7.61-59.11</td>
<td>44.87 ± 19.19</td>
</tr>
<tr>
<td>ASAT (L)</td>
<td>6.18 ± 4.08</td>
<td>1.50-22.64</td>
<td>14.26 ± 7.35</td>
</tr>
<tr>
<td>NASAT (L)</td>
<td>14.50 ± 7.55</td>
<td>5.95-36.47</td>
<td>30.62 ± 12.28</td>
</tr>
<tr>
<td>Internal (L)</td>
<td>6.51 ± 3.5</td>
<td>1.45-13.53</td>
<td>7.67 ± 3.08</td>
</tr>
<tr>
<td>IAAT (L)</td>
<td>3.72 ± 2.40</td>
<td>0.28-8.73</td>
<td>4.06 ± 1.91</td>
</tr>
<tr>
<td>NAIAT (L)</td>
<td>2.78 ± 1.20</td>
<td>1.17-6.47</td>
<td>3.61 ± 1.28</td>
</tr>
<tr>
<td>IAAT/ASAT</td>
<td>0.66 ± 0.32</td>
<td>0.12-1.57</td>
<td>0.31 ± 0.15</td>
</tr>
</tbody>
</table>

All data presented as mean ± SD and range. Where: WC: waist circumference; WHR: waist-to-hip ratio; WHtR: waist-to-height ratio; IHCL: intra-hepatocellular lipid; IPCL: intra-pancreatic cellular lipid, Adipose tissue deposits are in litres; TAT: total adipose tissue; SAT: subcutaneous; ASAT: abdominal subcutaneous; NASAT: non-abdominal subcutaneous; Internal: total internal; IAAT: intra-abdominal; NAIAT: non-abdominal internal. Male versus female data were analysed by unpaired Student’s t-test.
3.3.4.i Gender Differences in Adipose Tissue Content

As might be expected due to their greater sizes and perhaps age, female subjects had higher levels of adipose tissue in all depot measured when compared to male subjects, see Table 3.4. Interestingly, while these gender differences were significant for subcutaneous depots (SAT \( p<0.001 \), ASAT \( p<0.001 \), NASAT \( p<0.001 \)), they did not reach significance in internal depots (internal \( p=0.18 \), IAAT \( p=0.55 \)).

To account for the differences in size, adipose tissue depots were assessed as a proportion of total adipose tissue content, to determine whether there were differences in adipose tissue distribution independent of body size, see Table 3.5.
Table 3.5 Gender Differences in Adipose Tissue Depots as a Proportion of Total Adipose Tissue Content

<table>
<thead>
<tr>
<th></th>
<th>Male Subjects (n=37)</th>
<th>Female Subjects (n=24)</th>
<th>significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>% SAT</td>
<td>75.73 ± 6.22</td>
<td>84.94 ± 4.06</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>% ASAT</td>
<td>21.16 ± 4.13</td>
<td>26.10 ± 4.37</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>% NASAT</td>
<td>54.57 ± 5.58</td>
<td>58.84 ± 4.08</td>
<td>0.001</td>
</tr>
<tr>
<td>% Internal</td>
<td>24.27 ± 6.22</td>
<td>15.06 ± 4.06</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>% IAAT</td>
<td>13.25 ± 5.48</td>
<td>7.81 ± 2.75</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>% NAIAT</td>
<td>11.02 ± 2.48</td>
<td>7.25 ± 1.82</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

All data presented as mean ± SD. Adipose tissue depots are expressed as a percentage of total adipose tissue content. SAT: subcutaneous; ASAT: abdominal subcutaneous; NASAT: non-abdominal subcutaneous; Internal: total internal; IAAT: intra-abdominal; NAIAT: non-abdominal internal. Male versus female data were analysed by unpaired Student’s t-test.

From Table 3.5, it can be clearly seen that despite the differences in size, there are clear gender differences in adipose tissue distribution between the groups.

Male subjects carrying a significantly greater proportion of their adipose tissue internally (Male: 24.27 ± 6.22 vs. Female: 15.06 ± 4.06%, p<0.001) whereas female subjects carry a far higher proportion of their adipose tissue externally as subcutaneous adipose tissue (Female: 84.94 ± 4.06 vs. Male: 75.73 ± 6.22%, p<0.001).
3.3.4.ii Gender Differences in Liver Fat Content

There were no significant gender differences in IHCL measured by either MRS (Male: 8.16 ± 13.78 vs. Female: 8.91 ± 13.25 p=0.83) or ME imaging (Male: 6.80 ± 7.95 vs Female: 6.78 ± 7.00, p=0.99).

3.3.4.iii Gender Differences in Pancreatic Fat Content

There were no significant gender differences in IPCL between male and female subjects (Male: 5.18 ± 6.12 vs. 6.29± 5.96 p=0.48).

3.3.5 Interaction between Age, Gender and Adipose Tissue

The specific interaction between gender and age was further investigated using Multivariate linear regression analysis (AGE x GENDER). The following variables had a significant impact on the interaction between the two: BMI, Weight, WC, WHR, TAT, SAT, ASAT, NASAT (Table 3.6 and Table 3.7) (p<0.05 for all). However, the mean values of Internal and IAAT were not significantly different between the two groups in the regression analysis.
3.3.6 Interaction between Age and Gender and IHCL

Gender differences in IHCL content were detected in the multivariate analysis when age was adjusted for (Table 3.6 and Table 3.7), with male subjects having on average lower IHCL relative to female subjects, but this difference did not reach statistical significance (p=0.125).

3.3.7 Interaction between Age and Gender and IPCL

As previously observed in Table 3.3, there was a significant correlation between IPCL and age, however when the effect of Gender was adjusted for, the significance disappeared.
Table 3.6 Gender Specific Anthropometric Variable, Adiposity and Ectopic fat in Univariate Analysis

<table>
<thead>
<tr>
<th>Anthropometric Variables</th>
<th>GENDER β *</th>
<th>GENDER p</th>
<th>AGE β</th>
<th>AGE p</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age (yrs)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>-6.63±5.96</td>
<td>0.271</td>
<td>-0.14±0.21</td>
<td>0.52</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>-8.44±2.05</td>
<td>&lt;0.001</td>
<td>0.07±0.08</td>
<td>0.38</td>
</tr>
<tr>
<td>WC (cm)</td>
<td>-9.96±5.33</td>
<td>0.067</td>
<td>0.237±0.19</td>
<td>0.23</td>
</tr>
<tr>
<td>Hip (cm)</td>
<td>-18.43±3.85</td>
<td>&lt;0.001</td>
<td>0.16±0.15</td>
<td>0.31</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>16.02±1.74</td>
<td>&lt;0.001</td>
<td>-0.35±0.08</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>WHR</td>
<td>0.07±0.025</td>
<td>0.09</td>
<td>0.001±0.001</td>
<td>0.259</td>
</tr>
<tr>
<td>WHtR</td>
<td>-0.17±0.023</td>
<td>&lt;0.001</td>
<td>0.002±0.001</td>
<td>0.044</td>
</tr>
</tbody>
</table>

**Ectopic Liver Fat**

| MRS IHCL                  | -0.76±3.56 | 0.83     | 0.10±0.12 | 0.42 |
| ME IHCL                   | 0.02±1.99  | 0.99     | 0.08±0.07 | 0.23 |

**Ectopic Pancreas Fat**

| ME IPCL                   | -1.11±1.59 | 0.486 | 0.19±0.05 | <0.001 |

**Adiposity Stores**

| TAT (L)                   | -25.36±4.68 | <0.001 | 0.24±0.19 | 0.231 |
| SAT (L)                   | -24.20±4.01 | <0.001 | 0.15±0.17 | 0.40 |
| ASAT (L)                  | -8.08±1.55  | <0.001 | 0.05±0.06 | 0.417 |
| NASAT (L)                 | -16.12±2.54 | <0.001 | 0.10±0.11 | 0.399 |
| Internal (L)              | -1.16±0.87  | 0.187   | 0.09±0.028 | 0.003 |
| IAAAT (L)                 | -0.34±0.58  | 0.567   | 0.07±0.02 | <0.001 |
| NAIAT (L)                 | -0.83±0.32  | 0.013   | 0.02±0.01 | 0.076 |
| IAAT/ASAT                 | 0.35±0.07   | <0.001 | 0.006±0.003 | 0.049 |

The effect of age and gender on anthropometric variables, individual adiposity and ectopic fat stores was determined using univariate regression analysis. Anthropometric variables, ectopic fat and adiposity depots were the dependent variables with age (year) and gender (male = 1, female = 0) as the independent variables. β represents the regression coefficient for each independent variable included in the model ± standard error. * Gender β represents difference for male minus female. WC: waist circumference; WHR: waist-to-hip ratio; WHtR: waist-to-height ratio; IHCL: intra-hepatic lipids; IPCL: intra-pancreatic cellular lipid. Adipose tissue deposits are in liters (l); TAT: total adipose tissue; SAT: subcutaneous AT; ASAT: abdominal subcutaneous AT; NASAT: non-abdominal subcutaneous AT; Internal: total internal AT; IAAAT: intra-abdominal AT; NAIAT: non-abdominal internal AT.
Table 3.7 Gender Specific Anthropometric Variable, Adiposity and Ectopic Fat in Multivariate Analysis

<table>
<thead>
<tr>
<th>Anthropometric Variables</th>
<th>GENDER X AGE P-value</th>
<th>GENDER</th>
<th>AGE P-value</th>
<th>GENDER</th>
<th>AGE P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight (kg)ᵇ</td>
<td>0.020</td>
<td>-63.25±23.65</td>
<td>0.010</td>
<td>-1.07±0.41</td>
<td>0.012</td>
</tr>
<tr>
<td>BMI (kg/m²)ᵇ</td>
<td>0.024</td>
<td>-27.03±8.22</td>
<td>0.002</td>
<td>-0.30±0.14</td>
<td>0.041</td>
</tr>
<tr>
<td>WC (cm)ᵇ</td>
<td>0.026</td>
<td>-55.71±21.28</td>
<td>0.011</td>
<td>-0.58±0.37</td>
<td>0.123</td>
</tr>
<tr>
<td>Hip (cm)</td>
<td>0.116</td>
<td>-43.01±15.79</td>
<td>0.009</td>
<td>-0.40±0.28</td>
<td>0.149</td>
</tr>
<tr>
<td>Height (m)</td>
<td>0.940</td>
<td>14.81±6.57</td>
<td>0.028</td>
<td>-0.20±0.11</td>
<td>0.082</td>
</tr>
<tr>
<td>WHRᵇ</td>
<td>0.019</td>
<td>-0.14±0.10</td>
<td>0.147</td>
<td>-0.002±0.002</td>
<td>0.320</td>
</tr>
<tr>
<td>WHR</td>
<td>0.150</td>
<td>-0.30±0.09</td>
<td>0.002</td>
<td>-0.001±0.002</td>
<td>0.370</td>
</tr>
<tr>
<td>Ectopic Liver Fat</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MRS IHCL</td>
<td>0.280</td>
<td>-15.49±14.70</td>
<td>0.300</td>
<td>-0.14±0.56</td>
<td>0.580</td>
</tr>
<tr>
<td>ME IHCL</td>
<td>0.094</td>
<td>-12.50±8.03</td>
<td>0.125</td>
<td>-0.12±0.14</td>
<td>0.405</td>
</tr>
<tr>
<td>Ectopic Pancreas Fat</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ME IPCL</td>
<td>0.167</td>
<td>-7.39±5.83</td>
<td>0.210</td>
<td>0.08±0.10</td>
<td>0.464</td>
</tr>
<tr>
<td>Adiposity Stores</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TAT (L)ᵇ</td>
<td>0.010</td>
<td>-73.10±18.51</td>
<td>&lt;0.001</td>
<td>-0.75±0.32</td>
<td>0.023</td>
</tr>
<tr>
<td>SAT (L)ᵇ</td>
<td>0.008</td>
<td>-67.01±15.76</td>
<td>&lt;0.001</td>
<td>-0.75±0.28</td>
<td>0.009</td>
</tr>
<tr>
<td>ASAT (L)ᵇ</td>
<td>0.042</td>
<td>-20.85±6.25</td>
<td>0.001</td>
<td>-0.22±0.11</td>
<td>0.045</td>
</tr>
<tr>
<td>NASAT (L)ᵇ</td>
<td>0.003</td>
<td>-46.16±9.79</td>
<td>&lt;0.001</td>
<td>-0.53±0.17</td>
<td>0.003</td>
</tr>
<tr>
<td>Internal (L)</td>
<td>0.089</td>
<td>-6.09±3.34</td>
<td>0.074</td>
<td>-0.01±0.06</td>
<td>0.934</td>
</tr>
<tr>
<td>IAAT (L)</td>
<td>0.104</td>
<td>-3.21±2.15</td>
<td>0.141</td>
<td>0.02±0.04</td>
<td>0.687</td>
</tr>
<tr>
<td>NAIAT (L)</td>
<td>0.093</td>
<td>-2.88±1.31</td>
<td>0.032</td>
<td>-0.02±0.02</td>
<td>0.382</td>
</tr>
<tr>
<td>IAAT/ASAT</td>
<td>0.417</td>
<td>0.22±0.26</td>
<td>0.390</td>
<td>0.006±0.004</td>
<td>0.151</td>
</tr>
</tbody>
</table>

The effect of age and gender on anthropometric variables, individual adiposity and ectopic fat stores was determined using a multivariate regression analysis. Anthropometric variables, ectopic fat and adiposity deposits were the dependent variables with age (year), gender (male = 1, female = 0) and GENDER X AGE interaction as independent variables. β represents the regression coefficient for each independent variable included in the final model ± standard error. Gender β represents difference for male minus female. ᵜ represents all the gender x age interaction that was significant. WC: waist circumference; WHR: waist-to-hip ratio; WHtR: waist-to-height ratio; IHCL: intra-hepatocellular lipid; IPCL: intra-pancreatic cellular lipid. Adipose tissue deposits are in liters (L); TAT: total adipose tissue; SAT: subcutaneous AT; ASAT: abdominal subcutaneous AT; NASAT: non-abdominal subcutaneous AT; Internal: total internal AT; IAAT: intra-abdominal AT; NAIAT: non-abdominal internal AT. 129
3.3.8 Impact of Age and Gender on the Relationship between Anthropometric Measurements and Adipose Tissue Depots

Gender-specific correlation analysis for all anthropometric variables, adipose tissue (in litres) and ectopic fat stores (arbitrary units) are shown in Table 3.8 (Male) and Table 3.9 (Female).

Apart from a few exceptions (Height and the majority of IAAT/ASAT ratio), all variables correlated with each other to a significant degree (p<0.01).

3.3.8.1 Relationship between Anthropometric Measurements and Adipose Tissue Depots in Male Subjects

Most of the adiposity store variables correlated with the anthropometric parameters to a significant degree (p<0.01). BMI was the variable, which correlated to the greatest degree with all adiposity stores in male subjects (TAT: r=0.88, SAT: r=0.88, Internal: r=0.79, SAAT: r=0.90, IAAT: r=0.78, p<0.01, Table 3.8) followed by WC (TAT: r=0.87, SAT: r=0.84, Internal: r=0.83, SAAT: r=0.84, IAAT: r=0.81, all p<0.01)
Interestingly, Age was strongly associated with, Internal \((r=0.55, p<0.01)\), IAAT \((r=0.58, p<0.01)\), and IAAT/ASAT ratio \((r=0.52, p<0.01)\).

### 3.3.8.ii Relationship between Anthropometric Measurements and Adipose Tissue Depots in Female Subjects

In female subjects, BMI once again was the variable, which correlated to the greatest degree with all adiposity stores (TAT: \(r=0.94\), SAT: \(r=0.94\), Internal: \(r=0.78\), SAAT: \(r=0.91\), IAAT: \(r=0.69, p<0.01\), Table 3.9) followed by Hip (TAT: \(r=0.93\), SAT: \(r=0.93\), Internal: \(r=0.80\), SAAT: \(r=0.85\), and IAAT: \(r=0.76\), all \(p<0.01\)).

Age however lost its correlation in female subjects and only strongly associated with IAAT/ASAT ratio \((r=0.55, p<0.01)\).
Table 3.8 Linear Correlation Analysis between Anthropometric Measurements, lipid Stores and Body Fat stores in Male Participants.

<table>
<thead>
<tr>
<th>MALES</th>
<th>ANTHROPOMETRIC VARIABLES</th>
<th>ECTOPIC FAT STORES</th>
<th>ADIPOSITY STORES in LITRES</th>
</tr>
</thead>
<tbody>
<tr>
<td>n=37</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AGE</td>
<td>.126</td>
<td></td>
<td></td>
</tr>
<tr>
<td>WEIGHT</td>
<td>-.496**</td>
<td>.345*</td>
<td></td>
</tr>
<tr>
<td>HEIGHT</td>
<td>.465**</td>
<td>.772**</td>
<td>.016</td>
</tr>
<tr>
<td>HIP</td>
<td>.223</td>
<td>.824**</td>
<td>.023</td>
</tr>
<tr>
<td>WHR</td>
<td>.570**</td>
<td>.584**</td>
<td>-.213</td>
</tr>
<tr>
<td>WHR</td>
<td>.409*</td>
<td>.702**</td>
<td>-.016</td>
</tr>
<tr>
<td>BMI</td>
<td>.375*</td>
<td>.837**</td>
<td>-.175</td>
</tr>
<tr>
<td>MRS Liver</td>
<td>.363*</td>
<td>.588**</td>
<td>.112</td>
</tr>
<tr>
<td>ME total IHCL</td>
<td>.452**</td>
<td>.526**</td>
<td>-.008</td>
</tr>
<tr>
<td>ME total IPCL</td>
<td>.602**</td>
<td>.412**</td>
<td>-.148</td>
</tr>
<tr>
<td>TAT</td>
<td>.374*</td>
<td>.821**</td>
<td>-.006</td>
</tr>
<tr>
<td>SAT</td>
<td>.282</td>
<td>.815**</td>
<td>-.004</td>
</tr>
<tr>
<td>Internal</td>
<td>.550**</td>
<td>.719**</td>
<td>-.026</td>
</tr>
<tr>
<td>SAAT</td>
<td>.317</td>
<td>.827**</td>
<td>-.030</td>
</tr>
<tr>
<td>NASAT</td>
<td>.274</td>
<td>.779**</td>
<td>-.009</td>
</tr>
<tr>
<td>IAAT</td>
<td>.581**</td>
<td>.686**</td>
<td>-.087</td>
</tr>
<tr>
<td>NAIAT</td>
<td>.456**</td>
<td>.733**</td>
<td>.138</td>
</tr>
<tr>
<td>IAAT/ASAT</td>
<td>.520**</td>
<td>-.129</td>
<td>-.183</td>
</tr>
</tbody>
</table>

All data are presented as Spearman rank test correlation.* = p<0.05, ** = p<0.01. Abbreviations: WC: waist circumference; WHR: waist-to-hip ratio; WHtR: waist-to-height ratio; IHCL: intra-hepatocellular lipid; IPCL: intra-pancreatic cellular lipid, TAT: total adipose tissue; SAT: subcutaneous; SAAT: subcutaneous abdominal; NASAT: non abdominal subcutaneous; Internal: total internal; IAAT: intra-abdominal; NAIAT: non-abdominal internal
Table 3.9 Linear Correlation Analysis between Anthropometric Measurements, Lipid Stores and Body Fat Stores in Female Participants.

<table>
<thead>
<tr>
<th>FEMALES</th>
<th>ANTHROPOMETRIC VARIABLES</th>
<th>ECTOPIC FAT STORES</th>
<th>ADIPOSITY STORES in LITRES</th>
</tr>
</thead>
<tbody>
<tr>
<td>n=24</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AGE</td>
<td>WEIGHT</td>
<td>HEIGHT</td>
<td>WAIST</td>
</tr>
<tr>
<td>AGE</td>
<td>-0.392</td>
<td></td>
<td></td>
</tr>
<tr>
<td>WEIGHT</td>
<td>-0.316</td>
<td>0.196</td>
<td></td>
</tr>
<tr>
<td>HEIGHT</td>
<td>-0.222</td>
<td>0.78**</td>
<td>-0.131</td>
</tr>
<tr>
<td>HIP</td>
<td>-0.213</td>
<td>0.94**</td>
<td>0.058</td>
</tr>
<tr>
<td>WHR</td>
<td>-0.144</td>
<td>0.56**</td>
<td>-0.254</td>
</tr>
<tr>
<td>WHtR</td>
<td>-0.145</td>
<td>0.93**</td>
<td>-0.126</td>
</tr>
<tr>
<td>BMI</td>
<td>-0.300</td>
<td>0.94**</td>
<td>-0.074</td>
</tr>
<tr>
<td>MRS Liver</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ME total IHCL</td>
<td>-0.094</td>
<td>0.75**</td>
<td>0.012</td>
</tr>
<tr>
<td>ME total IPCL</td>
<td>0.237</td>
<td>0.300</td>
<td>-0.137</td>
</tr>
<tr>
<td>TAT</td>
<td>-0.380</td>
<td>0.98**</td>
<td>0.126</td>
</tr>
<tr>
<td>SAT</td>
<td>-0.420*</td>
<td>0.98**</td>
<td>0.167</td>
</tr>
<tr>
<td>Internal</td>
<td>-0.043</td>
<td>0.78**</td>
<td>-0.043</td>
</tr>
<tr>
<td>SAAT</td>
<td>-0.250</td>
<td>0.93**</td>
<td>0.057</td>
</tr>
<tr>
<td>NASAT</td>
<td>-0.444*</td>
<td>0.96**</td>
<td>0.152</td>
</tr>
<tr>
<td>IAAT</td>
<td>0.059</td>
<td>0.71**</td>
<td>-0.047</td>
</tr>
<tr>
<td>NAIAT</td>
<td>-0.160</td>
<td>0.79**</td>
<td>-0.084</td>
</tr>
<tr>
<td>IAAT/ASAT</td>
<td>0.549**</td>
<td>-0.316</td>
<td>-0.124</td>
</tr>
</tbody>
</table>

All data are presented as Spearman rank test correlation. *= p<0.05, **= p<0.01. Abbreviations: WC: waist circumference; WHR: waist-to-hip ratio; WHtR: waist-to-height ratio; IHCL: intra-hepatocellular lipid; IPCL: intra-pancreatic cellular lipid, TAT: total adipose tissue; SAT: subcutaneous; SAAT: subcutaneous abdominal; NASAT: non abdominal subcutaneous; Internal: total internal; IAAT: intra-abdominal; NAIAT: non-abdominal internal.
3.3.8.iii Relationship between Body Composition Measurements and IHCL in Male Subjects

In male subjects, internal adiposity correlated to the greatest degree with IHCL (IAAT: $r=0.73$, $p<0.01$; Internal $r=0.74$, $p<0.01$, Table 3.8).

There was also a strong correlation between IHCL and WHR ($r=0.70$, $p<0.01$). Furthermore, all anthropometric parameters were greatly associated with all individual ectopic fat depots, except for height.

3.3.8.iv Relationship between Body Composition Measurements and IHCL in Female Subjects

Female subjects had similar adiposity stores correlating to IHCL (IAAT: $r=0.79$ followed by Internal: $r=0.77$, $p<0.01$, Table 3.9). IHCL in females was also considerably associated with all anthropometric variables except for Age and Height, in particular, WC ($r=0.78$ p<0.01,) and Weight ($r=0.75$, p<0.01).
3.3.8. Relationship between Body Composition Measurements and IPCL in Male Subjects

In male subjects, internal adiposity was also correlated to the greatest degree with IPCL (Internal AT: \( r=0.60, p<0.01 \), IAAT \( r=0.61, p<0.01 \), Table 3.8).

WHR and age were also strongly associated with IPCL (WHR: \( r=0.67, p<0.01 \), age \( r=0.60, p<0.01 \)). There was a significant but weaker correlation between IPCL and IHCL \( (r=0.44, p<0.01) \).

3.3.8. vi Relationship between Body Composition Measurements and IPCL in Female Subjects

Female subjects also had the strongest correlations between IPCL and internal stores of adipose tissue, \( (IAAT: r=0.64, p<0.01, \text{ Internal}: r=0.59, p<0.01, \text{ Table 3.9}) \).

There was lack of association with IPCL and any of the anthropometric variables.
3.3.8.vii Impact of Correcting for Waist Circumference on the Relationship between Adiposity and Ectopic Fat

After adjusting for Waist circumference, the correlation between IAAT and IHCL was reduced in both male subjects ($r=0.43$, $p=0.009$) and female subjects ($r=0.33$, $p=0.13$).

This had no impact on the relationship between IAAT and IPCL in female subjects but the correlation between IAAT and IPCL was reduced in male subjects ($r=0.36$, $p=0.033$).

3.3.8.viii Impact of Correcting for Weight on the Relationship between Adiposity and Ectopic Fat

When Weight was adjusted for in female subjects, the relationship between IPCL and Internal adiposity stores was unchanged.

For males the association with IAAT with IHCL ($r=0.46$, $p=0.006$) and IPCL ($r=0.48$, $p=0.004$) was similar. Adjustment is for Waist or Weight, made little difference in either genders.
3.3.9 The Impact of Age on Body Composition

The age of subjects ranged from 18-65 years, with a mean age of 42 ± 15.4 years in men and 50.2 ± 11.1 years in women.

Age grouping was split for every 10 years (Thomas, Parkinson et al. 2012) which corresponded to the following ranges; 1: 16-25 years, 2: 26-35 years, 3: 36-45 years, 4: 46+ years. 15% of all subjects classified as group 1 (16-25 years old), 13% qualifying as group 2 (26-35 years old), 20% group 3 (36-45 years old) and 53% as group 4 (46+ years old), see Table 3.10

3.3.9.i The Impact of Age on Anthropometry

There was a tendency for BMI to increase with age, but this did not reach significance (p=0.59), see Table 3.10

Similarly there were no age related changes in any of the other anthropometric parameters measured: weight (p=0.72), WC (p=0.32), Hip (p=0.66), WHR (p=0.23) or WHtr (p= 0.13).
Table 3.10 Age Group Specific Summary Measures in Whole Cohort

<table>
<thead>
<tr>
<th>Age Group</th>
<th>Data for whole cohort</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age Group</td>
<td>16-25</td>
</tr>
<tr>
<td>number</td>
<td>9</td>
</tr>
</tbody>
</table>

**Anthropometric Variables**

<table>
<thead>
<tr>
<th>Variable</th>
<th>16-25</th>
<th>26-35</th>
<th>36-45</th>
<th>&gt;46</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>22.3 ± 2.7</td>
<td>30.9 ± 2.9</td>
<td>40.8 ± 2.3</td>
<td>56.9 ± 6.2</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>88.2 ± 20.6</td>
<td>99.3 ± 27.8</td>
<td>93.0 ± 27.6</td>
<td>89.6 ± 20.7</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>28.0 ± 8.5</td>
<td>30.3 ± 9.9</td>
<td>31.9 ± 10.6</td>
<td>32.5 ± 8.0</td>
</tr>
<tr>
<td>WC (cm)</td>
<td>92.2 ± 21.9</td>
<td>105.1 ± 20.9</td>
<td>97.4 ± 24.2</td>
<td>105.2 ± 18.8</td>
</tr>
<tr>
<td>Hip (cm)</td>
<td>104.2 ± 14.8</td>
<td>107.9 ± 12.2</td>
<td>107.5 ± 25.9</td>
<td>111.8 ± 15.0</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>178.9 ± 7.4</td>
<td>182.0 ± 7.4</td>
<td>171.4 ± 8.8</td>
<td>166.6 ± 9.0</td>
</tr>
<tr>
<td>WHR</td>
<td>0.88 ± 0.08</td>
<td>0.97 ± 0.10</td>
<td>0.91 ± 0.12</td>
<td>0.94 ± 0.10</td>
</tr>
<tr>
<td>WHtR</td>
<td>0.58 ± 0.10</td>
<td>0.59 ± 0.09</td>
<td>0.63 ± 0.17</td>
<td>0.67 ± 0.11</td>
</tr>
</tbody>
</table>

**Ectopic Liver Fat**

<table>
<thead>
<tr>
<th>Variable</th>
<th>16-25</th>
<th>26-35</th>
<th>36-45</th>
<th>&gt;46</th>
</tr>
</thead>
<tbody>
<tr>
<td>MRS IHCL</td>
<td>2.51 ± 4.41</td>
<td>7.05 ± 7.47</td>
<td>14.47 ± 21.95</td>
<td>8.22 ± 11.74</td>
</tr>
<tr>
<td>ME IHCL</td>
<td>2.64 ± 3.64</td>
<td>7.03 ± 5.55</td>
<td>9.03 ± 10.88</td>
<td>7.06 ± 7.09</td>
</tr>
</tbody>
</table>

**Ectopic Pancreas Fat**

<table>
<thead>
<tr>
<th>Variable</th>
<th>16-25</th>
<th>26-35</th>
<th>36-45</th>
<th>&gt;46</th>
</tr>
</thead>
<tbody>
<tr>
<td>ME IPCL</td>
<td>1.72 ± 1.30</td>
<td>2.61 ± 1.37</td>
<td>5.54 ± 6.41</td>
<td>7.49 ± 6.67</td>
</tr>
</tbody>
</table>

**Adiposity Stores**

<table>
<thead>
<tr>
<th>Variable</th>
<th>16-25</th>
<th>26-35</th>
<th>36-45</th>
<th>&gt;46</th>
</tr>
</thead>
<tbody>
<tr>
<td>TAT (L)</td>
<td>26.22 ± 21.46</td>
<td>35.13 ± 24.76</td>
<td>39.48 ± 26.78</td>
<td>39.87 ± 18.73</td>
</tr>
<tr>
<td>SAT (L)</td>
<td>21.87 ± 19.06</td>
<td>28.61 ± 21.87</td>
<td>32.80 ± 24.40</td>
<td>31.95 ± 16.78</td>
</tr>
<tr>
<td>ASAT (L)</td>
<td>6.25 ± 6.40</td>
<td>8.75 ± 7.13</td>
<td>10.14 ± 8.28</td>
<td>10.09 ± 6.85</td>
</tr>
<tr>
<td>NASAT (L)</td>
<td>15.62 ± 12.67</td>
<td>19.86 ± 14.85</td>
<td>22.66 ± 16.27</td>
<td>21.87 ± 10.22</td>
</tr>
<tr>
<td>Internal (L)</td>
<td>4.35 ± 2.58</td>
<td>6.52 ± 3.40</td>
<td>6.67 ± 3.54</td>
<td>7.92 ± 3.14</td>
</tr>
<tr>
<td>IAAT (L)</td>
<td>2.02 ± 1.55</td>
<td>3.28 ± 1.81</td>
<td>3.65 ± 2.23</td>
<td>4.59 ± 2.16</td>
</tr>
<tr>
<td>NAIAT (L)</td>
<td>2.32 ± 1.14</td>
<td>3.24 ± 1.65</td>
<td>3.03 ± 1.40</td>
<td>3.33 ± 1.16</td>
</tr>
<tr>
<td>IAAT/ASAT</td>
<td>0.41 ± 0.23</td>
<td>0.49 ± 0.24</td>
<td>0.50 ± 0.36</td>
<td>0.57 ± 0.34</td>
</tr>
</tbody>
</table>

WC: waist circumference; WHR: waist-to-hip ratio; WHtR: waist-to-height ratio; IHCL: intra-hepatocellular lipid; IPCL: intra-pancreatic cellular lipid. Adipose tissue deposits are in litres (L); TAT: total adipose tissue; SAT: subcutaneous; ASAT: abdominal subcutaneous; NASAT: non-abdominal subcutaneous; Internal: total internal; IAAT: intra-abdominal; NAIAT: non-abdominal internal. All data are presented as mean ± SD.
3.3.9.ii The Impact of Age on Adiposity

There was a trend of total and subcutaneous adipose stores to increase with age, but this did not reach significance: TAT (p=0.40), ASAT (p=0.53) and SAT (p=0.54). There was a significant increase in both Internal (p=0.04) and IAAT (p=0.01) with age.

3.3.9.iii The Impact of Age on Liver Fat Content

There was a trend for an increase in IHCL from the 16-25 age group to the 36-45 age group, after which there was no further increase, although none of these changes were statistically significant (p=0.28) (Table 3.10, Figure 3.4)
3.3.9.iv The Impact of Age on Pancreatic Fat Content

The mean value of IPCL, on the other hand, rose significantly (p=0.03) throughout the different age groups, (Table 3.10, Figure 3.5).

Figure 3.4 Change in IHCL with Age
Figure 3.5 Increasing IPCL with Age

P = 0.047
3.3.10 Impact of Body Mass Index (BMI) on Body Composition

BMI grouping corresponded to the following ranges; 1: 18-25 kg/m², 2: 25-30 kg/m², 3: 30-40 kg/m², 4: >40 kg/m². BMI ranged from 18.7 – 50.9 kg/m², with a mean of 28.1 ± 6.1 kg/m² in men and 36.5 ± 10.0 kg/m² in women. 32.8% of all subjects were classified as overweight (BMI: 25<30), 18.0% were classed as obese (BMI: 30<40) while 23% were morbidly obese (BMI: 40+), see Table 3.11.

3.3.10.i Impact of Body Mass Index (BMI) on Anthropometry

All anthropometric parameters increased significantly throughout the BMI groups Weight, WC, Hip, WHR and WHtr, (p<0.001 for all), see Table 3.11.
<table>
<thead>
<tr>
<th>Data for whole cohort</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>p</th>
</tr>
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<tbody>
<tr>
<td>BMI Group number</td>
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<td>20</td>
<td>11</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td><strong>Anthropometric Variables</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>36.1 ± 12.1</td>
<td>48.5 ± 14.60</td>
<td>54.3 ± 13.8</td>
<td>43.9 ± 11.6</td>
<td>0.01</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>71.9 ± 8.9</td>
<td>80.3 ± 8.1</td>
<td>97.4 ± 9.2</td>
<td>124.5 ± 16.6</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>23.3 ± 1.7</td>
<td>27.1 ± 1.4</td>
<td>32.6 ± 2.3</td>
<td>46.0 ± 2.9</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>WC (cm)</td>
<td>82.2 ± 4.5</td>
<td>92.1 ± 11.9</td>
<td>110.1 ± 8.7</td>
<td>131.4 ± 8.8</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Hip (cm)</td>
<td>96.0 ± 4.7</td>
<td>100.6 ± 12.1</td>
<td>113.8 ± 7.2</td>
<td>133.6 ± 9.7</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>175.6 ± 10.7</td>
<td>172.1 ± 8.9</td>
<td>172.8 ± 7.2</td>
<td>164.4 ± 11.0</td>
<td>0.02</td>
</tr>
<tr>
<td>WHR</td>
<td>0.86 ± 0.06</td>
<td>0.92 ± 0.11</td>
<td>0.97 ± 0.10</td>
<td>0.99 ± 0.08</td>
<td>0.001</td>
</tr>
<tr>
<td>WHtR</td>
<td>0.55 ± 0.04</td>
<td>0.59 ± 0.08</td>
<td>0.66 ± 0.06</td>
<td>0.82 ± 0.08</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>Ectopic Liver Fat</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MRS IHCL</td>
<td>0.54 ± 0.55</td>
<td>6.39 ± 10.94</td>
<td>14.47 ± 16.73</td>
<td>15.72 ± 16.60</td>
<td>0.004</td>
</tr>
<tr>
<td>ME IHCL</td>
<td>1.43 ± 0.62</td>
<td>5.81 ± 6.65</td>
<td>11.14 ± 9.34</td>
<td>10.90 ± 7.63</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>Ectopic Pancreas Fat</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ME IPCL</td>
<td>1.75 ± 0.92</td>
<td>5.70 ± 6.04</td>
<td>8.48 ± 7.18</td>
<td>7.66 ± 6.64</td>
<td>0.011</td>
</tr>
<tr>
<td><strong>Adiposity Stores</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TAT (L)</td>
<td>17.01 ± 5.00</td>
<td>26.66 ± 7.46</td>
<td>42.77 ± 7.94</td>
<td>70.78 ± 9.72</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>SAT (L)</td>
<td>13.52 ± 4.56</td>
<td>20.73 ± 7.12</td>
<td>33.32 ± 7.36</td>
<td>60.31 ± 10.72</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>ASAT (L)</td>
<td>3.41 ± 1.43</td>
<td>5.78 ± 2.08</td>
<td>10.29 ± 2.68</td>
<td>20.53 ± 4.32</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>NASAT (L)</td>
<td>10.11 ± 3.20</td>
<td>14.96 ± 5.17</td>
<td>23.04 ± 5.07</td>
<td>39.78 ± 7.73</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Internal (L)</td>
<td>3.48 ± 1.22</td>
<td>5.93 ± 2.10</td>
<td>9.45 ± 2.12</td>
<td>10.47 ± 2.24</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>IAAT (L)</td>
<td>1.58 ± 0.86</td>
<td>3.29 ± 1.63</td>
<td>5.90 ± 1.44</td>
<td>5.66 ± 1.50</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>NAIAT (L)</td>
<td>1.90 ± 0.43</td>
<td>2.64 ± 0.66</td>
<td>3.55 ± 0.86</td>
<td>4.81 ± 0.93</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>IAAT/ASAT</td>
<td>0.52 ± 0.30</td>
<td>0.64 ± 0.38</td>
<td>0.61 ± 0.22</td>
<td>0.30 ± 0.16</td>
<td>0.013</td>
</tr>
</tbody>
</table>

WC: waist circumferance; WHR: waist-to-hip ratio; WHtR: waist-to height ratio; IHCL: intra-hepatocellular lipid; IPCL: intra-pancreatic cellular lipid, Adipose tissue deposits are in litres (l); TAT: total adipose tissue; SAT: subcutaneous; ASAT: abdominal subcutaneous; NASAT: non abdominal subcutaneous; Internal: total internal; IAAT: intra-abdominal; NAIAT: non-abdominal internal. All data are presented as mean ± SD. BMI grouping corresponds to the following BMI ranges; 1: 18.5<25, 2: 25<30, 3: 30<40, 4: 40+. 
3.3.10.ii Impact of Body Mass Index (BMI) on Adiposity

As might be expected all depots increased with increasing BMI, TAT, SAT, ASAT, *Internal*, IAAT (p<0.001 for all), see Table 3.11.

