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SHORT COMMUNICATION

Low-carbohydrate diet induced reduction of hepatic lipid content observed with a rapid non-invasive MRI technique

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ABSTRACT. Low carbohydrate diets are currently fashionable for inducing weight loss, but the metabolic effects at organ level are not well understood, especially the effect on liver fat storage. Such studies require serial hepatic fat measurements, for which liver biopsy is impractical. In 10 healthy volunteers we demonstrate the use of rapid (total 2 min acquisition time, 10 min magnet room time), non-invasive, quantitative MRI to serially measure hepatic fat changes induced by following a low carbohydrate diet for 10 days. A significant ($p<0.01$) reduction in hepatic fat after 3 days of dieting was observed in 5 subjects. All subjects demonstrated significant ($p<0.01$) reductions in hepatic fat by day 10. A strong correlation ($\kappa=0.81$) existed between the initial fat content and the percentage fat content reduction in the first 3 days of the diet. All subjects lost weight (average 1.7 kg at day 3 and 3.0 kg at day 10), but this was not correlated with hepatic fat loss after 3 days or 10 days of dieting. The results presented illustrate the potential value of MR hepatic fat quantification in longitudinal studies of hepatic fat content.

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Hepatic steatosis is of particular interest in the western world owing to the increasing prevalence of the insulin resistance or ''metabolic'' syndrome. Hepatic steatosis and non-alcoholic steatohepatitis (NASH) are now considered a part of this condition and there is emerging evidence that, rather than being a consequence of systemic insulin resistance, they may have a causative role [1, 2]. As a result, therapeutic or dietary interventions to reduce hepatic steatosis may be more appropriate for treating insulin-resistance, rather than by treating the different aspects separately. It is estimated that 20–25% of the US population is obese and that among the obese group there is a high prevalence of non-alcoholic fatty liver disease (NAFLD). The risk of serious sequelae such as type II diabetes mellitus or progression to chronic liver disease means that this is an area requiring urgent research and assessment [3].

These types of studies require accurate serial quantification of hepatic steatosis, but currently liver biopsy (and related biochemical analysis) is the established method. As an invasive procedure, even with a minimal related morbidity and mortality, this is difficult to justify in healthy volunteers and impractical for serial measurements. Additionally the method samples only small volumes of tissue, which may lead to problems with regional fat variation and give unrepresentative results.

An alternative approach is to use a validated rapid imaging method that allows absolute hepatic fat (mainly intracellular triglyceride) estimation through fat specific chemical shift imaging corrected for T_2* variation [4, 5]. Previous studies have shown a good correlation between steatosis assessed by liver biopsy and by MRI methods [6], and excellent correlation exists between hepatic fat measured by liver histology, MRI and CT methods [7, 8].

The effect of low carbohydrate diets on weight loss, insulin resistance and serum triglyceride markers has recently been the focus of intensive research efforts [9, 10], but to the best of the authors' knowledge, the effect of such diets on hepatic fat has never been directly measured. The aim of this work was to monitor the hepatic fat response of healthy volunteers during the induction phase of a low-carbohydrate diet using the MRI method described above.

Materials and methods

The study was approved by the local ethics committee and informed consent was obtained from the volunteers after the procedure was fully explained. Participants received no monetary incentive. Given the lack of consensus on the normal hepatic lipid percentage range measured by MRI we used an arbitrary limit of a pre-diet hepatic fat estimate of 7.0% or above as the entry threshold for the study. This threshold was chosen as it was the median fat percentage observed in our previous work on healthy volunteers ($n=25$); a similar result has also been shown with this technique by Fishbein [4]. 10

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healthy volunteers (3 male, 7 female, age 32–56 years) were recruited from the community: the volunteers were not taking regular medication and had no history of hepatic or biliary disease. Five further volunteers were excluded from the study after an initial MRI examination showed their hepatic fat estimate to be below 7.0%.

The participants were asked to follow a low carbohydrate diet at home for 10 days, restricting carbohydrate intake to less than 20 g carbohydrate per day in the form of green salad or vegetables, but with no other restriction on total energy intake or food choice. The volunteers kept a diet diary and abstained from alcohol.

Hepatic fat measurement by MRI was performed at 4 time points; immediately pre-diet, at 3 days and 10 days on the diet and 7 days after reverting to their normal diet. The volunteers were examined at the same time of day on each occasion. The initial body mass index (BMI) was recorded and the weight of the volunteers was assessed on each visit to the MRI unit. Weight was measured with the subjects changed for the MRI examination and without shoes. Adherence to the diet was monitored by the patients maintaining a food record sheet and by urinary ketone assessment to ensure that ketosis was initiated and maintained. Ketones are usually not detectable in healthy volunteers following a balanced diet.

