The effect of the thiazolidinedione troglitazone and association of leptin on body composition in non-insulin dependent diabetes mellitus

IRENE ELIZABETH KELLY
BSc (Honours), State Registered Dietitian

A thesis submitted for the degree of Master of Science
to
The University of Glasgow
January 1998

From research conducted at the
Department of Human Nutrition, University of Glasgow
Glasgow Royal Infirmary
Glasgow, Scotland

CONTENTS

SUMMARY AND CONCLUSIONS OF THE FINDINGS IN THE PRESENT THESIS

The present study examined the effect of the thiazolidione troglitazone and the influence of serum leptin on body composition in patients with non-insulin dependent diabetes mellitus. The study was designed as a double blind, randomised, placebo-controlled, parallel group trial, where subjects were allocated to receive either troglitazone or matching placebo. Sixteen males and seven females were recruited from the Diabetes Centre at Glasgow Royal Infirmary. All subjects were treated by diet alone or diet and sulphonylureas and had a fasting glucose between 8 and 16mmol/l. The commonly used anthropometric measurements as well as a standard underwater weighing method were employed to determine percentage body fat. A novel magnetic resonance imaging technique was used to calculate the volume of total abdominal and intra-abdominal fat. Serum leptin was analysed using a radioimmunoassay kit.

The results of the study indicate that troglitazone had no significant effect on body weight of total body fat. However troglitazone did significantly reduce intra-abdominal fat. Analysis of leptin with measures of adiposity showed that leptin correlated significantly with body fat by underwater weighing and body mass index, but

correlated weakly with intra-abdominal fat. These results suggest that subcutaneous adipose tissue (which is the main site of body fat) rather than intra-abdominal fat, is associated with leptin in overweight subjects with NIDDM.

From the present study it is possible to suggest that:

1. Troglitazone significantly reduces intra-abdominal fat as measured by magnetic resonance imaging, but total body fat does not change significantly.

2. Troglitazone may preferentially increase insulin sensitivity and lipogenesis in tissues other than intra-abdominal fat, and the results would still be in keeping with minor (non significant) increases in other sites of adipose tissue.

3. A single magnetic resonance imaging scan of the intra-abdominal fat area at the intervertebral disc between L2 and L3 vertebrae offers a cheaper, faster and safer method, with high prediction of total-abdominal fat volumes.

4. Subcutaneous adipose tissue (the major body fat depot) rather than intra-abdominal fat, is associated with leptin in overweight diabetic subjects.

ACKNOWLEDGEMENTS

Research studies in the present thesis were guided by Professor Michael EJ Lean of the Department of Human Nutrition, Glasgow Royal Infirmary, University of Glasgow. Thank you for your patience and opportunity to develop my research skills further

Thanks are due to Mike Wallace of the Department of Biochemistry, Glasgow Royal Infirmary for his support of the analysis of Leptin.

Thanks to Professor Reginald Green and his staff, Department of Radiology, Health Care International, Glasgow for their support and guidance with magnetic resonance imaging.

Thanks are due to my colleagues at the Department of Human Nutrition but special thanks are due to Thang S Han, Catherine Hankey and Wilma Leslie – your patience is to be commended!

A special thanks is due to all volunteers!

Mum and Dad, thank-you for your endless encouragement and support.

This project was funded by Glaxo-Wellcome

ABBREVIATION USED IN THE PRESENT THESIS

AT	adipose tissue
BF	body fat
BMI	body mass index
CI	confidence interval
MRI	magnetic resonance imaging
NIDDM	non-insulin dependent diabetes
r	correlation coefficient
SD	standard deviation
UWW	underwater weighing
WHR	waist to hip ratio

Publications arising from the present thesis

(see Appendix Two for reprints)

Full papers

Han TS, **Kelly IE**, Walsh K, Greene RMF, Lean MEJ. Relationships between volumes and areas from single transverse scans of intra-abdominal fat by magnetic resonance imaging. *International Journal of Obesity* 1997;**21**:1161-66

Kelly IE, Han TS, Walsh K, Lean MEJ. The effect of troglitazone on body composition of patients with Type 2 diabetes. *Diabetes* (submitted for publication)

Kelly IE, Han TS, Wallace AM, Walsh K, Lean MEJ. The effect of serum leptin and body fat compartments in non-insulin dependent diabetes. (submitted for publication)

Abstracts

Kelly IE, Han TS, Walsh K, Lean MEJ. Serum leptin and body composition in non-insulin dependent diabetes. *International Journal of obesity* 1997;**21**:S98

Kelly IE, Han TS, Walsh K, Lean MEJ. The effect of troglitazone on intra-abdominal fat in type 2 diabetes. British Diabetic Association Conference Bournemouth October 1997 *Diabetic Medicine* 1997;**14**:S21

CHAPTER ONE

Introduction & Background:

Literature review

1.1 Body Composition and fat distribution of patients with non-insulin dependent diabetes

Historical perspectives

People with non-insulin dependent diabetes mellitus (NIDDM) have a distinctive body morphology, which was recognised by pioneering anthropologists such as Lister and Tanner (1955), who described the physique of NIDDM subjects as "roundness of the body contour, a tendency to obesity, smooth skin with fine hair, and short tapering limbs with small hands and feet."

Body fat distribution in diabetic subjects was first examined by Jean Vague in the nineteen forties (Vague 1949). His pioneering work described diabetic men and women as having an "android" body shape, later described as an apple shape by scientists in the eighties. These patients tend to accumulate relatively more body fat around and particularly within the abdomen.

The associations of body fatness as indicated by body mass index, with morbidities and mortalities are well established from epidemiological data (Lew and Garfinkle, 1979). Body shape and fat distribution are reflected by waist to hip ratio (WHR). WHR has been shown to be a predictor of the incidence of diabetes mellitus in both adult men (Larson *et al*, 1984) and women (Ohlson, 1985) independently of body mass index. WHR has also shown to relate to the ratio of intra-abdominal to abdominal subcutaneous fat mass (Ashwell *et al*, 1985).

Recent advances

Waist circumference is marginally less good than WHR in identifying NIDDM in cross-sectional surveys (Han *et al*, 1998). However, recent evidence has shown that waist circumference alone is a better predictor of intra-abdominal fat than WHR (Han *et al* 1997a). Several longitudinal studies have shown that waist circumference is better than WHR in predicting the development of NIDDM in men over 13.5 years (Ohlson *et al*, 1985), and these findings were confirmed by other recent prospective studies by Carey *et al* (1996) and Wei *et al* (1997). These findings suggest that it is possible that large waist circumference predicts the development of NIDDM, whilst muscle loss leads to decreased hip circumference measurement when NIDDM develops.

Muscle atrophy may also be one of the key factors associated with NIDDM and related metabolic disturbances. This is supported by a study of Seidell *et al* (1997) showing that NIDDM subjects have both larger waist and smaller hips than expected for their given BMI.

NIDDM has a strong familial pattern suggesting an important genetic predisposition but it has also been suggested as a consequence of development failure of vital organs in early life, such as the pancreas and the liver. This has been considered as malnutrition at a critical stage of fetal development (Barker, 1994). It can be mimicked by gestational protein deficiency in animals (Hales & Barker, 1992).

Another more secure indicator of poor nutrition in early life is short stature (Floud *et al* 1990). Disproportionate limb length relative to body length, including high ratio of lower leg length to height, which may reflect interrupted growth (McCance, 1968), has been shown to associate with NIDDM (Han *et al,* 1997b). Whether the individual's predisposition results from genetic factors or fetal insult, weight gain exceeding BMI 23kg/m^2 is necessary for developing NIDDM (Chan *et al,* 1994).

1.2 Methods of measuring body composition

Total Body Fat

Many techniques have been developed for assessing body composition in human subjects. The conventional "gold standard" laboratory method is under-water weighing (UWW) which measures body density, a two compartment model from which fat and lean mass contents are estimated by employing standard figures for density of fat (0.9kg/L) and fat free mass (1.1kg/L) (Siri, 1961).

Other "standard" methods for measuring body composition include total body potassium, measuring intracellular water in which potassium concentration present is constant. This method detects the naturally occurring radioactive ^{40}K isotope fraction, which can be extrapolated to estimate total body potassium, and hence total body water (Brodie *et al,* 1980). Deuterium ($^2H_2O_2$) labelled water can be ingested and its distribution gives a more distinct measure of total body water from which body fat can

be estimated from standard assumptions about water space (Schoeller *et al,* 1989). The principles of these techniques are described by Brodie (1980), Garrow, (1983) and Shephard, (1991). Body water measurement together with methods such as dual X-ray absorptiometry (DEXA) to estimate body calcium and bone mass, allow the development of more complicated 3 and 4 compartment models. These multicompartment models may have advantages in specific settings, but ultimately there is no independent "true" reference method.

Field methods of assessing body composition

The "standard" methods are rather complicated and expensive for everyday clinical use, therefore simple and cheaper anthropometric methods have been derived to estimate body composition and total body fat.

The skinfold technique uses skinfold calipers to measure subcutaneous fat at standard sites over the biceps, triceps, subscapular and suprailliac regions. The sum of these four skinfold thicknesses is used to predict body density measured by underwater weighing. Equations are available to relate skinfold thicknesses to percentage body fat (Durnin and Womersley, 1974).

Other simple anthropometric methods used to estimate body composition include the use of body mass index (Deuenberg *et al*, 1991) and more recently, equations employing waist circumference with or without tricep skinfold (Lean *et al* 1996) have been published, again these methods employed the use of regression equations derived from UWW.

All methods for assessing body composition introduce errors in subjects with unusual characteristics such as the elderly, extremely obese or with disease such as NIDDM and coronary heart disease (Lean *et al* 1996). The advantage of the waist method is that it takes account of variation in fat distribution and is thus preferred for subjects who may have altered fat distribution e.g. NIDDM patients (Lean *et al*, 1990)

1.3 Leptin and Body Fat

Leptin was identified in 1994 by Zhang *et al* (1994) as a product of a gene that is defective in an obese strain of ob/ob mice and soon after, its human homologue. Leptin is produced by adipocytes and their specific receptors are found in many tissues, including the hypothalamus in the brain, which is thought to be the most important feed back regulatory centre. Leptin's main action is believed to be the signal for negative feed back control on appetite and energy expenditure.