Interestingly although internal adipose tissue increased in a stepwise fashion between BMI groups, this was not true for IAAT.

There were significant increases from BMI groups 1-2, and 2-3 but not between BMI groups 3 and 4. This may reflect the high proportion of female subjects in BMI Group 4 (11 Females, 3 Males), compared with BMI group 3 (4 Females, 7 Males), as previously shown in Table 3.5 Female subjects carry most of their adipose tissue as subcutaneous adipose tissue.

3.3.10.iii Impact of Body Mass Index (BMI) on Liver Fat Content

Overall the mean value of IHCL increased significantly with BMI (p<0.001), Table 3.11 and Figure 3.6.
Interestingly IHCL increased significantly between groups 1-3, and 1-4, but not groups 1-2, 2-3 or 3-4.

Figure 3.6 Change in IHCL with BMI Group

3.3.10.iv Impact of Body Mass Index (BMI) on Pancreatic Fat Content

Overall the mean value of IPCL increased significantly with BMI (p<0.05), Table 3.11. Reflecting the changes observed in IHCL, IPCL increased significantly between groups 1-3, and 1-4, but not group 1-2, 2-3 or 3-4 (Table 3.11 and Figure 3.7).
3.3.11 Impact of BMI Group on the Relationship between Anthropometric Measurements and Adipose Tissue Depots

BMI group correlation analysis for all anthropometric variables, adipose tissue (in litres) and ectopic fat stores (arbitrary units) are shown in Tables 3.12 (Group 1 = 18<25 kg/m²), Table 3.13 (Group 2 = 25<30 kg/m²), Table 3.14 (Group 3 = 30<40 kg/m²) and Table 3.15 (Group 4 = 40+ kg/m²).

Figure 3.7 Change in IPCL with BMI Group
3.3.11.i BMI Group 1 - Relationship Between Anthropometric Measurements and Adiposity

In Group 1, apart from a few exceptions mentioned below, most variables did not correlate with each other.

Height was the variable, which correlated predominantly and negatively with a few adiposity stores (TAT: \( r = -0.52 \) (p<0.05), SAT: \( r = -0.53 \) (p<0.05), SAAT: \( r = -0.62 \) (p<0.05), Table 3.12.

*Internal* adiposity store was mainly associated with Waist (\( r = 0.69 \) (p<0.01), as well as IAAT (\( r = 0.67 \), p<0.01).

3.3.11.ii BMI Group 1 - Relationship Between Body Composition and Liver Fat

*Internal* adiposity store was the variable, which correlated to the greatest degree with IHCL (\( r = 0.68 \), \( P<0.01 \), Table 3.12). Waist circumference also correlated significantly with IHCL (\( r = 0.63 \), p<0.01)
3.3.11.iii BMI Group 1 - Relationship Between Body Composition and Pancreatic Fat

IPCL only correlated with age (r = 0.55, p<0.05, Table 3.12).
Table 3.12 Linear Correlation Analysis between Anthropometric Measurements, Lipid Stores and Body Fat Stores in BMI Group 1.

<table>
<thead>
<tr>
<th>BMI Group 1</th>
<th>ANTHROPOMETRIC VARIABLES</th>
<th>ECTOPIC FAT STORES</th>
<th>ADIPOSITY STORES in LITRES</th>
</tr>
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<td>n=16</td>
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<td>HEIGHT</td>
</tr>
<tr>
<td>AGE</td>
<td>- .624**</td>
<td>.784**</td>
<td>- .801**</td>
</tr>
<tr>
<td>WEIGHT</td>
<td>- .624**</td>
<td>.784**</td>
<td>- .801**</td>
</tr>
<tr>
<td>HEIGHT</td>
<td>- .624**</td>
<td>.784**</td>
<td>- .801**</td>
</tr>
<tr>
<td>HIP</td>
<td>- .624**</td>
<td>.784**</td>
<td>- .801**</td>
</tr>
<tr>
<td>WHR</td>
<td>- .624**</td>
<td>.784**</td>
<td>- .801**</td>
</tr>
<tr>
<td>BMI</td>
<td>- .624**</td>
<td>.784**</td>
<td>- .801**</td>
</tr>
</tbody>
</table>

All data are presented as Spearman rank test correlation. MRS IHCL (M:12 , F:4), ME total IHCL (M:12 , F:4 ) and ME total IPCL (M:12 , F:4); *= p<0.05,**= p< 0.01. Abbreviations: WC: waist circumference; WHR: waist-to-hip ratio; WHtR: waist-to-height ratio; IHCL: intra-hepatocellular lipid; IPCL: intra-pancreatic cellular lipid, TAT: total adipose tissue; SAT: subcutaneous; SAAT: subcutaneous abdominal; NASAT: non abdominal subcutaneous; Internal:total internal; IAAT: intra-abdominal; NAIAT: non-abdominal internal.
3.3.11.iv BMI Group 2 - Relationship between Anthropometric Measurements and Adiposity

In Group 2, WHtR was the variable, which correlated predominantly with adiposity stores; TAT ($r=0.67, p<0.01$), SAT ($r=0.71, p<0.01$), and SAAT ($r=0.61, p<0.01$), Table 3.13.

Followed by Hip circumference, TAT ($r=0.65, p<0.01$), SAT ($r=0.67, p<0.01$), and SAAT ($r=0.62, p<0.01$), Table 3.13. WHR also correlated with Internal adipose tissue ($r=0.68, p<0.01$), IAAT ($r=0.70, p<0.01$) and IAAT/ASAT ($r=0.80, p<0.01$).

3.3.11.v BMI Group 2 - Relationship between Body Composition and Liver Fat

There was a significant correlation between WHR and IHCL ($r=0.65, p<0.01$), see Table 3.13. There was also a significant correlation between IAAT/ASAT and IHCL ($r=0.63, p<0.01$) and IAAT ($r=0.55, p<0.05$, Table 3.12)
3.3.11.vi BMI Group 2 - Relationship between Body Composition and Pancreatic Fat

There was a significant correlation between height and IPCL (r = 0.48, p<0.05), see Table 3.13. IPCL was also strongly correlated with IAAT (r= 0.54, p<0.05), and Internal adiposity store (r= 0.46, p<0.05).
Table 3.13 Linear Correlation Analysis between Anthropometric Measurements, Lipid Stores and Body Fat Stores in BMI Group 2.

<table>
<thead>
<tr>
<th>BMI Group 2</th>
<th>ANTHROPOMETRIC VARIABLES</th>
<th>ECTOPIC FAT STORES</th>
<th>ADIPOSITY STORES in LITRES</th>
</tr>
</thead>
<tbody>
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<td>HEIGHT</td>
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<tr>
<td>AGE</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WEIGHT</td>
<td>-.171</td>
<td>.816**</td>
<td></td>
</tr>
<tr>
<td>HEIGHT</td>
<td>-.185</td>
<td>.307</td>
<td>-.008</td>
</tr>
<tr>
<td>HIP</td>
<td>.233</td>
<td>.387</td>
<td>.496*</td>
</tr>
<tr>
<td>WHR</td>
<td>.144</td>
<td>.366</td>
<td>.502*</td>
</tr>
<tr>
<td>BMI</td>
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<td>-.0325</td>
</tr>
<tr>
<td>MRS Liver</td>
<td>.062</td>
<td>.318</td>
<td>.577**</td>
</tr>
<tr>
<td>ME total IHCL</td>
<td>.077</td>
<td>.293</td>
<td>.524*</td>
</tr>
<tr>
<td>ME total IPCL</td>
<td>.377</td>
<td>.284</td>
<td>.479*</td>
</tr>
<tr>
<td>TAT</td>
<td></td>
<td>-.0299</td>
<td>-.322</td>
</tr>
<tr>
<td>SAT</td>
<td>.171</td>
<td>-.0318</td>
<td>-.0336</td>
</tr>
<tr>
<td>Internal</td>
<td>.381</td>
<td>.263</td>
<td>.339</td>
</tr>
<tr>
<td>SAAT</td>
<td>.147</td>
<td>-.238</td>
<td>-.0255</td>
</tr>
<tr>
<td>NASAT</td>
<td>.158</td>
<td>-.361</td>
<td>-.381</td>
</tr>
<tr>
<td>IAAT</td>
<td>.320</td>
<td>.270</td>
<td>.349</td>
</tr>
<tr>
<td>NAIAT</td>
<td>.361</td>
<td>.215</td>
<td>.330</td>
</tr>
<tr>
<td>IAAT/ASAT</td>
<td>.230</td>
<td>.267</td>
<td>.438</td>
</tr>
</tbody>
</table>

All data are presented as Spearman rank test correlation. MRS IHCL (M:15 , F:5), ME total IHCL (M:15 , F:5 ), and ME total IPCL (M:15 , F:5); *= p<0.05, **= p<0.01. Abbreviations: WC: waist circumference; WHR: waist-to-hip ratio; WHtR: waist-to-height ratio; IHCL: intra-hepatocellular lipid; IPCL: intra-pancreatic cellular lipid, TAT: total adipose tissue; SAT: subcutaneous; SAAT: subcutaneous abdominal; NASAT: non abdominal subcutaneous; Internal: total internal; IAAT: intra-abdominal; NAIAT: non-abdominal internal.
3.3.11.vii BMI Group 3 - Relationship Between Anthropometric Measurements and Adiposity

In Group 3, there was also a strong correlation between WHtR and adiposity; TAT (r=0.74, p<0.01), SAT (r=0.82, p<0.01), and SAAT (r=0.77, p<0.01) (Table 3.14) followed by Hip circumference SAT (r=0.74, p<0.01), and SAAT (r=0.77, p<0.01) (Table 3.14).

3.3.11.viii BMI Group 3 - Relationship Between Body Composition and Liver Fat

There were no significant correlations between IHCL and any of the anthropometric variables or adiposity stores in group 3. (Table 3.14)

3.3.11.ix BMI Group 3 - Relationship Between Body Composition and Pancreatic Fat

There was no association between IPCL and any of the anthropometric variables or adiposity stores in group 3. (Table 3.14)
Table 3.14 Linear Correlation Analysis between Anthropometric Measurements, Lipid Stores and Body Fat Stores in BMI Group 3.

<table>
<thead>
<tr>
<th>BMI Group 3</th>
<th>ANTHROPOMETRIC VARIABLES</th>
<th>ECTOPIC FAT STORES</th>
<th>ADIPOSITY STORES in LITRES</th>
</tr>
</thead>
<tbody>
<tr>
<td>n=11</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AGE</td>
<td>AGE</td>
<td>WEIGHT</td>
<td>HEIGHT</td>
</tr>
<tr>
<td>WEIGHT</td>
<td>-0.387</td>
<td>.700*</td>
<td></td>
</tr>
<tr>
<td>HEIGHT</td>
<td>.182</td>
<td>0.582</td>
<td>.427</td>
</tr>
<tr>
<td>HIP</td>
<td>-0.473</td>
<td>0.059</td>
<td>-0.178</td>
</tr>
<tr>
<td>WHR</td>
<td>.310</td>
<td>0.527</td>
<td>.373</td>
</tr>
<tr>
<td>BMI</td>
<td>-0.082</td>
<td>-0.245</td>
<td>-.645*</td>
</tr>
<tr>
<td>MRS Liver</td>
<td>.296</td>
<td>0.227</td>
<td>.055</td>
</tr>
<tr>
<td>ME total IHCL</td>
<td>.328</td>
<td>0.236</td>
<td>.145</td>
</tr>
<tr>
<td>ME total IPCL</td>
<td>.538</td>
<td>-0.491</td>
<td>-.409</td>
</tr>
<tr>
<td>TAT</td>
<td>-0.100</td>
<td>0.036</td>
<td>-0.409</td>
</tr>
<tr>
<td>SAT</td>
<td>-0.210</td>
<td>0.036</td>
<td>-0.327</td>
</tr>
<tr>
<td>Internal</td>
<td>0.433</td>
<td>0.209</td>
<td>0.045</td>
</tr>
<tr>
<td>SAAT</td>
<td>-0.337</td>
<td>0.073</td>
<td>-0.282</td>
</tr>
<tr>
<td>NASAT</td>
<td>-.137</td>
<td>-0.018</td>
<td>-0.382</td>
</tr>
<tr>
<td>IAAT</td>
<td>.333</td>
<td>0.382</td>
<td>0.191</td>
</tr>
<tr>
<td>NAIAT</td>
<td>.251</td>
<td>0.064</td>
<td>-0.009</td>
</tr>
<tr>
<td>IAAT/ASAT</td>
<td>.506</td>
<td>.236</td>
<td>.345</td>
</tr>
</tbody>
</table>

All data are presented as Spearman rank test correlation. MRS IHCL (M:7, F:4), ME total IHCL (M:7, F:4) and ME total IPCL (M:7, F:4); *= p<0.05,**= p<0.01. Abbreviations: WC: waist circumference; WHR: waist-to-hip ratio; WHR: waist-to-height ratio; IHCL: intra-hepatocellular lipid; IPCL: intra-pancreatic cellular lipid, TAT: total adipose tissue; SAT: subcutaneous; SAAT: subcutaneous abdominal; NASAT: non abdominal subcutaneous; Internal: total internal; IAAT: intra-abdominal; NAIAT: non-abdominal internal
3.3.11.x BMI Group 4 - Relationship Between Anthropometric Measurements and Adiposity

Hip was once again the variable, which correlated most strongly with adiposity; TAT (r=0.67, p<0.01), SAT (r= 0.64, p<0.05) and SAAT (r=0.72, p<0.01) followed by BMI; TAT (r=0.67, p<0.01), SAT (r= 0.65, p<0.05) and SAAT (r=0.71, p<0.01) (Table 3.15).

3.3.11.xi BMI Group 4 - Relationship Between Body Composition and Liver Fat

In group 4, IHCL was significantly correlated to NAIAT (r=0.81, p<0.01) and Internal adiposity (r = 0.59, p<0.05), see Table 3.15.

3.3.11.xii BMI Group 4 - Relationship Between Body Composition and Pancreatic Fat

In group 4, IPCL was negatively associated WHR (r= -0.54 p<0.05). There were no other significant correlations between IPCL and any other parameter, see Table 3.15.
Table 3.15 Linear Correlation Analysis between Anthropometric Measurements, Lipid Stores and Body Fat Stores in BMI Group 4.

<table>
<thead>
<tr>
<th>BMI Group 4</th>
<th>ANTHROPOMETRIC VARIABLES</th>
<th>ECTOPIC FAT STORES</th>
<th>ADIPOSITY STORES in LITRES</th>
</tr>
</thead>
<tbody>
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<td></td>
<td></td>
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<td>HEIGHT</td>
<td>WAIST</td>
</tr>
<tr>
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</tr>
<tr>
<td>HIP</td>
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<tr>
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<td>.407</td>
</tr>
<tr>
<td>BMI</td>
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<td>0.376</td>
<td>-0.187</td>
</tr>
<tr>
<td>MRS Liver</td>
<td>-0.058</td>
<td>-0.042</td>
<td>-0.046</td>
</tr>
<tr>
<td>ME total IHCL</td>
<td>-0.238</td>
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<td>.141</td>
</tr>
<tr>
<td>ME total IPCL</td>
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<td>-0.336</td>
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<tr>
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<td>IAAT/ASAT</td>
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<td>0.248</td>
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</table>

All data are presented as Spearman rank test correlation. MRS IHCL (M:3, F:11), ME total IHCL (M:3, F:11) and ME total IPCL (M:3, F:11); *= p<0.05, **= p<0.01. Abbreviations: WC: waist circumference; WHR: waist-to-hip ratio; WHtR: waist-to-height ratio; IHCL: intra-hepatocellular lipid; IPCL: intra-pancreatic cellular lipid, TAT: total adipose tissue; SAT: subcutaneous; SAAT: subcutaneous abdominal; NASAT: non abdominal subcutaneous; Internal: total internal; IAAT: intra-abdominal; NAIAT: non-abdominal internal.
3.3.12 Impact of Habitual Exercise on Body Composition

All participants filled a clinical health questionnaire prior to having the MR study, see Appendix 1. Within the questionnaire enquiries were made regarding habitual weekly exercise activity.

They were categorized as:

‘Low’: measured as 1-3 times per week

‘Moderate’: 3-5 times per week

‘High’: >5 times per week.

25% of all subjects were classified as having a high exercise level (93% of men, 7% women), 23% had a moderate exercise level (50% men, 50% women) while 52% reported a low exercise level (52% of men, 48% of women).
3.3.12.i Impact of Habitual Exercise on Adiposity

There were significant differences in adipose tissue volume between subjects who reported low/sedentary levels of physical activity, and those who reported both moderate (p<0.001) and high levels of activity (p<0.001). Interestingly, there were no significant differences between the high and moderate groups, see Table 3.16 and Figure 3.8.
Table 3.16 Impact of Exercise Level on Body Composition

<table>
<thead>
<tr>
<th>Exercise Level</th>
<th>Sedentary/Low</th>
<th>Moderate</th>
<th>High</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>number</td>
<td>31</td>
<td>14</td>
<td>15</td>
<td>p</td>
</tr>
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</table>

**Anthropometric Variables**

<table>
<thead>
<tr>
<th></th>
<th>Exercise Level</th>
<th>Sedentary/Low</th>
<th>Moderate</th>
<th>High</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yrs)</td>
<td>49.2 ± 13.7</td>
<td>45.8 ± 14.7</td>
<td>35.9 ± 11.8</td>
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<tr>
<td>Weight (kg)</td>
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<td>79.2 ± 16.8</td>
<td>77.9 ± 6.9</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>36.6 ± 9.3</td>
<td>27.1 ± 4.4</td>
<td>25.0 ± 2.3</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>WC (cm)</td>
<td>113.6 ± 20.9</td>
<td>94.0 ± 14.5</td>
<td>85.8 ± 6.4</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>Hip (cm)</td>
<td>117.6 ± 19.5</td>
<td>104.6 ± 9.8</td>
<td>97.0 ± 5.1</td>
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<tr>
<td>Height (cm)</td>
<td>169.5 ± 10.2</td>
<td>170.4 ± 9.7</td>
<td>176.9 ± 9.6</td>
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<tr>
<td>WHR</td>
<td>0.97 ± 0.11</td>
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<td>0.89 ± 0.05</td>
<td>0.006</td>
<td></td>
</tr>
<tr>
<td>WHtR</td>
<td>0.70 ± 0.14</td>
<td>0.61 ± 0.06</td>
<td>0.55 ± 0.04</td>
<td>&lt;0.001</td>
<td></td>
</tr>
</tbody>
</table>

**Ectopic Liver Fat**

<table>
<thead>
<tr>
<th></th>
<th>Exercise Level</th>
<th>Sedentary/Low</th>
<th>Moderate</th>
<th>High</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>MRS IHCL</td>
<td>12.84 ± 15.67</td>
<td>7.24 ± 12.34</td>
<td>1.06 ± 1.63</td>
<td>0.02</td>
<td></td>
</tr>
<tr>
<td>ME IHCL</td>
<td>9.91 ± 8.36</td>
<td>5.45 ± 6.57</td>
<td>1.95 ± 1.57</td>
<td>0.002</td>
<td></td>
</tr>
</tbody>
</table>

**Ectopic Pancreas Fat**

<table>
<thead>
<tr>
<th></th>
<th>Exercise Level</th>
<th>Sedentary/Low</th>
<th>Moderate</th>
<th>High</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>ME IPCL</td>
<td>7.10 ± 6.36</td>
<td>5.46 ± 7.00</td>
<td>2.95 ± 3.23</td>
<td>0.09</td>
<td></td>
</tr>
</tbody>
</table>

**Adiposity Stores**

<table>
<thead>
<tr>
<th></th>
<th>Exercise Level</th>
<th>Sedentary/Low</th>
<th>Moderate</th>
<th>High</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>TAT (L)</td>
<td>50.63 ± 21.40</td>
<td>28.75 ± 10.41</td>
<td>17.23 ± 5.60</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>SAT (L)</td>
<td>41.51 ± 20.00</td>
<td>23.10 ± 9.23</td>
<td>13.35 ± 4.77</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>ASAT (L)</td>
<td>13.27 ± 7.64</td>
<td>7.01 ± 3.46</td>
<td>3.49 ± 1.50</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>NASAT (L)</td>
<td>28.23 ± 12.75</td>
<td>16.09 ± 5.86</td>
<td>9.85 ± 3.33</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>Internal (L)</td>
<td>9.12 ± 2.88</td>
<td>5.65 ± 2.42</td>
<td>3.89 ± 1.49</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>IAAT (L)</td>
<td>5.12 ± 1.95</td>
<td>3.22 ± 1.87</td>
<td>1.96 ± 1.16</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>NAIAT (L)</td>
<td>4.00 ± 1.15</td>
<td>2.43 ± 0.60</td>
<td>1.93 ± 0.50</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>IAAT/ASAT</td>
<td>0.51 ± 0.30</td>
<td>0.53 ± 0.37</td>
<td>0.57 ± 0.31</td>
<td>0.82</td>
<td></td>
</tr>
</tbody>
</table>

WC: waist circumference; WHR: waist-to-hip ratio; WHtR: waist-to-height ratio; IHCL: intra-hepatocellular lipid; IPCL: intra-pancreatic cellular lipid, Adipose tissue deposits are in litres (L); TAT: total adipose tissue; SAT: subcutaneous; ASAT: abdominal subcutaneous; NASAT: non abdominal subcutaneous; Internal: total internal; IAAT: intra-abdominal; NAIAT: non-abdominal internal. All data are presented as mean ± SD.
3.3.12.ii Impact of Habitual Exercise on Liver Fat Content

IHCL in different exercise level groups are shown in (Figure 3.9). As expected, the higher the exercise level the lower the IHCL content, although there was no significant difference between the moderate and high intensity groups (p=0.36) or the moderate and low groups (p=0.12).
There was a significant difference between the high and low intensity groups (p=0.001).

**Figure 3.9** HCL distributions in the High, Moderate and Low Exercise Level Groups

3.3.12.iii Impact of Habitual Exercise on Pancreatic Fat Content

IPCL levels between the low and moderate, and between the moderate and high intensity exercise groups were not significantly different (p=0.67 and p=0.49 respectively), see Figure 3.10.
However, the difference in IPCL between the high and low intensity exercise groups approached significance ($P=0.074$).

**Figure 3.10** Pancreas Fat (IPCL) Distribution in the High, Moderate and Low Exercise Level Groups
3.3.13 Fat Distribution in the Liver

Currently, IHCL content is reported as part of a localized measurement (MRS) or by measuring the total IHCL content using different non-invasive methods. (Choji 1993, Vanhamme 1997, Szczepaniak, Nurenberg et al. 2005, Thomas, Hamilton et al. 2005, Cassidy, Yokoo et al. 2009, Lee, Park et al. 2010, Reeder, Cruite et al. 2011, Thomas, Parkinson et al. 2012, Angulo, Bugianesi et al. 2013, Thomas, Fitzpatrick et al. 2013) However, these approaches do not give any indication of the possible heterogeneity in the distribution of IHCL across this particular organ.

Therefore, in order to resolve this matter, I have taken a more in depth look at IHCL content by using standard clinical and surgical procedures to segment the liver in the Multi-echo images. Couinaud used the ramifications of the portal vein as the basis of division (Strasberg 2005, Couinaud 1957) whereby the liver is divided into eight functionally independent segments (Figure 3.11). Each segment has its individual vascular inflow, outflow and biliary drainage. In the midpoint of each segment there is a branch of the portal vein, hepatic artery and bile duct. In the periphery of each segment derives vascular outflow through the hepatic veins. (Fischer, Thorn et al. 2005, Fasel, Majno et al. 2010, Couinaud 1957)
Figure 3.11 Image taken from http://www.imaios.com/. There are 8 liver segments, which are numbered in a clockwise manner. Segment 1 (caudate lobe) is located posteriorly, and is not visible on a frontal view.

The same automated pixel-by-pixel analysis performed in the ME of the whole liver described in Chapter 2 was performed to obtain heat maps of segment (I, II & III, IV, V & VIII and VI & VII) respectively according to Couinaud classification (Soler, Delingette et al. 2001, Couinaud 1957, Varupula 2012) and mimicking the CT images of Surgery textbooks and reported studies. (Lafortune, Madore et al. 1991, Smith, Downey et al. 1998, Rieker, Klos et al. 2003, Townsend 2004, Kodama, Ng et al. 2007) Care was taken to avoid blood vessels, bile ducts, and artefacts (Figure 3.12 and Figure 3.13). Segments II & III, V & VIII and VI & VII refer to the superior (II, IV, VII and VIII) and inferior (III, IV, V and VI) plane of the liver (Couinaud 1957) (Figure 3.11).
Figure 3.12 Image taken from http://www.radgray.com showing Sequential CT scan through the liver with Couinaud’s segments divided and numbered
Figure 3.13 Heat maps generated from multi-echo images acquired at various segments throughout the liver showing variations in IHCL

3.3.14 Fat distribution in the pancreas

However, these approaches once again do not give any indication of the possible heterogeneity in the distribution of IPCL across this particular organ. Therefore, applying the same principle as for IHCL distribution to resolve this matter, I have taken a more in depth look at IPCL content by using standard clinical and surgical procedures to divide the pancreas into regions in the Multi-echo images. Surgeons typically describe the location of pathology within the pancreas in relation to four regions: the head, neck, body, and tail. (F. Brunicardi 2009)

The head of the pancreas was defined as the area of the pancreas nestled in the C-loop of the duodenum to the anatomic right of the superior mesenteric vein (F. Brunicardi 2009). The body was defined as the anatomic right half of the remaining pancreatic tissue and the tail was defined as the anatomic left half of the remaining pancreatic tissue. The body and tail of the pancreas lie anterior to the splenic artery and vein (F. Brunicardi 2009, N. S. Patel 2013), see Figure 3.14.
Figure 3.14 Anatomy of the pancreas. Image taking from the Dana-Fraber Cancer institute http://www.dana-farber.org The Pancreas has three areas: head, body, and tail.

Data obtained and analysis of the multi-echo imaging sequences applied the same procedures as the analysis of IHCL (see Chapter 2). Following the automated pixel-by-pixel analysis, heat-maps of total pancreas, as well as the head, body and tail of the pancreas were obtained to provide fat and water percentages (Figure 3.15).

Mean inter-examination coefficient of variation (CoV) $\text{CH}_2+\text{CH}_3/\text{water}+\text{fat}$ examination, was assessed by analysing each segment or region three times, and was found to be $<1\%$ for total and regional IHCL and IPCL.
Figure 3.15 Heat maps generated from multi-echo images acquired at various regions throughout the pancreas showing variations in IPCL
3.3.15 Regional Ectopic Fat Content in the Liver and Pancreas – Whole Cohort Analysis

A summary of the ectopic fat content and distribution for the whole cohort with the novel method in ME is shown in (Table 3.17).

Table 3.17 Whole Cohort Variable Data with Segmentation

<table>
<thead>
<tr>
<th></th>
<th>All Cohort (n=61)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± SD</td>
</tr>
<tr>
<td><strong>Liver Fat</strong></td>
<td></td>
</tr>
<tr>
<td>IHCL (MRS)</td>
<td>8.45 ± 13.47</td>
</tr>
<tr>
<td>Total IHCL (ME)</td>
<td>6.79 ± 7.53</td>
</tr>
<tr>
<td>Segment 1</td>
<td>5.98 ± 6.94</td>
</tr>
<tr>
<td>Segment 2&amp;3</td>
<td>6.22 ± 6.96</td>
</tr>
<tr>
<td>Segment 4</td>
<td>6.77 ± 7.28</td>
</tr>
<tr>
<td>Segment 5&amp;8</td>
<td>7.01 ± 8.31</td>
</tr>
<tr>
<td>Segment 6&amp;7</td>
<td>7.01 ± 8.43</td>
</tr>
<tr>
<td><strong>Pancreatic Fat</strong></td>
<td></td>
</tr>
<tr>
<td>Total IPCL</td>
<td>5.62 ± 6.03</td>
</tr>
<tr>
<td>Head</td>
<td>4.75 ± 5.22</td>
</tr>
<tr>
<td>Body</td>
<td>5.31 ± 6.56</td>
</tr>
<tr>
<td>Tail</td>
<td>4.86 ± 4.77</td>
</tr>
</tbody>
</table>

All data are presented as mean ± SD and range. IHCL: intra-hepatocellular lipid; IPCL: intra-pancreatic cellular lipid.
3.3.15.i Distribution of Fat Content across the Liver in the Whole Cohort

Segment 5&8 (7.01 ± 8.31) and Segment 6&7 (7.01 ± 8.43) had the highest mean values of IHCL compared to other segments in the liver, although none of these differences reach statistical significance (Table 3.17, Figure 3.16).

Figure 3.16 Liver Fat (IHCL) Distributions in Whole Cohort
3.3.15.ii Distribution of Fat Content across the Pancreas in the Whole Cohort

The Body has a higher level of IPCL (5.31 ± 6.56) compared with the Head (4.75 ± 5.22) and Tail (4.86 ± 4.77) regions, though this did not reach statistical significance (Table 3.17, Figure 3.17).

Figure 3.17 Pancreas Fat (IPCL) Distributions in Whole Cohort
3.3.16 Correlation Analysis for Whole Cohort Variable with Segmentation

Whole cohort correlation analysis for all anthropometric variables, adipose tissue (in litres) and ectopic fat stores (arbitrary units) with the novel method in ME are shown in Table 3.18.

All Adiposity store variables except for IAAT/ASAT ratio correlate with ectopic fat stores to a significant degree (p<0.01).

3.3.16.i Liver Fat Content – Relationship between Different Segments and their Association with Adiposity

Total Internal adiposity store was the variable, which correlated to the greatest degree with IHCL in all segments (segment 1: r= 0.69, segment 2&3: r= 0.68, segment 4: r= 0.76, segment 5&8: r= 0.73 and segment 6&7: r= 0.75 p<0.01).

Similar results were found with IAAT adiposity store (segment 1: r= 0.67, segment 2&3: r= 0.67, segment 4: r= 0.75, segment 5&8: r=0.72 and segment 6&7: r= 0.74 p<0.01), see Table 3.18. Moreover, IHCL in all segments remained highly correlated with all anthropometric parameters except for age and height.
3.3.16.ii Pancreatic Fat Content – Relationship between Different Segments and their Association with Adiposity

IAAT adiposity store was correlated most strongly with regional IPCL content (Head: $r=0.41$, Body: $r=0.47$ and Tail: $r=0.40$, $p<0.01$, Table 3.18) but less strongly than the correlation between IHCL and IAAT.

There was also a strong correlation between Internal adiposity and IPCL (Head: $r=0.37$, Body: $r=0.45$ and Tail: $r=0.36$, $p<0.01$).

Age was strongly associated with IPCL (Head: $r=0.39$, Body: $r=0.42$ and Tail: $r=0.38$, $p<0.01$).
Table 3.18 Linear Correlation Analysis between Anthropometric Measurements, Lipid Stores with Segmentation and Body Fat Stores in Whole Cohort.

<table>
<thead>
<tr>
<th>ALLCOHORT</th>
<th>ANTHROPOMETRIC VARIABLES</th>
<th>ECTOPIC FAT STORES</th>
<th>ADIPOSITY STORES in LITRES</th>
</tr>
</thead>
<tbody>
<tr>
<td>n=61</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AGE</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WEIGHT</td>
<td>-0.25</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HEIGHT</td>
<td>-0.40**</td>
<td></td>
<td></td>
</tr>
<tr>
<td>WAIST</td>
<td>0.35**</td>
<td>-0.15</td>
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<tr>
<td>HIP</td>
<td></td>
<td>0.87</td>
<td></td>
</tr>
<tr>
<td>WHR</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WHR</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMI</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MRS IHCL</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ME total</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IHCL</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ME segment 1 IHCL</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ME segment 2&amp;3 IHCL</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ME segment 4 IHCL</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ME segment 5&amp;8 IHCL</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ME segment 6&amp;7 IHCL</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ME total IPCL</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ME Head IPCL</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ME Body IPCL</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ME Tail IPCL</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SAT</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NTAT</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Internal</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TAT</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SAT</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NTAT</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

All data are presented as Spearman rank test correlation. MRS IHCL (M:37 , F:24), ME total IHCL (M:37 , F:24), segment 1 IHCL (M:37 , F:24), segment 2&3 (M:37 , F:24), segment 4 (M:37 , F:24), segment 5&8 (M:37 , F:24), segment 6&7 (M:37 , F:24), ME total IPCL (M:37 , F:24), head (M:37 , F:24) and tail (M:37 , F:24); *= p<0.05,**= p< 0.01. Abbreviations: WC: waist circumference; WHR: waist-to-hip ratio; WHR: waist-to-height ratio; IHCL: intra-hepato-cellular lipid; IPCL: intra-pancreatic cellular lipid; TAT: total adipose tissue; SAT: subcutaneous; SAAT: subcutaneous abdominal; NASAT: non abdominal subcutaneous; Internal: total internal; IAAT: intra-abdominal; NAIAT: non-abdominal internal.
3.3.17 Gender Differences in Regional Ectopic Fat Content

Gender specific characteristics with the novel method in ME are shown in (Table 3.19). Subjects were found to have an overall non significance in all ectopic fat depots and their segments.

