Title. Effects of short-term energy restriction on liver lipid content and inflammatory status in severely obese adults: results of a randomised controlled trial (RCT) using two dietary approaches.

Authors:

Emma L Baldry[1]

Guruprasad P. Aithal[2]

Philip Kaye[3]

Iskandar R Idris[4]

Andrew Bennett[6]

Paul C Leeder[5]

Ian A Macdonald[6]

[1] Faculty of Medicine & Health Sciences, University of Nottingham, University of Nottingham Medical School, Queen's Medical Centre, Nottingham, NG7 2UH

[2] National Institute for Health Research (NIHR) Nottingham Digestive Diseases Biomedical Research Unit (BRU), Nottingham University Hospitals NHS Trust and University of Nottingham, University of Nottingham Medical School, Queen's Medical Centre, Nottingham, NG7 2UH

[3] Department of Cellular Pathology, Nottingham University Hospitals NHS Trust, Nottingham Digestive Diseases Centre BRU, University of Nottingham, Queens Medical Centre, Nottingham, NG7 2UH

[4] MRC Musculoskeletal Physiology and Ageing, Division of Medical Sciences & Graduate Entry Medicine, Derby Teaching Hospital, University of Nottingham, DE22 3DT

[5] The East-Midlands Bariatric & Metabolic Institute (EMBMI), Derby Teaching Hospitals NHS Foundation Trust, Royal Derby Hospital, Uttoxeter Road, Derby, DE22 3NE

[6] Faculty of Medicine & Health Sciences, University of Nottingham, University of Nottingham Medical School, Queen's Medical Centre, Nottingham, NG7 2UH

Short title. Effects of two short-term pre-bariatric surgery diets.

Abstract word count:189

Main text word count:1776

Number of references: 15

Number of tables:2

Supplementary data

Address for correspondence:

Emma Baldry

Leicester Diabetes Centre, Leicester General Hospital, Gwendolen Road, Leicester LE5 4PW

E mail: emma.baldry@uhl-tr.nhs.uk

Abstract

Short-term very low energy diets (VLED) are used in clinical practice prior to bariatric surgery, however, regimens vary and outcomes for a short intervention are unclear. We examined the effect of two VLEDs; a food-based diet (FD) and meal replacement plan (MRP) (LighterLife) over two weeks in a randomised controlled trial (RCT). We collected clinical and anthropometric data, fasting blood samples, and dietary evaluation questionnaires. Surgeons took liver biopsies and made a visual assessment of the liver. We enrolled 60 participants and 54 completed (FD n=26, MRP n=28). Baseline demographic features, reported energy intake, dietary evaluation and liver histology were comparable between groups. Both diets induced significant weight loss. Perceived difficulty of surgery correlated significantly with the degree of steatosis on histology. Circulating inflammatory mediators: CRP, Fetuin-A and IL6 reduced pre to post diet. Diets achieved comparable weight loss and reduction in inflammatory biomarkers, perceived operative difficulty, and patient evaluation. NAFLD histology assessments post-diet were also not significantly different between diets. Results indicate effectiveness of short term very low energy diets and energy restriction irrespective of macronutrient composition although small sample size precluded detection of subtle differences between interventions.

Keywords

VLED, preoperative, bariatric, NAFLD, energy restriction

Introduction

Ectopic lipid accumulation is a common factor underlying non-alcoholic fatty liver disease (NAFLD), insulin resistance and Type 2 diabetes (DM) (1). Metabolic dysfunction is linked to pro-inflammatory mediators such as Interleukin-6 (IL6), Fetuin-A and C-reactive protein (CRP), which also contribute to progression to inflammatory non-alcoholic steatohepatitis (2-4).

An energy restricted diet is used prior to bariatric surgery to facilitate the laparoscopic approach to surgery via a reduction in liver size and improved liver flexibility, by reducing stores of lipid, enabling access to the upper stomach and oesophagus (5). In practice, this is achieved with the use of a short-term pre-operative very low energy diet (VLED), or "liver reducing" diet. Our survey of current practice in 2013 found significant variability in form, duration and application of the pre-operative diet used (6).