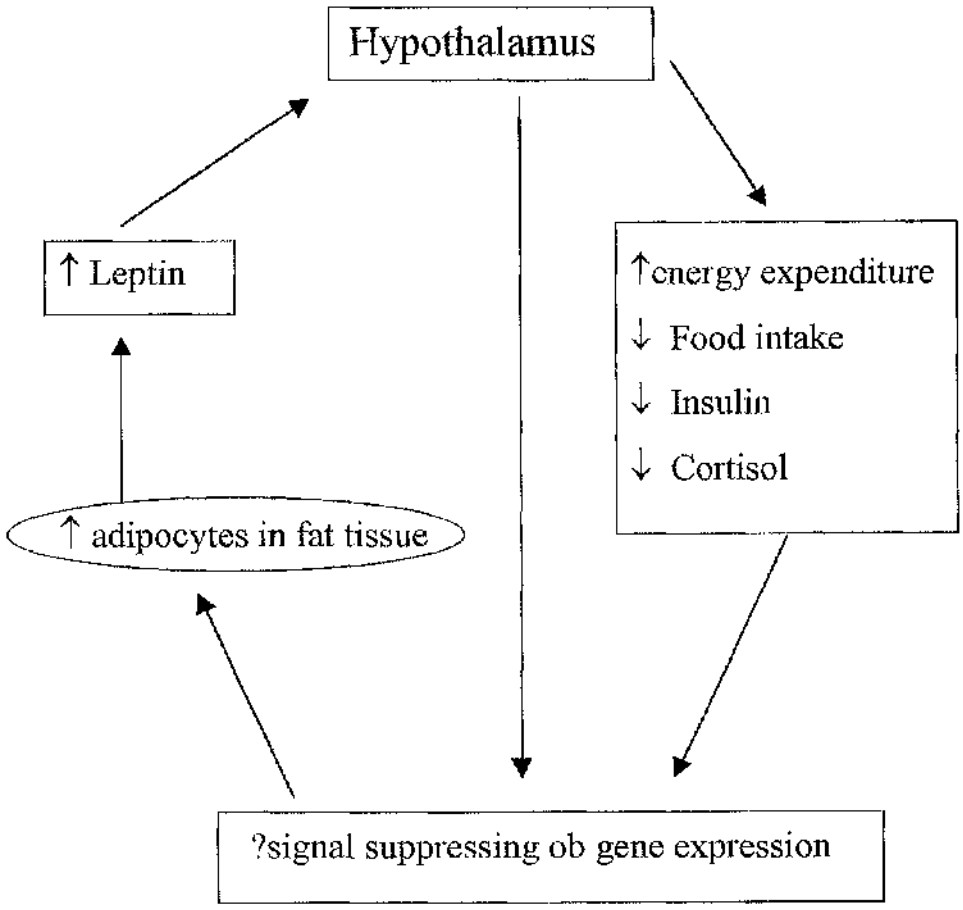


Figure 1.1 Proposed mechanisms of the effects of leptin on physiological responses and its negative feedback loop

Administration of recombinant leptin to hormone-deficient ob/ob mice results in weight loss through reduced food intake and increased energy expenditure. It also causes diet induced weight loss in normal lean and obese mice (Considine, 1996). Giving exogenous leptin to animals results in weight loss but this effect has not been shown in humans. BMI correlates with plasma leptin but when leptin values for men and women with the same BMI are compared, women have higher concentrations and this can be related to a relatively larger fat mass in women (Considine, 1996).

It is thought that one of leptin's main actions is to inhibit the synthesis and release of the hypothamlamic neuropeptide Y, which has the effect of decreasing food intake, increasing thermogenesis and decreasing concentrations of corticosteroids and insulin in plasma.

Caro *et al* (1996) have hypothesised that if leptin concentrations of the cerebrospinal fluid can be equated to the hypothalamic interstitial leptin concentration, then it is possible to understand why obese people do not have the expected response to their endogenous hyperleptinaemia as they are leptin resistant. Thus, in humans the possibility of leptin resistance makes it less suitable as a treatment for obesity.

1.4 Biochemical properties and clinical potential of troglitazone

Troglitazone is a thiazoladinedione compound, which enhances the effect of insulin action on peripheral tissues and the liver, which improves hyperglycaemia, hyperinsulinaemia. Troglitazone treatment also decreases low density lipoprotein and triglycerides, while increases high density lipoproteins in subjects with NIDDM (Ghazzi *et al* 1997). It is well tolerated at all doses and is being evaluated as a treatment for NIDDM and impaired glucose tolerance.

Licences were granted in 1997 for clinical use of troglitazone in NIDDM subjects but if it increases insulin sensitivity then an increase in fat deposition might be expected, which could be an understandable result.

This would be of particular importance for long term health if the internal intra-abdominal fat was to be expanded.

In rodents troglitazone has been found to cause a marked increase in fat proliferation. A two year histopathologic evaluation in mice has demonstrated vascular tumours found mainly in the subcutis and often associated with adipocytes (Glaxo Wellcome Internal Report, 1997). These tumours were associated with plasma concentrations 13 to 18 times the human therapeutic steady state concentration. Doses associated with plasma concentrations three times the anticipated human therapeutic steady-state concentration did not cause vascular tumours above the control background. No such tumours were found in the rat and it was therefore proposed that the induction of tumours in the mice was related to their genetic predisposition. An unusual feature of the tumour was the fatty changes observed in adjacent adipocytes. No similar effects were observed in a 12 months study of monkeys. Genetic toxicity tests were also found to be negative.

The mechanism of action of troglitazone has not yet been fully elucidated, however it appears to work by either mimicking or enhancing insulin action without any effects on β-cell insulin secretion (Fugiwara *et al*, 1988) with the end result of improving insulin-mediated glucose disposal and reducing hepatic glucose output, possibly involving an increase in clearance of circulating free fatty acids (Suter *et al*, 1992; Petrie and Donnelly, 1994).

Various *in vitro* and animal studies have demonstrated that troglitazone enhances insulin action at both the receptor and post receptor levels in both peripheral and hepatic tissues without the enhancement of insulin secretion (Fujiwara *et al*, 1988). Suter *et al* (1992) have demonstrated that troglitazone exerts its *in vivo* hypoglycaemic effect by improving peripheral insulin resistance and reducing hepatic glucose output, the circulating plasma insulin fell both in the fasting and postprandial states.

Antonucci *et al* (1997) have shown that troglitazone treatment for 12 weeks improved postglucose glycaemic response in patients with impaired glucose tolerance. Troglitazone has also been shown to activate the ligand sensitive transcription factor known as peroxisome proliferator activated receptor-gamma (PPAR-γ). Activation of these receptors promotes adipocyte differentiation and regulation of a number of gene encoding proteins that regulate lipid metabolism, suggesting that the PPAR-γ receptor may play a role in the adipogenic signalling cascade and lipid metabolism.

The action of troglitazone is in contrast to the effects of the traditional first-line hypoglycaemic drugs (sulphonylureas), which induce increased plasma insulin levels in certain NIDDM subjects by a direct effect on β cells (Oates *et al*, 1989). In a study of a small group of NIDDM subjects, troglitazone treatment was shown to substantially reduce peripheral insulin resistance, lowering the elevated rates of hepatic glucose production and decreasing the rates of insulin secretion.

In a study of meal tolerance test, the levels of free fatty acid and glucagon were shown to be significantly lower and remain reduced after treatment with troglitazone (Suter *et al*, 1992).

Evidence from the aforementioned studies suggest that troglitazone offers a new therapeutic option for the treatment of NIDDM. It is also possible that troglitazone could be combined with either sulfonylureas or small doses of exogenous insulin, which may provide an even more effective therapy for NIDDM patients. The possibility of enhancing insulin action in adipose tissue, which would increase fat deposition, might alternatively have a negative influence on diabetic control.

1.5 Research Questions of the present thesis

What is the effect of troglitazone on body fat and fat distribution in NIDDM patients?

What is the association of leptin with body fat and fat distribution in NIDDM patients?

1.6 Hypotheses to be tested

1. If Troglitazone increases insulin sensitivity in adipose tissue it will also enhance lipogenesis and promote fat accumulation.

2. This effect may vary in different sites of adipose tissue.

3. Troglitazone might increase intra-abdominal fat, and thereby ultimately have a negative effect on diabetic control.

4. Leptin is associated differently with body fat from different depots.

1.7 Aims of the present thesis

1. To investigate the effect of troglitazone on body composition by
    a. Underwater weighing - to quantify total fat
    b. Subcutaneous skinfolds - to quantify subcutaneous fat
    c. MRI - to quantify intra-abdominal fat.

2. To examine the associations of leptin with body fat from different sites.

CHAPTER TWO

METHODS

2.1 Study Design

This study was designed as a double blind, randomised, placebo-controlled, parallel group trial to determine the effect of troglitazone on body composition. Subjects were allocated randomly to receive either troglitazone or matching placebo. Randomisations were made in accordance with the code generated by Medical Data Sciences Clinical Pharmacology at Glaxo Wellcome. In addition to their usual treatment which was unchanged throughout the study, subjects in the treated group received three 200mg tablets of troglitazone (GR92132X) once daily and those in the placebo group received three identical placebo tablets (GR92132X) once daily for 12 weeks.

2.2 Subjects

Sixteen male and seven female subjects with NIDDM were recruited from the Diabetes Centre at Glasgow Royal Infirmary. All subjects gave informed consent before entry into the study. Subjects were aged between 44 - 74 years, treated by diet alone or diet plus sulphonylureas and were free from any active disease. Subjects selected for the present study had fasting blood glucose between 8 and 16mmol/l and body mass index (BMI) below 40kg/m^2, (the upper limit for MRI scanning).

Exclusion criteria included women of child bearing age, insulin treatment, cardiovascular disease, renal or hepatic disease, diabetic neuropathy, alcohol or substance abuse, participation in another clinical trial within the last three

months, concurrent medications which have changed or commenced within three months of starting the study: metformin, insulin, hypolipidaemic drugs or warfarin. Ethical approval was obtained from Glasgow Royal Infirmary Joint Ethics Committee and Health Care International Joint Ethical Committee.

2.3 Anthropometry

Height was measured using a fixed stadiometer (Castlemead, Herts, UK) to the nearest mm with the Frankfort plane horizontal. Measurements were made while subjects were wearing a swimming costume. Weight to the nearest 0.1kg was measured using regularly calibrated scales (Seca, Germany).

Body circumferences were measured according to standard recommendations (WHO, 1995) using flexible steel tape (Holtain Ltd, Crymch, UK). Waist circumference was measured midway between the lowest rib margin and the lateral iliac crest. Subjects were asked not to tuck their stomach in while being measured. Maximum hip circumference was measured over the greater trochanters with the measuring tape held horizontal. Measurements were made while subjects stood with their feet about 20-30cm apart. Mid-upper arm circumference was measured at the midpoint between the acromion process and the olecranon. All measurements were taken twice and the mean was used for analysis.

Subcutaneous skinfold thicknesses were measured at the sites of biceps, triceps, subscapular and supra-iliac using calipers (Holtain, Crymych, UK) to the nearest 0.2mm **(Figure 1)**. All the skinfold thicknesses were measured in triplicate and the mean values were used in analysis. The skinfold equations have been derived to predict total body fat determined by underwater weighing (Durnin and Womersley, 1974).

2.4 Measuring total body density and body fat by underwater weighing

Body density was determined by underwater weighing (UWW) **(Figure 2)** as described by Lean *et al* (1996). A metal chair was suspended in a hydrotherapy pool, which was kept at a constant temperature of 36.5°C. The chair was attached to a strain gauge (Mecmesin Ltd Horsham, United Kingdom) with a 20kg range and sensitivity of 0.01kg. Subjects sat in the chair with the water up to their neck. A nose clip sealed their nose. Subjects were encouraged to clear their lungs by blowing out as much as they could and asked to gently lower their head underwater. The underwater weight was recorded by a pen recorder, which was calibrated with known weights before the experiment.

The residual lung volume was simultaneously measured with UWW by a three-breath-nitrogen technique modified from Womersley (1974). Four anaesthetic bags (Ohmeda PLC, Hatfield, United Kingdom) were filled with a known volume of 100% oxygen measured with a dry rolling seal spirometer (PK Morgan, Kent, United Kingdom).