Table 3.19 Gender Differences in Regional Ectopic Fat Content

<table>
<thead>
<tr>
<th></th>
<th>Male (n=37)</th>
<th>Female (n=24)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± SD</td>
<td>Range</td>
<td>Mean ± SD</td>
</tr>
<tr>
<td>Liver Fat</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IHCL(MRS)</td>
<td>8.16 ±13.78</td>
<td>0.00 – 59.57</td>
<td>8.91 ± 13.25</td>
</tr>
<tr>
<td>Total IHCL</td>
<td>6.80 ± 7.95</td>
<td>0.70 – 32.27</td>
<td>6.78 ± 7.00</td>
</tr>
<tr>
<td>Segment 1</td>
<td>5.98 ± 7.36</td>
<td>0.16 – 29.42</td>
<td>5.99 ± 6.37</td>
</tr>
<tr>
<td>Segment 2&amp;3</td>
<td>6.02 ± 7.15</td>
<td>0.97 – 29.96</td>
<td>6.53 ± 6.79</td>
</tr>
<tr>
<td>Segment 4</td>
<td>6.97 ± 7.93</td>
<td>0.64 – 32.11</td>
<td>6.47 ± 6.30</td>
</tr>
<tr>
<td>Segment 5&amp;8</td>
<td>7.00 ± 8.80</td>
<td>0.44 – 33.69</td>
<td>7.03 ± 7.67</td>
</tr>
<tr>
<td>Segment 6&amp;7</td>
<td>6.85 ± 8.71</td>
<td>0.59 – 35.21</td>
<td>7.26 ± 8.16</td>
</tr>
<tr>
<td>Pancreatic Fat</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total IPCL</td>
<td>5.18 ± 6.12</td>
<td>0.40 – 24.18</td>
<td>6.29 ± 5.96</td>
</tr>
<tr>
<td>Head</td>
<td>4.34 ± 5.33</td>
<td>0.13 – 23.63</td>
<td>5.37 ± 5.08</td>
</tr>
<tr>
<td>Body</td>
<td>4.49 ± 6.00</td>
<td>0.13 – 25.53</td>
<td>6.57 ± 7.29</td>
</tr>
<tr>
<td>Tail</td>
<td>4.89 ± 5.23</td>
<td>0.14 – 25.29</td>
<td>4.80 ± 4.07</td>
</tr>
</tbody>
</table>

All data are presented as mean ± SD and range. IHCL: intra-hepatocellular lipid; IPCL: intra-pancreatic cellular lipid. Male versus female data was analysed by unpaired Student’s t-test.
3.3.17.i Gender Differences in Regional Liver Fat Content

Segment 5&8 had the highest mean value of IHCL in males compared to other segments (7.00 ± 8.80), whereas segment 6&7 had the highest level of IHCL in female subjects (7.26 ± 8.16) (Table 3.19).

When investigating for gender and age specific interactions using Multivariate linear Regression analysis (AGE x GENDER), the segments of the liver were not significant between gender once the effect of age was adjusted for (Table 3.20, Table 3.21).

3.3.17.ii Gender Differences in Regional Pancreatic Fat Content

In male subjects, the highest mean value of IPCL was found in the Tail compared to other regions (4.89 ± 5.23). In female subjects highest mean value of IPCL was found in the Body compared to other regions (6.57 ± 7.29), (Table 3.19).

A substantial association with age in univariate analysis was observed: Head (p=0.001), Body (p=0.007) and Tail (p=0.001) (Table 3.20). However, there were no difference between men and women in terms of IPCL content in different regions of the pancreas.
In the multivariate analysis, IPCL regions were no longer significant in age or gender (Table 3.21)

**Table 3.20** Gender specific Ectopic Fat with Segmentation in Univariate Analysis

<table>
<thead>
<tr>
<th>Ectopic Liver Fat</th>
<th>GENDER β</th>
<th>GENDER p</th>
<th>AGE β</th>
<th>AGE p</th>
</tr>
</thead>
<tbody>
<tr>
<td>MRS IHCL</td>
<td>-0.76±3.56</td>
<td>0.83</td>
<td>0.10±0.12</td>
<td>0.42</td>
</tr>
<tr>
<td>ME IHCL</td>
<td>0.02±1.99</td>
<td>0.99</td>
<td>0.08±0.07</td>
<td>0.23</td>
</tr>
<tr>
<td>ME segment 1 IHCL</td>
<td>-0.01±1.83</td>
<td>0.10</td>
<td>0.07±0.06</td>
<td>0.29</td>
</tr>
<tr>
<td>ME segment 2&amp;3 IHCL</td>
<td>-0.51±1.84</td>
<td>0.78</td>
<td>0.07±0.06</td>
<td>0.24</td>
</tr>
<tr>
<td>ME segment 4 IHCL</td>
<td>0.50±0.19</td>
<td>0.80</td>
<td>0.09±0.07</td>
<td>0.18</td>
</tr>
<tr>
<td>ME segment 5&amp;8 IHCL</td>
<td>-0.03±2.20</td>
<td>0.99</td>
<td>0.08±0.08</td>
<td>0.28</td>
</tr>
<tr>
<td>ME segment 6&amp;7 IHCL</td>
<td>-0.40±2.22</td>
<td>0.86</td>
<td>0.08±0.08</td>
<td>0.30</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Ectopic Pancreas Fat</th>
<th>GENDER β</th>
<th>GENDER p</th>
<th>AGE β</th>
<th>AGE p</th>
</tr>
</thead>
<tbody>
<tr>
<td>ME IPCL</td>
<td>-1.11±1.59</td>
<td>0.486</td>
<td>0.19±0.05</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>ME Head IPCL</td>
<td>-0.10±1.37</td>
<td>0.455</td>
<td>0.15±0.04</td>
<td>0.001</td>
</tr>
<tr>
<td>ME Body IPCL</td>
<td>-2.08±1.71</td>
<td>0.230</td>
<td>0.16±0.06</td>
<td>0.007</td>
</tr>
<tr>
<td>ME Tail IPCL</td>
<td>0.09±1.26</td>
<td>0.944</td>
<td>0.14±0.04</td>
<td>0.001</td>
</tr>
</tbody>
</table>

The effect of age and gender on ectopic fat stores was determined using univariate regression analysis. Ectopic fat was the dependent variables with age (year) and gender (male = 1, female = 0) as the independent variables. β represents the regression coefficient for each independent variable included in the model ± standard error. a Gender β represents difference for male minus female. IHCL: intra-hepatocellular lipid; IPCL: intra-pancreatic cellular lipid.
The effect of age and gender on ectopic fat stores was determined using a multivariate regression analysis. Ectopic fat was the dependent variables with age (year), gender (male = 1, female = 0) and GENDER X AGE interaction as independent variables. β represents the regression coefficient for each independent variable included in the final model ± standard error. Gender β represents difference for male minus female. b represents all the gender x age interaction that was significant. Statistical analysis was performed on log10 transformed variables. IHCL: intra-hepatocellular lipid; IPCL: intra-pancreatic cellular lipid.

### 3.3.18 Correlation Analysis Gender Differences in Regional Ectopic Fat Content

Gender-specific correlation analysis for all anthropometric variables, adipose tissue (in litres) and ectopic fat stores (arbitrary units) with the novel method in ME are shown in Table 3.22 (men) and Table 3.23 (women). Apart from a few exceptions, all variables correlated with each other to a significant degree (p<0.01).
Table 3.22 Linear Correlation Analysis between Anthropometric Measurements, Lipid Stores with Segmentation and Body Fat Stores in Male Participants.

<table>
<thead>
<tr>
<th>MELES</th>
<th>ANTHROPOMETRIC VARIABLES</th>
<th>ECTOPIC FAT STORES</th>
<th>ADIPOCYTES STORES in LITRES</th>
</tr>
</thead>
<tbody>
<tr>
<td>AGE</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>AGE</td>
<td>WEIGHT</td>
<td>HEIGHT</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>WAIST</td>
</tr>
<tr>
<td></td>
<td></td>
<td>HIP</td>
<td>WHR</td>
</tr>
<tr>
<td></td>
<td></td>
<td>WHRR</td>
<td>BMI</td>
</tr>
<tr>
<td></td>
<td></td>
<td>MRS HCL</td>
<td>ME total IHCL</td>
</tr>
<tr>
<td></td>
<td></td>
<td>ME segment 1 IHCL</td>
<td>ME segment 2&amp;3 IHCL</td>
</tr>
<tr>
<td></td>
<td></td>
<td>ME segment 4 IHCL</td>
<td>ME segment 5&amp;6 IHCL</td>
</tr>
<tr>
<td></td>
<td></td>
<td>ME segment 8&amp;9 IHCL</td>
<td>ME total IPCL</td>
</tr>
<tr>
<td></td>
<td></td>
<td>ME Head IPCL</td>
<td>ME Body IPCL</td>
</tr>
<tr>
<td></td>
<td></td>
<td>ME Tail IPCL</td>
<td>TAT</td>
</tr>
<tr>
<td></td>
<td></td>
<td>SAT</td>
<td>SAT</td>
</tr>
<tr>
<td></td>
<td></td>
<td>NAISAT</td>
<td>NAISAT</td>
</tr>
<tr>
<td></td>
<td></td>
<td>IAAT</td>
<td>IAAT</td>
</tr>
<tr>
<td></td>
<td></td>
<td>NAIAAT</td>
<td>NAIAAT</td>
</tr>
<tr>
<td></td>
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</tr>
<tr>
<td></td>
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<td></td>
</tr>
<tr>
<td></td>
<td>AGE</td>
<td>1.16</td>
<td>0.333*</td>
</tr>
<tr>
<td></td>
<td>WEIGHT</td>
<td>-0.80**</td>
<td>0.34*</td>
</tr>
<tr>
<td></td>
<td>WAIST</td>
<td>0.46**</td>
<td>0.72**</td>
</tr>
<tr>
<td></td>
<td>HIP</td>
<td>0.22</td>
<td>0.824**</td>
</tr>
<tr>
<td></td>
<td>WHR</td>
<td>0.570**</td>
<td>0.24**</td>
</tr>
<tr>
<td></td>
<td>WHRR</td>
<td>0.49**</td>
<td>0.702**</td>
</tr>
<tr>
<td></td>
<td>BMI</td>
<td>0.375**</td>
<td>0.837**</td>
</tr>
<tr>
<td></td>
<td>MRS HCL</td>
<td>0.365**</td>
<td>0.847**</td>
</tr>
<tr>
<td></td>
<td>ME total IHCL</td>
<td>0.455**</td>
<td>0.803**</td>
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<tr>
<td></td>
<td>ME segment 1 IHCL</td>
<td>0.611**</td>
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<td>ME segment 2&amp;3 IHCL</td>
<td>0.411**</td>
<td>0.850**</td>
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<td></td>
<td>ME segment 4 IHCL</td>
<td>0.456**</td>
<td>0.852**</td>
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<td>ME segment 5&amp;6 IHCL</td>
<td>0.489**</td>
<td>0.878**</td>
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<td>ME segment 8&amp;9 IHCL</td>
<td>0.431**</td>
<td>0.844**</td>
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<tr>
<td></td>
<td>ME total IPCL</td>
<td>0.602**</td>
<td>0.812**</td>
</tr>
<tr>
<td></td>
<td>ME Head IPCL</td>
<td>0.467**</td>
<td>0.806**</td>
</tr>
<tr>
<td></td>
<td>ME Body IPCL</td>
<td>0.424**</td>
<td>0.785**</td>
</tr>
<tr>
<td></td>
<td>ME Tail IPCL</td>
<td>0.507**</td>
<td>0.780**</td>
</tr>
<tr>
<td></td>
<td>SAT</td>
<td>0.374**</td>
<td>0.821**</td>
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<tr>
<td></td>
<td>NAISAT</td>
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<td>0.842**</td>
</tr>
<tr>
<td></td>
<td>IAAT</td>
<td>0.350**</td>
<td>0.884**</td>
</tr>
<tr>
<td></td>
<td>NAIAAT</td>
<td>0.471**</td>
<td>0.850**</td>
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<tr>
<td></td>
<td>RAISAT</td>
<td>0.520**</td>
<td>0.815**</td>
</tr>
</tbody>
</table>

All data are presented as Spearman rank test correlation. MRS IHCL (M:37), ME total IHCL (M: 37), segment 1 (M:37), segment 2&3 (M: 37 ), segment 4 (M:37 ), segment 5&8 (M:37 ), segment 6&7 (M:37 ), total IPCL(M:37 ), head (M:37 ), body (M:37 ) and tail (M:37); *p < 0.05,**p< 0.01. Abbreviations: WC: waist circumference; WHR: waist-to-hip ratio; WHR: waist-to-height ratio; IHCL: intra-hepaticcellular lipid; IPCL: intra-penguinocellular lipid; TAT: total adipose tissue; SAT: subcutaneous; SAAT: subcutaneous abdominal; NAASAT: non abdominal subcutaneous; Internal: total internal; IAAT: intra-abdominal; NAIAAT: non-abdominal internal.
Table 3.23 Linear Correlation Analysis between Anthropometric Measurements, Lipid Stores with Segmentation and Body Fat Stores in Female Participants.

<table>
<thead>
<tr>
<th>Females</th>
<th>Anthropometric Variables</th>
<th>Ectopic Fat Stores</th>
<th>Adiposity Stores in Litres</th>
</tr>
</thead>
<tbody>
<tr>
<td>n=24</td>
<td>AGE</td>
<td>WEIGHT</td>
<td>HEIGHT</td>
</tr>
<tr>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>AGE</td>
<td>-0.392</td>
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<td></td>
</tr>
<tr>
<td>WEIGHT</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>HEIGHT</td>
<td>-0.22</td>
<td></td>
<td></td>
</tr>
<tr>
<td>WAIST</td>
<td>-0.13</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HIP</td>
<td>-0.31</td>
<td></td>
<td></td>
</tr>
<tr>
<td>WHR</td>
<td>-0.14</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMI</td>
<td>-0.31</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

All data are presented as Spearman rank test correlation. MRS IHC (F: 24), ME total IHC (F: 24), ME segment 1 (F: 24), ME segment 2&3 (F: 24), ME segment 4 (F: 24), ME segment 5&6 (F: 24), ME total IPCL (F: 24), ME head (F: 24), body (F: 24) and tail (F: 24); p<0.05; **p<0.01. Abbreviations: WC: waist circumference; WHR: waist-to-height ratio; WHtR: waist-to-thigh ratio; IHCL: intra-hepato cellular lipid; IPCL: intra-pancreatic cellular lipid, TAT: total adipose tissue; SAT: subcutaneous; SAAT: subcutaneous abdominal; NASAT: non abdominal subcutaneous; Internal: total internal; IAAT: intra-abdominal; NAIAT: non-abdominal internal

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3.3.18.i Correlation Analysis Gender Differences in Regional Liver Fat Content

In male subjects, *Internal* adiposity store was the variable, which correlated to the greatest degree with IHCL in individual segments (segment 1: $r=0.68$, segment 2&3: $r=0.62$, segment 4: $r=0.75$, segment 5&8: $r=0.70$ and segment 6&7: $r=0.71$, $p<0.01$), followed by IAAT (segment 1: $r=0.65$, segment 2&3: $r=0.61$, segment 4: $r=0.74$, segment 5&8: $r=0.70$ and segment 6&7: $r=0.71$, $p<0.01$).

In addition, all anthropometric parameters correlated strongly with IHCL in different segments, except for height, see Table 3.22.

While in female subjects, IAAT had the strongest correlation with IHCL in all segments (segment 1: $r=0.70$, segment 2&3: $r=0.79$, segment 4: $r=0.78$, segment 5&8: $r=0.80$ and segment 6&7: $r=0.79$, $p<0.01$, Table 3.23), followed by *Internal* (segment 1: $r=0.70$, segment 2&3: $r=0.78$, segment 4: $r=0.77$, segment 5&8: $r=0.78$ and segment 6&7: $r=0.78$, $p<0.01$).

Moreover, all anthropometric parameters correlated strongly with IHCL in different segments, except for Age and Height.
3.3.18.ii Correlation Analysis Gender Differences in Regional Pancreatic Fat Content

In male subjects, IAAT was the variable, which correlated to the greatest degree with IPCL in individual regions (Body: $r=0.43$, $p<0.01$ and Head: $r=0.39$, $p<0.05$, Table 3.22) with the exception of (Tail: $r=0.30$). **Internal** adiposity store also correlated strongly with IPCL in individual regions (Body: $0.42$ $p<0.05$ and Head, $r=0.34$, $p<0.05$) with the exception once again of (Tail: $r=0.29$).

In addition, Age correlated strongly with IPCL in different pancreatic regions. (Head: $r=0.47$, Body: $r=0.42$ and Tail: $r=0.51$, $p<0.01$, Table 3.22)

In female subjects, IAAT had the strongest correlation with IPCL in individual regions (Head: $r=0.64$, Body: $r=0.52$ and Tail: $r=0.61$, $p<0.01$, Table 3.23), followed by **Internal** (Head: $r=0.59$ $p<0.01$, Body: $r=0.49$ $p<0.05$ and Tail: $r=0.54$ $p<0.01$, Table 3.23).

In female subjects, IPCL did not associate with any of the anthropometric parameters in different pancreatic regions.
3.3.18.iii Correlation Analysis Gender Differences in Regional Ectopic Fat Content – The Impact of Correcting for Weight and Waist

When Weight or Waist was adjusted for separately in male and female subjects, none of the liver segments nor the pancreatic regions correlated to a significant level with adiposity or anthropometric parameters. The exception was a strong association between IPCL in all regions and Age when Weight was adjusted for in men (Head: $r=0.52$, Body: $r=0.49$ and Tail: $r=0.53$, all $p=0.001$).

Similar correlations were found between Age and IPCL in the regions when Waist was adjusted for in men; the partial correlation was stronger when Weight was controlled for than when Waist was controlled for (Head: $r=0.480$, Body: $r=0.459$, and Tail: $r=0.509$, all $p<0.05$).
3.3.19 The Effect of Age on Regional Fat Content in the Liver

As seen previously (Table 3.10) the highest levels of total IHCL were seen in subjects between the ages of 36-45 years old (9.03 ± 10.88).

The same was observed for the regional analysis, with subjects aged between 36-45 years old having the highest level of IHCL in each liver segment, although these differences did not reach the level of significance, see Figure 3.18, and Table 3.24.

![Figure 3.18 Variation in Regional IHCL Content in Different Age Groups](image-url)
Table 3.24 Age Group Specific Summary measures in Whole Cohort with Segmentation

<table>
<thead>
<tr>
<th>Age Group</th>
<th>16-25</th>
<th>26-35</th>
<th>36-45</th>
<th>&gt;46</th>
</tr>
</thead>
<tbody>
<tr>
<td>number</td>
<td>9</td>
<td>8</td>
<td>12</td>
<td>32</td>
</tr>
</tbody>
</table>

Liver Fat

<table>
<thead>
<tr>
<th></th>
<th>Data for whole cohort</th>
</tr>
</thead>
<tbody>
<tr>
<td>IHCL (MRS)</td>
<td>2.51 ± 4.41</td>
</tr>
<tr>
<td>Total IHCL (ME)</td>
<td>2.64 ± 3.64</td>
</tr>
<tr>
<td>Segment 1-2&amp;3</td>
<td>2.39 ± 2.91</td>
</tr>
<tr>
<td>Segment 4</td>
<td>2.58 ± 3.42</td>
</tr>
<tr>
<td>Segment 5&amp;8</td>
<td>2.43 ± 3.93</td>
</tr>
<tr>
<td>Segment 6&amp;7</td>
<td>2.67 ± 4.67</td>
</tr>
</tbody>
</table>

Pancreatic Fat

<table>
<thead>
<tr>
<th></th>
<th>Data for whole cohort</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total IPCL</td>
<td>1.72 ± 1.30</td>
</tr>
<tr>
<td>Head</td>
<td>1.50 ± 0.84</td>
</tr>
<tr>
<td>Body</td>
<td>1.46 ± 1.39</td>
</tr>
<tr>
<td>Tail</td>
<td>2.28 ± 1.76</td>
</tr>
</tbody>
</table>

All data are presented as mean ± SD. IHCL: intra-hepatocellular lipid; IPCL: intra-pancreatic cellular lipid. Age ranges: 1: 16-25 years, 2: 26-35 years, 3: 36-45 years, 4: 46+ years old. Differences between age ranges were tested for using ANOVA and Post-hoc Turkey analysis.

3.3.20 The Effect of Age on Regional Fat Content in the Pancreas

There was a significant increase in total IPCL content across the different age groups (p<0.05), however whilst IPCL levels increased by age group in all three pancreatic levels, these differences did not quite reach statistical significance, see Table 3.24 and Figure 3.19.
Figure 3.19 Variation in Regional IPCL Content across Different Age Groups
3.3.21 The Effect of BMI on Regional Fat Content in the Liver

The mean value of liver ectopic fat (IHCL) increased significantly (p<0.001 for all) in the different BMI groups, see Table 3.25 and Figure 3.20.

Post hoc analysis revealed significant differences in IHCL content between BMI groups 1 and 3 (p<0.05) and also between BMI groups 1 and 4 (p<0.01) for all liver segments. Additionally for segment 2&3 there was an additional difference between BMI groups 2 and 4 (p=0.09).
Table 3.25 BMI Group Specific Summary measures with Segmentation in Whole Cohort

<table>
<thead>
<tr>
<th></th>
<th>Data for whole cohort</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>BMI Group</strong></td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td><strong>p</strong></td>
</tr>
<tr>
<td><strong>number</strong></td>
<td>16</td>
<td>20</td>
<td>11</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td><strong>Liver Fat</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IHCL (MRS)</td>
<td>0.54 ± 0.55</td>
<td>6.39 ± 10.94</td>
<td>14.47 ± 16.73</td>
<td>15.72 ± 16.60</td>
<td>0.004</td>
</tr>
<tr>
<td>Total IHCL (ME)</td>
<td>1.43 ± 0.62</td>
<td>5.81 ± 6.65</td>
<td>11.14 ± 9.34</td>
<td>10.90 ± 7.63</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Segment 1</td>
<td>1.15 ± 0.64</td>
<td>5.28 ± 5.91</td>
<td>9.60 ± 8.65</td>
<td>9.66 ± 7.60</td>
<td>0.001</td>
</tr>
<tr>
<td>Segment 2&amp;3</td>
<td>1.58 ± 0.70</td>
<td>5.30 ± 6.23</td>
<td>9.29 ± 9.16</td>
<td>10.41 ± 6.87</td>
<td>0.001</td>
</tr>
<tr>
<td>Segment 4</td>
<td>1.39 ± 0.86</td>
<td>5.78 ± 6.63</td>
<td>11.30 ± 8.50</td>
<td>10.78 ± 7.21</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Segment 5&amp;8</td>
<td>1.26 ± 0.62</td>
<td>5.94 ± 7.20</td>
<td>11.80 ± 10.43</td>
<td>11.37 ± 8.74</td>
<td>0.001</td>
</tr>
<tr>
<td>Segment 6&amp;7</td>
<td>1.23 ± 0.65</td>
<td>5.84 ± 7.12</td>
<td>11.83 ± 10.59</td>
<td>11.50 ± 9.10</td>
<td>0.001</td>
</tr>
<tr>
<td><strong>Pancreatic Fat</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total IPCL</td>
<td>1.75 ± 0.92</td>
<td>5.70 ± 6.04</td>
<td>8.48 ± 7.18</td>
<td>7.66 ± 6.64</td>
<td>0.01</td>
</tr>
<tr>
<td>Head</td>
<td>2.42 ± 1.99</td>
<td>5.04 ± 5.08</td>
<td>5.56 ± 7.01</td>
<td>6.34 ± 5.96</td>
<td>0.19</td>
</tr>
<tr>
<td>Body</td>
<td>1.83 ± 1.43</td>
<td>4.71 ± 5.72</td>
<td>7.38 ± 7.06</td>
<td>8.54 ± 8.93</td>
<td>0.02</td>
</tr>
<tr>
<td>Tail</td>
<td>2.37 ± 1.51</td>
<td>5.13 ± 5.68</td>
<td>6.86 ± 5.65</td>
<td>5.74 ± 4.34</td>
<td>0.07</td>
</tr>
</tbody>
</table>

IHCL: intra-hepatocellular lipid; IPCL: intra-pancreatic cellular lipid. All data are presented as mean ± SD. BMI grouping corresponds to the following BMI ranges: 1: 18.5<25, 2: 25<30, 3: 30<40, 4: 40+. Differences between BMI groups were tested for using ANOVA and Post-hoc Turkey analysis.
3.3.22 The Effect of BMI on Regional Fat Content in the Pancreas

The effect of BMI on regional differences in IPCL were less clear cut. There was a trend for IPCL to increase with increasing BMI group, but this only reached significance in the body of the pancreas (between BMI groups 1 and 4, \( p<0.05 \)), with no statistically significant differences in the head or tail regions, despite a strong trend, Table 3.25 and Figure 3.21).
3.3.23 Impact of BMI Group on Correlation Analysis of Body Composition and Regional Ectopic Fat Content

BMI group correlation analysis for all anthropometric variables, adipose tissue (in litres) and ectopic fat stores (arbitrary units) with the novel method ME segmentation analysis are shown in Table 3.26 (Group 1 = 18<25 kg/m²), Table 3.27 (Group 2 = 25<30 kg/m²), Table 3.28 (Group 3 =30<40 kg/m²) and Table 3.29 (Group 4 =40+ kg/m²).
In Group 1, apart from a few exceptions mentioned below, most variables did not correlate with each other.

3.3.23.i Correlation Analysis of Body Composition and Regional Liver Fat Content in BMI Group 1

*Internal* adiposity store was the variable, which correlated to the greatest degree with some individual segments in the liver (segment 5&8: r=0.65, segment 6&7: r=0.71, all p<0.01, segment 4: r=0.61, p<0.05, Table 3.26). Similarly there was a strong correlation between IAAT and regional IHCL (segment 5&8: r=0.55 and segment 6&7: r=0.68, all p<0.01, segment 4: r=0.51, p<0.05).

Waist was the only anthropometric variable that correlated to some degree to regional liver fat content (segment 4: r=0.52, segment 5&8: r=0.51 and segment 6&7: r = 0.60, p<0.05).
3.3.23.ii Correlation Analysis of Body Composition and Regional Pancreatic Fat Content in BMI Group 1

There were no significant correlations between regional IPCL and adiposity, although there was a significant negative association between Body IPCL and Weight ($r = -0.58$, $p<0.05$, Table 3.24) and between Body IPCL and Waist ($r = -0.52$, $p<0.05$, Table 3.26)
### Table 3.26 Linear Correlation Analysis between Anthropometric Measurements, Lipid Stores with Segmentation and Body Fat Stores in BMI Group 1.

<table>
<thead>
<tr>
<th>ME Group 1</th>
<th>ANTHROPOMETRIC VARIABLES</th>
<th>ECTOPIC FAT STORES</th>
<th>ADIPOSIY STORES in LITRES</th>
</tr>
</thead>
<tbody>
<tr>
<td>n=50</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

| | ME Total IHCL | ME segment 1 IHCL | ME segment 2 & 3 IHCL | ME segment 4 & 5 IHCL | ME segment 6 & 7 IHCL | ME Total IPCL | ME Head IPCL | ME Body IPCL | ME Tail IPCL | TAT | SAT | Internal | SAAT | NASAT | IAAT |
| | | | | | | | | | | | | | | | |
| AGE | .017 | .899* | .268 |               |               |               |               |               |               |               | .512* | .512* | .512* | .512* | .512* |
| WEIGHT | .247 | .046 | .244 | .235 |               |               |               |               |               |               | .268 | .321 | .321 | .321 | .321 |
| HIP | .372 | .009 .645** | .907* |               |               |               |               |               |               |               | .268 | .321 | .321 | .321 | .321 |
| WHR | .522** | .638** | .080 | .487 | .264 |               |               |               |               |               | .268 | .321 | .321 | .321 | .321 |
| BMI | .233 | .076 | .256 | .280 | .638** | .041 | .800* |               |               |               | .268 | .321 | .321 | .321 | .321 |
| ME Liver | .061 | .397 | .237 | .394 | .013 | .188 | .103 | .11 |               |               | .268 | .321 | .321 | .321 | .321 |
| ME total IHCL | .063 | .441 | .362 | .629** | .115 | .315 | .044 | .102 | .72** |               | .268 | .321 | .321 | .321 | .321 |
| ME segment 1 IHCL | .004 | .247 | .265 | .222 | .137 | .003 | .908 | .906 | .908** | .906** | .268 | .321 | .321 | .321 | .321 |
| ME segment 4 & 5 IHCL | -.009 | .173 | .341 | .523* | .187 | .160 | 0 | .067 | .664** | .664** | .321 | .321 | .321 | .321 | .321 |
| ME segment 6 & 7 IHCL | -.094 | .390 | .387 | .514** | .186 | .178 | .050 | .034 | .87** | .87** | .321 | .321 | .321 | .321 | .321 |
| ME total IPCL | .549 | .039 | .722 | .073 | .365 | .097 | .247 | .162 | .185 | .047 | .271 | .184 | .025 | .065 | .321 |
| ME Head IPCL | .445 | -.224 | .328 | .086 | .196 | .179 | .197 | .319 | -.086 | -.147 | -.044 | -.247 | -.077 | -.168 | -.147 | .739** |
| ME Body IPCL | .0145 | -.570* | -.356 | -.512* | -.108 | -.291 | -.003 | -.212 | -.009 | -.356 | .041 | -.237 | -.216 | -.191 | -.406 | .459 | .322 |
| ME Tail IPCL | .211 | .141 | -.059 | .185 | .169 | .132 | -.116 | -.329 | .008 | -.171 | -.108 | -.188 | -.012 | -.066 | -.510 | .474 | -.018 |
| TAT | .466 | -.229 | -.517* | .040 | .074 | -.309 | .92** | .585* | .15 | -.062 | .118 | -.241 | .059 | .125 | .115 | .171 | .12 | .009 | -.226 |
| SAT | .438 | -.228 | -.522* | -.089 | .458 | -.321 | .92** | .585* | .056 | -.018 | .035 | -.253 | -.001 | .054 | .044 | .147 | .074 | .053 | -.286 |
| Internal | .0528 | .135 | .040 | .690** | .211 | .353 | .309 | .285 | .212 | .679** | .456 | .515 | .612* | .645** | .712** | .138 | .147 | -.274 | -.103 | .432 | .524 |
| SAAT | .549 | -.318 | -.623* | -.684 | .302 | -.221 | .885** | .585* | .053 | -.074 | .015 | .3 | -.069 | .004 | .029 | .226 | .153 | .026 | -.191 | .929** | .957** | .282 |
| NASAT | .231 | -.366 | -.371* | -.638 | .468 | -.362 | .906** | .580* | .059 | -.012 | .064 | -.256 | .086 | .640 | .029 | .115 | .103 | .082 | -.297 | .899** | .986** | .624 | .554** |
| IAAT | .126 | .019 | .600** | .122 | .345 | .328 | .241 | .044 | .608 | .031 | .268 | .512* | .552* | .679** | .085 | .121 | -.408 | -.205 | .439 | .229 | .589** | .212 | .228 |
| NIAAT | .158 | .200 | .508* | .402 | .012 | .347 | .276 | .530* | .644** | .635** | .153 | .65* | .675** | .712** | .01 | .147 | -.194 | -.286 | .518 | .042 | .882** | .344 | .424 | .789** |
| KAT/SAT | -.064 | .380 | .446 | .665** | -.031 | .488 | -.271 | -.079 | .312 | .508 | .151 | .333 | .403 | .408 | .529* | -.106 | -.089 | -.465 | .118 | -.429 | -.034 | .659** | -.400 | -.315 | .774** |

All data are presented as Spearman rank test correlation. *= p<0.05, **= p< 0.01. Abbreviations: WC: waist circumference; WHR: waist-to-hip ratio; WHtR: waist-to-height ratio; IHCL: intra-hepatocellular lipid; IPCL: intra-pancreatic cellular lipid, TAT: total adipose tissue; SAT: subcutaneous; SAAT: subcutaneous abdominal; NASAT: non abdominal subcutaneous; Internal: total internal; IAAT: intra-abdominal; NIAAT: non-abdominal internal.
3.3.23.iii Correlation Analysis of Body Composition and Regional Liver Fat Content in BMI Group 2

In Group 2, from the anthropometric parameters, Height was greatly correlated with IHCL in different segments (segment 1: $r=0.53$, segment 5&8: $r=0.54$ and segment 6&7: $r=0.50$, all $p<0.05$, and segment 2&3: $r=0.69$, segment 4: $r=0.58$, both $p<0.01$, see Table 3.25). There were also significant correlations between WHR and regional IHCL content (segment 1: $r=0.57$, segment 2&3: $r=0.59$, segment 5&8: $r=0.57$ and segment 6&7: $r=0.56$, all $p<0.01$ segment 4: $r=0.56$, $p<0.05$).

The adipose variable which correlated most strongly with regional IHCL content in BMI group 2 was the IAAT/ASAT ratio, (segment 2&3: $r = 0.60$, $p<0.01$, segment 1: $r=0.55$, segment 4: $r=0.53$, segment 5&8: $r =0.54$ and segment 6&7: $r=0.51$, all $p<0.05$, Table 3.27).

3.3.23.iv Correlation Analysis of Body Composition and Regional Pancreatic Fat Content in BMI Group 2

IAAT, was the adipose depot, which correlated to the greatest degree with IPCL in the head and body of the pancreas, but not the tail (Head: $r=0.63$, $p<0.01$ and Body: $r=0.47$, $p<0.05$). There were no significant correlations between regional IPCL and anthropometric variables.
### Table 3.27 Linear Correlation Analysis between Anthropometric Measurements, Lipid Stores with Segmentation and Body Fat Stores in BMI Group 2.

<table>
<thead>
<tr>
<th>ME Group 2</th>
<th>ANTHROPOMETRIC VARIABLES</th>
<th>ECTOPIC FAT STORES</th>
<th>ADIPOSITY STORES in LITRES</th>
</tr>
</thead>
<tbody>
<tr>
<td>n=50</td>
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<td></td>
<td></td>
</tr>
<tr>
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<td></td>
</tr>
<tr>
<td>WEIGHT</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>HEIGHT</td>
<td>-0.385**</td>
<td>-0.346**</td>
<td></td>
</tr>
<tr>
<td>WAIST</td>
<td>0.213</td>
<td>0.387</td>
<td>-0.069*</td>
</tr>
<tr>
<td>HIP</td>
<td>0.260</td>
<td>0.803</td>
<td>-0.488</td>
</tr>
<tr>
<td>WHR</td>
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<td>0.366</td>
<td>0.502**</td>
</tr>
<tr>
<td>MHR</td>
<td>0.361</td>
<td>-0.351</td>
<td>-0.458*</td>
</tr>
<tr>
<td>BMI</td>
<td>-0.129</td>
<td>0.263</td>
<td>-0.325</td>
</tr>
<tr>
<td>ANS Liver</td>
<td>0.062</td>
<td>0.318</td>
<td>-0.577**</td>
</tr>
<tr>
<td>ME total IHCL</td>
<td>0.777</td>
<td>0.293</td>
<td>-0.524*</td>
</tr>
<tr>
<td>ME segment 1 IHCL</td>
<td>-0.178</td>
<td>0.244</td>
<td>-0.528*</td>
</tr>
<tr>
<td>ME segment 2 IHCL</td>
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<td>-0.684**</td>
</tr>
<tr>
<td>ME segment 4 IHCL</td>
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<td>0.255</td>
<td>0.579**</td>
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<tr>
<td>ME segment 5 &amp; 8 IHCL</td>
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<td>0.263</td>
<td>0.541*</td>
</tr>
<tr>
<td>ME segment 6 &amp; 7 IHCL</td>
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<td>0.228</td>
<td>0.477*</td>
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<tr>
<td>ME total IPCL</td>
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<td>0.284</td>
<td>0.479*</td>
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<tr>
<td>ME Head IPCL</td>
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<td>0.290</td>
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<tr>
<td>ME Body IPCL</td>
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<td>0.444</td>
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<td>ME Tail IPCL</td>
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<tr>
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<td>-0.336</td>
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<td>0.359</td>
</tr>
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<td>SAAT</td>
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<td>-0.255</td>
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<td>SAT/ASAT</td>
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<td>0.267</td>
<td>0.438</td>
</tr>
</tbody>
</table>

All data are presented as Spearman rank test correlation. * = p < 0.05, ** = p < 0.01. Abbreviations: WC: waist circumference; WHR: waist-to-hip ratio; WHtR: waist-to-height ratio; IHCL: intra-hepatoceleular lipid; IPCL: intra-pancreatic cellular lipid, TAT: total adipose tissue; SAT: subcutaneous; SAAT: subcutaneous abdominal; NASAT: non abdominal subcutaneous; Internal: total internal; IAAT: intra-abdominal; NAIAT: non-abdominal internal.
3.3.23.v Correlation Analysis of Body Composition and Regional Liver Fat Content in BMI Group 3

In Group 3, apart from IAAT/ASAT ratio which showed a strong correlation with IHCL in segment 4 of the liver ($r=0.71\ p<0.05$, Table 3.28) and segment 5&8 of the liver ($r= 0.63\ p<0.05$, Table 3.28) variables did not correlate with each other.

