Impact of interventions for obesity on insulin sensitivity using the leptin to adiponectin ratio.

A Thesis Submitted By

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Summary

During this work, I have explored the use of a milk-based meal replacement programme in the treatment of obesity and have examined the changes in insulin resistance via leptin to adiponectin ratio (LAR) as a novel marker of insulin resistance in patients who underwent weight-loss interventions in our service.

Firstly, I conducted a retrospective cohort study of patients with a history of severe obesity who underwent a milk-based meal replacement programme, explored its use as a weight loss intervention given its effect size in terms of reducing weight and the significant improvement in parameters such as ALT, lipid profile, Hba1c with significant reductions in the use of antihypertensives and antihyperglycemics in patients who completed this programme, all suggestive of changes in insulin sensitivity.

I then conducted a prospective cohort study of patients with severe obesity who underwent a milk-based meal replacement programme for weight loss and examined the changes in LAR, showing a significant reduction in weight and other metabolic parameters which were similar to the retrospective cohort along with a significant reduction in LAR, which was driven by a large reduction in leptin rather than a rise in adiponectin. We showed a significant correlation and association between the reduction in insulin resistance and weight loss.

Lastly, I conducted a prospective cohort study of patients with severe obesity who underwent sleeve gastrectomy for weight loss and examined the changes in LAR by measuring their levels on the day of surgery and at one-year follow-up, showing a significant reduction in weight, metabolic parameters, and LAR, driven primarily by a change or rise in adiponectin. We were the first group to examine changes in insulin resistance in the form of LAR in patients who underwent sleeve gastrectomy for weight loss, and we have shown an association between insulin resistance and change in weight.

Importance of the work arising from this thesis

- Chapter two is the first study to demonstrate the large effect size of milk-based meal replacement programme in terms of weight reduction.
- Chapter two is the first study to demonstrate the improvement in the anthropometric and metabolic profile of Irish adults with severe obesity post-milk-based meal replacement programme.
- Chapter 3 is the first study to demonstrate changes in leptin to adiponectin ratio post sleeve gastrectomy.
- Chapter 3 is the first study to demonstrate a correlation between weight loss and leptin to adiponectin ratio post sleeve gastrectomy.
- Chapter 4 is the first study to show changes in leptin to adiponectin ratio post-milk-based meal replacement programme in a cohort of Irish adults with severe obesity.
- Chapter 4 is the first study to demonstrate the correlation and association between weight loss and leptin to adiponectin ratio post a meal replacement programme in adults with severe obesity.

Author Contributions

Contributions of the author of this thesis

Chapter 2: Mohammed Faraz rafey drafted the manuscript, devised a data collection strategy, consented patients for the study, conducted data analysis and interpretation.

Chapter 3: Mohammed Faraz Rafey contributed to the study design, patient recruitment, and consented patients for the study, collected blood samples, conducted data analysis, and prepared the manuscript.

Chapter 4: Mohammed Faraz Rafey contributed to study design, patient recruitment, and consented patients for the study, collected blood samples, delivered the clinical intervention, data collection, conducted data analysis, and prepared the manuscript.

Contributions of Co-authors of the studies

Chapter 2: Connor Murphy helped draft the manuscript, devised a data collection strategy, helped with data analysis and interpretation. Niamh Beatty, Razk Abdalgwad, and Robert McGrath helped with data collection and revised the manuscript. Katriona Kilkelly, Helena Griffin, and Mary Hynes delivered the intervention, helped with data collection and revising the manuscript. Martin O'Donnell helped with study design, data analysis, and manuscript writing. Paula O'Shea processed biochemistry samples, collated metabolic data, and revised the manuscript. Chris Collins and Colin Davenport revised the manuscript. Francis Martin Finucane designed and supervised the study, drafted the manuscript, and is the guarantor for the study

Chapter 3: Clarissa Ern Hui Fang contributed to the study design, conducted data analysis, and prepared the manuscript. Iulia Ioana, Helena Griffin, Tim O'Brien, Paula O'Shea, and Mary Hynes contributed to the study design and revised the manuscript. Oliver McAnena and Chris Collins performed the surgical procedures, contributed to the study design, and revised the manuscript. Colin Davenport contributed to the data analysis and revision of the manuscript. Francis Martin Finucane supervised the drafting of the manuscript and the data analysis.

Chapter 4: Razk Abdalgwad contributed to patient recruitment, conducted data analysis, and helped in the preparation of the manuscript. Siobhan Foy and Brid Claffey contributed to patient recruitment and delivery of the clinical intervention. Paula O'Shea processed biochemistry samples, collated metabolic data, and revised the manuscript. Colin Davenport and Derek Timothy O'Keeffe contributed to the data analysis and revised the manuscript. Francis Martin

Finucane conceptualized the study design, led the clinical intervention, and supervised the data collection, drafting of the manuscript, and data analysis. All authors have given final approval of the version to be published and agree to be accountable for all aspects of the work.

List of Abbreviations

ABCA1	ATP-binding cassette transporter A1
ACC	American College of Cardiology
AGB	Adjustable Gastric Banding
AHA	American Heart Association
ALT	Alanine Aminotransferase
BMI	Body Mass Index
EBW%	Excess Body Weight percentage
FDA	Food and Drug Administration
FTO	Fat mass and obesity associated gene
GDPR	the General Data Protection Regulation
GIP	Glucose dependent Insulinotropic Polypeptide
GLP-1	Glucagon like peptide-1
Hba1c	Haemoglobin a1c
HBD	Hypo-energetic Balanced Diet's
HDL	High density lipoprotein
HOMA-IR	homeostasis model assessment of insulin-Insulin Resistance
HSE	Health Service Executive
IL-6	interleukin 6
LAR	Leptin to Adiponectin Ratio
LDL	Low Density Lipoprotein
LELDs	low energy liquid diets
NHS	National Health Service
NO	Nitric Oxide

OS	Obesity Society
PPAR-γ	Proliferator-Activated Receptor-γ
QALY	Quality adjusted life years
RBP-4	Retinol Binding Protein 4
RYGB	Roux-en-Y Gastric Bypass
SG	Sleeve Gastrectomy
SGLT2-I	Sodium Glucose Co-transporter-2 inhibitor
SSG	Salt Sensitivity Genes
STROBE	Strengthening the Reporting of Observational Studies in Epidemiology
TNF-α	Tumour Necrosis Factor-α
TWL%	Total weight loss percentage
T2DM	Type 2 Diabetes Mellitus
VLED's	Very Low Energy Diet's

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Publications and Presentations Arising from the Thesis

1. **Effects of a milk-based meal replacement program on weight and metabolic characteristics in adults with severe obesity.**

Presented as an Abstract poster in European Congress of Endocrinology in May 2017 and Irish Endocrine Society in October 2017

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3. **Changes in the Leptin to Adiponectin Ratio are Proportional to Weight Loss after Calorie Restriction.**

Mohammed Faraz Rafey, Razk Abdalgwad, Paula Mary O'Shea, Siobhan Foy, Brid Claffey, Colin Davenport, Derek Timothy O'Keeffe, Francis Martin Finucane

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Other Publications arising from this work:

1. **Long-Term Changes in Weight in Patients With Severe and Complicated Obesity After Completion of a Milk-Based Meal Replacement Programme.**

   Abdalgwad, R., **M. F. Rafey**, S. Foy, M. Newell, C. Davenport, D. T. O'Keeffe, and F. M. Finucane. "Long-Term Changes in Weight in Patients with Severe and Complicated Obesity after Completion of a Milk-Based Meal Replacement Programme." *Front Nutr* 7 (2020): 551068. https://dx.doi.org/10.3389/fnut.2020.551068.

2. **Changes in alanine aminotransferase in adults with severe and complicated obesity during a milk-based meal replacement programme.**

   Abdalgwad, R., **M. F. Rafey**, C. Murphy, I. Ioana, P. M. O'Shea, E. Slattery, C. Davenport, D. T. O'Keeffe, and F. M. Finucane. "Changes in Alanine Aminotransferase in Adults with Severe and Complicated Obesity During a Milk-Based Meal Replacement Programme." *Nutr Metab (Lond)* 17 (2020): 87. https://dx.doi.org/10.1186/s12986-020-00512-5.

3. **Utilising a milk-based meal replacement programme in a bariatric patient with poorly controlled type 2 diabetes mellitus.**

   Maher, M., **M. F. Rafey**, H. Griffin, K. Cunningham, and F. M. Finucane. "Utilising a Milk-Based Meal Replacement Programme in a Bariatric Patient with Poorly Controlled Type 2 Diabetes Mellitus." *Endocrinol Diabetes Metab Case Rep* 2019 (Apr 8 2019). https://dx.doi.org/10.1530/edm-19-0008.

4. **Prolonged acidosis is a feature of SGLT2-I induced euglycaemic diabetic ketoacidosis.**

   Rafey, M. F., A. Butt, B. Coffey, L. Reddington, A. Devitt, D. Lappin, and F. M. Finucane. "Prolonged Acidosis Is a Feature of Sglt2i-Induced Euglycaemic Diabetic Ketoacidosis." *Endocrinol Diabetes Metab Case Rep* 2019 (Sep 27 2019). https://dx.doi.org/10.1530/edm-19-0087.

5. **Risperidone-induced type 2 diabetes presenting with diabetic ketoacidosis**

   Hui Fang, C. E., **M. F. Rafey**, A. Cunningham, S. F. Dinneen, and F. M. Finucane. "Risperidone-Induced Type 2 Diabetes Presenting with Diabetic Ketoacidosis." *Endocrinol Diabetes Metab Case Rep* 2018 (2018). https://dx.doi.org/10.1530/edm-18-0031.

Declaration

I hereby declare that the thesis titled ''Mechanisms underlying changes in insulin resistance after bariatric interventions'' is all my own work and that I have not obtained a degree in this University or elsewhere on the basis of any of this work. I have taken reasonable care to ensure that the work is original to my best knowledge and has not been taken from other sources except where such work has been cited and acknowledged within the text.

Mohammed Faraz Rafey

31st August 2021

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Chapter One:

Introduction:

1.1: Obesity:

Obesity is a multifactorial disorder in origin, with its contributors being lack of physical activity[1], the role of genetic factors[2], behavioral disorders such as binge eating, emotional or comfort eating habits [3], and environmental factors such as an abundance of high-calorie food availability. The hallmark of obesity is the disparity between energy intake and energy expenditure[4, 5]. Obesity is associated with other co-morbidities such as Type 2 Diabetes (T2DM), hypertension, dyslipidemia, coronary artery disease, osteoarthritis, cerebrovascular events, and cancers[6-9], and it is significantly associated with poor quality of life and a decrease in life expectancy[10-12].

There is increasing evidence of the role of gut hormones and their central control in the development of obesity [13-15]. Although there are many gut peptides that play a role in the development of obesity, some of them are known to play a key role, such as Glucagon-like peptide 1 (GLP1), which is primarily produced in the small intestine in response to carbohydrate and fat intake [16], and it works by working on the centers of brain inducing satiety, increasing transit time for passage of food from the gut giving a feeling of fullness after meals, and it's a potent incretin effect hormone increasing insulin levels and decreasing glucagon levels after food intake[17], and its levels are known to be reduced in obesity[18]. Peptide YY is another peptide hormone produced from the small intestines in response to fat intake, it works by delaying gastric emptying giving a feeling of fullness, and its levels are thought to be reduced in obesity[19, 20]. Pancreatic polypeptide is another gut hormone released in response to a meal and hypoglycemia, and it controls appetite by decreasing gastric emptying rate and reducing gallbladder motility, and its levels are reduced in obesity[21, 22]. Cholecystokinin is another important gut hormone produced from the small intestines, it is expressed in a variety of centers in the brain, working as a neurotransmitter, and it also reduced appetite by delaying gastric emptying, and its levels are also thought to be decreased in obesity [23-25]. Ghrelin is a hormone of hunger produced from the stomach, and it plays an important role in appetite regulation, and its levels are thought to be less suppressed after a meal in obese individuals

compared to their lean counterparts[26, 27]. Alterations in the hypothalamic-pituitary axis have been associated with the development of obesity [28]. Increased stress and its effect on cortisol release via excessive release of cortisol and exogenous use of steroids have been associated with the development of obesity [29, 30]. We will describe the role of leptin and adiponectin in the development of obesity in further sub-chapters of this thesis.

Lack of physical activity is associated with the development of obesity [1]. In a systematic review of school children from 34 countries, low physical activity and high television viewing times suggest sedentarism was associated with the development of obesity [31]. In a study comparing Swiss children to the children of seven other European countries, the prevalence of obesity was significantly lower in Swiss children; However, the dietary habits were very similar, the difference in the prevalence of obesity was attributed to increased physical activity in the form of cycling and walking seen in the Swiss children compared to others[32]. In an Irish longitudinal cohort study of children with chronic illnesses, it was reported that children with chronic illnesses had decreased levels of physical activity and increased prevalence of obesity [33]. In a study of the Irish traveling community, low physical activity significantly correlated with the risk of cardiovascular disease [34]. The American Heart Association (AHA) /American College of Cardiology (ACC) /Obesity Society (OS) Guidelines recommends at least six months of high intensity, comprehensive lifestyle intervention, consisting of a reduction in daily calorie intake, increased physical activity combined with behavioral therapy in the management of obesity [35]. In a metanalysis of exercise interventions and their effect on weight loss and cardiovascular risk factors, it was shown that exercise interventions significantly reduce weight, Body mass index (BMI), waist circumference, insulin resistance, systolic and diastolic blood pressure, and lipid profile in obese and overweight individuals [36]. In a randomized control trial of women with obesity, utilizing a hypocaloric diet for weight reduction, moderate and high-intensity exercise groups were seen to be more effective in reducing body fat index and maintaining muscle mass [37].

Genes influence weight gain, maintenance, and weight regain after weight loss interventions[2]. Several single gene defects in the leptin-melanocortin pathway have been well described in the literature, which leads to congenital monogenic forms of obesity[38-40]. In a systematic review and metanalysis of 3133 obese and 3123 non-obese individuals, melanocortin receptor four gene and its single nucleotide variant rs17782313 is associated with a high risk of obesity, and

the risk of developing obesity is higher in individuals with homozygous mutation of this gene[41]. In a study exploring salt sensitivity genes (SSG)and their role in obesity, visceral adipose tissue samples were collected, and microarray expression data was used to construct a complex protein interaction network of the genes, which revealed 23 SSG's which are expressed predominantly in the adipose tissue, 15 of these 23 SSG's and eight other SSG's showed co-expression with obesity-related genes, which play a role in adipogenesis and adipocytokine signaling [42]. In a systematic review and metanalysis of vitamin D receptor gene (VDR), FOKL and TAQL polymorphisms were associated with a high risk for the development of obesity in states of homozygous mutations[43]. Fat mass and obesity-associated gene (FTO), is thought to play an important role in obesity through increment of energy intake[44], plays a role in central control of energy homeostasis[45]. An allele of FTO variant rs9939609 is associated with increased weight, fat mass, BMI, and energy intake in children [46]. In a study of a healthy middle eastern population, subjects with the FTO rs9939609 AA variant had higher carbohydrate and lower fat intake and significantly higher BMI compared to subjects without the mutation[47]. In a study evaluating gene expression profiles of subcutaneous versus visceral adipose tissue in obese and non-obese individuals, Phosphatidic Acid Phosphohydrolase Type 2c (PPAP2C), Cytochrome P450 Family 4 Subfamily A Member 11(CYP4A11) and Cytochrome P450 Family 17 Subfamily A Member 1(CYP17A1) gene upregulation was seen in visceral adipose tissue of obese individuals, which are thought to play an important role in development of obesity[48]. In a study of obese women who underwent bariatric surgery versus non-obese women, subcutaneous and visceral adipose tissue samples, significantly higher mRNA expression of C1q/TNF-related protein 12 (CTRP12) in subcutaneous adipose tissue samples and expression of interleukin 6(IL-6) and monocyte chemoattractant 1 (MCP-1) inflammatory genes in subcutaneous and visceral adipose tissue samples of obese patients was reported, which might play a role in glucose metabolism and obesity related inflammatory processes[49]. In a study of 68 obese and 48 non-obese individuals, methylation of Receptor activator of NF-κB ligand (RANKL) and C-FOS proto-oncogene had a significant influence on the circulating levels of adipocytokines in obese individuals[50]. In a study examining the expression of ATP-binding cassette transporter A1 (ABCA1), which plays a role in regulation of high-density lipoprotein (HDL) cholesterol and insulin sensitivity of the adipose tissue, obese and insulin-resistant individuals had significantly lower expression of ABCA1 gene in the visceral adipose tissue[51]. In a study evaluating the role of Fibroblast growth factor 21 (FGF21) and its gene expression in

children aged 8-11 years, it was noted that T allele of rs11665896 mutation in the FGF21 gene was associated with obesity, and subjects with TT genotype consumed more carbohydrates[52].

1.2: Prevalence of Obesity

There is an increase in the global prevalence of obesity [53], with an overall increase from 1980 to 2013 by 27.5% in adults and 47.1% for children[54], when compared between 1975 to 2016, prevalence increased from 3% to 11% among adult males, from 6% to 15% among adult females and in children from less than 1% in 1975 to between 6-8% in 2016 [55]. Between 1980 and 2008, the prevalence of obesity in adults aged greater than 20 increased, with an average BMI increase per decade in men of 0.4 kg/m^2 and 0.5 kg/m^2 in women, with 205 million men and 297 million women determined to be obese worldwide [56]. In 2015 a total of 107.7 million children and 603.7 million adults were deemed to be obese worldwide, with four million deaths accounted annually related to obesity, amongst which the majority were accounted to obesity-related cardiovascular disease[57]. In a large worldwide study of children and adolescents, 128.9 million participants were pooled from 2416 population-based studies, showed 50 million girls and 74 million boys aged 5 to 19 were obese worldwide, with 20% or more prevalence in Polynesia and Micronesia, the Middle East and North Africa, the Caribbean, and the USA in 2016[58]. As per a United States of America (USA) study conducted between 1990 and 2008 from the behavioral risk factor surveillance system database, a linear time trend forecast predicted that by the year 2030, 51% of the USA population to be obese, with an overall increase by 33% in the prevalence of obesity in the next two decades of its publication and an overall increase in severe obesity by 130%[59]. Prevalence of obesity was different in low income versus high-income countries; in low-income countries prevalence of obesity was higher in the population with higher levels of education, whereas in medium and high-income countries, its prevalence was associated with being higher with lower levels of education [60], if you analyze it in a different model, in the developing countries gross domestic product is positively associated with obesity prevalence, whereas in the developed world lower income population was associated with higher prevalence of obesity [61].

The prevalence of obesity with a BMI of more than or equal to 30 kg/m^2 varies from a particular country to another [58]. In the European data from 2008, the prevalence of obesity ranged from 6.2% to 36.5% in women versus 4% to 28.3% in men with higher prevalence in central, eastern, and southern Europe[62], and as per the 2015 data of 16 European countries 47.6% of adults

were overweight or obese, and an increase in prevalence in the northern European countries [63]. In terms of North America, in the USA, 34.9% of adults were deemed to be obese [64], in Canada, the prevalence was much lower between 25% to 27% of adults in the obesity range, and in Mexico, obesity prevalence is deemed to be around 30% [65]. In India, obesity prevalence is between 11.8% to 31.3%, with variance between urban and rural populations [66]. In China, the prevalence of obesity was 29.9% in 2002 [67], and in children and adolescents, the trend increased from 1.7% to 11.7% between 1991 to 2015[68]. The prevalence of obesity amongst women in Ghana was 35.4% in 2014 [69]; in Mozambique, the prevalence of obesity and overweight increased from 18.3% to 30.5% between 2005 and 2015[70]. In Argentina, the prevalence of obesity increased between 2008 to 2014 from 21.3% to 26.9% in males and from 15.2% to 23% in females [71]. In a systematic review of obesity in Ireland, amongst the primary school children, conducted between 2002 and 2012, the prevalence of obesity and overweight in all studies was between 20 to 34%[72] and when compared against 21 other European countries, Ireland ranked 10th in terms of prevalence of childhood obesity, with highest rates seen in Greece[73]. In conclusion, there is a difference in prevalence of obesity around the world but the one thing in common amongst all countries is the increase in prevalence of obesity.

1.3: Role of Medications, Dietary and lifestyle modification in the treatment of obesity

Medically supervised weight loss of 5% to 10% is accepted as being clinically meaningful in the management of obesity [35]. Patients with type 2 diabetes who lost 5% to 10% of weight had a reduction in their glycaemic control in terms of Haemoglobin a1c (Hba1c) of 0.5%, reduction in their systolic and diastolic blood pressure by five mmHg, a significant increase in their HDL cholesterol as well as a reduction in Triglyceride levels [74]. While dietary interventions are widely used worldwide to achieve meaningful weight loss and improvement of obesity-related co-morbidities[75], medications such as Semaglutide, which is a glucagon-like peptide 1 (GLP-1), are emerging as fierce competitors in achieving meaningful and sustained weight loss[76]. In this section, we will have an overview of various dietary and lifestyle interventions used globally to obtain a meaningful weight loss and use of licensed medications and their role in the treatment of obesity.

Lifestyle and behavioral interventions have been long prescribed as tools in the management of obese and overweight patients. Modifications of lifestyle behaviors related to nutrition and

physical activity can be associated with improvement in the underlying obesity and associated metabolic disorders[77]. Though the benefits of regular physical activity were known to ancient Greeks, it is in the last century that the scientific knowledge around this topic made enormous strides, even moderate levels of physical activity are known to give considerable improvements of health for patients suffering from excessive weight and are also known to prevent the development of obesity in subjects who have a normal weight[78]. The current trends in lifestyle modification therapy for overweight and obese patients combine specific recommendations on nutrition and physical exercise along with behavioral and cognitive procedures and strategies[79]. In the Da Qing study, diet and exercise intervention was successful in significantly decreasing the incidence of type 2 diabetes in patients with impaired glucose tolerance [80]. In the look-ahead trial, patients with a history of type 2 diabetes in the intensive lifestyle intervention group had better outcomes with weight, Hba1c, systolic and diastolic blood pressure, and HDL cholesterol level when compared to diabetes education and support group highlighting the importance of intensive lifestyle interventions[81]. In a cohort of severely obese 150 individuals, an eight-week supervised, structured lifestyle intervention programme showed significant improvements in weight, adiposity, fitness, diabetes control, and cardiovascular risk factors [82]. A systematic review and meta-analysis noted that behavioral therapy improves adherence to lifestyle interventions in adult obese patients, and this, in turn, is associated with improvements in overall health outcomes[83].

In the OPTIWIN study, 273 patients were randomized to either receive a total meal replacement dietary intervention of 800 to 1200 Kcal or a food-based dietary plan for weight loss in which the participants reduced their calorie intake by 500-750 kcal below estimated total energy expenditure, it was noted that at 26 weeks meal replacement arm achieved 12.4% ± 0.6% weight loss versus 6.0% ± 0.6% in the food-based dietary plan group and at 52 weeks weight loss in the meal replacement group was 10.5% ± 0.6% versus 5.5% ± 0.6% in the food-based dietary plan group showing a substantial difference in weight loss achieved with meal replacement strategy[84]. In a nutritionally balanced conventional meal replacement programme of 50 patients with obesity, in which participants received two meals rich in soy and pea protein 240kcal each along with one normal meal with a calorie restriction of 1500kcal for males and 1200kcal in females per day for eight weeks, showed substantial improvement in body weight, body fat composition, waist circumference, lipid profile, insulin resistance and an overall improvement in cardiovascular health[85]. In the CALARIE trial of young non-obese individuals, a

calorie deficit diet of 25% less than the daily requirements resulted in better cardiovascular outcomes, insulin sensitivity indexes, and risk of metabolic dysfunction compared to the control group [86]. A primary care led meal replacement randomized control trial of 306 individuals with type 2 diabetes who were subjected to a calorie-restrictive diet of around 850Kcal for two to five months followed by two to eight weeks of food re-introduction and structured dietary and lifestyle support for weight maintenance thereafter, showed that 24% of participants in the treatment arm had 15kgs or more of wight loss and diabetes remission in 46% at one year[87].

In a metanalysis of 17 randomized controlled trials with a minimum follow-up of eight weeks to analyze the differences between low carbohydrate versus low-fat diets, it was noted that both low-fat diet and low carbohydrate diet are effective strategies in weight loss therapy in individuals who are strictly adherent with the diet, and in comparison low carbohydrate diet was considered to be better in achieving modestly significant results compared to low-fat diet[88]. In another metanalysis of 29 studies comparing very-low energy diets (VLED's) using <800 Kcal versus hypo-energetic balanced diets (HBD's)with an average of 1000 Kcal, it was seen that VLED's had an average weight loss of 29% versus 17% in HBD's at 4 to 5 years of follow-up and the groups which exercised more were thought to have had more success at maintaining their weight lost[89]. Although dietary interventions are useful in short-term weight loss, their long-term efficacy and weight regain are something of concern [90]. Dietary interventions are not known to be cost-effective; for example, the cost per QALY gained for weight watchers is around 34,630$[91]. While some studies show reasonable retention rates[87], a high attrition rate is a well-known phenomenon in patients undergoing dietary interventions[92, 93].

In terms of drug therapy for weight loss, there are many food and drug association (FDA) approved medications available in the market today [94]. Orlistat, which works by inhibiting pancreatic and gastrointestinal lipase activity and therefore decreasing lipid absorption, showed a mean weight loss of 5.8kg in a four-year double-blind, randomized control trial and had shown to reduce the incidence of type 2 diabetes in patients who had impaired glucose tolerance at baseline [95]. Naltrexone/ Bupropion combination which suppresses appetite and increases satiety by working as opioid receptor antagonist and dopamine and norepinephrine reuptake inhibitor has shown to have a >5% weight loss at 56 weeks of intervention in a randomized control trial [96]. Lorcaserin, a selective serotonin 2C receptor agonist that works by reducing appetite, has shown to have a substantial weight loss of 10.9% at one year in a randomized control trial, and in conjunction with behavioral modification, they showed good weight

maintenance compared to placebo[97]. Phentermine/ Topiramate, which works as a norepinephrine agonist and GABA agonist by suppressing appetite, in a randomized control trial, have shown to decrease body weight by 5% at 56 weeks of intervention but had a series of adverse events reported with a high attrition rate in the trial[98]. GLP-1 agonist therapy, which works by stimulating insulin secretion, inhibiting glucagon secretion, decreasing gastric emptying, and increasing satiety after meals, is the present and future of medication-induced weight loss therapy. In a double-blind, randomized control trial of 3731 non-diabetic patients, liraglutide, a once-daily GLP-1 agonist at 3mg doses, showed a mean weight loss of 8.4±7.3 kg at 56 weeks, with 63.2% of patients losing 5% of body weight and 33.1% of patients losing 10% at 52 weeks[99], with the maintenance of weight loss for up to two years with continuous therapy[100]. In a recent double-blind, randomized control trial of once-weekly GLP1- agonist therapy of semaglutide, 1961 adults with obesity were randomized to either receive placebo or 3mg semaglutide, mean change in body weight in the treatment arm was 14.9% at 68 weeks with 86.4% of patients in treatment arm achieving 5% or more, 69.1% achieving 10% or more and 50.5% achieving 15% or more weight loss and participants in the treatment arm had better cardiovascular outcome and participants reported improvement in physical activity compared to baseline [101].

1.4: Role of Bariatric or Metabolic Surgery in treatment of Obesity

Surgical alteration of a normal bodily system to achieve a biological result for a potential health benefit has been described in the literature as metabolic surgery[102]. Bariatric surgery is a type of metabolic surgery where surgical procedures tend to result in weight loss that is necessary for the management of obesity that is not managed by conservative treatment options[103]. There has been an ever-growing need for bariatric surgeries is due to their safety[104, 105], effectiveness[106], and cost-benefit [107]in the management of obesity and type 2 diabetes. When compared with intensive medical therapy over a five-year follow-up, bariatric surgery was associated with a greater reduction in Hba1c, with a greater reduction in serum triglyceride levels, and better quality-adjusted life-years (QALY) compared to intensive medical therapy arm[106].In terms of cardiovascular outcome, a metanalysis of bariatric surgeries in 19,543 patients has shown to cause remission of hypertension in 63%, dyslipidemia in 65%, and type 2 diabetes in 73% of the study population[108]. Roux-en-Y gastric bypass (RYGB) surgery has been shown to significantly reduce albuminuria and increase adiponectin levels in patients with obesity and type 2 diabetes even as soon as six months post-surgery[109]. Bariatric surgeries

have been shown to decrease serum tumor necrosis factor α(TNF-α), decrease serum leptin levels and increase serum adiponectin levels, reducing the overall inflammome state in severely obese individuals[110]. In terms of Bone mineralization, bariatric surgery has been shown to increase bone formation and resorption from as early as six months post-surgery and up to seven years post-surgery[111].

There were an estimated 468,609 bariatric surgeries that were performed worldwide in 2013, with the highest number related to the North American region with the most commonly performed procedures internationally in order from RYGB followed by sleeve gastrectomy (SG) and adjustable gastric banding (AGB), respectively[112]. As per the 2019 data from the International Federation for the Surgery of Obesity and Metabolic Disorders from 51 different countries, 14 of which provided data from their national registries, RYGB comprised of (38.2%), SG comprised of (46.0%), one anastomosis gastric bypass comprised of (7.6%) and gastric banding operations (5.0%) as the primary surgical options since 2014 from the 188,162 procedures performed[113]. This shift towards SG from bypass surgeries is due to the fact that there are fewer post-operative complications with SG[114, 115], and there is no difference in absolute weight loss between the two surgeries[114], though RYBG remains the gold standard for treatment of obesity with type 2 diabetes (T2DM) and other metabolic complications such as hypertension and dyslipidaemia[116, 117]. Bariatric surgeries have been proven to be cost-effective, as per a study published in the National Health Service (NHS), which has a very similar approach to treatment options like the Health Service Executive (HSE), bariatric surgeries were associated with reduced mean costs to the health service by €2742 with an overall increase of 0·8 life-years and 4·0 QALY's over a lifetime compared with usual care[107].