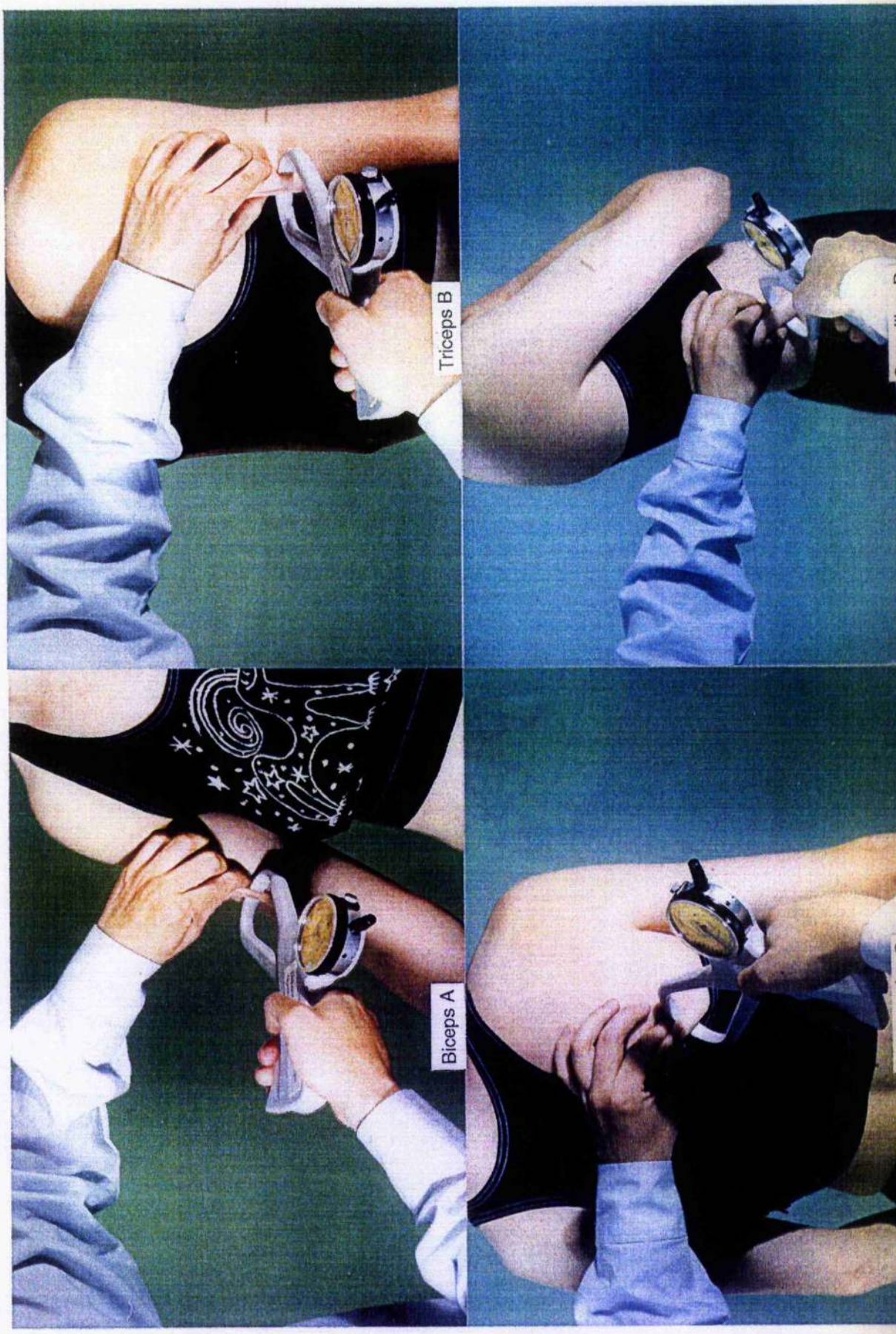


Figure 1 - Measuring skinfold thicknesses using skinfold calipers

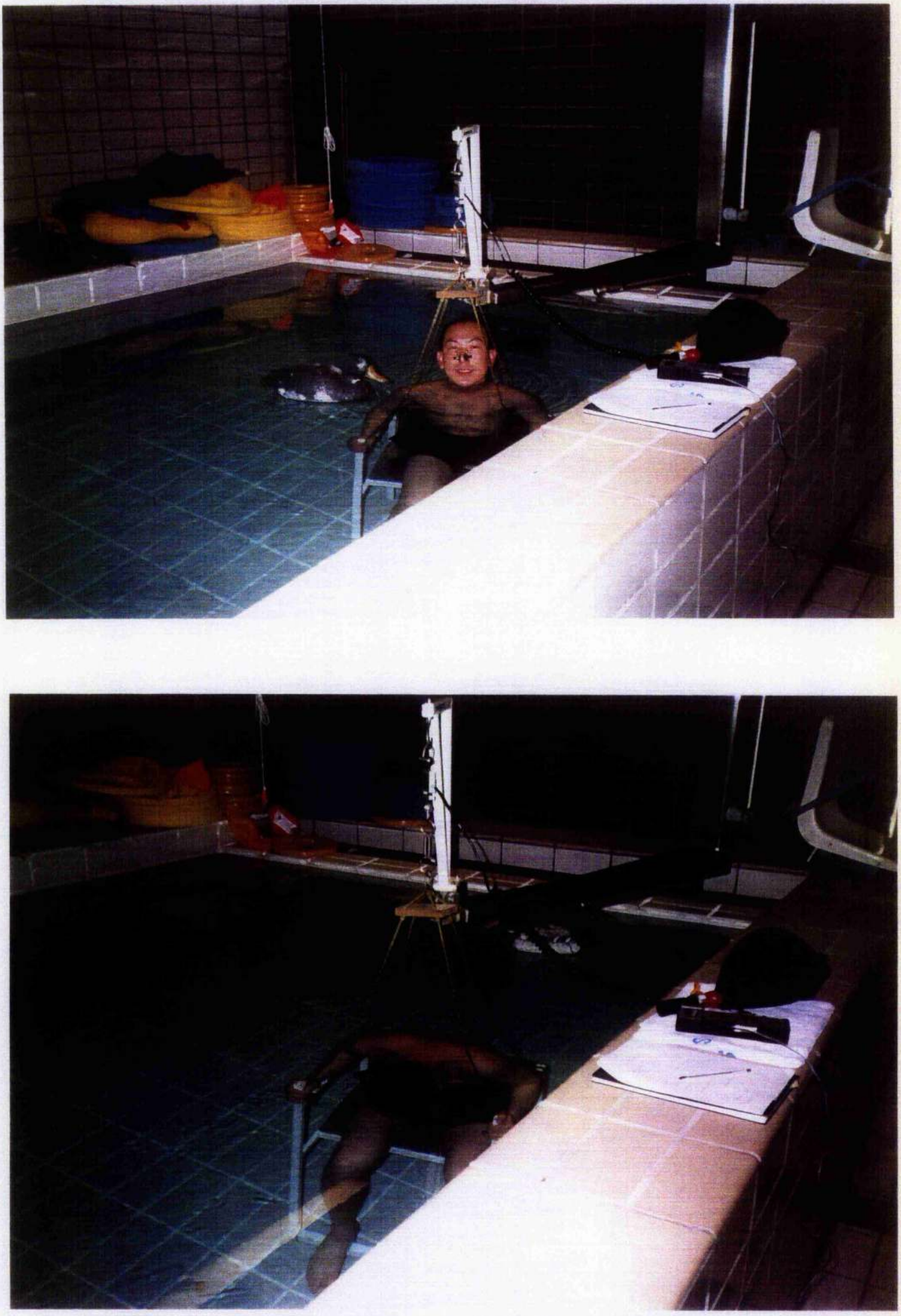


Figure 2 - Measuring total body density by underwater weighing

Approximately 3 litres for women and 4 litres for men (Wilmore *et al*, 1980). On raising their head, a 35ml mouthpiece connected to the anaesthetic bag was instantly inserted into the subject's mouth. The subject then breathed deeply in and out of the bag three times.

After the last complete expiration, the bag was sealed and the gas mix obtained was immediately analysed for oxygen (Polarographic, PK Morgan, Kent, United Kingdom) and carbon dioxide (Infa-red, PK Morgan) contents. Nitrogen content of the gas mix was calculated from the difference in oxygen and carbon dioxide.

Residual lung volume was calculated as:

$$RV = \frac{F \times N \times (V + 0.035l)}{80\% - N\%}$$

Where V is the initial volume of 100% oxygen in the gas bag, 0.035 L is the volume of the dead space in the mouthpiece, with nitrogen content of alveolar air before the test assumed to be 80%. A correction factor (Γ) was included for standard temperature and pressure in dry conditions (STD):

$$F = \frac{273 + Tb}{273 + Tg} \times \frac{Patm - Ps}{Patm - Pa}$$

Subjects were allowed to practise the techniques for holding their breath, bending down and up, and breathing into the mouthpiece outside the pool. They also practised in the water to familiarise themselves with the pool environment. Four measurements were made for each subject, and the pool was allowed to become absolutely calm before starting.

Body density was calculated using the equation:

$$BD = \frac{Wa}{(Wa - Ww) / Dw - RV}$$

Where BD is body density (kg/l), Wa and Ww are weight of the subject in air and water respectively, Dw is the density of water at 36.5°C, and RV is residual lung volume.

Total body fat was calculated from body density using Siri's equation (1961):

$$BF\% = \frac{4.95}{(BD - 4.5)} \times 100\%$$

Where percentage body fat (BF%) is in percentage of body weight.

2.5 Measuring Abdominal Fat by Magnetic Resonance Imaging (MRI)

Measuring total abdominal fat by magnetic resonance imaging (MRI) **(Figure 3)** requires a series of consecutive images in order to calculate a volume. In this study, extra and intra-abdominal fat volumes were calculated using multiple MRI scans at the levels between L1 and L5 vertebrae. The tissues of the abdomen were scanned using an MRI machine (General Electric corporation, Milauwkee, WI, USA) with a magnetic field strength of 1.5 Tesla. Ordinary spin echo sequences were used with a repetition time of 350ms and echo time of 12ms for the abdominal measurement.

Four sagittal images of the trunk were scanned to find the vertebral column **(Figure 4a)**. To obtain reproducible imaging volumes, the volumes of the abdomen were taken to extend from the bottom of the inferior plate of the L1 to the bottom of the inferior plate of the L5 vertebra. Data were collected from as many continuous 20mm thick sections as could completely fit within this interval. This imaging volume approximately corresponds with the levels of the xiphisternum to the anterior iliac crest (Han *et al*, 1997 a & c). Transverse sections (eight in women and nine in men) were then analysed individually **(Figure 4b)**.

