UNIVERSITY OF DERBY

INVESTIGATION OF THE METABOLIC EFFECTS OF LIRAGLUTIDE ON PATIENTS WITH OVERWEIGHT AND OBESITY

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List of Abbreviations

%	Percent
£	British Pound
<	Less than
>	Greater than
ΔCt	Delta threshold cycle
≤	Less than or equal to
≥	Greater than or equal to
°	degrees
µL	Microlitre
ADA	American Diabetes Association
AGE	advanced glycation end-products
AGI	Alpha-glucosidase inhibitors
AKT	protein kinase B
AKT1	Serine/threonine-protein kinase 1
AlbuminBCG	Albumin Assay Kit

AlkP	Alkaline phosphatase
ALT	alanine aminotransferase
AMPK	AMP-activated protein kinase
APOA1	Apolipoprotein AI
apoB	apolipoprotein B
AST	aspartate aminotransferase
ATP	Adenosine triphosphate
BAT	Brown Adipose Tissue
BCA	bicinchoninic acid
BDNF	brain derived neurotrophic factor
BMI	Body Mass Index
BMP	Bone Morphogenetic Proteins
BMR	Basal metabolic rate
BSA	bovine serum albumin
C	Celsius
C/EBP	CCAAT-enhancer binding protein
CART	cocaine-and amphetamine-regulated transcript
cDNA	Complimentary deoxyribonucleic acid
CENTRAL	Cochrane Central Register of Controlled Trials
CERS1	Ceramide synthase 1
Chol	Cholesterol
CI	Confidence interval
cm	Centimetre
CoA	Coenzyme A

CRP	C-Reactive Protein
Cu+	Copper ion
Cu2+	Copper (II) ion
CV	Cardiovascular
CXXC5	CXXC-type zinc-finger protein 5
DAG	diacylglycerol
dH2O	Distilled and autoclaved water
DM	Diabetes Mellitus
DNA	Deoxyribonucleic acid
DPP-4	dipeptidyl peptidase-4
eGFR	estimated glomerular filtration rate
ELISA	enzyme-linked immunosorbent assay
EMBASE	Excerpta Medica
ER	Endoplasmic reticulum
ERK	extracellular signal-regulated kinase
EU	European Union
FFA	free fatty acids
FOXO1	forkhead box O
g/dl	grams per decilitre
GAPDH	Glyceraldehyde 3-phosphate dehydrogenase
GDF	Growth differentiation factors
GDF15	Growth differentiation factor 15
GGT	gamma-glutamyltransferase
GIP	glucose-dependent insulinotropic polypeptide

GLP-1	Glucagon-like Peptide 1
GLP-1 RA	Glucagon-like peptide-1 receptor agonists
GLUT4	glucose transporter 4
GR	Good responder
GRB10	growth-factor-receptor-bound protein 10
GSK3B	glycogen synthase kinase 3β
HbA1c	haemoglobin A1c
HDL	High density lipoprotein
HFD	high fat diet
Hg	mercury
HOMA-IR	Homeostatic model assessment of insulin resistance
HOMA-S	Homeostasis Model Assessment of insulin sensitivity
ICF	Informed Consent Form
IGF1R	Insulin like growth factor1 receptor
IKK	inhibitor of kappa B kinase
IL-1b	Interleukin-1 beta
IL-6	interleukin-6
IL-8	Interleukin 8
INSR	Insulin receptor
IP3	inositol triphosphate
IRS1	Insulin receptor substrate 1
IRS-1	insulin receptor substrate-1
JAK	Janus kinase
JAK2	Janus kinase 2

JNK	c-Jun N-terminal kinase
JNK1	c-Jun N-terminal kinase 1
JNK2	c-Jun N-terminal kinase 2
JNK3	c-Jun N-terminal kinase 3
KATP	ATP-dependent potassium
kcal	Kilocalorie
kg	Kilogram
kg/m2	Kilogram per metre squared
L	Litre
LADA	latent autoimmune diabetes in adults
LDL	Low density lipoprotein
LEP	Leptin
LEPR	Leptin Receptor
LTBP3	Latent-transforming growth factor beta-binding protein 3
MAP2k1	Mitogen-activated protein kinase kinase 1
MAPK	mitogen-activated protein kinase
MAPKKK	MAPK kinase kinase
MAPKKK	MAPK kinase
MC4R	melanocortin receptor
MCP-1	Monocyte chemoattractant protein-1
MEDLINE	Medical Literature Analysis and Retrieval System Online
MeSH	Medical Subject Heading
mg	Milligram
mg	Milligram

mg/mL	Milligram per millilitre
miRNA	Micro RNA
mL	Millilitre
mM	Millimolar
mm	millimetre
mm Hg	millimetre of mercury
mmol	millimole
mmol/dl	millimole per decilitre
mmol/l	millimole per litre
mmol/mol	millimole per mole
MOAI	Monoamine oxidase inhibitors
MODY	maturity onset diabetes of the young
MRI	Magnetic Resonance Imaging
mRNA	Messenger ribonucleic acid
MRS	Magnetic Resonance Spectroscopy
mTORC1	mammalian target of rapamycin complex 2
mTORC2	mammalian target of rapamycin complex 2
NAFLD	non-alcoholic fatty liver disease
NF-kB	Nuclear factor kappa-light-chain-enhancer of activated B cells
NF-κB	nuclear factor kappa B
NHS	National Health Service
NICE	National institute for health and care excellence
nm	nanometer
nmole/nM	Nanomole

NPY	neuropeptide Y
NTRK2	Neurotrophic receptor tyrosine kinase 2
ob/ob	Obese mouse model
OD	Optical density
OMA	Obesity Medicine Association
OSBPL2	oxysterol-binding protein-like 2
p	Probability
PC	Phosphatidylcholine
PC1/2	proprotein convertase-1/2
PCOS	Polycystic ovary syndrome
PCOS	Polycystic ovary syndrome
PCR	polymerase chain reaction
PDK1	Phosphoinositide-dependent kinase-1
PE	Phosphatidylethanolamine
pH	Power of hydrogen
PI	Principal investigator
PI3K	phosphatidylinositol 3-kinase
PIP2	phosphatidylinositol-4,5-bisphosphate
PIP3	phosphatidylinositol-3,4,5-trisphosphate
PLA2G2A	Cytosolic phospholipase A2 group II
PLA2G4A	Cytosolic phospholipase A2 group IV
PLA2G6	Cytosolic phospholipase A2 group VI
PLA2G7	Cytosolic phospholipase A2 group VII
PLCD1	Phospholipase C Delta 1

PNPLA3	Patatin-like phospholipase domain-containing protein 3
POMC	Pro-opio mealocortin protein
PPARγ	peroxisome proliferator-activated receptor gamma
PPG	Post prandial glucose
PR	Poor responder
PRIMSA-P	Preferred Reporting Items for Systematic review and Meta-Analysis Protocols
PROs	patient-reported outcomes
PROSPERO	International Prospective Register of Systematic Reviews
PTPN3	Protein Tyrosine Phosphatase Non-Receptor Type 3
qRT-PCR	Quantitative real-time PCR
RAF1	Rapidly accelerated fibrosarcoma
RCT	Randomised controlled trial
RNA	Ribonucleic acid
rpm	Revolutions per minute
RYGB	Roux-en-Y gastric bypass
S1P	sphingosine-1-phosphate
S1PR1	Sphingosine-1-phosphate receptor 1
S473	serine 473
SD	standard deviation
SE	standard error
SGLT2	sodium-glucose cotransporter 2
SGPP1	Sphingosine-1-Phosphate Phosphatase 1
SH2B1	Src homology 2 B adapter protein
SHMT1	Serine Hydroxymethyltransferase 1

SI	Sub-Investigator
SIM1	Single-minded homologue of drosophila
SphK	sphingosine kinase
SPHK1	Sphingosine Kinase 1
SPHK2	Sphingosine Kinase 2
SR	Super responder
STAT	signal transducer and activator of transcription
STAT3	signal transducer and activator of transcription 3
STAT5	signal transducer and activator of transcription 5
T1D	Type 1 diabetes
T2D	Type 2 diabetes
T308	threonine 308
TAG	Triaglycerol
TCF7L2	Transcription factor 7-like 2
TGF-β	Transforming growth factor beta
TNFα	tumour necrosis factor alpha
TP1	Time point 1
TP2	Time point 2
TP3	Time point 3
Trig	Triglycerides
TSC1/2	TSC1/2, tuberous sclerosis protein complex 1 and 2
TZD	Thiazolidinedione
U/L	Units per litre
U/μL	Units per microlitre

UCP1	Uncoupling Protein 1
UHCW	University Hospitals Coventry & Warwickshire
UK	United Kingdom
USA	United States of America
VEGF	vascular endothelial growth factor
Vit	Vitamin
Vit D25OH	25-hydroxy vitamin D
VLDL	Very low density lipoprotein
WAGR	Wilms tumour, aniridia, genitourinary anomalies and mental retardation syndrome
WAT	White adipose tissue
WHO	World Health Organisation
WL	Weight loss
yr	years
ZNF1	Zinc cluster protein
α	alpha
β	Beta

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Preface

I declare that this thesis was composed by myself, and the work contained herein is my own. Part of this work has been published as part of conference abstracts for *Obesity 2021* and *Society for Endocrinology – British Endocrine Society 2021.* This work was formed as part of the original programme of research, with these published as later versions.

Citations and Abstracts:

Spencer, L., Dimitriadis, G. K., Duggirala, A., Bate, D., Davasgaium, A., Al-Hasani, W., ... & Tripathi, G. (2021, October). 3 mg Liraglutide ameliorates inflammation and improves hypothalamic regulation of energy homeostasis by modulation of Sphingosine-1-Phosphate signalling in super-responders. *In Endocrine Abstracts (Vol. 77)*. Bioscientifica.

Abstract

Background: Growing evidence suggests that hypothalamic lipid sensing plays a key role in controlling food intake, fat deposition and energy balance and that its dysregulation could lead to obesity and type 2 diabetes (T2D). Recent investigations reported that sphingosine-1-phosphate (S1P) is involved in the hypothalamic control of energy homeostasis. Intra-cerebroventricular administration of S1P decreased food intake and increased energy expenditure in rodents.

Methods: We conducted a 24-week, open-label real-world study involving 62 participants with a BMI >30kg/m^2, without T2D. Patients received once-daily subcutaneous liraglutide 3.0 mg, alongside a reduced calorie diet based on individual estimated basic metabolic rate. The primary outcome was change in body-weight. Secondary outcomes included changes in anthropometrics, proinflammatory cytokines (IL1b, IL6, IL-8 and TNFa) and plasma metabolome using Ultra Performance Liquid Chromatography-Mass Spectrometry (UPLC-MS) providing untargeted study of water-soluble metabolites (HILIC-LCMS) and lipid metabolites (C18 reversed-phase LCMS).

Results: Participants were aged 38.6±9.8 years (mean ± SD) and 87.1% of participants were women. They weighed 117.5±24.5kg with BMI of 41.33±6.9kg/m^2. At week 24, participants had lost 12.85±8.4 kg or 9.9±5.8 % body weight (P < 0.001). 55.1% of participants lost 5-10% and 18.4% lost >10% body weight (P < 0.001). According to weight loss (WL) response, participants were divided into non-responders (<5% WL,n = 21), good responders (5-10% WL,n = 19) and super-responders (>10% WL,n = 9). At week 24, oxidised lysoglycerophospholipid metabolic pathway was heavily enriched. Metabolites phosphatidylcholine and triglycerides were significantly (P < 0.001) downregulated, and S1P was upregulated (P < 0.005) comparing super-responders to non-responders. IL-6 had significant positive correlation (r=0.732, P < 0.001) with WL. In all super-responders, IL-6 concentration was significantly decreased and S1P expression was significantly higher.

Conclusions: In this study, administration of liraglutide was associated with upregulation of S1P and reduction of IL-6 in super-responders. S1P signalling may be key in determining response to treatment with liraglutide.

Dimitriadis, G.K., Spencer, L., Dimitria, D., Bate, D., Leca, B., Duggirala, A., Davasgaium, A., Aylwin, JB., Miras, A., Vincent, R., le Roux, C., Christian, M., Randeva, H., & Tripathi, G.. (2021, June). Liraglutide 3.0 mg within a NHS Tier-3/4 weight management service results in similar weight loss compared to regulatory trials – The LIPOSAX first UK real-world evidence study. *In Obesity Abstracts (Vol. 3).* Bioscientifica

Abstract

Liraglutide 3 mg daily is an approved, prescription injectable GLP-1 receptor agonist, which can reduce weight in patients with obesity, with or without obesity complications. We conducted a 24-week, open-label real-world study involving 62 participants with a BMI >30 kg/m^2 or >27 kg/m^2 if they had co-existing dyslipidaemia or hypertension. No patients had type 2 diabetes. Patients received once-daily subcutaneous liraglutide 3.0 mg, alongside NHS Tier-3 lifestyle advice. The reduced calorie diet was based on individual estimated basic metabolic rate. The primary end point was change in body weight. Secondary outcomes included changes in anthropometrics and circulating biomarkers of metabolism (metabolomics and miRNAs). For miRNA analysis, participants were categorised into responders (>5% weight loss) and non-responders (<5% weight loss). Ten miRNAs were analysed (including miR-424, miR-143,

miR-222, miR-103 and miR-146b). RNA was isolated using miRNeasy and reverse transcribed into cDNA using miRCURY. Cel-miR-39-3p and Unisp6 spike-ins were used as controls. Their geometric mean was used to normalise miRNA expression by the calculated 2^-Δct values. Final analysis included 49 patients. At baseline, 87.1% of participants were women, patients were aged 38.6±9.8 years (mean ± SD), weighed 117.5±24.5 kg, had fat-mass of 58.96±15.91 kg and BMI of 41.33±6.9 kg/m^2. Fasting glucose was 5.3±0.58 mmol/l, and ALT 24.9±12.6 U/l. At week 24, patients lost 12.85±8.4 kg or 9.9±5.8 % body weight (P<0.001) and fat-mass decreased by 11.27±7.88 kg (P<0.001). 55.1% of patients lost 5-10% and 18.4% lost >10% body weight (P<0.001). Fasting glucose reduced by 0.7±0.7 mmol/l (P<0.001) and ALT by 8.8±12 U/l (P<0.005). Good responders had downregulation of miR-424 (P<0.001) whilst poor responders had upregulation of miR-424 (P<0.01). There were no changes in other miRNAs. The most frequently reported adverse events were mild to moderate nausea and diarrhoea. There were no serious adverse events. In this study, 3.0 mg of liraglutide, as an adjunct to a reduced calorie diet and increased physical activity offered within a UK NHS Tier-3/4 weight management service, was associated with reduced body weight and improved metabolic control similar to what has been reported by regulatory trials.

Thesis Abstract

Obesity is a well-established risk factor for type 2 diabetes (T2D), a chronic metabolic disorder affecting millions worldwide. While lifestyle interventions such as diet and exercise are often the first-line treatment for obesity and T2D, pharmacological interventions can also play a crucial role in managing these conditions. Liraglutide is a glucagon-like peptide-1 receptor agonist that has been approved for the treatment of obesity and T2D. It works by reducing appetite, promoting weight loss, and improving glycemic control. However, the precise mechanisms underlying the beneficial effects of liraglutide on metabolism and glucose homeostasis are not fully understood.

The aim of this study was to investigate the beneficial metabolic sequelae of Liraglutide in patients with obesity or overweight, including changes in vital signs, anthropometric characteristics (weight, body mass index, and body composition), biochemical parameters, metabolomics, and miRNA molecules from blood tests. The study hypothesis was that treatment with Liraglutide 3mg daily would have beneficial effects on patients with overweight or obesity.

Firstly, a systematic review was conducted to examine the current knowledge base of GLP-1 receptor agonist treatment, focusing on metabolic changes associated with the treatment. A 24-week open-label real-world study was conducted, including 62 participants. Once-daily subcutaneous liraglutide 3.0 mg was administered alongside a reduced calorie diet. The study assessed the primary outcome of body weight change, as well as secondary outcomes of changes in anthropometrics, proinflammatory cytokines, and plasma metabolome using UPLC-MS. Total RNA was extracted from adipocytes, and gene expression profiles were compared between liraglutide-treated and non-treated cells to examine changes in metabolic pathways. In addition, PE, PC, and S1P assays were conducted to analyze the lipid metabolism based on the findings from human samples. Furthermore, miRNA analysis was performed to explore the potential molecular mechanisms underlying the metabolic effects of liraglutide.

The results of the systematic review found that Liraglutide alters metabolic markers in patients with obesity and T2DM. However, further trials were needed to detect the mechanism of GLP-1RA action on molecular pathways and determine novel biomarkers of treatment effects. The clinical study found that liraglutide was associated with upregulation of S1P and reduction of IL-6 in super-responders, with mild to moderate nausea and diarrhea being the most frequently reported adverse events. Additionally, liraglutide treatment led to reduced body weight and improved metabolic control, similar to findings in regulatory trials. The study's results provide evidence of the beneficial effects of liraglutide on lipid metabolism, insulin sensitivity, and inflammation. They suggest that gene expression profiling and miRNA expression may be useful in predicting liraglutide treatment response and identifying novel therapeutic targets for diabetes and obesity. Further research is required to confirm these findings and determine the molecular mechanisms involved in liraglutide's effects on adipose tissue function. Of particular interest is the relationship between the findings of S1P, ceramide, SPHK1 and SPHK2, all of which are involved in the S1P signalling pathway, which could be an important regulator of metabolic and immune function.

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In this section, I want to profoundly thank everyone who has played a significant role in shaping my academic and research journey. These have ranged from providing important experiences with regards to hands on training, reading my research, preparing for conferences, pastoral support, and a lot more. Below I would like to express my gratitude to as many people as possible.

Firstly, I would like to express my gratitude to my Director of Studies, Gyanendra Tripathi. I could not have asked for a better person to guide me along this journey, helping me make the change from Sport Science to Biomedical Science. He has allowed me to become more confident in my own abilities and trusted me to present our research at an international conference.

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Another person who has also been integral to the PhD project is Georgios Dimitriadis, one of our external collaborators. I would like to express my gratitude for the time he has made for me, even though he is always so busy! He again trusted me to present our research in his place at a conference and was able to prepare me for what this may entail.

In a collaborative project such as this, it is important to acknowledge specific areas where collaborators have had an input into the project. Firstly, the clinical trial was conducted by Dr. Georgios Dimitriadis at University Hospital Coventry & Warwickshire NHS Trust. For cell culture of the ChubS7 cell lines, credit goes to Dr. Alice Murphy at Nottingham Trent University.

For the Systematic Review, various people need to be acknowledged. Firstly, to Dr. Georgios Dimitriadis (UHCW) and Gyan Tripathi (University of Derby) for conceptualisation of the review. The designing of the protocol was completed with help from Dr. Georgios Dimitriadis, Dr. Aparna Duggirala and Bianca Leca (UHCW). The Literature search and data analysis was carried out by Eduard Oštarijaš (Institute for Translational Medicine, University of Pecs Medical School, Pecs, Hungary). Study screening and selection was performed with help from Dr. Aparna Duggirala, Asma Omran and Magdalena Lech (both Faculty of Life Sciences and Medicine, School of Life Course Sciences, King's College London, London, UK) with any conflicts completed by Georgios Dimitriadis. The final review was approved by all authors.

All assays and qPCR's were performed by myself under the guidance of Dr. Aparna Duggirala at the University of Derby, and initial training on how to perform these was given by Dr. Aparna Duggirala too. Mass Spectometry data was provided by the Birmingham Phenome Centre.

The technicians at the University of Derby have been nothing but patient and kind with me, especially early on when I was getting to grips with becoming a biomedical scientist. They taught me a wide variety of techniques in the lab, as well as giving up time out of their day to help me with experiments. From these, I would like to specifically thank Caroline Mills and Mary Bastidas, who have been such a good support team throughout my three years.

I would also like to thank the other students who are using the labs in their own PhD studies. Having people to talk to while waiting for the incubation stages of experiments to finish has been a lifesaver.

Finally, I would like to recognise the support of my parents and my girlfriend. You have all provided emotional support, listened to my ideas, and encouraged me during challenging times. I am so proud to be submitting a PhD thesis, and it could not have happened without the people listed above. Thank you.

Chapter 1 – Introduction

1.1 Obesity

1.1.1 Definition of Obesity

Obesity is a chronic, relapsing disease (Bray, Kim, Wilding, & World Obesity Federation, 2017), which has emerged as one of the major public health concerns in modern society. The progressive and relapsing nature of the disease means that obesity requires long-term management. Obesity has been defined by the Obesity Medicine Association (OMA) as "A chronic, progressive, relapsing, and treatable multi-factorial, neurobehavioral disease, wherein an increase in body fat promotes adipose tissue dysfunction and abnormal fat mass physical forces, resulting in adverse metabolic, biomechanical, and psychosocial health consequences." Obesity and diabetes together comprise a major global health problem. The term "diabesity" is used frequently to describe the twin epidemic of diabetes fuelled by obesity (McNaughton, 2013). This condition is multifaceted, encompassing physiological, social, and psychological implications that extend across various ages and socioeconomic groups.

1.1.2 Classification of Obesity

Currently, body mass index (BMI) provides the most convenient measure of population-level overweight and obesity and is sufficiently diagnostic for many patients. This is a simple calculation of weight (in kilograms) divided by the square height in meters (kg/m^2) (Nuttall, 2015). Normal weight is defined by a BMI ≥18.5 to 24.9 kg/m^2, overweight with a BMI ≥25 to 29.9 kg/m^2, and obese a BMI ≥30 kg/m^2. The obese BMI range is often split into 3 classes – class I with BMI 30 to 34.9 kg/m^2, class II BMI 35 to 39.9 kg/m^2, and class III BMI ≥40 (also referred to as severe, extreme, or massive obesity) (WHO, 2000). It is important to note that these classifications underestimate the obesity risk in Asian and South Asian populations, so their classifications are slightly altered, with overweight being BMI between 23 and 24.9 kg/m^2 and obese being BMI >25kg/m^2 (Consultation, 2004). BMI is a widely accepted measurement as it reflects total body fat and is extremely clinically relevant, finding that as BMI increases, so does the risk for diabetes (DeFronzo et al., 2015), gall bladder disease (Bray, 2011), cardiovascular disease and cancer (Katzmarzyk et al., 2012). Alongside this, a study of BMI and mortality found that the optimal BMI for lowest rate of mortality was between 22.5 and 25 kg/m^2 (Whitlock et al., 2009).

There are limitations to be considered with using BMI, and as such it should not be the sole method in diagnosing obesity. While BMI has been shown to strongly correlate with body fat by bioelectrical impedance (Ranasinghe et al., 2013), this relationship is curvilinear in nature

and is influenced by age, gender and race (Mills, Gallagher, Wang, & Heshka, 2007), making the measurement have the potential to be imprecise. The major limitation of BMI is that it cannot differentiate muscle mass to fat mass, and resulting distribution of fat mass (Huml & Schold, 2021). BMI fails to reflect adiposity and body composition, meaning "normal weight" obese patients may be missed (Renzo et al., 2006).

Regional distribution of body fat has been shown to be more important than excess adiposity as a risk factor for metabolic diseases (Després, 2012). Accumulation of adipose tissue in the abdominal region is associated with the development of obesity-related comorbidities (Goossens, 2017), whereas accumulation of adipose tissue in the gluteofemoral region is associated with a protected lipid and glucose profile and reduced risk of metabolic disease (Yusuf et al., 2005). Thus, various additional techniques can be used to diagnose obesity such as anthropometric measures (waist circumference, hip circumference, neck circumference), magnetic resonance imaging (MRI) and magnetic resonance spectroscopy (MRS). All methods come with their own advantages and disadvantages. However, one thing is clear – early and accurate diagnosis of obesity can play a crucial role in the prevention of complications associated with obesity, in turn reducing morbidity and mortality (Kumar, Acharya, Wanjari, & Sagar, 2021).

1.1.3 Global Epidemic of Obesity

The prevalence of overweight and obesity is increasing worldwide (Roberto et al., 2015). Since 1980, occurrence of obesity has experienced a twofold increase in over 70 countries since 1980, and it has consistently risen in the majority of other nations. In 2015, 107.7 million children and 603.7 million adults were obese (Tabarés Seisdedos, 2017). Obesity has been recognised by the World Health Organisation (WHO) as a global epidemic as early as 1997 (WHO, 2000), tripling worldwide between 1975 and 2016. This has largely been due to the effects of globalisation, with rapid urbanisation which has led to a major change in the behaviour of populations around the world. Overall, it is clear to see that obesity rates worldwide are rising (figure 1.1), with graphs showing significant increases in the total of men with overweight (figure 1.1A), women with overweight (Figure 1.1B), men with obesity (figure 1.1C) and women with obesity (figure 1.1D).

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Figure 1.1 – Trend of number of people with A) Men with BMI 30-34.9kg/m^2, B) Women with BMI 30-34.9kg/m2, C) Men with BMI ≥ 35kg/m^2 and D) Women with BMI ≥ 35kg/m^2. Graph images from http://www.ncdrisc.org/obesity-stacked-pop.html (NCD Collaboration, 2016).

In men, the average BMI worldwide, adjusted for age, rose from 21.7 kg/m^2 (with a 95% credible interval of 21.3–22.1) in 1975 to 24.2 kg/m^2 (with a range of 24.0–24.4) in 2014. Similarly, in women, the average BMI increased from 22.1 kg/m^2 (21.7–22.5) in 1975 to 24.4 kg/m^2 (24.2–24.6) in 2014 on a global scale (NCD Collaboration, 2016). Research from the WHO in 2018 shows the prevalence of obesity amongst adults is highest in the region of the Americas (29%), Europe (23%) and the Eastern Mediterranean region (21%). Worldwide, obesity rates range from as low as 8% collectively in China, Japan, India and certain African nations to over 60% in Nauru (World Health Organization, 2018). The prevalence of obesity in the United States is around 37%, Canada 31% and the United Kingdom at 30%, with France (23%), Germany (26%) and Australia (30%) following closely (World Health Organization, 2018).