Although weight loss via a VLED is associated with reversal of the obesity related complications due to the reduction of ectopic lipid deposits (7), there is limited evidence for the effects of the different pre-surgery VLEDs on outcomes related to liver histology, inflammatory markers and their effectiveness related to perceived operative difficulty.

We therefore examined the outcomes of two commonly used VLED regimens in severely obese adults prior to undergoing bariatric surgery. Our primary outcome was liver histology post-diet, with secondary outcomes including weight loss achieved, circulating inflammatory markers pre to post diet, and peri-operative visual assessment of the liver for perceived difficulty of surgery. We also asked participants to evaluate the diets.

Materials and Methods

Study design

The study was registered http://www.isrctn.com/, registration number: ISRCTN65605485.

We conducted the RCT between May 2012 and June 2014. Patients were eligible if they had a body mass index (BMI) >40kgm^2 and acceptance onto the bariatric surgical programme at Derby Hospitals NHS Foundation Trust following discussion at the bariatric MDT. Participant exclusion criteria included; age <18years, secondary NAFLD, Type 2 diabetes treated by medication other than Biguanides, excess alcohol intake, pregnancy, and exclusions linked to application of a restrictive dietary regime.

Participants were randomly allocated to receive 800kcal/d VLED for two weeks pre-operatively, either Derby Teaching Hospitals NHS Foundation Trust standard pre-bariatric surgery food-based diet (FD) or a Meal replacement plan diet using LighterLife nutritional supplements (MRP). Block randomisation was used with matched pairs, for gender and diabetes status. The participants, and study team who supplied the dietary information to participants, were not blinded to intervention.

Sampling and data collection

Clinical and anthropometric data were collected during visits and from patients' notes. A pre-intervention fasting blood test, weight and height, were taken at the time of the standard pre-operative appointment. On completion of the diet participants completed a short qualitative evaluation. Participants were weighed post-diet on the morning of their bariatric surgery. When the participant was cannulated for anaesthesia a further fasting blood sample was taken. Intra-operatively a liver biopsy was taken and the surgeon completed a visual assessment of perceived operative difficulty.

Outcome measures

Primary outcome was histological assessment of a liver biopsy performed at the time of bariatric surgery (post-diet), secondary outcomes were change in total body weight (pre to post diet), circulating inflammatory markers (pre to post-diet), peri-operative visual assessment of the liver for perceived difficulty of surgery and evaluation of dietary approach (post-diet).

Sample size

The sample size was based on an estimation of the difference in liver lipid after following FD or MRP. For 80% power (at $p=0.05$) 98 participants would need to be randomised (49 to each arm) to see a statistically significant difference in the level of steatosis (Instat Graph Pad V2.0 software).

Statistical methods

The data were analysed with IBM SPSS Statistics for WINDOWS version 22 (IBM Corporation). Two-tailed non-parametric statistical tests were used; Wilcoxon signed rank test, Spearman's Rho, Mann–Whitney U test, and Kendall's Tau C Test. Threshold of significance was defined at $P<0·05$.

Bonferroni correction for multiple comparisons was carried out where stated.

Further details of methods and data collection are in Supplementary data.

Results

196 potential participants were screened for eligibility over a 25 months period of recruitment. 105 verbally consented to the study, 60 completed informed consent, were randomised and started the trial, and 54 completed. Six participants did not complete the study; five due to operations being cancelled and one patient was started on insulin. One adverse event, and one serious adverse event, were reported but were not deemed to be related to the intervention.

Baseline data (Table 1)

Participants' BMI ranged from 41.5-66.8kg/m^2, with a median of 50.7kg/m^2. 81.5% of participants were female, which is consistent with the national average for patients undergoing bariatric surgery. Age ranged from 24 to 65 years, with median 45. There was no significant difference between diet groups demographically or anthropometrically (age P=0.245, BMI P=0.691, gender P=0.568, DM status P=0.860).

