PO Box 2345, Beijing 100023, China
www.wjgnet.com
wjg@wjgnet.com

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CLINICAL RESEARCH

Effect of nutritional counselling on hepatic, muscle and adipose tissue fat content and distribution in non-alcoholic fatty liver disease

E Louise Thomas, Audrey E Brynes, Gavin Hamilton, Nayna Patel, Adam Spong, Robert D Goldin, Gary Frost, Jimmy D Bell, Simon D Taylor-Robinson

E Louise Thomas, Audrey E Brynes, Gavin Hamilton, Nayna Patel, Adam Spong, Robert D Goldin, Gary Frost, Jimmy D Bell, Simon D Taylor-Robinson, The Robert Steiner MR Unit, MRC Clinical Sciences Centre (ELT, GH, NP, AS, JDB, STR), Department of Nutrition and Dietetics (AEB, GF) and Division of Medicine (Medicine A), Faculty of Medicine (GH, NP, AS, STR), Imperial College London, Hammersmith Hospital, Du Cane Rd, London W12 0HS, United Kingdom and Histopathology Department (RDG), Faculty of Medicine, Imperial College London, St Mary's Hospital, Praed Street, London W2 1NY, United Kingdom
Supported by the British Medical Research Council, United Kingdom, No. MRC CEG G99000178
Correspondence to: Dr. E Louise Thomas, Robert Steiner MR Unit, MRC Clinical Sciences Centre, Imperial College London, Hammersmith Hospital, Du Cane Rd, London W12 0HS, United Kingdom. louise.thomas@csc.mrc.ac.uk
Telephone: +44-20-83833772 Fax: +44-20-83833038
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Abstract

AIM: To assess the effectiveness of the current UK clinical practice in reducing hepatic fat (IHCL).

METHODS: Whole body MRI and ^{1}H MRS were obtained, before and after 6 mo nutritional counselling, from liver, soleus and tibialis muscles in 10 subjects with non-alcoholic fatty liver disease (NAFLD).

RESULTS: A 500 Kcal-restricted diet resulted in an average weight loss of 4% (-3.4 kg,) accompanied by significant reductions in most adipose tissue (AT) depots, including subcutaneous (-9.9%), abdominal subcutaneous (-10.2%) and intra-abdominal-AT (-11.4%). Intramyocellular lipids (IMCL) were significantly reduced in the tibialis muscle (-28.2%). Decreases in both IHCL (-39.9%) and soleus IMCL (-12.2%) content were also observed, although these were not significant. Several individuals showed dramatic decreases in IHCL, while others paradoxically showed increases in IHCL content. Changes in body composition were accompanied by improvements in certain liver function tests: serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT). Significant correlations were found between decreases in IHCL and reductions in both intra-abdominal and abdominal subcutaneous AT. Improvements in liver function tests were associated with reductions in intra-abdominal AT, but not with changes in IHCL.

CONCLUSION: This study shows that even a very modest reduction in body weight achieved through lifestyle modification can result in changes in body fat depots and improvements in LFTs.

Key words: Intra-abdominal adipose tissue; Intrahepatic fat; Intramyocellular lipids; Weight loss; Magnetic resonance imaging

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INTRODUCTION

The incidence of non-alcoholic fatty liver disease (NAFLD) has increased rapidly over the last few years and is now one of the commonest causes of abnormal liver function test (LFT) results in patients presenting to hepatology clinics in both Europe and the USA[1]. Hepatic steatosis may be prevalent in more than 30% of the population[2,3]. NAFLD encompasses a wide spectrum of liver diseases, from mild fatty infiltration, through to steatohepatitis, cirrhosis and fibrosis[4]. One of the major factors thought to be responsible for fat accumulation in the liver is obesity[3], with NAFLD being a key feature of insulin resistance and the metabolic syndrome[5]. Treatment is currently limited, although lifestyle modification including dietary change and increased exercise to promote weight loss are thought beneficial[6].