In England alone a 2019 survey found that 28% of adults in the country were obese, with a further 36.2% being overweight, meaning a total of 64.2% of the adult population with overweight or obesity (National Health Service, 2019). Of these adults with obesity, one in eight were morbidly obese, making a total of 3.3% of all adults. Men were also more likely to be overweight or obese (68.2% compared to 60.4%). Based on the Health Survey for England conducted in 2021, a higher percentage of men (69%) compared to women (59%) are classified

as overweight or obese. The difference between the sexes becomes more pronounced in middle-aged groups. For instance, among individuals aged 16 to 24, 32% of men and 24% of women fall into the overweight or obese category. Similarly, among those aged 45 to 54, 82% of men and 65% of women are classified as overweight or obese.

When it comes to obesity specifically, the prevalence is similar among men and women, with rates of 25% and 26% respectively. Obesity tends to increase with age, starting at 8% among adults aged 16 to 24, peaking at 32% among individuals aged 65 to 74, and then declining to 26% among those aged 75 and older.

It is important to identify countries which are most affected by obesity. It enables targeted public health interventions and resource allocation to address the significant health risks associated with obesity, such as heart disease and diabetes. By identifying these countries, policymakers can strategically allocate funding and implement tailored prevention and treatment programmes. Furthermore, it allows for comparative analysis of various contributing factors, fostering a deeper understanding of the complex causes of obesity. International collaboration and knowledge sharing become possible, leading to the development of global strategies and best practices. Lastly, recognising the economic impact of obesity helps governments and stakeholders prioritise prevention efforts and invest in long-term solutions. Overall, identifying countries most affected by obesity serves as a crucial step towards mitigating its health and economic consequences.

It has been found that eleven countries, namely the USA, China, India, Brazil, Mexico, Russia, Egypt, Indonesia, Iran, Turkey, and Pakistan, are home to half of all women currently living with obesity (World Obesity Federation, 2022). Similarly, nine countries, including the USA, China, India, Brazil, Mexico, Russia, Egypt, Germany, and Turkey, have half of all men living with obesity. The United Kingdom is ranked as the 14th highest country in terms of female obesity and the 10th highest for male obesity. This research highlights the global impact of the obesity epidemic.

In the African region, 1 in 13 men (7.76%) and 1 in 5 women (20.41%) are predicted to be obese in 2030, or around 27 million men and 74 million women at risk of the complications of obesity by 2030. This is a projected increase of 300% from 2010 to 2030. It is noted that the difference in men and women is particularly high for this region.

In the Americas, 1 in 3 men (34.41%) and 2 in 5 women (39.72%) are predicted to be obese by 2030. This amounts to 134 million men and 164 million women, showing a 1.5-fold increase over 2010 figures. It is of particular note that in men, USA has the highest obesity prediction by far, with USA having 47% obesity rate by 2030, with the next highest projections being Canada (39%) and Bermuda (37%).

For the Eastern Mediterranean region, 1 in 5 men (21.69%) and 1 in 3 women (33.15%) are predicted to be in the obese range in 2030, or approximately 58 million men and 84 million women. Obesity in this region will have more than doubled since 2010. Of note, 5 countries (Kuwait, Jordan, Egypt, Saudi Arabia and United Arab Emirates) have a predicted prevalence of over 50% in females.

Across Europe, in 2030 it is predicted to be around 1 in 3 men (29.42%) and women (29.97%) for obesity, around 102 million men and 113 million women. In 2010, 63 million men and 83.5 million women were obese.

For Southeast Asia, 1 in 20 men (5.15%) and 1 in 11 women (9.31%) are projected to be obese using a BMI of $\geq 30 kg/m^2$, equating to 39 million men and 69 women. This represents a twofold increase from 2010 figures.

The Western Pacific region has a wide range of obesity prevalence, with some pacific islands such as American Samoa, Nauru and Cook Islands having high levels of obesity. However, this region also includes some Asian countries which have low prevalence and high population, somewhat skewing the prevalence data. In the region, 1 in 10 men (9.66%) and 1 in 8 women (10.26%) women are projected to be obese in 2030, showing a twofold increase since 2010. To show the skewness of the data, some regions have very high obesity for both men and women, such as American Samoa (66% and 69%), Cook Islands (66% and 69%), Nauru (67% and 68%) and Palau (65% and 68%). Some of the projected lowest prevalence countries in both genders are Vietnam (3% and 4%), Japan (8% and 5%) and South Korea (6% and 6%).

While obesity prevalence differs over the regions of the world, it is clear to see that the obesity epidemic is worsening in every region. If post-2000 trends continue, the probability of meeting the global obesity target is virtually zero. By 2025, global obesity prevalence is predicted to

reach 18% in men and 21% in women, with severe obesity being 6% of men and 9% of women (NCD Collaboration, 2016), with other research suggesting that 20% of women will be obese by 2030, totalling 586 million globally (World Obesity Federation, 2022). The same research predicts that 15% of men will be obese, totalling 439 million. In total, the prediction is that 18% of all adults will be obese in 2030, for a total of 1.025 billion people. At present, no country in the world is on track to reach the WHO's 2025 target on obesity (World Obesity Federation, 2022).

1.1.4 Cost of Obesity

As obesity has reached epidemic proportions, there is growing concern about its substantial impact on health and the economy. The National Health Service (NHS) in the UK faces a significant financial burden from the associated disease and illness linked with obesity. In 2014/15, the NHS allocated £6.1 billion towards treating obesity-related illnesses. However, projections indicate that this figure will escalate to £9.1 billion annually by the year 2050. This highlights the escalating costs and long-term implications of obesity on healthcare expenditure in the UK. (Public Health England, 2017). Healthcare resource utilisation costs in the UK, defined as general practitioner contact, prescriptions and hospitalisations have been shown to increase as BMI increases (C. Le Roux, Chubb, Nørtoft, & Borglykke, 2018). Further analysis shows that the biggest driver in this increase is the increased usage of prescriptions in patients with overweight or obesity.

At the individual level, the relationship between obesity and healthcare costs is well established. A meta-analysis of 75 studies from 1990 to 2016 estimated associations between BMI and healthcare related costs (Kent et al., 2017). This study found that, when compared with healthy weight individuals, healthcare costs increased 12% for overweight individuals and 36% for obese individuals. The increased costs were highest for medications, with an 18% increase with overweight and 68% for obese. Increases were also seen in inpatient care at 12% overweight and 34% obese, and ambulatory care at 4% overweight and 26% obese. Overall, the cost of obesity is predicted to have serious financial implications for society and the UK health system (Keaver, Xu, Jaccard, & Webber, 2020).

1.1.5 Aetiology of Obesity

Obesity is a complex and multifactorial disease (Badman & Flier, 2005). Similar to all chronic conditions, numerous factors and influences (Figure 1.2) contribute to obesity development.

These include genetics, biology, access to healthcare, mental well-being, sociocultural elements, equity, consumption of ultra-processed foods, economic factors, commercial influences, and environmental determinants. These factors interact with one another and have the potential to exacerbate the condition. (World Obesity Federation, 2022).

Figure 1.2 – Obesity is a complex and multifactorial disease, with a wide range of drivers and determinants (Badman & Flier, 2005).

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1.1.5.1 Genetic causes of obesity

The role of genetic predisposition to obesity has been widely demonstrated. Through twin, family, and adoption studies, researchers have approximated the heritability of obesity to range from 40% to 70%(Loos & Yeo, 2022). In studies which focus on the comparison of monozygotic (100% shared genes) and dizygotic (50% shared genes) twins the heritability of obesity is very high, with values ranging from 50% to 90%, clustering between 70% to 80% (Bouchard, 2021). A Canadian study examined the increased caloric intake of 12 pairs of monozygotic male twins by increasing intake of 1000 kcal per day for 6 days a week over 100 days. This found that weight gain had a higher variation between different twin pairs rather than within the same pair, highlighting the potential for genetics to play a role in weight gain (Bouchard et al., 1990).

Another example of the role of genetics in obesity was in a study with 540 adopted adults who were adopted as children in Denmark (Stunkard et al., 1986). This study grouped adults into four BMI groups according to BMI and compared these with the BMI of their adopted and biological parents. There were no significant relationships between the adopted parents and the adoptees BMI, whereas there was a significant relationship between the adoptees and their biological parents, with biological mothers showing a closer relationship ($p<0.001$ compared

to $p<0.02$). Other studies have shown that genetics can influence obesity related behaviour such as eating patterns and exercise (Silventoinen, Rokholm, Kaprio, & Sørensen, 2010).

The above studies were some of the first to identify the role of genetics in obesity development. Additional studies have categorized the genetic factors contributing to obesity into three main classifications (Thaker, 2017). These classifications comprise monogenic factors, syndromic factors, and polygenic factors, all contributing to obesity. Monogenic factors pertain to obesity caused by a mutation in a solitary gene, typically within the leptin-melanocortin pathway. Syndromic obesity involves the presence of severe obesity alongside other characteristics like neurodevelopmental abnormalities and malformations in different bodily systems. Conversely, polygenic obesity arises from the combined influence of multiple genes, with their impact amplified in an environment that encourages weight gain.

Monogenic obesity is caused by a single gene mutation, many of the genes identified are those that disrupt the regulatory system of appetite and weight. Obesity caused by monogenic defects are rare, occurring in only 2-5% of individuals with obesity (Kleinendorst et al., 2020). The initial breakthrough in understanding the mechanism of obesity occurred with the discovery of ob/ob mice, which exhibited obesity as a result of a mutation in the leptin gene. This discovery opened doors to further research, leading to the identification of the melanocortin pathway. This pathway involves the signalling of an individual's satiety or fed state through the circulation of hormones like leptin and insulin. These hormones play a crucial role in regulating appetite and energy balance (Baldini & Phelan, 2019). Most of the known monogenic effects in these individuals are an effect on the leptin-melanocortin signalling pathway, which regulates hunger, satiety and energy expenditure (Baldini & Phelan, 2019). Monogenic obesity is seen through mutations in leptin (LEP), leptin receptor (LEPR), pro-opio melanocortin protein (POMC), neurotrophic receptor tyrosine kinase 2 (NTRK2), brain derived neurotrophic factor (BDNF), Src homology 2 B adapter protein (SH2B1) and deficiencies in proprotein convertase-1/2 (PC1/2), melanocortin receptor (MC4R), and Single-minded homologue of drosophila (SIM1) (Thaker, 2017). Most monogenic mutations have been identified in patients with severe and early onset (<10 years old) obesity (Loos & Yeo, 2022), often demonstrating an inheritance pattern (Farooqi & O'Rahilly, 2006).

Some other genetic conditions can lead to syndromic obesity due to single gene defects or multiple gene defects associated with phenotypes and early-onset severe obesity. Over 25 forms

of syndromic obesity have been recognised (Chung, 2012). The term "syndromic obesity" is employed to describe individuals, both children and adults, who are obese and exhibit additional characteristics such as cognitive delay, dysmorphic features, organ-specific abnormalities, hyperphagia (excessive hunger), and/or other indications of hypothalamic dysfunction. These syndromes involve a combination of obesity and various associated clinical features, indicating a complex relationship between genetic factors, hypothalamic functioning, and physiological abnormalities. (Farooqi & O'Rahilly, 2005). Bardet-Biedl syndrome and Prader-Willi syndrome are the two most prevalent types of syndromic obesity. Additional forms of syndromic obesity include Alström syndrome, Wilms tumor, aniridia, genitourinary anomalies, and mental retardation (WAGR) syndrome, Laurence-Moon-Beidl syndrome, Cohen syndrome, Albright's hereditary osteodystrophy, and Borjeson-Forssman-Lehmann syndrome. Syndromic obesity often manifests alongside cognitive impairments and atypical behaviors, ranging from mild to severe. Moreover, there is a higher correlation between obesity and other syndromes that impact cognition (Bell & Bhate, 1992; Thaker, 2017). The precise mechanism underlying weight gain in the majority of these syndromic conditions remains uncertain. The complexity of these disorders, coupled with the involvement of multiple genetic and physiological factors, makes it challenging to pinpoint a specific mechanism for weight gain. Further research is needed to uncover the precise mechanisms by which these syndromes contribute to weight gain and obesity (Omer, 2020).

1.1.5.2 Extragenetic causes of obesity

Dietary factors

There is ongoing debate regarding the role of high-fat and high-carbohydrate diets in the current increase in obesity prevalence. While it is true that both types of diets have been implicated, it is important to consider the overall dietary patterns, individual metabolic variations, and other lifestyle factors when assessing their impact on obesity. The macronutrient composition of diets such as carbohydrates, fat and protein have been considered for their potential relevance to the development of obesity (Kopp, 2020). High-fat as well as high-carbohydrate diets have been blamed for the current increase in the prevalence of obesity (Hall, 2018; Ludwig & Ebbeling, 2018).

High-fat diets have traditionally been held responsible for the obesity epidemic (Hall, 2018). However, many studies have shown that a high-fat, low-carbohydrate diet does not promote weight gain (Westman et al., 2007). Carbohydrates play a major role in the Western diet as it is

found in refined cereals, corn, potatoes, and sugar. In particular, sugar is consumed in large amounts, found in beverages, sweets, additives and ready meals (Kopp, 2020). Carbohydrates are indeed the macronutrients that have the most significant impact on postprandial blood glucose and insulin response. High-carbohydrate diets can cause a rapid rise in blood glucose levels, triggering the release of insulin to regulate blood sugar. Insulin promotes the storage of glucose as glycogen in the liver and muscles, and excess glucose is converted into fat for long-term storage.(Kopp, 2003; Ludwig & Ebbeling, 2018), It has been suggested that high-carbohydrate diets cause obesity, although research does not suggest this view. Rather, studies which had a high-carbohydrate low fat diet caused moderate weight loss (Poppitt et al., 2002).

As shown, neither carbohydrate nor fats are fattening on their own. It requires a mixed diet with high amounts of carbohydrates and fat, like current Western diets to produce weight gain through a glucocentric metabolism (Cordain et al., 2005).

Endocrinological Conditions & Medication

Endocrinological conditions can also contribute to obesity by affecting the way the body processes and stores fat, as well as regulate appetite and metabolism. Cushing's syndrome is one endocrinological condition that can contribute to obesity. This condition is caused by the overproduction of the hormone cortisol, which is produced by the adrenal gland. Cortisol plays a role in stress response and also helps to regulate metabolism. When there is an excess of cortisol in the body, it can lead to weight gain due to fat tissue redistribution, especially in the abdominal area, leading to central obesity (Ferrau & Korbonits, 2015).

Hypothyroidism refers to a medical condition in which the thyroid gland functions below the normal level, resulting in an underactive thyroid. This condition has been found to have a potential contribution to the development of obesity. When the thyroid gland does not produce enough thyroid hormones, the body's metabolism slows down. This reduced metabolic rate makes it harder for the body to burn calories efficiently, leading to weight gain and, in some cases, obesity (A. Verma, Jayaraman, Kumar, & Modi, 2008). Additionally, hypothyroidism can cause water retention and increase levels of certain lipids in the bloodstream (Rizos, Elisaf, & Liberopoulos, 2011), further contributing to weight gain. Individuals with hypothyroidism can also often experience fatigue and low energy levels (Vaidya & Pearce, 2008), which can lead to a sedentary lifestyle and decreased physical activity, exacerbating the weight gain associated with the condition. Whilst not everyone with hypothyroidism will become obese,

the hormonal imbalances and metabolic changes associated with the condition can make weight management more challenging for those affected.

Polycystic ovary syndrome (PCOS) is another endocrinological condition that can contribute to obesity. This condition is characterised by the development of cysts on the ovaries and an imbalance of hormones such as oestrogen and testosterone. One way that PCOS can contribute to obesity is through insulin resistance, which means that the cells do not respond properly to insulin and can lead to high levels of insulin in the body, causing the body to store more fat (Legro, 2012). In addition, PCOS can also contribute to obesity through appetite and metabolism changes, such as increased appetite and cravings for high-calorie, high-fat foods, and a slower metabolism.

Certain medications can contribute to weight gain and obesity. Certain antidepressants such as Amitriptyline (Average weight gain of 0.5 - >7.0 kg) and Nortriptyline (Average gain of up to 4 kg) are associated with weight gain, as well as Monoamine oxidase inhibitors (MOAIs) (weight gain 0.1 – 7kg). Induced weight gain and changes in metabolism are known as important side effects of antidepressants, with metabolic changes associated with weight gain include mainly being attributed to glucose and lipid metabolism (Himmerich & Minkwitz, 2015). Some medications used to lower blood sugar, such as insulin, sulfonylureas, and thiazolidinediones, as well as some antihypertensives like clonidine and atenolol, can also cause significant weight gain (Omer, 2020).

Individual Factors

Energy expenditure is determined by three main factors: the basal metabolic rate (BMR), the energy required for food digestion, and the energy used during physical activity. Basal metabolic rate (BMR) is the amount of energy that the body needs to maintain basic functions such as breathing, circulation, and cell production while at rest. BMR makes up a significant portion of an individual's total energy expenditure and tends to decrease with age and with weight loss. Research (Ying Zhang et al., 2016) has found that the BMR of people with overweight or obesity were significantly more than those of normal BMI ($p<0.001$). The BMR of people with obesity were significantly more than overweight ($p= 0.036$).

Socioeconomic factors play a significant role in contributing to obesity in the United Kingdom. Access to affordable, healthy food options is often limited in low-income areas, leading to a

reliance on cheaper, calorie-dense processed foods that are high in fat, sugar, and salt. Additionally, individuals from lower socioeconomic backgrounds may face barriers in accessing recreational facilities and opportunities for physical activity. Limited resources and time constraints can make it challenging to engage in regular exercise or participate in sports. Furthermore, stressors associated with lower socioeconomic status, such as financial difficulties and job insecurity, can contribute to emotional eating and unhealthy coping mechanisms. Addressing these socioeconomic disparities and promoting equitable access to healthy food choices and opportunities for physical activity are essential in combating the obesity epidemic in the UK. In the United Kingdom, socioeconomic disadvantage in childhood or adulthood is associated with higher BMI, which persists with age and over different generations (Bann, Johnson, Li, Kuh, & Hardy, 2017).

Environmental factors

The prevalence of obesity can be higher due to the workplace environment. Extended periods of work can lead to higher BMI as a consequence of limited opportunities for exercise and reduced engagement in physical activity (Omer, 2020). Furthermore, this situation can contribute to a higher prevalence of obesity as individuals tend to rely more on convenient processed meals and fast food options instead of preparing home-cooked meals. A study conducted in 2018 demonstrated a notable correlation between longer working hours and increased BMI (Cook & Gazmararian, 2018). Another study found that mothers who worked longer had higher risk of their children having obesity (G. Lee & Kim, 2013), highlighting the impact of the work environment on the well-being of children.

The modern-day environment can influence travel and commuting, again having an effect on obesity prevalence. One study showed there was a positive correlation between high BMI and vehicle miles travelled, as well as commute time, showing that driving more tends to lead to less physical activity and thus less calories spent (Yi Zhang, Wu, Li, Liu, & Li, 2014).

Social networks and the peer effect are also important facets of obesity. One study looked at the influence of weight gain on an individual's network of friends and family (Christakis & Fowler, 2007). This found that an individual's risk of weight gain increased by more than 50% if a friend became obese, and 30% if a partner became obese. Overall, it has been shown that 30% of obesity cases may be attributed to environmental factors (Martinez, 2000).

1.1.6 Prognosis of Obesity

Obesity is a serious health condition that is linked to numerous health complications. These complications can have substantial effects on an individual's overall health and quality of life, and they also elevate the risk of mortality. Studies have shown that overweight and obesity are associated with increased all-cause mortality, meaning the risk of death from any cause is higher among individuals who are overweight or obese. Figure 1.3 demonstrates that all-cause mortality tends to be lowest within the BMI range of 20 to 24.9 kg/m², indicating a lower risk of death for individuals within that range. This highlights the importance of maintaining a healthy weight and body mass index (BMI) in order to reduce the risk of mortality and improve overall health outcomes (De Gonzalez et al., 2010).

Figure 1.3 - Estimated Hazard Ratios for Death from Any Cause According to Body-Mass Index (De Gonzalez et al., 2010)

Research has consistently indicated that individuals with obesity face an increased likelihood of mortality from various causes, including heart disease, stroke, and specific cancer types. Obesity serves as a significant risk factor for heart disease, a leading global cause of death. The excessive body weight associated with obesity places strain on the cardiovascular system, potentially leading to the development of conditions such as coronary artery disease, high blood pressure, and heart failure which is the leading cause of death worldwide (Powell-Wiley et al., 2021). Obese individuals are more likely to develop high blood pressure, high cholesterol, and other risk factors for heart disease. In addition to its impact on various health conditions, obesity is also linked to an elevated risk of developing certain types of cancer, including colon, breast, and endometrial cancers. Research suggests that approximately 20% of all cancer cases

can be attributed to factors such as weight, weight gain, and obesity (Wolin, Carson, & Colditz, 2010). Historical data points to obesity as a cause for approximately 15% of cancer death in males, and 20% of cancer deaths in females (Calle, Rodriguez, Walker-Thurmond, & Thun, 2003), although this is now thought to be an underestimation due to the increased prevalence of obesity over the past two decades (Wolin et al., 2010). Generally, life expectancy decreases as BMI increases, with exception to underweight which also decreases life expectancy (Figure 1.4). A person with a healthy BMI range can expect an 80% change of reaching age 70, BMI of 35-40 reducing this chance to 60%, and BMI of 40-50 reducing this even further to around 50% (Prospective Studies Collaboration, 2009).

Figure 1.4 – Life expectancy data for participants at different BMI ranges
(Prospective Studies Collaboration, 2009)

Obesity is also associated with an increased risk of several other health conditions, and prevalence for each comorbidity increases as BMI increases (Must et al., 1999). Obesity is associated with disorders of almost every system in the body (Ghandehari, Le, Kamal-Bahl, Bassin, & Wong, 2009) (Figure 1.5). For example, obese individuals are more likely to develop high blood pressure, which can increase the risk of heart disease and stroke. Additionally, obesity is linked to an increased risk of osteoarthritis and gallbladder disease. Obese individuals are also more likely to develop sleep apnoea (Romero-Corral, Caples, Lopez-Jimenez, & Somers, 2010), a condition in which breathing is repeatedly interrupted during sleep. This can lead to daytime drowsiness, difficulty concentrating, and an increased risk of heart disease and stroke.

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Figure 1.5 – Obesity can be associated with many disorders of almost every system in the body (Guh et al., 2009).

Beyond its well-documented physical consequences, such as increased risk of chronic conditions, impaired mobility, and reduced overall health status, obesity also manifests in significant psychological and social ramifications. One prominent psychological aspect concerns the heightened prevalence of mental health disorders among individuals who are overweight or obese. Studies have consistently shown higher rates of anxiety, depression, and low self-esteem in this population (Puhl & Heuer, 2009). These psychological issues can create additional challenges for individuals striving to adopt and sustain healthy lifestyle changes, as they may undermine self-efficacy and motivation. Obesity also has a significant impact on an individual's quality of life. Many people who are overweight or obese experience psychological and social problems, such as anxiety, depression, and low self-esteem. These issues can make it more challenging for individuals to make healthy lifestyle changes. Obesity not only impacts life expectancy but also diminishes healthy life expectancy and adversely affects overall quality of life. Individuals with obesity are at a higher risk of developing co-morbidities at a younger age (Donini et al., 2020).

Furthermore, the social consequences of obesity cannot be overlooked. Obese individuals often face social stigmatization and discrimination, leading to negative social experiences and decreased social support. The weight bias prevalent in society contributes to a cycle of marginalization, impacting individuals' self-perception and interpersonal relationships (Puhl & Heuer, 2009). This social isolation can perpetuate feelings of loneliness, hinder participation in social activities, and impede the development of supportive networks, all of which are essential for maintaining a satisfying quality of life.

1.1.7 Pathways and Mechanisms of Obesity

Obesity is a widely prevalent disorder which affects a significant portion of the global population. The exact mechanisms underlying its development are not yet fully understood. However, it is widely recognised that obesity is regulated by multiple pathways, which operate in a heterogenous manner (Wen et al., 2022). Recent advancements in the understanding of the signalling pathways in obesity development have created new opportunities to combat this complex health issue with more precision. This section will review the various pathways currently linked with the development of obesity.

Figure 1.6 - Signalling pathways involved in pro-obesity and anti-obesity mechanisms (Wen et al., 2022).

MAPK pathway

Dysregulation of the mitogen-activated protein kinase (MAPK) pathway is linked to the pathogenesis of obesity (Figure 1.6, figure 1.8). The MAPK pathway is a complex signalling cascade that regulates various cellular processes, such as cell proliferation, differentiation, survival, and apoptosis (Yue & López, 2020). It is activated by extracellular stimuli, such as growth factors, cytokines, and stress signals, and it consists of a series of protein kinases that phosphorylate and activate each other. This acts through a three-tiered kinase cascade which connects extracellular stimuli to intracellular signals through MAPK kinase kinase (MAPKKK), a MAPK kinase (MAPKK), and the MAPK (Pudewell, Wittich, Kazemein Jasemi, Bazgir, & Ahmadian, 2021). The MAPK pathway is divided into three main branches:

the extracellular signal-regulated kinase (ERK), c-Jun N-terminal kinase (JNK), and p38 pathways. Dysregulation of these pathways has been implicated in the pathogenesis of several diseases, including obesity, through regulation of appetite, adipogenesis, glucose homeostasis and thermogenesis (Kassouf & Sumara, 2020).

Differentiation of preadipocytes into mature adipocytes, a process known as adipogenesis, is regulated by various signalling pathways, among them the MAPK pathway. The ERK and JNK pathways have been demonstrated to stimulate adipogenesis by promoting the expression of key transcription factors involved in adipogenesis, such as peroxisome proliferator-activated receptor gamma (PPARγ) and CCAAT-enhancer binding protein (C/EBP) β. Recent research studies have indicated a potential link between obesity and increased levels of p38 MAPK in the p38 pathway, suggesting that the p38 pathway may also play a role in the development of obesity. These findings suggest that multiple signalling pathways, including the ERK, JNK, and p38 pathways within the MAPK pathway, are involved in the regulation of adipogenesis and potentially contribute to the pathogenesis of obesity (Bashan et al., 2007).