A lump of lard (lipid) was placed next to a container of water in the MRI scanner to simulate lipid in adipose tissue (AT) and lean tissue. The scan was analysed to obtain the threshold value where only the fat in the lard could be imaged. Pilot tests determined the threshold value to be at 300

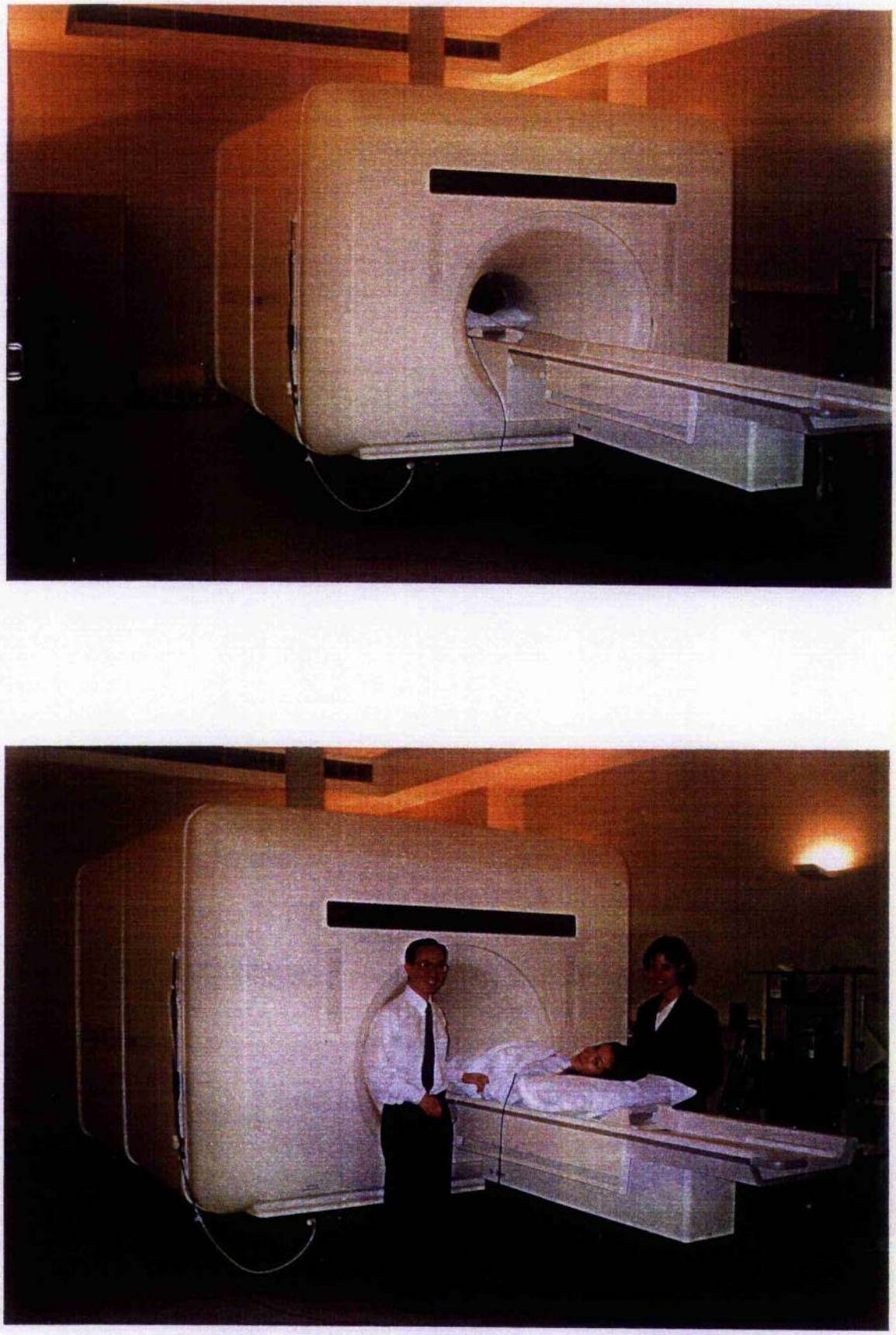
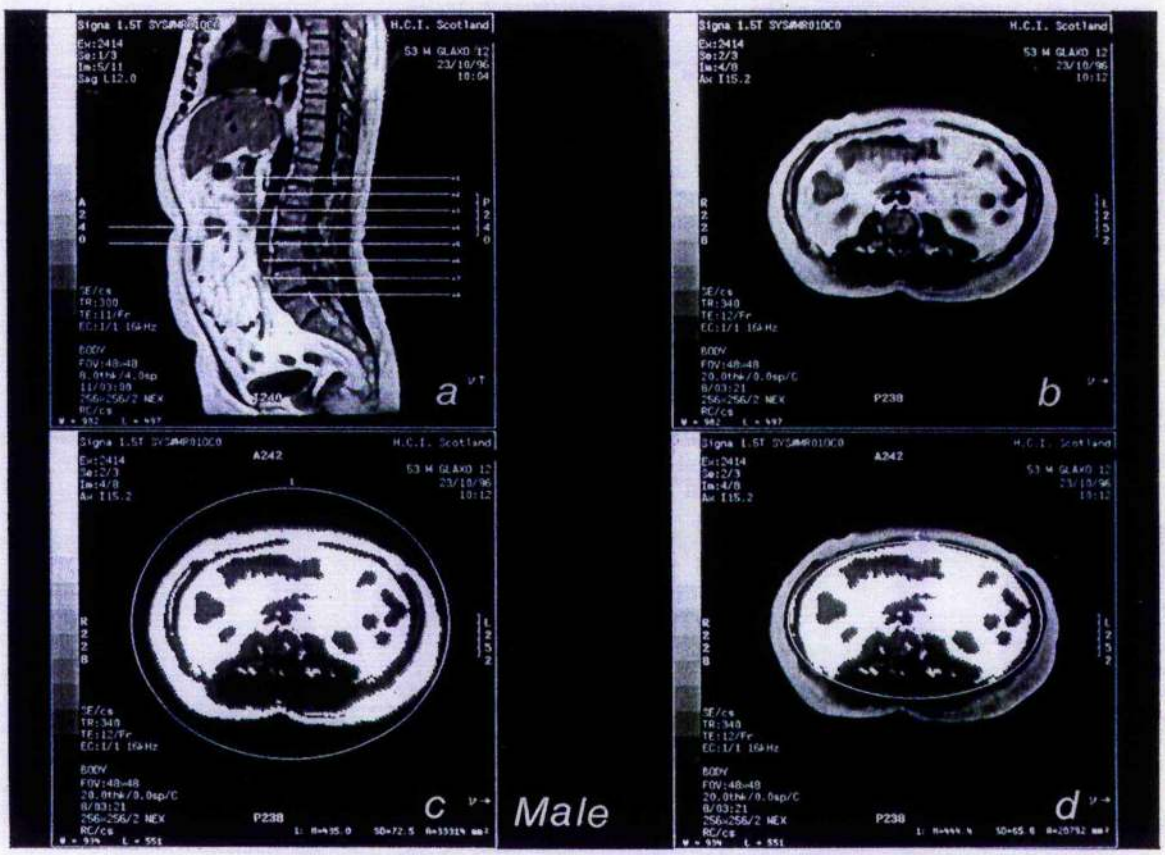


Figure 3 - Magnetic Resonance Imaging (MRI) for scanning body tissues



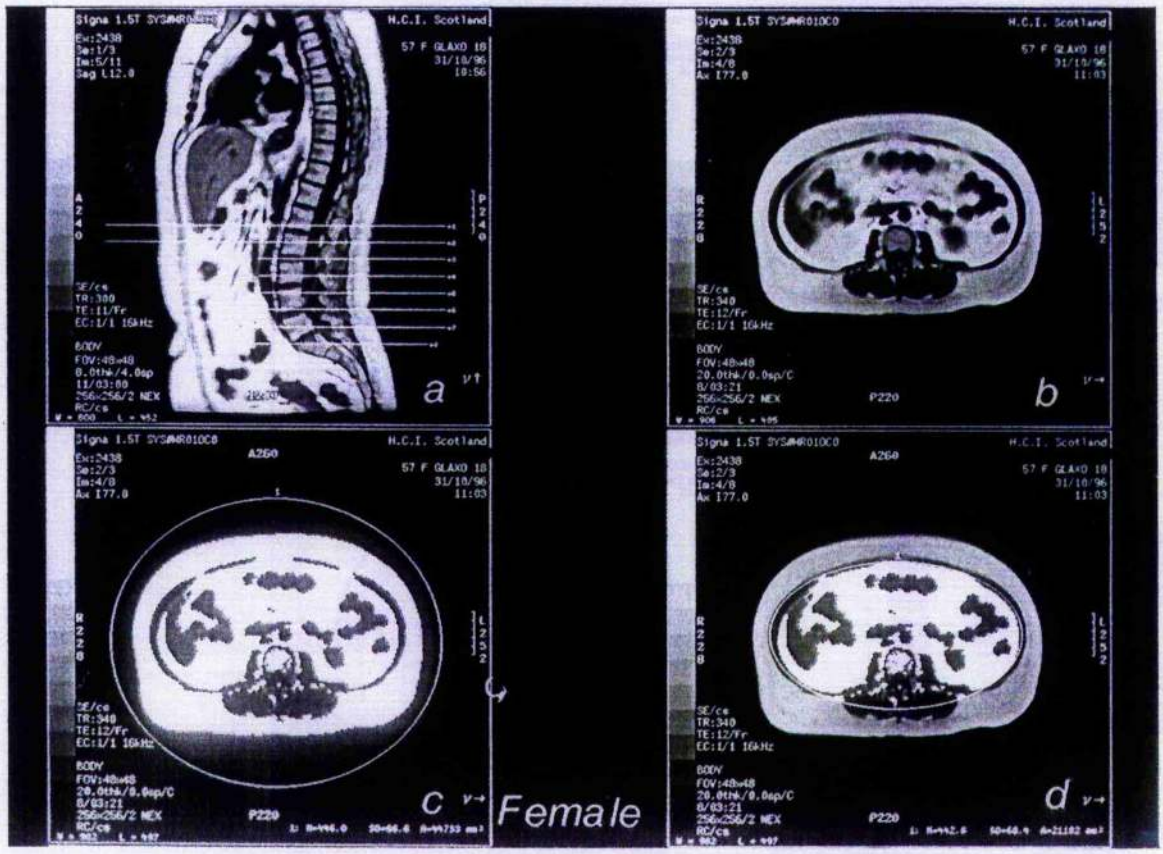


Figure 4 - Abdominal fat images from Magnetic Resonance Imaging (MRI)

(arbitrary units) for every subject for subsequent calculations.

This method may be scanner specific and thus may not apply to other scanners.

Calculations of intra-abdominal and extra-abdominal adipose tissue (AT) were carried out after setting the appropriate threshold value (as determined from the lard at 300 units) that separates AT from lean tissues. The number of pixels for total abdominal AT was obtained from the regions of interest (ROI) by encircling the whole abdominal contents with an ovoid line **(Figure 4c)**. To obtain the number of pixels for intra-abdominal AT, the ovoid line was then positioned at the fascial plane to separate the intra-abdominal and extra-abdominal AT **(Figure 4d)**. Total volume of abdominal AT (mm^3) was obtained by summing the AT areas in eight (or nine in eight men) transverse scans obtained from each subject, and multiplied by 20 mm which was the slice thickness.

The factor 10^{-6} was used to convert the volume of AT in mm^3 into litres, and then to volume of fat (kg) assuming AT contains 80% fat, 2% protein, 18% water with negligible minerals (Garrow, 1974). The average density of AT was assumed to be 0.9255kg/l (Siri, 1961), thus fat was converted from AT by a factor of 0.9255 x 80%. The difference between the total volumes of abdominal fat and intra-abdominal fat provides the total volume of extra-abdominal fat.

Table 2.1 MRI Intra-abdominal fat calculated by 2 different observers to calculate coefficient of variation

Subject	Total tsh a	Total iek b	Mean (a+b)/2	Diff a-b	% Error	Diff^2 $(a-b)^2$
1	166393	165116	165754.5	1277	0.77	160729
2	153143	154556	153849.5	-1413	-0.92	1996569
3	127105	131553	129329	-4448	-3.44	19784704
4	194444	194088	194266	356	0.18	126736
5	115414	117202	146308	-1788	-1.22	3196944
6	133727	133687	133707	40	0.03	1600
7	202956	203649	203302.5	-693	-0.34	480249
	156169	**157122**	**$\bar{x}$=156645**			**$\Sigma(a-b)^2 = 27217531$**

tsh = observer 1, Iek = observer 2, diff = difference

General equations 1-3 are for calculating coefficient of variation for repeated measurements.

Equation 1 $\bar{x} = \frac{(a_i + b_i)}{2}$

Where $\bar{x}$ is overall mean

Equation 2 $SD = \sqrt{\frac{\sum (a_i - b_i)^2}{2 \times n}}$

Where a is first observer and b is second observer, n is the number of subjects with repeated measurements and sd is the standard deviation of the differences in the repeated measurements.