1.5: Insulin resistance and measurement of insulin resistance

Glucose serves as a primary fuel utilized by the human cells, with its cellular uptake mediated by the pancreatic hormone Insulin. Reduction in insulin sensitivity in the target organs, thereby leading to insulin resistance, can therefore impact critical functions of many cells in the human body. Insulin resistance is the cause of various metabolic disorders, including T2DM, metabolic syndrome, impairments in insulin signaling, disrupting entry of glucose into the adipocytes and skeletal muscle cells. The exact mechanism of insulin resistance is complex to understand, but usually, it is mainly due to oxidative stress, inflammatory processes, mutations occurring in

Insulin receptors, mitochondrial dysfunctionality, and endoplasmic reticulum stress[118]. In the liver, insulin resistance is manifested by increased glucose production via gluconeogenesis in the presence of hyperinsulinemia[119]. In the skeletal muscle, insulin resistance leads to decreased uptake of glucose following carbohydrate ingestion leading to hyperglycemia [120]. With obesity and decreased physical activity contributing to insulin resistive states and in the presence of genetic predisposition [121], pancreatic beta-cell failure occurs with time leading to decreased insulin production and overt diabetes [122]. Decreased levels of GLP-1 and glucose-dependent insulinotropic polypeptide (GIP), mainly produced in the small intestine, leading to a reduction in incretin effect and insulin secretion in insulin resistive states [123]. Increased levels of sodium-glucose co-transporter in the proximal convoluted tubules lead to increased reabsorption of glucose from the kidneys in type 2 diabetes leading to hyperglycemia [124]. The brain plays a major role in inducing satiety in response to hyperinsulinemia[125], and it has been proven that hypothalamic dysfunction in obese individuals leads to altered levels of satiety, proving that there is resistance to insulin in the hypothalamus in obese and insulin-resistant individuals [126]. Unhealthy adipocytes play a major role in the development of insulin resistance[127] by increasing serum free fatty acid concentration leading to gluconeogenesis [128], with increased production of pro-inflammatory cytokines and decreased production of anti-inflammatory adipokines[129, 130]. Insulin resistance is a multifactorial pathology, and understanding the role of adipocyte-derived insulin resistance and its improvement with weight-loss interventions in this thesis will play a major role in the treatment of T2DM and obesity in the future.

Measuring insulin resistance can be very difficult, with the gold standard being the hyperinsulinaemic euglycaemic clamp, in which plasma insulin concentrations are raised by administering exogenous insulin while maintaining a euglycaemic state via administration of variable rate glucose infusion. In this state of euglycemia, the uptake of glucose in the tissues is considered as a measure of whole-body tissue sensitivity to exogenous insulin[131]. This is quite challenging with high technical demand and resource-intensive; hence there has always been a need for easier, robust, and cheap techniques to measure insulin resistance. HOMA-IR and HOMA-S or the homeostasis model assessment of insulin results use a technique in which fasting insulin and glucose levels are obtained and tallied with a computer-generated model which predicts the homeostatic concentrations of insulin and glucose which arise from varied beta-cell dysfunction and insulin resistance resulting in the values correlating the individual's insulin resistance, and this has been correlated with hyperinsulinaemic euglycaemic clamp technique

[132]. Retinol binding protein 4 (RBP4) is an adipokine that transports retinol from liver to skeletal muscle, its levels are increased in states of obesity and insulin resistance and play a role in skeletal muscle insulin resistance development, and its plasma concentration levels are considered as a marker of insulin resistance [133-135]. Chemerin is an adipokine that causes skeletal muscle insulin resistance by decreasing glucose uptake in the muscles, and its production is upregulated by TNFα and downregulated by PPAR-γ; it has been correlated with hyperinsulinaemic euglycaemic clamp technique as a robust marker of insulin resistance [136, 137]. Adipocyte fatty acid-binding protein 4, which is expressed predominantly in macrophages and adipose tissue, plays a role in free fatty acid storage and lipolysis, has been correlated with other insulin resistance measurement techniques, and is thought to be a biomarker of insulin resistance [138]. FBG-21 plays a role in the healthy expansion of subcutaneous adipose tissue increasing the storage capacity of adipose tissue in insulin-sensitive obese individuals, and it is negatively correlated with insulin resistance; its reduced levels are thought to be a marker of insulin resistance [139]. Myostatin, a myokine, which plays a role in restraining muscle growth has been recently correlated with other markers of insulin resistance and is thought to a novel biomarker of measurement of insulin resistance [140, 141]. Non-coding RNAs such as miRNAs (micro-RNA) and lncRNAs (long-non-coding RNA) are involved in the regulation of the hepatic insulin signaling cascade, and their dysregulation has been reported in the hepatocytes of insulin-resistant subjects, making them a potential biomarker of hepatic insulin resistance [142]. In patients with diabetic nephropathy, the proinsulin to insulin ratio is thought to be a good biomarker of insulin resistance [143]. Three hydroxybutyrate, which is a catabolic intermediate of circulating branched-chain amino acids metabolism, is considered a novel adipocyte-derived regulator of adipocyte subtype-specific functions and is strongly linked to obesity, insulin resistance, and type 2 diabetes mellitus, is thought to be a novel biomarker of insulin resistance [144]. Lastly, leptin to adiponectin ratio or LAR, these are two major adipokines circulating in large quantities in serum and its measurement which is correlated in large epidemiological studies with other methods of measurement of insulin resistance[145], is thought to be as robust clinical markers of insulin resistance and obesity related co-morbidities [146, 147]. We will describe the role of leptin and adiponectin further in this introduction, as we have evaluated the changes in leptin, adiponectin and LAR in our studies in this thesis as a measure of insulin resistance after weight loss interventions.

1.6: Leptin

Leptin, discovered in 1994[148], is a 167 amino acid adipokine with a four-helix bundle motif similar to that of a cytokine, with primary expression in adipocyte and its circulating levels usually indicates the energy stores of the body in the adipose tissue[149]. Leptin plays a role in activating several signal transduction pathways, of which the most important pathway is the activation of Janus kinase two signal transducer and activator of transcription 3 (JAK2/STAT3), which plays a major role in energy homeostasis by activation of proopiomelanocortin (POMC), which in-turn activates anorexigenic neuropeptides such as αmelanocyte-stimulating hormone (αMSH), in the arcuate nucleus of the hypothalamus, therefore inducing satiety [150, 151]. Leptin also plays an important role in several hypothalamic pathways involved in development and reproduction[152-155]. Leptin is directly associated with obesity-related hypertension, with its preserved ability in activating the sympathetic nervous system regardless of its metabolic actions[156]. Leptin is known to be an independent link between obesity and the development of arthritis, and its levels are found to be increased in the synovial fluid in obese individuals, which causes an increased production of matrix metalloproteinases (MMPs), pro-inflammatory mediators, and nitric oxide (NO) in chondrocytes, therefore inducing arthritic changes[157]. Leptin receptors are thought to be expressed in the hippocampus and are thought to play a role in hippocampal synaptic plasticity that ultimately affects hippocampal-dependent learning and cognitive function[158]. Factors that upregulate leptin production include excess fat storage in adipocytes, overfeeding, glucose intake, hyperinsulinemia, glucocorticoid excess, estrogen excess, and inflammatory cytokines such as TNF-α and IL-6 (in acute states) and factors that downregulate leptin production are fasting, low energy states (leanness), catecholamine and adrenergic agonists, thyroid hormone, androgens, Peroxisome Proliferator-activated Receptor-γ (PPAR-γ) agonists and inflammatory cytokines (TNF-α & IL-6 in prolonged states)[159]. Exogenous leptin therapy is used widely in congenital leptin deficiency disorders and lipoatrophic disorders in establishing satiety and improving their overall metabolic health by reduction of underlying insulin resistance[160, 161].

Leptin resistance or hyperleptinemia is a well-known phenomenon in obesity[162]; various mechanisms have been proposed in the development of leptin resistance. These include disruption of leptin signaling in the hypothalamic areas of the brain, impaired transport of leptin across the blood-brain barrier, inflammatory conditions of the hypothalamus, endoplasmic reticulum stress, and autophagy [163, 164]. In obese individuals, increased energy intake is

associated with a low-grade inflammatory response that leads to dysregulation of lipid storage and adipokine secretion; this, in turn, results in alterations in leptin sensitivity, defects in leptin transport across the blood-brain barrier, and post-receptor signaling[165]. Several studies have shown that a reduction in leptin levels after weight loss interventions is associated with an overall improvement in metabolic health in obese individuals[108, 110, 166-168].

1.7: Adiponectin

Adiponectin, first described in 1995 as adipocyte complement-related protein of 30 kDa or ACRP30, was thought to be a factor that participates in energy homeostasis[169], then in April 1996 described and registered as novel collagen-like secretory protein exclusively produced by the adipocytes apM1[170], in May 1996 described as adipoQ described as a polypeptide of 247 amino acids with a secretory signal sequence at the amino terminus, a collagenous region and a globular domain [171]and in October 1996 described as Gelatin-binding protein of 28 kDa containing all the sequences of apM1 protein, specific to its production in adipose tissue and was thought to play a role in lipid catabolism and storage and whole-body metabolism[172]. The three major oligomeric multimers of adiponectin have been described with a poorly differentiated biological role, and they include a low molecular weight trimer, a middle molecular weight hexamer, and a high molecular weight 12-18 multimer [173]. Adiponectin works mainly via two receptors, adipoR1 expressed in the skeletal muscles and adipoR2 which is predominantly expressed in the liver [174]. Increased AMP-activated protein kinase (AMPK) activity is achieved by activation of adipoR1 expression in liver and muscle, which increases fatty acid oxidation and glucose uptake, therefore causing the insulin-sensitizing effects of adiponectin[175]. AdipoR2 expression in the liver results in a decrease in gluconeogenesis and increased oxidation of fatty acids via AMPK and peroxisome proliferator-activated receptor (PPAR)-alpha pathways hence playing an essential role in insulin sensitization or working against hepatic insulin resistance [176].

In mice, recombinant adiponectin has been shown to improve hepatic steatosis, inflammation, and hepatomegaly and decrease alanine aminotransferase levels [177]. Adiponectin resistance is a phenomenon associated with diet-induced hyperlipidemia; leptin and adiponectin imbalances have been thought to play a role in abdominal obesity-related cardiovascular disease [178]. Decreased levels of adiponectin play a role in the development of insulin resistance[179], and

higher adiponectin levels are thought to play a protective role against acquiring type 2 diabetes [180]. Adiponectin plays an important role in the clearance of apoptotic cells by macrophages through receptor-dependent pathways involving calreticulin, therefore helping in reducing systemic inflammation[181]. In mice models, adiponectin is known to reduce atherosclerosis [182], and adiponectin levels were seen to be low in humans with coronary artery disease [183]. Adiponectin prevents endothelial cell apoptosis hence producing a vascular protective effect [184]. Adiponectin increases endogenous nitric oxide production, interferes with AMPK activation, mammalian target of rapamycin (mTOR), and nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κβ) signaling, therefore can play an important role theoretically in the treatment of pulmonary hypertension [185]. An animal model noted that adiponectin alleviates exacerbation of airway inflammation and oxidative stress in obesity-related asthma mice[186]. Adiponectin reduces CD-163, which is a pro-inflammatory monocyte-macrophage-specific receptor, and its levels are increased in type 2 diabetes and obesity; it can therefore reduce systemic inflammation[187]. Adiponectin is known to suppress saturated free fatty acid-dependent hypothalamic inflammation by suppressing the activation of microglia and therefore plays a vital role in energy homeostasis [188]. Antioxidant phenol hydroxy-tyrosol and monounsaturated fatty acid oleic acid present in olive oil prevents adiponectin downregulation [189]. PPAR-γ agonist therapy is known to increase circulating levels of adiponectin [190].

1.8: Leptin to adiponectin ratio

Leptin to adiponectin ratio or LAR is a robust marker of whole-body insulin resistance, as leptin is associated with creating a state of inflammation which leads to insulin resistance [191], and adiponectin with its adipoR1 and adipoR2 receptors play a role in the reduction of hepatic and skeletal muscle insulin resistance[174]. LAR has been correlated with markers of insulin resistance such as HOMA-S and hyperinsulinaemic euglycaemic clamp studies in a large cohort of individuals without prior history of T2DM[145], with hyperinsulinaemic euglycaemic clamp studies in patients with prior history of T2DM[192], with HOMA-IR in a cohort of adults with a prior diagnosis of T2DM [193, 194] and in children with no prior history of T2DM[195]. LAR is a predictive marker along with a high calf to thigh ratio in healthy individuals for the development of T2DM [196]. LAR has been correlated with HOMA-IR in pregnancy with a positive correlation with higher baseline BMI[197], and in patients with a history of the polycystic ovarian syndrome, it has been validated as a predictor of disease activity and has been positively correlated with

other markers of insulin resistance and BMI[198]. In children where a diagnosis of T2DM vs. T1DM is difficult, adiponectin to leptin ratio can be used with its levels being low in children with T2DM in comparison to children with T1DM [199]. In obesity and T2DM, leptin levels are known to be high with lower levels of adiponectin indicating a dysfunctional state in the adipose tissue secondary to the metabolic disorders, creating a state of chronic inflammation[200], fibrosis and impaired angiogenesis [201]. LAR, when measured inversely in the form of adiponectin to leptin ratio, has been negatively correlated with markers of inflammation such as c-reactive protein and serum amyloid A [202]. LAR is a predictive marker of carotid intima-media thickness [203]. With the above understanding of LAR so far and with insight into its role in T2DM after weight loss interventions, LAR could potentially serve as a marker of response to treatment and improvement in overall metabolic dysfunction.

1.9: Aims and objectives:

1. To explore the use of and to determine the effects of a 24-week milk-based meal replacement programme on anthropometric variables including weight, BMI, excess body weight % and metabolic variables including systolic and diastolic blood pressure, alanine aminotransferase (ALT), Hba1c and lipid profile, pre and at 24 weeks in a retrospective cohort study of adults with severe obesity.
2. To establish the changes in leptin, adiponectin, and leptin: adiponectin ratio (LAR), pre and post at one year, in a prospective cohort study of patients with severe obesity undergoing sleeve gastrectomy as a form of bariatric surgery. To compare the changes in leptin, adiponectin, and LAR with anthropometric variables including weight, BMI, and excess body weight % and metabolic variables including systolic and diastolic blood pressure and Hba1c and to determine a relationship between the change in insulin resistance in the form of change in LAR and weight loss achieved via bariatric surgery in the form of sleeve gastrectomy.
3. To determine the influence of a 24-week milk-based meal replacement programme on changes in leptin, adiponectin, and LAR, pre and post programme, in a prospective cohort study of patients with severe obesity. To compare leptin, adiponectin, and LAR changes to the anthropometric variables including weight, BMI, excess body weight %, and metabolic variables including systolic and diastolic blood pressure, and Hba1c. To determine a relationship between the change in insulin resistance in the form of change in LAR and weight loss achieved via a milk-based meal replacement programme.

Chapter Two:

Effects of a milk-based meal replacement programme on weight and metabolic characteristics in adults with severe obesity.

2.1: Background:

Due to the higher prevalence of severe obesity [204], the availability of effective interventions for affected patients is increasingly required. Undoubtedly, bariatric surgery is an efficacious and cost-effective intervention [205]. However, only 1 out of 10 patients with obesity who meet the criteria for bariatric surgery agree to have this option [206]. Other branches of bariatric care need to be improved and assessed. The benefits of structured lifestyle interventions have been established in many studies among different patient subgroups, including those with comorbidities such as non-diabetic hyperglycemia [207], prevalent cardiovascular disease [208] or established T2DM [209, 210]. However, meaningful, long-term weight loss is difficult to be maintained with lifestyle modification alone [211]. Recently, a large general practice-based cohort study of adults with severe obesity in the UK found that the proportion achieving 5% weight loss after one year was just about 12.5% for men and 14.3% for women [212]. In other words, most patients with severe obesity in the community attending primary care-based services do not manage to achieve even moderate weight loss. For some, it is believed that a significant improvement in health requires a weight loss of 10% [210]. However, the improvements in health in bariatric patients were observed after more moderate weight loss of 2.7% [213].

The use of low energy liquid diets (LELDs, ~1200 kcal/ day) as part of intensive lifestyle modification programmes for the treatment of obesity has been described in many studies. The ideal weight loss is approximately 10kg [209, 214]. Though, weight regains limits the longer-term efficacy of these interventions [211, 215]. Side effects of low energy diets are constipation, dizziness, alopecia, nausea, headache, diarrhoea, abdominal pain, and cholelithiasis [216]. These side effects are related to a reduction in fibre consumption, the diet itself, medications, inadequate fluid intake, or long intervals between meal replacements [216]. Implementing meal replacement interventional studies is challenging. Some of the main problems highlighted in the literature include maintaining sufficient trial numbers [209, 214], and combating attrition rates, as attrition rates can be as high as 50% [211, 217]. Another design limitation is the cost of the

intervention. Commercial meal replacement programmes can be expensive, with some analyses suggesting they are prohibitively cost-ineffective [218]. Semi-skimmed milk is a potential low-cost alternative to commercially produced meal replacement supplements. Milk whey protein attenuates muscle loss [219] and preserves myofibrillar protein synthesis [220] in adults with obesity during very low-calorie diets. Milk reduces appetite, calorie intake, and body weight [221] and alters post-prandial glucose and lipid metabolism [222] in men with obesity. In mice, milk casein-derived peptides reduce high-fat diet-induced adipose tissue inflammation [223]. A recent trial showed that drinking low fat milk made children feel fuller and eat less later in the day compared to juice or water [224].

There is limited data regarding the feasibility, efficacy, and safety of a semi-skimmed milk-based meal replacement programme for adults with severe obesity. In the bariatric clinic of University Hospital Galway, we introduced a milk-based LELD in 2013, providing patients with approximately 1200 kcal/day over eight weeks, with a subsequent 16-week period of food reintroduction, as described below. The aim of this study was to conduct a retrospective analysis of patient characteristics, key anthropometric and metabolic outcomes, and attrition rates in patients attending the programme, in order to inform the more robust design of prospective studies or potential randomized controlled trials for future evaluations of the efficacy and safety of a semi-skimmed milk-based meal replacement programme.

2.2: Methods:

2.2.1: Study design, population and setting:

This was a single-center, retrospective cohort study, conducted in accordance with the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guidelines for Observational study [225]. The study population included patients with a history of severe obesity who were referred to our milk-based meal replacement programme. During the programme patients attended the bariatric clinic every two weeks for 24 weeks (14 visits in total), met the nurse, a dietician, and physician at each visit, had periodic blood tests performed, and had weight, height, and blood pressure measurements taken. All baseline and follow-up measures for the programme were conducted in the Bariatric Medicine Clinic at the Centre for Diabetes, Endocrinology, and Metabolism in Galway University Hospitals (GUH).

2.2.2: Inclusion and Exclusion criteria:

Male and female patients aged 18 years or older referred to the bariatric service for assessment of severe obesity were included in the study. Severe obesity was defined as a BMI ≥40 kg m^{-2} (or ≥35 kg m^{-2} with co-morbidities such as T2DM or obstructive sleep apnea syndrome). Patients must have been willing to attend all the 14 scheduled study visits. Females of childbearing potential who were pregnant, breastfeeding, or planning to become pregnant or were not using effective contraceptive methods were not considered eligible for the programme. Patients with recent myocardial infarction (within six months), untreated arrhythmia, untreated left ventricular failure, recent cholelithiasis (within the past year), hepatic dysfunction defined as acutely deranged liver function tests of 15 times the upper limit of normal or established liver cirrhosis or renal dysfunction defined as an eGFR of <30ml/min, type 1 diabetes, untreated major psychiatric disorders, eating disorders, cancer, previous bariatric surgery, a BMI <35 kg m^{-2} or those deemed unlikely to attend for the full programme (e.g. frequent clinic non-attendance) were not considered as participants to the programme.

The milk-based meal replacement programme was a therapeutic option for all patients attending the service irrespective of their wish to participate in the research studies that were ongoing in the department, and patients included in this study were the ones who consented and were happy to be a part of the study. The patients who started the programme but did not complete it were followed similarly to any other patient attending the service with a usual six-monthly follow-up with an option to be seen earlier than this, depending on the clinical requirement. They had other therapeutic options such as a lifestyle intervention in the form of the Croi-Clann programme, which is an eight-week supervised lifestyle and dietary intervention, they had an option of medical treatment with either liraglutide or semaglutide, they had access to psychological assessment and treatment with our bariatric psychologist Dr. Mary Hynes, and they all had an option to be enrolled on the waiting lost for surgical intervention if they fulfilled the criteria and requirements for it.

2.2.3: Ethics approval:

The study was approved by the Galway University Hospitals' Central Research Ethics Committee in December 2017 (ref CA 1900). As the programme was part of standard clinical care for patients attending our service between 2013 and 2016 and was not a prospective research study, we did not prospectively obtain written informed consent from patients to use their data for research purposes. Considering recent changes in European legislation regarding the use of personal data (the General Data Protection Regulation (GDPR)), we have only used data in this

study from the subgroup of patients who agreed to this retrospectively and provided written informed consent.

2.2.4: Measurements:

Weight was measured on a Tanita® scale and height with a Seca® wall-mounted stadiometer, according to departmental standard operating procedures. Blood pressure was measured with an automated oscillometric device (Omron®) using a large cuff on the right arm after participants had been seated quietly for five minutes. Three measures were recorded at one-minute intervals. A 12-lead electrocardiogram was performed to exclude occult ischemic heart disease or cardiac arrhythmia. Blood was drawn after an overnight fast for glucose, renal, and lipid profiles. All blood samples were processed locally in the Galway University Hospitals' Department of Clinical Biochemistry (certified to ISO 15189 2007 accreditation standard). HbA1c was measured with HPLC (Menarini® HA8160 auto-analyzer). Total cholesterol was measured using the CHOP-PAP method. HDL-cholesterol and triglycerides were measured using the enzymatic and the GPO-PAP methods, respectively (COBAS® 8000 modular analyzer). Low-density lipoprotein (LDL)-Cholesterol was derived with the Friedewald equation. Information relating to antihypertensive, lipid-lowering, and antidiabetic medication use at baseline and at the end of 24 weeks was extracted from the medical records of each participant.

2.2.5: Intervention:

The milk-based LELD consisted of three continuous eight-week phases, each with fortnightly visits to the bariatric medicine clinic. During the first (weight loss) phase from weeks one to eight inclusive, an exclusively milk-based liquid diet was prescribed, consisting of approximately 2.5 liters per day of semi-skimmed milk divided into seven portions throughout the day into equal doses, with additional sodium replacement, vitamin, mineral, and fiber supplementation, equating to approximately 1200 kcal/day. The precise caloric content and volume of milk were determined by baseline body weight, according to a departmental standard operating procedure. Throughout this phase, renal and liver profiles were assessed every two weeks, and the patient was seen by the consultant endocrinologist, bariatric nurse, and dietitian at each visit. During the second phase (weight stabilization) from weeks nine to sixteen inclusive, there was a gradual re-introduction of low-calorie meals from a set menu over eight weeks, according to protocol under the supervision of the bariatric dietitian with fortnightly visits continuing. During the third phase (weight maintenance) from weeks 17 to 24 inclusive, the liquid

component of the diet was stopped completely, and a fully solid isocaloric diet was restarted, based on individualized meal plans, under the supervision of the bariatric dietitian.

2.2.6: Outcome Measures:

The primary outcome measure was body weight. Within the cohort, there were distinct subgroups of patients for whom specific outcomes were more relevant, such as those with type 2 diabetes. There were several secondary outcome variables, including BMI, percentage excess body weight, blood pressure, HbA1c, and lipid profiles. We set the threshold for an elevated HbA1c ("high HbA1c") as "yes" if HbA1c ≥ 48 mmol/mol. We derived an *a priori* categorical variable for prevalent "dyslipidaemia" as "yes" if LDL-Cholesterol was ≥1.8 mmol/l in patients with diabetes or ≥3.0 mmol/l in patients without diabetes at baseline, based on European Society of Cardiology guidelines [226]. Then we defined the presence of poor blood pressure control ("hypertensive") as "yes" if the systolic blood pressure (SBP) was ≥150 mmHg (in patients ≥60 years) or ≥140 mmHg (in patients <60 years) or if the diastolic blood pressure (DBP) was ≥ 90 mmHg (regardless of age) [227]. Of note, we were unable to classify patients as having hypertension or not as we did not prospectively record this in the medical notes in a consistent fashion, though we did record whether specific blood pressure medications were used at baseline and follow-up. We repeated the categorization of all the above variables at each time point over 8, 16, and 24 weeks. Lastly, we derived a categorical variable "achieved 10% weight loss" as "yes" or "no," depending on whether the total percentage of body weight loss was above this threshold at 8, 16, and 24 weeks.

2.2.7: Statistical methods:

Summary statistics (mean, standard deviation, range (or for categorical variables, the number, n, and proportion, %)) for age, sex, height, diabetes status, weight, BMI, severe BMI status, % EBW, SBP, DBP, high HbA1c status, hypertensive status, dyslipidemia status, achieved 10% weight loss, total-, LDL-, HDL-cholesterol and triglyceride and HbA1c were obtained for times 0, 8, 16 and 24 weeks. We derived a surrogate measure of insulin resistance from the triglyceride: HDL-cholesterol ratio [228, 229]. Triglyceride: HDL-cholesterol ratio was shown to be the best predictor of insulin resistance and myocardial infarction in dyslipidemic patients who are at high risk of cardiovascular disease [228, 229]. In order to convert our mmol/l values to the equivalent United States values (mg/dl), we applied a conversion factor of 38.67 for HDL-cholesterol and

88.57 for triglycerides. Information on reasons for withdrawal from the intervention was not routinely recorded.

Continuous explanatory variables were compared using the two-sample t-test or Mann Whitney Test as appropriate, while categorical explanatory variables were compared using the Person's Chi-Square test. For completers, repeated-measures ANOVA was used to determine whether there were statistically significant changes over time in outcome measures. All analyses were performed using SPSS version 24.

2.3: Results:

Between January 2013 and Oct 2018, 260 patients were enrolled into the milk-based meal replacement programme at the Bariatric Medicine clinic in Galway University Hospitals. Of these, 139 (53.5%) completed all 24 weeks of the intervention, with 121 (46.5%) discontinuing the intervention. From 139 completers, 105 (75.5%) agreed to participate in this study and provided written informed consent. Given that 1867 new patients were seen in our bariatric service over the six-year study period, 13.9% of newly referred bariatric patients ultimately participated in our milk programme.

The baseline characteristics of the 105 patients who completed the intervention and consented to study participation are described in table 1. Of these, 56 (53.3%) were female, and the mean age was 51.1±11.2 (range 18-71.6) years. Obesity-related Comorbidities were prevalent, with 35.2% of patients diagnosed with diabetes, 61.9% treated for hypertension, and 40.9% on lipid-lowering therapy. Changes in anthropometric and metabolic characteristics in intervention completers at 8, 16, and 24 weeks are shown in table 2.3.1. There was a 22.9±9.5 kg reduction in weight as anticipated (Figure 2.3.1), with a reduction in BMI of 8.0±3.2 kg m^{-2} (*P*<0.001). The proportion of patients losing 10% or more of their body weight at weeks 8, 16 and 24 was 59 %, 87.6 % and 86.7%, respectively (*P*=0.002), the proportion loosing 15% or more was 11.4%, 43.8%, 48.6% respectively (P=0.002).

There were no statistically significant changes in the systolic or diastolic blood pressures over time, but the number of completers taking antihypertensive therapy fell from 68 (64.7%) at baseline to 37 (35.2%) at 24 weeks, a reduction of 29.5% (*P*<0.001). Specifically, of 25 (28.5%) taking angiotensin receptor blockers at baseline, 15 (14.2%) remained on these at follow-up, a reduction of 40% (*P*<0.001).

Table2.3.1: Changes in anthropometric and metabolic variables over time in 105 patients completing the milk programme.

	Week 0	Week 8	Week 16	Week 24	P-value
Anthropometric characteristics					
Weight (Kg)	144 (27.6)	128.3 (25.2)	122.5 (24.4)	121.1 (25.0)	<0.001
BMI (kg/m^2)	50.6 (8.0)	45.1 (7.5)	43.1 (7.4)	42.6 (7.6)	<0.001
EBW (%)	102.5 (32.0)	80.4 (30.1)	72.4 (29.6)	70.4 (30.4)	<0.001
Severe Obesity: N (%)	102 (97.1)	90 (85.7)	75 (71.4)	68 (64.8)	0.017
Metabolic characteristics					
Systolic BP (mmhg)	127.5 (13.4)	123.3 (13.8)	124.2 (14.3)	122.9 (14.6)	0.073
Diastolic BP (mmhg)	70.0 (10.7)	69.4 (11.6)	68.9 (11.5)	70.7 (11.2)	0.348
Hba1c (mmol/mol)*	38.8 (3.7)	36.0 (3.9)	35.1 (3.2)	35.0 (3.4)	<0.001
Hba1c (mmol/mol)**	66.3 (13.0)	53.3 (14.0)	48.3 (13.7)	48.4 (13.5)	<0.001
ALT (i.u./L)	35.2 (25.4)	36.7 (20.7)	27.5 (12.1)	24.8 (13.2)	<0.001
Total Cholesterol (mmol/l)	4.6 (0.9)	3.9 (0.9)	4.2 (1.0)	4.4 (1.1)	<0.001
LDL-Cholesterol (mmol/l)	2.7 (0.8)	2.2 (0.8)	2.6 (0.9)	2.6 (0.9)	<0.001
High LDL-Cholesterol: N (%)	49 (46.7)	26 (24.8)	41 (39.0)	46 (43.8)	<0.001
HDL-Cholesterol (mmol/l)	1.2 (0.3)	1.1 (0.3)	1.1 (0.3)	1.2 (0.3)	<0.001
Triglycerides (mmol/l)	1.8 (0.7)	1.4 (0.5)	1.3 (0.5)	1.3 (0.5)	<0.001
Triglyceride:HDL ratio	3.7 (2.2)	3.0 (1.3)	2.8 (1.4)	2.6 (1.3)	<0.001

*Patients with no history of type 2 diabetes **patients with a history of type 2 diabetes. P values were calculated using repeated-measures ANOVA.