The nature and rapidity of weight loss has been shown to be important[7]. Previous studies have used gastric banding[8,9], or very low calorie diets[7,10] to promote significant, or rapid weight loss, resulting in reduced

intrahepatocellular lipid (IHCL) content. However, rapid reduction in weight may worsen underlying inflammation and fibrosis in patients with non-alcoholic steatohepatitis (NASH)[7,9]. Several studies have shown that liver fat content in NAFLD can be reduced through more moderate weight loss, achieved by dietary restriction, either alone[11,12], in combination with exercise[13-17], or using pharmacological intervention[18-26]. To date, no study has looked at the effects of first line treatment in clinical management algorithms offered to most people attending outpatient clinics with NAFLD. The aim of this study was therefore to assess the impact of current United Kingdom nutritional clinical practice to reduce body adiposity and its impact on liver fat content and measures of hepatic function.

MATERIALS AND METHODS

Written informed consent was obtained from all volunteers. Permission for this study was obtained from the Ethics Committee of Hammersmith Hospital, Imperial College London, (Rec. 93/4047; 93/3995). The study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki.

Subjects

Ten patients, referred to the Hepatology Outpatient Clinics at the Hammersmith and St Mary's Hospitals in London, were recruited (ST-R). All had unexplained abnormal liver function tests (LFTs) as a reason for referral with raised serum aspartate transaminase (AST) and/or gamma-glutamyl transpeptidase levels (γGT). All were clinically obese with a mean body mass index of 31.6 ± 4.6 kg/m^2. Three subjects had type Ⅱ diabetes diagnosed within the last 3 years, two were treated with diet only, and one subject took metformin. Five had a history of dyslipidaemia. No patient drank alcohol in excess of 20 g/d and none had a history of excess alcohol consumption. No co-existing reason for the LFT abnormalities was found on screening for viral hepatitis or autoimmune liver disease. Serum copper, caeruloplasmin and iron studies were normal in each patient. All had increased echogenicity on hepatic ultrasound examination, compatible with hepatic steatosis. This was confirmed on subsequent liver biopsy in four of the subjects (RDG). The characteristics of the study group at baseline are shown in Table 1.

Dietary Intervention

All patients were referred to the Hammersmith Hospital 'Lifestyle Clinic'. The aim of the treatment was a gradual weight loss of 5%-10% of initial body weight within 6 mo. Subjects attended seven appointments with a registered dietician (AEB) over 6 mo, with fortnightly phone calls between appointments. Reported energy intakes using 3 d diaries were 2464 ± 66 kcals, (of which 46% ± 4% carbohydrate, 35% ± 3% fat, 18% ± 1% protein), in line with typical British diets. All subjects were sedentary at baseline scoring 7.1 ± 0.4 using Baecke activity questionnaires. Subjects were given advice on modifying their diets, which centred on behaviour change around dietary intake in accordance with Hammersmith Hospitals Dietetic Department policy[27]. The aim was to induce a 500 kcal energy deficit in the diet. Activity was encouraged in the form of walking using the "10 000 steps a day" campaign and pedometers were advised to aid compliance.

Table 1 Anthropometry results before and after dietary intervention mean ± SD

	Pre-diet (n = 10)	Post-diet (n = 10)	P
Weight (kg)	96.3 (87.9-105.6)	93.0 (83.9-103.0)	0.006
BMI (kg/m^2)	31.3 (28.3-34.9)	30.2 (27.2-33.9)	0.006
Waist circumference (cm)	114.6 (107.8-121.4)	107.9 (101.6-114.3)	0.001
Systolic BP (mmHg)	133.4 (117.0-149.8)	129.8 (121.3-138.2)	0.62
Diastolic BP (mmHg)	79.4 (76.5-82.3)	77.6 (71.9-83.8)	0.50
Pulse (bpm)	67.3 (60.6-75.2)	66.3 (55.3-79.9)	0.60

Significance taken as $P < 0.05$. BMI: Body mass index; BP: Blood pressure; bpm: Beats per minute.