Equation 3 $CV = \frac{SD}{\bar{x}} \times 100\%$

Where the CV is the coefficient of variation.

Calculation of coefficient of variation for repeated intra-abdominal fat by 2 different observers using general equations above and data from table 2.1.

$\bar{x} = 156645$

$$SD = \sqrt{\frac{\sum (156169 \cdots 157122)^2}{2 \times 7}}$$

$SD = 1394.3$

$$CV = \frac{1394.3}{156645} \times 100\%$$

$\boldsymbol{CV = 0.89\%}$

2.6 Blood pressures and Heart rate

Subjects rested in supine position for at least 10 minutes on a couch in a thermoneutral environment (24°C). Systolic blood pressure (SBP) was measured as the first Korotkoff sound and diastolic (DBP) as the fifth Korotkoff sound, using a standard sphygmomanometer. Mean arterial blood pressure was calculated as 1/3 (SBP - DBP) + DBP (Vander et al, 1990). Heart rate was taken at the brachial or radial artery for one minute.

2.7 Biochemical Measurements

2.7.1 Blood Sampling

Venesection was performed to collect fasting blood samples at week 0 (baseline) and at week 12 (follow-up). Serum for measurement was collected by centrifugation of clotted blood at 3000g for 20 minutes at room temperature. Aliquots were pipetted into glass tubes, capped and stored at -70°C for later analysis.

2.7.2 Leptin Analysis

Leptin was analysed using a radioimunoassay (RIA) kit from Linco Research, USA. Test tubes containing standards, quality controls and samples tubes were all prepared in duplicate, mixed with assay buffer, label, leptin antibody, precipitating reagent. The tubes were vortexed and incubated overnight at 4°C.

The next morning, all the tubes were set on ice and 1.0ml of cold precipitating reagent was added to all the tubes (except the totals) and vortex again and incubated for 20 minutes at 4°C and centrifuged for 30 minutes at 2600rpm at 4°C to create a firm pellet. The supernatant was aspirated immediately leaving the pellet at the bottom of the tube, which was then counted using a gamma counter. A full account of the RIA kit procedure can be found in **Appendix One**.

2.8 Statistical Analysis

Statistical methods used to analyse the data in the present thesis were:

1. Linear regression analysis were used to determine the correlations between baseline variables e.g correlation between leptin (dependent) and body fat (independent).

2. Partial correlations were used to adjust for confounding factors e.g. correlations between leptin and body fat, with adjustments for gender and age.

3. Independent t-tests were used to detect the differences between 2 independent groups e.g. comparing the differences in intra-abdominal changes between the two study groups (treatment and placebo) from baseline to follow-up.

4. Paired t-tests were used to determine changes within each study group e.g. comparing the changes from baseline to follow-up in total body fat in each of the study groups.

Power calculations to determine the number of subjects recruited

With a view to use for the treatment of Type 2 diabetes or glucose intolerance, the present study was designed to have power to exclude an effect of 1.4% on percentage body fat which equated to an approximate 1kg effect on body weight in 12 weeks (90% power at $p<0.05$). (Bland, 1993).

CHAPTER THREE

BASELINE RELATIONSHIPS BETWEEN INDICES OF ADIPOSITY AND LEPTIN

Part of this chapter has been submitted as full paper for publication in a peer-reviewed journal as:

Kelly IE, Han TS, Walsh K, Lean MEJ. Relationships between serum leptin and body fat compartments in non-insulin dependent diabetes.

Part of this chapter has been presented at the European Congress of Obesity (Dublin, 1997) and published as an abstract:

Kelly IE, Han TS, Wallace AM, Walsh K, Lean MEJ. Body composition and leptin in non-insulin dependent diabetes. *International Journal of Obesity* 1997 Vol **21** (supplement 2) :S98.

3.1 RESULTS

Table 3.4.1 shows the characteristics of 16 men and 7 women at baseline. Compared to men, the women had tendency to be older, lighter, shorter, but they had a higher BMI, waist circumference, higher waist to hip ratio, more extra-abdominal and total body fat, higher concentration of serum leptin and less intra- abdominal fat.

Table 3.4.2 shows the correlation coefficients between serum leptin and indices of adiposity, with and without adjustments for sex and age. For all degree of adjustments, serum leptin correlated significantly with all indices of adiposity, except intra-abdominal fat. After adjustment for sex and age, intra-abdominal fat became significantly correlated with serum leptin while the significant associations with extra-abdominal fat and subcutaneous skinfolds were lost.

The relationships between serum leptin and intra-abdominal fat (Figure 3.5.1) and waist circumference (Figure 3.5.4) appear to be sex specific, whereas the relationship with total extra-abdominal fat showed no sex difference (Figure 3.5.2). Figure 3.5.3 shows that serum leptin correlated significantly to total body fat in men but there was no significant relationship in the women.

3.2 Discussion

Previous studies have related circulating leptin to body fat using derived measurements such as skinfolds or bioelectrical impedance (BIA). The present study used standard method to determine total body fat by UWW (the gold standard) and a novel application of MRI scanning techniques to quantify abdominal fat compartments by direct measurements. These measures of adiposity were related to serum leptin in men and women with NIDDM. It was shown that serum leptin correlated weakly with intra-abdominal fat and more strongly with other indices of body fatness, including total body fat by underwater weighing and anthropometric measurements particularly BMI and waist circumference.

3.3 Conclusions

Our results suggest that subcutaneous adipose tissue, which is the main site of adipose tissue rather than intra-abdominal fat, is associated with leptin in overweight NIDDM subjects. These findings have an important health implication. It seems possible to conclude from our data that "leptin resistance" in NIDDM may be most marked in those with an expanded subcutaneous fat mass - i.e. not particularly in those with a more central fat distribution, which is a characteristic of NIDDM.

If "leptin resistance" is important in the aetiology of obesity as has been suggested (Caro *et al*, 1996), then this conclusion would indicate no special role for leptin deficiency in patients who have or who develop NIDDM. Furthermore, treatment with exogenous leptin, if it is successful in overcoming insulin resistance and inducing weight loss, might therefore be expected to have its major effect on subcutaneous fat.

Our findings of sex specific relationships between serum leptin concentration with waist circumference are similar to previous results found by Zimmet *et al* (1996). Leptin treatment might thus have a lesser impact on diabetes control and related atherogenic factors than a treatment which reduces intra-abdominal fat. To test these new hypotheses which emerge from the present thesis, future research will require a similar study of non diabetic subjects, and detailed measurements of fat compartments during leptin treatment.

Table 3.4.1 Subject characteristics

	Men (n=16)		Women (n=7)	
	Mean	SD	Mean	SD
Age (years)	56.2	6.3	62.3	9.1
Weight (kg)	81.8	11.7	77.6	11.5
Height (m)	1.71	0.07	1.57	0.04
Body mass index (kg/m^2)	27.9	3.0	31.6	4.7
Waist (cm)	100.3	9.9	107.1	9.0
Waist to hip ratio	1.02	0.10	1.07	0.08
Extra-abdominal fat by MRI (kg)	1.56	0.38	3.53	0.67
Intra-abdominal fat by MRI (kg)	3.16	0.64	2.52	0.63
Percentage body fat by UWW	31.0	5.0	44.5	6.0
Leptin (ng/ml)	6.92	3.76	21.7	8.88

Table 3.4.2 Baseline relationships between indices of adiposity and leptin

	Unadjusted†	Sex adjusted‡	Sex and Age adjusted‡
BMI	0.707***	0.645**	0.632**
WHR	0.448*	0.446*	0.439*
Waist	0.645**	0.665**	0.668**
Sum of 4 skinfolds	0.650**	0.431*	0.425
Percentage body fat by UWW	0.782***	0.473*	0.661**
Extra-abdominal fat by MRI	0.816***	0.462*	0.398
Intra-abdominal fat by MRI	0.385	0.421	0.439*

*** $p<0.001$, **$p<0.01$, $p<0.05$.

† Bivariate correlations

‡ Partial correlations

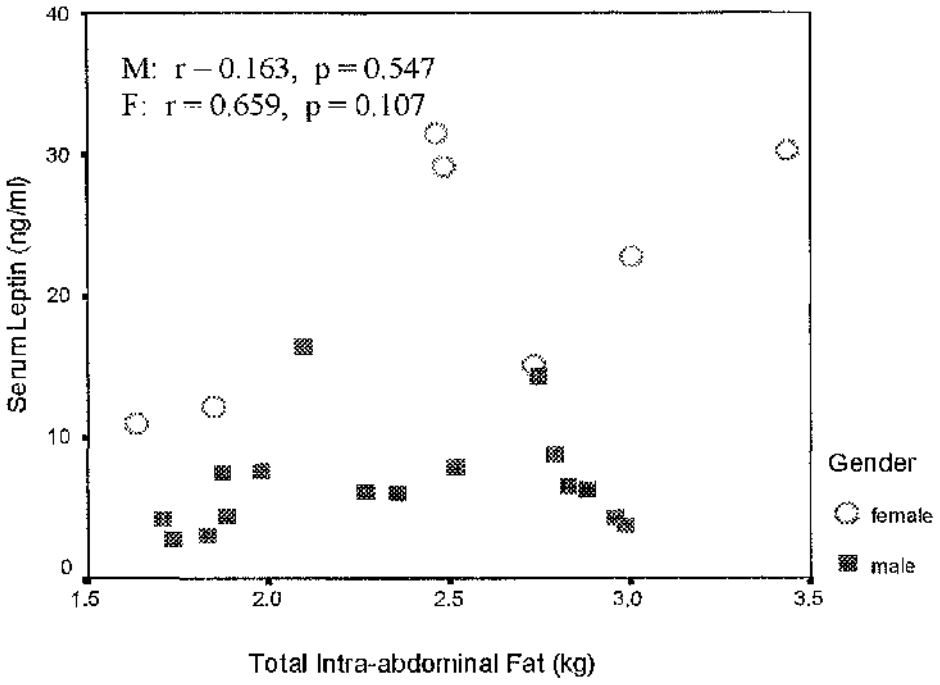


Figure 3.5.1 Relationship between serum leptin and intra-abdominal fat

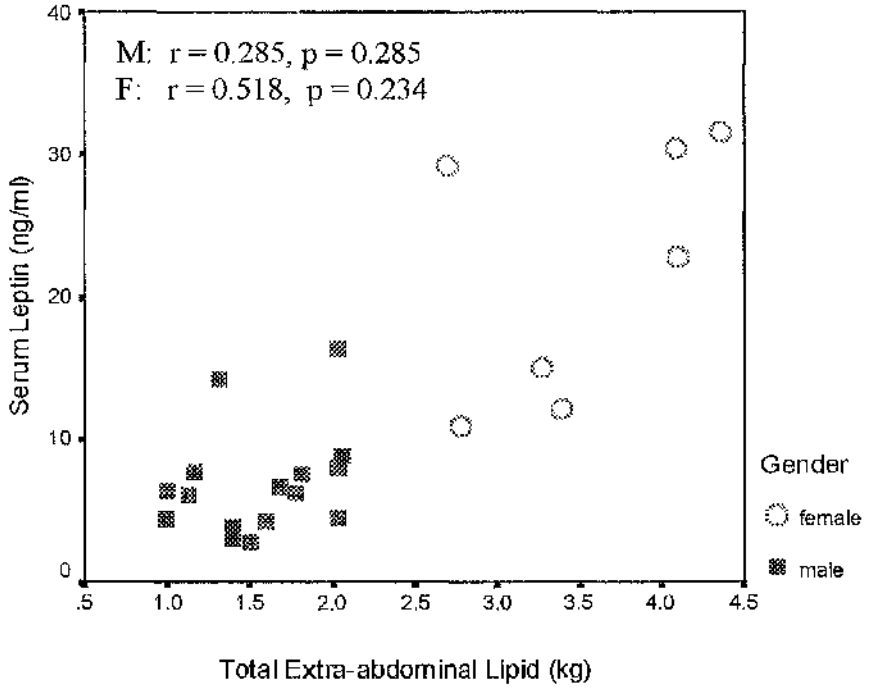


Figure 3.5.2 Relationship between serum leptin and total extra-abdominal lipid in 16 men and 7 women