	Week 0	week 8	p value	week 16	p value	week 24	p value
Anthropometric characteristics							
Weight (Kg)	144 (27.6)	128.3(25.2)	<0.001	122.5(24.4)	<0.001	121.1(25.0)	<0.001
BMI (kg/m2)	50.6 (8.0)	45.1(7.5)	<0.001	43.1(7.4)	<0.001	42.6(7.6)	<0.001
EBW (%)	102.5 (32.0)	80.4(30.1)	<0.001	72.4(29.6)	<0.001	70.4(30.4)	<0.001
% Total weight loss		10.9(3.7)	<0.001	14.9(4.4)	<0.001	15.9(6.6)	<0.001
Metabolic characteristics							
Systolic BP (mmhg)	127.0 (13.4)	123.9(13.8)	0.031	124.2(14.3)	0.048	122.9(14.6)	0.013
Diastolic BP (mmhg)	70.0 (10.7)	69.4(11.6)	0.579	68.9(11.5)	0.318	70.7(11.2)	0.556
Hba1c (mmol/mol)*	38.8 (3.7)	36.0(3.9)	<0.001	35.1(3.2)	<0.001	35.0(3.4)	<0.001
Hba1c (mmol/mol)**	66.3 (13.0)	53.3(14.0)	<0.001	48.3(13.7)	<0.001	48.4(13.5)	<0.001
ALT (i.u./L)	36.0 (25.5)	37.4(20.9)	0.515	28.5(13.4)	<0.001	24.7(13.1)	<0.001
Total Cholesterol (mmol/l)	4.6 (0.9)	3.9(0.9)	<0.001	4.2(1.0)	<0.001	4.4(1.1)	0.012
LDL-Cholesterol (mmol/l)	2.6 (0.8)	2.2(0.8)	<0.001	2.5(0.95)	0.36	2.6(0.9)	0.813
HDL-Cholesterol (mmol/l)	1.2 (0.3)	1.1(0.3)	<0.001	1.1(0.3)	0.001	1.2(0.3)	0.313
Triglycerides (mmol/l)	1.8 (0.7)	1.3(0.4)	<0.001	1.3(0.5)	<0.001	1.3(0.5)	<0.001

*Patients with no history of type 2 diabetes **patients with a history of type 2 diabetes. P values were calculated using the student t pair test.

Of 30 (26.4%) taking angiotensin-converting enzyme inhibitors at baseline, 21 (20%) remained on these at follow-up, a reduction of 30.0% (*P*<0.001). Of 24 (22.9%) taking calcium channel blockers at baseline, 6 (5.7%) remained on these at follow-up, a reduction of 75% (*P*<0.001). Of 31 (29.5%) taking beta-blockers at baseline, 28 (26.7 %) remained on these at follow-up, a reduction of 9.7 % (*P*<0.001). Of 31 (29.5%) taking diuretics at baseline, 16 (15.2%) remained on these at follow-up, a reduction of 48.4% (*P*<0.001). Of 6 (5.7%) taking alpha-blockers at baseline, 2 (1.9%) remained on these at follow-up, a reduction of 66.7 % (*P*<0.001).

There were statistically and clinically significant improvements in all components of the lipid profile, though unexpectedly, these were most pronounced at 8 weeks and were somewhat attenuated by 24 weeks, as shown in table 2.3.1, despite the progressive weight loss observed during that time. Moreover, the reduction in the proportion of patients with elevated LDL cholesterol from 46.7 % to 43.8 % between baseline and follow-up (*P*<0.001). We were careful to avoid either the introduction or cessation of any lipid-lowering therapy during the duration of the intervention. Of 39 (37.1%) completers who were taking statin therapy at baseline, all but one continued this throughout the intervention, with four patients also taking ezetimibe at baseline and follow-up. The reduction in the triglyceride: HDL cholesterol ratio was consistent with an increase in insulin sensitivity with the intervention.

I noted a significant reduction in HbA1c over time in the 37 patients with diabetes, equivalent to a 16.3±13.6 mmol/mol reduction by 24 weeks, with an effective normalization to the diagnostic threshold for diabetes of 48 mmol/mol (*P*<0.001). There was also a significant reduction in HbA1c in patients without prevalent T2DM. Of 10 patients requiring insulin at baseline, five (50%) had stopped it by 24 weeks (*P*<0.001). Of 15 patients taking sulphonylureas, 13 (86.7%) stopped these (*P*<0.001), while 14 (77.8%) of 18 patients taking GLP-1 receptor agonists had stopped these by 24 weeks (*P*<0.001). Similarly, seven of nine (77.8%) patients stopped dipeptidyl peptidase inhibitors (*P*<0.001), while one patient taking pioglitazone discontinued this at the start of the intervention. Three of six patients taking sodium-glucose co-transporter 2 (SGLT2) inhibitor drugs remained on these throughout the intervention (*P*<0.001), while 35 of 38 patients remained on metformin throughout (*P*<0.001). The number of patients on two or more antidiabetic medications came down from 32 to 9, a reduction of 71.9 % (*P*<0.001). In the five patients who remained on insulin, their dose came down from 123.1±21.6 to 28.7±13.4 units per day (*P*<0.001), a reduction of 76.7%.

In patients with diabetes who completed the programme were slightly older and with a lesser degree of obesity than completers without diabetes. I observed a worse lipid profile in patients without diabetes, which I think may be due to a higher prevalence of statin use in patients with

diabetes versus those patients without diabetes (64.9 versus 22.1 %, P<0.001). Notwithstanding the differences in adiposity at baseline, there was no difference in the anthropometric response to the intervention in completers with versus those without diabetes, as shown in figures 2.3.1-2.3.4.

To examine if changes in weight at different timepoints are predictive of future weight, I used linear regression analysis in SPSS 26, with weight at 24 weeks as the dependent factor and weight at eight weeks as an independent factor, and the result of the analysis showed a positive association with a β value of 0.947 and a positive correlation with an R^2value of 0.89 and findings were significant at a p-value of <0.001. I then analyzed if weight at 16 weeks predicted weight at 24 weeks using the same linear regression analysis, and the results were significant again at a p-value of <0.001, a positive association was seen with a β value of 1.0 and a positive correlation with an R^2value of 0.96. There was also a significant association between weight at 24 weeks as the dependent variable and % of total weight loss at 16 weeks as the independent variable with a βvalue of -1.9 with a p-value of 0.006. There was also a significant association seen between % total weight loss at 24 weeks as the dependent variable and % total weight loss at eight weeks with a β value of -.025 and p-value of 0.023 and with % total weight loss at 24 weeks as the dependent variable and % total weight loss at 16 weeks an independent variable with a β value of 1.2 with a p-value of <0.001. There was no association or correlation seen with weight at 0 weeks and the final weight, and there was no association or correlation seen with age or sex of the participants with the final weight.

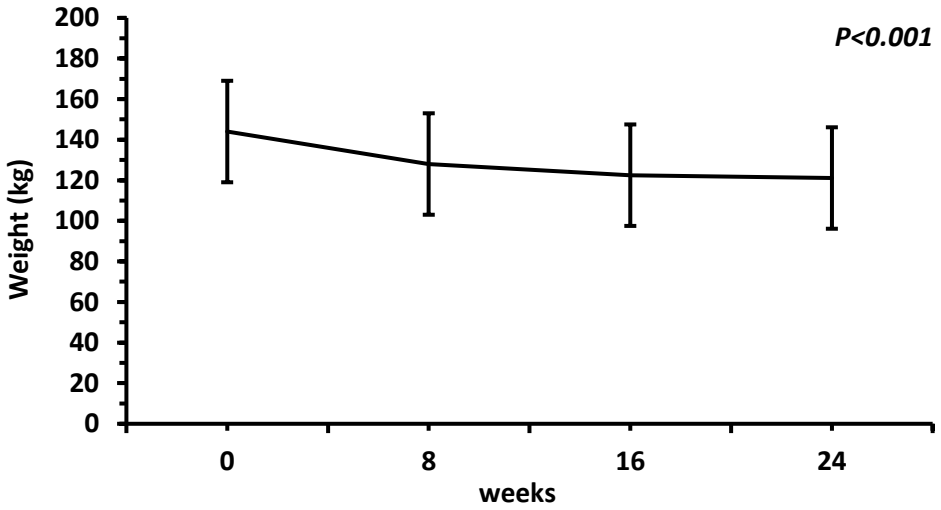


Figure 2.3.1a: Changes in weight over 24 weeks.

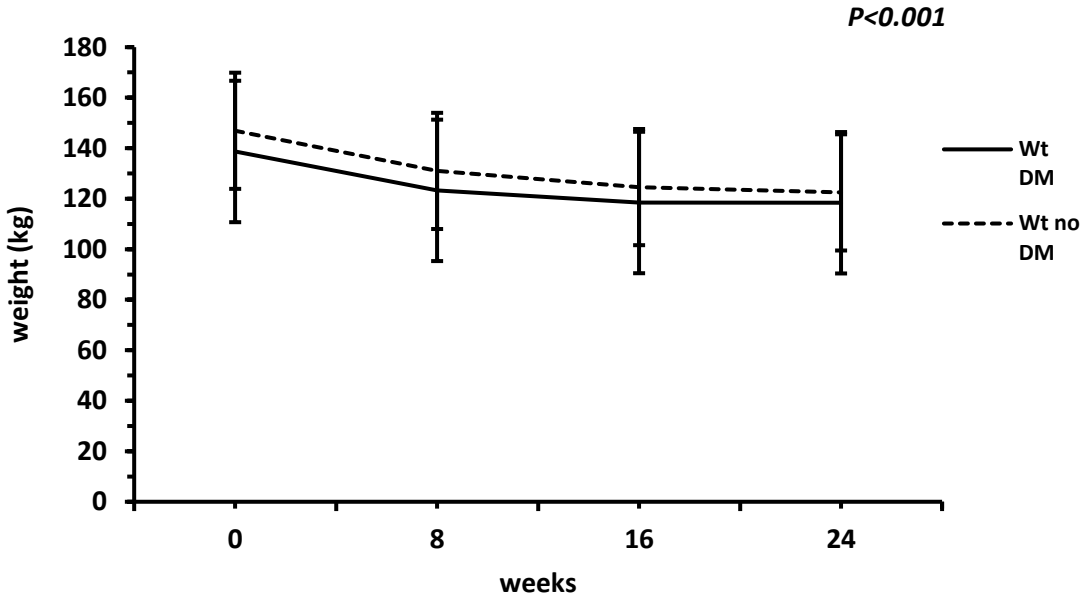


Figure 2.3.1b: Changes in weight by DM history.

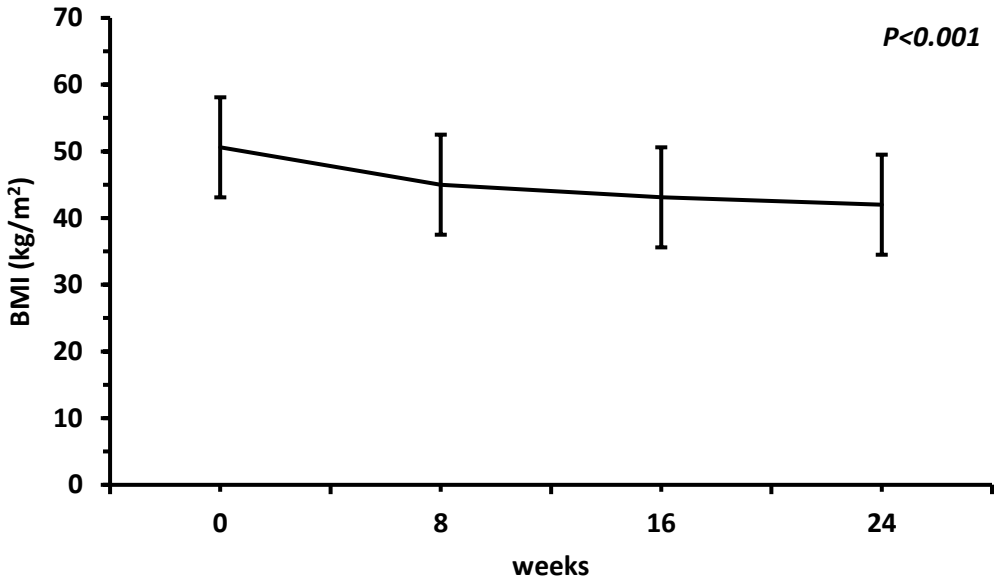


Figure 2.3.2a: Changes in BMI over 24 weeks.

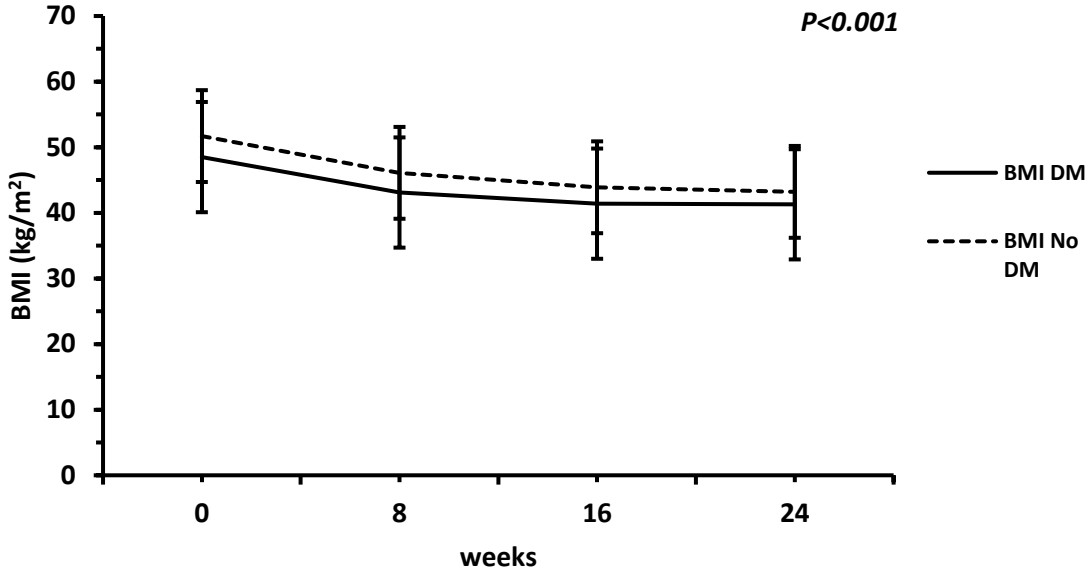


Figure 2.3.2b: Changes in BMI by DM history.

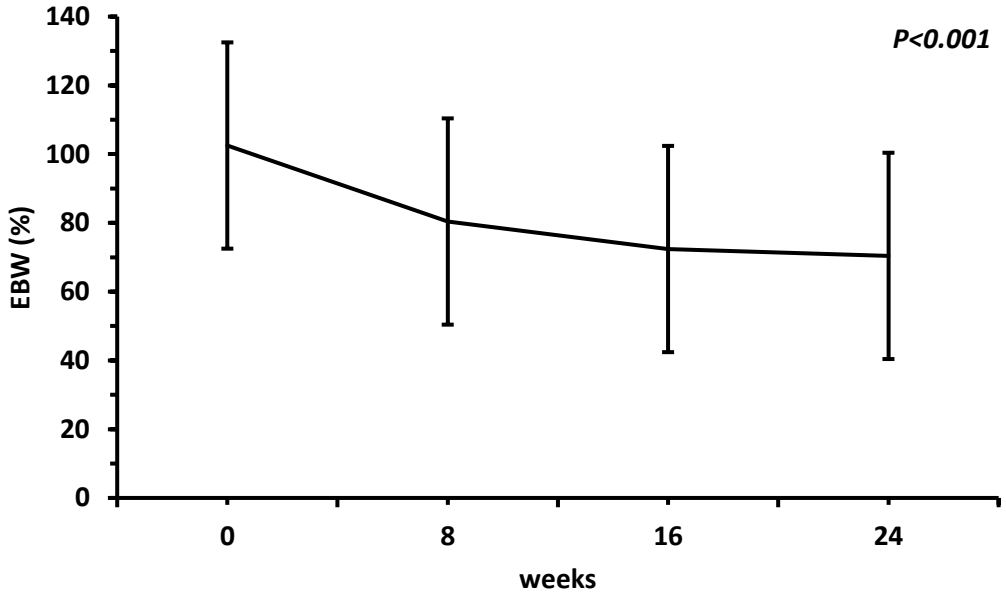


Figure 2.3.3a: Changes in EBW% over 24 weeks..

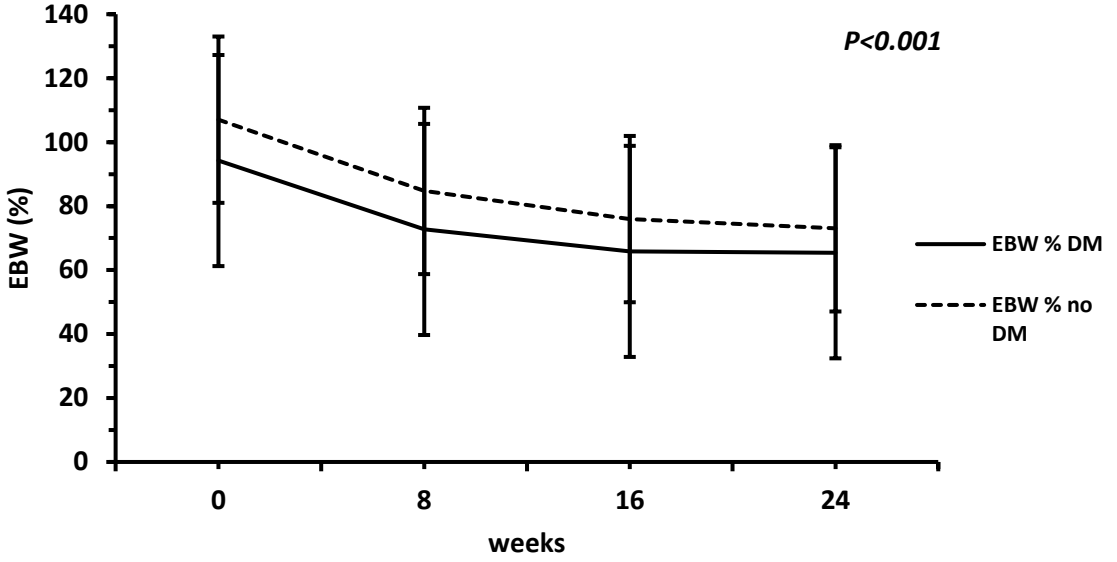


Figure 2.3.3b: Changes in EBW% by DM history.

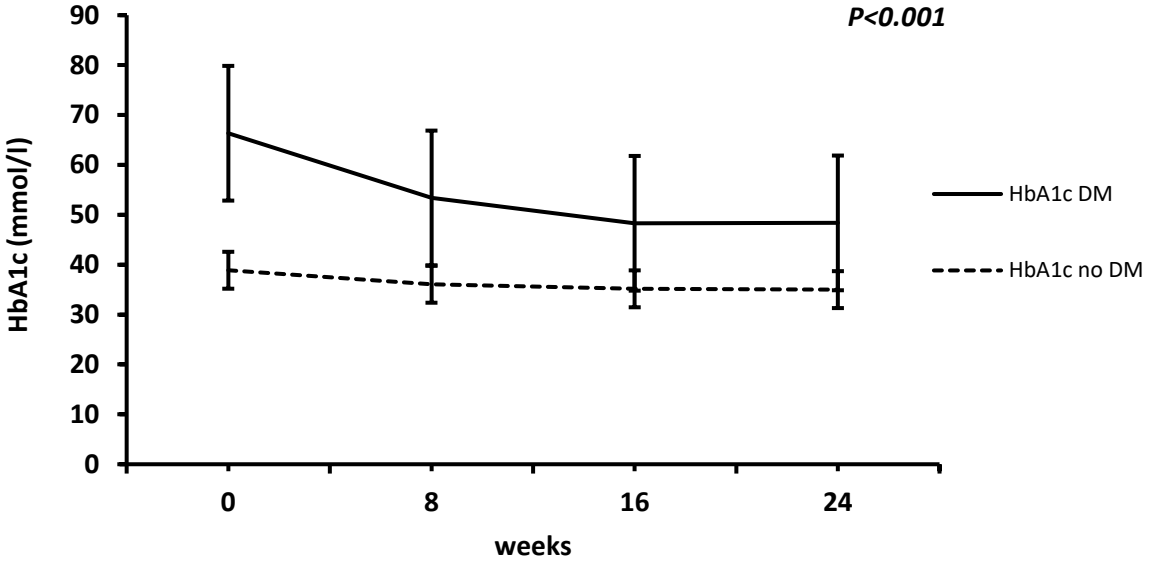


Figure 2.3.4: Changes in Hba1c over 24 weeks.

2.4: Discussion:

This study showed significant improvements in excess weight and associated anthro-metabolic characteristics. These changes were significantly greater than those observed with a more conventional lifestyle modification programme which is utilized in a similar patient population, where mean weight loss in completers of a ten-week structured diet and physical activity programme was 2.7kg [213].

Notwithstanding the relatively high drop-out rate, the fact that 54% of patients were able to tolerate the intensive schedule of clinic visits and the very significant curtailment of dietary intake, particularly in the first eight weeks, is surprising to many. The mechanisms underlying a likely reduction in appetite are unknown. Ketosis has been shown to attenuate the increases in ghrelin and appetite that occur with dietary restriction [230], and in recent years, there has been a growing interest in the role of therapeutic ketosis in weight loss interventions [231]. Unfortunately, ketones are not routinely measured as part of the milk intervention, so their contribution to the findings of this study is uncertain. These could and should be measured in future clinical trials of the intervention. It may be that the unique constituents of milk might account for some of the benefits seen in this intervention, though an aetiological trial with an equivalent non-milk-based meal replacement substrate would need to be done to clarify this issue.

I have probably underestimated the proportion of patients with dyslipidaemia by focussing only on LDL cholesterol to define this arbitrarily rather than applying cut-offs to total cholesterol and triglycerides also. However, I believe that the large reductions in these individual components of the lipid profile are convincing and compelling, particularly given that statin therapy was neither initiated nor stopped during the intervention.

A limitation of the study was using a convenience sample size without a prior sample size calculation; in order to overcome this limitation, I have done a retrospective sample size calculation. Retrospective sample size analysis was done by calculating effect size and power of the intervention with weight loss at 24 weeks as the predictor of effect size, using univariate analysis of variance on SPSS 26, the results showed a power of 1.0, with an effect size of 0.99 with a p-value of the calculation at <0.001 which was quite significant. I then used G power 3.1.9.7 to calculate the sample size required to achieve a positive result using student t pair, with the α set at 0.05 (5% chance of false-positive result), power set at 0.99, which will give is a β value of 0.01 (1% chance of false-negative result), and effect size set at 0.99 as calculated, the sample size required to achieve or replicate a similar result was found to be 21 with a critical t at 2.08. I then calculated the sample size with similar parameters of α, β, effect size, and power to achieve or replicate a similar result using

repeated measures ANOVA analysis, and we found a sample size requirement of 32 with an f value of 2.94.

Arguably the most important limitation of this study is the lack of follow-up data for the patients who started the intervention but dropped out. However, the focus of this study design was exclusively on patients who completed the intervention. Future trials will incorporate additional design elements to capture data on the duration of therapy intervention and dietary intervention on subjects that dropped out. It must be emphasized, therefore, that this work is not a description of the effectiveness or efficacy of the milk programme but serves as a useful estimate of the effect size of the intervention as well as the attrition rate to inform the development of future randomized controlled trials. It might be unlikely, but it is possible that some of those who dropped out may have had significant weight regain or serious adverse events that precluded continued participation, which was not pointed out, which is why I have avoided any attempt to compare data in completers and non-completers using last or mean observation carried forward analyses, for example. Nor was there evidence regarding the safety of the intervention, as this would require the prospective collection of information as well as ongoing follow-up in patients who dropped out of the intervention. While participant retention in some studies is good[214, 232], attrition rates similar to ours here are not uncommon [90, 217, 233]. Thus, our per-protocol analyses of outcome changes in intervention completers is likely to have introduced some bias, residual confounding, and possibly type 1 statistical error. However, even randomized controlled trials of meal replacement programmes have had methodological limitations. In one systematic review of 45 trials of non-surgical long-term weight loss maintenance interventions in adults with obesity, only 10 had robust allocation concealment, 17 described some form of blinding, and 25 were deemed to handle incomplete data well[234]. Other reviews have confirmed that poor allocation concealment and blinding are particularly prevalent in trials of LELDs [235]. Hence, there is scope for enhanced rigor in similar future trials in order to reduce the potential for bias, residual confounding, and type 1 errors. Clearly, it would be desirable to have information about safety outcomes and patients who drop out of the intervention in these trials, consistent with STROBE guidelines [225].

2.5: Conclusions:

Notwithstanding the limitations in this study, I believe that the findings are novel and important, particularly in the context of planning a more robust prospective observational and randomized controlled trial assessment of the milk diet intervention. Such studies will need to extend the follow-up period well beyond the completion of the intervention, as weight regain is a well-established

problem in the longer term [211, 215]. Ultimately this might help to broaden and refine the range of therapeutic options for adults affected by obesity.

Chapter Three:

The leptin to adiponectin ratio (LAR) is reduced by sleeve gastrectomy in adults with severe obesity: a prospective cohort study

3.1: Background:

Obesity is an important contributor to type 2 diabetes risk, primarily through its adverse effects on insulin sensitivity [236]. Bariatric surgery to treat severe obesity leads to significant improvements in diabetes control [237], cardiovascular risk [108, 238], microalbuminuria [109], and systemic inflammation [239]. However, studies of the effects of bariatric surgery on insulin sensitivity are limited [240, 241], partly because quantifying insulin sensitivity can be challenging. For example, the gold-standard hyperinsulinaemic-euglycaemic clamp technique allows precise quantification of hepatic and skeletal muscle insulin sensitivity [131] but is technically demanding, time-consuming and expensive. The leptin: adiponectin ratio (LAR) has been validated as a robust measure of whole-body insulin sensitivity in large epidemiological studies [145, 242]. Both of these hormones are adipokines secreted exclusively by adipocytes, and they are important regulators of metabolic homeostasis. Leptin acts on the hypothalamus to regulate food intake and energy expenditure [243], and individuals with obesity are known to have higher leptin levels [244]. Conversely, adiponectin increases tissue fat oxidation and reduces circulating free fatty acids, and is lower in individuals with obesity[245]. Several studies have described reductions in leptin and increases in adiponectin in adults with severe obesity undergoing bariatric surgery [168, 238-241, 246]. Recently, investigators described changes in the adiponectin: leptin ratio (ALR) in a cohort of 25 Spanish adults with type 2 diabetes who underwent Roux-en-Y gastric bypass [247]. To date, the influence of sleeve gastrectomy on the ratio of these two hormones has not been described. I sought to determine the effects of sleeve gastrectomy on leptin and adiponectin levels as well as the LAR in adults with severe obesity, and to determine whether the magnitude of weight loss after surgery influenced the change in LAR.

3.2: Methods:

I conducted a single-centre, interventional prospective cohort study of all patients undergoing laparoscopic sleeve gastrectomy at our hospital over four months between September and December 2016. I performed follow-up measures 12.1±1 months after surgery. All study participants provided written informed consent, and the study was approved by the Galway University Hospital's Research Ethics Committee (reference C.A. 2058). The study was conducted adhering to the STROBE guidelines [248].

Inclusion criteria for bariatric surgery at our institution are consistent with those internationally. I have defined severe obesity as BMI ≥40 kgm^{-2} (or ≥35 kgm^{-2} with co-morbidities such as type 2 diabetes and obstructive sleep apnea syndrome). Male and female patients aged 18 years or older, put forward for consideration for sleeve gastrectomy by the bariatric multidisciplinary team at our institution (nurse, dietitian, physician, psychologist, surgeon) were all eligible for inclusion. Our clinical practice is to refer these patients for surgical consideration after completion of a ten-week structured lifestyle modification programme that we have described in detail previously [82]. Patients must have undertaken a formal psychological assessment of the suitability of sleeve gastrectomy for their treatment. Those with a recent myocardial infarction (within six months), untreated arrhythmia, untreated left ventricular failure, recent cholelithiasis (within the past year), type 1 diabetes, untreated major psychiatric disorders, eating disorders, undergoing cancer treatment, or a BMI <35 kg m^{-2} or those deemed unlikely to attend for post-operative follow-up (e.g. frequent clinic non-attendance) were excluded from undergoing sleeve gastrectomy. The study population was a convenience sample, and its size was determined by the number of sleeve gastrectomies done during the study period. All metabolic and anthropometric baseline and follow-up measures were conducted at our bariatric out-patient clinic.

Weight was measured on a Tanita® scale and height with a Seca® wall-mounted stadiometer. Blood pressure was measured with an automated oscillometric device (Omron®) using a large cuff on the right arm after participants had been seated quietly for five minutes. Three measures were recorded at one-minute intervals, and the average of the three was recorded. Blood samples were drawn from patients in the fasted state on the morning of their sleeve gastrectomy. HbA1c was measured using High-Performance Liquid Chromatography (HPLC) on the Menarini® HA8160 analyzer. Leptin and adiponectin were measured using separate two-site microtiter plate-based DELFIA assays manufactured by R&D Systems Europe, Abingdon UK. After the collection of each sample in the clinical setting, samples were transferred to the lab by hand by the researchers. Within 20 minutes of obtaining the samples, they were centrifuged at 3000 rpm for 5 minutes to separate blood and

plasma, and plasma samples were then labeled and stored at -40 degrees Celsius. Frozen plasma samples were then transferred in the frozen state to Addenbrookes University laboratory for measurement. The adiponectin assay measured "total" adiponectin, and analyses have found a between batch imprecision of 5.4% at 3.6 µg/ml, 5.2% at 9.2 µg/ml, and 5.8% at 15.5 µg/ml, as previously described [249]. For leptin, the between batch imprecision was 7.1% at 2.7 ng/ml, 3.9% at 14.9 ng/ml and 5.7% at 54.9 ng/ml.