3.3.23.vi Correlation Analysis of Body Composition and Regional Pancreatic Fat Content in BMI Group 3

There was no association between regional IPCL and any of the adiposity depots in BMI group 3 (Table 3.28).

Head was only associated with Age ($r= 0.62,\ p<0.05$) and Body negatively with Weight ($r= -0.62,\ p<0.05$).
### Table 3.28 Linear Correlation Analysis between Anthropometric Measurements, Lipid Stores with Segmentation and Body Fat Stores in BMI Group 3.

<table>
<thead>
<tr>
<th>BMI Group 3</th>
<th>ANTHROPOMETRIC VARIABLES</th>
<th>ECTOPIC FAT STORES</th>
<th>ADIPOSITY STORES in LITRES</th>
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<tr>
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<tr>
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<td>.182</td>
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<tr>
<td>ME segment 2&amp;3 HIACL</td>
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<td>-.036</td>
<td>-.045</td>
</tr>
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<td>.082</td>
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<td>ME segment 5&amp;6 HIACL</td>
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<tr>
<td>ME segment 6&amp;7 HIACL</td>
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<tr>
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<tr>
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<td>.064</td>
<td>-.255</td>
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<tr>
<td>ME segment 5&amp;6 HIACL</td>
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<td>.464</td>
<td>.773**</td>
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<tr>
<td>ME segment 6&amp;7 HIACL</td>
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<td>-.258</td>
</tr>
<tr>
<td>ME total IPCL</td>
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<td>.071</td>
<td>-.071</td>
</tr>
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<td>TAT</td>
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<td>SAT</td>
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<td>.036</td>
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<tr>
<td>Internal</td>
<td>.473</td>
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<tr>
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<td>.382</td>
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<td>.382</td>
<td>.019</td>
</tr>
<tr>
<td>AAAT</td>
<td>.355</td>
<td>.064</td>
<td>.909</td>
</tr>
<tr>
<td>MRS IPCL</td>
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<td>.071</td>
<td>-.137</td>
</tr>
<tr>
<td>MRS Liver</td>
<td>.355</td>
<td>.071</td>
<td>-.109</td>
</tr>
<tr>
<td>ME total HIACL</td>
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<td>.064</td>
<td>.382</td>
</tr>
<tr>
<td>ME segment 1 HIACL</td>
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<td>.073</td>
<td>-.258</td>
</tr>
<tr>
<td>ME segment 2&amp;3 HIACL</td>
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<td>.073</td>
<td>-.258</td>
</tr>
<tr>
<td>ME segment 4 HIACL</td>
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<td>.073</td>
<td>-.258</td>
</tr>
<tr>
<td>ME segment 5&amp;6 HIACL</td>
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<td>.073</td>
<td>-.258</td>
</tr>
<tr>
<td>ME segment 6&amp;7 HIACL</td>
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<td>.073</td>
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<tr>
<td>ME total IPCL</td>
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<td>.064</td>
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<td>SAT</td>
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<td>.382</td>
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<tr>
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<tr>
<td>MRS IPCL</td>
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<tr>
<td>MRS Liver</td>
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<td>.071</td>
<td>-.109</td>
</tr>
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3.3.23.vii Correlation Analysis of Body Composition and Regional Liver Fat Content in BMI Group 4

In BMI Group 4, IHCL in all segments correlated to the greatest degree with NAIAT (segment 1: r=0.77, segment 2&3: r=0.69, segment 4: r=0.75, segment 5&8: r=0.79 and segment 6&7: r=0.79, all p<0.01, Table 3.29) followed by Internal adiposity store in certain segments (segment 2&3: r=0.54, segment 4: r=0.56 and segment 5&8: r=0.56, all p<0.05).

3.3.23.viii Correlation Analysis of Body Composition and Regional Pancreatic Fat Content in BMI Group 4

IPCL was negatively associated with WHR in the Head (r=-0.54 p<0.05) and Body (r=-0.59 p<0.05) and Tail (r= -0.55, p<0.05, Table 3.29) of the pancreas.

In addition, Head IPCL was significantly associated with WHtR (r= 0.56, p<0.05, Table 3.27) and Tail IPCL with IAAT/ASAT ratio (r= 0.60, p<0.05, Table 3.29).
**Table 3.29 Linear Correlation Analysis between Anthropometric Measurements, Lipid Stores with Segmentation and Body Fat Stores in BMI Group 4.**

<table>
<thead>
<tr>
<th>BMI Group 4</th>
<th>ANTHROPOMETRIC VARIABLES</th>
<th>ECTOPIC FAT STORES</th>
<th>ADIPOSITY STORES in LITRES</th>
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</thead>
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<td>AGE WEIGHT HEIGHT WAIST HIP WHR WHR BMI</td>
<td>MRS HCL ME total HCL ME segment 1 HCL ME segment 2&amp;3 HCL ME segment 4 HCL ME segment 5&amp;8 HCL ME total IPCL ME Head IPCL ME Body IPCL ME Tail IPCL TAT SAT Internal SAAT NASAT IAAT NAIAT</td>
<td></td>
</tr>
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<td>ME Tail IPCL</td>
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</tr>
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<td>0.164</td>
<td>0.187</td>
<td>0.319</td>
</tr>
<tr>
<td>NAIAT</td>
<td>0.108</td>
<td>0.213</td>
<td>0.297</td>
</tr>
<tr>
<td>SAT%SAAT</td>
<td>0.086</td>
<td>0.325</td>
<td>0.248</td>
</tr>
</tbody>
</table>

All data are presented as Spearman rank test correlation. *= p<0.05, **= p< 0.01. Abbreviations: WC: waist circumference; WHR: waist-to-hip ratio; WHRH: waist-to-height ratio; HICL: intra-hepatoceleular lipid; IPCL: intra-pancreatic cellular lipid, TAT: total adipose tissue; SAT: subcutaneous; SAAT: subcutaneous abdominal; NASAT: non abdominal subcutaneous; Internal: total internal; IAAT: intra-abdominal; NAIAT: non-abdominal internal.
3.3.24 Correlation Analysis and the Relationship between Regional and Total Liver Fat Content

The higher the BMI the stronger the correlation between ME total IHCL and ME segments IHCL, see Table 3.30.

Nevertheless, the IHCL content in segment 4 of the liver shows a near perfect correlation with total liver fat IHCL in all groups regardless of their BMI (r=0.96-0.99) (Table 3.30).

As for segment 2&3, it has the lowest association when compared to the other segments (r=0.74-0.96) (Table 3.30).
Table 3.30 BMI Group Specific Correlation Coefficients (r) of MRS and ME for estimating Liver Fat

<table>
<thead>
<tr>
<th>BMI Group</th>
<th>Correlation coefficients (r)</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>ME vs MRS IHCL</td>
<td></td>
<td>0.73</td>
<td>P=0.001</td>
<td>0.97 P&lt;0.001</td>
<td>0.86 P=0.001</td>
</tr>
<tr>
<td>ME total IHCL vs ME segment 1 IHCL</td>
<td></td>
<td>0.78</td>
<td>P&lt;0.001</td>
<td>0.90 P&lt;0.001</td>
<td>0.94 P&lt;0.001</td>
</tr>
<tr>
<td>ME total IHCL vs ME segment 2&amp;3 IHCL</td>
<td></td>
<td>0.74</td>
<td>P=0.001</td>
<td>0.88 P&lt;0.001</td>
<td>0.96 P&lt;0.001</td>
</tr>
<tr>
<td>ME total IHCL vs ME segment 4 IHCL</td>
<td></td>
<td>0.96</td>
<td>P&lt;0.001</td>
<td>0.96 P&lt;0.001</td>
<td>0.90 P&lt;0.001</td>
</tr>
<tr>
<td>ME total IHCL vs ME segment 5&amp;8 IHCL</td>
<td></td>
<td>0.84</td>
<td>P&lt;0.001</td>
<td>0.96 P&lt;0.001</td>
<td>0.96 P&lt;0.001</td>
</tr>
<tr>
<td>ME total IHCL vs ME segment 6&amp;7 IHCL</td>
<td></td>
<td>0.82</td>
<td>P&lt;0.001</td>
<td>0.94 P&lt;0.001</td>
<td>0.96 P&lt;0.001</td>
</tr>
</tbody>
</table>

Data was analysed by Spearman rank test correlation. Where MRS- Magnetic Resonance Spectroscopy, ME- Multi-echo

3.3.25 Correlation Analysis and the Relationship between Regional and Total Pancreatic Fat Content

In the pancreas there was a similar pattern as observed in the liver, the higher the BMI the greater the correlation between the regions (Table 3.31).

In BMI group 1 there was no significant correlation between total IPCL and the Body IPCL and Tail IPCL (r= 0.46, r=0.47) respectively (Table 3.31).
Table 3.31 BMI Group Specific Correlation Coefficients (r) of ME for estimating Pancreatic Fat

<table>
<thead>
<tr>
<th>BMI Group</th>
<th>Correlation coefficients (r)</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>ME total IPCL vs ME Head IPCL</td>
<td></td>
<td>0.74</td>
<td>0.82</td>
<td>0.91</td>
<td>0.93</td>
</tr>
<tr>
<td></td>
<td>P= 0.001</td>
<td></td>
<td>P&lt;0.001</td>
<td>P&lt;0.001</td>
<td>P&lt;0.001</td>
</tr>
<tr>
<td>ME total IPCL vs ME Body IPCL</td>
<td></td>
<td>0.46</td>
<td>0.87</td>
<td>0.87</td>
<td>0.98</td>
</tr>
<tr>
<td></td>
<td>P= 0.074</td>
<td></td>
<td>P&lt;0.001</td>
<td>P&lt;0.001</td>
<td>P&lt;0.001</td>
</tr>
<tr>
<td>ME total IPCL vs ME Tail IPCL</td>
<td></td>
<td>0.47</td>
<td>0.86</td>
<td>0.87</td>
<td>0.74</td>
</tr>
<tr>
<td></td>
<td>P= 0.064</td>
<td></td>
<td>P&lt;0.001</td>
<td>P&lt;0.001</td>
<td>P=0.003</td>
</tr>
</tbody>
</table>

Data was analysed by Spearman rank test correlation, Where ME- Multi-echo

3.3.26 Matrix Analysis and the Relationship between Regional and Total Ectopic Fat Content

When the BMI range is normal (Group 1=18<25 kg/m²) there appears to be a variation between the total and regional ectopic fat content in both the liver and pancreas (Figure 3.22).

As the BMI increases to an overweight range (Group 2: 25<30 kg/m², see Figure 3.23) there is less variation in regional and total fat content in both organs.

The correlations between total and regional IHCL continue to strengthen in both the obese (Group 3: 30<40 kg/m²) (Figure 3.24) and morbidly obese groups (Group 4: 40+ kg/m²)
(Figure 3.25). A similar result is found in the pancreas, with the exception of the tail IPCL in the pancreas. (Figure D).

**Figure 3.22** Matrix Correlations of Total Pancreas Fat, Head Pancreas Fat, Body Pancreas Fat and Tail Pancreas Fat with Total Liver Fat and Liver Segments in BMI group 1

Shaded boxes indicate BMI Group 1 specific variation in total Multi-echo imaging of liver fat (ME total IHCL), segment 1 liver fat Multi-echo (ME segment 1 IHCL), segment 2&3 liver fat Multi-echo (ME segment 2&3 IHCL), segment 4 liver fat Multi-echo (ME segment 4 IHCL), segment 5&8 liver fat Multi-echo (ME segment 5&8 IHCL), segment 6&7 liver fat Multi-echo (ME segment 6&7 IHCL) (A) and total Multi-echo imaging of pancreas fat (ME total IPCL) (B) Head Multi-echo imaging of pancreas fat (ME head IPCL) (C) Body Multi-echo imaging of pancreas fat (ME Body IPCL) (D) Tail Multi-echo imaging of pancreas fat (ME tail IPCL)
Figure 3.23 Matrix Correlations of Total Pancreas Fat, Head Pancreas Fat, Body Pancreas Fat and Tail Pancreas Fat with Total Liver Fat and Liver Segments in BMI group 2

Shaded boxes indicate BMI Group 2 specific variation in total Multi-echo imaging of liver fat (ME total IHCL), segment 1 liver fat Multi-echo (ME segment 1 IHCL), segment 2&3 liver fat Multi-echo (ME segment 2&3 IHCL), segment 4 liver fat Multi-echo (ME segment 4 IHCL), segment 5&8 liver fat Multi-echo (ME segment 5&8 IHCL), segment 6&7 liver fat Multi-echo (ME segment 6&7 IHCL) (A) and total Multi-echo imaging of pancreas fat (ME total IPCL) (B) Head Multi-echo imaging of pancreas fat (ME head IPCL) (C) Body Multi-echo imaging of pancreas fat (ME Body IPCL) (D) Tail Multi-echo imaging of pancreas fat (ME tail IPCL)
Figure 3.24 Matrix Correlations of Total Pancreas Fat, Head Pancreas Fat, Body Pancreas Fat and Tail Pancreas Fat with Total Liver Fat and Liver Segments in BMI group 3

Shaded boxes indicate BMI Group 3 specific variation in total Multi-echo imaging of liver fat (ME total IHCL), segment 1 liver fat Multi-echo (ME segment 1 IHCL), segment 2&3 liver fat Multi-echo (ME segment 2&3 IHCL), segment 4 liver fat Multi-echo (ME segment 4 IHCL), segment 5&8 liver fat Multi-echo (ME segment 5&8 IHCL), segment 6&7 liver fat Multi-echo (ME segment 6&7 IHCL) (A) and total Multi-echo imaging of pancreas fat (ME total IPCL) (B) Head Multi-echo imaging of pancreas fat (ME head IPCL) (C) Body Multi-echo imaging of pancreas fat (ME Body IPCL) (D) Tail Multi-echo imaging of pancreas fat (ME tail IPCL)
Figure 3.25 Matrix Correlations of Total Pancreas Fat, Head Pancreas Fat, Body Pancreas Fat and Tail Pancreas Fat with Total Liver Fat and Liver Segments in BMI group 4

Shaded boxes indicate BMI **Group 4** specific variation in total Multi-echo imaging of liver fat (ME total IHCL), segment 1 liver fat Multi-echo (ME segment 1 IHCL), segment 2&3 liver fat Multi-echo (ME segment 2&3 IHCL), segment 4 liver fat Multi-echo (ME segment 4 IHCL), segment 5&8 liver fat Multi-echo (ME segment 5&8 IHCL), segment 6&7 liver fat Multi-echo (ME segment 6&7 IHCL) (A) and total Multi-echo imaging of pancreas fat (ME total IPCL) (B) Head Multi-echo imaging of pancreas fat (ME head IPCL) (C) Body Multi-echo imaging of pancreas fat (ME Body IPCL) (D) Tail Multi-echo imaging of pancreas fat (ME tail IPCL)
3.3.27 Impact of Exercise Level Intensity on Regional Liver Fat Content

IHCL content in different the segments of the liver according to exercise level groups are shown in (Table 3.32 and Figure 3.26).

As expected, the higher the exercise level the lower the IHCL content in all regions of the liver. There were significant differences in IHCL content between the Sedentary/Low and High intensity groups in all regions (all p<0.01).

However there were no significant differences between the moderate and high intensity groups or between the Moderate and Low intensity groups.
Figure 3.26 Liver Fat Distributions in the High, Moderate and Low Exercise Level Groups
Table 3.32 Impact of Exercise Level Intensity on Regional Ectopic Fat Content

<table>
<thead>
<tr>
<th>Exercise Level</th>
<th>Sedentary/Low</th>
<th>Moderate</th>
<th>High</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>number</td>
<td>31</td>
<td>14</td>
<td>15</td>
<td></td>
</tr>
<tr>
<td><strong>Liver Fat</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IHCL (MRS)</td>
<td>12.84 ± 15.67</td>
<td>7.24 ± 12.34</td>
<td>1.06 ± 1.63</td>
<td>0.017</td>
</tr>
<tr>
<td>Total IHCL</td>
<td>9.91 ± 8.36</td>
<td>5.45 ± 6.57</td>
<td>1.95 ± 1.57</td>
<td>0.002</td>
</tr>
<tr>
<td>Segment 1</td>
<td>8.86 ± 7.83</td>
<td>4.57 ± 5.82</td>
<td>1.62 ± 1.25</td>
<td>0.002</td>
</tr>
<tr>
<td>Segment 2&amp;3</td>
<td>9.07 ± 7.75</td>
<td>4.73 ± 6.26</td>
<td>2.04 ± 1.30</td>
<td>0.003</td>
</tr>
<tr>
<td>Segment 4</td>
<td>9.96 ± 7.95</td>
<td>5.25 ± 6.32</td>
<td>1.93 ± 1.69</td>
<td>0.001</td>
</tr>
<tr>
<td>Segment 5&amp;8</td>
<td>10.36 ± 9.41</td>
<td>5.61 ± 6.93</td>
<td>1.82 ± 1.82</td>
<td>0.003</td>
</tr>
<tr>
<td>Segment 6&amp;7</td>
<td>10.38 ± 9.64</td>
<td>5.60 ± 6.90</td>
<td>1.74 ± 1.62</td>
<td>0.003</td>
</tr>
<tr>
<td><strong>Pancreas Fat</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total IPCL</td>
<td>7.10 ± 6.36</td>
<td>5.46 ± 7.00</td>
<td>2.95 ± 3.23</td>
<td>0.09</td>
</tr>
<tr>
<td>Head</td>
<td>5.80 ± 5.88</td>
<td>4.53 ± 5.74</td>
<td>3.03 ± 2.27</td>
<td>0.24</td>
</tr>
<tr>
<td>Body</td>
<td>7.05 ± 7.51</td>
<td>4.37 ± 6.29</td>
<td>2.85 ± 3.47</td>
<td>0.10</td>
</tr>
<tr>
<td>Tail</td>
<td>5.49 ± 4.59</td>
<td>5.00 ± 6.48</td>
<td>3.72 ± 3.09</td>
<td>0.51</td>
</tr>
</tbody>
</table>

IHCL: intra-hepatocellular lipid; IPCL: intra-pancreatic cellular lipid. All data are presented as mean ± SD. Differences between exercise levels were tested for using ANOVA and Post-hoc Turkey analysis.

3.3.28 Impact of Exercise Level Intensity on Regional Pancreatic Fat Content

There was a similar trend in the pancreas with the lowest IPCL content observed in the group which undertook the highest intensity exercise (Table 3.32 and Figure 3.27), however, none of these differences reach significance.
Figure 3.27 Pancreas Fat Distributions in the High, Moderate and Low Exercise Level Groups.
3.4 Discussion

In this chapter I have described the relationship between ectopic fat content in both the liver and pancreas and how this related to body composition, in particular adipose tissue content. I have also applied novel analysis techniques which have allowed me to map regional accumulation of ectopic fat in both the liver and pancreas, compared this with conventional MR methods of measuring ectopic fat content and seen how regional fat distribution related to body composition.

There was a wide range of variability in my cohort in liver ectopic fat content, with ~40% displaying elevated IHCL, coinciding with other population studies. (Donhoffer 1974, Seppala-Lindroos, Vehkavaara et al. 2002, Tarasow, Siergiejczyk et al. 2002, Thomas, Hamilton et al. 2005) This may be due to the relatively small number of subjects, and gender imbalance particularly in different BMI groups. By comparison, a slightly smaller proportion of the cohort had elevated pancreatic fat content (~33%); though a recent study by Wong et al. (Wong Vincent Wai-Sun 2014) claimed that the upper limit for normal IPCL content is 10.4% in healthy volunteers, generating yet a clinically unclear relevant cut-off point for IPCL.

In this study, ME of total IHCL displayed a generally good correlation and concordance with regard to the gold standard MRS IHCL (r =0.96), but not at high levels of IHCL. This is most
likely due to the different methods’ formulas rather than differences between MR techniques per se. (MRS= (CH$_2$/Water) vs. ME= CH$_2$+CH$_3$/water+fat)

When utilising the novel ME derived technique, that allows regional assessment of ectopic fat in liver, I found that overall, regardless of which segment of the liver I measured to determine the lipid content it correlated significantly (p<0.001) to total IHCL, with a reproducibility of <1%, although, there was quite an inter-individual variation.

The lack of significant overall regional variation between segments might be the outcome of a ‘diffuse fatty liver’ deposition in my cohort, which is the most prevalent form of NAFLD. (Cassidy, Yokoo et al. 2009, Decarie, Lepanto et al. 2011) The liver seems to be homogeneously involved by fat accumulation. (Thomas, Hamilton et al. 2005, Decarie, Lepanto et al. 2011, N. S. Patel 2013) However, studies have reported focal fatty sparing or infiltration at liver imaging. (Halvorsen, Korobkin et al. 1982, Kammen, Pacharn et al. 2001, Yoshimitsu, Honda et al. 2001)

A study from Capitan et al. (Capitan, Petit et al. 2012) demonstrated a heterogeneous effect at the segmental level, indicating a more geographical fat deposition. (Cassidy, Yokoo et al. 2009, Decarie, Lepanto et al. 2011, Capitan, Petit et al. 2012) This implies that ectopic fat in the liver have various atypical patterns of distribution that may be affected not only by fat infiltration
but also other metabolic conditions, (Mills, Doppman et al. 1977) hence, leading to misdiagnosis.(Capitan, Petit et al. 2012)

IPCL, alternatively, had an uneven accumulation of fat throughout its regions, although significantly associated (p<0.001) with a reproducibility of <1%, the Head and Body regions had the highest correlation with the total IPCL, when compared to the disengaged Tail region.

This uneven fatty infiltration may be caused by differences in the histological characteristics between the 2 embryological pancreatic buds. (Marchal, Verbeken et al. 1989, Donald, Shorvon et al. 1990, Jacobs, Coleman et al. 1994, Matsumoto, Mori et al. 1995) The pancreas forms from 2 separate buds: the dorsal and the ventral buds. Fusion of these 2 buds forms the resulting pancreas. The ventral bud forms the posterior parts of the head and the uncinate process while the larger dorsal bud forms the body, tail, and anterior part of the head. (Suda, Mizuguchi et al. 1981, Uchida, Takada et al. 1999, Borghei, Sokhandon et al. 2013)

There are reports that detected a markedly fat deposition in pancreas islet cells. (Noronha, Salgadinho et al. 1981, Tushuizen, Bunck et al. 2007) However, according to some studies with pathological and histological observations, pancreas lipid deposition occurred mainly in adipocytes within the exocrine tissue or in the adipose tissue in the interlobular space rather than in cells. (Tham, Heyerman et al. 1991, Ferrozzi, Bova et al. 1996, Nghiem, Olson et al.)
Ensuing that ectopic fat content in the pancreas is unlikely to serve as a biomarker of the endocrine function of the pancreas. (Szendroedi and Roden 2009)

3.4.1 Anthropometry, IHCL and IPCL

There were significant correlations between total and regional IHCL and most anthropometric measurements especially BMI, weight, WC (more significantly in females) and WHR (more significantly in males), in agreement with previous studies. (Flegal, Shepherd et al. 2009, Thomas, Parkinson et al. 2012)

Furthermore, Sijens et al. (Sijens, Edens et al. 2010) observed a strong relationship between IPCL and BMI. Interestingly, in my cohort only men mirrored this connection, more pronouncedly when looking at the pancreas fat content as a whole (ME total IPCL) less so in individual regions.

Whilst most variables, as expected, increased with increasing BMI group, the changes in IHCL and IPCL are less clear with an apparent rise from group 1-3 followed by a decrease in group 4. Further examination of the data suggests this may be related to the gender balance within these groups, where BMI Group 1 and 2 contain 75% male subjects, whilst BMI group 4 is
78% females. Therefore it is likely that gender differences in body composition and ectopic fat are masking differences related to BMI.

Men also showed a strong affiliation with WC and IPCL content. A few studies observed similar associations as well. (Lee, Kim et al. 2009, Heni, Machann et al. 2010, Wong Vincent Wai-Sun 2014) Nonetheless, more profoundly was the significant association of pancreatic fat content in men with WHR in all pancreatic regions (p<0.05). Though, intriguingly, female subjects in my study showed no association with WC and WHR in the pancreatic regions or with any of the anthropometric variables.

Thus WHR in my study, a measurement known to be more closely related to the intra-abdominal compartment (Borkan, Gerzof et al. 1982, Ashwell, Cole et al. 1985, Vanderkooy, Leenen et al. 1993), may be an appropriate anthropometric parameter for IHCL and IPCL content particularly in men.

### 3.4.2 Adiposity, IHCL and IPCL

Adiposity stores, in particular IAAT, had a profound effect on ectopic fat accumulation in the liver, independent of gender. Though, IHCL may be masked by gender differences in adiposity,
where despite a wide range of BMI, males had significantly higher proportion of total internal fat compared to females (p<0.001).

Furthermore, previous studies have shown clear gender differences in IHCL with significantly higher levels in male when compared with female subjects. (Schwimmer, McGreal et al. 2005, Rossi, Fantin et al. 2011, Thomas, Parkinson et al. 2012)

Nevertheless, my results are consistent with previous studies demonstrating that visceral abdominal adipose tissue is one of the determinants of liver fat content. (Nielsen, Guo et al. 2004, Thomas, Hamilton et al. 2005, Eguchi, Eguchi et al. 2006, Jakobsen, Berentzen et al. 2007, Rossi, Fantin et al. 2011) This may be due to the anatomical position of the liver, where the hepatic vascular network consists of a dual input: from the hepatic artery, which delivers ~30%, and the portal vein, which delivers ~70% of the blood reaching the liver. (Sase, Monden et al. 2002) Visceral fat venous blood is drained directly to the liver through the portal vein. This contrasts with subcutaneous fat where venous drainage is through systemic veins. (Ibrahim 2010) However, although visceral fat provides direct hepatic access to FFA and adipokines secreted by visceral adipocytes, (Ibrahim 2010) studies from patients with lipodystrophy suggest that even with little or no adipose tissue, fatty liver, and insulin resistance can still develop quite strikingly, (Garg 2004) which undermines the portal hypothesis.
Moreover, Nielsen et al. (Nielsen, Guo et al. 2004) have observed that the relative influence of portal vein FFA derived from visceral fat is minor when compared with FFA derived from subcutaneous fat; only about 5% and 20% in lean and obese subjects, respectively during post-absorptive conditions.

Therefore, the amount of fat present in hepatocytes arises from an imbalance between lipid acquisition and removal (Cohen, Horton et al. 2011) and represents a complex interaction of (1) adipose tissue lipolysis about 59% (2) de novo lipogenesis by 26% and (3) dietary chylomicrons (15%). (Donnelly 2005, Fabbrini, Sullivan et al. 2010, Cohen, Horton et al. 2011, Ghosh 2014)

Comparable to liver fat content, lipid accumulation in the pancreas was also affected by IAAT in both genders and more so in overweight participants (BMI: 25<30 kg/m²) where the Head and Body regions of the pancreas had the greatest influence. The Tail did not follow that pattern.

Though, once again similarly to IHCL, this may be masked by gender differences in adiposity, where males had significantly higher proportion of total internal fat compared to females.
Nevertheless, association between fatty pancreas and IAAT has been previously observed in some (Lee, Kim et al. 2009, Heni, Machann et al. 2010, Rossi, Fantin et al. 2011) but not all studies. (Hannukainen, Borra et al. 2011, van der Zijl, Goossens et al. 2011) Parallel to my results, Heni et al. (Heni, Machann et al. 2010) observed a significant association between pancreatic fat content and visceral fat in overweight participants, whilst van der Zijl et al. (van der Zijl, Goossens et al. 2011) and Hannukainen et al. did. (Hannukainen, Borra et al. 2011)

The use of different methods for measuring pancreatic lipid content as well as differences in the study population may partially explain inconsistency in the findings of these studies. (Heni, Machann et al. 2010, Hannukainen, Borra et al. 2011, van der Zijl, Goossens et al. 2011) Ordinarily, the peribiliary region (head and body), has been shown to be the most fat infiltrated, (Matsumoto, Mori et al. 1995, Saisho, Butler et al. 2007, Kawamoto, Siegelman et al. 2009) while other studies showed no significant difference in fat content across the regions. (Li, Xie et al. 2011, N. S. Patel 2013)

Ultimately, although a correlation between total IHCL and IPCL is present, it was weaker than might be expected, and less clear cut than the relationship between IHCL and IAAT.
3.4.3 Age, IHCL and IPCL

IHCL was shown to have an effect with age at (36-45 years old), explicitly in men in all age groups when it came to IPCL, when compared to other age groups (with no significance). These observations mirror the prevalence of NAFLD in the general population which increases 39% in those aged 40 to 50 years. (Kagansky, Levy et al. 2004, Adams, Angulo et al. 2005, Argo and Caldwell 2009, Okanoue, Umemura et al. 2011) As well as being more prevalent in men than women up to the age of 60 years. (Browning, Szczepaniak et al. 2004, Fan and Farrell 2009, Hashimoto and Tokushige 2011)

But at older ages the disorder is more prevalent in women. (Browning, Szczepaniak et al. 2004)

The underlying mechanisms for this gender dimorphism is currently unknown, but may be related to a decline in hepatic blood flow (by 33%), hepatic volume (up to 25%), and liver function with age. (Frith, Day et al. 2009) which follows identifiable prevalence trends of those for the metabolic syndrome, peaking in middle age, and decreasing in octogenarians. (Frith, Day et al. 2009, Gan, Chitturi et al. 2011)
Moreover, when age was taken into account in IPCL significant gender differences were noted in all three pancreatic regions, Saisho et al. noted similar results in men with the progression of fat deposition in the pancreas until the 4th decade of life, whereas the pancreatic fat volume in women remained unchanged from the 2nd to the 9th decade. (Saisho, Butler et al. 2007)

3.4.3 Exercise, IHCL and IPCL

Impact of exercise showed that participants with higher physical activity and fitness had lower hepatic fat content in all segments than less active and less fit individuals. This observation is consistent with previous studies demonstrating that this may be mediated, in part, by a reduction in hepatic lipogenesis. (Di Mauro, Pagano et al. 2009, Marques, Motta et al. 2010, Aoi, Naito et al. 2011, Hannukainen, Borra et al. 2011, Johnson, Keating et al. 2012)

Though, there is a lack of significance in IHCL levels between the moderate and high fitness groups. This may have been greatly affected by, once again, gender. Very few women reported high exercise levels (7% women), which accounts for the higher IAAT/ASAT ratio in this group, a similar analysis in a single sex cohort may produce different results.

Alternatively, physical activity through different intensities, showed no significant effect on IPCL content, mirroring a study by Hannukainen et al.(Hannukainen, Borra et al. 2011). A
significant decrease was only observed between the two extremities of low and high fitness groups, except in the Tail region of the pancreas, where it appeared unaltered.

It is challenging to arrive to clear conclusions with IPCL, due to the difficulty of tissue collection from the pancreas, which limits histological proof. Thus, using this non-invasive novel ME segmented technique may be an innovative approach in looking at challenging organs such as the latter and interpreting a more illustrative fatty infiltration.

In conclusion, overweight participants (Group 2: BMI: 25<30 kg/m²) presented the most significant correlations. In addition to having the strongest affiliation with different pancreatic regions and liver fat segments as well as having these two organs associated with each other in fat content solely in this group when compared to obese participants (BMI: 30< 40 kg/m²) and morbidly obese participants (BMI: +40 kg/m²). Height was unexpectedly greatly correlated in this group with Liver and its segments but only with pancreas fat content as a whole and not with individual regions.
Chapter IV: The Impact of Lifestyle Interventions on Liver and Pancreatic Fat Content and Distribution

4.1 Introduction

It has been shown that genetic, environmental, and behavioural factors determine the response of adipose tissue to excess energy intake over energy expenditure. (Snel, Jonker et al. 2012) Diet and lifestyle interventions are powerful tools in improving both ectopic fat deposition and the function of the organ in which the ectopic fat is accumulated. A lifestyle characterized by inactivity and a high-calorie diet is a known risk factor for obesity and Type 2 Diabetes as well as for impaired insulin sensitivity. (Schrauwen 2007, Knudsen, Hansen et al. 2012, Snel, Jonker et al. 2012)

Body composition, particularly the accumulation of visceral fat (Gastaldelli, Cusi et al. 2007, Goossens 2008, Usui, Asaka et al. 2010) and poor aerobic fitness (Eriksson and Lindgarde 1996) are key determinants of the metabolic syndrome. (Kissebah, Vydelingum et al. 1982, Krotkiewski, Bjorntorp et al. 1983) In humans, hepatic lipid content is the most easily modified by high fat feeding, followed by visceral fat. (Bachmann, Dahl et al. 2001, Snel, Jonker et al. 2012) Although, lifestyle modification such as calorie restriction and weight loss are the recommended therapy in NAFLD, (Westerbacka, Lammi et al. 2005, Zivkovic, German et al. 2007, Bellentani, Dalle Grave et al. 2008, Kirk, Reeds et al. 2009, Nicklas, Wang et al. 2009,
Musso, Gambino et al. 2010, Peng, Wang et al. 2011) there are currently no systematic evaluations of its efficacy. (Thoma, Day et al. 2012)

Moreover, accumulation of lipids in beta-cells has a negative effect on beta-cell function in rodents. Zucker (ZDF) rats on relatively high fat diets have shown excessive lipid inflow into the islets, the increased secretion pressure resulted in beta-cell deterioration and eventually apoptosis. (Pick, Clark et al. 1998) Conversely, restriction in food intake by pre-diabetic rats, counteracted the accumulation of fat and deterioration of beta-cells. (Ohneda, Inman et al. 1995)

In humans, recent studies have shown that lipid accumulation in the pancreas was increased in individuals with impaired glucose metabolism and T2DM. However, there were no clear association with beta-cell dysfunction in some studies. (van Raalte, van der Zijl et al. 2010, van der Zijl, Goossens et al. 2011) Whereas a study by Lim et al. (Lim, Hollingsworth et al. 2011) shows a significant decrease in pancreatic fat and a normalisation of beta cell function by acute negative energy balance alone.

The nature and rapidity of weight loss has also been shown to be important. (Andersen, Gluud et al. 1991) Previous studies have used surgical methods such as gastric banding (Raz, Eldor et al. 2005, Luyckx, Desaive et al. 1998, Dixon, Bhathal et al. 2004, Mattar, Velcu et al. 2005,
Shaffer 2006) and very low calorie diets (Andersen, Gluud et al. 1991, Petersen, Dufour et al. 2005) to promote significant rapid weight loss, resulting in reduced hepatic lipid content.

However, large and rapid reduction in weight may deteriorate underlying inflammation and fibrosis in patients with non-alcoholic steatohepatitis (NASH). (Andersen, Gluud et al. 1991, Luyckx, Desaive et al. 1998) Although weight loss induced by bariatric surgery decreases steatosis, inflammation, and fibrosis whilst having considerable beneficial metabolic effects in the liver by decreasing hepatic glucose production as well as hepatic VLDL-triglyceride secretion rate, and diminishing hepatic gene expression of factors that regulate hepatic inflammation and fibrogenesis. (Dixon, Bhathal et al. 2004, Clark, Alkhuraishi et al. 2005, Klein, Mittendorfer et al. 2006, Fabbrini, Sullivan et al. 2010)

Non-invasive techniques such as Proton (^1H)-magnetic resonance spectroscopy (MRS) and magnetic resonance imaging (MRI) are the norm in assessing hepatic TG content in nutritional interventions in obese and T2DM subjects. (Petersen, Dufour et al. 2005, Tamura, Tanaka et al. 2005, Thomas, Brynes et al. 2006, Thamer, Machann et al. 2007)

Consequently, I have used once again the Multi-echo (ME) Imaging based technique to look at fat content and distribution in the liver and pancreas in the overfeeding (Study 1), calorie restriction (Study 2) and bariatric surgery (Study 3) interventional studies (Chapter 2.6).
will also be incorporating the novel approach to map regional fat distribution in the liver and pancreas (as described in Chapter 3), to further define potential changes across these organs before and after intervention.