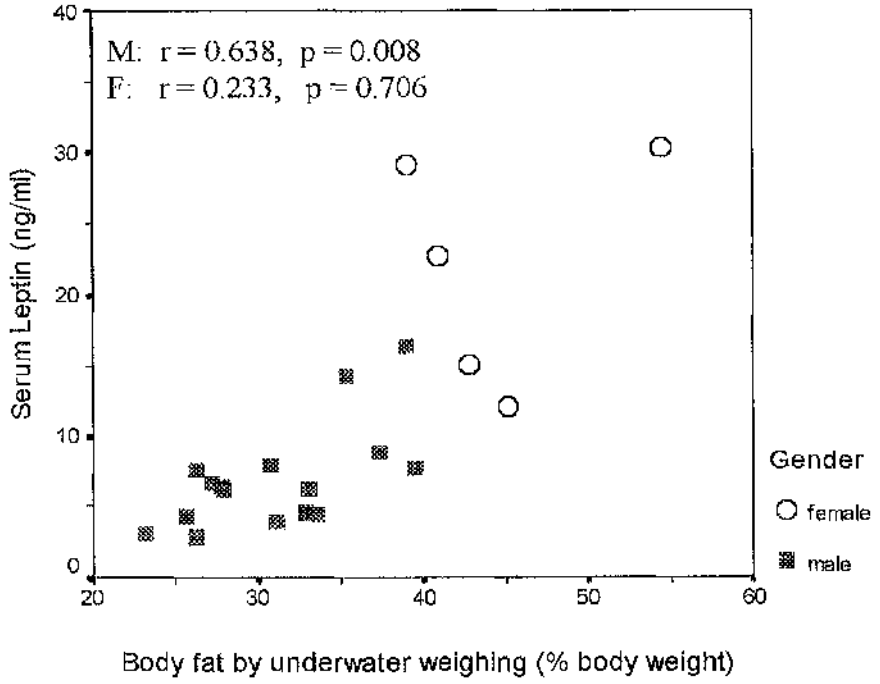


Figure 3.5.3 Relationship between serum leptin and body fat by underwater weighing

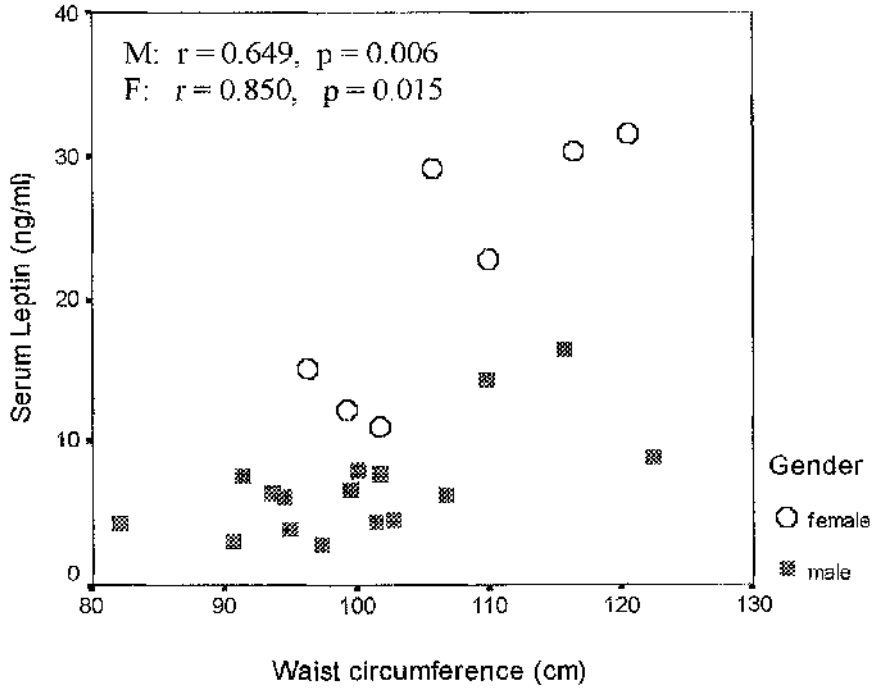


Figure 3.5.4 Relationship between serum leptin and waist circumference

CHAPTER FOUR

EFFECT OF TROGLITAZONE ON BODY COMPOSITION, PHYSIOLOGICAL AND BIOCHEMICAL MEASURES

Part of this paper was accepted by the British Diabetic Association for oral presentation and published as an abstract:

Kelly IE, Han TS, Walsh K, Lean MEJ. Effects of troglitazone on intra-abdominal fat in NIDDM patients. *Diabetic Medicine* 1997;**14** (suppl 4):S21

Part of this chapter has been submitted as a full paper for publication in a peer-reviewed journal as:

Kelly IE, Han TS, Walsh K, Lean MEJ. The effect of troglitazone on body composition in patients with type 2 diabetes.

4.1 Results

Table 4.4.1 shows the characteristics of 12 subjects who were treated with troglitazone and 10 subjects on placebo. There were no significant differences between the troglitazone treated and the placebo treated groups at baseline or at follow-up.

Table 4.4.2 shows the changes in body composition within treatment groups and between treatment groups. There was a significant ($p < 0.05$) decrease in intra-abdominal fat in subjects treated with troglitazone for the 12 weeks. There were no significant changes in all measures of body composition in subjects who were treated with placebo. There were no differences between the two treatment groups in other measures of body composition (**Figures 4.5.1 – 4.5.3**) Subjects who were treated with troglitazone lost significantly more ($p < 0.05$) intra-abdominal fat than the placebo treated group (**Figure 4.5.4**).

4.2 Discussion

Obesity and intra-abdominal fat accumulation are characteristics of NIDDM and the pre-diabetic state, and can be related to many of the metabolic abnormalities linked to diabetic complications and ill health.

It is now well established that intra-abdominal fat is associated with a variety of metabolic disorders including hyperlipidaemia, hypertension and diabetes (Bjorntorp *et al,* 1991), and that a decrease in intra-abdominal fat leads to decreases in total cholesterol, triglycerides, glucose, insulin and increases high density lipoproteins (Lemieux *et al,* 1996). The present study was not designed to have power to evaluate the effect of troglitazone on metabolic variables which have been demonstrated elsewhere (Ghazzi *et al,* 1997). However, the present study showed that after 12 weeks of troglitazone treated subjects lost significantly more intra-abdominal fat than those who were on placebo. There were no differences between the two treatment groups for the changes in other measures of body mass and fatness.

In vitro and animal studies indicate that troglitazone works by mimicking or enhancing insulin action at both receptor and post-receptor levels, in both peripheral and hepatic tissues, without any effect on pancreaticβ–cell secretion (Fugiwara *et al,*, 1988). Troglitazone thus works in contrast to the effects of certain commonly used hypoglycaemic drugs, which induce increased plasma insulin levels in certain Type 2 diabetic subjects (Oates *et al,* 1989). Increased insulin, or insulin sensitivity, enhances lipogenesis, and causes weight gain.

The gain in total body fat observed in animal studies may be due to activation of the ligand sensitive transcription factor known as the PPARγ. Activation of these receptors promotes adipocyte differentiation and regulation of a number of gene encoding proteins that regulate lipid metabolism which suggests that the PPAR-γ receptor may play a role in the adipogenic signalling cascade and lipid metabolism.

The decrease in intra-abdominal fat mass with troglitazone is reassuring as this effect probably has positive health implications such as inducing a favourable fat distribution towards a more peripheral fat deposition (more pear shaped).

The present study employed standard and highly reproducible methods for measuring body composition which included the gold standard UWW (Lean *et al*, 1996) to determine total body fat (repeatability <1%) and MRI to determine abdominal fat compartments. We have developed a novel method for calculating intra-abdominal and extra-abdominal fat (Han, Kelly, Lean, 1997) by taking advantage of the natural circular separation of intra-abdominal and extra-abdominal fat by the fascial plane (**Figure 3d**). We used ovoid lines to determine the regions of interest (ROI) in these abdominal fat compartments, and found this method to be highly reproducible with a coefficient of variation for repeated measurements by two different observers of 0.89% (Table 2.1).

Why intra-abdominal fat mass falls while total body fat is maintained cannot be answered directly by the present study, but clearly the effect of troglitazone on insulin sensitivity is less marked in the intra-abdominal fat. Evidence from the aforementioned studies suggest that troglitazone offers a new therapeutic option for the treatment of NIDDM. It is possible that troglitazone could be combined with either sulfonylureas or small doses of exogenous insulin, which may provide an even more effective therapy for NIDDM patients.

4.3 Conclusions

Total body fat and extra-abdominal fat from MRI measurements were unaltered and there was no change in total body fat measured by UWW. Non-insulin dependent diabetics treated with troglitazone have a significant reduction in their intra-abdominal fat, which suggests that, as well as an insulin sensitising agent, troglitazone might thus have health benefits related to other metabolic disorders by inducing a more favourable body fat distribution.

Troglitazone increases insulin sensitivity at a whole body level, but does not increase total body fat because intra-abdominal fat is reduced. This suggests that troglitazone increases insulin sensitivity in tissues other than intra-abdominal fat.

Table 4.4.1. Characteristics of 11 subjects who were treated with troglitazone and 10 subjects on placebo

	Troglitazone (n = 11)			Placebo (n = 10)			
	Mean	SD	Range	Mean	SD	Range	p†
Baseline							
Age (yrs)	58.0	8.6	44.1-68.7	58.6	7.50	48.4-74.1	0.78
Weight (kg)	78.9	11.2	61.7-102.0	82.1	12.2	61.8-107.2	0.88
Height (m)	1.66	0.09	1.49-1.78	1.69	0.09	1.59-1.85	0.54
BMI (kg/m^2)	28.7	3.9	22.9-37.1	28.6	3.76	24.0-35.6	0.71
Waist (cm)	100.1	10.3	82.1-122.5	102.7	9.1	90.6-116.5	0.79
Waist to hip ratio	1.03	0.12	0.90-1.36	1.02	0.06	0.93-1.12	0.82
Total Body fat (%)	34.5	7.1	25.6-45.1	33.4	9.2	23.2-54.4	0.89
Total Body fat (kg)	27.4	7.3	15.8-38.1	28.6	10.2	19.4-48.7	0.78
Extra-abdominal fat (kg)	2.11	1.0	1.00-4.10	2.00	0.91	1.00-4.09	0.50
Intra-abdominal fat (kg)	2.36	0.51	1.71-3.01	2.41	0.60	1.64-3.44	0.82
Follow-up data							
Weight (kg)	79.6	11.4	62.5-103.5	82.3	12.2	61.6-107.5	0.60
BMI (kg/m^2)	29.0	4.13	23.2-38.11	28.74	3.78	24.1-35.19	0.89
Waist (cm)	102.2	10.2	83.5-124.5	102.6	11.1	87.8-121.2	0.93
WHR	0.94	0.15	0.49-1.03	0.99	0.05	0.89-1.05	0.33
Total Body fat (%)	35.5	7.31	25.9-45.2	32.9	9.71	23.3-55.5	0.51
Total Body fat (kg)	28.5	8.0	16.5-39.07	28.23	10.5	19.1-49.03	0.95
Extra-abdominal fat (kg)	2.03	1.05	0.45-3.82	1.84	0.90	1.04-3.84	0.66
Intra-abdominal fat (kg)	1.90	0.73	0.19-2.78	2.36	0.73	1.42-3.43	0.16

†Independent t-tests for the differences in characteristics between groups.