All statistical analyses were conducted with SPSS® version 24. Changes in anthropometric and metabolic variables between baseline and follow-up were assessed using the student's paired t-test, assuming equal variances. The relationship between the degree of weight loss over 12 months and changes in LAR was determined using Pearson correlation and linear regression modeling.

3.3: Results:

Of 148 patients with severe obesity who were on the waiting list for sleeve gastrectomy at the time the study started, 22 were put forward for surgery (based on clinical prioritization and length of time waiting, under a short-term, government-funded "waiting list initiative"). All of these patients were invited to the study, and all agreed to participate. However, one patient subsequently died six months after sleeve gastrectomy from an aggressive oesophageal carcinoma with liver metastases which first presented three months after his sleeve gastrectomy. This was despite the patient having a normal barium swallow and ultrasound before his bariatric surgery. Four patients attended for bariatric follow-up at other institutions and were unable to attend within our specified window of 10-14 months post-surgery for follow-up measures. Thus, I report on 17 patients for whom baseline and 12-month follow-up measures were available. Twelve patients were female, and 12 had type 2 diabetes at baseline. The mean duration of diabetes was 9.9 (range 3-27) years. Mean age was 52.2±8.3 (range 39-71) years, with a follow-up interval of 12.1±1 (range 10-13) months. Baseline and follow-up anthropometric and metabolic characteristics are presented in table 3.3.1. There were substantial reductions in weight, BMI, and excess body weight over 12.1 months, as shown, with a mean weight loss of 33.0±21.6 kg and an absolute reduction in excess body weight percentage (EBW%) of 47.2±28.8 % (all $p<0.001$), equivalent to a percentage total weight loss of 24.3±12%.

There was a non-significant trend to reduced HbA1c overall after 12 months, which was more pronounced (though remained non-significant) in the subgroup of patients with diabetes. Two patients were taking insulin preoperatively, and both remained on insulin 12 months later. Of two patients taking sodium-glucose linked transported 2 (SGLT2) inhibitor therapy preoperatively, one remained on it, and a second had stopped it. A third of patients had started the drug by the time of

their follow-up. Of 12 patients taking metformin at baseline, three had stopped this by 12 months. Of five patients taking glucagon-like peptide 1 (GLP1) agonist therapy at baseline, one remained on this at follow-up. All three patients on gliptin therapy for diabetes had this stopped at the time of their surgery, while no patients were taking sulphonylureas or glitazones at baseline or follow-up. Ten patients were taking statin therapy at baseline, and this was continued in all patients routinely post-operatively. There was a reduction in the prevalence of patients taking antihypertensive therapy from 71 to 29 % over 12 months.

Overall, there was a (non-significant) trend to reduced leptin levels, while there was a more pronounced and statistically significant increase in adiponectin. This equated to an overall reduction in the LAR, which was consistent with a 70.9% increase in insulin sensitivity in the cohort 12 months after sleeve gastrectomy. When examining changes in the subgroup of patients with diabetes, results were similar to the group overall.

Next, I sought to determine the correlation between the percentage weight loss and changes in leptin, adiponectin, and the LAR. As outlined in figure 3.3.1(a-c), there was a modest correlation only between the percentage weight lost with leptin and with adiponectin individually, such that the percentage weight loss accounted for approximately 39.5% of the reduction in leptin (r^2=0.3948, p=0.007) and 49.6% of the increase in adiponectin (r^2=0.4961, p=0.002), respectively. However, there was a much stronger correlation seen for the LAR, such that approximately 82.2% of the reduction in LAR was accounted for by the percentage weight lost (r^2=0.8222, p<0.001).

Table 3.3.1: Baseline and follow-up anthropometric and metabolic characteristics in 17 patients, 12 months after sleeve gastrectomy.

*** Denotes subgroup of patients with type 2 diabetes at baseline.**

Variable	N	Baseline Mean	SD	Follow-up Mean	SD	p-value
Weight (kg)	17	130.6	±30.8	97.6	±21.6	<0.001
BMI (kg/m^2)	17	46.9	±7.8	35.3	±7.2	<0.001
EBW(%)	17	87.5	±31.3	41.3	±28.8	<0.001
% Total weight loss	17			24.2	±12.1	<0.001
SBP (mmHg)	17	124.4	±13.0	126.8	±18.4	0.067
DBP (mmHg)	17	72.2	±10.9	71.3	±12.5	0.83
HbA1c(mmol/mol)*	17	62	±13.2	53.3	±11.8	0.16
Leptin (ng/ml)	17	40.7	±24.9	30.9	±30.5	0.11
Adiponectin (µg/ml)	17	4.49	±1.6	8.93	±6.36	0.005
LAR (ng/µg)	17	8.89	±4.8	5.26	±6.5	0.036
LAR (ng/µg)*	12	8.2	±3.5	5.1	±3.2	0.038

Data are presented as mean ± standard deviation (or # median and interquartile range for LAR, which was not normally distributed).

All variables were compared using the paired t-test, except for LAR, which was compared using the Wilcoxon Signed Rank Test. % total weight loss was calculated using the one-sample student t-test.

BMI: Body Mass Index DBP: Diastolic Blood Pressure EBW: Excess Body Weight

LAR: Leptin: Adiponectin Ratio SBP: Systolic Blood Pressure

Fig:3.3.1a: Δ Leptin by Δ Weight

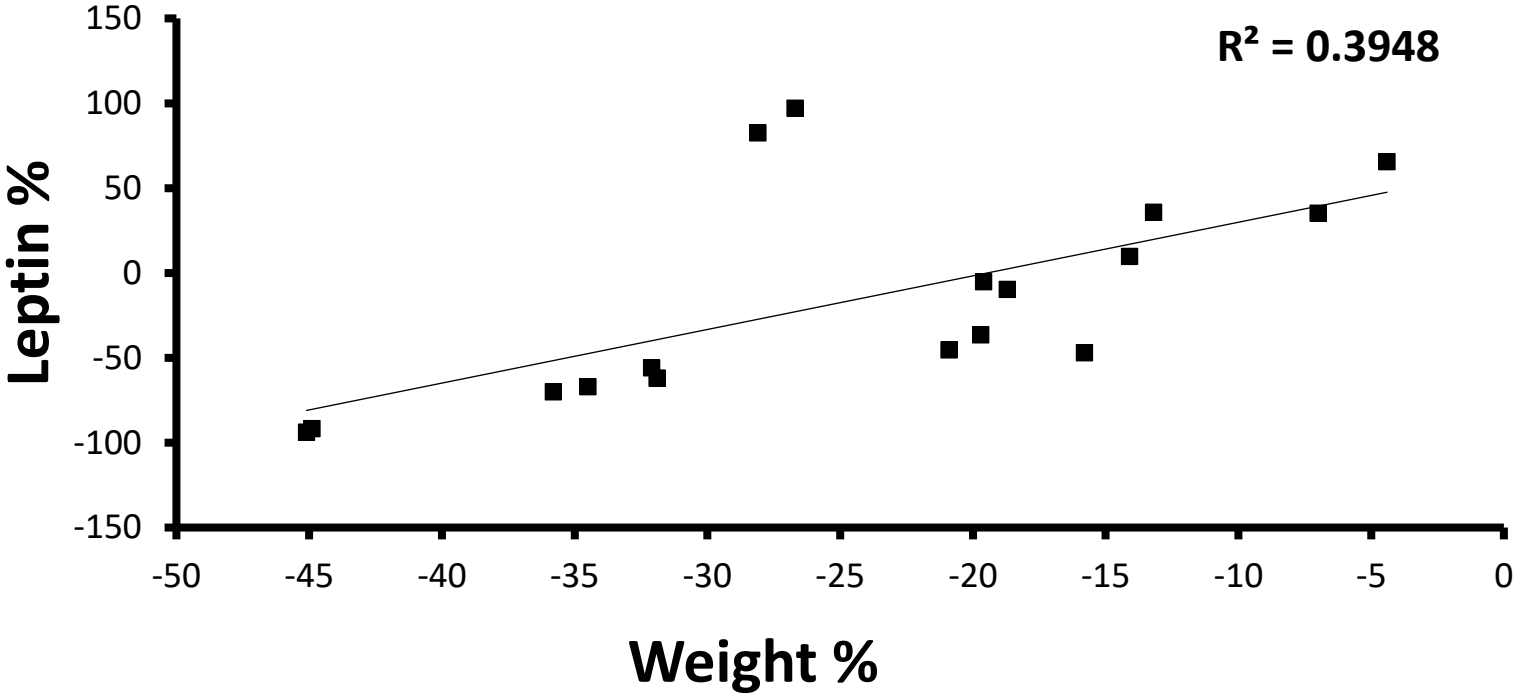


Fig:3.3.1b: Δ Adiponectin by Δ Weight

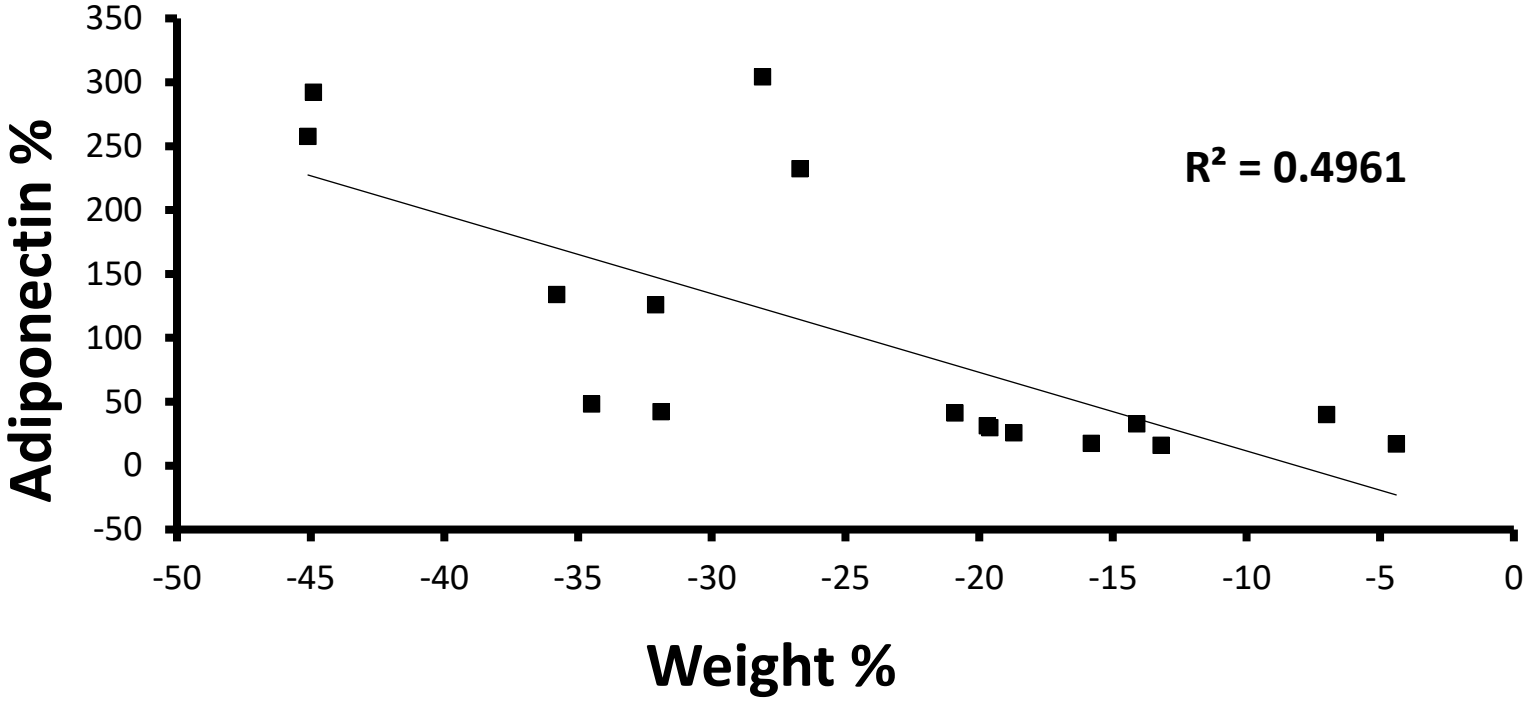
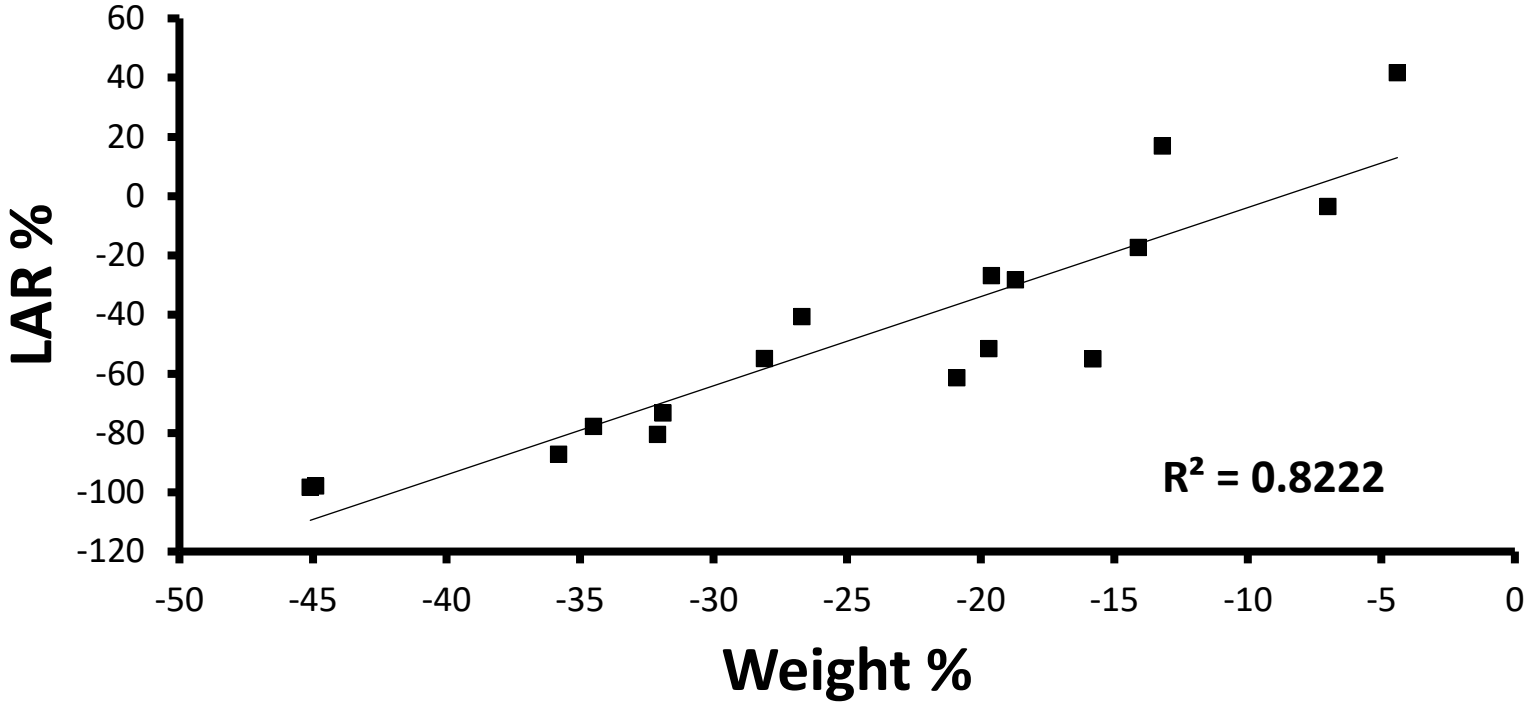


Fig 3.3.1c: Δ LAR by Δ Weight



3.4: Discussion:

I have shown that in a predominantly white cohort of adults with severe obesity who underwent laparoscopic sleeve gastrectomy, there was a substantial reduction in the LAR after 12 months, which may indicate an increase in insulin sensitivity. While several studies have measured leptin or adiponectin separately in patients after bariatric surgery [238-241, 246], I am aware of only one other study by Unamuno et al. that has considered them together as a ratio and as an indicator of adipocyte dysfunction [247]. This study, while describing an "inverted" ratio of adiponectin: leptin, found broadly similar effects on both hormones to ours, though all of their patients had type 2 diabetes, and all had undergone RYGB. Ours is the first study to consider these adipokines as a ratio in patients undergoing laparoscopic sleeve gastrectomy. I found that the reduction in LAR was driven primarily by an increase in adiponectin rather than a reduction in leptin, and these changes were proportional to the reduction in weight after surgery, again consistent with the Unamuno study. The magnitude of the changes we observed in leptin and adiponectin concentrations are broadly consistent with other studies, except that Roux-En-Y gastric bypass (RYGB) appears to have more pronounced effects on both hormones [238-240], and our findings are more consistent with those previously described after banding [241].

We can only speculate as to why we did not see a statistically significant reduction in leptin, but essentially this is either a true result, whereby sleeve gastrectomy really does not influence leptin levels, or it represents a type 2 statistical error, whereby we have failed to detect a statistically significant difference where one does in fact exist. I think the latter is more likely, and that our analysis was simply not adequately powered to detect this difference. One reason for this assertion is the relatively large reduction in leptin levels, approximately 25%, but with much larger standard deviations around each of the means (which are almost three times as large as the reduction seen) for leptin at baseline and follow-up. A second reason that we think the study was underpowered to detect a reduction in leptin was the recent publication of a randomized controlled trial confirming that leptin levels decrease after sleeve gastrectomy[250].

As the primary effect of leptin is to suppress appetite and defend against weight gain via hypothalamic signaling, it might seem counterintuitive to find a reduction rather than an increase after sleeve gastrectomy. However, this phenomenon is well described and may be due to 'leptin resistance,' whereby individuals with obesity demonstrate paradoxically high levels of circulating leptin but diminished leptin sensitivity [250]. Previous studies have suggested that decreased leptin levels after bariatric surgery do not attenuate weight loss because of compensatory increased leptin sensitivity in the hypothalamus [251]. The mechanisms by which leptin sensitivity might be restored

remain to be determined, but other adipokines such as fibroblast growth factor-21 (FGF-21) may mediate this effect via direct signaling in the central nervous system or through augmenting the secretion of adiponectin[252]. Another consideration is the interaction between leptin and adiponectin. For example, in normal-weight individuals, leptin enhances adiponectin secretion, but this effect is lost in patients with obesity through the action of caveolin-1, which attenuates leptin-dependant increases in adiponectin [253].

Increased circulating levels of adiponectin are not always indicative of improvements in health or reductions in cardiovascular risk: the so-called "adiponectin paradox" refers to the observation that while adiponectin mediates a variety of essentially beneficial effects and levels of adiponectin correlate positively with better long term cardiovascular outcomes in young, healthy populations they also correlate with increased risk of premature death in high-risk populations, especially older patients or those with ischemic heart disease, heart failure or renal failure [254]. The precise mechanisms underlying this paradox are not entirely understood, but it may be that it is unintentional or pathological rather than purposeful or therapeutic leads to a rise in adiponectin similar to that seen after bariatric surgery, with the underlying disease driving worse outcomes. Larger studies with longer follow-up could address this important consideration.

It is noteworthy that the mean HbA1c was low at baseline in our patients with diabetes. This is likely due to relatively short-term improvements in glycaemic control that occur with our pre-operative "high protein diet" that we use in order to reduce the size of the liver. Data regarding HbA1c in the period preceding surgery are not to hand. However, I don't think that this would affect our findings as it is likely that the pre-operative diet would have led to increased insulin sensitivity, reflected in a reduced LAR at baseline, thus, if anything attenuating our ability to detect a change in LAR after surgery. Put another way, the fact that we noted an increase in insulin sensitivity after surgery compared to a perioperative level that had almost certainly already been increased by the high protein diet arguably makes our findings more compelling. Future studies could incorporate measurement of HbA1c, leptin, adiponectin, and LAR just before the pre-operative high protein diet commences in order to determine the extent to which these variables change before surgery.

A key limitation of our study is its modest size (reflected in the borderline statistical significance of the reduction in LAR, for example), but the effects of bariatric surgery on metabolic outcomes and cardiovascular disease risk tend to be so profound that even in randomized controlled trials, surgical interventions require population sizes [237] that are orders of magnitude smaller than those in drug [255] or lifestyle intervention trials [256]. Our study population was a relatively heterogenous convenience sample, determined by the number of sleeve gastrectomies our institution could

perform over four months. Nonetheless, our cohort size is consistent with those in similar studies [238-241, 246], and our findings around the correlation between weight loss and reduced LAR are statistically robust. Rather than using LAR as the only index of insulin sensitivity, it would have been informative also to examine indices related to fasting glucose and insulin, HOMA[132]. I plan to do assess HOMA along with LAR in future studies in order to strengthen causal inference on the impact of bariatric interventions on insulin sensitivity.

As the limitation of the study was using a convenience sample size without a prior sample size calculation, in order to overcome this limitation, I have done a retrospective sample size calculation. Retrospective sample size analysis was done by calculating the effect size and power of the intervention with weight loss at 12 months as the predictor of effect size, using univariate analysis of variance on SPSS 26, the results showed a power of 0.85, with an effect size of 1.0 and a p-value of the calculation at 0.035 which was significant. Then G power 3.1.9.7 was used for sample size calculation to achieve a positive student t pair test, with α value set at 0.05 (5% chance of false-positive result), power of 0.85, which will give us a β value of 0.15 (15 % chance of a false-negative result) and the effect size calculated at 1.0, the sample size required to replicate a similar result was found to be 12 with a critical t at 2.21. I then calculated the sample size required to achieve a positive correlation using the spearman test as we did in our study; with similar effect size, α, β, and power, the sample size required was 15 with a critical f value of 3.88.

There were changes in diabetes medication usage within the cohort that are unavoidable in an observational study such as this, which might have influenced our results. For example, metformin is known to have a weak positive effect on insulin sensitivity [257] and potentially a lowering effect on circulating levels of leptin[202], so it is possible that the cessation of metformin diminished the reduction in the LAR observed in our patients. Also, GLP agonist therapy has previously been reported to attenuate the reduction in circulating levels of leptin after weight loss [258], so the cessation of GLP agonist therapy in four of our patients may have diminished the reduction in leptin that we observed. Similar considerations apply to the three patients who stopped gliptin medications, as these drugs decrease leptin and increase adiponectin[259]. SGLT2 inhibitors decrease leptin and increase adiponectin [260], so I do not think that initiation of this treatment in one patient and its cessation in another is likely to have led to the observed difference in LAR after the programme. Angiotensin-converting enzyme (ACE) inhibitors increase adiponectin[261], so I think their cessation would have attenuated rather than enhanced the observed reduction in LAR. Thus, we would expect that the overall reductions in the usage of these drugs would have led to attenuation, rather than an exaggeration of the measured differences in adipokines and LAR that we observed. Put another way, given that these drugs tend to “improve” leptin and adiponectin levels in

the same way that bariatric surgery does; their cessation might be expected to obscure any similar "improvements" seen after sleeve gastrectomy, rather than inducing them artefactually.

The correlation between % of the change in LAR bought upon by a relative change in % of weight loss was 82%, as evidenced in this chapter of the thesis indicating a reduction in insulin resistance; this information would compel us to explore other factors that would play a role in an overall improvement in metabolic health after bariatric surgery. These are the factors that mediate a change in energy intake and energy expenditure post-bariatric surgery. Changes in neural networks in the hypothalamus such as changes in activation or expression of agouti-related peptide (AgRP) neurons which promote hunger, and pro-opiomelanocortin (POMC) neurons which promote satiety, leads to decreased hunger, increased levels of satiety, and decreased feeling of reward associated with food intake, therefore decreasing energy intake [262, 263]. Increased glucose metabolism due to hypertrophied small intestine post-bariatric surgery and increase in physical activity leads to an overall increase in energy expenditure in patients who undergo bariatric surgery, causing an overall effect on their metabolic improvement [264, 265]. Changes in the elevation of gut hormones such as GLP-1 and peptide YY, which are produced from the L-cells of the small intestine, play an important role in inducing satiety post meals in patients after bariatric surgery[266]. Newer novel hormones such as oxyntomodulin and glicentin are also thought to induce the feeling of satiety post meals by increasing the production of GLP-1 and glucagon secretion [267]. Reduction in levels of ghrelin which is secreted in the stomach and plays a role in increasing hunger, is seen more after sleeve gastrectomy than after RYGB[268]. An increase in the secretion of bile acids and fibroblast growth factor 19 after bariatric surgery is thought to increase free fatty acid oxidation[269] and increase circulating levels of GLP-1 and peptide YY, therefore, playing a role in inducing satiety and inducing weight loss[270, 271]. Alteration in gut microbiota with increase in diversity is also associated with achieving weight loss and maintaining weight loss after bariatric surgery[272, 273]. Lastly genetic mutations can play a role in weight loss achievement and maintenance after bariatric surgery[274, 275].

Ultimately, this work could inform power calculations and sample sizes for future definitive interventions and aetiological trials in larger populations of patients to overcome these limitations and determine the efficacy of sleeve gastrectomy and other interventions to reduce insulin resistance. Whether LAR is a good way to identify "responders" to surgery or predict improvements in other obesity-related comorbidities such as type 2 diabetes or fatty liver disease remains to be determined in large, prospective studies.

3.5: Conclusions:

This single-centre, prospective cohort study of adults with severe and complicated obesity undergoing laparoscopic sleeve gastrectomy found that after twelve months, there was a substantial reduction in the leptin: adiponectin ratio and that this reduction was proportional to the amount of weight lost after surgery. Given the heterogenous nature and small size of the study population, the findings must be regarded as preliminary. Nonetheless, they suggest a change in adipocyte function consistent with improved insulin sensitivity. Further studies in larger, more specifically defined patient subgroups would help to elucidate further the relevance of adipokine measurement in these patients and the mechanistic basis for metabolic improvements after bariatric surgery.

Chapter four

Changes in the Leptin to Adiponectin Ratio are Proportional to Weight Loss after Calorie Restriction

4.1: Background:

Obesity is an important contributor to T2DM risk, primarily through its adverse effects on insulin sensitivity [236]. This is associated with a disruption of normal adipose tissue physiology, hyperplasia, and hypertrophy of adipocytes leading to a pro-oxidative, inflammatory thrombotic state, with altered production of adipokines such as leptin and adiponectin [276]. Both of these hormones are primarily and predominantly secreted by adipocytes, with some gastric production in the case of leptin [277], and they are important regulators of metabolic homeostasis. Leptin acts on the hypothalamus to regulate food intake and energy expenditure [243], and individuals with obesity are known to have higher leptin levels [244]. Conversely, adiponectin increases tissue fat oxidation and reduces circulating free fatty acids, and is lower in individuals with obesity [245]. Several studies have described changes in leptin and adiponectin levels in weight-loss interventions. A recent systematic review has shown that bariatric surgery leads to reduced leptin and increased adiponectin in adults with severe obesity [278]. Changes in these hormones have been shown to predict diabetes remission after surgery [279]. Similar reductions in leptin and increases in adiponectin have been described in adolescents [280] and adults [281] undergoing lifestyle modification for weight loss.

Calorie-restricted meal replacement programmes are a well-established treatment for T2DM and obesity [209, 214]. While improvements in glycaemic control after-meal replacement programmes are well described [87, 282-284], the mechanistic basis for these improvements is not completely understood. We have recently described changes in weight and metabolic characteristics in a retrospective cohort study of adults with severe and complicated obesity who completed an intensive six-month milk-based meal replacement programme in our regional bariatric centre [285]. Over 24 weeks, their mean body weight went from 144 to 121.1 kg, equivalent to 15.9% total body weight loss. Patients with diabetes had a reduction in HbA1c from 66.3 to 48.3 mmol/mol, and diabetes medication use decreased significantly. These changes were much larger than those we have observed with a more conservative lifestyle modification programme in our bariatric patients, where mean weight loss in completers of a ten-week structured diet and physical activity program was 2.7kg [213]. Potential benefits of using a milk-based meal replacement regimen include its relatively low cost compared to commercial meal replacement programmes [285], its suppression of appetite [221], and its enhancement of post-prandial glucose and lipid metabolism [222].

Studies of the effects of meal replacement programmes on insulin sensitivity are limited, partly because quantifying insulin sensitivity is challenging. For example, the gold-standard hyperinsulinaemic-euglycaemic clamp technique allows precise quantification of whole-body insulin sensitivity [286] but is relatively time-consuming and expensive. The leptin: adiponectin ratio (LAR) has been validated as a robust measure of whole-body insulin sensitivity in large epidemiological studies [145, 242]. To date, the influence of hypocaloric meal replacement on the ratio of these two hormones has not been described. We sought to determine the effects of the completion of a milk-based meal replacement programme on leptin and adiponectin levels as well as the LAR in adults with severe obesity and to determine whether the magnitude of weight loss after the programme influenced the change in LAR.

4.2: Methods:

We conducted a single-centre, prospective observational cohort study of bariatric patients who undertook and completed a milk-based meal replacement programme. As such, this study was a pre- and post-programme analysis focusing on completers only and without a separate control group. The study was conducted adhering to the STROBE guidelines [248]. Patients were approached and recruited between November 2017 and May 2019 when they attended the bariatric clinic in the Centre for Endocrinology, Diabetes, and Metabolism at Galway University Hospitals. During the out-patient, milk-based meal replacement programme, patients attended the bariatric clinic every two weeks for 24 weeks, with 14 visits in total. The study was approved by the Galway University Hospitals' Central Research Ethics Committee in December 2017 (ref CA 1802), with all participants providing written informed consent prior to participation in the study.