4.2 Statistical Analysis

All Data are presented as mean ± standard deviation in the tables. Differences related to groups were examined by paired or unpaired Student’s t test, mean ± standard errors of the differences are reported, as well as ANOVA to compare mean differences. The distributions of the obtained fat contents were not normal, thus non-parametric correlations were analysed using the Spearman rank test. Statistical significance was accepted at p<0.05.

4.3 Results

4.3.1 Study 1 – The Impact of Overfeeding on Body Composition

Subjects were asked to take 600 kcal / day in the form of 2 x 125ml Fortisip Compact drinks (250ml total) (nutritional content in Appendix 1) after breakfast on top of their own standard diet for a period of 6 days.
The control group, matched for gender and BMI were asked to drink a 0 kCal drink in the form of a 7UP soda (nutritional content in Appendix 1) for a period of 6 days.

Both groups were scanned immediately before and the morning after they concluded their intervention.

Participants were all male, with a mean age range of 19-47 years old. At baseline the 0kcal overfeeding group were older by 2 years and weighed 10kg less than the 600kcal overfeeding group (Table 4.0); these differences did not reach statistical significance.
Table 4.0 Baseline Characteristics of Study Participants

<table>
<thead>
<tr>
<th></th>
<th>0 kCal Group (n=6)</th>
<th>600 kCal Group (n=6)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± SD</td>
<td>Range</td>
<td>Mean ± SD</td>
</tr>
<tr>
<td><strong>Anthropometric Variables</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>34.2 ± 12.4</td>
<td>22-47</td>
<td>32.3 ± 10.3</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>73.9 ± 7.1</td>
<td>63.2-83.0</td>
<td>84.0 ± 10.4</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>24.7 ± 3.8</td>
<td>18.7-29.4</td>
<td>26.4 ± 2.4</td>
</tr>
<tr>
<td>WC (cm)</td>
<td>78.7 ± 12.5</td>
<td>63.0-92.0</td>
<td>89.0 ± 13.3</td>
</tr>
<tr>
<td>Hip (cm)</td>
<td>91.4 ± 4.1</td>
<td>84.0-95.0</td>
<td>112.7 ± 26.8</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>174 ± 7.3</td>
<td>165-184</td>
<td>178 ± 8.5</td>
</tr>
<tr>
<td>WHR</td>
<td>0.86 ± 0.08</td>
<td>0.75-0.97</td>
<td>0.92 ± 0.05</td>
</tr>
<tr>
<td>WHtR</td>
<td>0.46 ± 0.07</td>
<td>0.37-0.56</td>
<td>0.51 ± 0.06</td>
</tr>
<tr>
<td><strong>Ectopic Liver Fat</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MRS IHCL</td>
<td>0.41 ± 0.32</td>
<td>0.06-0.98</td>
<td>2.04 ± 2.73</td>
</tr>
<tr>
<td>ME IHCL</td>
<td>1.25 ± 0.36</td>
<td>0.76-1.81</td>
<td>3.11 ± 3.67</td>
</tr>
<tr>
<td>Segment 1</td>
<td>0.96 ± 0.44</td>
<td>0.26-1.57</td>
<td>2.64 ± 3.12</td>
</tr>
<tr>
<td>Segment 2&amp;3</td>
<td>1.49 ± 0.46</td>
<td>0.97-2.24</td>
<td>2.66 ± 2.21</td>
</tr>
<tr>
<td>Segment 4</td>
<td>0.98 ± 0.40</td>
<td>0.64-1.75</td>
<td>2.97 ± 3.40</td>
</tr>
<tr>
<td>Segment 5&amp;8</td>
<td>1.11 ± 0.46</td>
<td>0.63-1.98</td>
<td>3.43 ± 4.48</td>
</tr>
<tr>
<td>Segment 6&amp;7</td>
<td>1.20 ± 0.40</td>
<td>0.69-1.92</td>
<td>3.04 ± 3.59</td>
</tr>
<tr>
<td><strong>Ectopic Pancreas Fat</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ME IPCL</td>
<td>1.61 ± 0.71</td>
<td>0.56-2.66</td>
<td>3.20 ± 2.23</td>
</tr>
<tr>
<td>Head</td>
<td>2.18 ± 1.85</td>
<td>0.36-5.20</td>
<td>3.06 ± 1.82</td>
</tr>
<tr>
<td>Body</td>
<td>1.35 ± 0.90</td>
<td>0.13-2.33</td>
<td>3.18 ± 2.44</td>
</tr>
<tr>
<td>Tail</td>
<td>1.79 ± 1.35</td>
<td>0.25-3.96</td>
<td>3.77 ± 2.49</td>
</tr>
<tr>
<td><strong>Adiposity Stores</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TAT (L)</td>
<td>15.84 ± 4.26</td>
<td>10.62-21.26</td>
<td>21.37 ± 8.02</td>
</tr>
<tr>
<td>SAT (L)</td>
<td>11.9 ± 3.33</td>
<td>7.61-15.05</td>
<td>17.26 ± 6.39</td>
</tr>
<tr>
<td>ASAT (L)</td>
<td>2.95 ± 1.01</td>
<td>1.50-4.23</td>
<td>5.20 ± 2.42</td>
</tr>
<tr>
<td>NASAT (L)</td>
<td>8.94 ± 2.41</td>
<td>6.11-11.25</td>
<td>12.06 ± 4.04</td>
</tr>
<tr>
<td>Internal (L)</td>
<td>3.95 ± 1.80</td>
<td>1.97-6.45</td>
<td>4.11 ± 1.76</td>
</tr>
<tr>
<td>IAAT (L)</td>
<td>2.14 ± 1.46</td>
<td>0.58-4.32</td>
<td>1.97 ± 1.22</td>
</tr>
<tr>
<td>NAIAT (L)</td>
<td>1.82 ± 0.37</td>
<td>1.39-2.26</td>
<td>2.15 ± 0.63</td>
</tr>
<tr>
<td>IAAT/ASAT</td>
<td>0.74 ± 0.37</td>
<td>0.15-1.14</td>
<td>0.35 ± 0.14</td>
</tr>
</tbody>
</table>

All data are presented as mean ± SD. WC: waist circumference; WHR: waist-to-hip ratio; WHtR: waist-to-height ratio; IHCL: intra-hepatocellular lipid; IPCL: intra-pancreatocellular lipid, Adipose tissue deposits are in litres (L); TAT: total adipose tissue; SAT: subcutaneous; ASAT: abdominal subcutaneous; NASAT: non-abdominal subcutaneous; Internal: total internal; IAAT: intra-abdominal; NAIAT: non-abdominal internal. Baseline differences between 0 kcal 600 kcal groups were analysed by unpaired Student’s t-test.
4.3.1.i Changes in Body Composition following 0 Calorie Overfeeding

During the 6 day overfeeding period with 0 kcal, there was a small, but significant decrease in BMI (pre: 24.7 ± 3.8 vs. 24.6 ± 3.8 kg/m², p<0.05). There was also a decrease of 0.30 ± 0.30 kg in weight, though did not reach statistical significance (p=0.06), see Table 4.1.

4.3.1.ii Changes in Adiposity following 0 Calorie Overfeeding

There were small increases in most adipose tissue depots, but none of these changes reached statistical significance, see Table 4.1. There was no statistical difference between baseline and post overfeeding (0kcal) in all the adiposity stores (P>0.05 for all) (Table 4.1).

4.3.1.iii Changes in Liver Fat following 0 Calorie Overfeeding

There were small increases in both total and regional IHCL content between baseline and post overfeeding (0kcal), but none of these changes reached statistical significance, see Table 4.1. Interestingly, unlike other regions of the liver, there was a slight reduction in IHCL content in segment 2&3 0.25± 0.12, but this did not reach no statistical significance (p=0.09).

4.3.1.iv Changes in Pancreatic Fat following 0 Calorie Overfeeding

There was a trend for small increases in total, body and tail IPCL content following the 0 calorie intervention though none of these changes reached statistical significance, see Table 4.1. There was a non-significant decrease in head IPCL (p= 0.31).
Table 4.1 Impact of 0 kCal Supplementation on Body Composition

<table>
<thead>
<tr>
<th></th>
<th>0 kCal Baseline (n=6)</th>
<th>0 kCal Follow-up (n=6)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± SD</td>
<td>Range</td>
</tr>
<tr>
<td><strong>Anthropometric Variables</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>73.9 ± 7.1</td>
<td>63.2-83.0</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>24.7 ± 3.8</td>
<td>18.7-29.4</td>
</tr>
<tr>
<td>WC (cm)</td>
<td>78.7 ± 12.5</td>
<td>63.0-92.0</td>
</tr>
<tr>
<td>Hip (cm)</td>
<td>91.4 ± 4.1</td>
<td>84.0-95.0</td>
</tr>
<tr>
<td>WHR</td>
<td>0.86 ± 0.08</td>
<td>0.75-0.97</td>
</tr>
<tr>
<td>WHtR</td>
<td>0.46 ± 0.07</td>
<td>0.37-0.56</td>
</tr>
<tr>
<td><strong>Ectopic Liver Fat</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MRS IHCL</td>
<td>0.41 ± 0.32</td>
<td>0.06-0.98</td>
</tr>
<tr>
<td>ME IHCL</td>
<td>1.25 ± 0.36</td>
<td>0.76-1.81</td>
</tr>
<tr>
<td>Segment 1</td>
<td>0.96 ± 0.44</td>
<td>0.26-1.57</td>
</tr>
<tr>
<td>Segment 2&amp;3</td>
<td>1.49 ± 0.46</td>
<td>0.97-2.24</td>
</tr>
<tr>
<td>Segment 4</td>
<td>0.98 ± 0.40</td>
<td>0.64-1.75</td>
</tr>
<tr>
<td>Segment 5&amp;8</td>
<td>1.11 ± 0.46</td>
<td>0.63-1.98</td>
</tr>
<tr>
<td>Segment 6&amp;7</td>
<td>1.20 ± 0.40</td>
<td>0.69-1.92</td>
</tr>
<tr>
<td><strong>Ectopic Pancreas Fat</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ME IPCL</td>
<td>1.61 ± 0.71</td>
<td>0.56-2.66</td>
</tr>
<tr>
<td>Head</td>
<td>2.18 ± 1.85</td>
<td>0.36-5.20</td>
</tr>
<tr>
<td>Body</td>
<td>1.35 ± 0.90</td>
<td>0.13-2.33</td>
</tr>
<tr>
<td>Tail</td>
<td>1.79 ± 1.35</td>
<td>0.25-3.96</td>
</tr>
<tr>
<td><strong>Adiposity Stores</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TAT (L)</td>
<td>15.84 ± 4.26</td>
<td>10.62-21.26</td>
</tr>
<tr>
<td>SAT (L)</td>
<td>11.89 ± 3.32</td>
<td>7.61-15.05</td>
</tr>
<tr>
<td>ASAT (L)</td>
<td>2.95 ± 1.01</td>
<td>1.50-4.23</td>
</tr>
<tr>
<td>NASAT (L)</td>
<td>8.94 ± 2.41</td>
<td>6.11-11.25</td>
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<tr>
<td>Internal (L)</td>
<td>3.95 ± 1.80</td>
<td>1.97-6.45</td>
</tr>
<tr>
<td>IAAT (L)</td>
<td>2.14 ± 1.45</td>
<td>0.58-4.32</td>
</tr>
<tr>
<td>NAIAT (L)</td>
<td>1.82 ± 0.38</td>
<td>1.39-2.26</td>
</tr>
<tr>
<td>IAAT/ASAT</td>
<td>0.74 ± 0.37</td>
<td>0.15-1.14</td>
</tr>
</tbody>
</table>

All data are presented as mean ± SD. WC: waist circumference; WHR: waist-to-hip ratio; WHtR: waist-to-height ratio; IHCL: intra-hepatocellular lipid; IPCL: intra-pancreatic cellular lipid, Adipose tissue deposits are in litres (l); TAT: total adipose tissue; SAT: subcutaneous; ASAT: abdominal subcutaneous; NASAT: non-abdominal subcutaneous; Internal: total internal; IAAT: intra-abdominal; NAIAT: non-abdominal internal. Differences before and after the 0 kcal supplement were analysed by paired Student’s t-test.
4.3.1.v Changes in Body Composition following 600 Calorie Overfeeding

Following the overfeeding with 600 kcal, there was a trend to increase in body weight by (1%), BMI (1%) and waist (3%); though none of these changes reached statistical significance, see Tables 4.2 and 4.3.

4.3.1.vi Changes in Adiposity following 600 Calorie Overfeeding

Following the 600 kcal overfeeding intervention, there were small increases in most adipose tissue depots, although this only reached statistical significance in the subcutaneous AT depot (17.26 ± 6.39 vs. 17.82 ± 6.15 L, p=0.05), see Tables 4.2 and 4.3.
Table 4.2 Impact of 600 kCal Supplementation on Body Composition

<table>
<thead>
<tr>
<th></th>
<th>600 kCal Baseline (n=6)</th>
<th>600 kCal Follow-up (n=6)</th>
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<tbody>
<tr>
<td></td>
<td>Mean ± SD</td>
<td>Range</td>
</tr>
<tr>
<td><strong>Anthropometric Variables</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>84.0 ± 10.4</td>
<td>66.8-95.4</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>26.4 ± 2.4</td>
<td>24.0-30.5</td>
</tr>
<tr>
<td>WC (cm)</td>
<td>89.0 ± 13.3</td>
<td>66.8-104.0</td>
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<tr>
<td>Hip (cm)</td>
<td>112.7 ± 26.8</td>
<td>97.0-167.0</td>
</tr>
<tr>
<td>WHR</td>
<td>0.92 ± 0.05</td>
<td>0.83-0.98</td>
</tr>
<tr>
<td>WItR</td>
<td>0.51 ± 0.06</td>
<td>0.45-0.59</td>
</tr>
<tr>
<td><strong>Ectopic Liver Fat</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MRS IHCL</td>
<td>2.04 ± 2.73</td>
<td>0.08-6.43</td>
</tr>
<tr>
<td>ME IHCL</td>
<td>3.11 ± 3.67</td>
<td>0.70-9.92</td>
</tr>
<tr>
<td>Segment 1</td>
<td>2.64 ± 3.12</td>
<td>0.66-8.68</td>
</tr>
<tr>
<td>Segment 2&amp;3</td>
<td>2.66 ± 2.21</td>
<td>1.18-6.84</td>
</tr>
<tr>
<td>Segment 4</td>
<td>2.97 ± 3.40</td>
<td>0.75-8.89</td>
</tr>
<tr>
<td>Segment 5&amp;8</td>
<td>3.43 ± 4.48</td>
<td>0.44-11.55</td>
</tr>
<tr>
<td>Segment 6&amp;7</td>
<td>3.04 ± 3.59</td>
<td>0.59-9.35</td>
</tr>
<tr>
<td><strong>Ectopic Pancreas Fat</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ME IPCL</td>
<td>3.20 ± 2.22</td>
<td>0.73-6.69</td>
</tr>
<tr>
<td>Head</td>
<td>3.06 ± 1.82</td>
<td>1.35-5.60</td>
</tr>
<tr>
<td>Body</td>
<td>3.18 ± 2.44</td>
<td>0.81-7.86</td>
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<tr>
<td>Tail</td>
<td>3.77 ± 2.49</td>
<td>0.14-6.64</td>
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<tr>
<td><strong>Adiposity Stores</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TAT (L)</td>
<td>21.37 ± 8.02</td>
<td>9.79-32.7</td>
</tr>
<tr>
<td>SAT (L)</td>
<td>17.26 ± 6.39</td>
<td>8.34-26.4</td>
</tr>
<tr>
<td>ASAT (L)</td>
<td>5.20 ± 2.42</td>
<td>2.39-8.81</td>
</tr>
<tr>
<td>NASAT (L)</td>
<td>12.06 ± 4.04</td>
<td>5.95-17.62</td>
</tr>
<tr>
<td>Internal (L)</td>
<td>4.11 ± 1.76</td>
<td>1.45-6.27</td>
</tr>
<tr>
<td>IAAT (L)</td>
<td>1.97 ± 1.22</td>
<td>0.28-3.66</td>
</tr>
<tr>
<td>NAIAT (L)</td>
<td>2.15 ± 0.63</td>
<td>1.17-2.87</td>
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<tr>
<td>IAAT/ASAT</td>
<td>0.35 ± 0.14</td>
<td>0.12-0.48</td>
</tr>
</tbody>
</table>

All data are presented as mean ± SD. WC: waist circumference; WHR: waist-to-hip ratio; WItR: waist-height ratio; IHCL: intra-hepatocellular lipid; IPCL: intra-pancreatic cellular lipid, Adipose tissue deposits are in litres (l); TAT: total adipose tissue; SAT: subcutaneous; ASAT: abdominal subcutaneous; NASAT: non-abdominal subcutaneous; Internal: total internal; IAAT: intra-abdominal; NAIAT: non-abdominal internal. Differences before and after the 600 kcal supplement were analysed by paired Student’s t-test.
4.3.1 vii Changes in Liver Fat Content following 600 Calorie Overfeeding

There was a trend for total IHCL content to decrease following the 600 kcal overfeeding intervention. There were also changes in regional IHCL content with small decreases in segments 1, 4 and 5&6, whereas IHCL content increased in segments 2&3 and 6&7, however none of these changes reached statistical significance, see Tables 4.2 and 4.3.

An example of a subject with an increased in liver fat content is shown in Figure 4.0. In this volunteer, total IHCL content increased by 10% from baseline.

![Figure 4.0 Example of overfeeding variation of 600 kcal in 6 days in total liver fat](image)

A series of multi-echo (a & c) and corresponding heat-map images (b & d) from a 30 year old healthy male volunteer with a BMI of 25.60 kg/m² varying in levels of IHCL in the liver before & after overfeeding with 600 kcal.
Regional Changes are shown in Figure 4.1. In this example IHCL increased by 18.3% in segment 1, 15.8% in segment 2&3, 2% in segment 4, 9% in segment 5&8 and 13.1% in segment 6&7 from baseline during the 600kcal overfeeding.

**Figure 4.1** Example of overfeeding 600 kcal in 6 days in different segments of the liver

Multi-echo at baseline and post overfeeding with 600kcal and corresponding heat-map liver segment images from a 30 year old healthy male volunteer with a BMI of 25.60 kg/m² varying in levels of ectopic fat (IHCL) in the different segments of the liver before & after overfeeding with 600kcal.
4.3.1 Changes in Pancreatic Fat Content following 600 Calorie Overfeeding

There were no consistent changes in IPCL content following the 600kcal overfeeding intervention. There was a small increase in total IPCL (0.22 ± 0.71, p=0.48), but this did not reach statistical significance, see Tables 4.2 and 4.3.

An example of a subject with an increase in total IPCL content of 23.5% from baseline is shown in Figure 4.2.

**Figure 4.2** Example of overfeeding variation of 600 kcal in 6 days in total pancreas

A series of multi-echo (a & c) and corresponding heat-map images (b & d) from a 30 year old healthy male volunteer with a BMI of 25.60 kg/m² varying in levels IPCL before & after overfeeding with 600kcal.
There were also changes in regional IPCL content with an increase in Head (0.76± 1.62, \( p=0.30 \), Tables 4.2 and 4.3) and decreases in both the tail (0.20 ± 1.10, \( p=0.67 \)) and body (0.83 ± 0.86, \( p=0.07 \)) of the pancreas.

An example of a subject with changes in regional in IPCL content is shown in Figure 4.3. In this example, IPCL increased by 1.8% in the Head, 1.2% ME Body IPCL, and a decreased by 25.5% in the tail from baseline during the 600kcal overfeeding.

![Figure 4.3 Example of overfeeding variation of 600 kcal in 6 days in different regions of the pancreas](image)

**Figure 4.3** Example of overfeeding variation of 600 kcal in 6 days in different regions of the pancreas

Multi-echo at baseline and post overfeeding with 600kcal and corresponding heat-map pancreas region images from a 30 year old healthy male volunteer with a BMI of 25.60 kg/m² varying in levels of IPCL in the different regions of the pancreas before & after overfeeding with 600kcal.
4.3.1.ix Comparison of the Effects of 0 vs. 600 Calorie Overfeeding

A comparison of the changes from baseline to follow-up in the 0 kcal group vs. 600 kcal group is shown in Table 4.3. There were no significant differences between the groups following the intervention in any of the anthropometric measurements.

4.3.1.x Comparison of the Effects of 0 vs. 600 Calorie Overfeeding on Adiposity

Similarly there were no differences between the two groups in terms of changes in adiposity, although there was a non-significant trend for the 600kcal group to gain more adipose tissue compared with the 0kcal group.

4.3.1.xi Comparison of the Effects of 0 vs. 600 Calorie Overfeeding on Liver Fat Content

Paradoxically the 0kcal group appeared to have a tendency to increase their total and regional IHCL content, whereas the trend was for a corresponding reduction in the 600kcal group, although these differences did not reach statistical significance (Table 4.3).

4.3.1.xii Comparison of the Effects of 0 vs. 600 Calorie Overfeeding on Pancreatic Fat Content

There was a significant difference (p=0.03) in the response to the overfeeding intervention between the 0 kcal group and the 600 kcal group, in IPCL content in the body of the pancreas. This increased by 0.35 ± 0.75 in the 0kcal group and decreased by -0.83 ± 0.86 in the 600 kcal group (Table 4.3). There were no significant changes in total, head or tail IPCL content.

Table 4.3 Impact of 0 vs. 600 kCal Supplementation on Body Composition
<table>
<thead>
<tr>
<th></th>
<th>0 kCal (n=6)</th>
<th>600 kCal (n=6)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Anthropometric Variables</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>-0.30 ± 0.30</td>
<td>0.63 ± 0.92</td>
<td>0.06</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>-0.10 ± 0.10</td>
<td>0.20 ± 0.30</td>
<td>0.06</td>
</tr>
<tr>
<td>WC (cm)</td>
<td>5.22 ± 8.58</td>
<td>3.07 ± 6.35</td>
<td>0.63</td>
</tr>
<tr>
<td>Hip (cm)</td>
<td>2.08 ± 3.90</td>
<td>-12.85 ± 33.21</td>
<td>0.32</td>
</tr>
<tr>
<td>WHR</td>
<td>0.04 ± 0.07</td>
<td>0.005 ± 0.02</td>
<td>0.33</td>
</tr>
<tr>
<td>WHtR</td>
<td>0.03 ± 0.05</td>
<td>0.06 ± 0.01</td>
<td>0.32</td>
</tr>
<tr>
<td><strong>Ectopic Liver Fat</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MRS IHCL</td>
<td>0.27 ± 0.26</td>
<td>-0.27 ± 0.81</td>
<td>0.18</td>
</tr>
<tr>
<td>ME IHCL</td>
<td>0.01 ± 0.26</td>
<td>-0.16 ± 0.82</td>
<td>0.65</td>
</tr>
<tr>
<td>Segment 1</td>
<td>0.22 ± 0.42</td>
<td>-0.13 ± 1.17</td>
<td>0.52</td>
</tr>
<tr>
<td>Segment 2&amp;3</td>
<td>-0.25 ± 0.28</td>
<td>0.06 ± 0.34</td>
<td>0.12</td>
</tr>
<tr>
<td>Segment 4</td>
<td>0.18 ± 0.24</td>
<td>-0.02 ± 0.13</td>
<td>0.11</td>
</tr>
<tr>
<td>Segment 5&amp;8</td>
<td>0.19 ± 0.24</td>
<td>-0.14 ± 0.97</td>
<td>0.45</td>
</tr>
<tr>
<td>Segment 6&amp;7</td>
<td>0.24 ± 0.37</td>
<td>0.02 ± 0.68</td>
<td>0.51</td>
</tr>
<tr>
<td><strong>Ectopic Pancreas Fat</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ME IPCL</td>
<td>0.06 ± 0.55</td>
<td>0.22 ± 0.71</td>
<td>0.67</td>
</tr>
<tr>
<td>Head</td>
<td>-0.54 ± 1.17</td>
<td>0.76 ± 1.62</td>
<td>0.15</td>
</tr>
<tr>
<td>Body</td>
<td>0.35 ± 0.75</td>
<td>-0.83 ± 0.86</td>
<td>0.03</td>
</tr>
<tr>
<td>Tail</td>
<td>0.28 ± 1.87</td>
<td>-0.20 ± 1.10</td>
<td>0.60</td>
</tr>
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<td><strong>Adiposity Stores</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TAT (L)</td>
<td>0.21 ± 0.49</td>
<td>0.68 ± 0.84</td>
<td>0.28</td>
</tr>
<tr>
<td>SAT (L)</td>
<td>0.19 ± 0.24</td>
<td>0.57 ± 0.54</td>
<td>0.17</td>
</tr>
<tr>
<td>ASAT (L)</td>
<td>0.03 ± 0.11</td>
<td>0.14 ± 0.20</td>
<td>0.26</td>
</tr>
<tr>
<td>NASAT (L)</td>
<td>0.17 ± 0.24</td>
<td>0.43 ± 0.55</td>
<td>0.33</td>
</tr>
<tr>
<td>Internal (L)</td>
<td>0.02 ± 0.29</td>
<td>0.11 ± 0.43</td>
<td>0.67</td>
</tr>
<tr>
<td>IAAT (L)</td>
<td>-0.05 ± 0.12</td>
<td>0.006 ± 0.36</td>
<td>0.72</td>
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<tr>
<td>NAIAT (L)</td>
<td>0.07 ± 0.19</td>
<td>0.11 ± 0.12</td>
<td>0.73</td>
</tr>
<tr>
<td>IAAT/ASAT</td>
<td>-0.04 ± 0.06</td>
<td>0.014 ± 0.07</td>
<td>0.46</td>
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</tbody>
</table>

All data are presented as the mean ± SD of the change from baseline to follow-up for each group. WC: waist circumference; WHR: waist-to-hip ratio; WHtR: waist-to-height ratio; IHCL: intra-hepatocellular lipid; IPCL: intra-pancreatocellular lipid, Adipose tissue deposits are in litres (L); TAT: total adipose tissue; SAT: subcutaneous; ASAT: abdominal subcutaneous; NASAT: non-abdominal subcutaneous; Internal: total internal; IAAT: intra-abdominal; NAIAT: non-abdominal internal. Differences between groups were analysed by unpaired Student’s t-test.
4.3.2 Study 2 - The Effect of Calorie Restriction on Body Composition

Subjects were asked to fill in a 7-day diet diary which was subsequently used as the basis for individualised dietary counselling as part of the weight-loss strategy. This required for each volunteer to record all food items and drinks that they consumed over a period of 7 consecutive days (including a weekend). As part of the dietary assessment, volunteers were also asked to provide details regarding the method of food preparation and whether food was prepared at home or delivered/prepared, together with all available details of each brand, including packaging. Food diaries were analysed using DietPlan6 by a trained independent researcher competent in nutritional analysis.

DietPlan6 is a database with full set of UK food tables - all the foods and nutrients from the 5th and 6th Editions of McCance and Widdowson’s The Composition of foods plus all the published supplements, including Fatty acids and the Composition of Foods Integrated Data Set.

The results from these analyses were subsequently used to advice each subject to reduce ~500 calorie intake to achieve a desired weight loss. On average it was aimed for subjects to achieve a 3.0 kg reduction in weight at the end of a 12 weeks intervention period. Subjects were scanned at baseline and the morning after they concluded their intervention.

Participants (19 females and 16 males) had a mean age of 51.8 ± 13.02 years, range of 18-65 years. In addition to an overall BMI mean of 36.6 ± 8.4 kg/m² (range 25.6-50.9 kg/m²) at baseline (Table 4.4).
There were significant reductions in most anthropometric measurements following the 12 week intervention, see Table 4.4. The mean weight loss was 6.4%, with a 6.2% reduction in BMI, accompanied by reductions of 4.02% in waist and 3.6% in hip circumferences (Table 4.4).
Table 4.4 Calorie Restriction Specific Variable Data in Whole Cohort

<table>
<thead>
<tr>
<th></th>
<th>Baseline (n=35)</th>
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<th>Follow-up (n=35)</th>
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<td>Mean ± SD</td>
<td>Range</td>
<td>Mean ± SD</td>
<td>Range</td>
<td></td>
</tr>
<tr>
<td><strong>Anthropometric Variables</strong></td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>51.8 ± 13.02</td>
<td>18-65</td>
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<tr>
<td>Weight (kg)</td>
<td>103.4 ± 22.4</td>
<td>68.6-151.1</td>
<td>96.8 ± 21.8</td>
<td>66.5-147.1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>36.6 ± 8.4</td>
<td>25.6-50.9</td>
<td>34.3 ± 8.2</td>
<td>22.7-49.2</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>WC (cm)</td>
<td>114.5 ± 17.7</td>
<td>77.0-145.5</td>
<td>109.9 ± 17.6</td>
<td>78.0-141.0</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Hip (cm)</td>
<td>119.3 ± 14.4</td>
<td>98.2-147.0</td>
<td>115.0 ± 14.6</td>
<td>93.8-142.5</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>169 ± 10.0</td>
<td>141-190</td>
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<tr>
<td>WHR</td>
<td>0.96 ± 0.10</td>
<td>0.67-1.16</td>
<td>0.96 ± 0.09</td>
<td>0.75-1.14</td>
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<tr>
<td>WHtR</td>
<td>0.68 ± 0.12</td>
<td>0.45-0.92</td>
<td>0.66 ± 0.12</td>
<td>0.49-0.89</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>Ectopic Liver Fat</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MRS IHCL</td>
<td>12.13 ± 14.88</td>
<td>0.15-60.54</td>
<td>7.84 ± 12.26</td>
<td>0.19-50.04</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>ME IHCL</td>
<td>9.31 ± 8.01</td>
<td>0.88-32.27</td>
<td>5.93 ± 6.20</td>
<td>0.53-26.7</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Segment 1</td>
<td>8.37 ± 7.51</td>
<td>1.18-29.42</td>
<td>5.26 ± 5.85</td>
<td>0.46-26.89</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Segment 2&amp;3</td>
<td>8.50 ± 7.51</td>
<td>0.60-29.96</td>
<td>5.41 ± 5.69</td>
<td>0.78-25.23</td>
<td>&lt;0.001</td>
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<td>Segment 4</td>
<td>9.32 ± 7.60</td>
<td>0.93-32.11</td>
<td>5.92 ± 5.81</td>
<td>0.29-25.88</td>
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<tr>
<td>Segment 5&amp;8</td>
<td>9.62 ± 9.01</td>
<td>0.80-33.69</td>
<td>6.21 ± 7.16</td>
<td>0.39-29.15</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Segment 6&amp;7</td>
<td>9.78 ± 9.24</td>
<td>1.09-36.01</td>
<td>6.29 ± 7.59</td>
<td>0.18-33.28</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>Ectopic Pancreas Fat</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ME IPCL</td>
<td>8.01 ± 7.14</td>
<td>0.55-24.18</td>
<td>6.92 ± 5.67</td>
<td>0.24-20.88</td>
<td>0.21</td>
</tr>
<tr>
<td>Head</td>
<td>6.75 ± 6.82</td>
<td>0.38-23.63</td>
<td>6.29 ± 5.57</td>
<td>0.52-21.16</td>
<td>0.66</td>
</tr>
<tr>
<td>Body</td>
<td>7.62 ± 8.35</td>
<td>0.25-31.74</td>
<td>6.49 ± 5.86</td>
<td>0.32-25.29</td>
<td>0.28</td>
</tr>
<tr>
<td>Tail</td>
<td>6.43 ± 5.86</td>
<td>0.27-25.29</td>
<td>7.73 ± 7.19</td>
<td>0.45-28.74</td>
<td>0.18</td>
</tr>
<tr>
<td><strong>Adiposity Stores</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TAT (L)</td>
<td>50.25 ± 19.55</td>
<td>16.49-87.67</td>
<td>46.04 ± 19.62</td>
<td>12.80-82.50</td>
<td>&lt;0.001</td>
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<tr>
<td>SAT (L)</td>
<td>41.22 ± 18.42</td>
<td>12.45-76.64</td>
<td>37.89 ± 18.07</td>
<td>9.88-72.84</td>
<td>&lt;0.001</td>
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<tr>
<td>ASAT (L)</td>
<td>13.23 ± 7.05</td>
<td>2.67-28.24</td>
<td>12.05 ± 6.93</td>
<td>1.91-27.33</td>
<td>&lt;0.001</td>
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<tr>
<td>NASAT (L)</td>
<td>28.00 ± 11.75</td>
<td>9.79-51.30</td>
<td>25.84 ± 11.49</td>
<td>7.97-48.86</td>
<td>&lt;0.001</td>
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<tr>
<td>Internal (L)</td>
<td>9.03 ± 2.68</td>
<td>4.04-13.53</td>
<td>8.15 ± 2.73</td>
<td>2.92-12.92</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>IAAT (L)</td>
<td>5.14 ± 1.83</td>
<td>1.82-8.73</td>
<td>4.50 ± 1.76</td>
<td>1.13-8.06</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>NAIAT (L)</td>
<td>3.89 ± 1.11</td>
<td>2.22-6.47</td>
<td>3.59 ± 1.12</td>
<td>1.79-6.31</td>
<td>0.001</td>
</tr>
<tr>
<td>IAAT/ASAT</td>
<td>0.50 ± 0.32</td>
<td>0.17-1.57</td>
<td>0.48 ± 0.30</td>
<td>0.15-1.57</td>
<td>0.09</td>
</tr>
</tbody>
</table>

All data are presented as mean ± SD. WC: waist circumference; WHR: waist-to-hip ratio; WHtR: waist-to-height ratio; IHCL: intra-hepatocellular lipid; IPCL: intra-pancreatic cellular lipid. Adipose tissue deposits are in litres (L); TAT: total adipose tissue; SAT: subcutaneous; ASAT: abdominal subcutaneous; NASAT: non-abdominal subcutaneous; Internal: total internal; IAAT: intra-abdominal; NAIAT: non-abdominal internal. *Post calorie restriction ME total and regional IHCL (n=34), *WC (n=33), Hip (n=33), WHR (n=33) and WHtR (n=33). Baseline versus post calorie restriction data was analysed by paired Student’s t-test.
4.3.2.i Changes in Adiposity following Calorie Restriction

There were significant reductions in all adipose tissue depots following the 12 week intervention, see Table 4.4 and Figure 4.4. There was a 8.4% reduction in TAT, 8.1% reduction in SAT, 9.7% in internal AT (see Figure 4.5) and a 12.5% reduction in IAAT, all p<0.001.

![Figure 4.4 Reduction in Subcutaneous Adipose Tissue Depots following Weight Loss Intervention.](image)
4.3.2.ii Changes in Liver Fat Content following Calorie Restriction

Following the weight loss intervention there were large and highly significant reductions in both total and regional IHCL (all p<0.001), see Table 4.4 and Figure 4.6. In percentage terms, a greater proportion of IHCL was lost than adipose tissue, with a decrease in total IHCL of 36.3% and decreases in regional IHCL of between 35.4-37.2%.
Figure 4.6 Reduction in Total and Regional IHCL Content following Weight Loss

An example of a subject with a reduction in liver fat content is depicted in Figure 4.7. This subject had a decrease in total IHCL content of 24.3%.
Figure 4.7 Example of Calorie Restriction Variation in Total Liver Fat

A series of multi-echo (a & c) and corresponding heat-map images (b & d) from a 62 year old female volunteer with a BMI of 35.65 kg/m² at baseline and 34.49 kg/m² post calorie restriction. Varying in levels of IHCL are seen across the liver.