Biochemistry

Fasting blood samples were obtained for measurement of glucose, insulin, cholesterol, triglycerides and glycosylated haemoglobin (HbA_{1C}). AST, ALT, and γGT were determined as recommended by the European Committee for clinical laboratory standards.

Anthropometry

Body weight was measured to the nearest 100 g. Height was measured using a stadiometer to the nearest centimetre. Waist circumference was measured mid-way between the lowest rib and the iliac crest[28].

Total body adipose tissue content

Rapid T_1-weighted MR images (TR 36 ms, TE 14 ms) were acquired as previously described[29]. Subjects lay in a prone position with arms straight above the head, and were scanned from fingertips to toes, acquiring 10 mm-thick transverse images with 30 mm gaps between slices in the arms and legs, and 10 mm gaps between slices in the trunk. Images were analysed using SliceOmatic (Tomovision, Montreal, Quebec, Canada). Total and regional adipose tissue (AT) volumes were measured[29].

MRS of the liver

^{1}H MR spectra were acquired on a 1.5T Eclipse multi-nuclear system (Phillips Medical Systems, Cleveland, Ohio) using a flexible body coil. Spectra were obtained from the right lobe of the liver using a PRESS sequence (TR 1500 ms, TE 135 ms) without water saturation and with 128 signal averages. Intrahepatocellular lipids (IHCL) were measured relative to liver water content as previously described[30].

MRS of muscle

Intramyocellular lipids (IMCL) were measured in the soleus (S-IMCL) and tibialis (T-IMCL) muscles by ^{1}H MRS (TR 1500 ms, TE 135 ms, 256 averages). IMCL

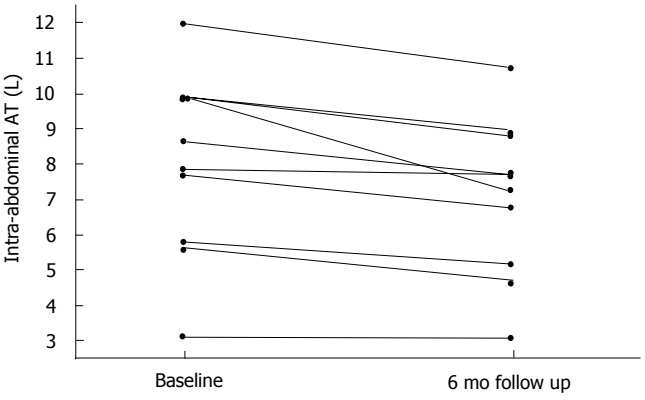

Figure 1 Changes in intra-abdominal adipose tissue content following six months dietary intervention.

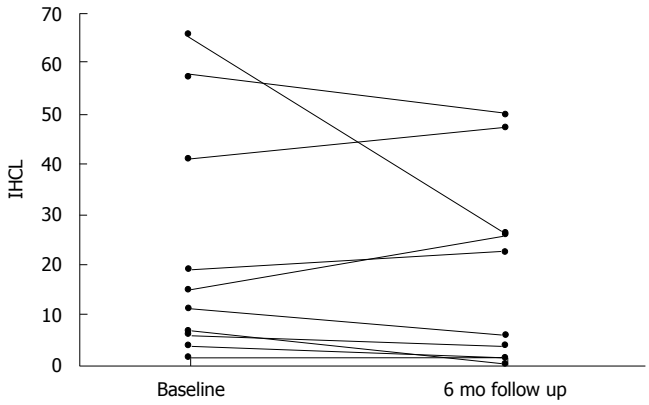

Figure 2 Changes in IHCL content following six months dietary intervention.