Table 4.4.2. Changes in body composition

	Troglitazone†		Placebo†		Differences‡ (treated - placebo)		
	Mean	95% CI	Mean	95% CI	Mean	95% CI	P
Weight (kg)	0.66	-0.71 to 2.04	0.25	-0.64 to 1.13	0.42	-1.15 to 1.98	0.58
BMI (kg/m^2)	0.25	-0.25 to 0.75	0.09	-0.23 to 0.40	0.16	-0.40 to 0.73	0.55
Waist (cm)	2.11	0.50 to 3.73	-0.05	-3.72 to 3.81	2.16	-1.5 to 5.84	0.23
WHR	-0.08	-0.20 to 0.04	-0.03	-0.08 to 0.02	-0.06	-0.18 to 0.07	0.37
Total Body Fat (%)	1.02	-1.13 to 3.17	-0.54	-1.68 to 0.60	1.56	-0.88 to 3.99	0.20
Total Body fat (kg)	1.11	-0.94 to 3.16	-0.35	-1.28 to 0.58	1.46	-0.82 to 3.73	0.20
Extra-abdominal fat (kg)	-0.08	-0.33 to 0.16	-0.09	-0.24 to 0.06	0.01	-0.28 to 0.26	0.94
Intra-abdominal fat (kg)	-0.47	-0.79 to -0.13	-0.06	-0.22 to 0.10	-0.41	0.05 to 0.77	0.03

† Paired t-test for the differences between baseline and 12 week follow-up data

‡ Independent t-test for the differences of changes in measures of adiposity between treatment groups

4.5 Effect of thiazolidinedione troglitazone on body composition

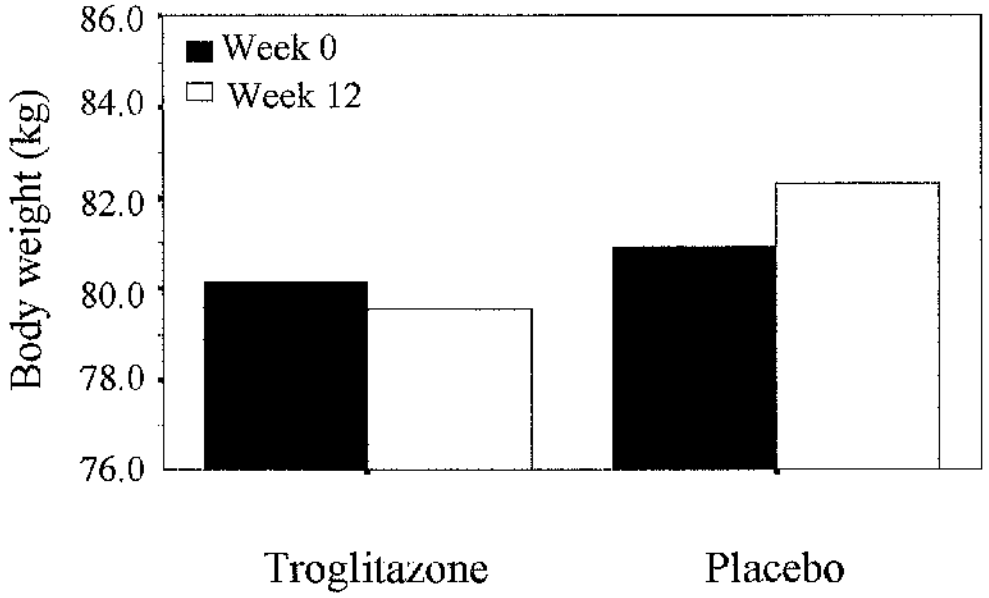


Figure 4.5.1 The Effect of troglitazone on body weight

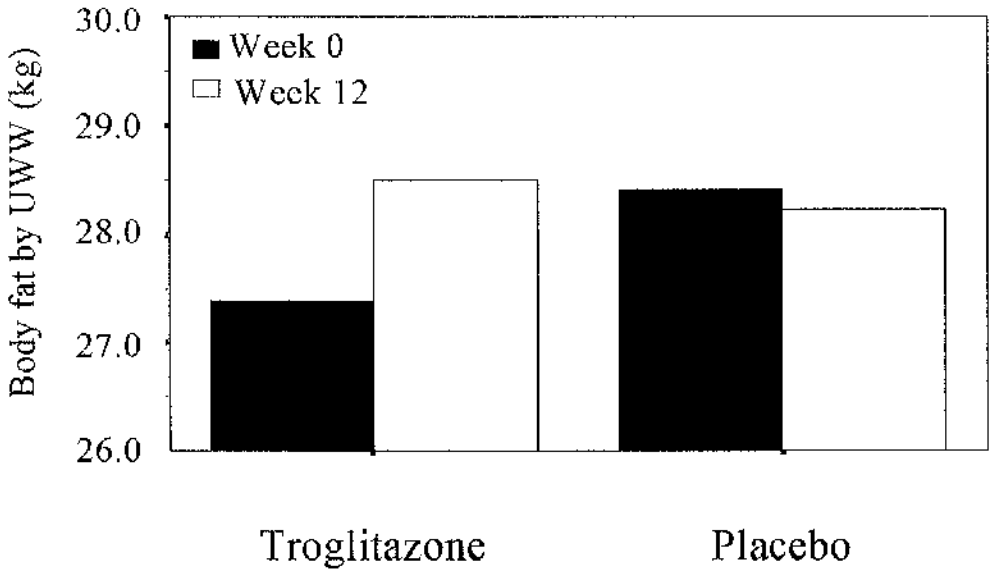


Figure 4.5.2 The Effect of troglitazone on body fat by UWW

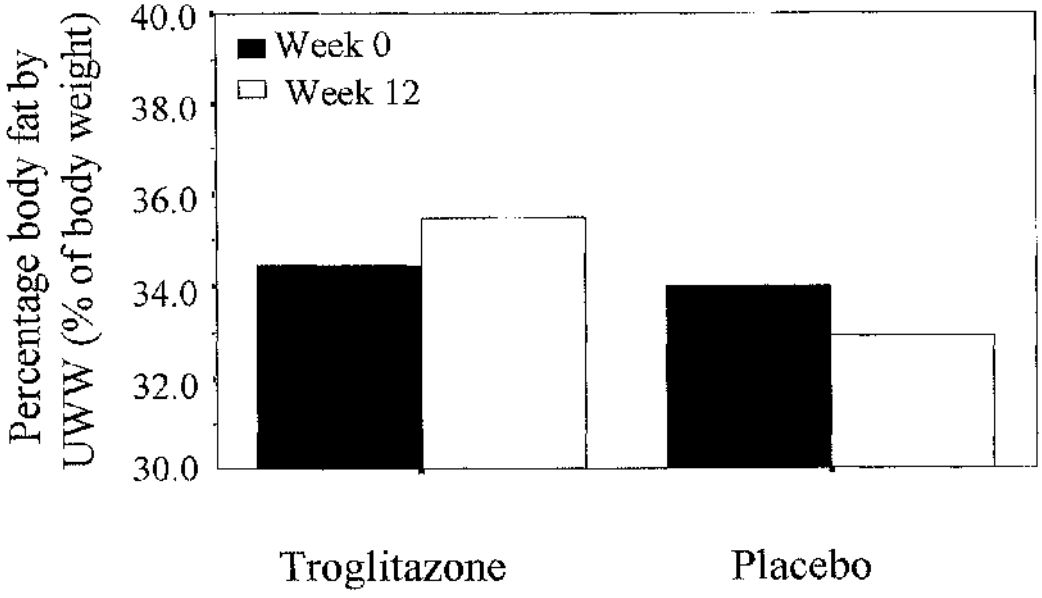


Figure 4.5.3 The effect of troglitazone on the percentage of body fat by UWW

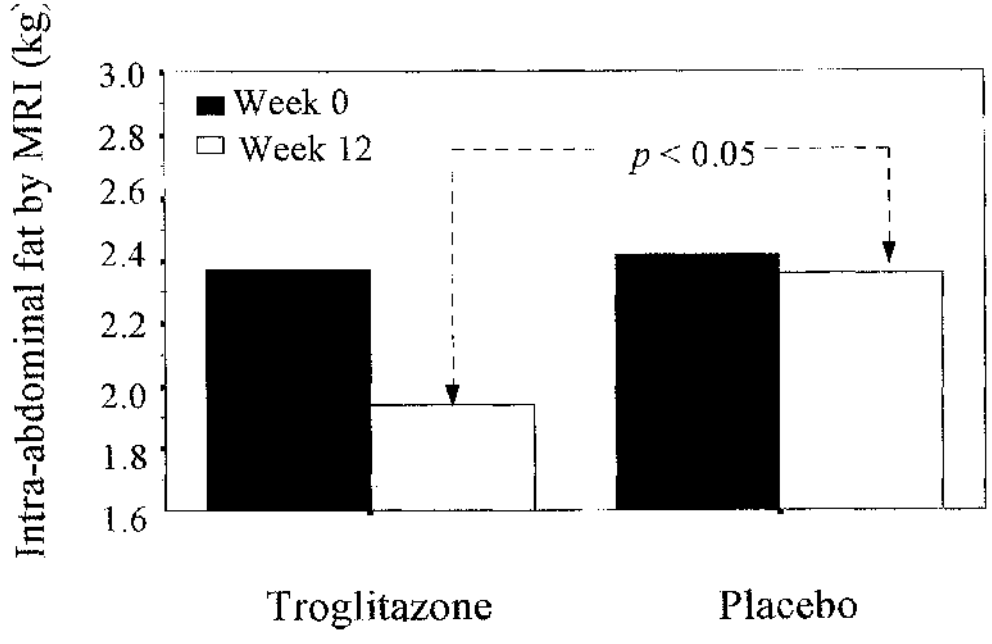


Figure 4.5.4 The effect of troglitazone on intra-abdominal fat over 12 weeks

CHAPER FIVE

GENERAL DISCUSSION AND CONCLUSIONS OF THE PRESENT THESIS

GENERAL DISUSSION

The results of the study indicate that troglitazone had no significant effect on body weight or total body fat. However troglitazone did significantly reduce intra-abdominal fat. This could be because troglitazone induced increased insulin sensitivity in tissues other than intra-abdominal fat, as total fat was not significantly increased. This study in the present thesis is unique in that it used the gold standard method of underwater weighing to determine total body fat, and a novel technique to measure abdominal fat compartments from MRI was developed. In the therapeutic situation the availability of MRI would be limited as most MRI machines are heavily used for diagnostic purposes. It became clear from the MRI analysis that a single scan at the area of the intervertebral disc between L2 and L3 offers a cheaper, faster and safer method with a high prediction of total abdominal fat volumes. This unique single scan could prove very useful as a research tool in future studies. This fast, safe and non-invasive technique would enable large numbers of subjects to be studied, providing a high prediction of their total abdominal fat mass.