There were also significant regional changes in the same subjects, which can be seen in Figure 4.8. 35.4% decrease in segment 1, 32% in segment 2&3, 12.3% in segment 4, 17.6% in segment 5&8 and 25.8% in segment 6&7 following weight loss.
Figure 4.8 Example Calorie Restriction Variations in Different Segments of the Liver

Multi-echo at baseline and post calorie restriction and corresponding heat-map liver segment images from a 62 year old female volunteer with a BMI of 35.65 kg/m² at baseline and 34.49 kg/m² post calorie restriction varying in levels of ectopic fat (IHCL) in the different segments of the liver.

4.3.2.iii Changes in Pancreatic Fat Content following Calorie Restriction

Despite a trend for a reduction in total IPCL (8.01 ± 7.14 vs. 6.92 ± 5.67, p=0.21) following the weight loss intervention, these did not reach statistical significance (Table 4.4).
Similar changes were observed in the head and body regions of the pancreas, see Figure 4.9, however there was a slight increase in tail post calorie restriction, but again this did not reach statistical significance (p=0.18).

**Figure 4.9** Reduction in Total and Regional IHCL Content following Weight Loss

An example of a subject with a reduction in total IPCL content is depicted in Figure 4.10. IPCL decreased by a substantial 74% in this subject.
Figure 4.10 Example of Calorie Restriction Variation in Total Pancreas Fat

A series of multi-echo (a & c) and corresponding heat-map images (b & d) from a 65-year old male volunteer with a BMI of 27.91 kg/m² at baseline and 27.22 kg/m² post calorie restriction varying in levels of IPCL.

Regional changes were also observed in this volunteer, see Figure 4.11. Head IPCL was reduced by 53.2%, Body by 6% and Tail by 38.2%.
Figure 4.11 Example of Reduced Calories Variation in Different Regions of the Pancreas

Multi-echo at baseline and post calorie restriction and corresponding heat-map pancreas region images from a 65 year old male volunteer with a BMI of 27.91 kg/m² at baseline and 27.22 kg/m² post calorie restriction varying in levels of IPCL in the different regions of the pancreas.
4.3.2.iv The Impact of Gender and Weight Loss on Liver Fat Content

Weight loss decreased in total IHCL by 33.9% from 8.03 ±1.72 to 5.31±1.32 (p<0.001) in females and by 37.2% from 10.55 ± 2.24 to 6.63 ± 1.73 (p=0.001) in male subjects, see Figure 4.12.

Figure 4.12 Reduction in Total IHCL in Female and Male Volunteers following Weight Loss.
4.3.2. Relationship between Weight Loss and Total Liver Fat Content

Interestingly, despite substantial reductions in both, there was no significant correlation between the reduction in IHCL and weight loss (female: $r=0.18$, $P=0.49$, male: $r=-0.003$, $p=0.99$), see Figure 4.13.

![Figure 4.13](image)

**Figure 4.13** Relation between the Absolute Change in Body Weight and IHCL in Male and Female Subjects.

Relation between the absolute change in body weight and IHCL content for male ($n=16$) (open circles) and female ($n=18$) (closed circles) subjects. Spearman correlation coefficients for the relation between the absolute change in body weight and IHCL are (A) $r=0.18$ ($p=0.49$) for females and $r=-0.003$ ($p=0.99$) for males.
4.3.2. vi Relationship between Weight Loss and Regional Liver Fat Content

There were significant reductions in regional IHCL content in all liver segments in both female and male volunteers (all \( p<0.001 \)). Despite an apparent greater reduction in male subjects (Figure 4.14), there were no differences in IHCL content related to gender.

![Graph showing reduction in regional IHCL content for male and female volunteers.](image)

**Figure 4.14** Reduction in Regional IHCL Content in Female and Male Volunteers following Weight Loss.

As in the case for total IHCL content there was no significant correlation between reduction in regional IHCL content and weight loss (Figure 4.15).
Figure 4.15 Relation between the Absolute Change in Body Weight and IHCL Content Male and Female Subjects in different Segments of the Liver

Relation between the change in body weight and regional IHCL for male (open circles) and female (closed circles) subjects. Spearman correlation coefficients for the relation between the change in body weight and IHCL in segment 1 (B) (female: r=0.04, p=0.88, male: r=-0.15, p=0.59); segment 2&3 (C) (female: r=0.24, p=0.35, male: r=0.02, p=0.96); segment 4 (D) (female: r=0.24, p=0.33, male: r=-0.03, p=0.92); segment 5&8 (E) (female: r=0.25, p=0.31, male: r=0.04, p=0.90); and segment 6&7 (F) (female: r=0.05, p=0.85, male: r=0.10, p=0.70).
4.3.2.vii The Impact of Gender and Weight Loss on Pancreatic Fat Content

Over all IPCL was reduced following weight loss by 0.71% (6.96 ±1.50 to 6.91±1.36, p=0.95) in female subjects and by 22% (8.87±2.03 to 6.92±1.44, p=0.13) in male subjects, although there was no significant effect of gender on the change in IPCL (p=0.21) (Figure 4.16).

There was no consistent weight loss changes in IPCL related to gender, with both genders increasing, or decreasing IPCL in different regions of the pancreas.

Interestingly there was a significant effect of gender (p=0.027) in the Head region, with an increase in IPCL in female, and a decrease in IPCL in male subjects.
Figure 4.16 – Reduction in Total and Regional IPCL Content in Female and Male Volunteers following Weight Loss.

As with the liver, there was no significant relationship between change in total (Figure 4.17) or regional IPCL (Figure 4.18) and change in body weight following weight loss period.
Figure 4.17 Relation between the Absolute Change in Body Weight and that in Pancreas Fat content for Male and Female Subjects in total IPCL

Relation between change in body weight and IPCL for male (open circles) and female (closed circles) subjects. Spearman correlation coefficients for the relation between the absolute change in body weight and IPCL are (A) \( r=0.17 \) (\( P=0.50 \)) for females and \( r=0.35 \) (\( P=0.19 \)) for males.
**Figure 4.18** Relation between the Absolute Change in Body Weight and IPCL Content for Male and Female Subjects in Variation in Different Regions of the Pancreas

Relation between change in body weight and regional IPCL content for male (open circles) and female (closed circles) subjects. Spearman correlation coefficients for the relation between the absolute change in body weight and IPCL are: (B) Head: female \( r=0.09, p=0.72 \), male \( r=0.30, p=0.26 \); (C) Body: female: \( r=0.14, p=0.57 \), male: \( r=0.46, p=0.08 \); (D) Tail: female: \( r=-0.03, p=0.90 \), male: \( r=0.23, p=0.40 \).
4.3.3 Study 3 - Impact of Bariatric Surgery Study on Body Composition

Morbidly obese subjects were scanned before and 6 months after undergoing bariatric surgery to determine its effect on ectopic fat content and distribution and whether it would differ from conventional calorie reduction intervention.

Besides the normal exclusion criteria, (Chapter 2) subjects were pre-selected based on their relative size due to the magnet-bore size. Subjects were scanned at baseline and 6 months following surgery. The standard protocol for bariatric surgery includes a short period of calorie-restriction prior to surgery in this case between 2 and 6 weeks of a low calorie diet. Therefore the changes between baseline and follow-up include the combined effects of the dietary restriction and surgical intervention.

Participants (83% female, 17% male) had a mean age range of 18-58 years with an overall mean BMI of 46.6 ± 2.6 kg/m² (range 42.8 – 50.9 kg/m²) at baseline and a mean BMI of 34.0 ± 2.4 kg/m² (range 30.4 – 37.2 kg/m²) 6 months post-surgery (Table 4.5).

During the 6 months weight loss period after bariatric surgery, subjects lost 27% of their body weight, had a 27% reduction in BMI, as well as reductions of 19% in waist and 18.5% in hip circumferences (Table 4.5).
Table 4.5 Impact of Bariatric Surgery on Body Composition

<table>
<thead>
<tr>
<th>Anthropometric Variables</th>
<th>Baseline (n=12)</th>
<th>6 months post-surgery (n=12)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± SD</td>
<td>Range</td>
</tr>
<tr>
<td>Age (yrs)</td>
<td>43.3 ± 12.1</td>
<td>18-58</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>127.3 ± 14.1</td>
<td>110.4-151.1</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>46.6 ± 2.6</td>
<td>42.8-50.9</td>
</tr>
<tr>
<td>WC (cm)</td>
<td>132.7 ± 8.2</td>
<td>114.1-145.5</td>
</tr>
<tr>
<td>Hip (cm)</td>
<td>135.9 ± 8.2</td>
<td>117.5-147.0</td>
</tr>
<tr>
<td>Height (m)</td>
<td>165.2 ± 8.9</td>
<td>154-185</td>
</tr>
<tr>
<td>WHR</td>
<td>0.98 ± 0.08</td>
<td>0.85-1.16</td>
</tr>
<tr>
<td>WHtR</td>
<td>0.80 ± 0.06</td>
<td>0.72-0.92</td>
</tr>
<tr>
<td>Ectopic Liver Fat</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MRS IHCL</td>
<td>13.27 ± 16.13</td>
<td>2.01-60.54</td>
</tr>
<tr>
<td>ME IHCL</td>
<td>10.08 ± 7.34</td>
<td>3.98-30.0</td>
</tr>
<tr>
<td>Segment 1</td>
<td>8.63 ± 6.90</td>
<td>2.17-26.77</td>
</tr>
<tr>
<td>Segment 2&amp;3</td>
<td>9.90 ± 6.83</td>
<td>3.57-28.1</td>
</tr>
<tr>
<td>Segment 4</td>
<td>9.79 ± 6.61</td>
<td>3.96-27.01</td>
</tr>
<tr>
<td>Segment 5&amp;8</td>
<td>10.43 ± 8.42</td>
<td>2.95-33.53</td>
</tr>
<tr>
<td>Segment 6&amp;7</td>
<td>10.69 ± 9.10</td>
<td>2.89-36.01</td>
</tr>
<tr>
<td>Ectopic Pancreas Fat</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ME IPCL</td>
<td>7.70 ± 6.72</td>
<td>1.26-21.62</td>
</tr>
<tr>
<td>Head</td>
<td>6.45 ± 6.17</td>
<td>0.59-17.56</td>
</tr>
<tr>
<td>Body</td>
<td>8.80 ± 9.43</td>
<td>0.32-31.74</td>
</tr>
<tr>
<td>Tail</td>
<td>5.21 ± 3.97</td>
<td>1.06-11.39</td>
</tr>
<tr>
<td>Adiposity Stores</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TAT (L)</td>
<td>73.80 ± 6.28</td>
<td>65.75-87.67</td>
</tr>
<tr>
<td>SAT (L)</td>
<td>63.83 ± 6.24</td>
<td>53.21-76.64</td>
</tr>
<tr>
<td>ASAT (L)</td>
<td>21.78 ± 2.81</td>
<td>18.38-28.24</td>
</tr>
<tr>
<td>NASAT (L)</td>
<td>42.05 ± 5.56</td>
<td>34.83-51.30</td>
</tr>
<tr>
<td>Internal (L)</td>
<td>9.97 ± 2.02</td>
<td>6.96-13.39</td>
</tr>
<tr>
<td>IAAT (L)</td>
<td>5.34 ± 1.36</td>
<td>3.51-7.56</td>
</tr>
<tr>
<td>NAIAT (L)</td>
<td>4.63 ± 0.90</td>
<td>3.21-6.47</td>
</tr>
<tr>
<td>IAAT/ASAT</td>
<td>0.25 ± 0.07</td>
<td>0.17-0.40</td>
</tr>
</tbody>
</table>

All data are presented as mean ± SD. WC: waist circumference; WHR: waist-to-hip ratio; WHtR: waist-to-height ratio; IHCL: intra-hepatocellular lipid; IPCL: intra-pancreatic cellular lipid, Adipose tissue deposits are in litres (l); TAT: total adipose tissue; SAT: subcutaneous; ASAT: abdominal subcutaneous; NASAT: non-abdominal subcutaneous; Internal: total internal; IAAT: intra-abdominal; NAIAT: non-abdominal internal. Baseline versus post-surgery data were analysed by paired Student’s t-test.
4.3.3.i Changes in Adiposity following Bariatric Surgery

There were significant reductions in adiposity 6 months after bariatric surgery. The mean reductions in adipose tissue were considerable, TAT 36.2%, SAT 37.4%, internal 28.1% and IAAT 35.2%, with the greatest percentage change in ASAT 39.8% (p<0.001 for all), see Table 4.5.

Interestingly there was a small increase in IAAT/ASAT ratio, it displayed a slight yet significant increase from 0.25 ± 0.07 to 0.27 ± 0.09 (p=0.05), reflecting the greater loss of ASAT compared with IAAT.

4.3.3.ii Changes in Liver Fat Content following Bariatric Surgery

There was a significant reduction in both total and regional IHCL content, see Table 4.5. Total IHCL was reduced by 61.4% (p<0.01) from 10.08 ± 7.34 to 3.89 ± 3.59.

IHCL was also significantly reduced in all but one segment of the liver; this did not reach significance in Segment 6&7 (see Figure 4.19).
An example of a subject with a significant reduction in IHCL is shown in Figure 4.20. In this individual, total IHCL content decreased by 70% from baseline.
Figure 4.20 Example of 6 Months Post-surgery Variation in Total Liver Fat

A series of multi-echo (a & c) and corresponding heat-map images (b & d) from a 43 year old female volunteer with a BMI of 45.05 kg/m² at baseline and 35.49 kg/m² post 6 months surgery varying in levels IHCL.

The same individual also showed significant reductions in regional IHCL content, see Figure 4.21. IHCL reductions of 73% were observed in segment 1, 81.9% segment 2&3, 78.3% segment 4, 82.4% segment 5&8 and 73% segment 6&7 from baseline post 6 months after surgery.
Figure 4.21 Example of 6 Months Post-surgery Variation in Different Segments of the Liver

Multi-echo at baseline and 6 months post-surgery and corresponding heat-map liver segment images from a 43 year old female volunteer with a BMI of 45.05 kg/m² at baseline and 35.49 kg/m² post 6 months surgery varying in levels IHCL in the different segments of the liver.

4.3.3.iii Changes in Pancreatic Fat Content following Bariatric Surgery

Following bariatric surgery, there was a trend for IPCL content to increase, total IPCL by 7.1%, Head IPCL by 18.8% and ME Tail IPCL by 23.6%, those these changes did not reach statistical significance.

A small non-significant decrease (1.7%) was observed in the Body pancreatic region (Table 4.5, and Figure 4.22).
An example of a subject with a reduction in IPCL content is depicted in Figure 4.23 and 4.24 were total IPCL decreased by 16.1% from baseline and 19.4% in Body.

There was an increase of 3.4% in Head IPCL and no alteration in the Tail from baseline to 6 months post-surgery.
Figure 4.23 Example of 6 months Post-surgery Variation in Total Pancreas Fat

A series of multi-echo (a & c) and corresponding heat-map images (b & d) from a 43 year old female volunteer with a BMI of 45.05 kg/m² at baseline and 35.49 kg/m² post 6 months surgery varying in levels of IPCL.
Figure 4.24 Example of 6 Months Post-surgery Variation in Different Regions of the Pancreas

Multi-echo at baseline and 6 months post-surgery and corresponding heat-map pancreas region images from a 43 year old female volunteer with a BMI of 45.05 kg/m² at baseline and 35.49 kg/m² post 6 months surgery varying in levels IPCL in the different regions of the pancreas.
4.3.3.iv Comparison of Changes in Body Composition following Weight Loss Induced by Dietary Restriction vs. Bariatric Surgery Study

As previously mentioned the data in section 4.3.3 looking at the effects of bariatric surgery on weight loss also contained a period of calorie-reduction prior to surgery as part of the standard protocol.

Therefore to determine whether diet and surgery resulted in different amounts or patterns of weight loss these individual were further examined at different time points – post diet but pre surgery, referred to as Baseline 2 and 6 months post-surgery (follow-up). This was compared with the changes pre and post caloric restriction in the wider cohort.

There were significant differences in the degree of change in body composition between the calorie-restriction and surgical groups with a mean weight loss of 6.4% in the calorie-restricted vs. 22.5% in the surgical intervention group (p<0.001).

Changes in waist circumference were also significantly different between the two groups with a mean weight loss of 4.02% in calorie restriction vs. 16.7% surgery p<0.001. Similar changes were seen for other anthropometric measurements (see Table 4.6).
### Table 4.6 Comparison of Calorie-Restricion and Bariatric Surgery on Body Composition

<table>
<thead>
<tr>
<th>Anthropometric Variables</th>
<th>Caloric Restriction (n=35)</th>
<th>Bariatric Surgery (n=12)</th>
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<tr>
<td></td>
<td>Baseline</td>
<td>Follow-up</td>
<td>Baseline</td>
</tr>
<tr>
<td>Age (yrs)</td>
<td>51.8 ± 13.02</td>
<td></td>
<td>43.3 ± 12.1</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>103.4 ± 22.4</td>
<td>96.8 ± 21.8</td>
<td>120.0 ± 14.1</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>36.6 ± 8.4</td>
<td>34.3 ± 8.2</td>
<td>43.9 ± 2.8</td>
</tr>
<tr>
<td>WC (cm)</td>
<td>114.5 ± 17.7</td>
<td>109.9 ± 17.6</td>
<td>129.0 ± 9.3</td>
</tr>
<tr>
<td>Hip (cm)</td>
<td>119.3 ± 14.4</td>
<td>115.0 ± 14.6</td>
<td>131.2 ± 7.8</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>169 ± 10.0</td>
<td></td>
<td>165.2 ± 8.9</td>
</tr>
<tr>
<td>WHR</td>
<td>0.96 ± 0.10</td>
<td></td>
<td>0.96 ± 0.09</td>
</tr>
<tr>
<td>WHtR</td>
<td>0.68 ± 0.12</td>
<td></td>
<td>0.66 ± 0.12</td>
</tr>
<tr>
<td>Ectopic Liver Fat</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MRS IHCL</td>
<td>12.13 ± 14.88</td>
<td>7.84 ± 12.26</td>
<td>9.13 ± 12.74</td>
</tr>
<tr>
<td>ME IHCL</td>
<td>9.31 ± 8.01</td>
<td>5.93 ± 6.20</td>
<td>6.61 ± 6.57</td>
</tr>
<tr>
<td>Segment 1</td>
<td>8.37 ± 7.51</td>
<td>5.26 ± 5.85</td>
<td>5.60 ± 6.05</td>
</tr>
<tr>
<td>Segment 2&amp;3</td>
<td>8.50 ± 7.51</td>
<td>5.41 ± 5.69</td>
<td>6.16 ± 5.86</td>
</tr>
<tr>
<td>Segment 4</td>
<td>9.32 ± 7.60</td>
<td>5.92 ± 5.81</td>
<td>6.12 ± 5.89</td>
</tr>
<tr>
<td>Segment 5&amp;8</td>
<td>9.62 ± 9.01</td>
<td>6.21 ± 7.16</td>
<td>7.17 ± 8.33</td>
</tr>
<tr>
<td>Ectopic Pancreas Fat</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ME IPCL</td>
<td>8.01 ± 7.14</td>
<td>6.92 ± 5.67</td>
<td>7.95 ± 6.71</td>
</tr>
<tr>
<td>Head</td>
<td>6.75 ± 6.82</td>
<td>6.29 ± 5.57</td>
<td>8.58 ± 6.91</td>
</tr>
<tr>
<td>Body</td>
<td>7.62 ± 8.35</td>
<td>6.49 ± 5.86</td>
<td>7.54 ± 5.88</td>
</tr>
<tr>
<td>Tail</td>
<td>6.43 ± 5.86</td>
<td>7.73 ± 7.19</td>
<td>8.60 ± 6.75</td>
</tr>
<tr>
<td>Adiposity Stores</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TAT (L)</td>
<td>50.25 ± 19.55</td>
<td>46.04 ± 19.62</td>
<td>69.32 ± 7.82</td>
</tr>
<tr>
<td>SAT (L)</td>
<td>41.22 ± 18.42</td>
<td>37.89 ± 18.07</td>
<td>59.80 ± 7.09</td>
</tr>
<tr>
<td>ASAT (L)</td>
<td>13.23 ± 7.05</td>
<td>12.05 ± 6.93</td>
<td>20.43 ± 2.97</td>
</tr>
<tr>
<td>NASAT (L)</td>
<td>28.00 ± 11.75</td>
<td>25.84 ± 11.49</td>
<td>39.36 ± 5.85</td>
</tr>
<tr>
<td>Internal (L)</td>
<td>9.03 ± 2.68</td>
<td>8.15 ± 2.73</td>
<td>9.53 ± 2.24</td>
</tr>
<tr>
<td>IAAT (L)</td>
<td>5.14 ± 1.83</td>
<td>4.50 ± 1.76</td>
<td>5.02 ± 1.40</td>
</tr>
<tr>
<td>NAIAT (L)</td>
<td>3.89 ± 1.11</td>
<td>3.59 ± 1.12</td>
<td>4.50 ± 0.99</td>
</tr>
<tr>
<td>IAAT/ASAT</td>
<td>0.50 ± 0.32</td>
<td>0.48 ± 0.30</td>
<td>0.25 ± 0.07</td>
</tr>
</tbody>
</table>

All data are presented as mean ± SD. WC: waist circumference; WHR: waist-to-hip ratio; WHtR: waist-to-height ratio; IHCL: intra-hepatocellular lipid; IPCL: intra-pancreatic cellular lipid. Adipose tissue deposits are in litres (L); TAT: total adipose tissue; SAT: subcutaneous; ASAT: abdominal subcutaneous; NASAT: non-abdominal subcutaneous; Internal: total internal; IAAT: intra-abdominal; NAIAT: non-abdominal internal. *Post calorie restriction ME total and regional IHCL (n=34), *WC (n=33), Hip (n=33), WHR (n=33) and WHtR (n=33), *Baseline 2 ME total and regional IHCL and IPCL (n=11). Differences between interventions were tested for using a student’s unpaired t-test on the difference between baseline and follow-up measurements for each group.
4.3.3.v Comparison of Changes in Adipose Tissue Content following Weight Loss Induced by Dietary Restriction vs. Bariatric Surgery Study

Total and regional adipose tissue content were significantly reduced following both interventions, however, bariatric surgery resulted in a far greater loss of adipose tissue compared with calorie-restriction alone, see Table 4.6 and Figure 4.25. Total adipose tissue was reduced by -8.4% in the calorie-restricted group, compared with -32.0% in the surgical group (p<0.001). These differences were reflected in most other adipose tissue depots, apart from the non-abdominal internal adipose tissue, where the differences between groups did not reach significance.

Figure 4.25 Comparison of Changes in Subcutaneous Adipose Tissue Depots following Calorie Restriction vs. Surgical Interventions
**Figure 4.26** Comparison of Changes in Internal Adipose Tissue Depots following Calorie Restriction vs. Surgical Interventions

**4.3.3.vi** Comparison of Changes in Liver Fat Content following Weight Loss Induced by Dietary Restriction vs. Bariatric Surgery Study

Weight loss decreased total IHCL content by 36.3% from 9.31 ± 8.01 to 5.93 ± 6.20 following calorie restriction and by 41.2% from 6.61 ± 6.57 to 3.89 ± 3.59 following bariatric surgery, although the difference between interventions was not statistically significant (p=0.99), see Table 4.6 and Figure 4.27. A similar pattern was repeated for regional changes following both interventions.
4.3.3.vii Comparison of Changes in Pancreatic Fat Content following Weight Loss Induced by Dietary Restriction vs. Bariatric Surgery Study

Changes in IPCL content following both caloric restriction and bariatric surgery were less clear cut, and there was a high degree of variability in the results.

Whilst caloric restriction resulted in a small decrease in total IPCL of $13.6\%$ from $8.01 \pm 7.14$ to $6.92 \pm 5.67$, bariatric surgery resulted in a small increase of $4.1\%$ from $7.95 \pm 6.71$ to $8.29 \pm 6.48$, there was no significant difference between the two interventions ($p=0.31$), see Table 4.6 and Figure 4.28.
Regarding regional changes in IPCL, both interventions resulted in small decreases in the Head.

The body and tail showed opposing changes with a decrease in the body and an increase in the tail following caloric restriction.

The exact opposite was found following bariatric surgery, an increase in the body and a decrease in the tail. In each of these cases the difference between interventions was not significant.
4.4 Discussion

4.4.1 Overfeeding study

In this interventional study there was a mean increase in weight of 1kg for 600 kcal subjects and a significant increase in BMI (1%) in only 6 days.

The composition of energy excess seems to have important effects on body energy storage, similar short term overfeeding studies found the same effect. (Diaz, Prentice et al. 1992, Klein and Goran 1993, Horton, Drougas et al. 1995) In fact, a study by Horton et al. (Horton, Drougas et al. 1995) observed how the excess of carbohydrate in a diet affects energy and nutrient balances differently than fat excess in a diet, where fat leads to more body fat accumulation than carbohydrate.

Although, some individuals seem better able to oppose weight changes with overfeeding. (Bouchard, Tremblay et al. 1990, Diaz, Prentice et al. 1992) This is possibly due to inter-individual variations in weight gain. (Dulloo and Jacquet 1999, Bray, Smith et al. 2012)

There were inconsistent changes in WC and hip circumferences in this study, where an increase of 6.2% was seen in the 0Kcal subjects and 3% was seen in the 600kcal subjects. As for Hip, an increase of 2.2% was seen in the 0Kcal subjects and a decrease of 11.4% was seen in the 600Kcal subjects. This may also be due to measurement errors where there were two different individuals taking the measurements from participants at different time points in this study.
Though perhaps the 0 kcal overfed group which showed unpredictably an increase in fat content was due to the content of the 7up free drink, which contained sweeteners such as Aspartame and Acesulfame K. Aspartame, which are used as sweeteners in the beverage industry mainly in diet sodas and 0kcal drinks. (Ferland, Brassard et al. 2007)

Observational studies showed that aspartame is almost 160 times sweeter than sugar and is absorbed from the intestine and metabolized by the liver to form phenylalanine, aspartic acid and methanol which can subsidize to weight gain, obesity, insulin resistance, and type 2 diabetes mellitus. (Hofmann, Dong et al. 2002, Vlassara, Cai et al. 2002, Schulze, Manson et al. 2004, Dhingra, Sullivan et al. 2007, Ferland, Brassard et al. 2007, Fowler, Williams et al. 2008)

Therefore, whether subjects where overfed with either 600 kcal or 0kcal drink for 6 days, there seem to be a non-significant increase in most of the adiposity stores (p>0.05) except for SAT where it had a significant increase in the 600kcal group (p=0.05).

IAAT had no significant effect or change in overfeeding. The variability of visceral fat during overfeeding has already been observed (Bouchard, Tremblay et al. 1990, Votruba and Jensen 2011, Alligier, Gabert et al. 2013) and can be attributed to a higher resistance to the dietary intervention.

Interestingly, IHCL content was affected differently in segments and in opposing ways whether subjects were overfed with the 600kcal drink or with the 0kcal drink. Segment 2&3 in the liver increased in fat content by 2% in 600kcal and 1 % in segment 6&7 in the liver while all other segments as well as total IHCL decreased with no significance. While in subjects having the 0
kcal drink, segment 2&3 in the liver decreased non significantly in fat content by 17% and increased in all other segments as well as in total IHCL with no significance.

Consistent opposition was also seen in IPCL content between groups, especially in the Body, where there was a significance difference between a decrease in IPCL content in the Body in 600kcal overfed participants by 26% and an increase of IPCL content in the Body in 0kcal overfed participants by 3% (p<0.05).

This may be due to the 0Kcal consumption once again, where diet soda consumption was observed having an increased risk on fatty liver due to lipid peroxidation, hepatic inflammation, and being a source of glycation end products. (Assy, Nasser et al. 2008) This mirrors the effect of the 7up free overfeeding in liver in this study, perceiving an increase of ectopic fat in all segments except for the lateral part of the liver (segment 2&3). The reason for this is unknown and has not been stated or studied previously.

Conversely, certain findings did not support that theory, and found no/ or a weight loss effect of diet sodas on fatness, ectopic fat, and metabolic factors. (Tordoff and Alleva 1990, Raben, Vasilaras et al. 2002, Maersk, Belza et al. 2012, Raben and Richelsen 2012).

On the other hand when looking at the effect of excess calories in the 600kcal overfed group, the main fat synthesis seemed to be in the adiposity stores, liver played measurably a minor role. A study by Aarsland et al. showed similar results. (Aarsland, Chinkes et al. 1997).

Once again, segment 2&3 in the liver was the only independent variable in liver fat content and increased with excess calories. The reason for this is unknown but a visible effect is seen in
these two segments depending on the type of excess energy in this study. Although, the inconsistent changes may partly be due to the small group in this study even with a reproducibility of <1% with the novel technique, this could be too small a challenge.

Several studies have shown the effect of different types of fat and their correlation to liver fat increase (Kechagias, Ernersson et al. 2008, Sobrecases, Le et al. 2010, Sevastianova, Santos et al. 2012, Rosqvist, Iggman et al. 2014) suggesting that the type of overfeeding diet seems to be an important determinant of liver fat accumulation, fat distribution, and body composition. (Rosqvist, Iggman et al. 2014)

Pancreas fat content, on the other hand, had a slight decrease in IPCL in its regions in the 600kcal group during weight gain. But a slight increase in total fat content of the pancreas.

The latest study by Rosqvist et al. (Rosqvist, Iggman et al. 2014) mirrored my results in the decrease of pancreatic fat during weight gain in a lean population, although the research did not look at regional variations.

Conversely, an increase in total IPCL in my overfed cohort has also been observed by Rossi et al. (Rossi, Fantin et al. 2011) which associated pancreas and liver fat accumulation with fat intake. Similarly, Pinnick et al. (Pinnick, Collins et al. 2008) observed pancreas fatty acid accumulation and composition in mice fed with high-fat diet.

In the 0kcal overfeeding intervention, the Head region decreased while total IPCL as well as the Body and Tail increased.
A study by El-Gamal et al. (El-Gamal 2012) in adult rats, observed a hyperstimulatory effect in β cells when administrated with aspartame, which may lead to the risk of the development of pancreatitis.

In the pancreas, a visible effect is seen the Body region depending on the type of excess energy in this study. But once again similarly to IHCL the inconsistent changes may partly be due to the small group in this study even with a reproducibility of <1% with the novel technique, this could be too small a challenge.

4.4.2 Calorie Restriction study

In this intervention study, there was a mean weight loss of 6.4%, with a 6.2 % reduction in BMI and reductions of 4.02 % in WC and 3.6% in hip circumferences. Furthermore, I found a significant decrease in all adiposity stores (p<0.001), particularly in IAAT (12.5%).

Visceral adipose tissue has been formerly perceived as more prone to weight reduction than subcutaneous adipose tissue. (Bjorntorp 1993) Adding that, all forms of weight loss affect visceral fat more than subcutaneous fat. (Armellini, Zamboni et al. 1991)

In addition, IHCL had a significant and similar reduction in all segments as well as the total liver fat content, even more pronounced than that of adiposity stores (35.4-37.2% vs. 8-12.5%).

Similar to my findings, previous studies addressing the effects of weight loss on liver fat have shown that even small decreases in body weight result in a considerable reduction in IHCL.
A non-significant reduction in IPCL content was also observed in this study from the effect of weight loss, except for the Tail region, which paradoxically appeared to increase. But the decrease in ectopic fat content was more pronounced in the liver (average of absolute change = 3.22) than in the pancreas (average of absolute change = 2.02).

Rossi et al. (Rossi, Fantin et al. 2012) also indicated how moderate weight loss can determine a significant decline in pancreas fat. Although, comparable to our study, he observed that the reduction of IHCL was greater than that of IPCL and visceral fat loss. This suggests that perhaps dietary restriction may mobilize more IHCL content than IPCL content. (Rossi, Fantin et al. 2012)

The incline of hepatic fat loss showed no significant differences between men and women in my findings, as observed in a study by de Souza et al. (de Souza, Bray et al. 2012)

In addition, according to Andersson et al., (Andersson, Sjostrom et al. 2010) women are more predominant in hepatic weight loss post the menopausal age, as seen in this study, which add to a reduced ability to increase adipose tissue blood flow leading to a high incidence of liver steatosis in this group.
In the pancreas, IPCL content altered greatly in men and women, where the absolute change in pancreas fat content had a non-significant but more positive correlation in men in most regions to the absolute weight change.

Pancreas fat loss in men decreased by 22% as for women, it was merely a reduction of 0.71%, showing once again a strong association between ectopic fat content in the pancreas and gender as seen in chapter 3.

Interestingly, the Head region increased significantly in IPCL content in female subjects and decreased in males. (p<0.05) The reason for this is unknown especially that most of the women in this study are post-menopausal but a visible and significant effect with gender is seen.

It is has been observed that the use of hormone replacement therapy in women post the menopausal age may elevate TG, (Rossouw, Cushman et al. 2008) which have been previously linked with increased risk of acute pancreatitis. (Lindkvist, Appelros et al. 2012) Exogenous oestrogen may also have harmful effects on the pancreas per se. as some cases stated oestrogen-induced pancreatitis in the absence of gallstones and hypertriglyceridemia. (Mungall and Hague 1975, Blake and Pitcher 2003) Unfortunately, hormone replacement therapy has not been reported in this intervention.

Nonetheless, the slight increase in the Tail region in this intervention study echoes the variation of the pancreatic fat content seen in this region when compared to the other pancreatic regions.
4.4.3 Bariatric surgery study

In this intervention study, there was a mean weight loss of 27%, with 27% reduction in BMI, as well as reductions of 19% in waist and 18.5% in hip circumferences. Furthermore, I found that bariatric surgery resulted in a far greater loss of AT when compared to calorie-restriction alone with significant and similar decrease in all adiposity stores post-surgery, (p<0.001) more so in ASAT (39.8%) than in IAAT (35.2%). This may be due to an original loss of IAAT post the induced diet before surgery, thus effecting ASAT changes secondarily.

Although, diet-induced weight loss revealed to affect visceral fat favourably, whereas with larger degrees of weight loss such as in this bariatric surgery intervention, the effect tends to be similar for both visceral and subcutaneous fat.(Chaston and Dixon 2008) Though some previous studies have shown a preferential loss of visceral fat in the early phases after bariatric surgery. (Busetto, Tregnaghi et al. 2000, Phillips, Lewis et al. 2005)

In this study we can see a significant increase in IAAT/ASAT ratio suggesting that body composition is not stable and may improve in time. In fact bariatric surgery is known to have favourable effects on appetite-mediating hormones and hunger, leading to a lower and healthier plateau of weight over time.(Sjostrom, Lindroos et al. 2004, le Roux, Aylwin et al. 2006)

In addition, IHCL content had a similar and extremely significant reduction in all segments as well as the total IHCL (p<0.05) less so in segment 6&7 in the liver (p= 0.27). This may be due to the higher amount of IHCL in segment 6&7 at baseline when compared to total IHCL and other segments’ IHCL content.
Other reports have also seen a significant difference between the right and left lobes, with the left lobe showing a significantly larger increase in attenuation than the right lobe in obese subjects. (Nomura, Ohnishi et al. 1987, Benjamino, Beglaibter et al. 2007)

Similar to my findings, previous studies showed the beneficial effects of weight loss on NAFLD after bariatric surgery. (Klein, Mittendorfer et al. 2006, Furuya, de Oliveira et al. 2007, Mummadi, Kasturi et al. 2008) Although some studies have shown a decrease in inflammatory mediators following surgically induced weight loss as well as hepatic injury and liver failure. (Kooby, Fong et al. 2003, Clark, Alkhuraishi et al. 2005, Fujioka 2005, Mattar, Velcu et al. 2005)

Interestingly, there was an increase in pancreas fat content in all regions except for the Body were there was a non-significant slight decrease.

In fact, an opposition was seen in total pancreas fat content (IPCL) decreasing in subjects with a diet induced weight loss but increasing in subjects that have undertaking surgery. The opposition persists in the different regions as well, with a non-significant increase in IPCL content in Body and a decrease in Tail post diet but an increase in Body and a decrease in Tail post-surgery.