Table 2 Whole body MRI before and after dietary manipulation (n = 10, geometric mean)

Adipose tissue (L)	Pre-diet	Post-diet	Change (%)	P (t-test)
Total	38.8 (32.1 to 46.9)	35.1 (28.5 to 43.4)	-9.5 (-14.4 to -4.4)	0.003
Subcutaneous	25.3 (20.8 to 30.8)	22.8 (18.1 to 28.7)	-9.9 (-15.5 to -3.9)	0.006
Internal	13.3 (10.6 to 16.7)	12.1 (9.6 to 15.2)	-9.3 (-14.1 to -4.2)	0.003
Sc abdominal	7.6 (5.8 to 10.1)	6.9 (5.1 to 9.3)	-10.2 (-18.6 to -0.9)	0.04
Sc peripheral	17.6 (14.9 to 20.8)	15.9 (12.9 to 19.5)	-9.7 (-14.9 to -4.3)	0.003
Intra-abdominal	7.6 (5.7 to 10.0)	6.7 (5.1 to 8.7)	-11.4 (-16.5 to -6.0)	0.001
Non-abdominal internal	5.7 (4.7 to 6.9)	5.4 (4.4 to 6.6)	-5.4 (-14.4 to 4.5)	0.24

t-tests were performed on log-transformed data, significance taken as $P < 0.05$. AT = adipose tissue; Sc = subcutaneous.

were measured relative to total muscle creatine signal, as previously described[31].

Statistical analysis

Statistical advice was provided by Dr Caroline Doré, MRC Clinical Trials Unit, London UK. The distribution of the data was tested for using the Shapiro-Wilk normality test. Normally distributed data are expressed as mean and 95% confidence interval (CI). Log_{10} transformation was used to correct variables that were not normally distributed. Results for log-transformed variables are presented as geometric mean and 95% CI. Comparison before and after lifestyle intervention were tested using a paired t-test. Associations between variables were assessed using Pearson's correlation coefficients. The level of significance was set at 5%. Stepwise multiple regression analysis was performed to predict changes in AST, ALT and IHCL. Given the small number of patients, only forward variable selection was used. Data were analysed using Unistat version 5.5 (Unistat Ltd, London, UK) and Stata (StataCorp 2001 Stata Statistical Software, Release 7.0; Stata Corporation, College Station, Texas, USA).

RESULTS

All patients who participated in this study lost weight, with a mean loss of -3.5 kg (range -0.6 to -10.0 kg, $P = 0.006$). Total AT content was also reduced, with a mean reduction of -3.5 litres (range -0.75 to -10.43 litres, $P = 0.003$). There was also a significant reduction in waist circumference -6.6 cm (range -2.5 to -13.0 cm, $P = 0.001$) (Table 1). There were significant decreases in most AT depots following this life style intervention (Table 2). The largest decrease was found in intra-abdominal AT (-11.4%) (Figure 1), with slightly smaller quantities of subcutaneous AT lost from abdominal (-10.2%) and peripheral areas (-9.7%). There was a strong relationship between the amount of abdominal AT lost subcutaneously and internally ($r = 0.81$, $P < 0.01$).

A reduction in AST and ALT was observed, although the latter did not reach significance (Table 3). There was no significant change in γGT. HbA_{1c} was also significantly reduced following the lifestyle intervention, suggesting an improvement in glycemic control. This may also be inferred by an improvement in insulin sensitivity observed in some individuals (HOMA %S), although as a group the increase did not reach significance.

The ^1H MRS findings are shown in Table 4. Although, seven of the 10 subjects showed marked reductions in IHCL (-57.2%), three subjects showed increases (33.2%), despite no significant difference between the groups in terms of weight loss (Figure 2). Thus, as a group the decrease in IHCL did not reach significance (-39.9%, $P = 0.12$). Interestingly, there was a significant correlation between changes in IHCL, weight loss ($r = 0.74$, $P < 0.01$) and intra-abdominal AT changes ($r = 0.83$, $P < 0.01$). This relationship was also significant for changes in IHCL and abdominal subcutaneous depot ($r = 0.76$, $P < 0.01$). There was a significant decrease in T-IMCL levels, but not for S-IMCL. Changes in T-IMCL were related to decreased

Table 3 Serum biochemistry results before and after dietary manipulation (n = 10)