In obesity, a characteristic feature is the development of insulin resistance, where the normal function of insulin signalling is impaired, resulting in decreased glucose uptake in target tissues such as skeletal muscle and adipose tissue. One mechanism that contributes to this impairment is the dysregulation of the MAPK pathway. Studies have demonstrated that dysregulation of the MAPK pathway can disrupt insulin signalling by phosphorylating insulin receptor substrate-1 (IRS-1). Phosphorylation of IRS-1 interferes with its normal function as a key mediator in insulin signalling, leading to reduced downstream signalling and impaired glucose metabolism. This disruption in insulin signalling further contributes to the development and progression of insulin resistance in obesity (Khoubai & Grosset, 2021), a key mediator of insulin signalling, on serine and threonine residues, leading to reduced insulin sensitivity. The JNK pathway has been implicated in the pathogenesis of insulin resistance by promoting the phosphorylation of IRS-1. However, JNK1 and JNK2 are shown to induce insulin resistance, while JNK3 may improve insulin sensitivity (Solinas & Becattini, 2017).

Inflammation is a key driver of metabolic dysfunction in obesity and has been linked to the dysregulation of the MAPK pathway (Bak et al., 2016). The JNK pathway has been shown to promote the secretion of pro-inflammatory cytokines, such as tumour necrosis factor alpha (TNFα) and interleukin-6 (IL-6) (Yung & Giacca, 2020), from adipocytes and macrophages.

This leads to the activation of pro-inflammatory signalling cascades, such as the nuclear factor kappa B (NF-κB) pathway, which further amplifies the inflammatory response.

In summary, the MAPK pathway plays a significant role in the pathogenesis of obesity by regulating adipogenesis, insulin signalling, and inflammation. Dysregulation of this pathway may contribute to the development of metabolic dysfunction and related diseases, such as type 2 diabetes.

PI3K/AKT pathway

The phosphatidylinositol 3-kinase (PI3K)/protein kinase B (AKT) (PI3K/AKT) signalling pathway (figure 1.7) plays a crucial role in regulating cell growth and proliferation. In the context of obesity development, the atypical activation of this pathway is considered significant in its pathogenesis. Abnormal activation of the PI3K/AKT pathway can disrupt the balance of cellular processes involved in energy regulation, leading to metabolic dysregulation, and potentially contributing to the development of obesity. Understanding and targeting this signalling pathway may offer potential avenues for therapeutic interventions and strategies in addressing obesity-related complications (X. Huang, Liu, Guo, & Su, 2018).

The PI3K/AKT pathway is a pivotal signalling cascade that orchestrates cellular responses to insulin stimulation. Upon binding of insulin to the insulin receptor (IR) on the cell membrane, autophosphorylation of specific tyrosine residues ensues, culminating in the activation of phosphoinositide 3-kinase (PI3K). Activated PI3K phosphorylates phosphatidylinositol-4,5-bisphosphate (PIP2), converting it into phosphatidylinositol-3,4,5-trisphosphate (PIP3). PIP3 serves as a secondary messenger, recruiting and translocating protein kinase B (AKT) to the plasma membrane (Corti et al., 2019).

Once localised at the plasma membrane, AKT undergoes phosphorylation at threonine 308 (T308) by phosphoinositide-dependent kinase 1 (PDK1) and at serine 473 (S473) by mammalian target of rapamycin complex 2 (mTORC2) (B. X. Huang, Lee, Akbar, & Kim, 2015). These phosphorylation events render AKT fully activated, enabling it to phosphorylate a multitude of downstream targets involved in diverse cellular processes. AKT activates the translocation of glucose transporter 4 (GLUT4), a pivotal part in glucose homeostasis, from intracellular storage compartments to the cell membrane, although the mechanism for this is unknown (Kupriyanova & Kandror, 1999)

Subsequently, translocated GLUT4 facilitates the transport of glucose across the cell membrane, increasing cellular glucose uptake (Navale & Paranjape, 2016). The interaction among insulin, PI3K, AKT, and GLUT4 helps control how the body processes glucose and is crucial for producing and using energy in tissues that respond to insulin, like muscles and fat. Overall, the PI3K/AKT pathway exerts influence over fundamental cellular processes, highlighting its significance in cellular homeostasis and metabolic regulation.

The dysregulation of the PI3K/AKT pathway in the context of insulin resistance significantly contributes to the development of obesity (X. Huang et al., 2018). In a healthy state, insulin binds to the insulin receptor (IR) on the cell membrane, initiating a cascade of events involving the PI3K/AKT pathway. However, in cases of insulin resistance, there are disruptions and abnormalities along this pathway.

In the context of insulin resistance, various factors interfere with the insulin signalling process, preventing proper activation and phosphorylation of AKT. As a result, AKT is unable to effectively phosphorylate its downstream targets, including GLUT4 (Abeyrathna & Su, 2015), which is responsible for facilitating the uptake of glucose into cells. Without the proper activation of GLUT4, cells become less responsive to insulin's signals and fail to take in glucose efficiently.

The impaired glucose uptake in insulin-resistant cells leads to elevated blood glucose levels, known as hyperglycaemia. Prolonged hyperglycaemia can have detrimental effects on various organs and tissues in the body, including the cardiovascular system (Davidson & Parkin, 2009). Elevated glucose levels contribute to the formation of advanced glycation end-products (AGEs) and oxidative stress (Singh, Bali, Singh, & Jaggi, 2014), both of which play a role in the development of cardiovascular complications.

Insulin resistance is often accompanied by compensatory hyperinsulinemia, where the body produces higher levels of insulin to overcome the resistance (Janssen, 2021). However, excessive insulin levels can further disrupt the delicate balance of the PI3K/AKT pathway and exacerbate insulin resistance. This creates a cycle of impaired glucose uptake, elevated blood glucose levels, and increased insulin production, which ultimately contributes to the development and progression of obesity.

In summary, dysregulation of the PI3K/AKT pathway is a key feature of obesity and related metabolic disorders. The development of insulin resistance, dysregulation of adipokine secretion, and alterations in nutrient availability and energy balance can all contribute to the dysregulation of this pathway.

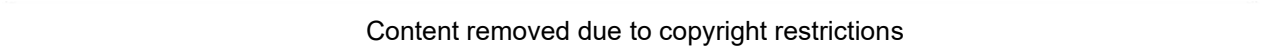

Figure 1.7 – Insulin Signalling in an Insulin Responsive Cell. Insulin binds to IRs, causing conformational change and autophosphorylation of tyrosine residues in cytoplasmic β subunits. Phosphorylated IRS binds PI3K, activating it and converting PIP2 to PIP3. PIP3 recruits Akt to the membrane, where it's phosphorylated and translocates GLUT4 for glucose entry. Akt inhibits GSK3B, promoting glycogen synthesis, and FOXO1, increasing lipoprotein lipase transcription. Phosphorylated Akt inhibits TSC1/2, activating mTORC1 for protein synthesis. mTORC1 phosphorylates IRS and GRB10, preventing insulin signaling (Acosta-Martinez & Cabail, 2022).

JAK/STAT pathway

The Janus kinase (JAK)/signal transducer and activator of transcription (STAT) (JAK/STAT) signalling pathway is a crucial pathway (Figure 1.8) in mediating the effects of various cytokines, hormones, and growth factors in the body (Shuai, 2000). The binding of these molecules to their respective receptors activates JAKs (Corry, Mott, & Owen, 2020), which then phosphorylate STAT proteins, allowing them to function as transcription factors by binding to specific DNA elements and regulating the transcription of targeted genes (Horvath, 2000). Dysregulation of the JAK/STAT pathway is associated with obesity (Gurzov, Stanley,

Pappas, Thomas, & Gough, 2016), either directly or by interacting with other signalling pathways like MAPK and PI3K.

The JAK/STAT pathway is closely linked to the melanocortin pathway, which regulates energy homeostasis through the hormone leptin (Harris et al., 2001). Leptin signalling involves the activation of JAK2 and phosphorylation of STAT3 and STAT5, which function as transcription factors (Banks, Davis, Bates, & Myers, 2000). The activation of STAT3/STAT5 is essential for controlling food intake (Liu, Du, Li, & Yang, 2021). The binding of leptin to its receptor also leads to downstream activation of Rho-kinase 1, which phosphorylates and activates JAK2 to maintain energy homeostasis (H. Huang et al., 2012). Additionally, it promotes the transcription of Proopiomelanocortin (POMC), deficiency of which is associated with obesity (Lindberg & Fricker, 2021), and increases the expression of carboxypeptidase, which suppresses food intake (Plum et al., 2009).

The JAK/STAT pathway also plays a role in regulating hepatic steatosis, a feature of obesity (Divella, Mazzocca, Daniele, Sabbà, & Paradiso, 2019). Research indicates that the specific lack of STAT3 in hepatocytes is associated with insulin resistance and heightened expression of genes involved in gluconeogenesis (Moh et al., 2008). Conversely, activation of STAT3 in hepatocytes has the potential to prevent steatosis, a condition characterised by the accumulation of fat in the liver. The hepatic growth factor–JAK2–STAT5–IGF1 axis also plays a key role in lipid metabolism (Dodington, Desai, & Woo, 2018), and low growth factor levels may contribute to obesity by decreasing lipolysis in adipose tissue and increasing hepatic steatosis. Additionally, mice with hepatocyte-specific deletion of JAK2 develop spontaneous steatosis but manifest protection against HFD-induced insulin resistance and glucose intolerance (S. Y. Shi et al., 2012).

Peripheral JAK/STAT signalling can be activated by leptin (Seth, Biswas, Ganguly, Chakrabarti, & Chaudhuri, 2021), and the secretion of leptin in adipose tissue induced by a high-fat diet (HFD) leads to an upregulation of the STAT3 target gene responsible for producing caveolin-1. This, in turn, reduces leptin signalling through a negative feedback mechanism (Picó, Palou, Pomar, Rodríguez, & Palou, 2022). STAT3 plays a role in promoting lipolysis, the breakdown of fats, while simultaneously inhibiting adipogenesis, the formation of new fat cells (Cernkovich, Deng, Bond, Combs, & Harp, 2008). In addition to STAT3, STAT4 also contributes to the pathophysiology associated with obesity. It does so by decreasing insulin

sensitivity and promoting inflammation within adipocytes (Dobrian et al., 2013). Obesity is further associated with adipocyte dysfunction and insulin resistance as a result of dysregulated JAK-STAT1 signalling (Richard & Stephens, 2014). Adipose function is influenced by the JAK proteins, and when JAK2 is specifically knocked out in adipocytes in mice, it leads to increased adiposity caused by impaired lipolysis (S. Y. Shi et al., 2014).

Overall, the JAK/STAT pathway plays a key role in the inflammatory response and metabolic dysregulation associated with obesity. Dysregulation of this pathway has been implicated in the development of insulin resistance, type 2 diabetes, cardiovascular disease, and other metabolic disorders. Targeting the pathway may be a potential therapeutic approach for the treatment of obesity and its associated metabolic disorders, although careful consideration must be given to the potential risks and benefits of such an approach. Further research is needed to fully understand the role of the JAK/STAT pathway in obesity and to develop safe and effective therapies targeting this pathway.

Figure 1.8 – The MAPK, PI3K/AKT and JAK/STAT signalling pathways in obesity pathogenesis (Wen et al., 2022).

TGF-β signalling pathway

The Transforming Growth Factor Beta (TGF-β) superfamily encompasses a collection of proteins with various members, such as TGF-β1-3, activins/inhibins, growth differentiation factors (GDFs), myostatin, and Bone Morphogenetic Proteins (BMPs). These proteins exert a wide range of functions in the regulation of appetite, lipid metabolism, and glucose homeostasis. Dysregulation of the TGF-β pathway has been implicated in several diseases, including obesity (Woo, Koziol-White, Panettieri, & Jude, 2021).

A member of the transforming growth factor-beta (TGF-β) superfamily, called Growth Differentiation Factor 15 (GDF15), has been recognised as a key regulator of appetite and a potential target for obesity treatment (Fung et al., 2021). Mice that lack GDF15 have been observed to develop obesity, while administering GDF15 to mice elicits a taste aversive response, implying that GDF15 plays a role in the regulation of energy balance. These findings suggest that manipulating GDF15 levels or targeting its signalling pathways may hold promise for the development of new therapeutic approaches to combat obesity (Satish Patel et al., 2019).

Elevated levels of TGF-β1 can be observed in individuals with non-insulin-dependent diabetes mellitus (Pfeiffer, Middelberg-Bisping, Drewes, & Schatz, 1996). The signalling pathway of TGF-β is involved in the regulation of glucose tolerance and the maintenance of energy balance. Studies have demonstrated that systemic inhibition of TGF-β/Smad3 signalling can safeguard mice against obesity, diabetes, and hepatic steatosis. This protection is achieved by promoting the expression of PGC-1α in adipose tissue. PGC-1α is a coactivator that plays a role in controlling energy metabolism and the generation of new mitochondria. Recent research suggests that aerobic exercise has the ability to suppress TGF-β activity, which can lead to improvements in insulin resistance. By modulating TGF-β signalling, aerobic exercise has the potential to enhance glucose regulation and increase insulin sensitivity (X. Wang, Yi, & Tang, 2022), and inhibition of TGF-β/Smad3 signalling has been found to have a preventive effect on β-cell apoptosis, the programmed cell death of pancreatic β-cells (J.-H. Lee et al., 2020), indicating that TGF-β/Smad3 antagonists hold promise as potential therapeutic agents for diabetes, as they have the ability to restore insulin sensitivity and promote β-cell homeostasis.

The role of TGF-β in energy expenditure has yielded inconsistent results in various studies. However, recent research has highlighted the involvement of Latent-transforming growth factor beta-binding protein 3 (LTBP3) in promoting the conversion of white adipose tissue (WAT) into brown adipose tissue (BAT). BAT is known to be more metabolically active and has the capacity to burn more calories. LTBP3 achieves this conversion by modulating the expression of Uncoupling Protein 1 (UCP1) and influencing mitochondrial oxygen consumption through TGF-β2 signalling. UCP1 is a key regulator of thermogenesis in brown adipocytes, enabling them to dissipate energy in the form of heat (Halbgebauer et al., 2021), thus showing the potential for obesity treatment (Machado et al., 2022).

AMPK pathway

AMP-activated protein kinase (AMPK) is an enzyme involved in sensing energy levels within cells and plays a vital role in regulating cellular metabolism. It responds to alterations in nutrient availability by being activated when the AMP/ATP ratio increases. This activation stimulates catabolic pathways while inhibiting anabolic pathways. By promoting the utilisation of stored nutrients like glucose and fatty acids for ATP production, AMPK activation enhances insulin sensitivity. This, in turn, improves the uptake and utilisation of glucose in peripheral tissues.

Obesity is characterised by an imbalance between the amount of energy consumed and the amount of energy expended, leading to the accumulation of adipose tissue. In the regulation of energy homeostasis and the development of obesity, AMP-activated protein kinase (AMPK) plays a crucial role. Recent studies have provided evidence linking AMPK dysfunction to the pathogenesis of obesity. AMPK is responsible for sensing and responding to changes in cellular energy status. Activation of AMPK promotes energy-producing processes such as glucose uptake and fatty acid oxidation while inhibiting energy-consuming pathways such as lipogenesis (Lindholm et al., 2013). In obese individuals, AMPK activity is decreased in adipose tissue, liver, and skeletal muscle (Xu et al., 2012), which contributes to the development of insulin resistance and metabolic dysregulation.

The molecular mechanisms underlying AMPK dysfunction in obesity are complex and not fully understood. However, several factors have been implicated in the regulation of AMPK activity, including inflammation, oxidative stress, and lipotoxicity. In obese individuals, chronic low-grade inflammation in adipose tissue can inhibit AMPK activity through the phosphorylation of serine residues on the α subunit by stress kinases such as c-Jun N-terminal kinase (JNK) and inhibitor of kappa B kinase (IKK) (Kusminski, Bickel, & Scherer, 2016). Additionally, oxidative stress can inhibit AMPK activity by promoting the oxidation of cysteine residues on the γ subunit, which disrupts the binding of AMP to the γ subunit (Hardie, Ross, & Hawley, 2012). Excessive accumulation of fatty acids in adipose tissue can lead to lipotoxicity, which impairs insulin signalling and AMPK activation (Eissing et al., 2013).

In conclusion, AMPK is a critical regulator of energy homeostasis, and dysregulation of AMPK activity in obesity contributes to the development of insulin resistance and metabolic dysregulation. Further research is needed to fully elucidate the molecular mechanisms

underlying AMPK dysfunction in obesity and to develop effective AMPK-targeted therapies for the treatment of metabolic disorders.

Wnt/β-catenin signalling pathway

The Wnt/β-catenin signalling pathway is a highly conserved pathway that regulates cell proliferation, differentiation, and cell fate determination. In recent years, it has been implicated in the pathogenesis of obesity. The pathway plays a critical role in adipogenesis, the process by which preadipocytes differentiate into mature adipocytes.

The Wnt/β-catenin pathway is a signalling pathway that has been suggested to play a negative role in adipogenesis and obesity (Xie et al., 2020). When activated, it induces the differentiation of mesenchymal stem cells into osteoblasts while suppressing the expression of adipocyte-related genes such as PPARγ and fatty acid synthase. (Matsushita et al., 2016). This results in the inhibition of adipogenesis, or the formation of fat cells.

Studies have shown that the protein β-catenin, which helps to mediate the function of the Wnt/β-catenin pathway, plays a critical role in the development of obesity (T. Wang et al., 2021). Studies have demonstrated that the knockout of oxysterol-binding protein-like 2 (OSBPL2), a transport protein involved in the functioning of β-catenin, can have significant effects on adipocyte development and obesity. Specifically, OSBPL2 knockout has been shown to promote the maturation of preadipocytes and lead to an obese phenotype. Conversely, when β-catenin is knocked out specifically in mature adipocytes, it has been found to confer resistance against adipose tissue expansion induced by a high-fat diet (HFD). However, this resistance is not observed in adipose tissue under regular chow-diet conditions (Chen et al., 2020).

The effects of the Wnt/β-catenin pathway on adipogenesis can vary depending on the type of fat cells, the diet, and the stage of fat cell development. Activation of the Wnt signalling pathway in adipose progenitor cells has been shown to have notable effects on adipose tissue composition in mice. Specifically, when Wnt signalling is activated within these cells, mice exhibit a significant reduction in visceral fat, which refers to the fat surrounding internal organs, and an increased degree of fibrosis in subcutaneous white adipose tissue (WAT) (Zeve et al., 2012). However, the stimulation of Wnt signalling within mature adipocytes in the same study did not yield the same result.

In mice fed a standard chow diet, the absence of β-catenin in adipocytes is detected and offset by stromal cells to preserve the balance of Wnt signalling throughout the adipose tissue, ensuring homeostasis (Bagchi et al., 2020). However, when subjected to a long-term high-fat diet (HFD), this compensatory mechanism is overruled, leading to an intensified impact of the pathway on adipogenesis.

The Wnt/β-catenin pathway also regulates insulin action and glucose homeostasis (Das, Das, Kalita, & Baro, 2021). TCF7L2, a Wnt protein, is linked to susceptibility to type 2 diabetes (Jin, 2016). In obesity-related diabetes, there is a downregulation of the Wnt/β-catenin signalling pathway. However, inhibiting the activity of CXXC-type zinc-finger protein 5 (CXXC5), a negative regulator of Wnt signalling, has been shown to improve the phenotype associated with this condition (Seo et al., 2022). Wnt signalling induces incretin synthesis and is linked to type 2 diabetes (García-Martínez, Chocarro-Calvo, Moya, & García-Jiménez, 2009). Wnt5a, a Wnt protein, contributes to inflammation in white adipose tissue and glucose dysregulation via JNK (Fuster et al., 2015). These show that the Wnt/β-catenin pathway is a potential target for treating metabolic diseases.

The Wnt/β-catenin pathway is also involved in energy homeostasis and its signalling is linked to leptin, neuroendocrine regulation, and browning of adipocytes. Wnt signalling was shown to be downregulated in leptin-deficient mice, and this was reversed by leptin treatment (Benzler et al., 2013). Wnt/β-catenin signalling is also associated with neuroendocrine regulation of body weight (Boucsein et al., 2016). It also plays a role in preadipocyte differentiation, as it promotes browning of WAT (F. Guo et al., 2021). The pathway has been found to be downregulated in leptin-deficient mice and inhibits browning of adipocytes.

In summary, the Wnt/β-catenin pathway plays a complicated role in the development of fat cells and obesity, with the effects varying depending on the type of fat cells, the diet, and the stage of adipogenesis. The protein β-catenin and other proteins in the pathway interact with other signalling pathways to regulate the development of fat cells, highlighting the complexity of the mechanisms underlying obesity.

Sphingosine Pathway

The sphingosine pathway is a complex signalling network that plays an important role in regulating various cellular processes, including cell growth, differentiation, and apoptosis (Roszczyc-Owsiejczuk & Zabielski, 2021). In recent years, the sphingosine pathway has emerged as a key player in the pathogenesis of obesity and type 2 diabetes.

The sphingosine pathway is initiated by the synthesis of sphingolipids, a class of membrane lipids. Sphingolipids are synthesised de novo from the condensation of serine and palmitoyl-CoA, leading to the formation of 3-ketosphinganine, which is subsequently reduced to sphinganine (Pralhada Rao et al., 2013). Sphinganine is then acylated to form dihydroceramide, which is converted to ceramide by the action of ceramide synthase (Sugimoto, Sakoh, & Yamada, 2004). Ceramide can be further metabolised into other bioactive sphingolipids, such as sphingosine and sphingosine-1-phosphate (S1P), by the action of specific enzymes.

Ceramide has been implicated in the pathogenesis of obesity through its ability to induce insulin resistance and inflammation in various tissues (Chavez & Summers, 2012), such as liver, muscle, and adipose tissue. Ceramide accumulation in these tissues is thought to impair insulin signalling by activating protein kinases, such as JNK and IKK (Boucher, Kleinridders, & Kahn, 2014), which phosphorylate serine residues on insulin receptor substrate-1 (IRS-1), leading to its degradation and decreased insulin signalling. In addition, ceramide can activate inflammatory pathways, such as NF-kB and MAPKs, leading to the production of pro-inflammatory cytokines, such as TNF-alpha, IL-6, and MCP-1 (Rivas et al., 2012). These cytokines can further impair insulin signalling and promote insulin resistance.

Sphingosine-1-phosphate (S1P), on the other hand, has been shown to have anti-obesity effects by promoting adipocyte differentiation and reducing adipose tissue inflammation (Chakrabarty et al., 2022). S1P is generated by the phosphorylation of sphingosine by sphingosine kinase (SphK), and it acts through specific receptors, such as S1P1 and S1P3, to regulate various cellular processes. S1P has been shown to promote adipogenesis by activating PPAR-γ and C/EBP-α, two transcription factors that are critical for adipocyte differentiation. In addition, S1P has been shown to reduce adipose tissue inflammation by inhibiting the production of pro-inflammatory cytokines and chemokines, such as TNF-alpha and MCP-1 (Guitton et al., 2020).

In conclusion, the sphingosine pathway is a complex signalling network that plays an important role in the pathogenesis of obesity. Ceramide accumulation in various tissues can induce insulin resistance and inflammation, while S1P can promote adipocyte differentiation and reduce adipose tissue inflammation. Understanding the molecular mechanisms underlying the sphingosine pathway may lead to the development of novel therapeutic strategies for the prevention and treatment of obesity and its associated metabolic disorders.

1.2 Type 2 Diabetes Mellitus

1.2.1 Definition of Diabetes Mellitus

Diabetes mellitus (DM) is a heterogeneous disease, which means it lacks a precise and uniform definition. The clinical characteristics of DM exhibit substantial variations both within and among different populations (Olaogun, Farag, & Hamid, 2020). DM can be defined as "a general term for heterogeneous disturbances of metabolism for which the main finding is chronic hyperglycaemia. The cause is either impaired insulin secretion or impaired insulin action or both" (Petersmann et al., 2019). Diabetes mellitus (DM) encompasses two primary types: Type 1 Diabetes (T1D) and Type 2 Diabetes (T2D). T1D is a chronic condition distinguished by the destruction of pancreatic β-cells, resulting in a complete deficiency of insulin. Immune mechanisms usually mediate the destruction of these cells. Another subtype of T1D, known as latent autoimmune diabetes in adults (LADA), is also classified under type 1 diabetes. LADA typically manifests later in life and progresses at a slower pace compared to typical T1D (Petersmann et al., 2019). The main cause of type 1 diabetes (T1D) is considered to be the autoimmune destruction of the insulin-secreting β cells located in the pancreas. In contrast, type 2 diabetes (T2D) is frequently linked to obesity and is characterised by a combination of diminished insulin secretion from the pancreatic β cells and insulin resistance in peripheral tissues (Taylor, Yazdi, & Beitelshees, 2021). Type 2 diabetes (T2D) is a progressive condition that encompasses a range of disorders. It can manifest as a state where insulin resistance is predominant, accompanied by a relative deficiency of insulin. Alternatively, it can present as a primary defect in insulin secretion alongside insulin resistance. T2D is the most prevalent type of diabetes, accounting for over 90% of all cases of diabetes mellitus (Stumvoll, Goldstein, & Van Haeften, 2005). This particular type of diabetes is frequently linked to other disorders, including the metabolic syndrome, which encompasses conditions such as obesity, hypertension (high blood pressure), and abnormal lipid (cholesterol) levels. It is commonly diagnosed in adulthood and is often the result of a combination of genetic

susceptibility and lifestyle factors, such as an unhealthy diet and sedentary behaviour with limited physical activity (Petersmann et al., 2019).