Liver tissue histology (Table 2)

Following the intervention, 12 of 24 participants in the FD group (50%), and 18 of 28 participants in the MRP group (64%) had histological evidence of steatosis, which were not significantly different. Three of 24 participants in the FD group (12.5%) and four of 28 participants in the MRP group (14.3%) showed evidence of moderate or severe steatosis (steatosis grade 2-3). 37% of participants in the FD group, and 39% of participants in the MRP group, showed evidence of significant portal or periportal fibrosis; but the difference between groups was not statistically significant. No significant difference was found between diet groups for steatosis grade (P=0.349), liver cell injury (P=0.567), portal inflammation (P=0.611), lobular inflammation (P=0.455), or fibrosis stage (P=0.605).

Weight change and self-reported intake (Table 1)

Median total body weight loss was 3.6% in the FD group, and 3.4% in the MRP group. Results were not significantly different between diet groups, for body weight loss or percentage weight loss ($P=0.494$ and $P=0.993$ respectively).

Median energy intakes (kcal/d) were not statistically different between diet groups ($P=0.311$). Macronutrient energy intake (% total energy intake) differed between the two diets, with protein, fat and carbohydrate intakes being significantly different between groups ($P<0.001$ Mann-Whitney U-Test Bonferroni correction α error probability threshold 0.016). Fluid intake recommendations were a minimum of 2L/d, with median reported intakes of 2.2L/d FD, and 2.5L/d MRP.

Surgeons' assessment

In 22 of the 54 cases, a surgeon completed a visual assessment of 1) access to the gastro-oesophageal junction, 2) liver volume and 3) liver rigidity. Increasing perceived difficulty was associated with higher percentages of steatosis (as assessed histologically). There was a correlation between visual assessment by the surgeons and the three assessment categories (above). There was no significant difference between diets for the three visual analogue assessments; gastric access ($P=0.513$), liver volume ($P=0.891$), liver rigidity ($P=0.702$).

Blood results

Concentrations of CRP and Fetuin-A reduced significantly after both diets. IL6 reduction was not significant.

Dietary evaluation

Participants' self-reported evaluation showed overall satisfaction with the diets was 92% and 85% satisfied or very satisfied. 82% in the FD group and 93% on the MRP reported that they found the diet easy to follow. 19% in the FD and 15% in the MRP reported always feeling hunger, whereas 8% and 11% respectively never felt hungry. There were no significant differences between evaluation responses for satisfaction, ease of use or hunger frequency between diets.

Further results in Supplementary data.

Discussion

This RCT examined two energy restrictive dietary regimens aiming for 800kcal/d for a period of two weeks prior to bariatric surgery. We found that both dietary approaches achieved equivalent and significant outcomes. In the two week period of intervention, patients in both groups lost about 3.5% of their total body weight, alongside a reduction in CRP, and Fetuin-A . This reflects an improvement in obesity related systemic inflammation which requires further work to evaluate clinical significance.

Macronutrient composition between FD and MRP were significantly different with 52% of total energy intake from carbohydrate in the FD compared to 38% in MRP group. A previous study has demonstrated that a reduced carbohydrate diet with moderate energy restriction leads to significantly more reduction in liver lipid at 48hours than a higher-carbohydrate diet (8). Our results demonstrate that when VLED interventions are considered over a period of two weeks, both FD and MRP were effective irrespective of macronutrient composition.

The main objective of a pre-operative energy restrictive dietary intervention is to improve intra-operative conditions for a laparoscopic surgical procedure. At the time of surgery, we found a lower than expected proportion of patients had histological evidence of NAFLD on liver biopsy (9) and steatosis levels were associated with visual assessment by the surgeons of operative difficulty. Both of the pre-operative diets could therefore be reasoned to have enabled surgery, from the perspective of reducing difficulty of the surgery, due to limiting steatosis.