The lack of association between pancreas fat content and weight loss with bariatric surgery might be due once again to the ratio of post-menopausal women to men in this study (83%), when taking into account the strong correlation of fatty pancreas with men seen in the previous chapter and the probable effect of hormone replacement therapy in women post the menopausal age.
In addition, the increase in pancreas fat content as opposed to an expected decrease might reflect a disturbance in pancreas metabolism. In fact, some studies have shown the possibility of pancreatic nesidioblastosis, (Chauhan, Vaid et al. 2010, Bal, Finelli et al. 2012) where the cause of hyperinsulinemic hypoglycemia is characterized by an acquired β cell hyperplasia most commonly found in patients post gastrointestinal surgery.(Clancy, Moore et al. 2006, Raffel, Krausch M et al. 2007)

However, Kashyap et al. (Kashyap, Bhatt et al. 2010) indicated that bariatric surgery seems to uniquely restore pancreatic β cell function and resurrect a failing pancreas.
Chapter V: Summary

The original motivation for this thesis was the inquisition of the accurate representation of the whole organ when assessing ectopic fat depots with current non-invasive methodologies. Unanswered questions surrounding the fat distribution of particular tissues and their effects behind their physiological patterns on certain chronic diseases captured my imagination and provided a focus for this work to be carried out.

The work central to this thesis sought to add further insight on non-invasive methodologies in assessing ectopic fat, by implementing a surgical angle and taking into account the anatomy of a particular organ.

In treating each region within the organ as a self-contained functional independent unit with specific vascularization and physiological patterns as well as using the novel MR-based method, the series of studies in this thesis were undertaken to explore further the complexity and heterogeneity of fat distribution in the liver and pancreas in the adult.

Key findings from the present thesis have demonstrated that:
1. The age range in my cohort (35-45 years) had the greatest increase in ectopic fat content in the liver and Age in general had a high correlation with IPCL in all regions especially in men. This may be due to the groups being skewed by small numbers. Although, aging is generally not only associated with increased adiposity, but also a redistribution in the pattern of adiposity.

Subsequently, the rises in liver and pancreas fat in my cohort may be due, in part, to an age-related deregulation of lipid metabolism in subcutaneous adipocytes, which leads to the accumulation of FFA in ectopic sites (Cree, Newcomer et al. 2004, Despres and Lemieux 2006, Li, Xie et al. 2011) before declining in older populations. (Carmelli, McElroy et al. 1991, Baumgartner, Stauber et al. 1995) The reason for this lack of association in the elderly is unknown, undefined age-related mechanisms possibly have a greater contribution in fat accumulation. It has been observed that at older ages, subdued responses to oxidative stress and altered endocrine functioning may affect the mechanisms of fatty infiltration.(Lamberts, van den Beld et al. 1997, Paolisso, Tagliamonte et al. 1998)

Furthermore, the available data suggests that there are effects of age and adiposity on pancreas volume. For example, anatomical studies reported decreased pancreas volume with aging in humans and histological studies report atrophy, fibrosis, and fatty infiltration of the pancreas in the aging population.
Saisho et al. (Saisho, Butler et al. 2007) observed that pancreatic fat volume increases with age in adults. In males, pancreatic fat volumes increase in the third and fourth decade and then remain constant until the seventh decade. In contrast, in females, pancreatic fat volumes remain remarkably unchanged.

2. Height correlated greatly with total and regional ectopic fat content in the liver with a weaker association with total ectopic fat content in the pancreas in overweight participants (25-30 kg/m²). When looking at the data in this group we can see that it constitutes of mostly men who are normally taller, BMI does not accurately control for large differences in height between individuals, and it may be skewed by high muscle mass.

Additionally, even though, that Height per se. is not usually seen as a biomarker for ectopic fat content in the liver, adult height do reflect the interaction of genetic and numerous early-life experiences and exposures (such as foetal, dietary, social and psychological circumstances) on several major adult-onset diseases.(Ben-Shlomo and Kuh 2002, Silventoinen 2003)

Adult leg length has been considered as a biomarker of early childhood exposures, as leg growth justifies the increase in total height in the pre-pubertal period. (Gunnell 2002) A study by Fraser et al.(Fraser, Ebrahim et al. 2008) observed that leg length, a marker
of childhood nutrition, was inversely associated in women with adult levels of liver enzymes such as alanine aminotransferase (ALT), gamma-glutamyl transferase (GGT) and Alkaline phosphatase (ALP) (ALT being the liver enzyme most closely associated with liver fat content). (Westerbacka, Corner et al. 2004) Therefore concluding that women with shorter legs may have an increased risk of liver disease.

Alternative possibilities may be that linear skeletal growth in itself influences the development of the liver and better linear growth leads to a larger liver, resulting in lower levels of liver enzymes and a better ability to withstand liver insults. (Fraser, Ebrahim et al. 2008) In addition, low bone mineral density has been recognised as a potential health problem in both men and women suffering from the metabolic syndrome. (Yaturu, Humphrey et al. 2009, Kim, Choe et al. 2010, Jeon, Lee et al. 2011)

Recent years have witnessed an increased awareness of the clinical and epidemiological association between liver fat and bone health in terms of reduced bone mineral density and an increased risk of osteoporosis in both children and adults. (Pirgon, Bilgin et al. 2011, Campos, de Piano et al. 2012, Moon, Lee et al. 2012, Pardee, Dunn et al. 2012) Thus lipids and lipoproteins are evolving as important regulators of skeletal physiologic characteristics and have been shown to inhibit osteoblast and enhance osteoclast differentiation and survival. (Parhami, Tintut et al. 2001, Tintut, Morony et al. 2004)

In fact, a recent study by Bredella et al. (Bredella, Gill et al. 2013), observed that IHCL is positively associated with bone marrow fat in young obese subjects, independent of
insulin resistance and exercise status. This supports the notion that ectopic fat content levels may exert negative effects on bone or are influenced by the same additional factors as the latter.

3. Liver fat distribution is homogeneous overall but seem to variable opposite effects in different segments in the overfeeding intervention study depending on the type of energy excess. As seen in chapter 4, segment 2&3 appeared differently compared to other segments. This may be due to the higher amount of IHCL in segment 2&3 at baseline when compared to total IHCL and other segments’ IHCL content. Although the reason for the opposite reaction to the two different overfeeding drinks is unknown, but an effect is seen in the left lobe of the liver depending on the type of excess energy. This may be due to the anatomical position of these segments and the position of the left lobe in regulating and maintains body functions. (Sherlock and Dooley 2008, Couinaud 1957)

4. Total and regional pancreas fat content in my cohort was significantly correlated with men with and had opposite effects on the Body region in the overfeeding intervention study, depending on the type of energy excess. As mentioned in chapter 3; this gender dimorphism has been observed previously.(Saisho, Butler et al. 2007) A recent study by Horng-Yih et al. (Ou, Wang et al. 2013) demonstrated that fatty pancreas was independently associated with β-cell dysfunction in males but not in females. In fact, pancreatic fat is not an important cause of β-cell dysfunction at the population level,
but it further increase impaired insulin secretion compared to visceral fat in subjects with pre-diabetes. (Heni, Machann et al. 2010)

Furthermore, Wong et al. (Wong, Wong et al. 2014) observed that the fatty pancreas was affected by mild iron overload and high serum ferritin mostly in men. This phenomenon may clarify the very low occurrence of fatty pancreas in women. In effect, ferritin is an acute-phase protein and is upregulated by pro-inflammatory cytokines such as tumor necrosis factor-alpha (TNF) and nuclear factor kappa light-chain enhancer of activated B cells (NF-κB). (Kowdley, Belt et al. 2012) Moreover, iron overload is a well-known cause of pancreatic dysfunction and may cause diabetes via hemochromatosis when the overload is extreme. (Pietrangelo 2007)

As seen in chapter 4, the Body in the pancreas appeared differently compared to other regions. The reason for this is unknown but a visible effect is seen in this region depending on the type of excess energy. This may be due to its anatomical position and being the largest part of the pancreas, thus more affected. As seen in chapter 3 the Head and Body regions tend to be the fattiest in IPCL content.

5. The Tail pancreatic region seemed to be an independent unit when compared to the Body and Head regions in the pancreas. It remained unaltered with exercise status, did not correlate with IAAT and increased in IPCL following weight loss.
This finding may not only be due to the distinct anatomical location and histological characteristics of the Tail,(Marchal, Verbeken et al. 1989, Jacobs, Coleman et al. 1994, Matsumoto, Mori et al. 1995, Kelsen 2008, Mulholland, Lillemoe et al. 2012) but also to the presence of a higher population density of islets in this region than in the head and body of the pancreas .(Hellman 1959, Wittingen and Frey 1974, Rahier, Guiot et al. 2008)

In fact, microscopically, IPCL is seen predominantly in the pancreatic parenchyma,(Olsen 1978) the islets of Langerhans are usually not affected by mild fatty infiltration. (Patel, Bellon et al. 1980, Hague and Amin 2006) This is perhaps the cause of a more focal fatty infiltration in the head and body pancreatic regions in my cohort, as seen in previous studies.(Atri, Nazarnia et al. 1994, Jacobs, Coleman et al. 1994, Matsumoto, Mori et al. 1995) However, an extreme degree of fatty infiltration may cause abnormal dilatation of the pancreatic duct, indicating an obstruction of the normal flow of pancreatic secretions found in symptoms such as acute pancreatitis and diabetes.(Patel, Bellon et al. 1980, Hadidi 1983, Lozano, Navarro et al. 1988, Chao, Lin et al. 2000)

6. Increase in total and regional pancreatic fat content post 6 months of bariatric surgery and in the Head of the pancreas after weight loss in women. As mentioned previously in chapter 4, in addition to the post-menopausal effect or the occurrence of nesidioblastosis, participants in this intervention undertook a dietary restriction before the surgery and might still be in the process or have already lost some ectopic fat content.
A surgical restriction of the stomach, comprising the regulation of food intake, transit, and absorption by the gastrointestinal system, the liver, and the brain, may cause hormonal imbalances in the pancreas till weight loss stabilises.(Cummings and Overduin 2007).

However, it has been proven that Bariatric surgery is a highly effective mean for restoring β-cell dysfunction and insulin resistance in very obese patients with type 2 diabetes (Mingrone, Panunzi et al. 2012, Schauer, Kashyap et al. 2012), although, there has been no records on the time span of the ‘remission’ and if this his improvement in diabetes is simply attributable to weight loss or other mechanisms.
5.1 Limitations

During the research of this thesis there were limitations. In addition to the limitations already mentioned in the discussion of the previous chapters, the lack of statistical significance in certain fat mass and ectopic fat contents throughout the thesis is limited by the relatively small number of subjects, and gender imbalance particularly in different BMI groups, overfeeding and bariatric intervention studies, which reduced the statistical power.

In addition, changes in body weight are not good indicators of energy gains or losses over short periods. Intervention studies, in particular the overfeeding study, should have been extended for as long as possible in order to reduce the influence of methodological errors. Additional studies need to be performed to further describe the heterogeneity of fat distribution especially in lifestyle modifications.

Measurements of ectopic fat content in the different segments and regions of the liver and pancreas measurements have not been correlated pathologically. Although it is unethical and invasive to conduct such tissue studies in healthy volunteers especially in the pancreas, it is possible to test the occurrence of the effect of IPCL on insulin resistance and β cell function, especially that we now recognize and uneven physiological pattern in this organ.

Moreover, further longitudinal studies are needed using this novel MRI-based method to provide further insights on the authenticity of its measurement especially in a small and challenging organ such as the pancreas, where a time consuming difficulty was encountered in
determining the boundary between the body and tail of the gland, since there was no satisfactory landmark available for this division, although, CoV was very low (<1%). Additionally, these longitudinal studies may assist in determining the threshold at which pancreatic fat accumulation leads to adverse outcomes.

Measurements of serum insulin level, blood lipids, and other metabolites including liver function tests and ferritin levels as an indicator of fatty pancreas would have been an important complement to findings of this thesis particularly in lifestyle intervention studies.

In addition, the importance of ethnic differences in the cut off threshold in fat distribution and ectopic fat deposition in addition to the effect of gestational age would have been a likely contribution to the regional heterogeneity in both organs. More studies on this topic are needed.

I also acknowledge that an MRI slice thickness of 10 mm using the novel MRI-based method may not provide optimal spatial resolution for measurement of fat in the pancreas. While the spectral model of fat in human pancreatic tissue is likely to be similar to that in liver tissue, this has not yet been technically verified. A refinement for future studies will be the incorporation of a spectral model of fat derived from human pancreas in vivo if possible.

3-dimensional (3D) techniques can also be developed using this novel MRI based method for estimating ectopic fat content in different segments and regions. One potential advantage of 3D imaging for measuring pancreatic fat is that it may allow acquisition of thinner slices, which would reduce potential contamination.
VI: References


Bonora, E., R. Micciolo, A. A. Ghiatas, J. L. Lancaster, A. Alyassin, M. Muggeo and R. A. DeFronzo (1995). "Is it possible to derive a reliable estimate of human visceral and
subcutaneous abdominal adipose tissue from simple anthropometric measurements?" Metabolism: clinical and experimental 44(12): 1617-1625.


Ding, D. and K. Gebel (2012). "Built environment, physical activity, and obesity: what have we learned from reviewing the literature?" *Health & place* 18(1): 100-105.


Appendix 1
STUDY PROTOCOL:
The effects of overfeeding on body fat and eating behaviour in preterm adults

Date: 09/08/2011, version 1.1, REC no: 11/LO/1097

STUDY MANAGEMENT GROUP

Chief Investigator: Dr. Tony Goldstone

Co-investigators: Prof. Jimmy Bell, Prof. Gary Frost, Dr. Louise Thomas and Ms. Nauf Al Saud.

Clinical queries should be directed to Dr. Tony Goldstone.

1. BACKGROUND

1.1 Introduction

The intra-uterine environment and early post-natal life are now recognized as key determinants of diseases risk later in life. The exposure to an unfavourable environment during these crucial periods is hypothesized to results in compensatory developmental changes that become permanent (Barker et al., 1993).

Previously work by the Metabolic and Molecular Imaging Group has developed methods for magnetic resonance (MR) imaging of the whole body and 1H MR spectroscopy of liver to measure total and regional fat deposits in adults (Thomas et al., 2005a).

We have demonstrated how the amount and distribution of fat in the body is influenced by diet, ethnicity and various genes. We have demonstrated the benefits of moderate exercise, moderate restriction of calories in the diet, and exercise training to alter body fat and improve health.

Preterm infants usually have an altered body composition, demonstrating decreased lean body mass and significantly greater fat deposition in deep subcutaneous and internal abdominal compartments as well as a significantly greater liver fat (IHCL) when compared to their full-term born counterparts (Uthaya et al., 2005).

Increased waist circumference, higher fasting glucose levels, increased blood pressure have been recorded in young adults born prematurely or with a very low birthweight (Euser et al., 2005; Irving et al., 2000; Johansson et al., 2005) Premature children also show increased levels of the hormone ghrelin in the blood, a hormone that increases hunger by acting on the brain including food reward systems (Darendeliler et al., 2009; Malik et al., 2008; Goldstone et al. 2010).

A recent study ("The preterm baby as a young adult", 07/H011/118) performed by our group in the Robert Steiner MRI Unit has shown that premature adults (<33weeks gestation, aged 19-27 years, n=13) have increased visceral fat (P=0.01), liver fat (P=0.02)
and muscle fat (P<0.05) compared to control adults born at term (n=10). It is currently unknown if abnormalities in brain food reward systems predisposing to overeating might be responsible for the increased risk of adiposity associated with prematurity.

Human physiology needs to be well adapted to cope with major disruptions in both the supply of and demand for energy. This adaptability requires 'a clear capacity to utilize lipid and carbohydrate fuels and to transition between them' (Kelley et al., 2002). Such aptitude characterizes a healthy state and can be termed 'metabolic flexibility'. However, increasing evidence points to metabolic inflexibility as a key dysfunction of the metabolic syndrome in obese and diabetic individuals (Storlein et al., 2004).

Therefore, we plan to approach this novel concept by studying the effects of a hyper-energetic diet on the metabolic plasticity of preterm adults, when compared to full term adults. Our previous studies have shown that 3 days of overfeeding by 250 kCal to normal adults increased weight from 82.3 ± 11.1 to 82.5 ± 10.8 kg, and % liver fat from 0.39 ± 0.36 to 0.64 ± 0.46 (n=6). We hypothesize that the increase in liver fat and intra-abdominal (visceral) fat during a short period of overfeeding will be greater in preterm than term adults.

Studies in humans using the functional neuroimaging techniques of positron emission tomography (PET) and fMRI have shown changes in the activity of various brain regions at rest or in response to food stimuli, e.g. viewing food pictures, smelling, tasting or imagining food, between the fasted hungy and fed satiated states. (Small and Prescott, 2005; Tataranni and DelPargi, 2003; Hintona, 2004; Kringelbach and Rolls, 2004; Simmons et al., 2005; Beaver et al., 2006; Malik et al., 2008; Goldstone et al., 2009).

Previous studies have shown that 2 days of overfeeding by 30% of basal energy requirements in thin individuals resulted in reduced hunger ratings and increased satiety as well as an attenuation of the activation in the insula (part of brain reward systems) in response to pictures of high-calorie foods (Cornier et al., 2009). Furthermore, this attenuation by overfeeding was blunted in overweight individuals (Cornier et al., 2009). We hypothesize that a short period of overfeeding in preterm adults will lead to blunted attenuation in brain reward system activation compared to term adults.

We therefore aim to use both the whole body MRI for measurement of liver fat as well as functional MRI to image brain reward systems before and after a short period of overfeeding to assess metabolic flexibility in term and preterm adults.

1.2 Hypothesis

Preterm adults and control full term adults differ in their metabolic flexibility in response to 10 days of overfeeding. This will be reflected in differences in the changes in hepatic fat and fat distribution, insulin sensitivity and lipids, and may be mediated via changes in brain reward systems and appetite regulating hormones.

2. STUDY OBJECTIVES

The aim of this study is to investigate changes in hepatic fat during 10 days of extra energy loading of 600 kcal/day, on top of their standard diet, in preterm compared to full term adults.

2.1 Study Outcome Measures

PRIMARY OUTCOMES

- Changes in hepatic fat as determined by MR spectroscopy scan before and after the
dietary intervention.

SECONDARY OUTCOMES

- Changes in body weight, percentage body fat and fat distribution as determined by whole body MRI scan and bioelectrical impedance analysis before and after dietary intervention.
- Changes in waist and hip circumference before and after dietary intervention.
- Changes in the activation in brain reward systems in response to viewing food and non-food stimuli, measured by functional magnetic resonance imaging (fMRI) before and after dietary intervention.
- Change in activation in brain reward systems in anticipation and receipt of non-food reward using fMRI before and after dietary intervention.
- Changes in appetite and gut hormone levels before and after dietary intervention.
- Changes in food intake (ad libitum test meal and 3-day home dietary records).
- Changes in insulin sensitivity (measured using homeostasis model assessment HOMA) and blood lipids, and other metabolites.
- Changes in appeal scores of food and non-food pictures.
- Changes in appetite measured by Visual Analogue Scale (VAS).
- Changes in serum metabolic profiles.
- Epigenetic changes (methylation, acetylation, histone modification) in DNA

3. PARTICIPANT ENTRY

80 male healthy male volunteers (40 preterm adults, 40 term adults), aged 18 - 50 years will be recruited for this randomized, single-blinded placebo controlled trial. Females are being excluded because of the difficulties in controlling for change in reward over the menstrual cycle. Pending age and BMI matched randomization:

- 20 Term male adults will be given a 0 calorie drink
- 20 Term male adults will be given a 600 kCal/day drink
- 20 Pre-term male adults will be given a 0 calorie drink
- 20 Pre-term male adults will be given a 600 kCal/day drink

Previous participants in MRI body fat studies of term and preterm adults (07/Q0411/19 and 07/H011/118) who have consented to being contacted when new research studies arise will be invited to consider whether they wish to take part in this study.

Others will be recruited via public advertisements, email mailing lists and Imperial College Healthcare NHS Trust websites. Targeting of preterm adults will also be achieved through advertisement in newspapers, and through BLISS the national UK premature baby charity (www.bliss.org.uk).

A participant’s information sheet (Appendix 2) will be given to volunteers who show interest in participating in the study. Participants will be given time to consider whether they wish to take part.

No procedures or measurements outside of normal routine care will be obtained prior to obtaining freely written informed consent from the participant (Appendix 3). Once this has been obtained, participants proceed with their screening visit at the Clinical Investigation Ward, Sir John McMichael Unit, Hammersmith Hospital.

3.1 Inclusion Criteria

- Gender: male to avoid the effect of menstrual cycles on food reward
- Age: 18 to 50 years (inclusive)
• BMI: 20 - 30 kg/m²
• Normal or corrected-to-normal vision
• Assessed as healthy, based on a pre-study examination
• Willingness and ability to give written informed consent and willingness and ability to understand, to participate and to comply with the study requirements
• Born at term (38-42 weeks gestation) or preterm (at or below 33 weeks gestation)

3.2 Exclusion Criteria

• Claustrophobia
• Pacemaker, metal implant, clips, implanted device, shrapnel or bullets, metal in eyes that precludes magnetic resonance imaging
• Treatment with any medication that might affect the study outcome (e.g., medication that is affecting neural activity, appetite regulation and/or blood flow)
• Haemorrhagic disorders and anticoagulant treatment
• Hepatic impairment as defined by screening visit liver function tests (aspartate aminotransferase (AST) or alanine aminotransferase (ALT) or gamma-glutamyl transpeptidase (GGT) of three times the upper normal reference limit)
• Significant intercurrent disease or history of clinically significant disease of any type, in particular liver, kidney, or heart disease, any form of diabetes mellitus or psychiatric illness (including Depression as defined by BDI–II score above 28)
• History of cancer, excluding skin cancer
• History of severe or multiple allergies, severe adverse drug reaction or leucopenia
• Smokers
• Regular drinkers of more than average three units of alcohol daily (1 unit = 300 ml beer, 1 glass wine, 1 measure spirit)
• History of, or current evidence of, abuse of alcohol or any drug substance, licit or illicit.
• Regular intake of over-the-counter (OTC) medication (other than the occasional paracetamol/aspirin)
• Poor compliers or subjects unlikely to attend
• Blood donation within the 12 week period before the initial study dose

3.3 Withdrawal Criteria

• Significant deviation from the approved protocol
• Significant non-compliance in taking either the 0 calorie or 600 kcal drink
• The participant no longer satisfies the inclusion and exclusion criteria set out by the approved protocol
• Significant incidental structural abnormalities on MRI whole body or brain scan
• The participant withdraws consent

3.4 Screening

Following the participant providing informed consent, they will undergo a health screening. This will take approximately 2-3 hours. Participants will be asked to come overnight fasted. They will be asked about their medical, drug, dietary and weight, family, and gestational history. They will have a blood sample taken to check urea and electrolytes, liver function, thyroid function, blood cell counts, iron status, glucose, lipids levels and HbA1c. Assays will be performed by the Department of Chemical Pathology at Imperial College Healthcare NHS Trust.

They will also have their blood pressure and pulse measured, height, weight and body fat by impedance measured.

They will also be asked to complete several validated questionnaires to assess the
participant’s eating behaviour, attitudes towards food, personality and mood (see Section 7.1), as well as a 15 min computerised food choice task to assess subjects’ liking and wanting for different foods. A series of pictures will be presented for rating and in a forced choice procedure, which also identifies food preferences (Finlayson et al., 2008). It is hypothesized that preterm subject may have increased liking and wanting particularly of high fat sweet foods than term subjects.

Subjects will also perform computerised or paper-based tasks including the (i) Cambridge gambling task: to assess decision-making, including risk taking and evaluation of rewarding events (Deakin et al., 2004; Clark et al., 2008); (ii) Stop signal task; (iii) 5 choice serial reaction time (5-CSRT): to assess impulsivity (Perry and Carrol, 2008; Nederkoorn et al., 2007); (iv) Wechsler Abbreviated Scale of Intelligence (WASI): to document intellectual and cognitive level (Berger S. Appl Neuropsychol. 5:37-42, 1998).

Additionally, the participant will also be asked to taste and rate their liking of a range of ready meals to see which one they find most palatable for use in the study days. They will also be given 2 three-day food diaries to take away and complete: one immediately and just before visit 1 (functional MRI).

3.5 Participant reimbursement

Subjects will not be paid for taking part in this study to avoid any possible feelings of coercion. However, in recompense for travel expenses, loss of earnings and the burden of repeated trips to the hospital, £110 per subject will be offered to every subject (£10 per screening visit and £20 per MRI visit). This will be paid at the end of the study. If subjects withdraw early they will be paid proportionately to the visits they have attended.

4. TREATMENTS

4.1 Drinks

Subjects will be asked to take 600 kcal / day (2 x 125ml Fortisip Compact drinks = 250 ml total) after breakfast on top of their usual standard diet, or a 0 kcal fruit flavoured drink. Fortisip Compact is a high energy drink (2.4 kcal/ml) with nutritional content as follows: protein 9.6g/100ml, carbohydrate 29.7g/100ml, fat 9.3g/100ml.

4.2 Ingredients

Fortisip Compact contains the following ingredients:

Water, glucose syrup, milk proteins, vegetable oils, tri potassium citrate, emulsifier (soy lecithin), flavour (vanilla), magnesium hydrogen phosphate, choline chloride, potassium chloride, acidity regulator (citric acid), tri sodium citrate, sodium L-ascorbate, ferrous lactate, zinc sulphate, nicotinamide, DL-a-tocopheryl acetate, colouring (curcumin), retinyl acetate, copper gluconate, sodium selenite, manganese sulphate, chromium chloride, calcium D-pantothenate, D-biotin, cholecalciferol, pteroylmonoglutamic acid, thiamin hydrochloride, pyridoxine hydrochloride, sodium molybdate, sodium fluoride, riboflavin, phytomenadione, potassium iodide, cyanocobalam.
It should also be stored in a dry, cool place (5-25°C). Once opened, bottles should be stored in a refrigerator for a maximum of 24 hours.

4.4 Safety

- Not for intravenous use.
- Not suitable for patients with galactosaemia.

4.5 Dispensing and accountability

Drinks will be blinded to the participants and therefore will be given in anonymised format and coded for each participant. Dispensing to the participant will be performed by the investigative team.

The date and drink given will be recorded in a dispensing log. When subjects return their empty packets at periodic intervals, this will also be recorded in the log in order to assess compliance to the given drink.

5. ADVERSE EVENTS

5.1 Definitions

An Adverse Event (AE) is defined as any untoward medical occurrence in a patient or clinical study subject. A Serious Adverse Event (SAE): any untoward and unexpected medical occurrence that:

- Results in death
- Is life-threatening – refers to an event in which the subject was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe
- Requires hospitalisation, or prolongation of existing inpatients’ hospitalisation
- Results in persistent or significant disability or incapacity
- Is a congenital anomaly or birth defect

Medical judgement should be exercised in deciding whether an AE is serious in other situations. Important AEs that are not immediately life-threatening or do not result in death or hospitalisation but may jeopardise the subject or may require intervention to prevent one of the other outcomes listed in the definition above, should also be considered serious.

5.2 Reporting Procedures

All adverse events will be reported. Depending on the nature of the event the reporting procedures below should be followed. Any questions concerning adverse event reporting will be directed to the Chief Investigator in the first instance.

5.3 Non serious AEs

All such events, whether expected or not, will be recorded.

5.4 Serious AEs

An SAE form will be completed and faxed to the Chief Investigator within 24 hours. All SAEs
will be reported to the Joint Research Office, Imperial College, London where in the opinion of the Chief Investigator (Prof. Jimmy Bell) and Dr Tony Goldstone the event was:

- ‘related’, i.e. resulted from the administration of any of the research procedures; and
- ‘unexpected’, i.e. an event that is not listed in the protocol as an expected occurrence

Reports of related and unexpected SAEs will be submitted within 15 days of the Chief Investigator becoming aware of the event, using the relevant SAE reporting form.

SAEs will also be reported to the Imperial College Healthcare NHS Trust Research and Development Office and the ethics committee through which permission for the study was originally granted.

6. STUDY PROTOCOL

Subjects will have 5 study visits (3 for body fat MRI and 2 for brain functional MRI) over a 28 day period. At the first MRI fat visit (day 0) subjects will be randomized and matched for age and BMI to receive the 0 kCal or 600 kCal per day dietary intervention to take for 10 days until Visit 4.

They will be given a supply of the drink to take home with instructions for them to take it each day at the end of breakfast.

An overview of the entire study can be seen in Figure 1.

Subjects will be asked to attend each MRI study day following an overnight fast. They will be asked to refrain from taking strenuous exercise and drinking alcohol for twenty-four hours before the study visit. They must not consume any food or drink with the exception of water after eating supper at 8pm on the evening prior to any study day.

For the three days preceding each fMRI study day as well before the last MRI fat study visit (day 28) they must also complete a food diary and bring it along with them to the study day. Subjects will be asked to consume identical evening meals at 8pm on the evening before each fMRI study visit.
Figure 1- Schematic representation of the study

6.1 STUDY VISITS 1 and 4 (between Day -6 and -4, and Day 10): Functional MRI

Subjects will be asked to complete a food diary for three days at home to obtain baseline food intake data before each fMRI visit. The subject will arrive at the Sir John McMichael Centre in the morning after an overnight fast.

Subjects will have their height and weight measured and bioelectrical impedance analysis to measure body fat. This is a painless, safe procedure that involves lying on a bed, having two sticky pads placed on a hand and foot, while attached to a Bodystat 1500 unit (Bodystat Ltd., Isle of Man, UK) and lying still for 1 minute, or standing on a metal plate for a minute.

The subject will have a cannula placed into their arm for serial blood sampling before being taken to the Robert Steiner MRI unit or Molecular and Translational Imaging Centre where they will undergo fMRI scanning on a 3T MRI scanner.

Subjects will have a brain magnetic resonance (MR) scans for up to 75 mins on each study visit. None of the MR techniques to be used employs ionizing radiation or intravenous contrast agents. Subjects lie supine in the scanner with their head placed in a padded head
coil for support. While in the scanner volunteers will have access to a buzzer to sound an alarm, and will be able to hear and respond to instructions from the scanning console. While in the scanner subjects view a mirror reflecting a computer screen mounted above or behind the head coil. Subjects can respond to instructions using a keypad or joystick held in their hand. Subjects can rate their hunger and mood at various times while in the scanner using this device.

The following anatomical brain scans will be collected at one or more of the visits:

(i) Anatomical T1 and T2 weighted MR scans to provide structural data on which to overlay the functional data.

(ii) Diffusion tensor imaging to examine white matter tracts.

The following resting state functional brain scans will be collected at one or more of the visits:

(iii) Arterial spin labeling to measure resting regional blood flow (Petersen et al., 2006; Paiva et al., 2007). A radiofrequency (RF) pulse is applied to the neck so as to 'magnetically-label' blood in vessels that send blood to the brain. Subsequent MR scanning of the brain is therefore able to detect blood flow changes as the labeled blood circulates.

(iv) Resting state BOLD functional MRI to measure regional connectivity in activity in different brain regions at rest (Damoiseaux et al., 2006).

One or more of the following task-related functional MRI scans will be collected at each visit:

(v) Food pictures: subjects view a variety of different pictures (e.g. food, household objects, animals, blurred pictures as a baseline) and rate how 'appealing' the pictures are using the keypad (Goldstone et al., 2009).

(vi) Monetary incentive delay (MID) task: a game in which subjects need to press a button during a specific time window when given a cue on the computer screen in order to win hypothetical monetary prizes of differing amounts to assess reward responsivity and ventral striatum function (Horn et al., 2003; Knutson et al., 2001; Knutson et al., 2000).

(vii) Go/NoGo Task: to assess impulsivity via effects on response inhibitory control mediated in the prefrontal cortex striatum (Horn et al., 2003; Yan et al., 2009; Chao HH et al., 2009; Li et al., 2008; Perry and Carrol, 2008). The task contrasts brain activation during responses to infrequent no go signals (e.g. ‘do not press’ button when viewing an infrequent red arrow) compared to an implicit go baseline (e.g. ‘do press’ button when viewing a frequent green arrow).

(viii) Control cognitive tasks: subjects undertake simple tests (e.g. viewing a 4Hz flashing checkerboard, pressing a button, reading, listening, speaking, recalling, and thinking about words or numbers, looking at pictures or faces)

The fMRI protocol is identical to several fMRI studies which already have ethical approval (07/Q0406/19, 08/H0707/163, 08/H0707/139, 10/H0707/60).

After the scan, subjects will be given a meal of known nutrient composition and will be asked to eat until comfortably full. The quantity of food eaten will be used as a quantitative assessment of appetite.

Appetite and mood will also be measured throughout the morning using visual analogue scales (VAS). Blood will be taken at various time points for measurement of hormones,

such as ghrelin, insulin, PYY and GLP-1, glucose and other metabolites.

At the end of the first fMRI visit subjects will be asked to complete a short questionnaire to find out their dietary likes and dislikes by rating the food pictures.

6.2 VISITS 2, 3 and 5 (Day 0, 7 and 28): Whole body MRI fat scan

The subject will arrive at the Robert Steiner MRI Unit, Hammersmith Hospital in the morning after an overnight fast.

Body mass (kg), height (cm), waist circumference (WC) (cm), and hip circumference (cm) will be measured and BMI (kg/m²) and waist-to-hip ratio (WHR) calculated. Anthropometric measurements including skinfold thicknesses measurement will also be taken. Subjects will complete the International Physical Activity Questionnaire.

They will then have their MRI body scan. The MRI fat protocol is identical to several studies which already have ethical approval (06/Q0411/173, 07/H011/118, 07/Q0411/19, 08/H0309/56, 09/H0709/18, 09/H0707/30).

Subjects will be in the MRI scanner for up to 1 hour. Scanning will be performed on a 1.5T Achieva scanner, Phillips Medical Systems, at the Robert Steiner MR Unit to determine total and regional adipose tissue content and magnetic resonance spectroscopy (MRS) will be performed to measure lipid content in organs including the liver (IHCL), muscles (IMCL) and pancreas (IPCL) (Rico-Sanz et al., 1999; Thomas et al., 2005b).

Subjects will lie supine or prone in the scanner and will be automatically moved through the scanner. They will have access to a buzzer to sound an alarm, and will be able to hear and respond to instructions from the scanning console.

Subjects will then be taken to the Sir John McMichael Centre Clinical Investigation Ward, Hammersmith Hospital. A cannula will be placed in their arm to allow serial blood sampling. Samples will be taken at regular intervals before and up to 3 hours after a fixed test meal.

Measurement of routine blood biochemistry, plasma metabolites including fasting glucose, total cholesterol, triglycerides, high-density lipoprotein, low density lipoprotein, adipocytokines such as adiponectin, and hormones such as leptin, ghrelin, PYY, GLP-1 and insulin will be performed by standard immunoassay. Insulin sensitivity will be assessed from concentrations of glucose and insulin using the quantitative insulin sensitivity check index (QUICKI) (Katz et al., 2000) and homeostasis model assessment (HOMA) of insulin resistance (Matthews et al., 1985). Assays will be performed by the Department of Chemical Pathology at Imperial College Healthcare NHS Trust and using commercial kits for radio-immunoassay and ELISA. Subjects will also have blood taken for assessment of epigenetic changes (methylation, acetylation, histone modification) in DNA from white blood cells.

Subjects will also be asked to complete Visual analogue scales (VAS) at several time points to measure appetite before and after the meal.

For visit 5 (day 28) subjects will be asked to complete a food diary for three days at home to obtain baseline food intake data before the study day.

6.3 Dietary Intervention

At the first MRI fat visit (day 0) subjects will be randomized to receive the 0 kCal or 600 kCal per day dietary intervention to take for 10 days until Visit 4, the second fMRI visit.
They will be given a supply of the milkshake or juice drink to take home with instructions for them to take it each day at the end of breakfast.