Examinations were performed on a 1.5 T whole body MRI (Excite, GEHT, Milwaukee) with an 8-channel body array. In and out of phase gradient echo scans (matrix 256×128, section (slice) thickness 10 mm, gap 1.5 mm, repetition time (TR)/echo time (TE)=180/2.2 ms (out of phase)/4.4 ms (in phase)) were acquired axially at two different flip angles (20° and 70°) and a T_2* map of the liver was obtained using a location-matched, multisection, multiecho gradient sequence (TR=120 ms, 16 equally spaced echoes, TE1=2.2 ms, TE2=4.4 ms). These required a total of three 20 s breath-holds in addition to an initial 20 s breath-hold study for checking the positioning. The T_2* data was used to correct the in-phase and out-of-phase images intensities for T_2* relaxation. The fat percentage was calculated by:

$$\mathrm{FP} = 100(S_{\mathrm{in}} - S_{\mathrm{out}})/2S_{\mathrm{in}} \qquad (1)$$

where FP is the fat percentage, S_{in} is the intensity from the in-phase image, S_{out} is the intensity from the out-of-phase image (both values corrected for T_2* relaxation). The acquisition of in-phase and out-of-phase images with different flip angles (and hence T_1 weighting) allows us to distinguish whether fat or water is the majority species [4, 5]: this resolves the ambiguity that occurs in Equation (1) for fat percentage greater than 50%.

Four sections centrally placed in the liver were analysed by a single operator and three circular regions of interest (ROI) with fixed area (5 cm^2) were positioned over the liver parenchyma on each section, avoiding large vessels and the gallbladder. The 12 ROIs for each individual were then averaged to give a result for that time point. Significant changes between time points were assessed using a paired *t*-test (SPSS 12.0). Hepatic fat percentage was the primary outcome measure, as assessed from the MR images. Correlation coefficients (Pearson) were calculated between the initial fat measurement and the percentage reduction in fat after 31 days and 10 days of the diet, and also between the percentage weight reduction and the percentage reduction in hepatic fat at days 3 and 10 of the diet.

Results

The diet records indicated that all the volunteers maintained the diet successfully and this was confirmed by evidence of ketosis on urine testing. All volunteers had ''negative'' ketone readings before the diet, and these all increased to at least ''moderate'' by day 3 and for the duration of the diet. The initial BMI of the volunteers (range 23–32, median 28) demonstrated that the volunteers were, at worst, moderately overweight. Table 1 shows the changes in hepatic fat percentage for the 10 individuals, at all the time-points in the trial. Figure 1 shows the relative change (compared with the pre-diet measurement) in the hepatic fat percentage graphically. Figure 2 illustrates the time course of results and error bars for subjects 1 and 2. There was no significant variation of the measured fat percentage with the location of the ROI. Five of the subjects experienced a significant ($p<0.01$) decrease in hepatic fat in the first 3 days of dieting. Two of these subjects displayed a further significant change between day 3 and day 10 of the diet. All 10 subjects experienced a significant

Table 1. Selected BMI, hepatic fat and weight data for the volunteers

Subject	Initial BMI (kg m^{-2})	Initial hepatic fat (%)	3 day hepatic fat (%)	10 day hepatic fat (%)	17 day hepatic fat (%)	Weight loss (kg) on completion of diet (day 10)	Weight change (kg) 1 week after diet (day 17)
1	30	12.1	10.0[a]	9.4[a]	9.9	4	+0.5
2	32	8.5	7.1[a]	7.4[a]	6.7	4	+2.0
3	29	11.8	9.0[a]	6.7[a]	6.4	4.5	−1.5
4	27	7.0	7.0	5.6[a]	8.4[b]	2	−1.5
5	23	9.0	7.9[a]	7.5[a]	7.7	3	0
6	28	7.2	6.9	6.4[a]	–	3	–
7	26	11.2	9.8	9.0[a]	9.9	3	+1.0
8	32	8.8	8.2	7.0[a]	8.6[b]	3	+0.5
9	26	7.7	7.1	5.0[a]	6.4[b]	1	+0.5
10	28	10.2	9.1[a]	6.1[a]	5.0[b]	2	−2

[a]$p<0.01$ compared with initial measurement.
[b]$p<0.01$ compared with previous measurement (17 days measurement only).
BMI, body mass index.