Diabetes encompasses various specific types, including conditions affecting the exocrine pancreas like pancreatitis, cystic fibrosis, and hemochromatosis. Endocrinopathies, which are disorders of the endocrine system, such as Cushing syndrome, acromegaly, and pheochromocytoma, can also lead to diabetes. Drug-induced diabetes can arise as a result of certain medications, including glucocorticoids, neuroleptics, alpha-interferons, and pentamidine. Genetic defects related to beta-cell function, such as maturity onset diabetes of the young (MODY) forms, as well as genetic defects of insulin action, can contribute to diabetes development. Various genetic syndromes, including Down syndrome, Turner syndrome, and Wolfram syndrome, may also be associated with diabetes. Additionally, certain infections like cytomegalovirus and congenital rubella can cause diabetes. Lastly, gestational diabetes refers to impaired glucose tolerance that appears or is diagnosed for the first time during pregnancy (Petersmann et al., 2019).

In the United Kingdom, Diabetes UK follows the World Health Organization (WHO) diagnostic criteria for diabetes mellitus. These criteria, which are widely used, include specific thresholds for blood glucose levels. According to these criteria, a diagnosis of diabetes can be made if the fasting plasma glucose level is equal to or greater than 7.0 mmol/dl (126 mg/dl), the random plasma glucose level is equal to or greater than 11.1 mmol/l (200 mg/dl), or if the plasma glucose level exceeds 11.1 mmol/l (200 mg/dl) two hours after a glucose challenge test. Additionally, Diabetes UK has incorporated the use of HbA1c testing for diabetes diagnosis since 2011. An HbA1c level of 48 mmol/mol (6.5%) is recommended as the cut-off point for diagnosing diabetes. These diagnostic criteria provide standardised guidelines for healthcare professionals to identify individuals with diabetes based on their blood glucose levels, whether through fasting plasma glucose, random plasma glucose, or HbA1c measurements. This facilitates accurate diagnosis and allows for appropriate management and treatment strategies to be implemented.

1.2.2 Pathophysiology of Type 2 Diabetes

Type 2 diabetes mellitus (T2D) involves a range of underlying biological mechanisms. These include peripheral insulin resistance, where the body's tissues become less responsive to the effects of insulin. Additionally, there is impaired regulation of hepatic glucose production,

which results in increased glucose output by the liver. Over time, there is a gradual decline in β-cell function, the cells responsible for producing insulin. This progressive decline can ultimately lead to β-cell failure, further exacerbating the impairment in glucose regulation seen in T2D (Muoio & Newgard, 2008).

Insulin resistance is a state where the body's insulin is not as effective as it should be based on the amount present in the blood (Ormazabal et al., 2018). The reduced effectiveness of insulin in key organs like the liver and muscles is a typical characteristic of type 2 diabetes. Insulin resistance develops and becomes more severe before disease onset (Janssen, 2021). In the initial phase of the disease, a decrease in insulin sensitivity causes the beta cells to work harder to produce more insulin to maintain normal blood sugar levels. This results in higher levels of insulin (hyperinsulinemia) and prevents high blood sugar (Banday, Sameer, & Nissar, 2020). However, over time, the beta cells' increased insulin production is not enough to make up for the decreased sensitivity to insulin. Additionally, the beta cells' function begins to deteriorate and eventually leads to a shortage of insulin. As a result, normal blood sugar levels can no longer be maintained and high blood sugar (hyperglycaemia) develops (Cerf, 2013).

1.2.3 Clinical Sequelae of Type 2 Diabetes

The clinical sequelae of type 2 diabetes are numerous and can lead to serious complications if not properly managed. In order to maintain glycaemic control and prevent pathophysiology, patients with T2D must balance their diet and clinical interventions to prevent hyperglycaemia or hypoglycaemia. Suboptimal glycaemic control can lead to increased risk for various cardiovascular diseases, amongst other complications (Dempsey, Owen, Yates, Kingwell, & Dunstan, 2016).

One of the most common and significant complications of type 2 diabetes is cardiovascular disease. Patients with T2D have 10% greater risk of coronary artery disease, 53% higher risk of myocardial infarction, 58% higher risk of stroke, and 112% increased risk of heart failure than those without T2D (Einarson, Acs, Ludwig, & Panton, 2018). This is due to the fact that diabetes can cause damage to the blood vessels, making them more prone to narrowing and blockage. The underlying mechanism for this association is multifactorial, including the presence of traditional cardiovascular risk factors such as hypertension, hyperlipidaemia, and inflammation, as well as metabolic abnormalities such as insulin resistance and hyperglycaemia (Leon & Maddox, 2015).

Another common complication of type 2 diabetes is diabetic neuropathy, which involves nervous system damage and occur in up to half of all individuals with diabetes (Callaghan, Price, Chen, & Feldman, 2015). This can lead to numbness, tingling, and pain in the extremities, as well as an increased risk of foot injuries and amputations. Diabetic neuropathy can also affect the autonomic nerves, leading to problems with digestion, sweating, and blood pressure regulation (Vinik, Maser, Mitchell, & Freeman, 2003). The underlying mechanism for diabetic neuropathy is thought to be related to chronic hyperglycaemia, which leads to the formation of advanced glycation end products (AGEs) and the activation of the polyol pathway, resulting in oxidative stress and inflammation (Yagihashi, Mizukami, & Sugimoto, 2011).

Diabetes can also lead to diabetic nephropathy, which is also referred to as diabetic kidney disease. This condition is a prevalent cause of end-stage renal failure and occurs due to damage to the blood vessels in the kidneys. This damage leads to impaired kidney function. The underlying pathophysiology of diabetic nephropathy is believed to be associated with persistent hyperglycaemia, which causes the formation of advanced glycation end-products (AGEs) and the activation of the renin-angiotensin-aldosterone system, thus resulting in glomerular hypertension and injury (Giunti, Barit, & Cooper, 2006).

Diabetic retinopathy, characterised by the harm to the retinal blood vessels, is another complication of diabetes that can result in vision loss and even blindness if left untreated. This condition is also believed to be a result of persistent hyperglycaemia, which leads to the formation of AGEs and the activation of the vascular endothelial growth factor (VEGF) pathway (Vujosevic et al., 2020), causing neovascularization and inflammation.

In addition, type 2 diabetes is associated with a higher risk of certain types of cancer, such as liver, pancreas, and endometrial cancer. In males with T2D, the risk of cancer of the prostate, blood (leukaemia, lymphoma), skin, thyroid, kidney, liver, pancreas, lung, colorectum, and stomach is increased significantly, and in females with T2D, there is a significantly increased risk of cancer of the nasopharynx, liver oesophagus, thyroid, lung, pancreas, blood (lymphoma, leukaemia), uterus, colorectum, breast, cervix, and stomach (Qi et al., 2019). Mechanisms for this association is not fully understood, but it is thought to be related to chronic inflammation, insulin resistance, and hyperinsulinemia.

Overall, a substantial portion of individuals with T2D experience physical and mental effects on their quality of life (Slagter et al., 2015). It is also associated with premature mortality and increased risk of morbidity from complications including cardiovascular disease, retinopathy, neuropathy and nephropathy (Cowie et al., 2018).

1.2.4 Epidemiology of Type 2 Diabetes

Type 2 diabetes is indeed a significant health concern worldwide, with a high prevalence observed across the globe. Current estimates indicate that approximately 462 million individuals are affected by type 2 diabetes, representing around 6.28% of the world's population (Khan et al., 2020). The global prevalence of type 2 diabetes is not only persistently high but also continuing to rise without any signs of stabilization. In 2017 alone, more than 1 million deaths were attributed to type 2 diabetes, making it the ninth leading cause of mortality. This represents a significant increase compared to its ranking as the eighteenth leading cause of death (Khan et al., 2020). In 1990, there were 0.61 million deaths due to diabetes, while by 2017, that number had escalated to 1.37 million, indicating an overall increase of 125.5%.

If effective prevention methods are not implemented, projections indicate that the rates of type 2 diabetes will further rise to reach 693 million individuals by 2045. These alarming statistics highlight the urgent need for adopting and implementing effective prevention strategies to curb the escalating prevalence of type 2 diabetes (Cho et al., 2018).

The prevalence of type 2 diabetes is slightly higher in males than in females, with 6219 cases per 100,000 among males and 5898 cases per 100,000 among females. This difference is within the range of uncertainty. The age at which new diagnoses are made is slightly earlier for males, and the incidence of the disease increases with age, peaking at 55-59 years. There does not appear to be a significant change in the age distribution from 1990 to 2017 (Khan et al., 2020).

According to Diabetes UK, the cost of diabetes to the NHS is over £1.5m an hour. Overall, the cost of Type 2 Diabetes treatment in the UK for 2012 was £11.718 billion (Kanavos, van den Aardweg, & Schurer, 2012). Breaking this number down further, drugs to treat diabetes directly equates to £0.712 billion, non-diabetes drugs £1.81 billion, inpatient costs £8.038 billion, outpatient excluding drugs at £1.158 billion. Alongside this, the NHS is spending another £10 billion a year on the NHS Diabetes Prevention Programme (NHS DPP), which equates to 10% of the NHS yearly budget, although the NHS DPP is projected to be highly cost effective over

the long term. According to data in 2010/11, he cost burden associated with diabetes is significant, with it accounting for approximately 10% of the total NHS resource expenditure. Without any changes in the treatment and management of diabetes, it is projected that this cost burden will increase to around 17% by the year 2035/36. This rise in costs is estimated to amount to over £22 billion in that fiscal year (Hex, Bartlett, Wright, Taylor, & Varley, 2012).

Type 2 diabetes remains a critical public health issue, with its prevalence, incidence, and impact on human health and mortality steadily increasing. Despite significant efforts in clinical care, research, and public health interventions, there are no indications of a slowdown in the rate of the disease's progression. Certain regions, such as Western Europe and Pacific Island states, are facing a disproportionately high burden of type 2 diabetes.

Various factors contribute directly to the increasing prevalence of type 2 diabetes. These factors include changes in the ratio of diagnosed to undiagnosed cases, an aging population, a younger age of onset of diabetes, improved survival rates among individuals with diabetes, and a rising incidence of new cases of diabetes.

The combination of these factors underscores the need for continued attention and efforts to address the growing challenges posed by type 2 diabetes. It is crucial to implement effective strategies for prevention, early detection, and management of the disease to mitigate its impact on individuals, communities, and healthcare systems (Colagiuri, Borch-Johnsen, Glümer, & Vistisen, 2005).

1.2.5 Treatment Options for Type 2 Diabetes

By maintaining glycated haemoglobin (HbA1c) at or below the goal value specified for each individual patient, alongside effective blood-glucose control, and other treatment, the risk of long-term microvascular and macrovascular problems is reduced. According to the American Diabetes Association (ADA), “strategies may include dietary changes, physical activity, behavioural therapy, pharmacologic therapy, medical devices, and metabolic surgery. The latter three strategies may be prescribed for carefully selected patients as adjuncts to dietary changes, physical activity, and behavioural counselling” (American Diabetes Association, 2021). Overall, the main aim of treatment of T2D is the prevention of long-term complications of the disease.

Bariatric Surgery

Bariatric surgery is a surgical intervention designed to aid in the management of obesity and its associated metabolic comorbidities, including type 2 diabetes (Jackson, Anekwe, Chang, Haskins, & Stanford, 2019). It is a type 2 diabetes treatment only for patients with morbid obesity ((BMI >40 kg/m^2 or > or =35 kg/m^2 with obesity-related comorbidity) (Monteforte & Turkelson, 2000). This intervention involves the alteration of the gastrointestinal anatomy and physiology to induce weight loss and metabolic changes. The success of bariatric surgery in promoting weight loss and improving metabolic health has been well-established in numerous studies. These studies suggest that bariatric surgery is associated with lower mortality (Doumouras et al., 2021), improved glucose control (Mingrone & Castagneto-Gissey, 2009), and reduced health care costs in patients with type 2 diabetes (Keating, 2009; Sjöholm et al., 2013), often leading to remission and better overall prognosis (J. Yu et al., 2015).

Bariatric surgery encompasses various procedures, such as gastric bypass, sleeve gastrectomy, and adjustable gastric banding, each targeting weight loss in different ways. Gastric bypass, also known as Roux-en-Y, involves the creation of a small pouch at the top of the stomach, with the small intestine being rerouted to directly connect to this pouch. Sleeve gastrectomy involves the removal of a portion of the stomach, while adjustable gastric banding entails placing an inflatable band around the upper part of the stomach to create a smaller pouch. All of these surgical procedures lead to weight loss through a combination of reduced food intake and/or malabsorption of nutrients. By restricting the capacity of the stomach or altering the digestive process, these surgeries result in significant weight loss for individuals who undergo them. It's important to note that bariatric surgery is typically recommended for individuals with severe obesity who have not achieved sustainable weight loss through other interventions (Kleinman et al., 2009).

The mechanisms by which bariatric surgery leads to weight loss are multifactorial. First, bariatric surgery reduces the capacity of the stomach to hold food, thereby inducing satiety and limiting food intake (Ionut & Bergman, 2011). Additionally, the re-routing of the intestine can lead to changes in the secretion of hormones involved in appetite regulation (Beckman, Beckman, & Earthman, 2010), leading to further reductions in food intake. Furthermore, bariatric surgery can promote changes in gut microbiota (Ulker & Yildiran, 2019), which may contribute to improvements in metabolic health.

While bariatric surgery has been found to be effective in promoting weight loss and improving metabolic health, it is not without risks. Complications associated with bariatric surgery include nutritional deficiencies, gastrointestinal problems, and psychological issues (Lupoli et al., 2017). Additionally, some patients may experience weight regain or complications in the long-term.

It is important to note that bariatric surgery should not be considered a standalone treatment for obesity and type 2 diabetes. Rather, it should be viewed as one component of a comprehensive approach to weight loss and metabolic health. In some cases, adjunctive therapies such as medication or behavioural interventions may be necessary to achieve optimal outcomes.

Metformin

Metformin is widely regarded as a first-line treatment option for Type 2 Diabetes and has been found to be effective both as a standalone medication (monotherapy) and in combination with other glucose-lowering drugs This recommendation is in line with the guidance provided by the International Diabetes Foundation's Clinical Guidelines Task Force in 2006, which advised the use of metformin as the first-choice therapy for T2D management (IDF Clinical Guidelines Task Force, 2006).

The origin of metformin can be traced back to a plant source and its mode of action has been determined to stem from its ability to reduce glucose production in the liver through the inhibition of gluconeogenesis (Baker et al., 2021). Gluconeogenesis is a group of metabolic processes in the cytosol and mitochondria that regulate the blood glucose levels during periods of fasting (Chourpiliadis & Mohiuddin, 2021). By decreasing glucose production, metformin leads to lower blood glucose levels and improved insulin sensitivity. Additionally, the drug enhances the uptake of glucose by muscle and fat tissue, thereby further reducing blood glucose levels (Polianskyte-Prause et al., 2019).

Metformin also has been observed to improve insulin sensitivity by activating the enzyme adenosine monophosphate-activated protein kinase (AMPK), which helps to regulate glucose metabolism in the body, working as a “fuel gauge” that becomes activated during energy consumption, resulting in inhibition of gluconeogenesis and increased fatty acid oxidation

(Zhou et al., 2001). This is thought to be the reason why metformin has been found to have a positive impact on cardiovascular health. Research by Buse (Buse et al., 2016) suggests that the primary glucose-lowering effect of metformin takes place in the gastro-intestinal tract, and other studies have also shown the metformin increased GLP-1 secretion in the gut microbiome, thus improving glucose homeostasis (Rena, Hardie, & Pearson, 2017). Overall, studies suggest that metformin treats T2D by controlling adiponectin expression improving glucose and lipid metabolism (Ismail, Soliman, & Ismail, 2013), reducing all-cause mortality (Mangold, Rawls, Wall, Gosmanova, & Canada, 2008), and potentially enhancing cognitive function (Q.-Q. Zhang et al., 2020) and gut based pharmacology (Napolitano et al., 2014). Metformin has been proven to be effective in treating and preventing type 2 diabetes by studies such as the United Kingdom Prospective Diabetes Study and the Diabetes Prevention Program. It is a safe and cost-effective option, and it continues to be the primary form of diabetes therapy when combined with diet and exercise (Andújar-Plata, Pi-Sunyer, & Laferrere, 2012).

Insulin

Insulin therapy is a viable treatment option for individuals diagnosed with type 2 diabetes (T2D). Insulin, a hormone naturally produced by the pancreas, plays a crucial role in regulating blood sugar levels by facilitating the uptake, storage, and utilisation of glucose in cells throughout the body. However, in T2D, the body develops a resistance to the effects of insulin, leading to inadequate blood sugar control. Insulin therapy is employed to address this insulin resistance and restore optimal blood sugar management. The goal of insulin therapy is to supplement or replace the body's own insulin production, ensuring that blood sugar levels are maintained within the target range.

Insulin works by binding to insulin receptors on the cell surface which initiates a cascade of intracellular chemical reactions. This process is called insulin signalling and it leads to the activation of specific intracellular molecules, called IRS1 (Insulin receptor substrate 1) and PI3K (phosphatidylinositol 3-kinase) which eventually activate downstream molecules such as GLUT4 (glucose transporter type 4) to be translocated to the cell membrane (Saltiel, 2021). This action results in an increase in glucose uptake by muscle and fat cells, and a decrease in glucose production by the liver.

There are several different forms of insulin therapy available, each with their own unique mechanisms of action. Rapid-acting insulin, also known as bolus insulin, is typically

administered before meals, and is designed to work quickly to lower blood sugar levels. This type of insulin is usually given as an injection or a pump. Bolus insulin resembles the physiological insulin increases stimulated by food (Wallia & Molitch, 2014). In comparison to conventional insulin, the utilisation of rapid-acting insulin analogues has been demonstrated to result in a reduction of postprandial hyperglycaemia and a decrease in the incidence of late postprandial hypoglycaemia (Donner & Sarkar, 2015).

Long-acting insulin, also known as basal insulin, is designed to provide a steady, low-level insulin supply throughout the day. This type of insulin is usually given as an injection once or twice a day (Cunningham & Freeman, 2022). Long-acting insulin has a slower onset and a longer duration of action than rapid-acting insulin and is primarily used to maintain consistent blood sugar levels.

Combination therapy, which involves using both rapid-acting and long-acting insulin, called Basal-Bolus regimen, is also an option for some individuals with T2D. This type of therapy is designed to mimic the body's natural insulin production and provide both a rapid and sustained effect on blood sugar levels. Research suggests a Basal-Bolus regimen in T2D can be more efficient in glucose control for non-critically ill patients when compared to basal insulin alone (Umpierrez et al., 2007)

Sulphonylureas

Sulphonylureas work by stimulating the pancreas to produce more insulin. They do this by binding to a specific receptor on the beta cells of the pancreas called the ATP-dependent potassium (KATP) channel. When sulphonylureas bind to this receptor, they cause the KATP channel to open, which leads to an influx of potassium ions into the beta cells (Al-Saleh et al., 2021). This causes the beta cells to depolarize and release more insulin into the bloodstream.

In addition to increasing insulin secretion, sulphonylureas also increase insulin sensitivity in peripheral tissues by increasing the number and sensitivity of insulin receptors (Blumenthal, 1977; J.-K. Park, Kim, & Song, 2008). This helps to improve glucose uptake and utilisation in these tissues, leading to lower blood sugar levels.

Sulphonylureas have been used for many years in the treatment of type 2 diabetes, as they are relatively inexpensive and have been proven to be effective in lowering blood sugar levels

(Tomlinson, Patil, Fok, Chan, & Lam, 2022). sulfonylureas and glinides are medications used in the treatment of type 2 diabetes mellitus (T2D) that have multiple hypoglycaemic effects. In addition to their role in stimulating the release of insulin from pancreatic cells, these drugs exhibit various actions that contribute to lowering blood sugar levels. Among their hypoglycaemic effects, sulfonylureas and glinides are known to decrease the clearance rate of insulin in the liver. This results in increased levels of insulin in the bloodstream, facilitating the uptake of glucose into cells and aiding in glucose regulation. These medications have the ability to reduce the secretion of glucagon, a hormone that typically raises blood sugar levels. By inhibiting glucagon release, sulfonylureas and glinides help to counteract the excessive release of glucose from the liver, contributing to better blood sugar control. Sulfonylureas and glinides can enhance the sensitivity of peripheral tissues to insulin. This means that the body's cells become more responsive to the effects of insulin, allowing for improved uptake and utilisation of glucose from the bloodstream (Lv, Wang, Xu, & Lu, 2020).

While sulphonylureas are generally well tolerated, they have some side effects, such as the risk of hypoglycaemia and weight gain. Sulfonylureas act directly on β-cells, leading to progressive dysfunction and worsening of insulin secretion (Sola et al., 2015). Thus, despite better glycaemic control in the short term, diabetes could worsen in the long term. Research among type 2 diabetes mellitus patients treated with sulfonylurea-based regimens suggested glycaemic levels were relatively well controlled, but hypoglycaemia remained a prevalent side effect (Y. S. Kim et al., 2019). Alongside this, sulphonylureas could add to the risk of fractures among the old with type 2 diabetes (Tao, E, Shi, & Zhang, 2021). Sulphonylureas are generally considered as second-line therapy after metformin and DPP-4 inhibitors.

Alpha-Glucosidase Inhibitors

Alpha-glucosidase inhibitors (AGIs) are a class of drugs used to treat type 2 diabetes by slowing the absorption of carbohydrates from the gut. When carbohydrates are eaten, they are broken down into simpler sugars, such as glucose, in the small intestine. Alpha-glucosidase is an enzyme that is responsible for breaking down complex carbohydrates into simpler sugars, so they can be absorbed into the bloodstream (Kalra, 2014). AGIs function by inhibiting the activity of the enzyme alpha-glucosidase. They achieve this by binding to the active site of the enzyme, preventing it from breaking down carbohydrates. As a result, the breakdown of carbohydrates is slowed down, leading to a reduction in the amount of glucose absorbed into the bloodstream. The delayed absorption of carbohydrates through the action of AGIs leads to

a decrease in postprandial (after-meal) blood glucose concentrations. On average, AGIs have been observed to lower postprandial blood glucose levels by approximately 3 mmol/l. This effect helps to regulate blood glucose levels and can be beneficial for individuals with conditions such as diabetes (Akmal & Wadhwa, 2022).

AGIs are typically taken before a meal and can be used in combination with other diabetes medications, such as metformin or sulfonylureas, to achieve optimal blood sugar control. AGIs have been found to be relatively safe and well-tolerated, with the most common side effects including diarrhoea, flatulence, and abdominal discomfort (Van de Laar, 2008). However, these side effects are usually mild and short-lived.

The use of α-glucosidase inhibitors offers several notable benefits in managing type 2 diabetes mellitus, especially in addressing postprandial hyperglycaemia (high blood glucose levels after meals). This is particularly significant in individuals who are newly diagnosed with type 2 diabetes and experience excessive postprandial glucose (PPG) levels.

AGIs exert their effect by controlling the release of glucose from complex carbohydrates and disaccharides, thereby helping to regulate blood glucose levels. They have demonstrated effectiveness and safety as both monotherapy and as an adjunct to other anti-diabetic medications. In cases where diet and exercise alone are insufficient in managing newly diagnosed type 2 diabetes, AGIs can be used as a first-line treatment option.

Furthermore, AGIs can be combined with various oral anti-diabetic drugs and insulin if monotherapy with these medications fails to achieve the desired targets for glycated haemoglobin (HbA1c) and PPG. This versatility and compatibility make AGIs a valuable option in the overall management of type 2 diabetes, providing an additional tool for healthcare professionals to tailor treatment plans and optimize glycaemic control for their patients (Derosa & Maffioli, 2012).

α-glucosidase inhibitors can be used in combination with other anti-diabetic drugs such as sulfonylureas, insulin, or metformin. In fact, they are recommended to be used in combination with other drugs if monotherapy with these drugs fails to achieve the targets for HbA1c and PPG. The potential benefits of using α -glucosidase inhibitors in combination with other anti-diabetic drugs include improved glycaemic control and reduced intraday and interday glucose

variability compared to other anti-diabetic drugs (Derosa & Maffioli, 2012). There may be an increased risk of hypoglycaemia when used in combination with sulfonylureas or insulin. However, this risk can be minimized by adjusting the dosage of these drugs accordingly. A review (SS Patel, 2016) suggests that α-glucosidase inhibitors could potentially be beneficial in lowering the risk of cerebrovascular events in individuals with diabetes. The study focuses on investigating the evidence indicating the significant involvement of postprandial hyperglycaemia in causing vascular damage, as well as the intricate interplay between hyperglycaemia and concurrent risk factors. The review suggests that α-glucosidase inhibitors, a class of medications that inhibit the action of certain enzymes involved in carbohydrate digestion, may have a positive impact on reducing the occurrence of cerebrovascular events. This is attributed to their ability to regulate postprandial hyperglycaemia, which refers to the elevated blood sugar levels after meals. By slowing down carbohydrate digestion and absorption, α-glucosidase inhibitors help to reduce postprandial hyperglycaemia and therefore may help to prevent vascular damage. Additionally, α-glucosidase inhibitors have been shown to have other beneficial effects on the cardiovascular system, such as improving lipid profiles and reducing blood pressure (SS Patel, 2016).

These studies suggest that alpha-glucosidase inhibitors help with type 2 diabetes by controlling hyperglycaemia (Derosa & Maffioli, 2012) and reducing the risk of cardiovascular events (SS Patel, 2016).

Thiazolidinediones

Thiazolidinediones (TZDs) are a class of compounds that enhance insulin sensitivity by targeting intracellular metabolic pathways, thereby improving insulin action in key organs (Yamanouchi, 2010). Additionally, TZDs have additional effects on metabolic processes. They increase the levels of adiponectin, a cytokine released by adipose tissue, which in turn enhances insulin sensitivity. TZD treatment also decreases hepatic gluconeogenesis, the process by which the liver produces glucose, leading to a reduction in blood glucose levels. Moreover, TZDs promote insulin-dependent glucose absorption in lean muscle and fat tissues. Additionally, these medications stimulate fatty acid oxidation, which contributes to the utilisation of stored fats for energy. These combined effects of TZDs play a role in improving insulin sensitivity and glucose metabolism in individuals with conditions such as type 2 diabetes (Gor et al., 2020).