4.2.1: Inclusion and Exclusion criteria:

Male and female patients aged 18 years or older referred to the bariatric service for assessment of severe obesity were eligible for inclusion. Our clinical practice is to define severe obesity as a BMI ≥40 kg m^{-2} (or ≥35 kg m^{-2} with co-morbidities such as type 2 diabetes or obstructive sleep apnea syndrome). Patients must have been willing to attend all of the 14 scheduled study visits. Female patients of childbearing potential who were pregnant, breastfeeding, or intended to become pregnant or were not using adequate contraceptive methods were not considered eligible for the programme. Those with a recent myocardial infarction (within six months), untreated arrhythmia, untreated left ventricular failure, recent cholelithiasis (within the past year),), hepatic dysfunction defined as acutely deranged liver function tests of 15 times the upper limit of normal or established liver cirrhosis or renal dysfunction defined as an eGFR of <30ml/min, type 1 diabetes, untreated

major psychiatric disorders, eating disorders, cancer, previous bariatric surgery, a BMI <35 kg m^{-2} or those deemed unlikely to attend for the full programme (e.g. frequent clinic non-attendance) were excluded from the programme.

4.2.2: Measurements:

Weight was measured on a Tanita® scale and height with a Seca® wall-mounted stadiometer. HbA1c was measured using High-Performance Liquid Chromatography (HPLC) on the Menarini® HA8160 analyzer. Leptin and adiponectin were measured at the start of the intervention and again after 24 weeks using separate two-site microtiter plate-based DELFIA assays manufactured by R&D Systems Europe, Abingdon UK. The adiponectin assay measures "total" adiponectin with inter-assay imprecision of 5.4% at 3.6 µg/ml, 5.2% at 9.2 µg/ml and 5.8% at 15.5 µg/ml, as previously described [249]. For leptin, the between batch imprecision at concentrations of 2.7 ng/ml, 14.9 ng/ml and 54.9 ng/ml was 7.1%, 3.9% and 5,7% respectively. The between run imprecision at a mean HbA1c concentration of 36 and 71 mmol/mol was <1.4%.

4.2.3: Intervention:

The milk-based meal replacement programme consisted of three continuous eight-week phases, each with fortnightly visits to the bariatric medicine clinic. During the first (weight loss) phase from weeks one to eight inclusive, an exclusively milk-based liquid diet was prescribed, consisting of approximately 2.5 liters per day of semi-skimmed milk divided into seven portions throughout the day in equal doses, with additional sodium replacement, vitamin, mineral and fiber supplementation, equivalent to approximately 1200 kcal/day of energy, with 130 g of carbohydrates and 40 g of fat intake per day. The volume of milk was titrated according to estimated total daily protein requirements (of 1.1 grams of protein per kilogram body weight per day, equivalent to 0.17 grams of nitrogen, with 75% of the estimated daily requirement replaced for patients with a BMI ≥35 kg m^{-2} and 65% replaced for those with a BMI ≥50 kg m^{-2}) according to a departmental standard operating procedure [285]. Throughout this phase, renal and liver profiles were assessed every two weeks, and the patient was seen by the consultant endocrinologist, bariatric nurse, and dietitian at each visit. During the second phase (weight stabilization) from weeks nine to sixteen inclusive, there was a gradual re-introduction of low-calorie meals under the supervision of the bariatric dietitian with fortnightly visits continuing. During the third phase (weight maintenance) from weeks 17 to 24 inclusive, the liquid component of the diet was stopped completely, and a fully solid isocaloric diet was restarted, based on individualized meal plans, under the supervision of the bariatric dietitian.

4.2.4: Statistical methods:

All statistical analyses were conducted with SPSS® version 26. The Shapiro-Wilk test was used to determine whether variables were normally distributed. Changes in anthropometric and metabolic variables between baseline and completion of the programme were assessed using the student's paired t-test. Where variables were not normally distributed, the Wilcoxon signed-rank test was used. Categorical variables were compared using the Chi-Square test. Correlation between the degree of weight loss and changes in leptin, adiponectin, and LAR was determined using Spearman correlation analysis. Associations between percentage change in weight (as the independent variable) and percentage change in leptin, adiponectin, and LAR (as dependent variables) were determined by linear regression analysis.

4.3: Results:

Of the 120 patients who started our milk-based meal replacement programme between November 2017 and May 2019, 52 (43.3 %) completed all 24 weeks of the programme and were included in the study analysis. Of the completers, 29 (55.8%) were female, and the mean age was 50.3±11.2 (range 18-74) years. The mean height was 1.67±0.09 m. The majority of our study population was ethnically "White Irish" (n=44, 84.6%), n=3, 5.76% were "Polish White" and n=1, 1.92% each were of "Irish traveller", "German Jewish," "German White," "Italian White" and "Pakistani Asian" ethnicity. Obesity-related comorbidities were highly prevalent, with 20 (38.5%) patients having T2DM, 37 (71.2%) being treated for hypertension, and 19 (36.5%) taking lipid-lowering therapy. Changes in anthropometric and metabolic characteristics are shown in table 4.3.1. There was a 22.8±9.7 kg reduction in weight, with a reduction in BMI of 8.0±3.2 kgm^{-2} and a reduction in excess body weight percentage (EBW%) of 32.1±12.9 % (all $p<0.001$), equivalent to a percentage total weight loss of 15.3±6.0 %. We found that 50 of the 52 patients (96.2%) achieved a total weight loss of 10% or more after 24 weeks.

There was a significant reduction in HbA1c in the group overall, both in patients with T2DM and those without diabetes, as shown in table 4.3.1. Of those patients with T2DM, 14 out of 20 (70 %) had an Hba1C less than 48 mmol/mol at the end of the program, and 8 out of 20 (40%) had one or more anti-hyperglycaemic drugs stopped during the programme (p=0.007). Six (30%) patients were taking insulin prior to the start of the programme, while four (20%) remained on it at completion ($p<0.001$). Six (30%) patients taking a sodium-glucose linked transported 2 (SGLT2) inhibitor

remained on this drug at the end of the programme. Seventeen (85%) patients who were on metformin remained on it throughout the programme. Nine (45%) patients were on glucagon-like peptide 1 (GLP1) agonist therapy prior to the start of the programme, and 7 (35%) remained on it at completion ($p<0.001$). Of the two (10%) patients who were on sulphonylurea therapy at baseline, both discontinued it by completion ($p<0.001$), and the one (5%) patient on a dipeptidyl peptidase 4 (DPP4)-inhibitor stopped this at the start of the programme. In total, 37 (71.1%) patients were on one or more anti-hypertensive agents at baseline, and 18 (34.6%) remained on this treatment at completion ($p=0.002$), whereas 19 (36.5%) were on lipid-lowering therapy, and all remained on those drugs at completion.

There were reductions in leptin and increases in adiponectin, with a significant reduction in LAR, as shown in table 4.3.1. The magnitude of the changes in these variables was similar in the subgroup with T2DM to the overall group. In linear regression analyses, with percentage weight loss as the independent variable, the magnitude of the percentage changes in leptin and LAR was strongly associated with weight loss. However, the magnitude of the percentage change in adiponectin was only associated with weight loss in patients with diabetes, not in patients without diabetes, as shown in table 4.3.2. So, for example, in patients with type 2 diabetes, for every percentage reduction in body weight, there was a 4.4% decrease in leptin, a 3.9% increase in adiponectin, and a 5.9% reduction in LAR. These results were similar after adjusting for age, sex, and baseline BMI. We then sought to determine if there was any association between the percentage change in LAR as the independent variable and the percentage change in HbA1c as the dependent variable. I have found no association between the two in patients with T2DM (ß=-0.57 [-1.6,0.5], $p=0.285$) or in patients without diabetes (ß=-0.75 [-2.0,0.4], $p=0.224$). When I excluded the patients with T2DM who had stopped diabetes medications, I found that there was a statistically significant association between the change in LAR and the relative change in HbA1c in the T2DM subgroup who had no changes to medications (ß= 5.7 [0.2, 11.1], $p=0.041$).

There were significant correlations between percentage weight loss and percentage change in leptin and LAR, but not adiponectin, in the group overall, as shown in figure 4.3.1 (a-c), such that 47% of the relative change in leptin and 34% of the relative change in LAR was accounted for by the relative change in weight. In the subgroup of patients with T2DM (figure 4.3.2 (a-c)), results were similar, but there was also a correlation between weight change and change in adiponectin levels.

Table 4.3.1: Changes in Anthropometric Variables, Leptin, Adiponectin and the LAR after Completion of the Milk-Based Meal Replacement Programme.

Variable	Pre	Post	P value
Weight (kg)	148.2±39.6	125.4±34.8	<0.001
BMI (kg m^{-2})	52.4±11.1	44.3±9.8	<0.001
EBW (%)†	103.1 [78.7, 138.0]	73 [47.2, 96.0]	<0.001
% Total weight loss		15.3±5.3	<0.001
Systolic Blood Pressure (mmHg)	126.5±14.3	122.7±13.7	0.048
Diastolic Blood Pressure (mmHg)	68.9±11.3	69.2±10.3	0.843
HbA1C (mmol/mol)**	38.2±3.5	35.0±3.1	<0.001
HbA1C (mmol/mol)*	60±17.4	47.5±15.5	0.001
Leptin (ng/ml)†	87.2 [48.6, 132.7]	39.1 [21.0, 76.4]	<0.001
Leptin (ng/ml)†**	92.8 [60.3,153.7]	42.8 [27.3,77.9]	<0.001
Leptin (ng/ml)†*	74.9 [35.9,111.7]	44.5 [17.6,60.8]	0.001
Adiponectin (µg/ml)†	5.6 [4.5, 7.5]	7.1 [5.5, 8.5]	<0.001
Adiponectin (µg/ml)†**	6.2 [5.0,8.6]	8.1 [6.5,10.6]	<0.001
Adiponectin (µg/ml)†*	4.8[3.1,3.6]	6.0[4.2,7.6]	0.004
LAR (ng/µg)†	15.0 [8.4, 22.4]	5.7 [3.0, 9.1]	<0.001
LAR (ng/µg)†**	11.7 [7.8,25.6]	5.6 [3.0,8.7]	<0.001
LAR (ng/µg)†*	16.0 [12.1, 21.2]	6.0 [3.0, 10.1]	0.002

All variables are presented as means ± standard deviation or for non-normally distributed data, medians and [interquartile ranges]

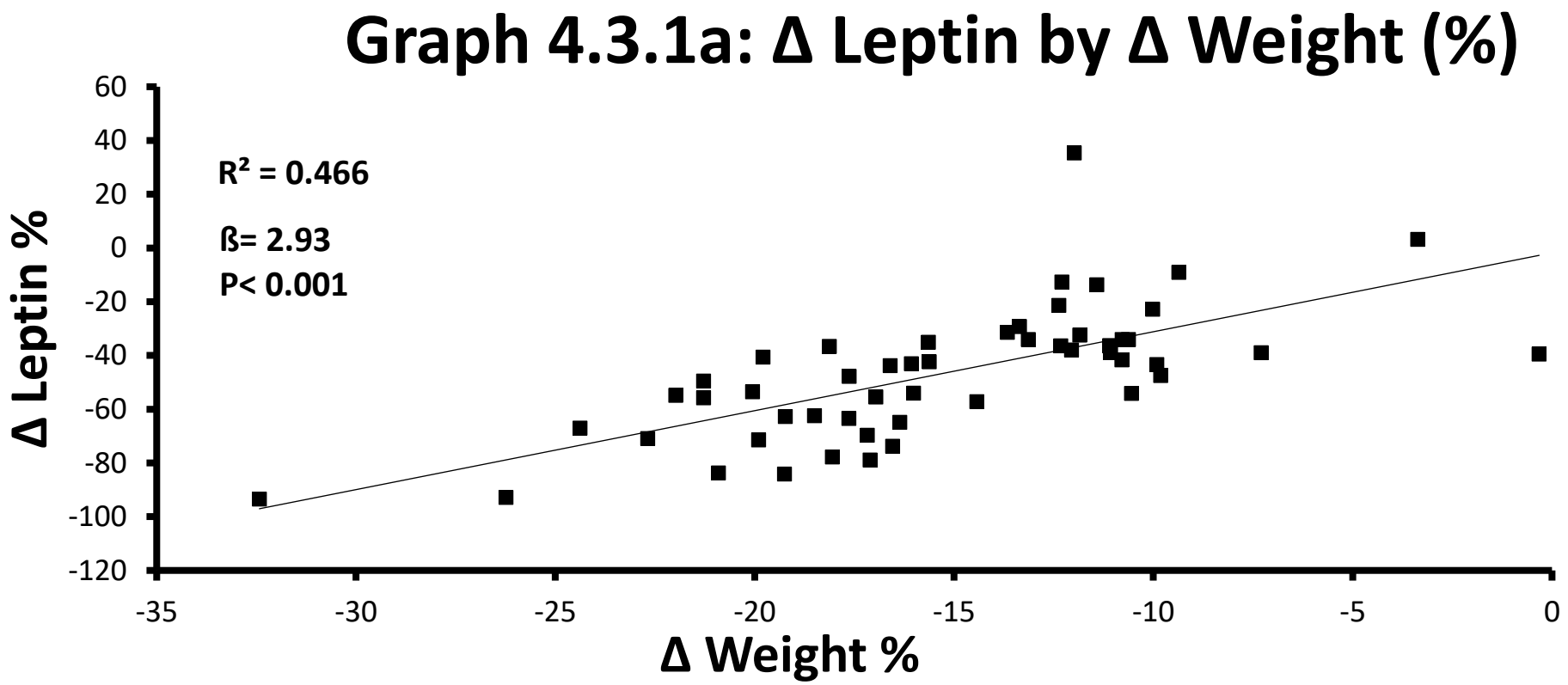
All variables were compared using the paired t-test, except for LAR, which was compared using the Wilcoxon Signed Rank Test. % total weight loss was calculated using the one-sample student t-test.

Table 4.3.2: Relationship Between Percentage Change in Weight and Percentage Change in Leptin, Adiponectin and the LAR after Completion of the Milk-Based Meal Replacement Programme.

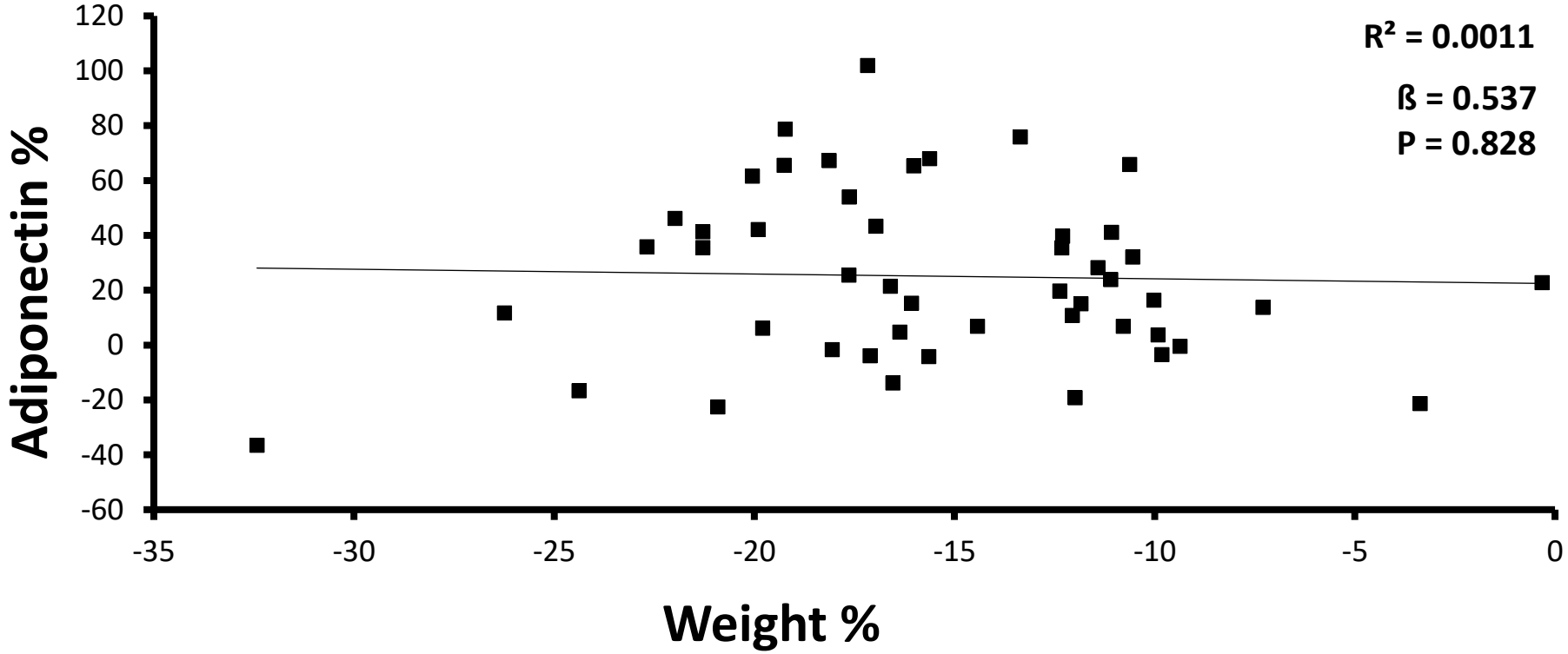
	B	95% CI	P
Entire group (n=52):			
Δ Leptin%	**2.9**	**[2.0,3.8]**	**<0.001**
Δ Adiponectin %	**0.4**	**[-4.3,5.1]**	**0.537**
Δ LAR %	**2.9**	**[1.7,4.1]**	**<0.001**
T2DM patients (n=20):			
Δ Leptin%	**4.4**	**[2.0,6.8]**	**0.001**
Δ Adiponectin %	**-3.9**	**[-7.4, -0.5]**	**0.02**
Δ LAR %	**5.9**	**[2.6,9.1]**	**0.001**
Patients without DM (n=32):			
Δ Leptin%	**2.4**	**[1.6,3.2]**	**<0.001**
Δ Adiponectin %	**2.1**	**[-4.9,9.2]**	**0.537**
Δ LAR %	**1.8**	**[1.0,2.7]**	**<0.001**

β denotes the beta coefficient and [confidence interval] for the estimate of the strength of the association between percentage weight change as the independent variable and changes in leptin, adiponectin and LAR as the dependent variables, in unadjusted linear regression analyses. Results were similar after adjusting for age, sex and baseline BMI.

Figure 4.3.1 (a-c): Correlations Between Percentage Change in Weight and Percentage Change in Leptin (a), Adiponectin (b) and the LAR (c) after Completion of the Milk-Based Meal Replacement Programme.



Graph 4.3.1b: Δ Adiponectin by Δ Weight (%)



Graph 4.3.1c: Δ LAR by Δ Weight (%)

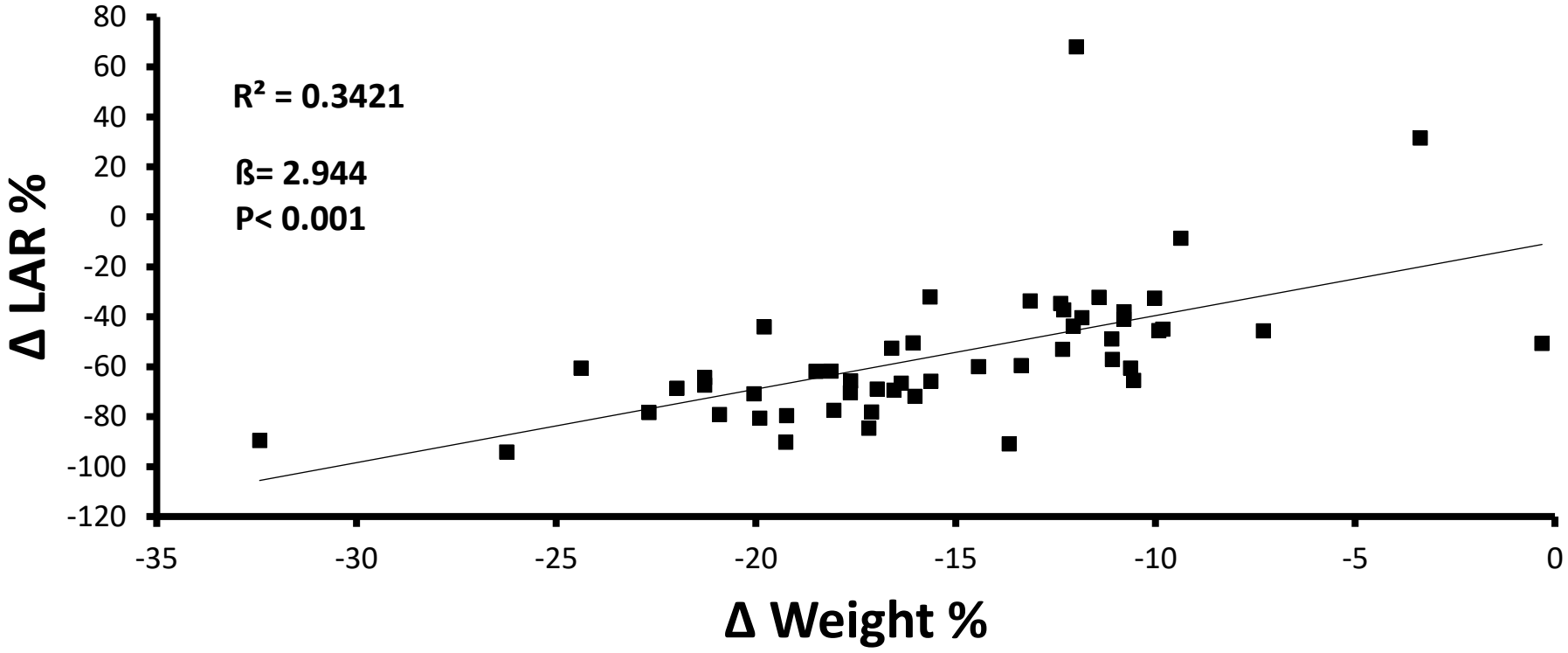
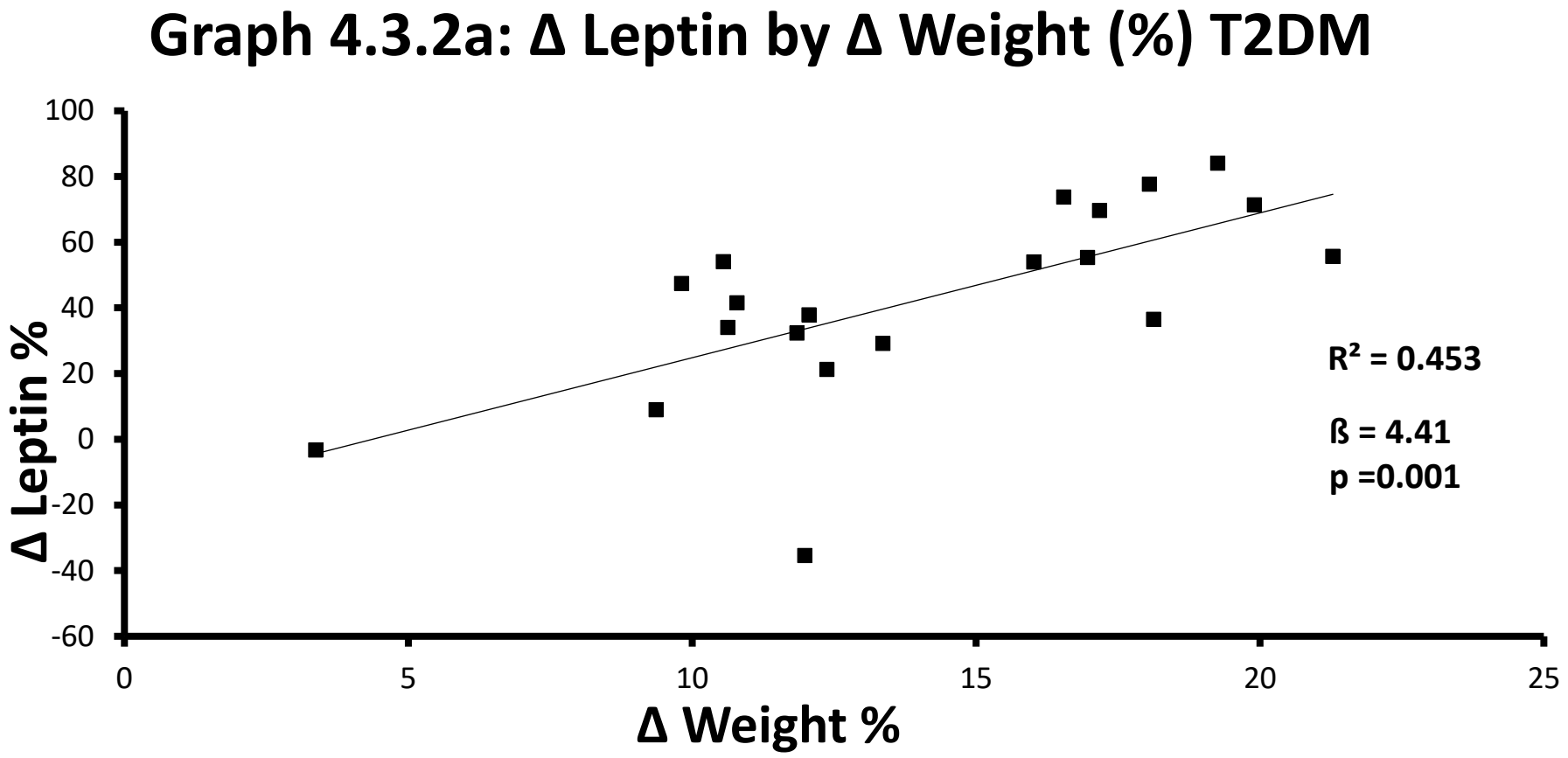
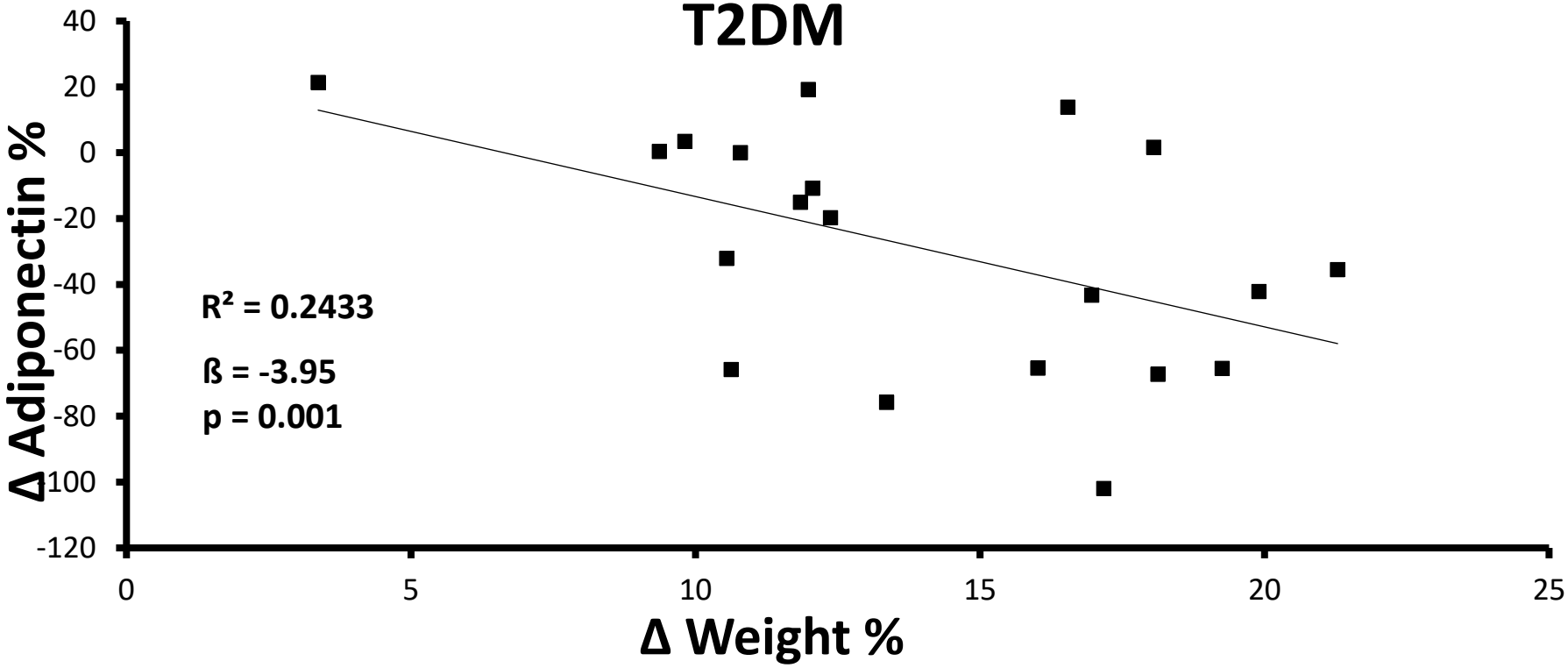


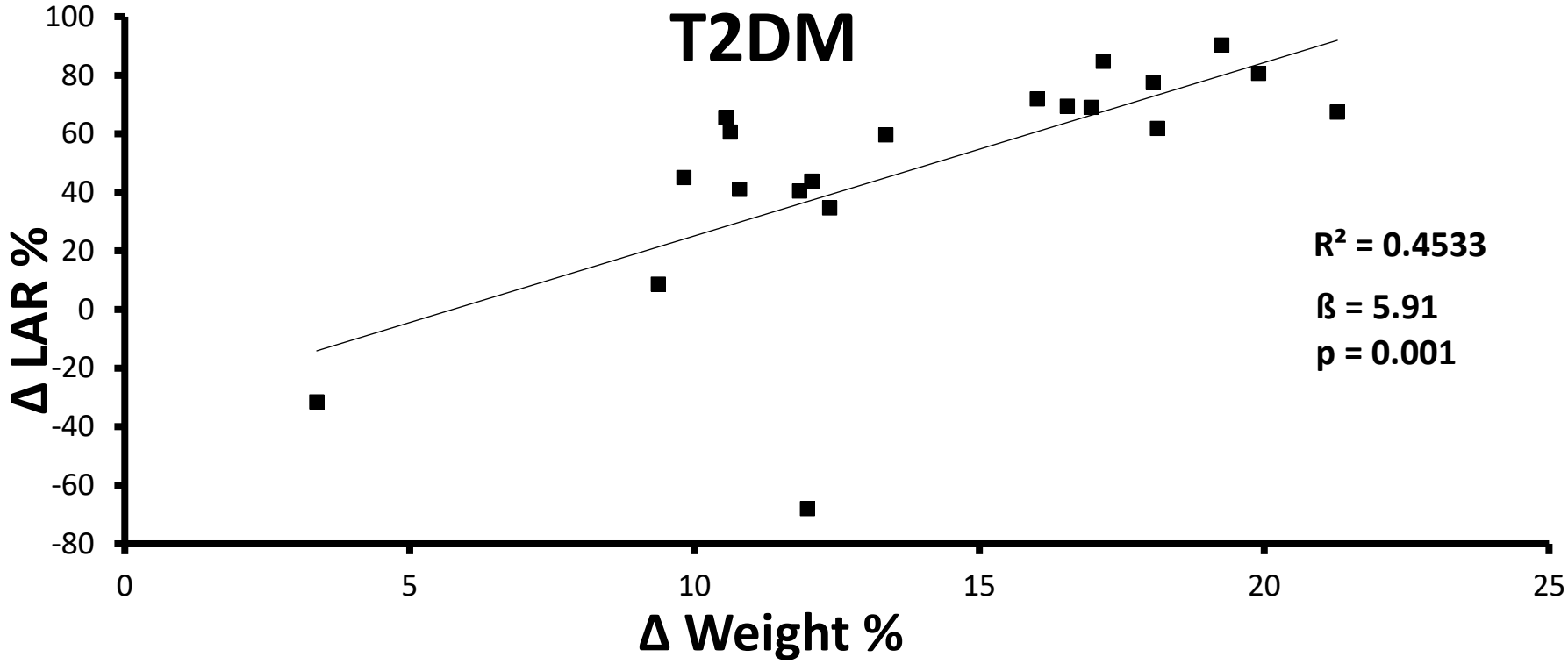
Figure 4.3.2 (a-c): Correlations Between Percentage Change in Weight and Percentage Change in Leptin (a), Adiponectin (b) and the LAR (c) after Completion of the Milk-Based Meal Replacement Programme in Subgroup of Patients with T2DM.