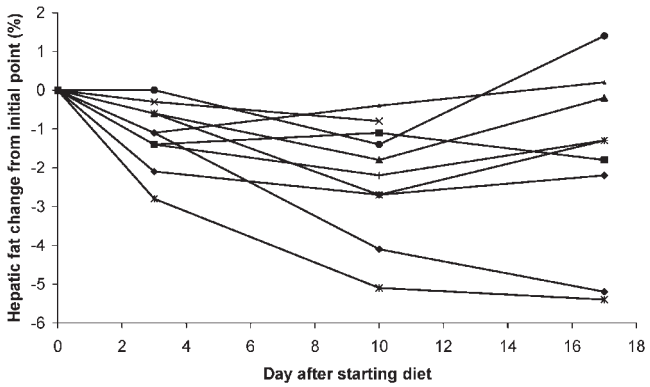

Figure 1. Relative change in hepatic fat for all 10 healthy volunteers compared with hepatic fat percentage at day 0.

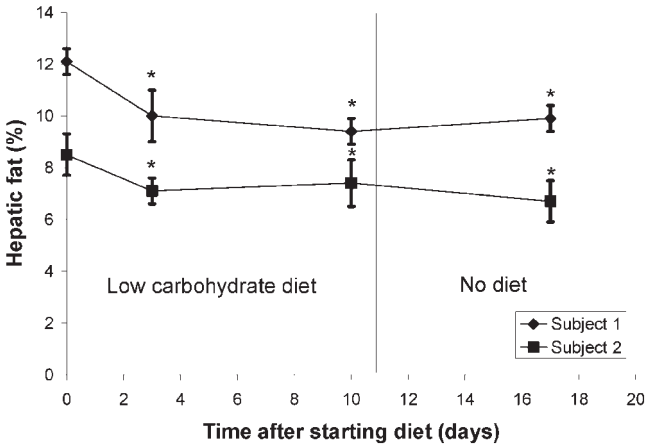

Figure 2. Plot of individual hepatic fat measurements for subjects 1 and 2 with error bars.

($p<0.01$) decrease in hepatic fat within 10 days of starting the diet. It was noted that the volunteers with the highest initial fat content had the greatest percentage decrease during the first 3 days of the diet ($\kappa=0.81$, range 0–24%), although the correlation at day 10 was weaker ($\kappa=0.42$, range 11–43%). All the subjects lost weight (mean weight loss: 1.7 kg at day 3 and 3.0 kg at day 10) but this was not correlated with changes in hepatic fat percentage. 1 week after stopping the diet, five subjects gained weight, three lost weight and one maintained the same weight (one was lost to follow up after cessation of the diet), though there was no correlation with hepatic fat change in the week after cessation of the diet.

Discussion

This study evaluates hepatic fat response to a low carbohydrate diet in 10 healthy volunteers and demonstrates a significant reduction of hepatic fat levels. To the best of our knowledge this is the first study to use quantitative image-based MR to demonstrate a significant reduction in hepatic fat during a low carbohydrate diet intervention. The dominance of fat and protein in this diet means that a decrease in liver fat reflects mobilization of hepatic lipid stores as an energy source and a contributor to the related ketosis. All subjects lost weight, demonstrating that either the diet itself or its appetite suppressant effect is hypocaloric.

The limitations of this pilot study were the small number of subjects studied and the lack of biochemical blood correlates, in particular the evaluation of insulin resistance by clamping methods. Although all subjects experienced reduced hepatic fat levels during the study, there was variation in the percentage of initial fat lost by day 10 (range 11–43%). Although there was an empirical correlation between initial fat percentage and the percentage of hepatic fat lost by day 3, evaluation of the insulin resistance changes of the subjects may elucidate the reason for the differing degrees of response, particularly since there was no correlation between the percentage hepatic lipid reduction and the reduction in weight. This would also provide insight into whether a similar response could be expected from the different patient groups with hepatic steatosis. A non-dieting (control) group was not assessed in this study but previous work on observing liver fat changes in healthy, non-dieting volunteers has shown weekly changes of no more than approximately 1% [11]. There was also no detailed study of the subjects dietary and alcohol habits for more than 3 days before commencing the diet: such a standardization step may be important in drawing full quantitative conclusions from a diet study of short duration. Future work in larger volunteer and patient groups will address these limitations.

There have been few comparable studies as the majority of metabolic studies do not measure hepatic fat content directly (presumably owing to the ethical difficulty of performing serial liver biopsies), choosing rather to measure serum triglycerides. In a study of overweight subjects fed a high fat (56% of calorie intake) diet (with carbohydrate) for a period of 2 weeks the liver accumulated fat (average 35% of the initial fat content) [12] compared with an isocaloric, low fat (16% of calorie intake) where there was a reduction in liver fat (average 20% of the initial fat content). A study of the effect of a hypocaloric low carbohydrate diet followed for 14 days by patients with type II diabetes found that there was an improvement in insulin resistance and a decrease in plasma triglycerides, though liver fat was not directly measured [10]. Other authors have reported evidence that low carbohydrate diets can alter body fat composition [13]. This is of importance given the emerging literature on the role of hepatic fat in the development of systemic insulin resistance, leading to type II diabetes mellitus, hyperlipidaemia, hypertension and increased artherosclerotic risk. This pilot study suggests that a low carbohydrate diet may have a role in modifying hepatic fat and hence insulin resistance.