TZDs exert their effects by modulating gene expression through their binding to peroxisome proliferator-activated receptor-gamma (PPAR-gamma). PPAR-gamma is a type of transcription factor that belongs to the family of hormone receptors responsible for regulating energy balance within the body. By binding to PPAR-gamma, TZDs activate this transcription factor, leading to the regulation of various genes involved in maintaining energy homeostasis. These genes are responsible for controlling processes related to glucose and lipid metabolism, insulin sensitivity, and the differentiation of adipocytes. (Vieira et al., 2019). The genes activated by the PPAR-gamma subtype are present in muscle, fat, and liver, regulating glucose metabolism, fatty acid storage, and adipocyte differentiation (Tyagi, Gupta, Saini, Kaushal, & Sharma, 2011). By raising adiponectin and glucose transporter type 4 (GLUT4) expression and reducing TNF-alpha's impact on adipocytes, TZDs reduce insulin resistance. Adipocytes and skeletal muscle cells' increased GLUT4 expression will boost their capacity to absorb glucose in response to insulin (Eggleton & Jialal, 2021).

Dopamine agonists

Dopamine receptor agonists, initially utilized for the treatment of prolactinomas and pituitary tumours, have garnered attention as a therapeutic option for type 2 diabetes (T2D) following the approval of bromocriptine in 2009 as a T2D treatment (Lamos, Levitt, & Munir, 2016). Dopamine agonists, being a class of drugs that imitate the actions of dopamine, a neurotransmitter involved in several physiological processes including movement, mood, and cognition (Choi & Horner, 2019) are believed to exert their effects in T2D through multiple pathways.

One of the principal mechanisms involves activation of dopamine receptors in the hypothalamus, regulating the secretion of insulin and glucagon, hormones responsible for controlling blood glucose levels (Vicchi et al., 2016). The sensitivity of insulin receptors is also believed to be increased by dopamine agonists, resulting in improved glucose uptake and decreased blood glucose levels (García-Tornadu et al., 2010).

Another mechanism through which dopamine agonists may offer benefit in T2D is by reducing inflammation and oxidative stress (Cincotta et al., 2022), which are considered significant drivers of insulin resistance and T2D This reduction is achieved through activation of anti-inflammatory pathways, such as the nuclear factor-kappa B (NF-kB) pathway (Nolan et al., 2020), and by increasing antioxidant production.

SGLT2 Inhibitors

SGLT2 inhibitors are a class of medications designed for the treatment of type 2 diabetes. They function by blocking the activity of a protein called sodium-glucose cotransporter 2 (SGLT2), which is responsible for the reabsorption of glucose from the renal tubules in the kidneys. Through the inhibition of SGLT2, these drugs decrease the amount of glucose that is reabsorbed into the bloodstream, resulting in an elevation of glucose excretion in the urine (Plosker, 2014). This mechanism of action is unique compared to other diabetes medications, as it targets glucose reabsorption in the kidney rather than insulin production or insulin sensitivity in the body.

SGLT2 inhibitors also decrease insulin resistance (Hosokawa & Ogawa, 2020), which is a common problem in patients with type 2 diabetes. This is because glucose is excreted in the urine, so the body's insulin requirements decrease, which leads to an improvement in insulin sensitivity.

SGLT2 inhibitors also have an effect on appetite and body weight. They have been shown to decrease appetite and lead to weight loss (Tahara, Kondo, Takasu, & Tomiyama, 2018), which is beneficial for patients with type 2 diabetes who are overweight or obese.

In addition to these benefits, SGLT2 inhibitors have also been shown to have positive effects on cardiovascular health. Studies have shown that these drugs can decrease the risk of cardiovascular disease and heart failure in patients with type 2 diabetes. This is likely due to the decrease in blood pressure and weight loss that occurs with SGLT2 inhibitors due to the downregulation of sympathetic activity which reduces the heart’s preload and afterload (Lytvyn, Bjornstad, Udell, Lovshin, & Cherney, 2017).

Incretin Mimetics

Incretin mimetics are a class of drugs used to treat type 2 diabetes, and the incretin system has become an important target in the treatment of T2D in recent years (Baggio & Drucker, 2007). They work by imitating the effects of incretins, hormones produced by the gut that stimulate insulin secretion and reduce glucagon secretion in response to food intake (Hansen, Vilsbøll, & Knop, 2010).

The regulation of blood glucose levels is significantly influenced by incretins. In people with type 2 diabetes, the incretin system is compromised, resulting in inadequate insulin release and increased glucagon secretion, leading to elevated blood sugar levels. Two primary classes of incretin mimetics exist: glucagon-like peptide-1 (GLP-1) receptor agonists and dipeptidyl peptidase-4 (DPP-4) inhibitors. GLP-1 receptor agonists replicate the functions of the GLP-1 hormone, which stimulates insulin secretion and reduces glucagon secretion (Hinnen, 2017). DPP-4 inhibitors prevent the breakdown of naturally occurring incretins, prolonging their effects on insulin and glucagon secretion (Godinho et al., 2015). GLP-1 is of particular interest for its glucose-lowering effects (Meier & Nauck, 2004), as well as its ability to slow gastric emptying and suppress secretion of glucagon (Baggio & Drucker, 2007).

The incretin effect refers to the stimulation of insulin secretion in response to meals that occurs as a result of increased release of incretin hormones from the gut. Incretins, such as GLP-1 and GIP, enhance insulin secretion in a glucose-dependent manner and suppress glucagon secretion (Hinnen, 2017). This results in increased insulin secretion and decreased glucose production by the liver, leading to improved glucose control and reduced blood glucose levels after meals. The incretin effect is impaired in individuals with type 2 diabetes (Knop et al., 2007), contributing to their high blood glucose levels.

Incretin mimetics are used as a second-line therapy for type 2 diabetes, after lifestyle changes and metformin have failed to control blood glucose levels adequately. They are usually administered as injections or pills and have been shown to improve glucose control, lower HbA1c levels, and promote weight loss (Cernea, 2011).

Inhibitors of Dipeptidyl Peptidase 4 (DPP-4 Inhibitors)

DPP4 inhibitors, also referred to as Gliptins, are a group of drugs utilized for managing type 2 diabetes by lowering elevated blood sugar levels. Their mechanism of action involves targeting the activity of an enzyme called dipeptidyl peptidase-4 (DPP4). This enzyme is involved in the breakdown of incretin hormones, particularly glucose-dependent insulinotropic polypeptide (GIP) and Glucagon-like peptide-1 (GLP-1). By inhibiting DPP4, these medications enhance the effects of GIP and GLP-1, leading to increased insulin secretion from the pancreas in a glucose-dependent manner. This ultimately helps regulate blood sugar levels in individuals with type 2 diabetes (Gautier, Fetita, Sobngwi, & Salaün-Martin, 2005). They stimulate insulin

secretion and inhibit glucagon secretion, leading to a decrease in blood glucose levels. DPP-4 is an enzyme which acts upon these hormones naturally, degrading the hormone immediately due to their short half-life. DPP-4 inhibitors work by blocking the DPP-4 enzyme, which leads to an increase in the levels of GLP-1 and GIP. This in turn causes an increase in insulin secretion by beta cells in the pancreas, ultimately reducing high blood sugar levels after eating and when fasting (Capuano et al., 2013).

DPP-4 inhibitors have been shown to have several benefits in the treatment of type 2 diabetes. They have been shown to improve glycaemic control, lower HbA1c levels, and reduce the risk of hypoglycaemia (Gallwitz, 2019). They also have been shown to have a neutral effect on body weight (Foley & Jordan, 2010). In addition, they have been shown to have a beneficial effect on cardiovascular risk factors, such as reducing blood pressure and lipid levels (Papagianni & Tziomalos, 2015).

GLP-1 Receptor Agonists

Glucagon-like peptide-1 receptor agonists (GLP-1 RAs) are a class of medications that play a crucial role in the management of type 2 diabetes. GLP-1 RAs mimic the effects of the naturally occurring hormone GLP-1.

Glucagon-like peptide-1 (GLP-1)

Glucagon-like peptide-1 (GLP-1) hormone is a critical regulatory hormone released by intestinal L cells in response to nutrient ingestion, especially carbohydrates and fats (D. J. Drucker & Nauck, 2006). It is a member of the glucagon family of hormones and is involved in regulating glucose metabolism and energy balance. The hormone's release is regulated by various factors, such as nutrients, gastrointestinal hormones, and neurotransmitters. The presence of food in the small intestine enhances GLP-1 hormone release, proportional to the caloric content of the meal. Neural signals, such as the parasympathetic nervous system, also enhance its release. GLP-1 plays a significant role in the management of type 2 diabetes (figure 1.9) and has been the subject of much research in recent years.

GLP-1 is an incretin hormone, which means it stimulates the release of insulin from pancreatic beta-cells in response to nutrient ingestion. GLP-1 hormone also suppresses glucagon secretion from pancreatic alpha-cells, reduces gastric emptying, and enhances satiety (Drucker, 2013). This results in improved glucose control and a decrease in hyperglycaemia. The incretin effect

of GLP-1 hormone is responsible for up to 70% of the insulin response to oral glucose intake. This incretin effect is reduced or absent in patients with type 2 diabetes, leading to impaired insulin secretion and glucose intolerance.

Figure 1.9 – GLP-1 target organs and its action (Kalra et al., 2019).

GLP-1 also slows gastric emptying, which increases satiety and decreases food intake (Deane et al., 2010). GLP-1 is released in response to food intake (figure 1.10), as shown in research over 24 hours across three different meals (Ørskov et al., 1996). The postprandial response of glucagon-like peptide 1 (GLP-1) is linked to the activation of certain brain regions involved in the regulation of appetite and food intake. When there is a peak increase in plasma GLP-1 concentrations after a meal, it is associated with corresponding increases in regional cerebral blood flow in specific areas of the brain. These areas include the left dorsolateral prefrontal cortex and the hypothalamus, which play crucial roles in appetite control and the regulation of food intake. This correlation suggests that the release of GLP-1 during the postprandial period may contribute to the modulation of appetite and the brain's response to food stimuli (Pannacciulli et al., 2007). These brain regions have previously been implicated in satiety and food intake regulation (Gautier et al., 2001; Le et al., 2006).

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Figure 1.10 – GLP-1 release in response to food intake (Ørskov, Wettergren, & Holst, 1996).

The dual effect on insulin and glucagon secretion, as well as gastric emptying, make GLP-1 a powerful tool in managing type 2 diabetes and other conditions related to glucose metabolism and energy balance.

The GLP-1 hormone has also been shown to have cardiovascular benefits, including a reduction in blood pressure, improvement in endothelial function, and a decrease in oxidative stress (J. Li et al., 2017). It also has a positive effect on lipid metabolism, including a reduction in plasma triglycerides and an increase in HDL cholesterol.

The half-life of endogenous GLP-1 hormone is short, typically less than two minutes (Seino, Fukushima, & Yabe, 2010). The rapid degradation of GLP-1 hormone is due to the action of the enzyme dipeptidyl peptidase-4 (DPP-4), which cleaves the GLP-1 peptide chain into inactive metabolites (Deacon, 2019). This rapid degradation of GLP-1 hormone limits its therapeutic potential in the treatment of diabetes, as it requires frequent dosing to maintain its biological activity. This frequent dosing is usually obtained through treatment with GLP-1 receptor agonists.

Current GLP-1 Receptor Agonists

Several GLP-1 receptor agonists (GLP-1 RAs) are approved for use in the treatment of type 2 diabetes: exenatide twice daily, exenatide once weekly, lixisenatide, dulaglutide, semaglutide and liraglutide (Madsbad, 2016). One other GLP-1 RA, albiglutide, has been previously approved but then discontinued for economic reasons. GLP-1 RAs have a proven efficacy for lowering glycated haemoglobin and decreasing weight in T2D, with reduced risk of hypoglycaemia compared with insulin or sulphonylureas (Inzucchi et al., 2015). The GLP-1RA advantage of not being associated with weight gain and very often leading to weight loss is of particular relevance given the frequency of obesity among type 2 patients (Home et al., 2014). The available GLP-1 RAs can be broken down into two categories: short-acting and long-acting compounds. Short-acting compounds include exenatide twice daily and lixisenatide. Long-acting compounds include once daily liraglutide, once weekly exenatide, dulaglutide, and semaglutide (Nauck, Quast, Wefers, & Meier, 2021).

Exenatide

Exenatide can be administered via subcutaneous injection twice daily or once weekly, having an elimination half-life of 3.3-4 hours (Nauck et al., 2021). It has been approved for use twice daily since 2005 in the USA, and 2006 in Europe (Kolterman et al., 2005). The approval for once weekly Exenatide came in 2012. Exenatide boosts glucose-dependent insulin secretion, slows gastric emptying, curbs food intake, and suppresses glucagon secretion by binding to pancreatic GLP-1 receptors (Bridges, Bistas, & Jacobs, 2021).

Compared to other diabetic treatments, exenatide has a lower risk of causing hypoglycaemia due to glucose-dependent insulin release (Chakraborti, 2010). Exenatide has been found to restore insulin response in type 2 diabetes patients and prolong insulin release in response to high glucose levels.

Exenatide treatment reduces glucagon release during hyperglycaemia, lowering hepatic glucose production and insulin need. Slowing gastric emptying slows glucose absorption into the bloodstream. Insulin release and glucagon suppression occur during both hyperglycaemic and euglycemic conditions. Exenatide alone reduces the chance of hypoglycaemia in diabetic patients. If used with other hypoglycaemia-causing drugs, the risk of hypoglycaemic episodes increases (Knop, Brønden, & Vilsbøll, 2017).

Lixisenatide

Lixisenatide is another short-acting compound, first approved in 2013 in Europe then in 2016 in the USA (Becker, Stechl, Steinstraesser, Golor, & Pellissier, 2015). It has a half-life of 2.6 hours and is administered once daily (Nauck et al., 2021). Lixisenatide, a GLP-1 RA, has been found to have several beneficial effects in the management of diabetes. It increases glucose-dependent insulin release, which helps regulate blood sugar levels. Additionally, lixisenatide decreases the secretion of glucagon, a hormone that raises blood sugar levels, and slows down gastric emptying, leading to a more controlled and gradual release of nutrients into the bloodstream. These combined actions contribute to improved glycaemic control in individuals with diabetes. Due to its 4-fold higher binding affinity for the GLP-1 receptor compared to native GLP-1, lixisenatide is able to be dosed once daily, despite its short half-life. This enhanced binding affinity allows for sustained activation of the GLP-1 receptor and prolonged therapeutic effects, making it an effective treatment option for individuals with diabetes. The ability of lixisenatide to slow gastric emptying allows for increased satiety, contributing to weight loss. Clinical trial results show that lixisenatide can reduce body weight by 1 to 5 kg (Shaefer Jr, 2016), and the GETGOAL-X study showed mean body weight reduced from baseline with lixisenatide from 94.5kg to 91.7kg (Rosenstock et al., 2013).

Dulaglutide

Dulaglutide is a long-acting compound, approved in 2014 (Geiser et al., 2016). It has a half-life of 4.7-5.5 days, and is administered once weekly (Nauck et al., 2021). The main mechanism of action of dulaglutide is to enhance insulin secretion in response to elevated glucose levels, reduce the secretion of glucagon, and slow down the emptying of the stomach. These effects work together to lower postprandial glucose levels. By increasing insulin secretion, dulaglutide helps to facilitate the uptake of glucose by cells for energy production and storage. Simultaneously, it decreases the release of glucagon, which helps to prevent excessive glucose production by the liver. Additionally, by slowing gastric emptying, dulaglutide can help to regulate the rate at which glucose is absorbed into the bloodstream, further contributing to the control of postprandial glucose levels. Overall, these actions of dulaglutide aim to improve glycaemic control and help individuals with conditions such as type 2 diabetes better manage their blood glucose levels after meals (Grunberger et al., 2012). Recent research has shown that dulaglutide outperforms alternative antidiabetic medications, exhibiting a reduction in HbA1c levels of close to or over 1% (Wysham et al., 2014). Dulaglutide stands out as the sole GLP-1 agonist that has undergone a successful head-to-head trial, demonstrating noninferiority when compared to liraglutide. (Farrokhi, Smiley, & Umpierrez, 2011). Dulaglutide has been shown

to have beneficial effects on body weight in patients with T2D. In a clinical trial, dulaglutide recipients experienced weight loss at 26 weeks, whereas patients in the insulin glargine group had a weight gain (Tuttle et al., 2018). These beneficial effects of dulaglutide on body weight were sustained at 52 weeks, and both dosages of dulaglutide were statistically significantly better than the insulin glargine group. Weight loss with dulaglutide were also seen incrementally at 1.5, 3.0 and 4.5 mg doses regardless of baseline BMI (Bonora et al., 2021). In addition to weight loss, dulaglutide has been shown to improve glycaemic control, reduce HbA1c levels, and lower fasting plasma glucose levels in patients with T2D. Dulaglutide also generally improved patient-reported outcomes (PROs), including total treatment satisfaction and decreased perceived hyperglycaemia at 26 and 52 weeks relative to metformin (M. Yu, Van Brunt, Varnado, & Boye, 2016). In the REWIND CV outcomes trial, dulaglutide was associated with a significant reduction in the risk of a major adverse cardiac event at a median of 5.4 years' follow-up (Gerstein et al., 2018).

Semaglutide

Semaglutide is another long-acting compound, approved in 2017 in the USA and 2019 in Europe (Marbury, Flint, Jacobsen, Derving Karsbøl, & Lasseter, 2017). It has a half-life of 5.7-6.7 days, and is administered once weekly (Nauck et al., 2021). An oral administered version of Semaglutide was approved in 2020, which is long-acting but administered once daily (Granhall et al., 2019). Semaglutide is a medication that has been shown to improve several health markers. After using it for 12 weeks, it has been seen to decrease HbA1c, systolic blood pressure, and body weight (Andreadis et al., 2018). This is due to the medication's ability to increase insulin production and reduce glucagon secretion, leading to lower fasting and post-meal glucose levels. Additionally, semaglutide has a positive impact on cardiovascular health by decreasing fasting triglycerides and VLDL cholesterol (Ahrén et al., 2018).

A systematic review and meta-analysis examined effectiveness of semaglutide in comparison to a placebo and other antidiabetic agents with respect to reducing HbA1c levels (Andreadis et al., 2018). The results indicated that semaglutide 0.5 mg and 1 mg were more effective than the placebo, resulting in reductions of HbA1c by 1.01% (95% CI 0.56 to 1.47) and 1.38% (95% CI 1.05 to 1.70), respectively. These findings remained consistent in sensitivity analyses that included only trials with low risk of bias and excluded a study using lower semaglutide doses (0.4 and 0.8 mg). Additionally, both semaglutide doses demonstrated significant reductions in HbA1c levels when compared to other antidiabetic agents, with semaglutide 0.5 mg showing a

weighted mean difference (WMD) of -0.63% (95% CI -0.95 to -0.31) and semaglutide 1 mg showing a WMD of -0.84% (95% CI -1.23 to -0.44). Notably, semaglutide 1 mg exhibited marginally better efficacy in reducing HbA1c compared to other GLP-1 receptor agonists.

Semaglutide consistently and significantly reduced body weight compared to comparators across the SUSTAIN trials, regardless of background medications or BMI subgroups. Subjects on semaglutide 0.5 mg lost 2.5 to 5.7 kg, and those on 1.0 mg lost 2.0 to 7.9 kg, while comparators showed weight gains or smaller losses. In most cases, semaglutide showed greater weight loss than comparators, with significant differences observed. Insulin glargine had a different pattern with weight increase independent of BMI (Ahrén et al., 2018).

Liraglutide

Liraglutide is a long-acting compound, approved in 2009 in Europe and 2010 in the USA (Damholt et al., 2006). It has a half-life of 12.6-14.3 hours, and is administered once weekly (Nauck et al., 2021). Liraglutide is a human GLP-1 analogue, with 97% amino acid homology to human GLP-1 (Knudsen et al., 2000). Liraglutide works by improving insulin production in the pancreas and controlling excess glucagon levels. Additionally, it slows down stomach emptying and boosts feelings of fullness through its impact on the hypothalamus. Liraglutide has a dose-related impact on weight loss, which is thought to result from its combined effects on the brain and digestive system. The GLP-1 receptor in the brain, particularly in the hypothalamus, plays a role in regulating appetite. Furthermore, the GLP-1 hormone produced in the gut and released by L cells can decrease food intake through signals sent to the brain via the vagal nerve and by slowing down stomach emptying and promoting feelings of fullness (Nuffer & Trujillo, 2015).

In accordance with the early Liraglutide trials involving rodents (Larsen, Fledelius, Knudsen, & Tang-Christensen, 2001), human trials looking at how liraglutide works showed that a single injection led to lower fasting and post-meal blood glucose levels, enhanced insulin secretion, improved beta cells' ability to respond to increasing glucose concentrations, and slower stomach emptying. Further clinical trials in the early stages of liraglutide's development consistently showed improvements in HbA1c levels (Chia & Egan, 2008). A large scale evaluation of data involved 6 randomised-controlled trials of liraglutide in patients with poorly controlled diabetes was performed, called the “Liraglutide Effect and Action Diabetes” LEAD program (Madsbad, 2009). The program highlighted 26-52 weeks of treatment with liraglutide

1.2mg or 1.8 mg daily results in significant HbA1c of 0.9-1.5% and 1.0-1.5%, of which the findings enabled liraglutide to be licensed for use in poorly controlled T2D.

Liraglutide also has positive effects on weight, and as such is a promising therapy for obesity. The LEAD program showed weight loss ranging from 0.2-3.2kg from 1.8mg therapy over 6 months. When dosage is increased to 3.0mg per day, as shown in the SCALE trial, weight loss increases in a dose-dependent manner (Pi-Sunyer et al., 2015). This showed that, at week 56, patients in the liraglutide group had lost a mean of 8.4±7.3 kg of body weight, and those in the placebo group had lost a mean of 2.8±6.5 kg (a difference of −5.6 kg). The mechanism by which liraglutide works to reduce body weight is well understood. Appetite is regulated in the hypothalamus. Here, the neurons pro-opiomelanocortin (POMC) and cocaine-and amphetamine-regulated transcript (CART) and neuropeptide Y (NPY) and agouti-related peptide (AgRP) in the arcuate nucleus regulate signals that controls appetite and food intake. Liraglutide accesses the hypothalamus directly, which was demonstrated in one study by dosing fluorescently labelled liraglutide to mice, and looking at its distribution in the brain (Secher et al., 2014). This showed liraglutide was clearly measurable in the mice, and neuronal activation saw a pattern after dosing which was consistent with increased satiety and fullness from POMC/CART neurons, and reduced hunger from NPY/AgRP neurons. Overall, liraglutide helps to induce and sustain weight loss in patients with obesity. Its efficacy is comparable to other available agents but it offers the unique benefit of improved glycaemic control (Mehta, Marso, & Neeland, 2017).

1.3 Obesity and Type 2 Diabetes Mellitus

Type 2 diabetes has been identified as a significant consequence of obesity (Al-Goblan, Al-Alfi, & Khan, 2014). This manifestation of diabetes arises from an impaired ability to utilize insulin effectively, which is a hormone responsible for maintaining proper glucose levels in the blood. Obesity has been widely recognised as a key risk factor for the development of type 2 diabetes, with a substantial proportion of individuals suffering from the condition exhibiting overweight or obesity (Apovian, Okemah, & O'Neil, 2019). Numerous studies have consistently demonstrated the correlation between obesogenic characteristics, such as high body mass index (BMI) and waist circumference, and the incidence of type 2 diabetes (S. Li et al., 2011). Excessive adiposity, particularly in the abdominal region, can impede the effective utilisation of insulin by the body (Hardy, Czech, & Corvera, 2012). In the context of obesity, the cells within the body develop insulin resistance, requiring increased insulin production to

maintain normal glucose levels. However, if the pancreas becomes unable to produce sufficient insulin to keep pace with the body's requirements over an extended period, hyperglycaemia, or elevated blood sugar levels, may occur (Taylor et al., 2021).

The excess fat in the body also leads to chronic inflammation which contributes to insulin resistance (Tack, Stienstra, Joosten, & Netea, 2012). Adipose tissue, commonly known as body fat, secretes signalling molecules called adipokines, which are involved in the regulation of glucose and lipid metabolism. When there is an excessive amount of adipose tissue present, these adipokines can contribute to chronic inflammation (J. Shi, Fan, Su, & Yang, 2019), which can further contribute to the development of insulin resistance.

However, not all individuals who have Type 2 Diabetes have obesity in terms of height and weight, and a BMI of less than 30 kg/m^2. The number of patients without obesity but with T2D is on the rise (Olaogun et al., 2020), with incidence of T2D without obesity being higher in older adults than younger adults (Cowie et al., 2009). The pathophysiology of type 2 diabetes (T2D) in older adults without obesity remains poorly understood. It is important to note that these individuals have a normal body mass index (BMI), indicating that risk factors other than obesity-related inflammation may play a significant role in the development of T2D in this population (Ellulu, Patimah, Khaza'ai, Rahmat, & Abed, 2017).

1.4 Summary

Obesity is a widespread and significant issue affecting people worldwide, and its consequences can have a profound impact on individuals' health and well-being. Both adults and children are affected by this problem. Notably, there has been a substantial increase in the prevalence of overweight and obesity among children and adolescents aged 5-19 over the years. In 1975, the prevalence was only 4%, but by 2016, it had surged to just over 18%. This alarming rise underscores the gravity of the obesity epidemic and the urgent need for effective measures to address and mitigate its consequences. Obesity is a major risk factor for non-communicable diseases such as: cardiovascular diseases, type 2 diabetes mellitus (T2D), musculoskeletal disorders, some forms of malignancies and infertility in both women and men. Therefore, finding a viable therapy which not only treats diseases but also helps weight loss is of paramount. Liraglutide is a derivative of human incretin glucagon-like peptide-1 (GLP-1) that stimulates insulin secretion. Liraglutide is licenced in USA, UK and EU for the management

of T2DM and obesity. This study will be the first study providing real-life data on the effects of 3mg liraglutide on anthropometric parameters and specific markers of metabolism.