6.4 **Dietician Review**

At Visit 5 (day 28), after the third MRI fat scan subjects will have an individual consultation with the study dietician giving them dietary advice for healthy eating and as necessary to help them lose any excess weight they have gained and not lost since stopping the dietary intervention.

6.5 **Physical Activity Monitoring**

Subjects will be given a physical activity monitoring device, the size of a matchbox, to wear continuously on their waist (on belt, trousers or skirt) or wrist for the 10 days of the dietary intervention.

At Visit 4 (day 10), the second fMRI visit, they will return this device and be given another device to wear to return on Visit 5 (day 28), the third MRI fat scan.

6.6 **Additional Participant Contact**

In addition to participants attending for the study days described previously, there will also be frequent brief telephone calls (at most every other day) and emails made by the study team to promote adherence to the diet prescribed, and ensure there have been no problems or adverse events. These will be approximately 5 minutes in duration on each occasion.

7. **DETAILS FOR SCREENING AND STUDY VISITS**

7.1 **Questionnaires**

At the screening visit as in our previous protocols (08/H0707/139, 09/H0707/30, 09/H0709/16, and 10/H0707/60), subjects will complete the following questionnaires to assess eating behaviour and personality measures related to reward sensitivity and mood:

1. Dutch Eating Behaviour Questionnaire (DEBQ) – to measure restraint, emotional and external influences on eating behaviour (Wardle, 1987).

2. Eating Disorder Examination Questionnaire (EDE-Q) – to screen for existing eating disorder (Fairburn and Beglin, 1994).


4. Behavioural Inhibition and Activation System (BIS / BAS) scales – to measure punishment and reward sensitivity, which have previously been correlated with fMRI responses to food pictures (Carver and White, 1994; Beaver et al., 2006).

5. Eysenck Personality Questionnaire (EPQ-R) – to measure extraversion that correlates with reward drive and sensitivity (Eysenck et al., 1985; Carver and White, 1994).

6. Beck Depression Inventory (BDI-II) and Anxiety Inventory – to measure levels of depression and anxiety (Beck et al., 1996).

7. Barratt Impulsivity Scale – to measure impulsivity which has been linked to overeating (Patton et al., 1995; Yeomans et al., 2008).
8. Smoking and recreational drug questionnaires to assess past and present smoking and addictive behaviour.

9. Three Factor Eating Questionnaire (TFEQ) to measure restraint, disinhibition and hunger (Stunkard and Messick, 1985).

10. Magnetic resonance imaging metal check list to ensure compatibility with MRI scanning.

11. Handedness inventory to measure degree of right or left handedness (Oldfield, 1971).

12. Disgust scale, which alters brain response to viewing food pictures (Olatunji et al., 2007; Calder et al., 2007).

At each of the fMRI study days, subjects will complete the following questionnaires:

1. Positive and Negative Affect Schedule (PANAS) - to measure tendency to experience positive and negative affect, which have previously been correlated with fMRI responses to food pictures (Watson et al., 1988; Killgore et al., 2006).

2. Mood/Depression Assessment Questionnaire – to screen for development of depression. If score is 2 or more, this will be followed by completion of the Beck Depression Inventory (BDI–II). Subjects with severe depression as indicated by a BDI–II score above 28 will be excluded since this can reduce appetite.

At each of the MRI fat study days, subjects will complete the following questionnaire:

1. International Physical Activity Questionnaire (Hagestroemer, 2006) to assess baseline levels of activity and to ensure they remain constant throughout the study duration (Craig et al., 2003).

7.2 Blood Sampling

Up to 20ml blood will be taken at the screening visit. On each of the 3 MRI fat study days up to 145ml in blood will be taken, and up to 120ml will be also be taken at each of the 2 fMRI study days. 10mls will also be taken for DNA and RNA analysis. Therefore in total over the 35 weeks study duration, up to 705ml of blood will be taken.

Plasma levels of glucose and other metabolites, insulin, ghrelin, leptin and other hormones will be measured by immunoassay. Lymphocyte DNA and histone methylation and modification will be measured to look at epigenetic changes.

7.3 Scoring of dietary likes and dislikes

After the test meal on the first fMRI study day, subjects will be asked to rate each of the food pictures as to how much they usually like to and how often they eat the foods shown. This will enable comparison to be made between subjects to ensure that differences in fMRI activation are not due to differences in the food preference of the pictures which may be present between the dietary intervention and control groups as well as preterm and full term individuals.

7.4 Visual Analogue Scales

Visual analogue scales will be used to assess subjective feelings of hunger, nausea, fullness, food palatability, sleepiness and mood at intervals throughout the study days.
7.5 **HOMA Index calculation**

The Homeostasis Model Assessment derives an estimate of insulin sensitivity from the mathematical modelling of fasting plasma glucose and insulin concentrations of the participants (Matthews et al., 1985). HOMA index values have been shown to be an accurate correlate of the gold-standard glucose clamp technique for assessment of insulin sensitivity without the large financial expense (Bonora et al., 2000).

Fasting insulin and plasma samples will be taken from participants on the MRI fat study day and will be used to calculate a HOMA index value for each participant at baseline and 7 days after the intervention period. Insulin resistance is an important component in the aetiology of the metabolic syndrome and type 2 diabetes mellitus.

7.6 **Food Diary Analysis**

For three days before the first fMRI study day as well as the last MRI study day (28 days), participants will be instructed to complete a food diary. This will allow for a comparison of energy and macronutrient intake at baseline and 10 and 28 days after dietary intervention. The diaries will be analysed using dietary analysis software.

7.7 **DNA sampling**

Common variations in several genes, such as the melanocortin-4 receptor and FTO, have been associated with obesity and altered function of pathways involved in appetite regulation (Xiang et al., 2006; Frayling et al., 2007; Dina et al., 2007; Young, 2007).

With specific consent, we therefore wish to collect saliva or blood for DNA and RNA at the start of the investigation on the first fMRI study day to enable for the examination of variations in genes related to obesity and appetite regulation in collaboration with Dr. Alexandra Blakemore and Prof. Philippe Froguel, Department of Genomic Medicine, Imperial College, Hammersmith Hospital and Prof. Mark McCarthy, Oxford Centre for Diabetes, Endocrinology and Metabolism. Epigenetic modifications will be studied in collaboration with the Epigenetics Section, MRC Clinical Sciences Centre.

7.8 **Metabolomic Investigations**

Metabolomics is the systematic study of the unique chemical fingerprints that specific cellular processes leave behind - specifically, the study of small-molecule metabolite profiles. The metabolome represents the collection of all metabolites in a biological fluid. Metabolic profiling can, thus give a 'snapshot' of the physiology of the organism. Before and after 10 days of dietary intervention, metabolite profiles in plasma will be investigated. These profiles can then be correlated with clinical end points assessed in the study.

Samples for metabolomic analyses will be collected on each of the three fat MRI study days. For this purpose, 2 x 2.5 mL heparin plasma (one sample for analysis and one as a backup) per visit is required. Therefore, 10 mL blood will be drawn into heparin-containing vacutainers before separation and storage.

Samples will be sent securely to an external laboratory for analysis that will also be blinded to the study treatments and will only receive anonymised samples. Any samples remaining will be discarded.
8. REGULATORY ISSUES

8.1 Safety and Protection of Participants

There are minimal risks associated with taking the 600 kCal drink for 10 days. The expected increase in body weight is about 4% of body weight. It is expected that weight should return to normal after the dietary intervention is finished. Nonetheless, subjects will receive dietary and physical activity advice from a qualified dietician at the end of the study to help with any weight that has not been lost.

The MRI scanner contains a very strong magnet. Therefore, individuals may not be able to have the MRI if they have any metal implanted in their body, for example any pacing device (such as heart pacer), any metal in their eyes, or certain types of heart valves or brain aneurysm clips.

None of the MR techniques to be used employs ionising radiation or intravenous contrast agents and all techniques mentioned may be performed during a single imaging session. Subjects will be in the MR scanner for 60-75 minutes on each study day.

There is not much space inside the MRI scanner and therefore some people may find this unpleasant or ‘claustrophobic’. This is therefore an exclusion criterion for recruitment to the study. As some of our volunteers will have participated in previous MRI studies, we can be sure that they tolerate the MR scanning from the point of view of claustrophobia and lying still. If subjects experience discomfort within the scanner despite the measures taken to ensure patient comfort, the patient may request immediate cessation of the procedure with withdrawal from the scanner by ringing the patient alarm bell.

The MRI produces a ‘hammering noise’ but subjects wear earplugs and headphones to prevent discomfort or damage to hearing.

The risks of placing a needle to draw blood from a vein include minor discomfort at the site of the puncture; possibly bruising and swelling around the puncture site; rarely, infection or faintness during the procedure.

A data monitoring committee consisting of Dr. Tony Goldstone, Consultant Endocrinologist, Imperial College Healthcare NHS Trust; Prof. Jimmy Bell, Group Head, Metabolic and Molecular Imaging Group, MRC Clinical Sciences Centre; Prof. Gary Frost, Professor of Nutrition and Dietetics Group, Section of Investigative Medicine, Imperial College London and as external member Dr. Anne Dornhorst, Consultant Diabetologist, Imperial College Healthcare NHS Trust will meet every 4 months to monitor the study and safety of participants.

8.2 Consent

All researchers have completed Good Clinical Practice Training and are proficient in taking informed consent from participants.

A face-to-face discussion will take place prior to the taking of informed consent at the Screening Visit so the participant can have the study fully explained to them by a researcher and have opportunity to ask any questions they may have. Using open-style questions, this will also give the researchers opportunity to ensure the participant has sufficient understanding and capacity to give consent. Written consent will be obtained from the participant using the consent form.

The right of the participant to refuse to participate without giving reasons will be respected. All participants will be made aware that they are free to withdraw at any time from the protocol treatment without giving reasons and without prejudicing further treatment.
8.3 **Data Handling and Record Keeping**

Personal contact details are required for communication with the subjects participating in the study. This information is held solely for communication between the researchers and participants. Personal details will only be stored on NHS password protected computers, and will only be accessed by the researchers involved in the study. No personal data will be kept on University computers, only anonymised data.

Information held on NHS computers is solely for the purpose of hospital bookings and routine sample collections and analysis (e.g. for medical screening). This information is password protected in a similar manner to that of other hospital patients.

Subjects will be given a personal study code number which will be used throughout the study and in the analysis of data.

Coded samples will be treated with confidentiality, similarly to patient samples, when undergoing analysis both in the department and in the hospital laboratory. Any samples sent to external laboratories for analysis will be fully anonymised and specific consent for this is sought on the consent form.

Analysis of results will take place in the MRC Clinical Sciences Centre and Section of Investigative Medicine and be carried out by the researchers themselves. The data will be kept in a secure environment in these departments, under the authority of Dr. Tony Goldstone and Prof. Jimmy Bell. The data will be stored for 20 years after completion of the study.

Authorized representatives of the Research and Development Department, Imperial College Healthcare NHS Trust may also be granted access on request.

If a patient withdraws their consent at any point during the active trial period data already collected shall not be used in any analysis or possible publication. The recorded data shall be held in accordance with the data handling and record keeping methods already specified.

8.4 **Unblinding**

It is not anticipated that premature unblinding will be required in this study due to the established safety profile of the dietary intervention. However, in the event that a participant has a side-effect considered serious, unexpected and possibly, probably or definitely related to the dietary intervention, the participant’s allocation will be unblinded.

8.5 **Indemnity**

Imperial College London holds Public Liability ("negligent harm") and Clinical Trial ("non-negligent harm") insurance policies which apply to this trial. In addition, volunteers are registered as patients so that they have a right to all the associated health benefits this confers. Therefore, in addition to the Imperial College "no fault" indemnity scheme, normal NHS indemnity rules will apply.

8.6 **Sponsorship**

Imperial College London will act as the main sponsor for this study. Delegated responsibilities will be assigned to the NHS trusts taking part in this study.
8.7 Funding

The study will be funded using reserve research funds by the Department of Investigative Medicine, Imperial College and core funding from the Metabolic and Molecular Imaging Group, MRC Clinical Sciences Centre at Hammersmith Hospital.

This protocol describes the study ‘The effects of overfeeding on body fat and eating behaviour in preterm adults’ and provides information about procedures for entering participants. Every care was taken in its drafting, but corrections or amendments may be necessary. These will be circulated to investigators in the study. Problems relating to this study should be referred, in the first instance, to the Chief Investigator.

This study will adhere to the principles outlined in the NHS Research Governance Framework for Health and Social Care (2nd edition). It will be conducted in compliance with the protocol, the Data Protection Act and other regulatory requirements as appropriate.

8.8 Audit and Inspection

The study may be subject to inspection and audit by Imperial College London under their remit as sponsor, the Study Coordination Centre and other regulatory bodies to ensure adherence to good clinical practice (GCP).

8.9 Trial Management

A Trial Steering Group (TSG) will be appointed and will be responsible for overseeing the progress of the trial at regular intervals. This will include the principal investigator and all other investigators named in the protocol.

8.10 Ethics Approval

The Chief Investigator has obtained approval from the London Queens Square Research Ethics Committee. The study must be submitted for Site Specific Assessment (SSA) at each participating NHS Trust. The Chief Investigator will require a copy of the Trust R&D approval letter before accepting participants into the study. The study will be conducted in accordance with the recommendations for physicians involved in research on human subjects adopted by the 18th World Medical Assembly, Helsinki 1964 and later revisions. Any amendments to the protocol should be approved by the sponsor and have ethical and Trust R&D approval before implementation.

9. POWER CALCULATIONS

Power calculations have been performed using a previous MRI body fat studies of term and preterm adults (07/Q0411/19 and 07/H011/118).

10. REFERENCES


361


magnetic resonance imaging and proton magnetic resonance spectroscopy study. Gut 54, 122-127.


INFORMATION SHEET FOR RESEARCH PARTICIPANTS

You will be given a copy of this Information Sheet and a signed copy of your consent form to keep, should you decide to participate in the study.

STUDY TITLE: THE EFFECTS OF OVERFEEDING ON BODY FAT AND EATING BEHAVIOUR IN PRETERM ADULTS

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

If you do decide to take part, please let us know beforehand if you have been involved in any other study during the last year. You are free to withdraw at any time without explanation. Thank you for reading this.

WHAT IS THE PURPOSE OF THIS STUDY?

Adults who were born prematurely (before 33 weeks of gestation) may be at increased risk of having higher amounts of fat in their abdomen and liver than adults who were born after a normal duration of pregnancy (approximately 40 weeks). Adults who were born prematurely may also be at increased risk of developing high blood pressure and sugar diabetes.

The aim of this study is to further investigate this and see if this may happen by studying the effects of a short 10 days of overfeeding in adults who were born prematurely (preterm) or after a normal period of pregnancy (term).

Participants will undergo whole body fat scans using magnetic resonance imaging (MRI), as well as brain scans while looking at pictures of food or performing simple tasks, complete questionnaires and measure your appetite and hormones. This allows us to investigate how your body fat and eating behaviour change and what are the causes.

This study is an important step towards finding out why preterm adults and full term adults differ in the way that they store fat.

WHY HAVE I BEEN INVITED?

You have been invited to take part in this study because you meet the following criteria:

Appendix 2. Participant Information Sheet v1 19 Aug 2011
• You are male aged 18-50 years
• You were born preterm or at full term
• You are of normal body weight or are overweight (but not obese) (body mass index between 20 and 30 kg/m²)

You should not take part in this study if you:

1) Have any illnesses which we feel make you unsuitable

2) If you take any medication that we feel makes you unsuitable

3) If you have donated blood in the last three months

4) You have claustrophobia or certain types of metal in your body that do not allow MRI scans to be performed

5) You are a current smoker

DO I HAVE TO TAKE PART?

It is up to you to decide whether or not to take part. If you do decide to take part you will be given this information sheet to keep and be asked to sign a consent form. If you decide to take part you are still free to withdraw at any time and without giving a reason.

WHAT WILL HAPPEN TO ME IF I TAKE PART?

In total you will be asked to attend for up to 6 visits: 1 screening visit (3 hours), 5 scanning study visits (each 5 hours). The day before and day of each visit you will be asked to avoid strenuous exercise or alcohol. If you only want or are able to attend for some of the scanning visits you may still be able to participate. At the end of this information sheet is a diagram showing a summary of what the study involves over time.

Screening visit

If you agree to take part in this study, you will be asked to attend the Clinical Investigation Ward at Hammersmith Hospital for up to 3 hours after an overnight fast. You will be asked some questions about your medical health, family health and medications. You will have blood samples taken (no more than 20ml blood equivalent to around one and a half tablespoons) to look at your kidney, liver, cholesterol, sugar, thyroid hormones.

With your permission, we will also take a sample of DNA and RNA from blood or saliva to look for changes in your genes that may be involved in the how the body controls appetite, brain function, metabolism and body weight. You will not be informed of the results of these genetic tests as all data will be analysed anonymously.

You will also have measurements of your height and weight. You will also have your height and
weight taken and your body fat content measured using a 'bio-electrical impedance' machine. This is a painless safe method that involves measuring the electrical current from your body and takes only about 5 minutes.

You will be asked to complete some questionnaires about your eating and exercise habits, personality and mood which will take 20 minutes. This information will be related to the results from your brain scans. You will also be asked to complete some simple tasks using a computer to test your reaction time, decision making and food preferences which will take up to 45 minutes to complete. A researcher will be available to assist you with this if necessary.

You will also be asked to taste the meal that will be used later on in the study. We will also ask you to keep a record of all food and drink consumed for three days at home to send back to us as part of the screening.

As long as these medical checks are satisfactory and you are still happy to participate, you will then be asked to attend for future study visits.

**Number of subsequent visits**

Following your first screening visit you will be asked to attend the Clinical Investigation Ward at Hammersmith Hospital as an outpatient on up to another 5 occasions for scanning study visits over a period of 5-6 weeks. These will be completed on dates convenient to you and the investigators. There will be 3 visits for whole body fat scans and 2 visits for brain scans.

**Dietary Intervention**

At the first MRI fat visit (day 0) you will be randomized to receive either a 0 calorie or 600 calorie drink to take each day for 10 days until Visit 4. You will be given a supply of the drink to take home with instructions to take it each day at the end of your usual breakfast. The order of these visits will be:

1. Brain scan
2. Body fat scan about 1 week later
   - Start drink supplement
3. Body fat scan 7 days later
4. Brain scan 3 days later
   - Stop drink supplement
5. Body fat scan 10 days later

At the end of the study you will have a consultation with the study dietician. They will give you dietary advice for healthy eating and if necessary to help you lose any excess weight you may have gained and not lost since you stopped taking the drink at day 10.

**Study visits**

On each of the 5 study visits, you will be asked to attend the Clinical Investigation Ward at Hammersmith Hospital in the morning. Each visit will last around 5 hours. You will be asked to abstain
from alcohol and strenuous exercise for 24 hours before the visit. After eating supper at 8pm the night before each study visit, you will be asked to have nothing to eat and only water to drink until you are given lunch on the day of the study visit at around 1-2pm.

Before 3 of the visits (Study Visits 1, 4 and 5) we will also ask you to keep a record of all food and drink consumed for the previous 3 days.

You will also have your height, weight, hips and waist measured. On some visits, you will also have your body fat content measured using a ‘bio-electrical impedance’ machine. This is a painless safe method that involves measuring the electrical current from your body and takes only about 5 minutes.

On each study day you will have a small plastic cannula tube inserted into a vein in one arm. A vein is the type of blood vessel commonly used for taking blood samples. You may feel some discomfort whilst the cannula is being inserted. After the cannula tube has been inserted this will be used to take blood samples.

Over the course of the visit, we will regularly ask you to score how you are feeling (e.g. rating your hunger) by placing a mark on a line called a visual analogue scale.

You will be given lunch to eat after the scan on each study visit.

**Blood tests**

Blood samples will be taken from the cannula in your arm. The total amount of blood taken on each study visit will not be more than 150 ml (about 10 tablespoons). The total amount of blood taken over all your visits over the study will not be more than 705ml (under a pint and a half). During blood testing, you will be seated or lying on a couch and can read or watch television. The blood samples will be used to measure the levels of sugar, fat and hormones in your blood, and also how the proteins that are attached to your DNA may be altered by the change in your diet.

**Brain scans (Study Visits 1 and 4)**

You will also complete questionnaires about your mood on each brain scan study visit which should take up to 20 minutes to complete.

At the brain scan visits you will have magnetic resonance imaging (MRI) brain scans by lying in an MRI scanner for up to 75 minutes. This will take place in the Robert Steiner Magnetic Resonance Imaging Unit or the Clinical Imaging Centre, nearby the Clinical Investigation Ward. This will enable us to look at the structure and activity of your brain. While in the scanner you will view a computer screen in a mirror above your head and have a keypad in your hand so that you can make button responses. While in the scanner your heart rate will be monitored.

During the functional brain scans we look at the activity of the brain at rest, while you look at a variety of different pictures on a screen (e.g. food, animals, household objects, faces, mildly stressful images
of war or accidents), or perform simple tasks like viewing a flashing light, pressing a button, reading, listening, speaking, recalling, thinking about words or numbers, playing games to win hypothetical prizes. You may be asked to make responses to either pictures or instructions on the screen e.g. rate how hungry you feel the appeal of pictures or taste, indicate the gender of faces, or press different buttons depending on what signal is shown on the screen.

You may have the opportunity to practice some of the tasks outside of the scanner or at the beginning of your time in the scanner. This will enable us to ensure that you can follow the instructions and lie still while in the scanner.

At one of the visits, you will also be asked to complete a short questionnaire to find out your dietary likes and dislikes that will take 15-20 mins.

**Body fat scans (Study Visits 2, 3 and 5)**

On the Fat scanning study visits you will have a whole body magnetic resonance imagining (MRI) scan in the Robert Steiner MRI Unit, Hammersmith Hospital. This takes around 40 minutes to 1 hour and will enable us to look at the fat content of your body, and measure how much fat is located in particular places such as the abdomen, liver and muscle. You will need to lie still on a trolley in the scanner which will move to scan you from your head to your toes and you will have contact with a researcher at all times throughout the scan.

You will also complete a short questionnaire about how physically active you are.

**Other Contact**

In addition to you attending for the study days, one of the study team will also contact you frequently to ensure that you are taking the drink supplement every day during the 10 day period and have not had any problems. This will be done by brief telephone calls (at most every other day) and email.

**WHAT DO I HAVE TO DO?**

To take part in this study you must be willing to do the following things:

1. Take the drink every day after breakfast for 10 days.
2. Attend the hospital on a screening visit and 5 separate study mornings.
3. Refrain from taking strenuous exercise and drinking alcohol for 24 hours before each study visit.
4. Have nothing to eat and only water to drink from 8pm on the night before each visit until lunch on the day of the study visit at 1-2pm.
5. Keep a record of all food and drinks consumed over three days on 4 occasions.

**WHAT ARE THE SIDE EFFECTS OF TAKING PART?**

MRI is a powerful, diagnostic body scanning technique, which is used in hospitals worldwide to create images of the inside of the body. MRI has been used safely for several decades and has no known
side-effects.

The drink supplements have not found to be linked to any serious side effects.

**WHAT ARE THE POSSIBLE DISADVANTAGES AND RISKS OF TAKING PART?**

Insertion of the cannula into your arm on each of the study days may cause minor discomfort or superficial bruising.

MRI scanning is a procedure that allows doctors to look inside the body by using a scanner that sends out a strong magnetic field and radio waves. MRI does not use X-rays. This procedure is used routinely for medical care and is very safe for most people, but you will be monitored during the entire MRI scan in case any problems occur. The risks of having an MRI scan are:

- The MRI scanner contains a very strong magnet. Therefore, you will not be able to have the MRI if you have any type of metal implanted in your body, for example, any pacing device (such as a heart pacemaker), any metal in your eyes, or certain types of heart valves or brain aneurysm clips. Someone will ask you questions about this before you have the MRI.
- There is not much room inside the MRI scanner. You may be uncomfortable if you do not like to be in close spaces (“claustrophobia”). During the procedure, you will be able to talk to and hear the MRI staff through a speaker and earphone system, and, in the event of an emergency, you can tell them to stop the scan. You will be closely monitored and repeatedly checked on to make sure you are as comfortable as possible. While your head is in the scanner, we will support it, so you can’t move it. If this upsets you, you will be able to signal and speak to the investigator and stop the scan through the use of a radio system and a signalling button. You will have the opportunity during the first MRI scan to ensure that you can tolerate having the scan before the next five scans required for the study are performed.
- The MRI produces a “hammering noise”. You will wear earplugs and headphones to prevent discomfort or damage to hearing. The headphones will also allow you to be able to hear us talk to you.
- You will be fully awake during the MRI scan and will not be sedated at any time. We will make every effort to ensure your comfort during this experiment.

It should be noted that the MRI brain and body scan cannot be viewed as a comprehensive health screening procedure or ‘health check-up’. However, very rarely, unexpected information can be detected which may need further investigation. In this event, you will be informed and a report will be sent to your GP, who will arrange further tests and coordinate your further care. In the rare event that we find a significant abnormality on your scans on the first visit this may exclude you from continuing with the rest of the study.
You may increase your body weight by a small amount (estimated to be on average only 4%) if you receive the high-calorie drink for 10 days. Your liver fat may also increase. However these changes should be reversible after you stop the drink. At the end of the study you will be given advice by a dietician about healthy eating, and especially if your body weight and liver fat have not returned to normal over the 18 days since you stopped the drink.

WHAT ARE THE POSSIBLE BENEFITS OF TAKING PART?
There are no direct benefits to you in taking part in this study. However the information that we get from this study will help us to better understand body fat distribution and appetite regulation. If any of the screening questionnaires or blood tests reveal any medical problems (e.g. depression, diabetes, high cholesterol, thyroid, kidney or liver problems, or abnormalities on scanning), your GP will be informed by the Investigators so that your GP can coordinate your further care, arrange any further tests, and refer you on to Hospital Doctors if necessary.

WHAT IF NEW INFORMATION BECOMES AVAILABLE?
Sometimes during the course of a research project, new information becomes available about the theme that is being studied. If this happens, your research doctor will tell you about it and discuss with you whether you want to continue in the study. If you decide to continue in the study you will be asked to sign an updated consent form. Also, on receiving new information your research doctor might consider it to be in your best interests to withdraw you from the study.

WHAT HAPPENS WHEN THE RESEARCH STUDY STOPS?
Once the study has finished, the results of the study can be made available to you and/or your GP should you wish. If you have any problems immediately following the study, then you should contact one of the research doctors on the numbers provided below.

WHAT IF SOMETHING GOES WRONG?
Imperial College London holds insurance policies which apply to this study. If you experience serious and enduring harm or injury as a result of taking part in this study, you may be eligible to claim compensation without having to prove that Imperial College is at fault. This does not affect your legal rights to seek compensation.

If you are harmed due to someone’s negligence, then you may have grounds for a legal action. Regardless of this, if you wish to complain, or have any concerns about any aspect of the way you have been treated during the course of this study then you should immediately inform the Principal Investigator (Dr. Tony Goldstone 020 3313 5856). The normal National Health Service complaint complaints mechanisms are also available to you. If you are still not satisfied with the response, you may contact the Imperial AHSC Joint Research Office.
WILL MY TAKING PART IN THIS STUDY BE KEPT CONFIDENTIAL?
All information which is collected about you during the course of the research will be kept strictly confidential. Any information about you which leaves the hospital will have your name and address removed so that you cannot be recognised from it. It is a requirement that your GP is informed, with your consent, of your participation in this study, at the start of the study.

WHAT WILL HAPPEN TO THE RESULTS OF THE RESEARCH STUDY?
The results are likely to be published in the year following the study. Your confidentiality will be ensured at all times and you will not be identified in any publication. At the end of the study, the results of the study can be made available to you and/or your GP should you wish. Research data generated from this study will be kept for 20 years.

WHO IS ORGANISING AND FUNDING THE RESEARCH?
This study is being organised and funded by the Metabolic and Molecular Imaging Group, MRC Clinical Sciences Centre and the Section of Investigative Medicine, Imperial College London. This study will also form part of a research thesis for a PhD student.

EXPENSES
You will receive a fixed payment to cover expenses including travel costs at the end of the study. This will be £10 for the screening visit and £20 per MRI visit (5 in total). Therefore the maximum amount claimable for the entire study is £110.

WHO HAS REVIEWED THE STUDY?
This study has been reviewed by the London Queens Square Research Ethics Committee (no. 11/LO/1097).

CONTACT FOR FURTHER INFORMATION
If you have any questions regarding the study please contact the researcher (Nauf Al Saud) on 020 3313 3772 or via email on premature@imperial.ac.uk.
If you would specifically like to talk to the doctor involved in the study, Dr Goldstone, he will be available by telephone during working hours (020 3313 5856).
The hospital switchboard (020 8363 1000) has home and mobile phone numbers for all the doctors involved in the study and can contact them at any time outside normal working hours.
If you agree to take part in the trial, you will also be given the mobile phone numbers of the researchers.
If you experience any problems during this study, you may withdraw at any stage.

Thank you for taking the time to read this.
Diagram showing what the study involves.
Participant Consent Form

STUDY TITLE: THE EFFECTS OF OVERFEEDING ON BODY FAT AND EATING BEHAVIOUR IN PRETERM ADULTS

Name of Principal Investigator: Dr. Tony Goldstone  Please tick and initial each statement:

1. I confirm that I have read and understand the participant information sheet dated 09/08/2011 version 1.1 for the above study.

2. I have had the opportunity to ask questions and discuss this study.

3. All my questions have been answered fully.

4. I have received enough information about the study.

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

6. I understand that my images and sections of any of my medical notes may be looked at by responsible individuals from Imperial College London and Imperial College Healthcare NHS Trust or from regulatory authorities where it is relevant to my taking part in this research.

7. I give permission for these individuals to access my records that are relevant to this research.

8. I give permission for my General Practitioner to be informed of my participation in this study and the results of any medical tests from my visits and brain scans.

9. I give permission for my images to be used for research by responsible individuals from Imperial College and Hammersmith Hospital so long as they do not contain identifying personal information.

10. I agree for a DNA/RNA sample to be taken and stored to look for changes that may be involved in obesity, brain function and the control of appetite and hormones, and understand that the results will not be fed back to me.

11. I give permission for my blood or DNA/RNA samples to be sent to laboratories in the United Kingdom or abroad for analysis so long as all personal information is removed from them.

12. I give permission for any stored samples that I give during this study, to be used in future studies which have been granted suitable ethical approval.

13. The compensation arrangements have been discussed with me.

14. I agree to take part in the above study.

15. I agree to be contacted again by the Investigators to participate in future research.

Name of Subject (block capitals)  Signature  Date

Principal Investigator  Signature  Date

Name of Person taking consent  (if different from Principal Investigator)  Signature  Date

Fat Imaging Data Sheet

Name ____________________________ Patient Number ________________________

Ethnicity (see guide) ____________________________

1. Asian or Asian British: Indian, Pakistani, Bangladesi, Other Asian background
2. Black or Black British: Caribbean, African, Other Black background
3. Chinese or Chinese British: Chinese, Other Chinese
4. mixed: White & Asian, Black and Black African, White and Caribbean, Other Mixed background
5. White: British, Irish, Other White background
6. Other: Other Ethnic background

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<tr>
<th>Date</th>
<th>Scan Number</th>
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<td>Weight</td>
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<td>Height</td>
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<td></td>
<td>Blood Pressure (BP)</td>
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<td></td>
<td>Resting HR (bpm)</td>
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<td>Waist</td>
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<td>Hip</td>
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<td>Dress Size</td>
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<td></td>
<td>VO2 (ml/kg/min)</td>
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Diet Type (Basic): Standard, Vegetarian, Vegan, Pescetarian (Mediterranean etc)??

Athlete □ Fit □ Active □ Sedentary □

ACTIVITY: <1, 1-3, 3-5, >5 hrs/wk

EXERCISE Programmed classes? Y __ N ____

Level: Lo □ Mod □ Hi □ V.Hi __________

(type/freq/dur): Aero / Resist, ________mins/ sess, ______day/wk

Birth weight: __________ Pre-term / Term / + Term

Gestational Age: __________ weeks

Delivery: standard / cesarean / forceps Breast/Formula fed

Smoker (past/current) / Non-smoker
The Robert Steiner Magnetic Resonance Unit,  
MRC Clinical Sciences Centre,  
Hammersmith Hospital,  
Du Cane Road,  
London W12 OHS

NAME ..........................................................................................................................................

**IMAGING SAFETY CHECK LIST**  
If the answer to any of the above questions is “YES” please give details.

<table>
<thead>
<tr>
<th></th>
<th>YES</th>
<th>NO</th>
<th>detail</th>
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<tbody>
<tr>
<td>Cardiac pacemaker</td>
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<tr>
<td>Mechanical heart valve</td>
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<tr>
<td>Any surgery</td>
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<tr>
<td>Cosmetic surgery/implants</td>
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<tr>
<td>History of foreign body in eye</td>
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<tr>
<td>Occupation as metal worker, grinder, welder</td>
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<tr>
<td>Metallic implant, metal prosthesis, orthopaedic plates, screws, dental implant etc.</td>
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<td>Piercing</td>
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<td>Shrapnel</td>
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<tr>
<td>Aneurysm clip/haemostatic clip</td>
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<tr>
<td>Ear Implants</td>
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<tr>
<td>Artificial Eye</td>
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<tr>
<td>Coloured contact lenses</td>
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<tr>
<td>Interventional radiological devices</td>
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<tr>
<td>Pregnancy</td>
<td></td>
<td>L.M.P.</td>
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<tr>
<td>IUCD (Intra-uterine Contraceptive Device)</td>
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<tr>
<td>Implantable pumps/ neurostimulators</td>
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<tr>
<td>Allergies</td>
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<tr>
<td>Are you wearing a watch?</td>
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<tr>
<td>Are you wearing any jewellery?</td>
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<tr>
<td>Is there anything in your pockets such as keys, etc.?</td>
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</table>

**NB: A device not known to be safe must be assumed to be unsafe.**  
In the case of devices operated by microprocessors (e.g. implantable pumps) device malfunction caused by field effects or circuitry must be considered. Other devices such as ventricular shunts, orthodontic braces may be safe, but may degrade image quality significantly. Images may also be degraded by metal containing tattoos or cosmetics. (see AJR 1988;151:811)

This is to confirm that the above checklist has been completed.

Signed ..................................(participant)  Date:..............................

Signed ..................................(MR Unit Personnel)  Date:..............................
7Up Free 600ml

**Product Details**
- Bigger bottle better value
- Sugar, colouring, caffeine and preservative free
- Low calorie lemon and lime flavoured soft drink with sweeteners.
- Put some pop into your everyday. Escape to a carefree world at facebook.com/7UPUK for fun and chances to Win. Don't grow up. 7UP.

**Other Information**
- Additives: Free From Artificial Colours
- Additives: Free From Artificial Preservatives
- Recycling Info: Recyclable Pack

**Ingredients**
- Carbonated Water, Citric Acid, Natural Lemon and Lime Flavouring, Malic Acid, Acidity Regulator (Sodium Citrate), Sweeteners (Aspartame, Acesulfame K), Potassium Chloride, Fecitin, Contains a source of Phenylyalanine

**Nutrition**

<table>
<thead>
<tr>
<th>Typical Values</th>
<th>Typical values per 100ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy</td>
<td>8kJ (1 kcal)</td>
</tr>
<tr>
<td>Protein</td>
<td>0.1g</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>0.1g</td>
</tr>
<tr>
<td>- of which Sugars</td>
<td>NIL</td>
</tr>
<tr>
<td>Fat</td>
<td>NIL</td>
</tr>
<tr>
<td>- of which Saturates</td>
<td>NIL</td>
</tr>
<tr>
<td>Fibre</td>
<td>NIL</td>
</tr>
<tr>
<td>Sodium</td>
<td>Trace</td>
</tr>
<tr>
<td>*equivalent as Salt</td>
<td>Trace</td>
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</tbody>
</table>