Biochemistry	Pre-diet	Post-diet	*P*
Glucose (mmol/L)	5.7 (5.2-6.2)	5.5 (4.8-6.1)	0.55
Insulin (mIU/L)	13.4 (8.4-18.4)	13.9 (8.2-19.5)	0.82
HOMA %S	424.04 (225.2-737.5)	540.1 (38.0-1400.8)	0.33
HOMA %B	28.5 (17.5-43.4)	25.2 (16.2-37.3)	0.50
HbA_{1c}	6.0 (5.7-6.3)	5.6 (5.2-6.1)	0.03
Cholesterol (mmol/L)	5.3 (4.6-6.1)	5.0 (4.6-5.5)	0.24
Total Chol:HDLc	4.4 (3.7-5.4)	4.0 (3.5-4.7)	0.22
TG (mmol/L)	1.9 (1.3-2.6)	1.9 (1.4-2.4)	0.88
AST (U/L)	32.2 (26.5-37.9)	28.4 (24.0-32.8)	0.02
ALT (U/L)	42.4 (29.1-55.7)	36.6 (22.5-50.7)	0.08
γGT (U/L)	39.0 (18.0-60.0)	40.2 (21.6-58.8)	0.34

HOMA: Homeostatic model assessment, %S estimates insulin sensitivity; %B estimates β-cell function; Chol: Cholesterol; HDL: High density lipoprotein; TG: Triglyceride; AST: Serum aspartate aminotransferase; ALT: Alanine aminotransferase; γGT: Gamma-glutamyl transpeptidase.

Table 4 Hepatic and Muscle fat before and after dietary modification (n = 10, geometric mean, t-test)

	Pre-diet	Post-diet	Change (%)	*P*
[1]IHCL	13.3 (5.8-30.9)	8.0 (2.4-26.4)	-39.9 (-69.5-18.4)	0.12
[1]IMCL soleus	19.6 (10.3-37.2)	17.2 (10.8-27.3)	-12.2 (-53.0-64.2)	0.65
[1]IMCL tibialis	11.6 (7.6-17.7)	8.3 (5.4-12.8)	-28.2 (-48.5-0.03)	0.05

[1]Measured in arbitrary units. IHCL: Intrahepatocellular lipid content; IMCL: Intramyocellular lipid content.

Table 5 Relationship between changes in liver biochemistry and adiposity

Change	Hepatic fat		AST		ALT	
	r	*P*	*r*	*P*	*r*	*P*
Body weight	0.74	0.01	0.16	0.7	0.32	0.4
Total AT	0.70	0.02	0.42	0.22	0.49	0.15
Abdominal sc AT	0.76	0.01	0.57	0.09	0.54	0.11
Peripheral sc AT	0.45	0.2	0.15	0.7	0.17	0.6
Intra-abdominal AT	0.83	0.003	0.71	0.02	0.83	0.003
Non-abdominal internal AT	0.28	0.4	0.23	0.5	0.35	0.3
hepatic fat	-	-	0.51	0.13	0.62	0.05
Soleus IMCL	0.50	0.14	0.27	0.4	0.21	0.6
Tibialis IMCL	0.53	0.12	0.42	0.22	0.56	0.09

Pearson product movement correlation coefficients (r) for the relationship between AST, ALT and hepatic fat and measures of total and regional adiposity and IMCL, performed using log transformed data. AT: Adipose tissue; IMCL: intramyocellular lipid content; Sc: Subcutaneous.

intra-abdominal AT (r = 0.73, P < 0.02), whereas changes in S-IMCL were related to decreased subcutaneous AT in both abdominal (r = 0.81, P < 0.01) and peripheral regions (r = 0.69, P < 0.05).