1.5 Research hypothesis, aims and objectives

1.5.1 Research Hypothesis

The research hypothesis for this study is treatment with Liraglutide 3mg daily will yield beneficial effects on patients with overweight or obesity. It is anticipated that Liraglutide will have positive impacts on metabolic pathways in patients, even though the specific pathway remains undetermined at present.

1.5.2 Research Aims

In this study we will investigate the beneficial metabolic sequelae of Liraglutide in patients with obesity or overweight, including changes in vital signs, anthropometric characteristics (weight, body mass index and body composition), biochemical parameters, metabolomics, and miRNA molecules from blood tests.

1.5.3 Research objectives

To assess:

The first objective of this research was to assess changes in body composition that occur as a result of Liraglutide treatment. Body composition refers to the relative proportions of different tissues in the body, including fat mass, lean mass, and distribution of adipose tissue. Changes in chemical processes involving products of metabolism while on treatment. Assessing changes in body composition related to treatment is essential for several reasons. Firstly, it provides valuable insights into the effectiveness of the treatment in altering body composition, which is a critical aspect of overall health and well-being. Understanding how the treatment influences fat mass, lean mass, and adipose tissue distribution can help identify whether it leads to desirable changes such as reductions in unhealthy fat accumulation and preservation of lean muscle mass. Additionally, changes in body composition can serve as important markers of metabolic health. Excessive fat accumulation, especially in visceral areas, is associated with increased risks of various chronic diseases, including cardiovascular disease, type 2 diabetes, and certain cancers. Monitoring changes in body composition allows researchers and healthcare professionals to evaluate the treatment's impact on mitigating these risks and improving metabolic health.

The second objective was to assess changes in chemical processes involving products of metabolism while on treatment. This objective aims to investigate how the treatment influences the metabolism of various substances, including carbohydrates, lipids, proteins, and other metabolites. Assessing changes in chemical processes involving products of metabolism related to treatment is crucial for understanding the treatment's impact on metabolic pathways and overall metabolic health. Metabolism plays a fundamental role in energy production, nutrient utilisation, and maintenance of homeostasis in the body.

The third objective was to assess changes in biochemical parameters related to treatment. Biochemical parameters include various markers, such as hormone levels, enzyme activities, inflammation markers, and other relevant biochemical indicators. Changes in biochemical parameters can serve as indicators of treatment efficacy and safety. For instance, alterations in hormone levels may reflect improvements in hormonal regulation, which is particularly relevant in conditions such as diabetes or thyroid disorders. Similarly, changes in enzyme activities can signify improvements in metabolic function or organ health.

The fourth and final objective was to identify novel metabolic pathways for new drug target discovery. This aims to explore and uncover previously unrecognised metabolic pathways and their associated molecular components, with the goal of identifying new therapeutic targets for the development of innovative treatments. Identifying novel metabolic pathways for new drug target discovery is crucial for advancing the field of medicine and improving treatment options for various diseases. Metabolic pathways are intricate networks of biochemical reactions that regulate the flow and utilisation of metabolites within cells and tissues. Dysregulation of these pathways is often associated with the development and progression of diseases, including metabolic disorders, cancer, and neurodegenerative conditions.

Chapter 2 – Subjects, Materials and Methods

2.1 Clinical Study Methodology

The clinical trial was led by Dr. Georgios Dimitriadis at University Hospitals Coventry & Warwickshire (UHCW) NHS Trust. The UHCW Trust carried out all anthropometric data collection. Data analysis was conducted by Lewis Spencer and the team at the University of Derby.

2.1.1. Ethical Approval

The clinical study received ethical approval from the NHS Regional Ethics Committee. Every participant willingly provided written and informed consent, and the study adhered to the guidelines set forth by the 18th World Medical Assembly's recommendations for physicians conducting research on human subjects, as adopted in Helsinki in 1964 and subsequent revisions. Funding for the project was provided by Kingston University London.

2.1.2 Subjects for Clinical Study

For the clinical study, a cohort of 63 adults (9 male, 54 female) with obesity (BMI≥30 kg/m^2) were recruited prospectively from the weight management and Polycystic ovary syndrome (PCOS) clinics at University Hospitals Coventry & Warwickshire (UHCW) NHS Trust. Inclusion criteria for the study was based on 3mg Liraglutide clinical indication. Specifically: Adult participants [age ≥18 years old without upper age limit (to the discretion of the investigators)], Body mass index (BMI) ≥30 kg/m^2 without coexisting comorbidities or BMI ≥27Kg/m^2 with comorbidities like hypertension, hyperlipidaemia, prediabetes or obstructive sleep apnoea and were willing to comply with study requirements and able to give informed consent. Exclusion criteria included: Type 1 or Type 2 diabetes mellitus, a history of any medical, psychological or other condition, or use of any medications, including over-the-counter products, which, in the opinion of the investigators, would either interfere with the study or potentially cause harm to the volunteer. These included: previous gastrointestinal surgery that could affect the outcomes of treatment with Liraglutide, including Billroth-2, Roux-en-Y gastric bypass, vertical sleeve gastrectomy or adjustable gastric band or other variations of these procedures. Other exclusion criteria include: history of chronic or acute pancreatitis, known active hepatitis or active liver disease, symptomatic gallstones or kidney stones, acute cholecystitis or history of duodenal inflammatory diseases including Crohn's Disease and Celiac Disease, persistent anaemia, defined as haemoglobin<10 g/dl, chronic or acute renal impairment (eGFR <30 ml/min/1.73m^2), active systemic infection, active malignancy within the last 5 years, including any form of thyroid cancer (including sporadic or familial medullary thyroid cancer) or personal, or family history of Multiple Endocrine

Neoplasia type 2, active illicit substance abuse or alcoholism, medications affecting insulin sensitivity (oral steroids, metformin, thiazolidinediones, atypical antipsychotics, hormonal contraceptives, weight loss medication) at screening or 6 months previously or current pregnancy or breastfeeding at screening or 6 months previously, no access at home to a telephone or other factor likely to interfere with ability to participate reliably in the study, donated blood during the preceding 3 months or intention to do so before the end of the study, any other mental or physical condition which, in the opinion of the Investigator, made the subject a poor candidate for clinical trial participation, were on any GLP-1 analogues or having used within 6 months preceding treatment initiation, known or suspected allergy to liraglutide. After dropouts, 49 participants were included in statistical analysis (6 male, 43 female).

2.1.3 Objectives

The primary objective of this study was to assess the effect of 3mg Liraglutide taken once daily on weight loss in patients with overweight (BMI: $\geq 27 Kg/m^2$) or obesity (BMI: $\geq 30 Kg/m^2$).

The secondary objectives of this study were to assess the effect of Liraglutide on:

a) Body composition, biochemical and immunological parameters

b) Metabolomic changes and its associated molecular pathways

c) Differentially expressed genes and small noncoding RNA

2.1.4 Trial Design

This clinical trial was a prospective open label study. It took place at a tertiary obesity, metabolic medicine, and diabetes center. Patients were recruited from the weight management and PCOS clinics at UHCW NHS Trust and received treatment with 3mg Liraglutide once daily as part of their clinical management plan. In addition to this medication, patients also received standard NHS Tier 3 lifestyle advice and support for a period of six months. Lifestyle modification aimed at weight loss was delivered by a dietician or other trained healthcare professional in individual sessions for six months. The length of treatment with Liraglutide continued for as long as it was clinically required, and patients were followed for the study for a total of six months after treatment initiation. However, patients who did not achieve at least a 5% weight loss after four months of treatment based on the specific product characteristics for 3mg Liraglutide once daily were not offered further treatment and were withdrawn from

the study. Data collected up to this point was used for analysis. All patients had the option to withdraw from treatment and/or the study at any time without giving any explanation, and this would not impact their clinical management. Liraglutide was offered to patients as per clinical need and the decision to treat them was made before their potential participation in the study. Therefore, patients did not receive treatment due to their participation in the study.

2.1.4.1 Screening Visit

This procedure was performed after the research team made first contact with the participant as part of their planned clinical management. All participants had confirmed during this visit their agreement to be initiated on treatment with 3mg Liraglutide once daily as per clinical management and were asked to read again and discuss the Informed Consent Form (ICF) with the Principal Investigator (PI) or with the Sub-Investigator (SI) and then sign it. A copy of the ICF was given to each participant. They were then screened to assess whether they met the inclusion criteria, and this process comprised of a medical history, routine physical examination, and the investigations that were performed routinely as part of clinical management. These included Full blood count, urea and electrolytes, liver function tests, renal function tests, thyroid function tests, HbA1c, lipid profile and fasting plasma glucose.

2.1.4.1 Baseline Visit

This visit took place at a date and time that was predefined by the patient and study team. Patients were asked to attend the WISDEM centre for this visit after having fasted from 10pm the night before and only consuming water from that time forward. They attended the WISDEM centre clinical trials unit at UHCW NHS Trust. The baseline visit involved performing 2 urine pregnancy tests for female patients of child-bearing potential to exclude pregnancy, followed by a blood test. This involved collecting 1 purple top vial (EDTA) to be spun, aliquoted, and stored in a -80°C freezer and 2 yellow top vials (Gel); one to be spun, aliquoted, and stored in a -80°C freezer, and another sent to UHCW pathology labs for analysis.

Patients had their weight, height, blood pressure, and pulse measured using WISDEM Tier 3/4 obesity service equipment, which was the same equipment used for clinical purposes. BMI was calculated from the participants' weight and height measurements using the NHS BMI Calculator website. This was followed by an assessment of body composition analysis using the BodPod.

There was then a discussion with an investigator regarding the use of 3mg Liraglutide once daily, which involved education on the use of the pen and lifestyle advice. Patients were also informed about any potential side effects from the use of their medication and how to effectively manage them. They were given the opportunity to use tester pens until they felt comfortable with their use. Finally, they were given all the necessary consumables required to use the Liraglutide 3mg once daily pen and a prescription to collect their medication from a pharmacy of their choice.

2.1.4.2 2-month Visit

This involved the same procedures as the baseline visit but additionally, investigators reviewed the patients' injecting technique and commented on their weight loss progress on 3mg Liraglutide once daily and lifestyle changes. They were encouraged to make the clinically necessary changes to achieve their goals.

2.1.4.3 4-month Visit

This involved the same procedures as previous visits. However, patients who did not achieve at least a 5% weight loss from baseline were withdrawn from treatment and the study as per the licencing indications for 3mg Liraglutide once daily for overweight or obesity. Successful patients continued with treatment and the study if they were still interested and willing.

2.1.4.4 6-month Visit

This involved the same procedures as previous visits. However, at this point, the patients completed the study and were thereafter followed in an outpatient clinic.

2.1.4.5 Body Composition Analysis

At baseline visit and every other follow up visit, participants had the body composition analysed by using the BodPod. The technique of body composition analysis from a BodPod is based on air displacement plethysmography (Cosmed Inc., USA) (Fields, Goran, & McCrory, 2002) . The BodPod is a non-invasive and highly accurate means of assessing body fat mass, body fat percentage and lean body mass using air displacement. Assessment of body composition using the BodPod took approximately 1 minute.

2.2 ChubS-7 Preadipocytes Cell Culture

ChubS7 is a preadipocyte cell line, which was purchased from the Nestlé Research Centre (Darimont et al., 2003). The composition of cell culture media and the method for ChubS7 pre adipocytes was described in previous studies by our team (Alhusaini et al., 2010). Briefly, preadipocytes were cultured into T75 cell culture flasks until confluent, and then trypsinised to obtain cells to carry out the study. The preadipocytes from the same passage were grown in 6-well plates (104 cells/well in 2 ml media) to confluence in DMEM/Ham's F-12 phenol-free medium (Invitrogen, UK) containing 10% FCS, penicillin (100 U/ml), streptomycin (100 µg/ml), and transferrin (5 µg/ml). At confluence, preadipocytes were differentiated in differentiation media (Promocell, Germany) containing biotin (8 µg/ml), insulin (500 ng/ml), Dexamethasone (400 ng/ml), IBMX (44 µg/ml), L-Thyroxin (9 ng/ml) and Ciglitazone (3 µg/ml) for 48 h. After this period, the cells were grown in nutrition media containing DMEM/Ham's F-12, 3% FCS, D-biotin (8 µg/ml), insulin (500 ng/ml) and Dexamethasone (400 ng/ml) until fully differentiated (10–14 days). Differentiated adipocytes (day 14) 24 h prior to treatments were switched to detoxification media (DMEM/Ham's F-12 phenol-free medium containing only 2% serum) to remove effects of differentiation components such as insulin and dexamethasone in nutrition media. The treatments were then placed in the fresh detoxification media. The cells were treated with 100 nm Liraglutide (dissolved in DMSO) while the control group was treated with the same volume of DMSO for 24 hour. For protein analysis cells were washed with cold PBS and harvested in lysis buffer (20 mM Tris–HCl, pH 7.5; 137 mM NaCl; 1 mM EGTA, pH 8; 1% Triton X-100; 10% glycerol; 1.5 mM MgCl2) containing protease and phosphatase inhibitors (10 mM NaF; 1 mM PMSF; 1 mM sodium metavanadate; 5 µg/ml aprotinin; 10 µg/ml leupeptin) (Sigma–Aldrich, UK) and stored at −80°C until required as described in Alhusaini et al. (2010).

2.3 RNA extraction and real-time polymerase chain reaction

Total RNA was extracted from adipocytes (RNeasy Lipid Tissue Mini Kit, Qiagen), according to manufacturers' instruction. RNA was quantified using a spectrophotometer (Nanodrop ND-1000, Labtech, UK), measuring at an absorbance of 260 nm. The ratios between absorbances 260/280 nm and 260/230 nm were measured to give an estimate of RNA purity. A value between 1.8 and 2.1 for both ratios was accepted as suitable RNA purity for use. Due to technical difficulties, RNA purity and integrity was additionally measured using a benchtop fluorometer (Qubit 4, Invitrogen, UK) with Qubit RNA High Sensitivity Assay Kit (#Q32852). RNA was stored at -80°C until use.

Complimentary DNA (cDNA) was synthesised using a High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems by ThermoFisher Scientific, # 4368814) was used. After allowing kit components to thaw on ice, RT master mix was made with 2 µL of 10X RT buffer, 0.8 µL 25X dNTP mix (100mM), 2 µL 10X Random Primers, 1 µL Multiscribe Reverse Transcriptase, 1 µL RNase Inhibitor, 3.2 µL Nuclease free water to give a total volume of 10 µL per reaction, with additional reactions factored into calculations to allow for volume loss. This was then gently mixed and placed on ice until needed. To prepare the cDNA RT reactions, 100ng of RNA, diluted with water to 10 µL total, based on spectrophotometer quantification, was used to make cDNA. RNA was pipetted into a sterile microcentrifuge tube (200 µL capacity). Samples were then vortexed and centrifuged briefly. Samples were then mixed with 10 µL reverse transcription mastermix, giving a final volume of 20 µL. Each sample was mixed thoroughly, briefly centrifuged, and incubated at room temperature for 5 minutes and then transferred to a thermocycler. Samples were heated to 25°C for 10 minutes, 37°C for 120 minutes, 85°C for 5 minutes and then held at 4°C until removed from the thermocycler. The final product was stored at -20°C until use.

Quantitative real-time PCR (qRT-PCR) was performed using an ABI 7500 standard Sequence Detection System (Applied Biosystems, UK). Reactions were carried out at 50 C for 2 minutes, 95 C for 10 minutes, and then 40 cycles of 95 C for 15 seconds then 60 C for 1 min. Reactions were prepared to 10 µL volumes in a 96 well plate. Pre-designed gene specific Taqman probes and primers were used in a reaction mix containing TaqMan universal PCR master mix (Applied Biosystems, UK). The list of Taqman probes and primers are as follows (Table 2.1): Insulin receptor substrate 1 (IRS1): Hs00178563_m1; Serine Hydroxymethyltransferase 1 (SHMT1_: Hs00541038_m1; Sphingosine Kinase 2 (SPHK2): Hs01016543_g1; Sphingosine-1-Phosphate Phosphatase 1 (SGPP1): Hs04189357_m1; Cytosolic phospholipase A2 group IV (PLA2G4A): Hs00233352_m1; Rapidly accelerated fibrosarcoma (RAF1): Hs00234119_m1; Phosphoinositide-dependent kinase-1 (PDK1): Hs01561847_m1; Protein Tyrosine Phosphatase Non-Receptor Type 3 (PTPN3): Hs00985058_m1; Cytosolic phospholipase A2 group II (PLA2G2A): Hs00179898_m1; Sphingosine-1-phosphate receptor 1 (S1PR1): Hs05018096_m1; Zinc cluster protein (ZNF1): Hs00932789_m1; Patatin-like phospholipase domain-containing protein 3 (PNPLA3): Hs00228747_m1; Phospholipase C Delta 1 (PLCD1): Hs00979908_m1; Sphingosine Kinase 1 (SPHK1): Hs00184211_m1; Serine/threonine-protein kinase 1 (AKT1): Hs00178289_m1; Insulin receptor (INSR): Hs00961557_m1; Mitogen-

activated protein kinase kinase 1 (MAP2K1): Hs00605615_mH; Glyceraldehyde 3-phosphate dehydrogenase (GAPDH): Hs99999905_m1; Cytosolic phospholipase A2 group VI (PLA2G6): Hs00185926_m1; Cytosolic phospholipase A2 group VII (PLA2G7): Hs00173726_m1; Insulin like growth factor1 receptor (IGF1R): Hs00609566_m1 and Ceramide synthase 1 (CERS1): Hs00193796_m1.

All reactions were multiplexed with the housekeeping gene glyceraldehyde 3-phosphate dehydrogenase (GAPDH), provided as a pre-optimised control probe (Applied Biosystems, UK) enabling calculation of delta threshold cycle (ΔCt) values (where ΔCt = Ct of GAPDH subtracted from Ct of gene of interest). Data is expressed as 2 raised to the power of the difference between control ΔCt and test ΔCt (ΔΔCt method). To exclude potential bias due to averaging, data was transformed through the Power equation 2^DDCt.

Table 2.1: Primers and Taqman Probe Details for genes used in qRT-PCR analysis.

Gene Symbol	**Gene Name**	**Taqman Probe ID**
IRS1	Insulin receptor substrate 1	Hs00178563_m1
SHMT1	Serine Hydroxymethyltransferase 1	Hs00541038_m1
SPHK2	Sphingosine Kinase 2	Hs01016543_g1
SGPP1	Sphingosine-1-Phosphate Phosphatase 1	Hs04189357_m1
PLA2G4A	Cytosolic phospholipase A2 group IV	Hs00233352_m1
RAF1	Rapidly accelerated fibrosarcoma	Hs00234119_m1
PDK1	Phosphoinositide-dependent kinase-1	Hs01561847_m1
PTPN3	Protein Tyrosine Phosphatase Non-Receptor Type 3	Hs00985058_m1
PLA2G2A	Cytosolic phospholipase A2 group II	Hs00179898_m1
S1PR1	Sphingosine-1-phosphate receptor 1	Hs05018096_m1
ZNF1	Zinc cluster protein	Hs00932789_m1
PNPLA3	Patatin-like phospholipase domain-containing protein 3	Hs00228747_m1
PLCD1	Phospholipase C Delta 1	Hs00979908_m1
SPHK1	Sphingosine Kinase 1	Hs00184211_m1
AKT1	Serine/threonine-protein kinase 1	Hs00178289_m1
INSR	Insulin receptor	Hs00961557_m1
MAP2K1	Mitogen-activated protein kinase kinase 1	Hs00605615_m1
GAPDH	Glyceraldehyde 3-phosphate dehydrogenase	Hs99999905_m1
PLA2G6	Cytosolic phospholipase A2 group VI	Hs00185926_m1
PLA2G7	Cytosolic phospholipase A2 group VII	Hs00173726_m1
IGF1R	Insulin like growth factor1 receptor	Hs00609566_m1
CERS1	Ceramide synthase 1	Hs00193796_m1

2.4 Assays

2.4.1 Bicinchoninic Acid (BCA) Assay (Protein Quantification)

The BCA (bicinchoninic acid) assay (Thermo Scientific, #23225) is a widely used method for quantifying protein concentrations in solution. The assay relied on the reduction of Cu2+ ions to Cu+ by proteins in the presence of bicinchoninic acid, resulting in the formation of a purple-coloured complex. The intensity of the colour was proportional to the amount of protein present in the sample. The samples used in the assay were Liraglutide 100nM and a control sample with no Liraglutide. To start the procedure, samples were homogenised and kept on ice until needed. 120µl of copper (II) sulphate was added to 6ml of BCA reagent. In a 96 well plate, 0, 2, 4, 6, 8 and 10 µl of bovine serum albumin (BSA) protein standard (1mg/ml) were pipetted in triplicates, corresponding to 0, 0.2, 0.4, 0.6, 0.8 and 1.0mg/ml protein, respectively. The protein standard was made up to 10µl with diluent as appropriate. Cell lysate was prepared in ultrapure water a 1:5 dilution. The cell lysate samples of water and Liraglutide treated samples or water and non-treated samples (10µl) were pipetted to independent wells in the plate, also in triplicates. Then, 200µl of BCA-copper sulphate solution was added to all wells. The plate was mixed thoroughly and left to incubate at 37°C for 30 minutes. Absorbance was read at 562nm from a spectrophotometer. The absolute protein standard concentration was calculated by deducting the blank reading from all readings. A protein standard curve (0-1mg/ml BSA) was produced and the concentration of the extracted protein from the sample was extrapolated.

2.4.2 Phosphatidylethanolamine (PE) Assay

The quantitative measurement of PE was determined using a competitive ELISA kit from Sigma-Aldrich (MAK361). The samples used in the assay were Liraglutide 100nM and a control sample with no Liraglutide. Before the procedure, the preparation instructions were followed. The PE Assay Buffer and Probe were warmed to prior to use. PE converter and Enzyme Mix were reconstituted in 220 µL of PE Assay Buffer before use. PE developer was thawed on ice before use. PE standard was thawed in a water bath at 45 °C for 15-20 minutes. The procedure is as follows. 2 to 10 µL of samples were added into wells of a 96 well clear plate. Two wells were prepared for each sample, named "Sample background control" and "Sample". The volume in the "Sample" wells was brought to 50 µL, and the volume in the "Sample background control" wells was brought to 70 µL using PE Assay Buffer. For the standard curve preparation, the 1 mM PE Standard was diluted 10-fold with PE Assay Buffer

to obtain a 100 µM PE solution. The solution was incubated at 45°C for 30 minutes. The Phosphatidylethanolamine (PE) Standards were prepared in a clear 96 well plate. For the converter mix, enough reagent was mixed for the number of assays that were to be performed. For each sample and standard well, 20 µL was prepared by mixing 18 µL of PE Assay Buffer and 2 µL of PE Converter. The converter mix was added to wells containing the samples and standards and mixed well. The convertor mix was not added to "Sample background control" wells. The plate was incubated at 45°C for 1 hour. For the reaction mix, enough reagents were mixed for the number of assays that were to be performed. For each well, 30 µL was prepared by mixing 21 µL of PE Assay Buffer, 5 µL of PE Developer, 2 µL of PE Enzyme Mix, and 2 µL of PE Probe. 30 µL of the reaction mix was added to all wells and mixed well. The plate was incubated at 45°C for 3 hours. Fluorescence in end-point mode was recorded at λ_{ex} = 535 nm/λ_{em} = 587 nm

2.4.3 Phosphatidylcholine (PC) Assay

The quantitative measurement of PC was determined using a competitive ELISA kit from Sigma-Aldrich (MAK049). The samples used in the assay were Liraglutide 100nM and a control sample with no Liraglutide. Before starting the procedure, the PC Assay Buffer was bought to room temperature. The PC Hydrolysis Enzyme and PC Development mix were both reconstituted with 200 µL of PC Assay Buffer. The PC Standard was reconstituted in 200 µL of water to generate a 50 mM (50 nmole/µL) PC Standard solution. The PC Standard for Colorimetric Detection was created by diluting 10 µL of the 50 mM (50 nmole/µL) PC Standard Solution with 990 µL of water to prepare a 0.5 mM (0.5 nmole/µL) standard solution. Added 0, 2, 4, 6, 8, 10 mL of the 0.5 mM PC standard solution into a 96 well plate, generating 0 (blank), 1, 2, 3, 4, and 5 nmole/well standards. PC buffer was added to each well to bring the volume to 50 µL. The procedure was followed according to manufacturer guidelines. The master reaction mix was set up according to manufacturer scheme. 50 µL of the mix was required for each well. In the sample blank wells, this was created with 46 µL PC Assay Buffer, 2 µL of PC Development mix and 2 µL Fluorescent Peroxidase Substrate. For samples and standard wells, this was 44 µL PC Assay Buffer, 2 µL PC Hydrolysis Enzyme, 2 µL PC Development Mix and 2 µL Fluorescent Peroxidase Substrate. This was then incubated for 30 minutes at room temperate. The plate was protected from light during this incubation period by aluminium foil and being placed in a dark cupboard. The absorbance was measured at 570 nm (A_{570}).

2.4.4 Sphingosine-1-Phopshate (S1P) Assay

The quantitative measurement of S1P was determined using a competitive ELISA kit from Abbexa (abx585002), according to the manufacturer's instructions. The samples used in the assay were Liraglutide 100nM and a control sample with no Liraglutide. The first step was reagent preparation, which is as follows. The Standard was prepared with 0.5 ml of Standard Diluent buffer to make the 1000 ng/ml Standard Solution, which was the highest standard. The reconstituted standard was allowed to sit for 10 mins, with gentle agitation prior to carrying out the serial dilutions, and foaming or bubbles were avoided. Tubes were labelled in preparation for the serial dilutions. Next, 0.6 ml of the Standard Diluent Buffer was aliquoted into each tube (apart from the highest standard tube). Then, 0.3 ml of the highest standard solution was added into the 1st tube and mixed thoroughly. Subsequently, 0.3 ml was transferred from the 1st to 2nd tube, mixed thoroughly, and so on. The standard was not vortexed as this would destabilise the protein. Next, the concentrated Wash Buffer was diluted 30-fold (1/30) with distilled water by adding 20 ml of concentrated wash buffer into 580 ml of distilled water. Next, Detection Reagents A and Reagent B were diluted 100-fold with their respective diluents and mixed thoroughly.