We also observed reduction in the biomarkers of obesity related systemic inflammatory response. Fetuin-A is an endogenous ligand for Toll-like receptor-4 through which free fatty acids induce inflammatory signalling and insulin resistance (10). Patients with severe obesity have markedly raised Fetuin-A levels. Markers of systemic inflammation, such as CRP, have been found to be determinants of risk for development of DM (11). Although our findings in the RCT are in line with observations elsewhere, where these markers correlate with measures of obesity, and reduce with significant weight loss (12-14), the majority of these observations were after longer periods of energy restriction and greater weight loss. The novelty of our findings were that the observed reduction in pro-inflammatory cytokines occurred after only two weeks and 3.5% weight loss. The main limitation to this work is the failure to reach the sample size. However, the results for NAFLD are similar to observations elsewhere after an energy restrictive diet when applied in the severely obese population (15). Further, we were unable to obtain liver biopsies or perform non-invasive estimation of hepatic lipid content using MRI both prior to and after the interventions, which would have allowed for more conclusive outcome appraisal. Randomisation however appears to have been effective and no significant differences in demographic or anthropometric profile were observed between the two groups at baseline.

In conclusion, our results indicate that energy intake of 715kcal/d for two weeks improves operative conditions regardless of the macronutrient composition. Patients were satisfied with VLEDs, which they also found easy to use. The fatty liver disease we found was in line with similar studies and is suggestive of an effect of both diets in reducing liver lipid. Reductions in inflammatory biomarkers require further investigation. We were unable to demonstrate a difference in either case between diets, and small sample size may have precluded detection of subtle differences.

Statement of authorship:

Guruprasad P Aithal, Andrew Bennett and Ian Macdonald all contributed to the conception and design of the study. Ian Macdonald, Paul Leeder and Iskandar Idris supported the organisation of the study and acquisition of data/samples. Philip Kaye completed histological assessment of liver biopsies. Emma Baldry collected, processed and analysed all data/samples. All authors contributed to interpretation of data and the drafting of the article.

Acknowledgements:

Thanks to LigherLife for providing the meal replacement products used in the study free of charge.

References

1. Virtue S, Vidal-Puig A. Adipose tissue expandability, lipotoxicity and the Metabolic Syndrome — An allostatic perspective. Biochimica et Biophysica Acta (BBA) - Molecular and Cell Biology of Lipids. 2010;1801(3):338-49.
2. Wieckowska A, Papouchado BG, Li Z, Lopez R, Zein NN, Feldstein AE. Increased Hepatic and Circulating Interleukin-6 Levels in Human Nonalcoholic Steatohepatitis. Am J Gastroenterol. 2008;103(6):1372-9.
3. Yoneda M, Mawatari H, Fujita K, Iida H, Yonemitsu K, Kato S, et al. High-sensitivity C-reactive protein is an independent clinical feature of nonalcoholic steatohepatitis (NASH) and also of the severity of fibrosis in NASH. Journal of Gastroenterology. 2007 2007//;42(7):573-82.
4. Trepanowski JF, Mey J, Varady KA. Fetuin-A: a novel link between obesity and related complications. Int J Obes. [Review]. 2015.
5. Colles SL, Dixon JB, Marks P, Strauss BJ, O'Brien PE. Preoperative weight loss with a very-low-energy diet: quantitation of changes in liver and abdominal fat by serial imaging. The American Journal of Clinical Nutrition. 2006 August 1, 2006;84(2):304-11.
6. Baldry E, Leeder P, Idris I. Pre-operative Dietary Restriction for Patients Undergoing Bariatric Surgery in the UK: Observational Study of Current Practice and Dietary Effects. Obes Surg. 2014 2014/03/01;24(3):416-21.
7. Steven S, Taylor R. Restoring normoglycaemia by use of a very low calorie diet in long- and short-duration Type 2 diabetes. Diabetic medicine : a journal of the British Diabetic Association. 2015 Feb 12.
8. Kirk E, Reeds DN, Finck BN, Mayurranjan MS, Patterson BW, Klein S. Dietary Fat and Carbohydrates Differentially Alter Insulin Sensitivity During Caloric Restriction. Gastroenterology. 2009;136(5):1552-60.
9. Machado M, Marques-Vidal P, Cortez-Pinto H. Hepatic histology in obese patients undergoing bariatric surgery. Journal of Hepatology. 2006 Oct;45(4):600-6.
10. Pal D, Dasgupta S, Kundu R, Maitra S, Das G, Mukhopadhyay S, et al. Fetuin-A acts as an endogenous ligand of TLR4 to promote lipid-induced insulin resistance. Nat Med. [Research Support, Non-U.S. Gov't]. 2012 Aug;18(8):1279-85.
11. Pradhan AD, Manson JE, Rifai N, Buring JE, Ridker PM. C-reactive protein, interleukin 6, and risk of developing type 2 diabetes mellitus. JAMA. 2001;286(3):327-34.
12. Brix JM, Stingl H, Höllerl F, Schernthaner GH, Kopp H-P, Schernthaner G. Elevated Fetuin-A Concentrations in Morbid Obesity Decrease after Dramatic Weight Loss. J Clin Endocr Metab. 2010 November 1, 2010;95(11):4877-81.
13. Bastard J-P, Jardel C, Bruckert E, Blondy P, Capeau J, Laville M, et al. Elevated Levels of Interleukin 6 Are Reduced in Serum and Subcutaneous Adipose Tissue of Obese Women after Weight Loss. The Journal of Clinical Endocrinology & Metabolism. 2000;85(9):3338-42.
14. Moschen AR, Molnar C, Geiger S, Graziadei I, Ebenbichler CF, Weiss H, et al. Anti-inflammatory effects of excessive weight loss: potent suppression of adipose interleukin 6 and tumour necrosis factor α expression. Gut. 2010 September 1, 2010;59(9):1259-64.
15. Lewis M, Phillips M, Slavotinek J, Kow L, Thompson C, Toouli J. Change in liver size and fat content after treatment with Optifast (R) very low calorie diet. Obes Surg. 2006 JUN 2006;16(6):697-701.