The nocturnal rise in leptin has been well documented (Sinha et al 1996). During the present study leptin analyses was performed in subjects following an overnight fast. This consistent approach has minimised the variability associated with leptin measurements made throughout the day. The leptin analysis showed that leptin correlated to body fat by underwater weighing and body mass index.

It is interesting that leptin only correlated weakly with intra-abdominal fat, which suggests that subcutaneous adipose tissue, which is the main site of adipose tissue, rather than intra-abdominal fat is associated with leptin in overweight NIDDM subjects.

This finding is supported Montague et al (1997) who have shown that mRNA appears to be expressed predominately by subcutaneous adipocytes, particularly in women.

These findings have important health implications as it seems possible to conclude from our data that "leptin resistance" in NIDDM may be most marked in those with an expanded subcutaneous fat mass i.e. not those with a more central fat distribution – which is a characteristic of NIDDM.

If leptin resistance is important in the aetiology of obesity as it has been suggested, then this conclusion would indicate no special role for leptin deficiency in patients who have or who develop NIDDM. It is also possible that treatment with exogenous leptin, if it is successful in overcoming insulin resistance and inducing weight loss, might therefore be expected to have a major effect on subcutaneous fat.

OVERALL CONCLUSIONS

Leptin

1. There is a sex specific relationship between waist and serum leptin

2. Serum leptin correlates weakly with intra-abdominal fat and more strongly with other indices of body fatness, including underwater weighing and anthropometric measurements, particularly, BMI and waist circumference.

3. The major depot of body fat, subcutaneous adipose tissue, rather than intra-abdominal fat is associated with leptin in overweight NIDDM subjects.

Troglitazone

Troglitazone has no significant effect on body weight.

1. Total body fat was not significantly changed when measured using the gold standard of underwater weighing.

2. Troglitazone significantly reduced intra-abdominal fat mass measured by novel MRI technique.

3. It can be suggested that troglitazone increases insulin sensitivity in tissues other than intra-abdominal fat, as total body fat does not increase significantly.

Methodology

1. A single MRI scan of the intra-abdominal fat area at the intervertebral disc between L2 and L3 vertebrae offers a cheaper, faster and safer method, with high prediction of total intra-abdominal fat volumes measured by multiple cuts.

2. Intra abdominal fat can be measured with high precision and coefficient of variation 0.89% using a novel multiple cut MRI technique.

FUTURE DIRECTIONS

Possible further steps following this research:

1. Repeat study with glucose intolerant subjects to investigate the effects of thiazolidinedione drug on body composition and metabolic improvements at an earlier stage in the disease

2. Further develop the use of MRI to investigate the long-term effects of thiazolidinedione drug on adipose tissue depots and skeletal muscle mass.

REFERENCES

Antonucci T, Whitcomb R, McLain R, Lockwood D. Impaired glucose tolerance is normalised by treatment with the thiazolidinedione troglitazone. *Diabetes Care* 1997;**25**:188-93

Ashwell M, Cole TJ, Dixon AK. Obesity: new insight into the anthropometric classification of fat distribution shown by computed tomography. *Br Med J* 1985;**250**:1692-94

Barker DJP. *Mothers, babies and disease in later life*. London: BMJ Books, 1994

Bland M. *An Introduction to medical statistics.* Oxford: Oxford University Press, 1993

Brodie DA. Techniques of measuring body composition, part 1. *Sports Medicine* 1980;**5**:11-40

Carey VJ, Walters EE, Colditz GA, Soloman CG, Willett WC, Rosner BA, *et al.* Body fat distribution and risk of non insulin dependent diabetes mellitus in women. The nurses' health study. *Am J Epidemiol* 1996;**145**:614-619

Caro JF, Kolacynski JW, Nyce MR, Ohannesian JP, Opentanova I, Goldman WH, Lynn RB, Zhang P, Singh MK, Considine RV. Decreased cerebrospinal-fluid/serum leptin ratio in obesity: a possible mechanism for leptin resistance. *Lancet* 1996;**348**:159-161

Chan JM, Rimm EB, Colditz GA, Stampfer MJ, Willett WC. Obesity, fat distribution, and weight gain as risk factors for clinical diabetes in men. *Diabetes Care* 1994;**17**:961-69

Considine RV, Sinha MK, Heiman ML, Kriauciunas A *et al.* Serum immunoreactive-leptin concentrations in normal-weight and obese humans. *N Engl J Med* 1996;**334**:292-295

Deurenberg P, Weststrate JA, Seidell JC. Body mass index as a measure of body fatness age- and sex- specific prediction formulas. *Br J Nutr* 1991; **65**:105-14

Durnin JVGA, Womersley J. Body Fat assessed from total body density and its estimation from skinfold thickness measurements on 481 men and women aged from 16 to 72 years. *British Journal of Nutrition* 1974;**32**:77-97

Fujiwara T, Yoshioka S, Yoshioka T, Ushiama I, Horikoshi H. Characterisation of a new oral antidiabetic agent CS-045: studies in KK and ob/ob mice and Zuckers fatty rats. *Diabetes*1988;**37**:1549-58

Garrow JS. Indices of Adiposity. *Nutr Abstr Rev* 1983;**53**:697-708

Ghazzi MN, Perez JE, Antonucci TK, Dirscoll JH, Huang SM, Faja BW, Whitcomb RW. Cardiac and Glycaemic benefits of troglitazone treatment in NIDDM. *Diabetes* 1997;**46**:433-439

Hales CN. and Barker DJ. Type 2 (non-insulin-dependent) diabetes mellitus: the thrifty phenotype hypothesis. *Diabetologia* 1992;**35**:595-601

Han TS, McNeill G, Seidell JC, Lean MEJ. Predicting intra-abdominal fatness from anthropometric measures:The influence of stature. *International Journal of Obesity* 1997a;**21**:587-594

Han TS, Hooper JP, Morrison CE, Lean MEJ. Skeletal proportions and metabolic disorders in adults. *Eur J Clin Nutr* 1997b;**51**:804-09

Han TS, Kelly IE, Walsh K, Greene RME, Lean MEJ. Relationships between volumes and areas from single transverse scans of intra-abdominal fat by magnetic resonance imaging. *International Journal of Obesity* 1997c;**21**:1161-66

Han TS, Feskens EJM, Lean MEJ, Siedell JC. Associations of stature, body mass and fat distribution with non-insulin dependent diabetes mellitus. *Diabetic Medicine* 1998 (in press)

Jackson AS and Pollock ML. Generalised equations for predicting body density of women. *Med Sci Sports Exer* 1980;**12**:175-82

Larsson B, Svärdsudd K, Welin L, Wilhelmsen L, Björntorp P, Tibblin G. Abdominal adipose tissue distribution, obesity, and risk of cardiovascular disease and death: 13 year follow-up of participants in the study of men born in 1913. *Br Med J* 1984;**228**:1401-04.

Lean MEJ, Han TS, Deurenberg . Predicting body composition by densitometry from simple anthropometric measurements. *Am J Clin Nutr* 1996;**63**:4-14

Lean MEJ, Powrie JK, Anderson AS, Garthwaite PH. Obesity, weight loss and prognosis in type 2 diabetics. *Diabetic Medicine* 1990;7:228-33

Lew EA,Garfinkle L. Variations in mortality by weight amoung 750 000 men and women. *J Chron Dis* 1979;**32**:563-76

Lister J, Tanner JM. The physique of diabetes. *Lancet* 1955;**ii**:1002-1004

McCance RA. *The effect of calorie deficiencies and protein deficiencies on final weight and stature*. In: Calorie deficiencies and protein deficiencies. McCance RA, Widdowson EM (eds). London: Churchill, 1968:319-328

McNeill G, Fowler PA, Maughan RJ *et al*. Body fat in lean and overweight women estimated b six methods. *Br J Nutr* 1991;**65**:95-103

Oates JA, Wood AJJ, Gerich JE: Medical intelligence: drug therapy: oral hypoglycemic agents. *N Engl J Med* 1989;**321**:1231-45

Ohlson LO, Larsson B, Svardsudd K, Welin L, Eriksson H, Wilhelmsen L et al. The influence of body fat distribution on the incidence of diabetes mellitus - 13.5 years of follow-up of the participants in the study of men born in 1913. *Diabetes* 1985;**34**:1055-58

Pertrie JR, DonnellyR. New pharmacological approaches to insulin and lipid metabolism. *Drugs* 1994;**47**:701-10

Reilly JJ, Murray LA, Wilson J, Durnin JVGA. Measuring the body composition of elderly subjects: a comparison of methods. *Br J Nutr* 1994;**2**:33-44

Schoeller DA, Van Santen E, Paterson DW, Dietz W, Jaspan J, Klein PD. Total body water measurement in humans with ^{18}O and ^{2}H labelled water. *Am J Clin Nutr* 1980;**33**:2686-93

Scottish Intercollegiate Guidelines Network. *Obesity in Scotland. Integrating prevention with weight management*. Edinburgh: Scottish Intercollegiate Guidelines Network, 1996.

Scottish Office. '*Scotland's health. A challenge to us all*'. Edinburgh: HMSO, 1992.

Seidell JC, Han TS, Feskens EJM, Lean MEJ. Narrow hips and broad waist circumferences independently contribute to increased risk of NIDDM. *J Intern Med* 1997;**242**:401-406

Shepherd RJ. *Body composition in biological anthropometry*. Cambridge: *Cambridge University Press*, 1991

Siri WE. *Body composition from fluid spaces and density analysis of methods*. In: Technique for measuring body composition. Brozek J, Henschel A, eds. Washington DC: Natural Academy of Sciences, 1961:223-244

Suter SL, Nolan JJ, Wallace P, Gumbiner B, Olefsky JM: Metabolic effects of new oral hypoglycaemic agent CS-045 in NIDDM subjects. *Diabetes Care* 1992;**15**:193-203

Vague J. Le diabete de la femme androide. *Presse Med* 1949;**57**:835

Vander AJ, Sherman JH, Luciano DS. *Human Physiology: The Mechanisms of body function*. New York: McGraw-Hill, 1990

Wei M, Gaskill SP, Haffner SM, Stern MP. Waist circumference as the best predictor of non insulin dependent diabetes mellitus (NIDDM) compared to body mass index, waist/hip ratio and other anthropometric measurements in Mexican Americans – a 7 year prospective study. *Obesity Research* 1997;**5**:16-23

Wilmore JH, Vodak PA, Parr RB, Grendda RN, Biling JE Further simplification of a method for determination of residual lung volume. *Med Sci Sports Exerc* 1980;**12**:216-18

World Health Organisation. Expert Committee on Physical Status: *The use and interpretation of anthropometry: report of a WHO expert committee*. Geneva: World Health Organisation Tech Rep Ser, 1995

Womersley J. 1974 Obesity its assessment, significance and control. PhD Thesis. University of Glasgow, 1974

Zimmet P, Hodge A, Nicolson M, Staten M, De Courten M, Moore J, Morawiecki A, Lubina J, Collier G, Alberti G, Dowse G. Serum leptin concentration, obesity, and insulin resistance in Western Samoans: cross sectional study. *Br Med J* 1996;**313**:965-69

Montague CT, Prins JB, Sanders L, Digby JE,O'Rahilly S. Depot- and sex specific differences in Human Leptin mRNA expression. *Diabetes* 1997;**46**:342-347

Sinha MK, Ohannesian JP,Heiman ML, Kriauciunas A, Stephens TW, Magosin S, Marco C, Caro JF. Nocturnal rise of leptin in lean, obese, and non-insulin dependdent diabetes mellitus subjects. *J Clin Invest* 1996; **97** (5): 1344-1347