Alternative non-invasive methods such as ultrasound and CT have been proposed, but ultrasound is limited by lack of specificity and CT by the use of ionizing radiation, which is difficult to justify in healthy individuals. The method used in this study can be implemented on the majority of currently installed MRI systems, and is simpler to implement than fat measurement by proton MR spectroscopy, which requires longer acquisition times and specialist analysis [6, 12]: spectroscopy methods cannot easily be used to sample the entire liver and cannot detect focal fat variation in the liver in a short examination. One study [14] finds that carbon-13

spectroscopy of hepatic lipids is in excellent agreement with morphometric analysis of biopsy specimens. However, this is principally a research technique and is not available in the vast majority of clinical settings.

This pilot study demonstrates the practical implementation and utility of MRI fat quantification as a tool in serial studies of hepatic fat content. In healthy volunteers the method demonstrated significant hepatic fat reduction resulting from a low carbohydrate dietary intervention.

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References

1. Samuel VT, Liu ZX, Qu X, Elder BD, Bilz S, Befroy D, et al. Mechanism of hepatic insulin resistance in non-alcoholic fatty liver disease. J Biol Chem 2004;279:32345–53.
2. Tiikainen M, Hakkinen AM, Korsheninnikova E, Nyman T, Makimattila S, Yki-Jarvinen H. Effects of rosiglitazone and metformin on liver fat content, hepatic insulin resistance, insulin clearance, and gene expression in adipose tissue in patients with type 2 diabetes. Diabetes 2004;53:2169–76.
3. Saadeh S, Younossi ZM, Remer EM, Gramlich T, Ong JP, Hurley M, et al. The utility of radiological imaging in nonalcoholic fatty liver disease. Gastroenterology 2002;123:745–50.
4. Fishbein MH, Gardner KG, Potter CJ, Schmalbrock P, Smith MA. Introduction of fast MR imaging in the assessment of hepatic steatosis. Magn Reson Imag 1997;15:287–93.
5. Hussain HK, Chenevert TL, Londy FJ, Gulani V, Swanson SD, McKenna BJ, et al. Hepatic fat fraction: MR imaging for quantitative measurement and display – early experience. Radiology 2005;237:1048–55.
6. Thomsen C, Becker U, Winkler K, Christoffersen P, Jensen M, Henriksen O. Quantification of liver fat using magnetic resonance spectroscopy. Magn Reson Imag 1994;12:487–95.
7. Longo R, Ricci C, Masutti F, Vidimari R, Croce LS, Bercich L, et al. Fatty infiltration of the liver: quantification by ^{1}H localized magnetic resonance spectroscopy and comparison with computed tomography. Invest Radiol 1993;28:297–302.
8. Joy D, Thava VR, Scott BB. Diagnosis of fatty liver disease: is biopsy necessary? Eur J Gastro Hepatol 2003;15:539–43.
9. Boden G, Sargrad, K, Homko C, Mozzoli M, Peter Stein T. Effect of a low-carbohydrate diet on appetite, blood glucose levels, and insulin resistance in obese patients with Type 2 diabetes. Ann Intern Med 2005;142:403–11.
10. Yancy WS, Olsen, MK, Guyton JR, Bakst RP, Westman EC. A low-carbohydrate, ketogenic diet versus a low-fat diet to treat obesity and hyperlipidemia. Ann Int Med 2004;140:769–77.
11. Lomas DJ, Black RT, Pinney J. Hepatic steatosis quantification by MRI: serial measurement and normal variation. Proc Intl Soc Magn Reson Med 2003;11:1424.
12. Westerbacka J, Lammi K, Hakkinen AM, Rissanen A, Salminen I, Aro A, et al. Dietary fat content modifies liver fat in overweight nondiabetic subjects. J Clin Endocrin Metab 2005;90:2804–9.
13. Dansinger ML, Gleason JA, Griffith JL, Selker HP, Schaefer EJ. Comparison of the Atkins, Ornish, Weight Watchers, and Zone diets for weight loss and heart disease risk reduction: a randomized trial. JAMA 2005;293:43–53.
14. Petersen KF, West AB, Reuben A, Rothman DL, Schulman GI. Non-invasive assessment of hepatic triglyceride content in humans with carbon-13 nuclear magnetic resonance spectroscopy. Hepatology 1996;24:114–7.