Changes in LFTs showed significant association with alterations in body composition, (Table 5). A significant correlation was found between reduction in intra-abdominal AT and changes in both ALT (r = 0.83, P < 0.01) and AST (r = 0.71, P < 0.05). A weaker correlation was found between changes in ALT and IHCL (r = 0.62, P = 0.05). Stepwise multiple regression analyses were performed to predict changes in AST, ALT and IHCL. Variables considered for inclusion in this model were changes in abdominal and peripheral subcutaneous AT, intra-abdominal and non-abdominal internal AT, weight and IMCL in the soleus and tibialis muscles. Only changes in intra-abdominal AT were able to predict changes in AST, ALT and IHCL. Changes in AST and ALT were unable to predict changes in IHCL.

DISCUSSION

In this study we have shown that modest weight loss, obtained through routinely available hospital dietetic clinical care, can have worthwhile effects on whole body adiposity, hepatic and muscle fat content and LFTs in NAFLD patients. These findings indicate that current UK clinical practice is effective in promoting a lifestyle change that has a positive effect on reducing adiposity. Huang *et al* used a similar nutritional method to that employed in the current study to promote weight loss and looked at changes in liver histology on biopsy. They found histological improvement in patients with a weight loss of 7%, but not in those who lost only 2%[12]. Similar findings were reported by Tikkainen *et al* in patients with an 8% weight loss[11]. Tamura *et al* using diet with or without exercise in a strictly controlled study, reported decreases in body fat of 9.6% and 8.2%, with a 25%-30% reduction in IHCL. IMCL also decreased, but only with exercise[13]. Hickman, reported an improvement in steatosis and liver histology following 12 wk of diet and exercise, with a weight loss of 6.6%[14,15]. In our study, subjects lost on average 4% of their body weight, with variable effects on IHCL and IMCL levels. It is possible that a greater weight loss than this is necessary to have a significant effect on IHCL, or it may be that the nature/stage of the disease is important for overall reduction in steatosis. For example, in the present study, while several individuals had dramatic decreases in IHCL, others paradoxically showed increases in IHCL. The reasons for this are not immediately apparent. Recent studies have suggested that IHCL content may be altered by a single meal[32], however we scanned our subjects following overnight fast, to minimise any potential effects. There were no obvious phenotypical or clinical differences between those subjects who responded to the weight loss with reductions in IHCL and the non-responders, who lost similar levels of body weight, but increased their IHCL content. However, where biopsy data were available (4/10 subjects), responders showed mild steatohepatitis, whereas non-responders additionally showed signs of more severe inflammation and fibrosis. Thus, IHCL reduction, as a result of dietary intervention, may be hampered in individuals whose fatty infiltration has already begun to progress to fibrosis, compared to those who solely have steatosis. Larger scale studies are clearly required to elucidate this further.

Very-low calorie formula diets or gastric banding has been used to promote more significant or rapid weight

loss to reduce hepatic fat content[7-10]. Andersen *et al* placed morbidly obese subjects on a very low calorie liquid diet (388 kcal/d), resulting in a weight loss of 34 kg, and significant reduction in hepatic fat[7]. Similarly, type-2 diabetic patients on very low fat (3%) liquid diets for 3-12 wk, lost 8 kg of body weight, with an 81% decrease in hepatic fat. Others have shown significant improvements in liver histology following large weight losses of over 30 kg[8,9]. However, morbidly obese patients undergoing rapid weight reduction, may develop portal inflammation, fibrosis and hepatitis in addition to the decrease in hepatic fat content[7,9]. Drug interventions have also been used to reduce hepatic fat and improve liver histology[18-26]. Orlistat and metformin in combination with diet, can reduce in both weight and hepatic fat[18,19], while metformin alone reduces body fat content (specifically subcutaneous AT), but has no effect on hepatic fat[20]. Reductions in hepatic fat have been observed following treatment with pioglitazone[21,22], rosiglitazone[20,23-25] and pantethine[26], despite the increases in body weight associated with glitazone therapy.