Graph 4.3.2b: Δ Adiponectin by Δ Weight (%)



Graph 4.3.2c: Δ LAR by Δ Weight (%)



4.4: Discussion:

I have shown that in a cohort of adults with severe obesity who completed a 24-week milk-based meal replacement programme, there was a substantial increase in adiponectin and reductions in leptin and LAR, consistent with a likely increase in insulin sensitivity. While a number of studies have described reductions in leptin and increases in adiponectin after dietary interventions [280, 281] and after bariatric surgery [238, 239], to date, none has considered changes in LAR per se. In a cohort of patients with T2DM undergoing bypass bariatric surgery, investigators described an increase in an "inverted" ratio of adiponectin: leptin, with broadly similar effects on both hormones to those we found [241]. Ours is the first study to consider these adipokines as a ratio in patients undergoing hypocaloric meal replacement. I found that the reduction in LAR was driven primarily by the reduction in leptin rather than the increase in adiponectin and that the change in LAR was proportional to the reduction in weight after the programme, again consistent with the findings described in gastric bypass patients [241]. The magnitude of the changes we observed in leptin and adiponectin concentrations are broadly consistent with other studies, such as with a commercial meal replacement programme (Optifast®) [287].

The amount of weight loss observed in our cohort appears to compare favorably with previously published descriptions of other meal replacement programmes [87, 288, 289]. It is important to note, however, that this study only focused on completers of a six-month period of intensive therapy and did not attempt to characterize whether weight loss or changes in the LAR were maintained in the longer term in these patients. Within these limitations and during the time period studied, we report almost all of our patients lost 10% or more of their body weight, the threshold considered by some to be required for meaningful improvements in metabolic health and cardiovascular risk [290]. This is consistent with the reductions I found in systolic (but not diastolic) blood pressure and antihypertensive therapy use as well as the significant and substantial reduction in HbA1c and diabetes medication use. I was surprised, therefore, to find no association between the magnitude of the change in LAR and the change in HbA1c. Previous studies in patients undergoing bariatric surgery have found that the magnitude of changes in adipokines predicts diabetes remission [279, 291]. Our results may be due to a lack of statistical power, but I am inclined to reject this as the only reason we did not find an association. It may be that the improvement in HbA1c in our cohort arose from a mechanism that occurs independently of a change in adipocyte function. Improvements in glucose metabolism with dietary restriction and weight loss are known to arise from reduced insulin resistance and enhanced beta-cell insulin secretion [292]. In particular, changes in liver fat content

and hepatic insulin resistance that arise from weight loss [293] are intricately linked with improvements in beta-cell function [294]. Hypocaloric meal replacement in patients with T2DM has been shown to decrease ectopic fat in the liver and pancreas, but a critically important factor necessary for the remission of diabetes is the concomitant restoration of beta-cell function [295]. It may be that the improvements we observed in glycaemic control in our patients arose from reduced hepatic and pancreatic ectopic fat and enhanced beta-cell function. Thus, it is a limitation of our study that we have not measured these specifically, especially given a growing realization that "adipose tissue" and "whole body" insulin resistance are distinct pathophysiological entities [296]. We note also that while measurement of the LAR in obesity therapy is not part of the standard clinical evaluation it does offer a relatively accessible method by which these complex metabolic issues can be included and explored in future research cohorts where the performance of clamp studies may not be logistically viable.

Another limitation of our study is its relatively modest size, though the large changes induced by the intervention allow a much smaller population with which to demonstrate an effect than many other weight loss interventions such as drugs [255] or lifestyle interventions [256]. Our study population was also a relatively heterogenous convenience sample, determined by the number of patients recruited to the milk-based meal replacement programme at our institution over the study period. Nonetheless, our findings around the correlation between weight loss and reduced LAR are statistically robust. There were changes in diabetes medication usage within the cohort that are unavoidable in an observational study such as this, which might have influenced our results. Metformin is known to have a weak positive effect on insulin sensitivity [257] and potentially a lowering effect on circulating levels of leptin [202], but there was no change in its usage in the study. SGLT2 inhibitors decrease leptin and increase adiponectin [260], but their use was also unchanged. GLP1 agonist therapy has previously been reported to attenuate the reduction in circulating levels of leptin after weight loss [258], and DPP4 inhibitors decrease leptin and increase adiponectin [259], so the reductions we observed in the use of these drugs would have led to an attenuation rather than an exaggeration of the change in LAR with weight loss. Angiotensin-converting enzyme (ACE) inhibitors increase adiponectin [261], so I think their cessation would have attenuated rather than enhanced the observed reduction in LAR.

Notwithstanding the metabolic improvements seen in patients in this study, there are several reasons to caution against considering our findings as definitive proof that the intervention will benefit patients. As with similar studies of meal-replacement programmes, our attrition rate was very high, with more than half of patients dropping out [87, 288]. Moreover, weight regain in the medium term often limits the longer-term efficacy of these interventions [211, 215]. Side effects of

hypocaloric meal replacement programmes are common and include constipation, dizziness, alopecia, nausea, headache, diarrhoea, abdominal pain, and cholelithiasis [216]. Some health economic analyses suggest that meal replacement programs are prohibitively cost-ineffective [218]. Nonetheless, milk is relatively inexpensive and accessible. It may be that the unique constituents of milk might account for some of the benefits seen in our intervention. As noted earlier, milk whey proteins attenuate muscle loss [219] during hypocaloric diets. Milk reduces appetite, calorie intake and body weight [221] and alters post-prandial glucose and lipid metabolism [222] in men with obesity. In mice, milk casein-derived peptides reduce high-fat diet-induced adipose tissue inflammation [223], though the impact on adipokines has not been described.

A limitation of the study was using a convenience sample size without a prior sample size calculation; in order to overcome this limitation, I have done a retrospective sample size calculation. Retrospective sample size analysis was done by using the calculated effect size and power of the intervention with weight loss at 24 weeks done for chapter 2. To calculate the change in means using the student t pair test, the sample size of 21 was obtained from chapter 2. To achieve significant results of correlation using spearman analysis and association using linear regression analysis, α was set at 0.05 (5% chance of false-positive result), β was set at 0.01 (1% chance of false-negative result), with power at 0.99, with an effect size of 0.99 as obtained in chapter 2, the sample size required to achieve similar results was 25, with a critical f value of 3.44.

Arguably our most important limitation is the lack of follow-up data for the patients who started the intervention but dropped out. It must be emphasized, therefore, that this work is not a description of the effectiveness or efficacy of the milk programme to reduce LAR, but rather it serves as a pilot study providing a useful estimate of the effect size of the intervention as well as the attrition rate, to inform the development of future randomized controlled trials. Though unlikely, it is possible that some of those who dropped out may have had significant weight regain or serious adverse events that precluded continued participation, which we would not be aware of, which is why I have avoided any attempt to compare data in completers and non-completers using last- or mean-observation carried forward analyses. With regards to any potential bias, it is possible that those patients who did not complete the intervention could have differed from completers in the pathophysiology of their obesity in such a way that would have led to differing LAR responses to changes in weight. I feel, however, that such a bias is unlikely given the magnitude of the LAR change exhibited by completers in our study, along with the observation that the changes in adipokines we report are broadly similar to those reported in response to other weight loss interventions as previously discussed [238, 239, 280, 281].

4.5: Conclusion:

Patients with severe obesity who completed a milk-based hypocaloric meal replacement programme had a substantial increase in their adiponectin and reductions in their leptin and LAR, consistent with decreased insulin resistance, which was directly proportional to the amount of weight lost, although the substantial improvements in glycaemic control in the subgroup of patients with T2DM were not proportional to the changes in LAR.

Chapter five

General discussion and conclusion of thesis

5.1: General discussion and conclusion:

I have examined the effect size of two of the most important interventions of our bariatric medicine service in this thesis, the milk-based meal replacement programme in chapters two and four of this thesis [285] and bariatric surgery in the form of sleeve gastrectomy in chapter three of this thesis [297]. Other interventions in our service in Galway include lifestyle intervention in the form of the CROI- CLANN programme [82], provision of medical therapy in the form of Semaglutide, and psychological interventions, which plays an important role in the form of group-based compassion focused therapy for binge eating and emotional eating disorders associated with obesity. We have had detailed insight into the weight loss and significant improvements in cardiovascular risk factors, insulin resistance measured in the form of LAR, and its correlation and association with weight loss via two different methods of weight-loss strategies.

I have seen a significant reduction in weight in patients who underwent the milk-based meal replacement programme, and the results were similar in the retrospective study as seen in chapter two with a total weight loss of 23.9 kgs in 105 patients versus 22.8 kgs seen in 52 patients, in the prospective study as seen in chapter four of this thesis. The amount of weight lost was significantly better than a comparative Cambridge, milk-based intensive weight loss intervention [288], in terms of total weight loss and the number of patients achieving >10% of weight loss, 69 % in the Cambridge population versus 86.7% in the retrospective study (chapter 2) versus 96.2% in the prospective study (chapter 4). The amount of weight lost after sleeve gastrectomy in our study (chapter 3) at 12 months of 33.0±21.6 kgs was on par with most centres around the world [298-300].

Weight loss achieved during meal replacement programmes is thought to be short-term with a concern of weight regain post-completion [89, 234, 301]. To examine this issue, I conducted a study during my time as an MD student in NUIG to study long-term changes in weight in patients after finishing a milk-based meal replacement programme [302]. In this longitudinal retrospective cohort study of 105 patients who completed milk-based meal replacement programme, 78 patients were included in long term analysis, excluding 17 patients who received GLP-1 agonist therapy, eight who received bariatric surgery, and two who received both after completion of programme and till the time of follow-up. 52.6% of the population was female and average age was 51.6±12 years, and the baseline weight was 144±26 kg with a baseline BMI of 50.5±7.6 kg/m^2 with a mean follow-up of the

entire cohort of 34±19.8 months. There was a significant reduction in weight at six months of completion of milk-based meal replacement programme from 144±26 kg to 121.2±24 kg at six months, the trend in weight regain was non-significant in the first two years with mean weight at one year of 129±29 kg (p=0.07) and at two years of 123.4±29 kg (p=0.17). Significant regain in weight was seen in the third year with a mean weight of 131.0±22.3 kg (P<0.001), and at the fourth year of follow-up, a similar significant weight regain was seen with a mean weight of 139.8±35.4 kg (p<0.001). Although we saw a significant weight regain at four years post-completion of the milk-based meal replacement programme, there was still a median net weight loss of 4.7 [9.5, 0.21] kg and 7.0 [13.9, 0.26] kg at three and four years of follow-up compared to baseline with a p-value of 0.04, with a relative percentage weight regain of 27.3 %, 32.1%, 81% and 66.2% at one, two, three and four years of follow-up. If we put it in other words, 87.2% of 78 patients achieved >10% weight loss at the completion of the programme, and one year 43.5%, at two years 42.9%, at three years 8.3% and at four years 31% of the population still had >10% weight loss from baseline prior to starting the milk-based meal replacement programme. Using multivariate regression models, an inverse association was seen between the weight regain and the amount of weight lost at completion, age, and duration of follow-up with a β value of 1.2 [0.46, 1.9] and a p-value of 0.002.

I saw a non-significant decline in systolic blood pressure in the retrospective cohort of patients who underwent milk-based meal replacement programme from 127.5±13.4 mmHg to 122.9±14.6 mmHg (P=0.73), but a significant decline in systolic blood pressure in the prospective cohort, from 125±14.3 mmHg to 122.7±13.7 mmHg, whereas diastolic blood pressure changes were non-significant in both cohorts with a significant decline in antihypertensive medications in both cohorts, with a decline from 64.7% to 35% (p<0.001) in the retrospective cohort and a decline from 71.1% to 34.6% (P=0.002) in the prospective cohort of patients. There was a similar and significant decline in Hba1c in patients with a history of T2DM in both cohorts, from 66.3±13 to 48.4±13.5 mmol/mol (p<0.001) in the retrospective cohort and from 60±17.4 to 47.5±15.5 mmol/mol (p=0.001) in the prospective cohort, with both cohorts reaching a range of Hba1c in the non-diabetic range with a significant decline in anti-hyperglycaemic medications.

I saw a significant decline in ALT levels in patients undergoing the milk-based meal replacement programme. In another study conducted during my time as a MD student in NUIG, I reported a transient rise in ALT levels during the first eight weeks of the programme, with its levels stabilizing at eight weeks and following a declining path thereafter; this shows a worsening of hepatic inflammation caused by a potential influx of non-esterified fatty acids from adipose tissue in response to calorie deficit diet or increased gluconeogenesis in the liver in response to low-calorie intake causing a transient rise in ALT levels. In patients with a high baseline of ALT, suggesting the

presence of non-alcoholic steatohepatitis, the rise in ALT levels was greater than the patients with normal ALT levels at baseline, helping us understand that in patients with higher ALT levels at baseline, there is a higher risk of hepatic inflammation during weight-loss interventions in early phases with overall improvement with continued weight loss [303].

Leptin levels in the patients who underwent sleeve gastrectomy were lower, to begin with (chapter 3) with a mean value of 40.7±24.9 ng/ml compared to patients who underwent the milk-based meal replacement programme (chapter 4), with median levels of 87.2 [48.6,132.7], and the changes in leptin were not significant in the surgical cohort with a decline from 40.7±24.9 ng/ml to 30.9±30.5 ng/ml with a p-value of 0.11 compared with a significant decline in the meal replacement group of patients from 87.2[48.6,132.7] ng/ml to 39.1[21.0, 76.4] p-value of <0.001. In terms of adiponectin levels, they were similar, to begin with in both cohorts, with the surgical cohort mean adiponectin levels of 4.49±1.6 µg/ml compared to the meal replacement cohort with a median of 5.6 [4.5, 7.5] µg/ml, the rise in adiponectin level was seen to be superior in the surgical cohort from a mean of 4.49±1.6 µng/ml to 8.93±6.36 µg/ml, compared to the meal replacement cohort rise from 5.6 [4.5, 7.5] ng/ml to 7.1 [5.5,8.5] ng/ml. In terms of LAR as a marker of insulin resistance, the surgical cohort had a lower LAR at baseline in comparison to the meal replacement cohort, with a median of 7.16 [5.21,10.59] ng/µg in all patients and in patients with a history of T2DM the baseline levels were a bit higher compared to all patients at 8.54 [5.09, 10.37] ng/µg, with median levels in the meal replacement cohort at 15.0 [8.4, 22.4] ng/µg in all patients and slightly higher in patients with a history of T2DM at 16.0 [12.1, 21.2] ng/µg, representing almost twice as high at baseline compared to the surgical cohort.

Reduction in insulin resistance measured via LAR was similar in all patients, with the surgical cohort's median reduction in LAR in all patients from 7.15 [5.21, 10.59] ng/µg to 2.59 [1.14, 7.44] ng/µg p-value of 0.02, an absolute reduction of 63.9%, compared to the meal replacement cohort, with a median reduction from 15.0 [8.4, 22.4] ng/µg to 5.7 [3.0, 8.7] ng/µg, an absolute reduction of 62%. When compared in patients with a history of T2DM, there was a superior reduction in insulin resistance seen in the meal replacement cohort, with the median reduction in the surgical cohort from 8.54 [5.09,10.37] ng/µg to 4.2 [2.24, 7,56] ng/µg with a p-value of 0.006, an absolute reduction of 50.6%, compared to the meal replacement cohort from 16.0 [12.1, 21.2] ng/µg to 6.0 [3.0, 10.1] ng/µg with a p-value of 0.002, an absolute reduction of 62.5%. There are a few plausible reasons for the discrepancy in the leptin, adiponectin, and LAR differences seen between the surgical cohort and meal replacement cohort. The most probable reason is the use of a high protein, low-calorie liver shrinkage diet [304], used prior to sleeve gastrectomy in the surgical cohort, and as these levels were measured at the day of the surgery, we do not consider the changes that occurred due to this

diet which could explain the high leptin levels at baseline in the surgical cohort of patients. The changes in the LAR seen pre and post interventions were measured at the different time frames in the surgical cohort at one year versus six months in the meal replacement cohort; although the magnitude of reduction in LAR was similar in both cohorts in all patients, the difference in reduction seen in the patients with T2DM could be explained by this time difference in measurement, although unlikely as substantial changes in leptin and adiponectin levels were seen in bariatric surgery cohorts as early as six months post-op [305]. Patients in the surgical cohort had exposure to lifestyle intervention in the form of the CROI-CLANN programme [82], and were seen by psychologists for behavioral therapy prior to undergoing surgery, in comparison to the patients in the meal replacement programme, in which the majority were not exposed to either lifestyle intervention or psychological input, giving rise to an argument that the patients in the meal replacement cohort had a higher metabolic risk profile, giving rise to higher leptin levels and greater reductions in LAR seen in the meal replacement cohort with a history of T2DM.

In terms of correlation between % of the change in LAR versus % of the change in weight, the surgical cohort had a far superior correlation of 82% with a p-value of <0.001 compared to the meal replacement cohort of 32% with a p-value of <0.001, but there was no significant association seen between % of the change in weight and % change in LAR in the surgical cohort in comparison to the meal replacement cohort with a significant association, with a β value of 2.944 and p-value of <0.001. This gives rise to the fact weight loss was accounted for 82% of the change in insulin resistance in the surgical cohort, but this accounted for only 32% of the change in the meal replacement cohort, with other factors playing a role in the change in insulin resistance via meal replacement which needs further investigation in future. The lack of association between % of the change in LAR versus % of the change in weight in the surgical cohort could be due to its modest size of n=17 and since the study was not numbered to achieve a positive association.

In summary, strengths of the second chapter of the thesis would be that I have now shown that in a population of severely obese individuals, participation in a milk-based meal replacement programme can have significant improvement in their body weight, BMI, EBW%, ALT, lipid profile, and hba1c, with a significant decline in the use of anti-hyperglycaemic therapy and antihypertensive therapy. We have proven to be better at achieving significant weight loss in comparison to other similar intensive weight loss interventions around the globe [87, 288], yet possibly being cost-effective with a total cost of 1088 € [87, 91]. In terms of limitations, retrospective nature of the study, but I have shown the effects of the programme in a prospective study in chapter four, high attrition rate, lack of follow-up of patients who dropped out of intervention, lack of long term follow-up data (which we

have addressed in a subsequent study [302]), lack of accountability of physical activity changes during the programme are a few to be mentioned.

In summary, the strengths of the third chapter of the thesis would be that we were the first study to have successfully shown the reduction in insulin resistance measured via LAR in patients undergoing sleeve gastrectomy as a form of weight loss surgery. I demonstrated a significant correlation between insulin resistance in the form of LAR and the amount of weight lost, with significant improvement in adiponectin as a driving factor for the change in LAR. In terms of limitations, there were many which need to be addressed in future randomized controlled trials, which include a modest cohort size although, there was a similar number of patients seen in a similar study that used an inverse of LAR in patients who underwent RYBG surgery [247], non-significant reductions in leptin levels, hba1c, lack of data on the effects of liver shrinkage diet which should be included as a baseline for obtaining blood samples including LAR, followed by levels at day of surgery and at one-year follow-up, and use of medications such as metformin which might have an effect on LAR levels.

In summary, the strengths of the fourth chapter were, we are the first study to show an improvement in insulin resistance after a meal replacement programme to be measured via LAR as a marker of insulin resistance. We are the first study to report a significant correlation and association between the percentage of change in LAR and the percentage of weight lost, with a reduction in leptin as a major driving factor in the change of LAR after a meal replacement programme, which is different from the surgical cohort where adiponectin change was the driving factor, and this is something which can be investigated in future head-to-head trials. In terms of limitations, there were a few such as high attrition rates, which were similar to the retrospective cohort, lack of data on patients who dropped out and were lost to follow-up, influence of medications on LAR changes, and a modest, convenient sample of 52 completers of programme, although we did achieve statistically significant results for the hypothesis and aim of the study.

In conclusion, I have successfully demonstrated significant effect size on weight loss and changes in metabolic parameters in patients who completed our milk-based meal replacement programme, I have successfully demonstrated the changes in insulin resistance via LAR in patients who underwent sleeve gastrectomy in our unit along with significant correlation between LAR and weight loss, and I have successfully demonstrated the reduction in LAR and its significant correlation and association with weight loss in a patient who underwent the milk-based meal replacement programme in a prospective cohort study. I believe that milk diet can play a key role in reducing metabolic risk in patients who are on the waiting list of bariatric surgeries with current waiting times, in excess of three years in Ireland, in treatment of patients who suffer from chronic venous ulcerations, in

treatment of polycystic ovarian syndrome for which I have recently established a link between University Hospital Galway and Beaumont Hospital's fertility specialist teams. I believe that the changes were seen in LAR and its correlation and association with weight loss has given us a great understanding of the pathophysiology of insulin resistance in the adipose tissue, and this will help us in designing randomized controlled trials in the future to assess the changes in insulin resistance in different weight-loss interventions. An example of a randomised controlled trial will be as described below

Population: Adults with a history of severe obesity defined as a BMI >40kg/m^2or >35 kg/$m^2$with co-morbidities such as Type 2 diabetes or Non-alcoholic steatohepatitis or sleep apnoea.

Intervention: To be randomised to either receive sleeve gastrectomy or milk-based meal replacement programme. As per sample size analysis with the known effect size of the interventions from work arising from this thesis, we would aim to recruit 25 patients in the surgical arm versus 50 patients in the milk-based meal replacement and control group as the attrition rate to follow-up is high as seen in the milk-based meal replacement with up to 50 % attrition demonstrated in this thesis.

Control: To be randomised to receive standard healthy eating and dietary advice.

Outcome: change in insulin resistance measured via leptin to adiponectin ratio and to demonstrate the difference in the change in adipokines after different bariatric interventions.

Outcome measures: All patients be followed up every two weeks to obtain anthropometric measures such as weight, height, and waist circumference, metabolic profile such as renal, liver, lipid, and Hba1c, and marker of insulin resistance in the form of leptin to adiponectin ratio. Optional inclusion of other markers of insulin resistance, including hyperinsulinaemic euglycaemic clamps, HOMA-IR etc, for comparison and correlation between LAR and other markers of insulin resistance. Measurement of other factors playing a key role in weight loss and weight maintenance and improvement in insulin resistance such as GLP-1, Peptide YY levels, Ghrelin, and FGF-19 levels, obtaining a stool sample every month to follow changes in gut microbiota associated with the two interventions.

Timeframe: Patients to be followed up every two weeks up to 1-year post sleeve gastrectomy and 1-year post starting of the milk-based meal replacement programme. The other option would be to be followed up until patients achieve a minimal weight loss of 15% as the mean weight loss observed in milk-based meal replacement programme is 15%, and to compare the changes in outcome variables at the time when patients in both interventions achieve a similar weight loss.

Supplementary Data

Protocol:

Intensive Weight Management Programme (IWMP)

Outline:

The Bariatric Medicine & Surgery (BMS) service in Galway University hospital provides a multidisciplinary team (MDT) approach for the management of severe obesity (BMI >40kg/m2). There are a variety of interventions that are open to patients attending the service, including a diet & physical activity programme (CROI CLANN) as well as Bariatric surgery for suitable candidates; however, these do not meet the needs of all patients. The aim of this intervention is to support patients in the improvement of medical risk factors through an intensive weight management programme over a period of 24 weeks.

This document outlines the standard operating procedures underlying the roles of the multidisciplinary team, which consists of consultant/registrar, obesity CNS & dietitian responsible for the management of the BMS service.

Definitions and abbreviations:

- Bariatric Medicine & Surgery (BMS)
- Galway University Hospital (GUH)
- Multidisciplinary Team (MDT)
- Body Mass Index (BMI)

General protocol overview:

Initial medical assessment

- Baseline bloods taken include, but are not restricted to: Urea & electrolytes, liver function tests (LFT), renal profile (RP), bone profile, fasting glucose, HbA1c, thyroid function tests (TFT), full blood count (FBC), fasting lipids, vitamin B_{12}, folate
- The patient is commenced on other medication as appropriate (e.g. anti-hypertensives, nicotine patches).
- The need for oral hypoglycaemic agents or insulin in patients with Type 2 diabetes is monitored. As a general guide, insulin sensitizers (e.g., metformin) may be continued. However, if the patient is currently taking sulphonylureas (e.g., diamicron MR), insulin secretagogues (e.g., replagnanide), or insulin, the dose should be reduced by 50% as soon as the patient commences the liquid low-calorie diet. Blood glucose should then be monitored pre-meal and at bedtime (qds) for three days with a view to discontinuing these medications if any blood sugars are ≤5mmol/L. *Note:* it is not unusual for insulin doses in excess of 100u per day to be discontinued in these patients once the liquid low-calorie diet regime has begun.
- Referral to other clinical teams is made as appropriate, e.g., cardiology, surgical.

Dietary strategy: meals and supplementation:

- The patient is commenced on a low-calorie milk-based diet (see Appendix 3).

Other food-based options (a conventional calorie-controlled diet) may be considered if the milk diet is not tolerated by individuals.

- Nutritional supplements are advised to ensure the liquid low-calorie diet is nutritionally complete, which are:

1. Multi-vitamin (Sona Balance, Sona Multiplus, or Boots A-Z multivitamin & mineral) – once daily
2. Omega 3,6,9 fatty acids (Omecor) – once daily

- In addition, patients are recommended to drink a salty drink daily (e.g., Stock cube or ½ teaspoon salt in water) to meet sodium requirements
- Patients are also commenced on a bulking laxative (e.g., Fybogel) in an effort to prevent constipation. If patients are already on laxative therapy, this should be continued.

Programme inclusion criteria and specifications:

- Of note, all cases being considered for commencement are discussed by the Bariatric MDT.
- BMI >35 kg/m^2
- A maximum of three patients may be commenced on the milk diet at each bi-weekly clinic.

Exclusion criteria:

- Age <18 or >75 years
- Pregnancy or breastfeeding
- Significant renal disease
- Significant cardiac disease
- Recent MI or CVA
- Uncontrolled hypothyroidism
- Significant learning difficulties
- Significant psychiatric disorders
- Inability to commit to clinic visit schedule
- Inability to tolerate milk

Initiation of IMWP: procedures:

- Body weight, height, blood pressure, baseline ECG were recorded.
- Full history and physical examination by medical team, CNS & Dietitian, including a comprehensive nutrition history.
- Programme protocol discussed with the patient, including potential risks and benefits.
- Nutritional requirements are calculated, and a dietary regimen is devised for the patient by a dietitian.
- Coping strategies and distraction techniques with respect to hunger are discussed with the patient prior to initiation. Written information is provided.

- Weight loss goals and time frames are discussed with the patient.
- Baseline blood tests were taken.
- Smoking cessation advice and resources given, when appropriate.

Ongoing monitoring (weight loss phase – weeks 1 to 8):

- Patient on low-calorie milk-based diet only, as described below.
- Fortnightly visits to clinic, with body weight and blood pressure monitoring.
- Medication reviewed by the medical team.
- The patient is reviewed by the Dietitian.
- The weight loss phase is used as an opportunity to provide education on various topics such as:
    - Record keeping
    - Physical activity
    - Environmental control
    - Food labeling
    - Calories in foods
    - Healthy eating
    - Weight loss maintenance
- A blood test taken, which include (but are not restricted to) urea & electrolytes, LFT, renal profile, FBC, TFT's, lipid profile, HbA1c.