Tables

Table 1 Baseline data; Body mass index (BMI), gender, age and diabetes status for the 54 participants that completed the study, separated by dietary intervention. There were no statistically different results between diet groups.
Post-diet results for body weight loss (%), self-reported energy intake (kcal/d), and macronutrient composition of energy intake (kcal/d). There was no statistically significant difference between weight loss (P=0.993) or estimated energy intake (P=0.311), however all macronutrient intakes were significantly different (P<0.001).
All data shown as median (range) unless stated otherwise.

		FD (n=26)	MRP (n=28)
Gender: female/ male (n)		22/4	22/6
No. with Type 2 Diabetes (%)		8 (31)	8 (29)
Age in years		47 (40)	42 (35)
BMI kg/m^2		51.1 (25.4)	50.1 (21.4)
Weight Loss %		3.6 (3.0)	3.4 (3.7)
Estimated energy intake kcal/d		715 (558)	715 (275)
Macronutrient % total energy intake	**Carbohydrate**	52 (21.5)	38 (7.8)
	Protein	30 (14.7)	37 (7.2)
	Fat	19 (18.8)	25 (14.3)

Table 2
Liver histology results from time of surgery (post-diet).
Steatosis results for grade and location, liver cell injury results including lobular and portal inflammation and fibrosis stage (CRN) for both dietary approaches.
Shown as count (n).

		FD (n=24)*	**MRP (n=28)**
Steatosis grade 0-3	0	12	10
	1	9	14
	2	2	2
	3	1	2
Steatosis location 0-3	0	22	27
	1	0	0
	2	2	1
	3	0	0
Liver cell injury (ballooning/ apoptosis) 0-3	0	18	19
	1	6	9
	2	0	0
Lobular inflammation 0-3	0	12	19
	1	10	5
	2	0	2
	3	1	2
Portal inflammation 0-3	0	4	2
	1	11	15
	2	9	11
Fibrosis Stage (detailed CRN definitions)	None	8	6
	Mild zone 3 perisinusoidal (1a)	0	2
	Portal/periportal (1c)	12	14
	Perisinusoidal and portal/periportal (2)	2	6
	Bridging fibrosis (3)	0	0
	Cirrhosis (4)	2	0

* Two participants in the FD group were unable to have liver biopsies taken at the time of surgery.