Only a few studies have compared changes in hepatic fat with changes in regional AT depots. Tiikkainen *et al* using diet restriction to reduce hepatic fat found that AT was lost from both subcutaneous and IAAT[11]. However, unlike the present study they found no correlation between changes in liver fat and the changes in subcutaneous and IAAT. Carey *et al*, using rosiglitazone found an increase in subcutaneous AT, with no change in IAAT[24]. Osono *et al* found decreased liver fat with pantethine, accompanied by a decrease in IAAT and an increase in subcutaneous AT[26]. It is clear from the differences between these studies that diet and drug interventions may result in quite different mechanisms for the clearance of fat from the liver and reduction in AT volume. We found significant correlations between improvements in hepatic fat and reductions in both subcutaneous abdominal and IAAT. There were also strong correlations between decreased IAAT and decreased AST and ALT. However, regression analyses suggested that only reduction in IAAT could predict improvements in liver function and hepatic fat. Weight loss from other depots may well have other benefits, but not directly on hepatic function. A correlation was found between reduction in subcutaneous abdominal AT and S-IMCL, which may have implications for improving insulin sensitivity, since insulin resistant individuals have elevated IMCL[33-35].

Several studies have looked at the effect of dietary restriction on AT content and distribution. A review of intervention strategies to reduce AT, suggests that most dietary interventions report a preferential loss of IAAT[36]. Most papers included used a greater degree of calorie restriction than the present study and generally used females[36]. In a study comparable to our own, Ross *et al* placed male subjects on a 700 kcal/d calorie restriction for 12 wk[37]. They reported reductions of 0.7 kg and 0.8 kg from the abdominal subcutaneous and IAAT depots respectively[37]. In our study, we found that similar proportions of AT were lost from both IAAT and subcutaneous abdominal depots, suggesting modest weight loss, achieved gradually, results in a non-selective loss of AT from both IAAT and subcutaneous abdominal depots. A significant reduction in T-IMCL, but not in S-IMCL, was observed. Previous studies have shown decreases in S-IMCL in response to large weight loss (24%)[38], but not to smaller reductions (8%-9%)[10,11,38]. The tibialis may be more sensitive to weight change than the soleus. Indeed, T-IMCL may be more sensitive to catabolic lipid metabolism than the soleus[39], which may explain its more significant response to intervention in this and previous studies[40-43].

Both serum AST and ALT were reduced following weight loss, though only AST reached significance. Most studies looking at the relationship between liver enzymes and adiposity have focused on ALT, since elevated ALT is associated with obesity and insulin resistance[44]. The lack of significance in the reduction in ALT observed in the present study could be explained by the suggested insensitivity of ALT to detect low levels of hepatic fat[45]. Alternatively, although patients were referred to the study with elevated LFTs, the levels had improved somewhat by the time of inclusion in the study. Therefore despite ultrasound and subsequently MRS showing elevated liver fat, baseline LFT values in several subjects, although elevated, were not significantly outside the normal range. A significant decrease in LFTs within a 'normal' range may be difficult to achieve, this also indicates that normal LFTs are not necessarily a good indicator of liver fat content. Huang *et al* found no change in either AST or ALT following a similar study of nutritional counselling, despite improvements in liver histology[12]. Interestingly, when their subjects were subdivided into responders and non-responders, the former showed a significant reduction in both ALT and AST[12]. We observed similar effects, subjects showing reduced IHCL also showed a significant reduction in ALT. However, regression analyses suggest that changes in AST and ALT are only associated with modulation of IAAT during weight loss. Clearly, any relationship between hepatic steatosis and LFTs must include potential effects of other fat depots, especially IAAT. Several of the results in this study approached significance or were not significant, despite many suggesting a trend of 'improvement'. This is likely due to the small number of subjects (n = 10) included in this study, therefore type-2 statistical errors must be considered as confounding factors.

In summary, we have shown that modest weight loss achieved through UK clinically-implemented nutritional counselling regimens can result in worthwhile changes in regional adiposity, improvements in liver function, and reductions in both hepatic and tibialis muscle fat content. This study used clinically available, standard nutritional regimens as such, and our results are representative of clinical practice for NAFLD patients within the UK. Although there were some individual decreases in hepatic fat content, a more significant weight loss may be required to ensure consistent changes.

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