Food reintroduction phase (weeks 8 to 16):

Weeks 1 & 2: Patients are asked to include a meal (dinner) consisting of a specific quantity of protein food as well as a specific quantity of vegetables. This meal should be weighed using digital food scales. The milk volume allocation should be reduced accordingly. The protein requirements of the patient should continue to be met by the diet. Monitoring should otherwise continue as in phase 1.

Weeks 3 & 4: Patients are asked to include two meals per day (lunch & dinner) consisting of 2 portions of protein foods, four portions of vegetables, and two portions of carbohydrate foods. All foods should continue to be weighed. The milk volume is reduced substantially at this stage. Monitoring should otherwise continue as in phase 1.

Weeks 5 & 6: Patients are asked to include three meals per day (breakfast, lunch & dinner). Individual preferences should be taken into account. Again milk volume should be reduced as appropriate. Monitoring should otherwise continue as in phase 1.

Weeks 7 & 8: Patients can introduce some low-calorie snacks into their diet. The full food reintroduction phase is now complete. Milk can continue to be included within healthy eating guidelines. At this stage, a healthy eating regimen should be established in line with healthy eating guidelines. Monitoring should otherwise continue as in phase 1.

Weight maintenance phase (week 16-24):

- Patients continue to attend every two weeks.
- Monitoring continues as per phases 1 & 2.
- Issues such as relapse management, planning meals in advance, managing difficult meal timing and goal setting. Self-monitoring is vital at this stage, and participants are encouraged to continue this practice on discharge.

Ongoing Support:

On completion of the IWMP, patients are followed up to ensure continued support and to aid with relapse management. Upon completion, they are offered to follow up appointments after eight weeks, 16 weeks, 24weeks, and afterward if requested.

Premature discharge of participant from IWMP:

- Self-discharge
- Not tolerating milk volume
- Adverse effects as a result of programme
- Non-compliance with milk/laxatives/multivitamins
- Multiple lapses resulting in participants consuming excess calories
- Non-attendance at appointments
- On instruction from the medical team

Low calorie milk-based diet outline:

Time	**Allowance**
8am	325ml semi-skimmed milk
10am	325ml semi-skimmed milk
12pm	325ml semi-skimmed milk
2pm	Either: 1 stock cube in a glass of water or 1 small sachet of salt in a glass of water
3pm	325ml semi-skimmed milk
6pm	325ml semi-skimmed milk
8pm	325ml semi-skimmed milk
10pm	325ml semi-skimmed milk

Supplements:

- Centrum Advance or Boots A-Z multivitamin (one a day)
- Omega 3,6,9 supplement.
- Fybogel 1-2sachets per day.

Notes:

- Unrestricted: Water, Tea, Coffee (no sugar; milk used from allowance above)
- Maximum 500mls “diet” minerals.

Summary:

Daily dietary intake	**Quantity**	**Electrolytes**	**Quantity**
Fluid volume	2275 ml	Sodium	53 mmol
Energy	1045 kcal	Potassium	87 mmol
Protein	75 g	Calcium	68 mmol
Fat	36 g		
Carbohydrate	113 g		

Nutritional intervention details:

Composition of the milk-based low-calorie diet (Luton & Dunstable hospital low-calorie liquid diet)

	2272 ml (4 pints) Semi-skimmed milk	**SonaBalance or Boots A-Z multivitamin (1 dose)**	Total	**Irish RDA 1999 (18-64yr)**	
				Male	Female
Energy	1045 kcal	0 kcal	1045 kcal		
Protein	75g	0 g	75 g	0.75g/kg/day	
Fat	36g	0 g	36 g		
n-6 PUFA	Trace	0		2 % of dietary energy	
n-3 PUFA	Trace	0		0.5% of dietary energy	
Carbohydrate	113g	0 g	113g		
Sodium	53 mmol	0 mmol	53 mmol		
Potassium	87 mmol	0	87 mmol	79 mmol	79 mmol
Calcium	2726 mg	162 /200mg	mg	800 mg	800 mg
Magnesium	272 mg	100 /60mg	372/332 mg		
Phosphorus	2135 mg	109 /0mg	mg	550 mg	550 mg
Iron	1.36 mg	18 /14mg	mg	10 mg	14 mg
Zinc	9 mg	15 /10mg	24 /19mg	9.5 mg	7 mg
Vitamin D	22.7 ug	10/5 ug	32.7/27.7 ug	0-10 ug	0-10 ug
Vitamin K		25/75ug	25/75 ug		
Vitamin A		1050 /400ug	1050/400ug		
Vitamin E	68 mg	30/12 mg	98/80 mg		
Vitamin C	22.7 mg	60/80 mg	82.7/102.7mg	60 mg	60 mg
Vitamin B6	1.36 mg	2/1.4 mg	3.36/2.76 mg	15 ug / g protein	
Thiamine	0.9 mg	1.5 /1.1mg	2.4/2 mg	100 ug / MJ	
Riboflavin	3.86 mg	1.7/1.4 mg	5.6/5.26mg	1.6 mg	1.3 mg
Niacin	2.27 mg	20/16 mg	22.27/18.27 mg	1.6 mg/MJ	
Vitamin B12	9.08 ug	6/2.5 ug	11.58 ug	1.4 ug	1.4 ug
Folate	113 ug	400/200 ug	513/313 ug	300 ug	300 ug

RDA, recommended daily allowance;

PUFA, polyunsaturated fatty acids.

Low calorie dietary regimen following food re-introduction in phase two/ three:

Meal	**Food**	**Kcals**	**Protein g**
Breakfast	• 2 slices of brown / white Bread • 1 pat of Low Low • Tea / coffee (no sugar)	140 25	4 0
Mid-morning	• 1 medium piece of fruit	40	0
Lunch	• 1 scoop of mashed potato • 2 slices of meat / 1 breast of chicken (no breadcrumbs or batter) / 1 fillet of fish (~ 3oz) • 4 tablespoons veg of the Day • 200ml low fat milk • 1 diet yoghurt	70 130 – 180 40 90 60	2 21–30 1 7 7
Mid-afternoon	• 1 medium piece of fruit	40	0
Tea	• 2 slices of white / brown Bread • 1 pat Low-Low • 1 small tin of tuna / 2 oz hard cheese / 2 slices of ham / 1 chicken breast / 2 boiled eggs • Heaped plate of lettuce / tomato / onion / sweetcorn / beetroot / mixed bean salad NO coleslaw/potato salad/mayonnaise/ dressing • Tea / coffee (no sugar)	140 25 180 – 240 40	4 0 14–21 1 – 7
Before bed	• 1 diet yoghurt	60	7
Total		1134	75

Chart 1: Milk Diet Programme Weeks 0-8: Weight loss phase

Based on:

total protein requirements per day = $0.17N_2$/kg×6.25 (g)

If BMI > 30 kg/m^2= Patients require 75% of total protein requirement during the programme

If BMI > 50 kg/m^2= Patients require 65% of total protein requirement during the programme

100 ml of semi skimmed milk contains

- 3.5 g of protein
- 45 Kcal of carbohydrates
- 1.5 g of fat
- 5 g of carbohydrates

During this phase the total protein requirements are based on BMI as above and are replaced completely by semi skimmed milk which is recommended to be consumed at three-hour intervals during the day.

Example:

patient X: weight 120 kgs, height 1.70 cm and BMI 41.5 kg/m^2

Total Protein requirement = 127.5 g/day

Total Protein requirement calculated as per BMI = 127.5 × 75% = 95.6 g/day

Total Volume of milk per day = 95.6 × 100 ÷ 3.5 = 2731 ml/day

Total Calorie intake per day (Kcal) = 2731 × 45 ÷ 100 = 1228 Kcal/day

Total Carbohydrate intake per day (g) = 2731 × 5 ÷ 100 = 136.5 g/day

Total Fat intake per day (g) = 2731 × 1.5 ÷ 100 = 40.9 g/day

Other Important Dietary Components

- Oxo/ Stock Cube × 1 per day
- Standard Multivitamin × 1 per day
- Omega 3 × 1 per day
- Fybogel Mebeverine × 2 sachets per day

Unlimited intake allowed: Water and tea/coffee (no sugar, milk),500 ml of diet minerals per day and no added sugar squash in water if desired.

Chart 2: Milk Diet Programme Weeks 8-10: Weight stabilization phase

Based on:

total protein requirements per day = $0.17N_2$/kg×6.25 (g)

If BMI > 30 kg/m^2= Patients require 75% of total protein requirement during the programme

If BMI > 50 kg/m^2= Patients require 65% of total protein requirement during the programme

100 ml of semi skimmed milk contains

- 3.5 g of protein
- 45 Kcal of carbohydrates
- 1.5 g of fat
- 5 g of carbohydrates

During this phase the total protein requirements are based on BMI as above and are replaced by introduction of main meal and semi skimmed milk which is recommended to be consumed at three-hour intervals during the day except during the main meal.

Components of main meal = 75 g cooked weight meat/chicken/fish + 150 g of vegetables/salad + 1 oval wholemeal pitta bread or 1 large wholemeal wrap.

Example:

patient X: weight 120 kgs, height 1.70 cm and BMI 41.5 kg/m^2

Total Protein requirement = 127.5 g/day

Total Protein requirement calculated as per BMI = 127.5 × 75% = 95.6 g/day

Main meal protein requirement: 33 % of total protein intake= 31.5 g/day

Total volume of milk per day = 63.0 × 100 ÷ 3.5 = 1802 ml/day

Total Energy intake = 1228 kcal/day

Other Important Dietary Components

- Oxo/ Stock Cube × 1 per day
- Standard Multivitamin × 1 per day
- Omega 3 × 1 per day
- Fybogel Mebeverine × 2 sachets per day

Unlimited intake allowed: Water and tea/coffee (no sugar, milk),500 ml of diet minerals per day and no added sugar squash in water if desired.

Chart 3: Milk Diet Programme Weeks 10-12: Weight stabilization phase

Based on:

total protein requirements per day = 0.17N_2/kg×6.25 (gm)

If BMI > 30 kg/m^2= Patients require 75% of total protein requirement during the programme

If BMI > 50 kg/m^2= Patients require 65% of total protein requirement during the programme

100 ml of semi skimmed milk contains

- 3.5 g of protein
- 45 Kcal of carbohydrates
- 1.5 g of fat
- 5 g of carbohydrates

During this phase the total protein requirements are based on BMI as above and are replaced by introduction of two meals and semi skimmed milk which is recommended to be consumed at three-hour intervals during the day except during the meals.

Components of meal 1 = 75 g cooked weight meat/chicken/fish + 150 g of vegetables/salad + 1 oval wholemeal pitta bread or 1 large wholemeal wrap.

Components of meal 2 = 125 g meat/chicken/fish + 150 g of vegetables + 150 g of boiled or steamed potato or 100 g of boiled pasta/rice.

Example:

patient X: weight 120 kgs, height 1.70 cm and BMI 41.5 kg/m^2

Total Protein requirement = 127.5 g/day

Total Protein requirement calculated as per BMI = 127.5 × 75% = 95.6 g/day

Main meal protein requirement: 66 % of total protein intake= 63 g/day

Total volume of milk per day = 33% of total protein intake=31.5 g= 31.5 × 100 ÷ 3.5 = 900 ml/day

Total Energy intake = 1228 kcal/day

Other Important Dietary Components

- Oxo/ Stock Cube × 1 per day
- Standard Multivitamin × 1 per day
- Omega 3 × 1 per day
- Fybogel Mebeverine × 2 sachets per day

Unlimited intake allowed: Water and tea/coffee (no sugar, milk),500 ml of diet minerals per day and no added sugar squash in water if desired.

Chart 4: Milk Diet Programme Weeks 12-14: Weight stabilization phase

Based on: total protein requirements per day = $0.17N_2$/kg×6.25 (g)

If BMI > 30 kg/m^2= Patients require 75% of total protein requirement during the programme

If BMI > 50 kg/m^2= Patients require 65% of total protein requirement during the programme

100 ml of semi skimmed milk contains

- 3.5 g of protein
- 45 Kcal of carbohydrates
- 1.5 g of fat
- 5 g of carbohydrates

During this phase the total protein requirements are based on BMI as above and are replaced by introduction of two meals, snacks in between meals and semi skimmed milk which is recommended to be consumed in two divided doses during the day.

Components of meal 1 = 75 g cooked weight meat/chicken/fish + 150 g of vegetables/salad + 1 oval wholemeal pitta bread or 1 large wholemeal wrap.

Components of meal 2 = 125 g meat/chicken/fish + 150 g of vegetables + 150 g of boiled or steamed potato or 100 g of boiled pasta/rice.

Components of Snacks = 3 pieces of fruit per day + 6 dessertspoons oats or 2 weeta-bix + 125 g of diet yoghurt.

Example:

patient X: weight 120 kgs, height 1.70 cm and BMI 41.5 kg/m^2

Total Protein requirement = 127.5 g/day

Total Protein requirement calculated as per BMI = 127.5 × 75% = 95.6 g/day

Main meal protein requirement: 66 % of total protein intake= 63 g/day

Snack protein requirement: 10 % of total protein intake = 9.56 g/day

Total volume of milk per day= 23 % of total protein intake = 21.9 g= 21.9 × 100 ÷ 3.5= 625 ml/day

Total Energy intake = 1228 kcal/day

Other Important Dietary Components

- Oxo/ Stock Cube × 1 per day
- Standard Multivitamin × 1 per day
- Omega 3 × 1 per day
- Fybogel Mebeverine × 2 sachets per day

Unlimited intake allowed: Water and tea/coffee (no sugar, milk),500 ml of diet minerals per day and no added sugar squash in water if desired.

*This meal plan is continued till the end of the programme.

Patient Information Leaflet

The **GERONIMO** Study

Genetic **E**ffects on the **R**esponse to an **O**utpatient Intensive **N**utritional **I**ntervention in **M**edically Complicated **O**besity

You have been invited to take part in this study because you were previously treated with the 'MILK DIET' at the Bariatric Medicine Clinic in University Hospital Galway.

While attending this clinic, information about your medical history, medications, blood test results, and measurements such as weight and height were routinely recorded. These records are confidentially and securely stored within Galway University Hospitals. As part of the GERONIMO study, we would like to analyze this information to assess the effectiveness of the care we provide to patients. Our aim is to inform better care for individual patients in the future. By using data from individual patients in this way, we can conduct valuable and important medical research that will ultimately lead to more effective and efficient patient care.

We would also like to investigate if genes influence weight loss response to the 'MILK DIET'. We are asking all patients who completed the 24-week programme to provide a blood sample from which we will obtain DNA in order to better understand the genetic influences on weight loss. We would like to measure leptin and adiponectin, the hormones produced by adipocytes/ fat cells, as they are involved in inflammation and insulin resistance in some patients. We would also like to measure blood ketone levels as a standard blood test in all our patients during the milk programme. No data that we use will ever be personally identifiable, nor will participating or declining this opportunity influence the nature of the care that you receive in any way, now or in the future. If you agree to take part in the study, you will be asked to come to the Clinical Research Facility in University Hospital Galway for a 10-minute appointment. **You will need to fast for 12 hours prior to this appointment.**

When you first arrive at the hospital, you will meet one of our research fellows and will be given the opportunity to ask any questions you may have about the study. If you are happy to proceed, you will be asked to sign a consent form. You will be provided with a copy of this consent form for your own personal records.

We will measure your height and weight and will estimate your total body fat composition. We will also take measurements of your waist, thigh, and hip. We would also like to measure your blood pressure.

Please note that the doctors involved in this study are covered by standard medical malpractice insurance. Nothing in this document or in the consent form for the study restricts or curtails your rights in any way.

Thank you for reading this information leaflet. Please note that it is intended to complement the other information you have received, and we would be delighted to discuss any queries you might have at any time.

Genetic **E**ffects on the **R**esponse to an **O**utpatient Intensive **N**utritional **I**ntervention in **M**edically Complicated **O**besity: The **GERONIMO** Study.

While attending the bariatric services in the hospital, information about your medical history, medications, blood test results (this will include blood ketone levels), and measurements such as weight and height are routinely recorded. These records are confidentially and securely stored, indefinitely, within Galway University Hospitals. As part of a research study, we would like to analyze this information to assess the effectiveness of the care that we provide to patients. Our aim is to inform better care for individual patients in the future. In particular, we would like to learn more about how patients respond to the "milk diet." By using data from individual patients in this way, we can conduct valuable and important medical research that will ultimately lead to more effective and efficient patient care. Secondly, we would like to see whether genes influence how well individuals respond to the milk diet. We are asking all patients who completed the programme to provide a blood sample, from which we will obtain DNA, in order to better understand the genetic influences on weight loss. We would also like to measure leptin and adiponectin levels; these are hormones produced by the adipocytes/fat cells and are involved in the process of inflammation in our body. No data that we use will ever be personally identifiable, nor will participating or declining this opportunity influence the nature of the care that you receive in any way, now or in the future.

Please initial each box

1. I confirm that I have read and understood the information detailed above and have been given the opportunity to ask questions. ☐

2. I agree to have information relating to my medical history, medications, blood tests, and body size measurements recorded during my visit and for this information to be confidentially and securely stored, indefinitely, within Galway University Hospitals. ☐

3. I consent to the results of any analysis of these data being published for scientific purposes, either in print media or electronically online, now or in the future. ☐

4. I understand that the data recorded during my visit will be anonymized whenever they are used for research purposes. ☐

5. I consent for a sample of blood to be analyzed for genes that have been identified now or will be in the future that are associated with obesity and diabetes. I understand that the nature of this research means that genetic information obtained from these tests will not be made available to me personally, or to my doctor, or any third party now or at any time in the future. ☐

6. I understand that I am free to withdraw consent for my data being used for research purposes at any time, without giving any reason, and without my medical care or legal rights being affected in any way, now or in the future. ☐

----------	----------	----------Name
of Patient (BLOCK CAPITALS)	Date	Signature
----------	----------	----------Name
of Researcher	Date	Signature

Response to reviewers for journal publication/ Rebuttal for journal publication

For Chapters 2 and 3 of the thesis

Dove press Milk Diet Paper Rebuttal for Diabetes, Metabolic Syndrome and Obesity: Targets and Therapy publication: Chapter 2 of thesis

Reviewer #1:

Comment 1:

I would recommend adding the nutrient profile of the first eight weeks of the trial to elucidate the detail of the dietary intervention. I would also suggest adding the detail of dietary intake for the remaining phases in a table presenting the foods, timing of consumption, etc.

Response to comment 1:

We have included supplementary charts of dietary intervention with the rebuttal, which includes all the details and calculations required for individualizing the dietary intake according to body weight and BMI (supplementary chart 1-4); this elucidates the dietary intake in detail and changes made during the course of the intervention.

Comment 2:

Did you control for their physical activity level throughout the study? if not, this should be acknowledged as a limitation of this study.

Response to comment 2:

This is a very interesting thought, and we will consider measurement of physical activity in our prospective studies, but unfortunately, we do not control their physical activity, and we have acknowledged this as a limitation of the study, which read as “there was no control for the physical activity levels throughout our study; therefore we cannot comment on physical activity energy expenditure levels during our intervention, and this should be addressed in our future studies.”

Comment 3:

Do you have any data on body composition profile (e.g., % fat-free mass, % Fat mass)? If not, why didn't the authors decide not to measure these outcomes?

Response to comment 3:

In terms of anthropometric measurements, we measured weight, BMI, and EBW% (Excess body weight %), but we did not measure any body composition profile. We do not have a reasonable explanation for this, but we would consider it in our future prospective studies.

Comment 4:

Referring to my previous question, how could authors conclude the weight loss is equivalent to fat loss without taking into consideration muscle mass, body water, bone content, etc.)

Response to comment 4:

We have never mentioned in our manuscript that weight loss is equivalent to fat loss; we do understand that one of the limitations of this study is the lack of body composition measurement, and we would like to address this in our future studies. We have added a limitation in our discussion describing this, which reads as “body composition measurements were not performed during this study and due to such a limitation, we cannot say that weight loss in our cohort was strictly limited to fat loss.”

Comment 5:

LINE 175- What is EBW? Is it Expected body weight? This needs to be reported in full when it is used for the first time in the manuscript. The same applies to the rest of the abbreviations.

Response to Comment 5:

We do apologize for not reporting this when we mentioned it the first time in our manuscript. We have carefully amended the manuscript and described all the abbreviations. EBW stands for Excess Body Weight, and we calculated excess body weight % using the formula (BMI÷25) ×100-100.

Comment 6:

it's unclear how you measure the adherence of participants in every three phases. Please explain in more detail and report data in case you have any.

Response to Comment 6:

We did not measure for adherence in our retrospective study, which is presented in this manuscript. We did ask the patients themselves regarding their adherence to diet based on weight loss profile in each visit during the programme, and those patients who were not adherent to the dietary protocols were stopped from continuing in the programme; this has played a major role in our attrition rates. To address the measurement of adherence in our programme we have started measuring ketone levels in our prospective studies, a subset of which was presented as a poster in the European Congress of Obesity in April 2019; we wish to complete this study by December 2019.

Reviewer #2:

Comment 1:

The rationale for the use of a solely milk-based diet in the first 8 weeks is not so clear for me. I understand that in comparison with commercial meal replacements, milk is low in costs. However, commercial meal replacements are most of the time also based on milk products, fortified with micronutrients. In this study, the diet was supplemented with sodium, vitamins, minerals and fiber as well, with accompanied costs. Moreover, the 14 sessions with health care professionals incorporated in the program is a quite intensive and costly part. Perhaps the authors could include a discussion of the costs of the intervention, so readers are able to compare that to conventional diets

Response to Comment 1:

We would like to thank the reviewer for the interesting yet difficult comment. We have mentioned the benefits of milk-based dietary intervention in the introduction section “Milk whey protein attenuates muscle loss and preserves myofibrillar protein synthesis in adults with obesity during very-low-calorie diets. Milk reduces appetite, calorie intake, and body weight and alters post-prandial glucose and lipid metabolism in men with obesity. In mice, milk casein-derived peptides reduce high-fat diet-induced adipose tissue inflammation. A recent trial showed that drinking low-fat milk made children feel fuller and eat less later in the day compared to juice or water”.

We do understand that most commercial meal replacement programmes are based on milk products fortified with micronutrients, but let us give you an example of the diet used in DiRECT Trial[87]; the intervention cost 1913 sterling pounds[306], which was based on a dietary intervention called counterweight plus, the cost for the product on their website for losing 15 kgs via their meal replacement programme and dietitian input for 12 months is stated as 2200 euros, which we believe is far expensive than the costs of our programme which has shown better results with a mean weight loss of 23.9 kgs in the six months, but we definitely lack data at 12 months in this study. In

fact, as stated by the reviewer the most expensive part of our delivery of the programme would be the 14 visits to the bariatric unit where the patients are seen by a nurse, dietitian and a doctor along with admin charges which accounted to approximately XXX euros. The cost of milk for six months as per the latest costs in Ireland is 290 euros, cost of multivitamins which would be the micronutrient supplementation is 36 euros for six months, cost of Oxo cube used as a salt replacement for six months is 23.1 euros and the cost of fybogel for fibre replacement equates to 87.3 euros for six months, omega 3 supplement costs 10 euros for six months, average cost of the meal recommendation in the weeks 8-24 approximates to 500 euros, so these components in total equates to approximately 950 euros for six months of the intervention, which is far cheaper than most commercial meal replacement programme. We do forget that this is far lower than the average grocery costs for one individual in Ireland would be approximately 400 euros per month which equated to 2400 euros for six months.

Comment 2:

Why choose for a milk-based diet rather than a normal diet? 1200 kcal/day is not that low and could easily be provided via a diet according to the standard nutritional guidelines with solid foods. Please, improve the rationale in the introduction and comment on this rationale in the discussion section.

Response to Comment 2:

This is a very interesting comment from the reviewer. Normal dietary advise is more than often not enough to treat patients with severe obesity[87]. 73 (68.9%) of the patients in our cohort had undergone a lifestyle and dietary modification programme of eight weeks [82] in which they had been individually given dietary and exercise advise, but this was not sufficient, and they have required interventions including a meal replacement programme. It is proven that the annual probability of maintaining 5 % weight loss in a large cohort of obese individuals who underwent community-based dietary advice or interventions was 1 in 8 in males and 1 in 7 in females[307]. Only 10 % of eligible patients avail bariatric surgery as a treatment option; therefore other domains of bariatric care such as meal replacements of some sort or another need to be developed and evaluated. Low energy liquid diets have been validated as a better option when compared to dietary advice or exercise alone [90]; therefore a diet with standard nutritional guidelines with solid foods is not going to be sufficient in obtaining at least 10 % weight loss which is considered meaningful [290]. We have described in detail the benefits of milk, which is used as a main dietary component in our diet, and therefore we believe that it is important for patients to have meal replacements other than

normal solid food as per nutritional guidelines; this might also have a placebo or psychological role in adherence to dietary advice. We have included this data in the discussion of our paper.

Comment 3:

In the discussion, the authors mention that a possible mechanism underlying a likely reduction in appetite is ketosis. However, one would not expect ketosis following a diet with more than 50 grams of carbohydrates a day. A quick calculation shows that 2.5 l of milk will deliver 125 gr of carbohydrate.

Response to Comment 3:

This is a very interesting consideration from the reviewer that milk-based meal replacement is not a ketogenic diet. We do agree with the reviewer that this is not a ketogenic diet, but in our discussion section(line 260-266) we commented saying that we do not understand the mechanism behind the suppression of appetite in patients undergoing this programme, but one plausible explanation maybe ketone production of certain degree that might contribute to it but again having said that we did not measure ketone levels and so we cannot conclude that this might be the underlying mechanism. We will, however consider measuring ketone levels in future studies in our intervention to elucidate our thinking and this has been mentioned in the discussion.

Comment 4:

One of the limitations of the study that is not sufficiently mentioned in the discussion, is the short duration of the intervention. Although the authors do mention the need for studies with longer follow-up, this limitation should be discussed further.

Response to Comment 4:

We do agree that there is no follow-up data demonstrated for the purpose of this study, and we have recognized this as the most important limitation in our discussion from lines 275 to 290 in our paper. We will definitely consider the demonstration of a longer duration of follow-up in our future studies.

Comment 5:

Of interest to mention in the discussion is the need for studies that also take into account the possible effects of this diet intervention on the results/complications of the bariatric surgery afterward. How many of these participants went for bariatric surgery after the diet intervention finished?

Response to Comment 5:

This is an interesting thought and would be considered during the examination of the long-term effects of milk-based dietary intervention. Ten patients (9.4%) in our cohort underwent bariatric surgery in the form of sleeve gastrectomy in the years following the milk-based diet intervention.

Responses to reviewers/ Rebuttal for Scientific Reports publication for Rafey et al, SREP-19-42186-T: Chapter 3 of thesis

Reviewer #1:

Comment 1:

Rafey and colleagues assessed the effect of Laparoscopic Sleeve Gastrectomy on the Leptin-to-Adiponectin Ratio (LAR) in adults with severe obesity. Globally the article is well written, the English is acceptable, and the objectives, methods and results are clearly presented. Nevertheless, some revisions might be needed to ameliorate the manuscript.

Concerning the form, the authors frequently used abbreviations without a prior signification in the text (HbA1c, STROBE, SGLT-2, GLP-1...).

Response 1:

We are grateful to this reviewer for their careful consideration of our manuscript and for their positive and constructive feedback, which has certainly allowed us to improve the paper. We have carefully reviewed the manuscript and made the abbreviation definitions clearer, as highlighted in the revised manuscript. STROBE refers to the "Strengthening the Reporting of Observational Studies in Epidemiology" guidelines. HbA1c refers to "glycosylated haemoglobin". SGLT2 refers to "sodium-glucose linked transported 2" drugs. GLP1 refers to "glucagon-like peptide 1".

Comment 2:

Page 2, line 3 to 5, (as well as in page 3, line 15 to 18 and in page 7, line 5 to 7) the authors highlight the fact that the influence of Bariatric surgery on the Leptin to Adiponectin ratio has not been previously described but there's one study realized by Unamuno X and collaborators, published in September 2019, which aimed to study in an the effects of Roux-en-Y gastric bypass (RYGB) on the circulating levels of adiponectin, leptin, and the adiponectin/leptin (Adpn/Lep) ratio in patients with obesity and type 2 diabetes (T2D). They found an increase of the Adpn/Lep ratio. Considering that, authors could reformulate the sentence and include the fact that a study on the influence of Bariatric surgery on Adpn/Lep ratio has been published (check the reference below).

[Unamuno X, Izaguirre M, Gómez-Ambrosi J, Rodríguez A, Ramírez B, Becerril S, et al. Increase of the Adiponectin/Leptin Ratio in Patients with Obesity and Type 2 Diabetes after Roux-en-Y Gastric Bypass. Nutrients. 3 sept 2019;11(9).]

Response 2:

We are extremely grateful to the reviewer for highlighting this important and relevant paper, which we have now incorporated into our manuscript. We have amended the start of the abstract to state specifically that the influence of sleeve gastrectomy on the ratio of the two adipokines has not previously been described. We have amended the introduction as follows: "Recently, investigators described changes in the adiponectin: leptin ratio (ALR) in a cohort of 25 Spanish adults with type 2 diabetes who underwent Roux-en-Y gastric bypass [247]. To date, the influence of sleeve gastrectomy on the ratio of these two hormones has not been described."

We have also amended the discussion to "tone down" the interpretation of the novelty and significance of our results, in the context of the important findings in the Unamuno paper, thus: "We are aware of only one other study by Unamuno et al. that has considered them together as a ratio and as an indicator of adipocyte dysfunction [247]. This study, while describing an "inverted" ratio of adiponectin: leptin, found broadly similar effects on both hormones to ours, though all of their patients had type 2 diabetes and all had undergone RYGB. Ours is the first study to consider these adipokines as a ratio in patients undergoing laparoscopic sleeve gastrectomy. We found that the reduction in LAR were driven primarily by an increase in adiponectin rather than a reduction in leptin, and these changes were proportional to the reduction in weight after surgery, again consistent with the Unamuno study."