Experiments were conducted as per manufacturers protocol. The pre-coated plate was used to set the standard, test sample, and control (zero) wells, with their positions recorded. The solution was added to the bottom of each well without touching the side walls, and the standards and samples were mixed by pipetting them up and down before being added to the wells. Care was taken to avoid foaming or bubbles. Next, 50 µl of the diluted standards were aliquoted into the standard wells, and 50 µl of Standard Diluent buffer was aliquoted into the control (zero) well. 50 µl of appropriately diluted sample was aliquoted into the test sample wells, and the plate was gently tapped or shaken to mix. Detection Reagent A working solution (50 µl) was immediately aliquoted to each well, and the plate was again gently tapped or shaken to mix. The plate was covered with a plate sealer and incubated for 1 hour at 37°C. After incubation, the cover was removed, and the solution was discarded. The plate was washed 3 times with 1X Wash Buffer, with each well being filled completely with Wash buffer (350 µl) and 2 minute soaking period was given. Complete removal of liquid at each step was essential for good performance. After the final wash, any remaining Wash Buffer was removed by aspirating or decanting. The plate was then inverted and blotted against clean absorbent paper towels. Detection Reagent B working solution (100 µl) was added to each well, and the plate was sealed and incubated for 30 minutes at 37°C. After incubation, the cover was removed, the

solution was discarded, and the wash process was repeated 5 times. Then, 90 µl of TMB Substrate was added to each well, and the plate was covered with the plate sealer and gently tapped to mix thoroughly. The plate was incubated at 37°C for 10 minutes, with care taken to avoid exposure to light. Finally, 50 µl of Stop Solution was added to each well, and the Stop Solution was mixed quickly and uniformly throughout the microplate to inactivate the enzyme completely. The plate was checked for fingerprints or water on the bottom, and the fluid in the wells was checked for bubbles. The OD was immediately measured at 450 nm using the FLUROstar Omega microplate reader (BMG Labtech).

2.5 Statistical Analysis

General statistical analysis was undertaken using Microsoft Excel, IBM SPSS Statistics 23.0 and GraphPad Prism 9. Data was examined for normality using the Shapiro-Wilk test. Data is expressed as mean value ± standard error (SE) or mean value ± standard deviation (SD) as noted. $P<0.05$ was considered statistically significant and significance levels are indicated as follows; *$P<0.05$, **$P<0.01$, ***$P<0.001$. Data was analysed using the two-tailed paired sample t-test for comparison of baseline to endpoint data or the comparison of treated and non-treated cells. Analysis of metabolomics data was undertaken using MetaboAnalyst 5.0 and R Studio Desktop in collaboration with the Birmingham Phenome Centre. Homeostasis Model Assessment (HOMA) analysis was conducted using the University of Oxford HOMA2 Calculator, which was then statistically analysed using the methods described above.

Chapter 3 – Metabolic effects of GLP-1RAs on type 2 diabetes and obesity: a systematic review and meta-analysis

3.1 Introduction

Obesity is a growing pandemic, with the World Health Organisation (WHO) estimating over 1.9 billion adults were overweight (Body Mass Index [BMI] ≥25 kg/m^2) in 2016, of these over 650 million having obesity (BMI ≥30 kg/m^2) (World Health Organisation, 2020). It is a chronic, relapsing, multifactorial disease (Heymsfield & Wadden, 2017), and is now considered an international public health challenge (James, 2018) due to the increased association with type 2 diabetes mellitus (T2DM). The risk of developing T2DM increases with the amount of excess body weight, increasing threefold with a body mass index of 25.0 to 29.9 and 20-fold with an index of 35 of higher compared with healthy body indexes (Field et al., 2001). Co-existence of obesity and type 2 diabetes may contribute to medical complications, such as cardiovascular, cerebrovascular, renal, and lower extremity complications (Potts et al., 2015). Weight reduction is therefore a key intervention goal for people with type 2 diabetes (Wilding, 2014). Under the National Health Service (NHS) in the United Kingdom, weight loss is achieved through the NHS Tiered Care Weight Management Pathway. Tier 1 focuses on behavioural – universal interventions through the reinforcement of healthy eating and physical activity, including public health and national campaigns, delivered by local and regional public health teams. Tier 2 involves multicomponent overweight and obesity weight management intervention, delivered in groups with healthy weight specialists. Tier 3 uses specialist services, in which higher grades of obesity requiring a wide range of interventions, such as GLP-1 receptor agonist treatment, are considered. Tier 4, the final tier, requires bariatric surgery for people with comorbidities in which weight reduction is the highest priority in their care management (Health, 2013).

GLP-1 Receptor Agonists (GLP-1RA's) belong to the tier 3 weight management pathway available for patients. So far there are seven approved GLP1RAs; liraglutide once daily, exenatide twice daily, lixisenatide once daily, exenatide once weekly, dulaglutide once weekly, semaglutide once weekly, and oral semaglutide once daily (Trujillo, Nuffer, & Smith, 2021). GLP-1 Receptor Agonists mimic the effects of endogenous GLP-1, which stimulates the secretion and biosynthesis of insulin and inhibits the secretion of glucagon, thereby regulating plasma glucose levels, as well as decreasing food intake and delaying gastric emptying (Doyle & Egan, 2007).

GLP-1 acts on the hypothalamus to promote satiety, the stomach to inhibit gastric emptying, adipose tissue. It increases insulin sensitivity and secretion, which in turn increases uptake of fatty acids and glycogen synthesis in the skeletal muscle, reduces adipose lipolysis and

increases fatty acid uptake in adipose tissue. This lowers fatty acid delivery to the liver, in turn decreasing hepatic gluconeogenesis and increasing glycogen synthesis and hepatic de novo lipogenesis (Akhtar, Iqbal, Vazquez-Montesino, Dennis, & Ahmed, 2019). GLP-1 also has cardiovascular benefits on blood pressure, vascular endothelium, atherosclerosis progression and inflammation, myocardial ischemia and heart failure (Coke, Deedwania, Hinnen, Magwire, & Miller, 2022). In T2DM and obesity, these processes can become slower or even stopped, thus the need for GLP-1 Receptor Agonist treatment arises, where the half-life of the respective agonists vary between 2-3 hours to several days, compared to the half-life of endogenous GLP-1 of about 2 minutes (S. Lee & Lee, 2017).

Studies find GLP-1RA treatment directly reduces cholesterol (Du, Wang, Yang, Zhao, & Han, 2014), triglycerides (Dallinga-Thie & Nieuwdorp, 2015; Du et al., 2014), lipid profile and body weight (Muzurović & Mikhailidis, 2020), as well as reducing liver enzymes alanine aminotransferase (ALT) (Cuthbertson et al., 2012; Fan, Pan, Xu, & Yang, 2013), aspartate aminotransferase (AST) (Fan et al., 2013) and gamma-glutamyltransferase (GGT) (Cuthbertson et al., 2012; Fan et al., 2013). Obesity is characterised with low-grade chronic inflammation, making markers of inflammation important to identify. C-Reactive Protein (CRP) has been found to be the strongest marker of inflammation associated with obesity, with chronic elevation of CRP causing obesity (Q. Li et al., 2020). Treatment with the GLP-1RA Liraglutide has been shown to reduce C-Reactive Protein, albeit with a small sample of 28 (Anholm et al., 2019).

Biomarkers of type 2 diabetes have been previously identified such as: specific genes such as insulin like growth factor 1 (IGF1) and insulin receptor substrate 1 (IRS1) among others (Wang-Sattler et al., 2012), carbohydrates (glucose and fructose) (Menni et al., 2013) or inflammatory biomarkers (C-reactive protein, interleukin-6, and tumour necrosis factor-α) (Winter et al., 2018). Previous studies on GLP-1RA treatment have only independently examined the effects of the treatment on one metabolite such as c-reactive protein (Mazidi, Karimi, Rezaie, & Ferns, 2017), adiponectin (Simental-Mendía, Sánchez-García, Linden-Torres, & Simental-Mendía, 2021) and adipokines (Yaribeygi, Maleki, Atkin, Jamialahmadi, & Sahebkar, 2021), rather than multiple metabolites. Systematic reviews have been published before, although these examine the effects of GLP-1 RA treatment on cardiovascular outcomes (F. Sun et al., 2015) or kidney and mortality outcomes (Kristensen et al., 2019; Sattar et al., 2021) in type 2 diabetes. A systematic review has been published identifying metabolomics

signatures in T2DM (Y. Sun, Gao, Fan, He, & Yan, 2020). The paper discusses the use of metabolomics as a tool for identifying biomarkers associated with T2D and its complications, as well as for gaining insights into the underlying pathophysiology of the disease. However, the paper does not provide any information on how the metabolic data changes when treated with T2DM medication. To the best of our knowledge, there are no studies indicating the metabolic changes when obese patients are treated with GLP-1RA. This meta-analysis provides a comprehensive collection of the metabolic changes occurring in patients treated with GLP-1RA. In the current systematic review, we aim to conduct a meta-analysis of the metabolic changes caused by treatment with GLP-1 RA on obese and type 2 diabetes patients.

3.2 Methodology

This protocol was developed in accordance with the Preferred Reporting Items for Systematic Review and Meta-Analysis Protocols (PRIMSA-P) and followed methods outlined in The Cochrane Handbook for Systematic Reviews of Interventions (Higgins JPT, 2022). This systematic review has been registered with PROSPERO (International Prospective Register of Systematic Reviews) with the registration number of CRD42021229468.

3.2.1 Search Strategy

One author (E.O) conducted systematic searches of eligible studies published up to 5 August 2021 through the following databases: Medical Literature Analysis and Retrieval System Online (MEDLINE), Excerpta Medica (EMBASE), Cochrane Central Register of Controlled Trials (CENTRAL) and SCOPUS (title + abstract + keywords). The key MeSH (Medical Subject Heading) search terms were: *(exenatide OR liraglutide OR lixisenatide OR albiglutide OR dulaglutide OR semaglutide) AND (diabetes AND obesity) AND random**. Only Randomised Controlled Trials (RCTs) were included to narrow down the search and increase the quality and further calculations of the included studies. In addition, reference lists of selected articles and other literature sources were browsed to ensure a comprehensive literature search was completed.

3.2.2 Study Selection

Inclusion criteria included studies on adults (18 years or older) with type 2 diabetes and obesity. Observation studies, cohort studies, clinical studies and intervention studies were included in the search. No restrictions were made regarding the intervention type, where a study took place, the number of participants or the duration of follow up. All studies had to be peer-reviewed.

Exclusion criteria were single case reports, expert opinion manuscripts, letters to the editor, commentaries, conference papers, animal studies, meta-analyses, narrative review articles and articles not in English. The patients with the following conditions were excluded: type 1 diabetes, obesity without type 2 diabetes and type 2 diabetes without the presence of obesity. An initial evaluation was performed to eliminate duplicate publications. Studies were assessed independently and in parallel by two reviewers against the inclusion criteria, initially based on titles and abstracts and then by reviewing the full text of the articles retained in the first step. Any disagreements were solved by discussion, and if consensus is not reached, arbitration by a third reviewer was required. The systematic reviews software Covidence was used to manage the study selection process, access to which was provided by UHCW.

3.2.3 Data Extraction

Data was extracted independently by three reviewers following the Cochrane Public Health Group Data Extraction and Assessment Template to create a data template which was pilot-tested and used for each article. Examples of data extracted included: study description (e.g. title, primary author, year of publication, country, intervention, follow-up duration, number of participants, aims), participant characteristics (e.g. mean age), patient outcomes (BMI, weight, haemoglobin A1c (HbA1c), fasting plasma glucose, fasting insulin, cholesterol, triglycerides, High Density Lipoprotein, Low Density Lipoprotein, heart rate, insulin resistance (HOMA-IR), insulin sensitivity (HOMA-S), systolic blood pressure, diastolic blood pressure, weight circumference, fat mass, fat free mass, ALT, AST, amylase, creatinine, C-Reactive protein, adiponectin, c-peptide, apolipoprotein B (apoB), and leptin), and quality assessment. Data extraction template creation and subsequent extraction were conducted on Microsoft Excel.

3.2.4 Quality Assessment

Quality appraisal was performed independently by three reviewers. Any disagreements were solved by discussion or, if necessary, with the arbitration of a third reviewer. Quality assessment of experimental studies was conducted in line with the Cochrane collaboration's risk of bias tool. Bias is assessed as a judgement (high, low, or unclear) for individual elements from five domains (selection, performance, attrition, reporting, and other).

3.2.5 Data Synthesis and Statistical Analysis

All meta-analytical calculations were carried out by a statistician using R statistical software (v4.2.0) with meta package (v6.1-0). Forest plots were created with meta package (v6.1-0).

Pooled mean differences were calculated from the extracted continuous data with 95% confidence intervals (CI) using the inverse variance method, with the DerSimonian-Laird random-effects model used in all calculations. Heterogeneity was assessed using Cochran's Q and I^2 statistics.

3.3 Results

3.3.1 Search Results

The search results were combined into EndNote reference management software, resulting in 2869 records (Medline 344, Embase 1175, Cochrane Central 821, Scopus 529). After running a duplicate removal tool, 1449 records were left which were then imported into Covidence, which removed a further 24 results. Five reviewers screened 1425 studies for title and abstracts, and 775 were excluded. Five reviewers screened the full text of the remaining 650 studies, with 637 studies excluded. The Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA-P) flow diagram is shown in figure 3.1, outlining the outcomes of the screening process. Thirteen studies were included in the synthesis and meta-analysis. General characteristics of included studies are presented in Table 3.1.

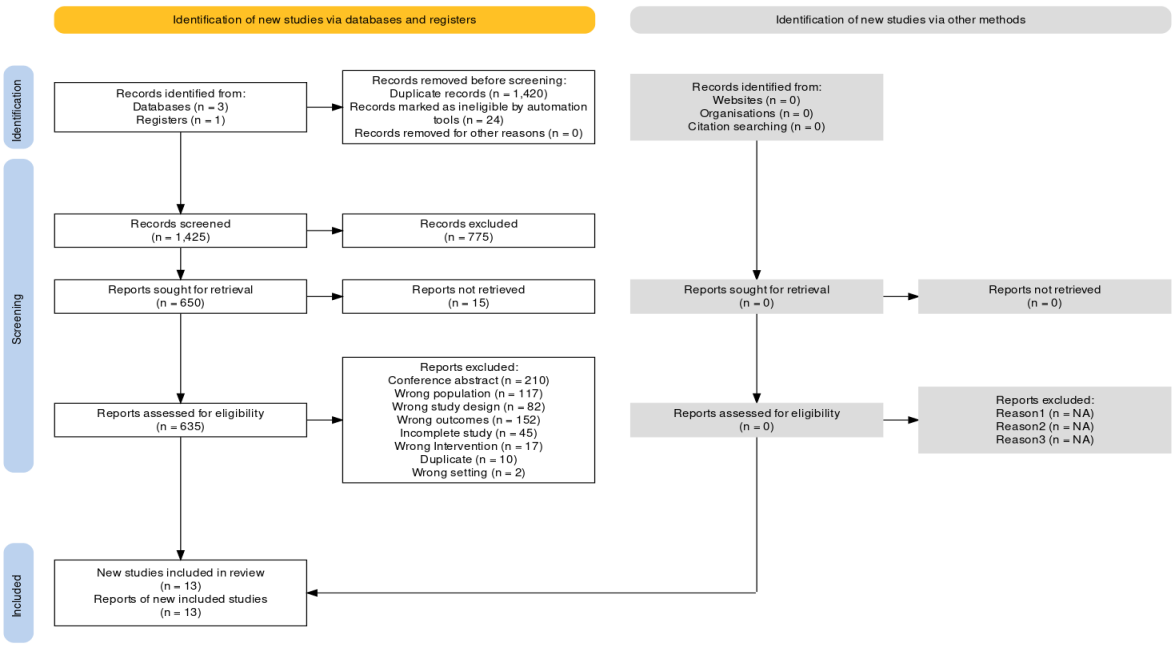


Figure 3.1 - PRISMA flow diagram for studies assessed for eligibility from Page et al.26 Generated with R package and Shiny App (Haddaway, Page, Pritchard, & McGuinness, 2022).

Table 3.1 – Characteristics of Included Studies Table. All listed studies are included in the meta-analysis.

Study	Country	Study Design	Primary Outcome	Follow-up	Treatment Sample size (n)	Population age (years)	Gender	GLP-1 RA Treatment	Treatment Dosage	Population Description
Abreu, 2019	USA	Prospective, randomised, open-label, parallel group, non-inferiority trial	Change in HbA1c	6 months	59	46.7 ± 9	40 female, 19 male	Liraglutide	1.8mg daily	Patients with type 2 diabetes with a confirmed HbA1c ≥10% on any treatment except for current use (within the prior 30 days) of prandial insulin (excluding premix insulin), dipeptidyl peptidase-4 (DPP-4) inhibitors or GLP1RAs
Ahmadi, 2019	Sweden	Multicentre, double blind, placebo controlled	Change in sagittal abdominal diameter, hip circumference, waist circumference, waist-to-hip ratio and adiponectin levels	24 weeks	63	63.8 ± 8.2	23 female, 40 male	Liraglutide	1.8mg daily	Patients with type 2 diabetes with a BMI 27.5–45.0 kg m^2, HbA1c ≥58 mmol mol^1 (7.5%) and ≤102 mmol mol^1 (11.5%), fasting C-peptide ≥0.1 nmol L^1 and ongoing treatment with multiple daily insulin injections
Anholm, 2017	Denmark	Randomised, double-blind, placebo-controlled, cross-over trial	Treatment effect on beta cell function	26 weeks	39	62.3 ± 7.6	31 male, 8 female	Liraglutide	1.8mg daily	Patients with newly diagnosed type 2 diabetes with BMI >25kg/m^2, >18 and <85 years old, with anti-diabetic medications discontinued 2 weeks before the first baseline visit

Study	Country	Study Design	Primary Outcome	Follow-up	Treatment Sample size (n)	Population age (years)	Gender	GLP-1 RA Treatment	Treatment Dosage	Population Description
Ariel, 2014	USA	RCT	Lipoprotein Profile	14 weeks	23	58 ± 7	33% male	Liraglutide	1.8mg daily	Volunteers with prediabetes, recruited from advertisements in newspapers, over the age of 40, both genders, overweight, good general health without known disease, at risk of type 2 diabetes
Bouchi, 2017	Japan	Single-center, randomised, open-label, comparative study	Change in visceral fat area	36 weeks	8	57 ± 16	63% male	Liraglutide	0.9mg daily	Patients with type 2 diabetes older than 20 years, who regularly visited Tokyo Medical and Dental University Hospital and had been treated with insulin. Had type 2 diabetes according to Japan Diabetes Society criteria, BMI>25kg/m^2.
Dandona, 2018	USA	Single-center, randomised, placebo-controlled, single-blind (patient) prospective study.	Effect of exenatide on interleukin-1 receptor antagonist (IL-1RA) plasma concentration	12 weeks	12	56 ± 10.4	Not included	Exenatide	10µg twice daily	Patients with obesity and type 2 diabetes with glycated haemoglobin A1c (HbA1c) values ranging between 7.5% and 9%. All patients were receiving stable doses of oral antidiabetic medications and insulin and had stable weight during the 4 weeks before the study.

Study	Country	Study Design	Primary Outcome	Follow-up	Treatment Sample size (n)	Population age (years)	Gender	GLP-1 RA Treatment	Treatment Dosage	Population Description
Davies, 2021	Argentina, Canada, Germany, Greece, India, Japan, Russia, South Africa, Spain, UAE, UK, USA	Phase 3, randomised, double-blind, double-dummy, placebo-controlled, multicentre superiority study	Coprimary endpoints were percentage change in bodyweight and achievement of weight reduction of at least 5%	68 weeks	404	55 ± 11	50.9% female	Semaglutide	2.4mg weekly	Adults with a body-mass index of at least 27 kg/m² and glycated haemoglobin 7–10% (53–86 mmol/mol) with type 2 diabetes at least 180 days before screening. Recruited from 149 outpatient clinics in 12 countries across Europe, North America, South America, the Middle East, South Africa, and Asia.
Dutour, 2016	France	Prospective randomised controlled study	Change in Epicardial adipose tissue, myocardial, hepatic and pancreatic triglyceride content	26 weeks	22	51 ± 2	59% male	Exenatide	10µg twice daily	Obese subjects with T2D and glycated haemoglobin (HbA1c) levels of 6.5–10%, all uncontrolled on oral antidiabetic therapy [metformin ± sulphonylureas ± dipeptidyl peptidase-4 (DPP-4) inhibitors]
Farr, 2016	USA	Randomised, placebo-controlled, cross-over study	Change in GLP-1, leptin and GIP levels	17 days	20	49.7 ± 2.4	11 male, 9 female	Liraglutide	1.8mg daily	Male and females with type 2 diabetes mellitus (DM; defined as fasting plasma glucose >125 mg/dL and/or HbA1c > 6.5%).
Guo, 2020	China	Single-center, prospective, randomised placebo-controlled stud	Changes in intrahepatic content of lipid , abdominal adiposity [subcutaneous adipose tissue (SAT) and visceral adipose tissue (VAT)]	26 weeks	31	53.1 ± 6.3	52% male	Liraglutide	Variable (based on blood glucose)	Patients with type 2 diabetes and NAFLD, aged 30-60 years having a diagnosis of type 2 diabetes inadequately controlled (HbA1c > 6.5%) under metformin monotherapy (at a stable dose of 20000 mg/day for at least 3 months)

Study	Country	Study Design	Primary Outcome	Follow-up	Treatment Sample size (n)	Population age (years)	Gender	GLP-1 RA Treatment	Treatment Dosage	Population Description
Harder, 2004	Denmark	Randomised, double-blind, parallel-group, placebo-controlled	Effect of liraglutide on glycaemic control	8 weeks	21	59.9 ± 10	11 male, 10 female	Liraglutide	0.6mg daily	Patients with type 2 diabetes, HbA1c 7-12%, BMI >27kgm^2
Yin, 2018	China	Prospective RCT	Change in body composition	16 weeks	19	45.2 ± 12.1	12 male, 7 female	Exenatide	5 µg twice daily (4 weeks), then 10 µg twice day	Overweight and obese T2DM patients with poor glycemic control despite metformin monotherapy were randomised using arbitrary computer-generated numbers to receive exenatide or glargine treatments for 16 weeks in addition to their current metformin treatment.
Taskinen , 2021	Sweden	Single blinded RCT	Postprandial apoB48 and apoB100 metabolism after treatment	16 weeks	14	30-75 (no specific age)	Male and Female (not specific)	Liraglutide	1.8mg per day	Patients with T2D, body mass index (BMI) of 27–40 kg/ m^2 , waist >88 cm in women and >92 cm in men, age 30–75 years, HbA1c of 42–75 mmol/mol (6%–9%), plasma triglyceride 1.0–4.0 mmoL/L, and LDL cholesterol <4.5 mmoL/L

3.3.2 Risk Of Bias Assessments

The bias assessment of the listed studies revealed very little overall significant risk of bias for all trials, all of which were graded by two independent reviewers. Bias assessment can be seen as a traffic light plot (Figure 3.2A) and as a summary plot (Figure 3.2B), generated by robvis (McGuinness & Higgins, 2021). In figures 3.2A and 3.2B, a green colour indicates low risk of bias, yellow indicates unclear bias, and red indicates high risk of bias.

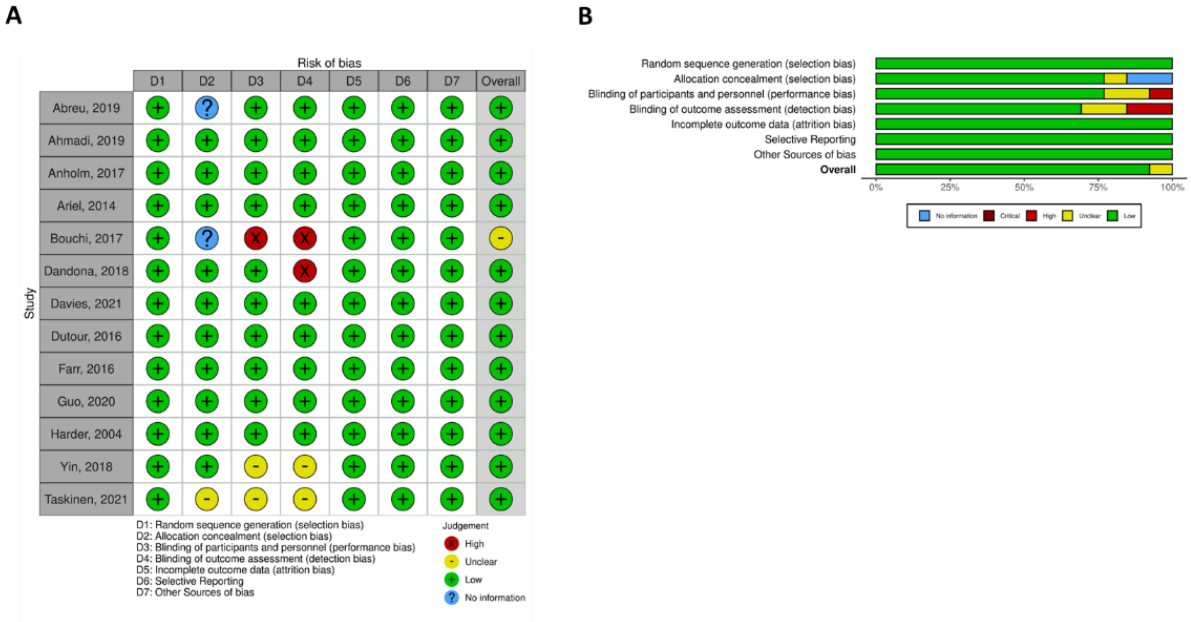


Figure 3.2 - Assessment of Risk of Bias using A) RoB Traffic Light Plot and B) RoB Summary Plot. Both generated by robvis (McGuinness & Higgins, 2021)

3.3.3 Meta-Analysis of Outcomes

The mean difference in fasting insulin was calculated from 7 studies as -3.9063 pmol/L (95% CI [-17.8909; 10.0783], p=0.58, I^2 = 70%, Figure 3.3A). The 7 included studies combined for 282 total observations, showing a minor reduction after treatment.