Comment 3:

Page 2, line 21 to 23 (conclusion section of the abstract), the authors concluded to an increased insulin sensitivity and a reduced cardiovascular risk. We think that they should revise this statement and be more cautious since they didn't directly assessed cardiovascular risk and insulin sensitivity. Even in the "discussion section" (page 7, line 3 and 4) you first concluded to a "substantial increases in insulin sensitivity after 12 months" before underlining the reduction in LAR. We think this could also be reformulate since your study firstly targeted the LAR.

Response 3:

We agree with the reviewer that more a more measured interpretation of the findings is needed here. We have amended the abstract conclusion as follows: “Patients undergoing laparoscopic sleeve gastrectomy had a substantial reduction in their LAR after 12 months which was proportional to the amount of weight lost. This may indicate an improvement in insulin sensitivity and a reduction in cardiovascular risk.”

We have also amended the very start of the discussion section as follows: “We have shown that in a predominantly white cohort of adults with severe obesity who underwent laparoscopic sleeve gastrectomy, there was a substantial reduction in the LAR after 12 months, which may indicate an increase in insulin sensitivity.”

Comment 4:

Page 4, line 4, the authors said that they performed follow-up measures 12 months after surgery but in the abstract (methods’ section) they précised 12.1±1 months. This should be harmonized.

Response 4:

We have amended the methods section accordingly and clarified that the mean follow-up duration was 12.1±1 months.

Comment 5:

Even though the authors did a prospective follow-up, the study is an interventional one. We think this should be précised in the “method” section.

Response 5:

We have amended the start of the methods section to note that this was a single-centre, interventional prospective cohort study, and we appreciate the opportunity to clarify this.

Comment 6:

Page 5, line 33, the authors talked of “borderline statistical significance”. We think that the term “borderline” is not necessary. Either there’s significance or not.

Response 6:

We agree, and we have amended that statement as follows: “This equated to an overall reduction in the LAR which was consistent with a 70.9% increase in insulin sensitivity in the cohort 12 months after sleeve gastrectomy.”

Comment 7:

Can it be a specific pathophysiological mechanism explaining the lesser influence of the Laparoscopic Sleeve Gastrectomy you used for severely obese patients on leptin compared to adiponectin? If yes this should be detailed in the discussion.

Response 7:

We agree that this interesting observation warrants further discussion and have revised the discussion accordingly. We noted already that while leptin decreased by approximately 25% in the year after surgery (from 40.7±24.9 to 30.9 ±30.5 ng/ml), this difference was not statistically significant. We think that this lack of statistical significance (p=0.11) was likely due to the large degree of variation in circulating concentrations of leptin that was observed both pre and post-gastrectomy, as opposed to a physiological effect that is specifically absent after sleeve gastrectomy. We also think that with larger numbers within the study, the difference would be statistically significant. In fact, this was recently reported by Kalinowski et al. [308] in a randomized control trial of bariatric surgery that included measurements of leptin. We have now referenced this paper in the discussion. We would emphasize thought that our primary objective was not to assess changes in leptin concentrations in isolation but in the context of concomitant changes in adiponectin also.

Comment 8:

8. Authors should add a “conclusion” section within the main text.

Response 8:

We agree and have added a conclusion to the end of the paper as follows: “This single-centre, prospective cohort study of adults with severe and complicated obesity undergoing laparoscopic sleeve gastrectomy found that after twelve months there was a substantial reduction in the leptin: adiponectin ratio and that this reduction was proportional to the amount of weight lost after surgery. Given the heterogenous nature and small size of the study population, the findings must be regarded as preliminary. Nonetheless, they suggest a change in adipocyte function consistent with improved insulin sensitivity. Further studies in larger, more specifically defined patient subgroups

would help to further elucidate the relevance of adipokine measurement in these patients and the mechanistic basis for metabolic improvements after bariatric surgery."

Reviewer #2:

Comment 9:

This article is a prospective cohort of patients who underwent gastric sleeve. The authors have measured leptin:adiponectin ratio before and after the gastric sleeve. The study group was heterogenous, and the results are borderline significant.

Response 9:

We are grateful to this reviewer for their careful consideration of our paper. We have sought the assistance of an additional expert in adipokine physiology and bariatric clinical care, who is now an additional co-author on the study. We have made our very best collective efforts as a research team to consider and respond carefully to each point made below. We have taken the comments in a constructive spirit.

Comment 10:

It is great that the authors have included LAR as a measure after bariatric surgery, as it is a good and sensitive measure of insulin sensitivity. However, I have several concerns. The most prominent one is the heterogenous study group, exclusion and inclusion criteria, and how this affects the adipokine measurements and results.

Response 10:

There are several points to address here. The reviewer is entirely correct in noting the heterogeneity in the study group. We have included this in the section describing key limitations of the study in the discussion and have amended the (newly included) conclusion section to highlight this limitation. The heterogeneity arises out of necessity – we do not think that the validity or integrity of the study would be enhanced by examining only a subgroup, especially when the number of patients is so small.

We also think that the heterogeneity that exists in our population would increase "random error" in our analysis and would diminish our statistical power, rather than biasing our results or leading to

type 1 statistical error. In other words, the fact that we have found a reduction in LAR in this group despite its heterogeneity suggests the reduction is real and that our assertion that this is a valid and novel scientific observation is a valid one. Finally, in relation to heterogeneity, we think that we have described the variation in characteristics of the patients as well as we possibly can.

We apologize for not including more information on inclusion and exclusion criteria and have added the following text to the methods: "Male and female patients aged 18 years or older, put forward for consideration for sleeve gastrectomy by the bariatric multidisciplinary team at our institution (nurse, dietitian, physician, psychologist, surgeon) were all eligible for inclusion. Our clinical practice is to refer these patients for surgical consideration after completion of a ten-week structured lifestyle modification programme that we have described in detail previously [82]. Patients must have undertaken a formal psychological assessment of the suitability of sleeve gastrectomy for their treatment. Those with a recent myocardial infarction (within six months), untreated arrhythmia, untreated left ventricular failure, recent cholelithiasis (within the past year), type 1 diabetes, untreated major psychiatric disorders, eating disorders, undergoing cancer treatment, or a BMI <35 kg m-2 or those deemed unlikely to attend for post-operative follow-up (e.g. frequent clinic non-attendance) were excluded from undergoing sleeve gastrectomy."

We acknowledge that the broad inclusion criteria we have adopted will have led to variations in the concentrations of the adipokines we have measured, and indeed variations in the degree to which these will change after sleeve gastrectomy. We hope the amendments here and elsewhere in the manuscript have addressed the reviewer's concerns in this regard.

Comment 11:

Please comment why you did not also use HOMA-IR and/or WBISI to detect insulin sensitivity, in addition to LAR. This is a weakness of the study. The authors should also present fasting insulin and c-peptide, as this is connected to insulin sensitivity.

Response 11:

We agree entirely that quantifying insulin sensitivity using different methods would enhance the level of causal inference that we can make around the impact of sleeve gastrectomy on insulin sensitivity. We have toned down our assertion in the manuscript (abstract, the discussion also in

line with comment 3 from Reviewer 1) to state that the reduction in LAR "may" represent a reduction in insulin resistance, rather than conclusively proving that it does so.

We are familiar with other methods of quantifying insulin sensitivity, including the hyperinsulinaemic euglycaemic clamp [286] and those indices such as WBISI [309] and HOMA [132]. While the WBISI is less complicated and expensive than the clamp, it still requires the administration of a standard oral glucose tolerance test. We were reluctant to do this in the current study because of the resource implications of trying to do these tests on a surgical ward on the day of surgery and also because in general, we do not perform these tests in patients in whom the diagnosis of diabetes has already been established. In other words, we consider the "participant burden" of an oral glucose tolerance test in those with diabetes to be unacceptably high for the purposes of this study. We did not think doing the oral glucose tolerance test would be feasible. However, this is certainly something we will consider in more substantive studies, particularly in subgroups of patients without diabetes.

We accept entirely that measures of fasting glucose, insulin, and c-peptide would have provided valuable information about "fasting" insulin sensitivity using HOMA [132]. We did not include these at the time the study was devised because we thought that changes in diabetes medication usage might confound any observed changes in glucose and insulin levels to a greater extent than changes in medication usage would influence adipokines. Nonetheless, we accept that this is a weakness, not just a limitation in the paper and have acknowledged that now in the discussion, which we have amended thus: "Rather than using LAR as the only index of insulin sensitivity, it would have been informative also to examine indices related to fasting glucose and insulin (such as the homeostasis model assessment, HOMA [132]). We plan to do assess HOMA along with LAR in future studies, in order to strengthen causal inference on the impact of bariatric interventions on insulin sensitivity."

Comment 12:

You refer to leptin and adiponectin as molecules. It is more suitable to call them hormones derived from fat, in particular.

Response 12:

We have made this clear in the amended introduction.

Comment 13:

I do not recommend using wording as «none has described LAR in bariatric patients. Please be a little bit more humble, and use wording like «there is limited data of what happens to the LAR after bariatric surgery.

Response 13:

We acknowledge that our description of the significance of our results should be more impartial and objective and have amended the manuscript accordingly – consistent with response 2 above to reviewer 1 also, where we've outlined the changes made.

Comment 14:

The article needs better language. I recommend a professional proofreading service of the whole manuscript, as it needs more scientific language, and spellings.

Response 14:

We have carefully reviewed each and every word in the manuscript for any typographical, grammatical, or spelling errors. In addition, we have had the article proof-read by a native English-speaking expert who is not an author on the paper.

Comment 15:

You do not mention leptin resistance and what this means for obese people.

Response 15:

We are grateful for the opportunity to include a consideration of leptin resistance in the revised paper and agree that it is relevant to our findings. We have now added to the discussion section a description of leptin resistance in the disease of obesity and its relevance to the post-bariatric surgical weight loss response, as follows: "As the primary effect of leptin is to suppress appetite and defend against weight gain via hypothalamic signaling, it might seem counterintuitive to find a reduction rather than an increase after sleeve gastrectomy. However, this phenomenon is well described and may be due to 'leptin resistance', whereby individuals with obesity demonstrate paradoxically high levels of circulating leptin but diminished leptin sensitivity [250]. Previous studies have suggested that decreased leptin levels after bariatric surgery do not attenuate weight loss because of compensatory increased leptin sensitivity in the hypothalamus [251]."

Comment 16:

Please reflect on the age of the participants, as higher age might affect the levels of adipokines.

Response 16:

Given we sought to determine changes in the measures of the two adipokines (and their ratio) over time, we did not think that the age of the study participants could somehow account for the changes we observed, as the duration of follow-up was consistent for each individual. We think that our study is far too small to conduct a sub-analysis to determine whether age influences the magnitude of the reduction in LAR with sleeve gastrectomy. This is a fascinating hypothesis, but one that we did not set out to address in our current paper. We would like to include the consideration in future larger studies. We would respectfully suggest that this does not diminish the quality or the integrity of the work we have presented in this paper, which is addressing a separate scientific question. We have conducted an analysis of any correlation between age and baseline leptin, adiponectin, and LAR levels, and there was no correlation whatsoever (with r^2 values of -0.107, -0.158, and -0.030, respectively (p=0.682, 0.544, and 0.908)). We have not made any amendments to the manuscript but can do so if it is deemed essential or would enhance the paper in any way.

Comment 17:

Please discuss how FGF-21, adiponectin and leptin work together, to explain potential ways this works. Also why leptin might not be so much changed.

Response 17:

We thank the reviewer for drawing our attention to the relevance of FGF-21, which has been reported to promote insulin sensitivity, thermogenesis, and energy expenditure, with overall beneficial effects on glucose metabolism and bodyweight, albeit with some discrepancies between murine and primate models. With regards to adiponectin and FGF-21 specifically, while this is an evolving field of research, increased FGF-21 activity is associated with higher adiponectin levels, which represents one potential mechanism whereby FGF-21 may enhance insulin sensitivity [252] (although this explanation has been challenged within the literature). With regards to the effects of FGF-21 on leptin, it has been shown that administration of FGF-21 improves sensitivity to leptin which in turn may mediate some of the weight loss effects observed with this adipokine. Within this context, it is noteworthy that Khan et al. [310] have previously reported that FGF-21 levels increased in the first months after sleeve gastrectomy, and this increase was strongly associated with observed weight loss. We did not include measurement of FGF-21 levels in our study as it was primarily an exploration of changes in LAR after sleeve gastrectomy, but we agree that this relationship merits

further study as a potential mechanism underlying the changes we observed. We have amended the discussion thus: “The mechanisms by which leptin sensitivity might be restored remain to be determined, but other adipokines such as fibroblast growth factor-21 (FGF-21) may mediate this effect via direct signaling in the central nervous system or through augmenting the secretion of adiponectin [252].”

With regards to why circulating levels of leptin did not decrease to a significant degree, we believe this is likely related to study power limitations and have emphasized that observation within the text of the discussion accordingly.

Comment 18:

You need to describe in further detail what kind of essays you are using for the adipokine measurement—producer, country, number of essay and so on. Please describe in further detail when and how you took the adipokine measurements. Please explain what kind of adiponectin you analyzed. Total or high molecular weight (HMW)? The high molecular weight is the more active form.

Response 18:

Thanks for the opportunity to provide further clarity on the assays we used to measure leptin and adiponectin. To note, we did not measure high molecular weight adiponectin in this study, only “total” adiponectin. We have amended the methods section as follows: “Leptin and adiponectin were measured using separate two-site micro titre plate-based DELFIA assays manufactured by R&D Systems Europe, Abingdon UK. The adiponectin assay measured “total” adiponectin and our “in-house” analyses have found a between batch imprecision of 5.4% at 3.6 µg/ml, 5.2% at 9.2 µg/ml and 5.8% at 15.5 µg/ml, as previously described [249]. For leptin, the between batch imprecision was 7.1% at 2.7 ng/ml, 3.9% at 14.9 ng/ml and 5.7% at 54.9 ng/ml.”

Comment 19:

Please specify the time of the day that you did the adipokine measurements, as adiponectin for example has shown to have diurnal rhythms.

Response 19:

All patients had the blood samples drawn for leptin and adiponectin measurement in a fasted state on the morning of their operation. We have amended the methods section to highlight this important clarification.

Comment 20:

Please describe what you did if the data was not normally distributed. If all data were normally distributed, also please write that.

Response 20:

Once again, we are grateful for the astute guidance of this reviewer. We rechecked each variable for normality and found that they were normally distributed except for LAR. Therefore rather than using the paired t-test to compare differences in means before and after surgery, we used the Wilcoxon Signed Rank Test and have expressed the values for LAR in table 1 as medians and interquartile ranges, as highlighted.

Comment 21:

Please also present data on fatpercent, fatweight (kg), and abdominal fat percent and weight, and correlate this to the adipokines. Subcutaneous fat have been shown to be different from visceral fat when it comes to secretion and handling of the adipokines. Please analyze this, and discuss it. Please also make a graph about this. Remember to do one in women, one in overall group and one in dia 2 group.

Response 21:

What the reviewer is asking for here is a description of body composition, which would clearly add a great deal of value to our study, along with a subgroup analysis of men versus women and patients with diabetes versus those with none. However, we did not assess body composition in this study. Nor is it adequately powered to examine differences by sex or diabetes status (or age for that matter), notwithstanding how valid those suggestions are and how interesting the results would be. These are aspects of this research field that need to be prioritized in future studies. However, we would implore the reviewer not to consider the absence of these data and analyses as a "weakness" of our paper. We did not set out to address these hypotheses, which we think are entirely separate to our original scientific question.

Comment 22:

You have a small study group, and the majority of them are women and have type 2 diabetes. Please do all the statistics with only women also, as women have shown to have different adipokines than men. Also please do all the statistics with the type 2 diabetes patients. Remember to exclude the ones who are using medications that can affect the adipokines. I suggest the readers also have a look and discuss other reports that includes both metabolic healthy obese, and obese with a metabolic disturbance.

Response 22:

We have reflected carefully as a group on this comment and find it hard to know how best to respond to it. While the study group is small, the impact of sleeve gastrectomy, even in our heterogenous patient group, on the LAR is so significant that we have been able to demonstrate it convincingly with 17 patients. The finding is novel and valid. What we did not set out to do was determine whether males and females, young and old or those with versus those without diabetes, responded differently. These are entirely valid, exciting scientific questions, but they were not what we sought to "discover." Rather, these questions need to be addressed in future studies. We feel very disappointed not to be able to incorporate the reviewer's guidance into this particular aspect of the paper, but we feel that trying to include everything requested here would diminish rather than enhance the paper. We have, of course, still done these analyses and present them in the table below. (We used Wilcoxon Signed Rank Test to compare pre- and post- surgery in the specific groups below.) If the reviewer or the editor insists, we can include these findings in the paper, but we would rather leave them out.

Rebuttal table: Subgroup changes in LAR.

	LAR Pre	LAR Post	p value
Male (n=5)	9.9[5.5,10.3]	2.2[0.19,5.7]	0.043
Male (n=4) with DM	8.5[4.6,9.9]	3.3[0.7,6.3]	0.068
Female (n=12)	6.8[5.1,10.6]	3.1[1.3,8.2]	0.015

Female (n=8) with DM	7.8[5.0,10.6]	5.5[2.2,8.5]	0.025
>50 years (n=7)	10.1[5.35.10.7]	2.4[1.0,4.5]	0.018
< 50 years (n=10)	7.0[5.0,11.6]	4.5[1.1,8.4]	0.059
>50 years (n=5) with DM	10.0[5.3,10.3]	3.8[1.7,6.6]	0.043
<50 years (n=7) with DM	7.1[4.9,10.4]	6.9[2.2,7.6]	0.063

Comment 23:

The report should include how many of the patients that have metabolic syndrome, as this might affect the adipokines.

Response 23:

We did not dichotomize patients on the basis of whether or not they had the metabolic syndrome, as we did not measure all of the components of the metabolic syndrome, nor was it part of our hypothesis. This is something that could be included in larger studies in the future.

Comment 24:

The study group is very heterogenous, and most of them take different medications that can affect adiponectin, leptin, in addition to leptin resistance and adiponectin resistance. This makes it very difficult to draw any conclusions at all in this report. The authors have to address this very seriously and reflect on this. The study group should be more heterogenous when having such a small study group. I believe this is one of the reasons why leptin is varying so much in the study group. The exclusions and the inclusion criteria of the study should be more carefully thought about to be able to draw any conclusions. The information about who stopped and who continued on medicines is good, but it also draws the attention to that the study group is very heterogenous and that some had so good «effect» that they stopped on medicines, while some had not. As the study is now, the only conclusion you can draw is that in a mixed group, most of them taking medications that affect the adipokines, the adipokines are improved. I suggest that the authors can try to take out all the

diabetic patients and do statistics on them to see where this goes, but I still think this study group is to heterogenous and varying for this sample size. My best recommendation is to include more patients with more clear inclusion and exclusion criteria.

Response 24:

We do not think that the heterogeneity of the patient group in any way diminishes the validity of our findings, and while we agree entirely that it is a limitation, we do not think it is a weakness, and the distinction between the two is an important one. We strongly reject the notion that patient heterogeneity "makes it very difficult to draw any conclusions at all in this report." The heterogeneity will have introduced random error, as opposed to systematic error or bias, into our study, and this would be expected to diminish our ability to detect a true difference in LAR after surgery. The very fact that we have found one in spite of the heterogenous nature of the patient group makes the results more plausible, not less plausible. The small study size and heterogeneity make subgroup analysis a less desirable and more challenging option, yet this is what the reviewer seems to want us to do. We respectfully suggest that our original scientific hypothesis was that (any) patients undergoing the specific intervention of laparoscopic sleeve gastrectomy would have a reduction in the specific outcome of interest to the study, which was the LAR, and that is what we have convincingly shown. We need to build this scientific knowledge now with the next generation of studies, like the ones the reviewer proposes, but we need to have the opportunity to publish the founding observations first, where they are robustly described and novel, as we think the case is here.

The reviewer has raised a concern about the potential confounding effects of medication changes on the LAR. We agree that this is important and warrants a review of each individual medication and its likely effects on the LAR. In total, 12 patients were taking metformin at the start of the study, and three of these had stopped it by the end of the programme. As metformin is known to have a weak positive effect on insulin sensitivity [257] and potentially a lowering effect on circulating levels of leptin [202], then it is possible that the cessation of metformin diminished the reduction in the LAR observed in our patients. If anything, however, this effect would only strengthen the primary observation of the study, which reported a significant decrease in the LAR after bariatric surgery. Therefore, we feel that the cessation of metformin in those three patients is unlikely to have accounted for the observed reduction in LAR and, if anything, may have attenuated it. Four patients taking GLP-1RA injections at baseline had stopped doing so by the end of the study. We note that

the administration of GLP-1 RA therapy has previously been reported to diminish the reduction in circulating levels of leptin that typically accompanies weight loss [258]. As a result, it is possible that the cessation of GLP-1 RA therapy in those four patients diminished the reduction in circulating levels of leptin that we observed. As with metformin, this effect only strengthens the primary finding of the study that LAR decreased after bariatric surgery, with this finding remaining significant despite any potential confounding effect from changes with this medication. Similar principles apply to the three patients who stopped gliptin medications during the programme, as medications from this class seem to decrease leptin and increase adiponectin [259]. With regards to SGLT2 inhibitor therapy, we report that one patient stopped their SGLT-2 inhibitor during the study while another patient was commenced on one. Again, the current evidence suggests that SGLT2 inhibitors decrease leptin and increase adiponectin [260], so we do not think that initiation of this treatment in one patient and its cessation in another is likely to have led to the observed difference in LAR after the programme. Finally, eight patients stopped antihypertensive medications during the programme. Angiotensin-converting enzyme (ACE) inhibitors have been reported to increase circulating levels of adiponectin [261], so we think their cessation would have attenuated rather than enhanced the observed reduction in LAR. We have added the above information and references to the second last paragraph of the discussion section.

Comment 25:

In the results section, please write the values (p-value, r, values, mean, CI and so on), when you present the results.

Response 25:

We have presented the means and standard deviations (or medians and interquartile ranges) for the anthropometric and metabolic variables a baseline and follow-up in table 1, along with p-values for the comparisons between the two. We have added the r-squared values from the tables into the text of the results section. We have presented the LAR results as median and interquartile range. We are not clear what other results need to be presented in the text. If the reviewer would like us to duplicate results presented in the table in the text of the results section, we can do that, but we are not clear if this is what is required.

Comment 26:

The authors have to be a lot more careful in their wording when talking about these results, in light of the small sample size and not carefully selected study group. I would say that there is difficult to draw any conclusions from this paper, but as advised, try to analyze females.

Response 26:

We agree entirely, and we have extensively revised the tone of the language describing the implications of our findings throughout the manuscript, as highlighted and in the new “conclusion” section.

Comment 27:

Please describe what blood pressure measurement of the three you used was used in the study.

Response 27:

We are grateful for the opportunity to clarify this important point and have amended the methods section as follows: “Three measures were recorded at one-minute intervals, and the average of the three was recorded.”

Comment 28:

The relationship between leptin and adiponectin has been shown to be of importance. Please discuss leptin signaling in relation to increased caveolin-1-expression in obesity, and how adiponectin may be altered because of this in obese subjects, and how this could affect your results. Also calculate leptin and adiponectin ratio (L:A ratio) for all subjects, and include this in the analysis as an independent mediator.

Response 28:

We must admit that we were unaware of this intriguing mechanism and are grateful for the opportunity to refer to it in the paper – we have amended the discussion thus: “Another consideration is the interaction between leptin and adiponectin. For example, in normal-weight individuals, leptin enhances adiponectin secretion, but this effect is lost in patients with obesity through the action of caveolin-1, which attenuates leptin-dependant increases in adiponectin [253].”

Comment 29:

Please discuss how and why female subjects often have different leptin and adiponectin secretion than men, and how this can affect your results. Testosteron has shown to inhibit the secretion of HMW adiponectin. The men also had 3 times more diabetes with fasting glucose above 7. Please elaborate how this can affect your results, when comparing men and women. The men in the study had more than double the alcohol consumption than the women in the study. Please comment how this may affect your results. Please perform the analysis with men and women separately, and do the analysis with only healthy subjects (no diabetes or insulin resistance). Please do the total analysis with the correction for sex and age. It would also be of importance to compare individuals with BMI above or below 30.

Response 29:

As the reviewer notes, differences in circulating levels of leptin and adiponectin between males and females have previously been reported, with females typically demonstrating higher levels of circulating leptin and adiponectin when compared to males [311, 312]. A variety of mechanisms may explain these differences, including but not limited to testosterone directly reducing adiponectin levels in males [313]. As noted by the reviewer, alcohol can also affect adiponectin levels with an increase in circulating adiponectin correlating with increased alcohol consumption as reported by other groups. However, while these observations may certainly be of relevance to baseline differences in leptin and adiponectin and the LAR in our study, we found no evidence that gender at baseline or alcohol consumption influenced the change in LAR in the aftermath of the intervention, i.e., sleeve gastrectomy. Both males and females demonstrated a significant and consistent decrease in LAR post sleeve gastrectomy. Thus, while we certainly agree that a study cohort with different characteristics to our own in terms of gender might have different baseline leptin and adiponectin levels, our results suggest that a decrease in LAR would be observed post-bariatric surgery regardless of baseline gender mix. With regards to the repeat analyses that the reviewer suggested, we have performed the following analyses below to examine the changes in LAR between males and females, patients with and without diabetes, and in those above and below 50 years of age. It should be noted, however, that the numbers of patients in these subgroup analyses are very small, and our study was not designed to address these considerations. While the reduction in LAR appears consistent across the entire cohort, it was not statistically significant in all subgroups, probably because of low power. The overall trend, however, strongly supports the primary finding of the study that LAR decreases post sleeve gastrectomy. With regards to the reviewer's request to divide the cohort above and below a BMI of 30, we were not able to perform this analysis as this is a

bariatric surgery population, and as such, the mean baseline BMI was 46. We have added the table below as a supplementary table to the manuscript, given its limitations in terms of patient numbers.

	LAR Pre	LAR Post	p value
Male (n=5)	9.9[5.5,10.3]	2.2[0.19,5.7]	0.043
Male (n=4)*	8.5[4.6,9.9]	3.3[0.7,6.3]	0.068
Female (n=12)	6.8[5.1,10.6]	3.1[1.3,8.2]	0.015
Female (n=8)*	7.8[5.0,10.6]	5.5[2.2,8.5]	0.025
>50 years (n=7)	10.1[5.35.10.7]	2.4[1.0,4.5]	0.018
< 50 years (n=10)	7.0[5.0,11.6]	4.5[1.1,8.4]	0.059
>50 years (n=5)*	10.0[5.3,10.3]	3.8[1.7,6.6]	0.043
<50 years (n=7)*	7.1[4.9,10.4]	6.9[2.2,7.6]	0.063

*stands for patients with a history of T2DM

Comment 30:

Please mention how the liver is of importance in the physiology of this. Do you have any measurements on Ultrasound of liver or ASAT, ALAT and so on?

Response 30:

We are sorry to inform the reviewer that we did not collate data on liver physiology during this study, and we respectfully suggest that while interesting, those data are well beyond the scientific scope of the paper and could be addressed in future studies.

Comment 31:

Please discuss your results in view of the adiponectin-paradox. Your study group had a quite high age, most of the >55 years old. In the older age groups adiponectin might have a different role. Please elaborate about this, and diseases such as heart failure, CVD and kidney failure in your study subjects, in view of the adiponectin-paradox.

Response 31:

The adiponectin paradox refers to the observation that while adiponectin mediates a variety of essentially beneficial effects and levels of adiponectin correlate positively with better long term cardiovascular outcomes in young, healthy populations, they also correlate with increased risk of premature death in other populations, and especially the elderly cohort with ischemic heart disease, heart failure or renal failure [254]. The precise mechanisms underlying this paradox are not entirely understood, but it may be that weight loss secondary to pathological, catabolic processes leads to a rise in adiponectin in a similar manner that bariatric surgery leads to a rise in adiponectin, with the reason behind the weight loss the main factor that influences the subsequent clinical outcome. An analysis of this phenomenon, however, was beyond the scope of the present study, and we agree this merits further study in future research of this nature and have adjusted the text of the manuscript to take these observations into account. We have added a paragraph on this in the discussion.

Comment 32:

Can you please review your results in light of adiponectin resistance, the connection with leptin, and the FGF-21-adiponectin axis.

Response 32:

In light of this and the comments above, we have extensively revised the manuscript to reflect in more detail the intricacies of the interaction of these adipokines. We acknowledge the relevance and importance of these mechanisms, but an analysis of their role in changes in adipokines after sleeve gastrectomy is beyond the scope of this study. Hopefully, the significant enhancements brought about by the reviewers' feedback will encourage future research in these important areas.

Comment 33:

Several studies show that N-3-PUFA and the consumption of fish affect adiponectin. Can you include information about the intake of N-3-PUFA and fish and elaborate on how this can affect your results.

Response 33:

We did not measure dietary patterns in our patients relating to N-3-PUFA and fish consumption.

Comment 34:

It is great that you use the LAR to measure insulin sensitivity and include it in your bariatric programme. However, I do recommend more care in selecting the study group, as many things can affect these adipokines.

Response 34:

We reiterate our gratitude to this reviewer for all of their constructive and helpful comments and for helping us to make this a better, more balanced, and informative paper. We hope that our responses have adequately reassured them as to the novelty and validity of our findings.

Editorial Board Member comments:

Comment 35:

We thank the authors for submitting their interesting study to Scientific Reports, and for their patience during the review process. While there is interest among the reviewers/editors in this study, the reviewers raise substantial concerns which should be addressed in a major revision. If the authors are able to address the caveats/questions raised by the reviewers, we would be pleased to consider a revised manuscript.

Response 35:

We really appreciate the opportunity to improve the manuscript, which has had an increase of 50% in the word count and 70% in the number of references.

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