The mean difference (MD) in Alanine Transaminase (ALT) pre- and post-intervention was calculated from 4 studies as -3.3274 U/L (95% CI [-7.6746; 1.0198], p=0.14, I^2 = 0%, Figure 3.3B). The 4 studies that included ALT combined for 184 observations, showing a reduction in ALT with GLP-1RA treatment.

Aspartate Transaminase mean difference was calculated from the same 4 studies as -1.0614 U/L (95% CI -3.7857; 1.6630], p=0.45, I^2 = 0%, Figure 3.3C). Again GLP-1RA treatment showed a reduction over 184 total observations after treatment.

The MD of Gamma-glutamyl Transferase was calculated from 2 studies as -6.4460 U/L (95% CI [-28.3184; 15.4264], p=0.56, I^2 = 0%, Figure 3.3D). GGT showed an overall reduction with treatment, from 84 observations.

Creatinine mean difference was calculated from 2 studies as 6.3485 µmol/L (95% CI [-3.4914; 16.1884], p=0.21, I^2 = 61%, Figure 3.3E). Creatinine was increased overall based on 159 observations from the 2 included studies.

The mean difference in adiponectin pre- and post-intervention was calculated from 3 studies as 0.8205 µg/mL (95% CI [-0.3441; 1.9850], p=0.17, I^2 = 27%, Figure 3.3F). The 3 studies included combined for 207 observations, showing a minor increase in adiponectin after treatment with GLP-1RA.

The C-peptide mean difference was calculated from 4 studies as 11.0208 pmol/L (95% CI [-14.3811; 36.4227], p=0.96, I^2 = 0%, Figure 3.3G). This was calculated from 213 total observations, showing an increase change in C-peptide after treatment.

Apolipoprotein B was reduced in 2 studies by -6.1485mg/dL (95% CI [-13.5033; 1.2062], p=0.1, I^2 = 0%, Figure 3.3H). The 2 studies included 74 total observations, and combined for a low heterogeneity, showing a total reduction in Apolipoprotein B after treatment.

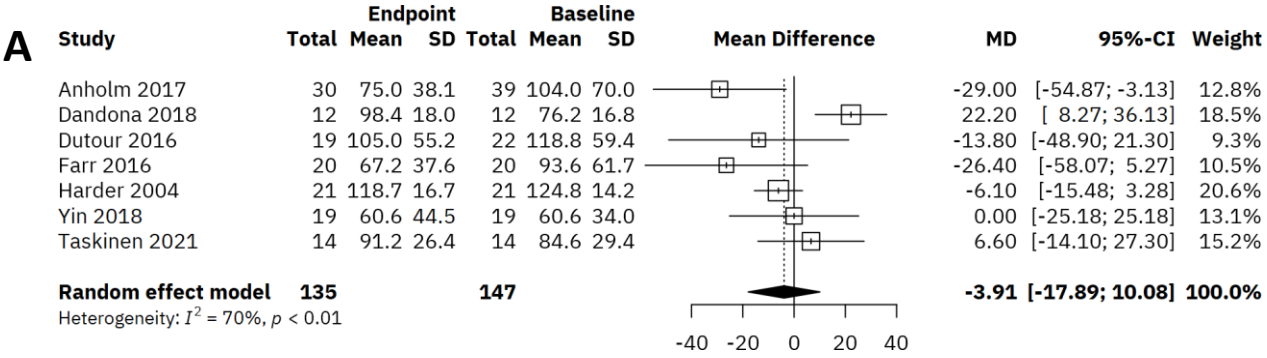
A similar trend was seen in leptin from 2 studies as a difference of -5.5784 ng/ml (95% CI [-13.8968; 2.7399], p=0.19, I^2 = 0%, Figure 3.3I). Leptin studies had 81 total observations, and a low level of heterogeneity, showing a total reduction with GLP-1RA treatment.

Mean difference in C-reactive protein was seen from 3 studies as -1.1329 mg/L (95% CI [-1.7316; -0.5343], p=0.0002, I^2 = 0%, Figure 3.3J). The 3 studies combined for 813 observations and had a low level of heterogeneity. C-reactive protein showed a significant reduction after GLP-1RA treatment.

The mean difference of fructosamine from 2 studies was calculated as -27.1986 µmol/L (95% CI [-31.1335; -23.2638], p<0.0001, I^2 = 0%, Figure 3.3K). The 2 included studies had 82

combined observations, with low heterogeneity. The decrease in fructosamine was significant after GLP-1RA treatment.

A



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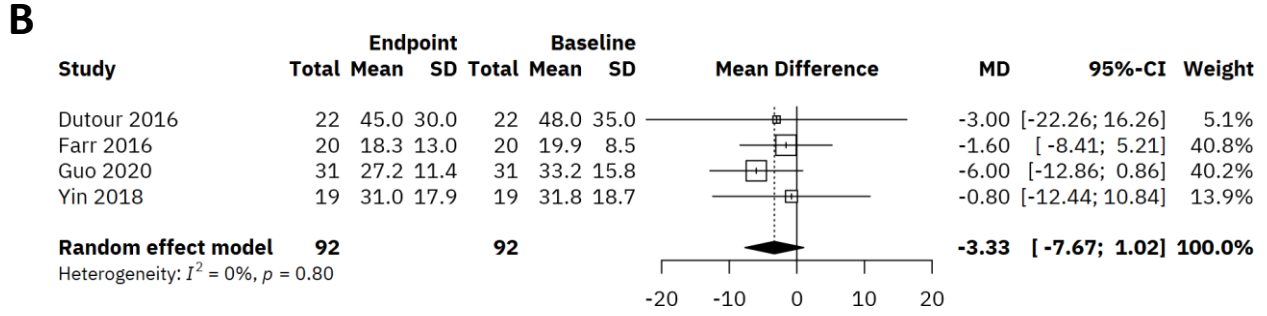

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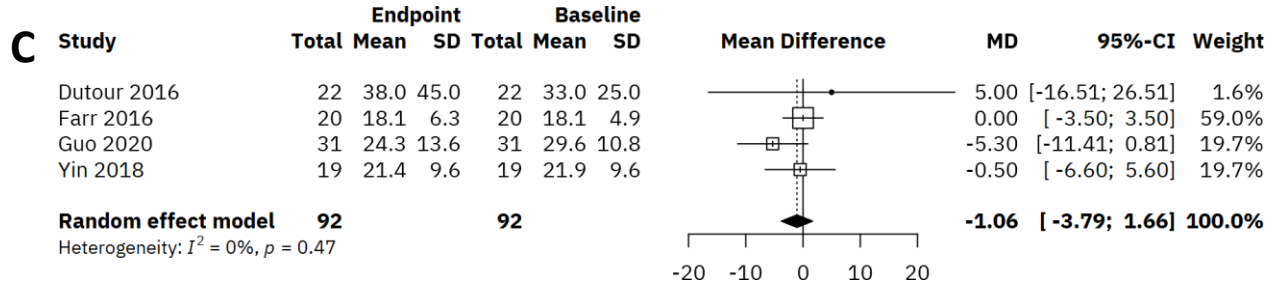

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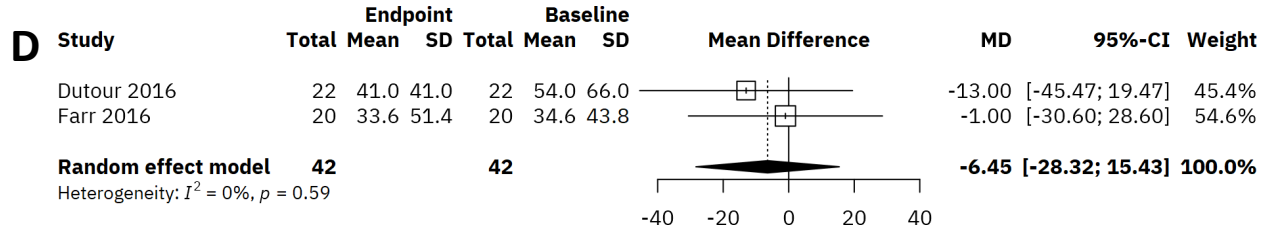

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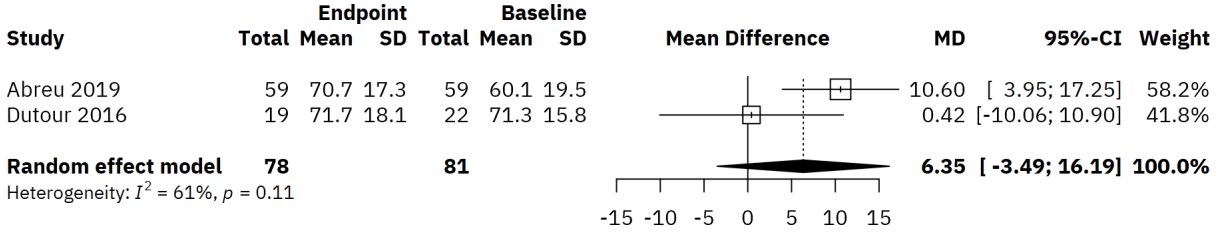
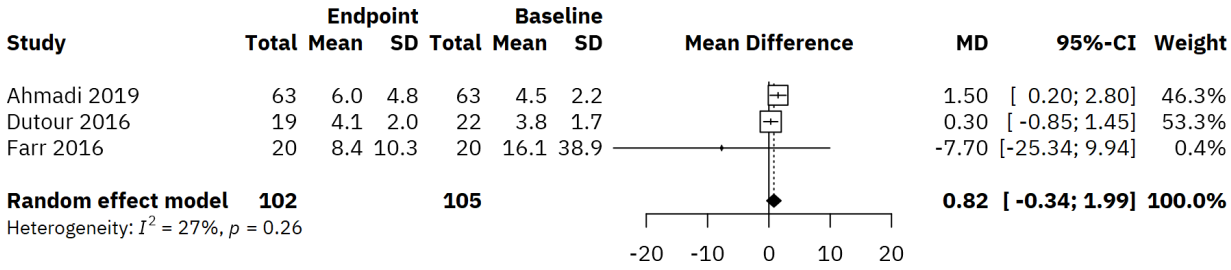
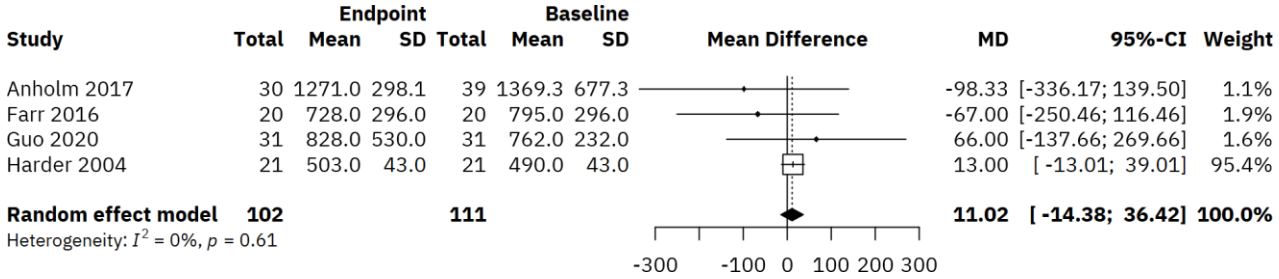

Study | Endpoint Total Mean SD | Baseline Total Mean SD | Mean Difference | MD 95%-CI Weight
Abreu 2019 59 70.7 17.3 59 60.1 19.5 10.60 [3.95; 17.25] 58.2%
Dutour 2016 19 71.7 18.1 22 71.3 15.8 0.42 [-10.06; 10.90] 41.8%
Random effect model 78 81 6.35 [-3.49; 16.19] 100.0%
Heterogeneity: I^2 = 61%, p = 0.11
-15 -10 -5 0 5 10 15

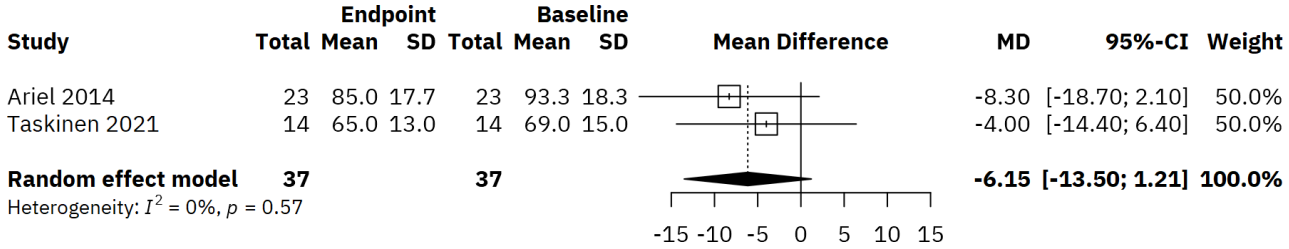
F


Study | Endpoint Total Mean SD | Baseline Total Mean SD | Mean Difference | MD 95%-CI Weight
Ahmadi 2019 63 6.0 4.8 63 4.5 2.2 1.50 [0.20; 2.80] 46.3%
Dutour 2016 19 4.1 2.0 22 3.8 1.7 0.30 [-0.85; 1.45] 53.3%
Farr 2016 20 8.4 10.3 20 16.1 38.9 -7.70 [-25.34; 9.94] 0.4%
Random effect model 102 105 0.82 [-0.34; 1.99] 100.0%
Heterogeneity: I^2 = 27%, p = 0.26
-20 -10 0 10 20

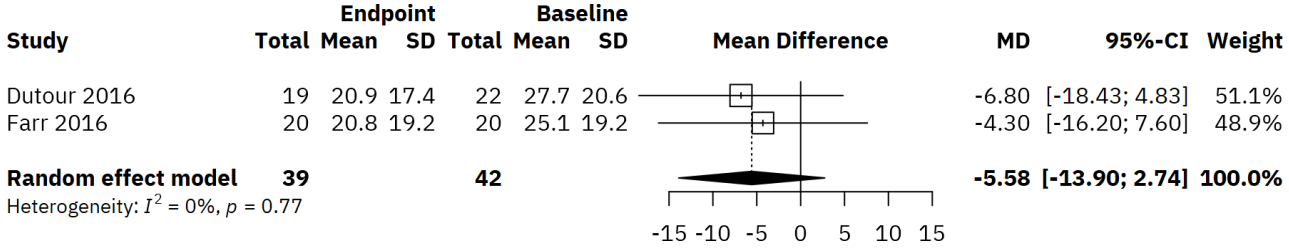
G


Study | Endpoint Total Mean SD | Baseline Total Mean SD | Mean Difference | MD 95%-CI Weight
Anholm 2017 30 1271.0 298.1 39 1369.3 677.3 -98.33 [-336.17; 139.50] 1.1%
Farr 2016 20 728.0 296.0 20 795.0 296.0 -67.00 [-250.46; 116.46] 1.9%
Guo 2020 31 828.0 530.0 31 762.0 232.0 66.00 [-137.66; 269.66] 1.6%
Harder 2004 21 503.0 43.0 21 490.0 43.0 13.00 [-13.01; 39.01] 95.4%
Random effect model 102 111 11.02 [-14.38; 36.42] 100.0%
Heterogeneity: I^2 = 0%, p = 0.61
-300 -100 0 100 200 300

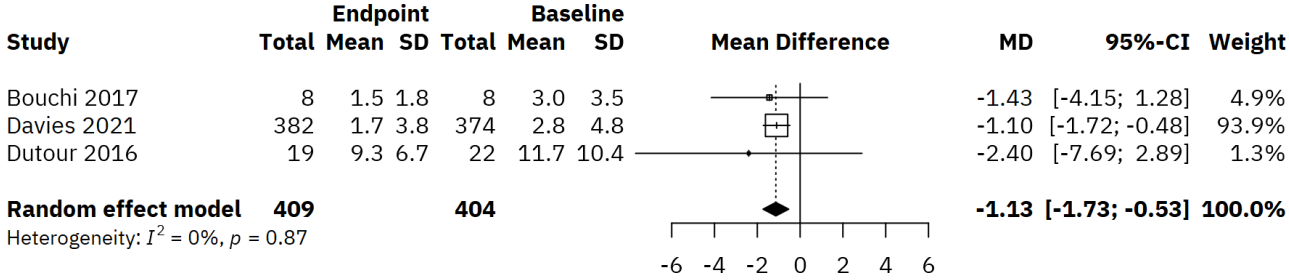
H


Study | Endpoint Total Mean SD | Baseline Total Mean SD | Mean Difference | MD 95%-CI Weight
Ariel 2014 23 85.0 17.7 23 93.3 18.3 -8.30 [-18.70; 2.10] 50.0%
Taskinen 2021 14 65.0 13.0 14 69.0 15.0 -4.00 [-14.40; 6.40] 50.0%
Random effect model 37 37 -6.15 [-13.50; 1.21] 100.0%
Heterogeneity: I^2 = 0%, p = 0.57
-15 -10 -5 0 5 10 15

I


Study | Endpoint Total Mean SD | Baseline Total Mean SD | Mean Difference | MD 95%-CI Weight
Dutour 2016 19 20.9 17.4 22 27.7 20.6 -6.80 [-18.43; 4.83] 51.1%
Farr 2016 20 20.8 19.2 20 25.1 19.2 -4.30 [-16.20; 7.60] 48.9%
Random effect model 39 42 -5.58 [-13.90; 2.74] 100.0%
Heterogeneity: I^2 = 0%, p = 0.77
-15 -10 -5 0 5 10 15

J


Study | Endpoint Total Mean SD | Baseline Total Mean SD | Mean Difference | MD 95%-CI Weight
Bouchi 2017 8 1.5 1.8 8 3.0 3.5 -1.43 [-4.15; 1.28] 4.9%
Davies 2021 382 1.7 3.8 374 2.8 4.8 -1.10 [-1.72; -0.48] 93.9%
Dutour 2016 19 9.3 6.7 22 11.7 10.4 -2.40 [-7.69; 2.89] 1.3%
Random effect model 409 404 -1.13 [-1.73; -0.53] 100.0%
Heterogeneity: I^2 = 0%, p = 0.87
-6 -4 -2 0 2 4 6

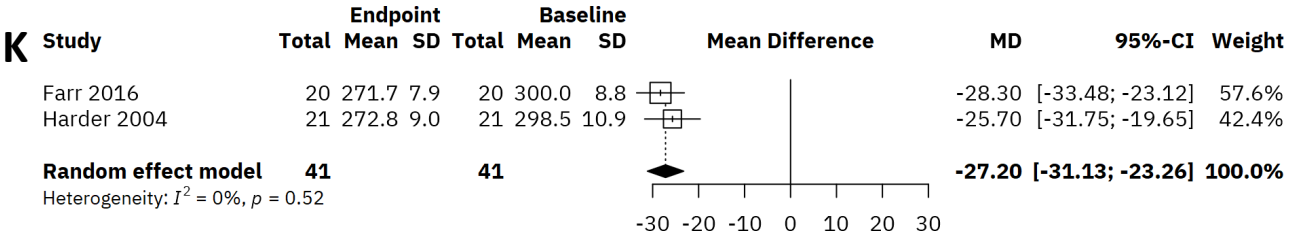


Figure 3.3 – Meta-analysis of the effect of GLP-1 receptor agonist treatment in patients with obesity and type 2 diabetes on metabolites A) Fasting Insulin, B) Alanine Aminotransferase, C) Aspartate Aminotransferase, D) Gamma-glutamyl Transferase, E) Creatinine, F) Adiponectin, G) C-peptide, H) Apolipoprotein B, I) Leptin, J) C-Reactive Protein, K) Fructosamine

3.4 Discussion

The primary objective of this systematic review and meta-analysis was to evaluate the metabolic effects of GLP-1 receptor agonist treatment for patients with obesity and type 2 diabetes. The meta-analysis included a total of 13 randomised controlled trials (RCTs) with 713 participants. Overall, the results showed that GLP-1 RA treatment led to reductions in ALT, AST, GGT, fasting insulin, adiponectin, apolipoprotein B, leptin, c-reactive protein and fructosamine. Of these, c-reactive protein and fructosamine were significantly decreased ($p<0.05$). Conversely, treatment with GLP-1RA increased creatinine and c-peptide, with none of the variables being significant.

Fasting insulin was decreased with treatment. An abnormally high insulin level (hyperinsulinemia) has been hypothesised to be a risk marker of metabolic diseases (Ling et al., 2016). Studies have found obesity was a predictor of fasting hyperinsulinemia (M. K. Kim, Reaven, & Kim, 2017). The effect of GLP-1 for weight loss could be a potential reason for the reduction in fasting insulin, as weight loss of 5% to 10% body mass decreases insulin resistance (Khoo, Hsiang, Taneja, Law, & Ang, 2017).

The meta-analysis suggests GLP-1 agonist treatment reduces the liver enzymes ALT ($p=0.06$), AST ($p=0.2$) and GGT ($p=0.5$). Research shows the mean level of serum ALT, AST and GGT are significantly higher in obese groups of patients when compared to normal BMI (Ali et al., 2021). One systematic review found GLP-1 receptor agonist treatment significantly reduces ALT and GGT, although AST showed no change (Rezaei et al., 2021). Excess fat accumulation in the liver may be one cause for increased levels of these enzymes in the blood, as obesity has been associated with high levels of circulating enzymes (Laine et al., 2021). Thus, the effects

of GLP-1RA treatment in reducing body fat and weight may be linked to the reduction of the liver enzymes AST, ALT and GGT (Yudan Zhang et al., 2019).

The meta-analysis revealed creatinine levels were increased (p=0.07) in the selected studies. Research by Harita shows lower levels of serum creatinine can be associated with an increased risk of type 2 diabetes (Harita et al., 2009). Creatinine concentration is a direct reflection of skeletal muscle mass. As skeletal muscle mass is associated with type 2 diabetes (type 2 diabetes lowers skeletal muscle mass (K. S. Kim et al., 2014)), serum creatinine may also be associated with type 2 diabetes.

Adiponectin was decreased (p=0.5) after GLP-1RA treatment in the meta-analysis, although a considerable heterogeneity (I^2=96%) must be noted. A recent systematic review found GLP-1RA treatment significantly increased adiponectin levels (Simental-Mendía et al., 2021). However, this review also found liraglutide had a significant effect on adiponectin, while exenatide treatment did not affect the concentrations. Adiponectin is an anti-inflammatory adipokine regulates a number of metabolic pathways involved in glucose and lipid homeostasis, and research has shown obesity decreases plasma adiponectin levels, while aerobic exercise, caloric restriction and weight loss has the opposite effect (Ghoshal & Bhattacharyya, 2015). While the meta-analysis overall found a slight decrease in adiponectin levels, the high heterogeneity could be a possible explanation, with 2 studies finding a slight increase in adiponectin, and 2 studies finding a decrease, contributing to the finding being insignificant.

C-peptide levels were increased (p=0.4) after treatment with GLP-1RAs. C-peptide is a widely used measure of pancreatic beta cell function (Leighton, Sainsbury, & Jones, 2017), and is widely associated with both type 1 diabetes (too little c-peptide) and type 2 diabetes (too much c-peptide). Due to this, c-peptide would be expected to reduce in type 2 diabetes, which has not been the case in the present systematic review, and research shows it is type 1 diabetes treatment which should lead to c-peptide increasing (Kuhadiya, Prohaska, Ghanim, & Dandona, 2019). However, it should be noted the result of the meta-analysis was not close to being significant (p=0.4), with major decreases being seen in one study which were not seen in the other 3 included. C-peptide levels have been shown to be significantly higher with higher levels of BMI (Irwin et al., 2005), with research by Anholm indicating a significant decrease in c-peptide levels after Liraglutide treatment (Anholm et al., 2017).

Apolipoprotein B levels were decreased (p=0.1) after treatment. Obesity contributes to the pathogenesis of cardiovascular disease, with an altered composition of many lipids, which includes increased levels of Apolipoprotein B. Measurement of ApoB levels provides information about all atherogenic lipoproteins (very-LDL, LDL and IDL), rather than measuring all separately. Liraglutide has been shown to reduce ApoB levels (Engelbrechtsen et al., 2017), as has exenatide (Schwartz et al., 2010), which is in line with the findings of the meta-analysis (Ariel et al., 2014; Taskinen et al., 2021).

The meta-analysis also revealed leptin was decreased (p=0.09) after GLP-1 agonist treatment. Leptin is sometimes known as the "satiety hormone", affecting energy balance by control of appetite (Friedman, 2019). The loss of leptin signalling to the brain is associated with food intake and obesity by controlling energy intake. One such example of this is a study which showed GLP-1 administration reduced serum leptin levels in mice (Goldsmith et al., 2015). This effect has also been shown in humans with both liraglutide (Frøssing et al., 2018) and exenatide (L. Shi et al., 2017) administration.

C-reactive protein significantly (p=0.0002) decreased after GLP-1RA treatment. This is congruent with other studies, with one meta-analysis of 7 treatment arms revealing a significant reduction in CRP levels following treatment with GLP-1 receptor agonists (Mazidi et al., 2017). CRP measures the amount of a protein in the blood that signals acute inflammation (Collins & Costello, 2021), with inflammation being linked to complications of obesity. It has been shown there is a significant association between elevated CRP levels and glucose control in type 2 diabetes (Tan et al., 2003). Another more recent systematic review examined the effect of GLP-1RAs on biomarkers of inflammation and oxidative stress in T2DM patients, finding GLP-1RA treatment versus standard diabetes treatment or placebo significantly reduced CRP levels, supporting the anti-inflammatory effects of GLP-1RAs (J. J. Bray et al., 2021).

Fructosamine was also significantly decreased (p<0.0001). Fructosamine is a marker of glucose control, reflecting the average glycaemic level of the previous 2-3 weeks (Nansseu et al., 2015). The results of the systematic review agree with recent research, which found serum levels of fructosamine were significantly lower in db/db mice compared to a non-treated control group when treated with a GLP-1RA, trending even lower when the dosage was increased (Ren et al., 2019). Other research also shows GLP-1RA treatment significantly decreases plasma fructosamine levels in diabetic monkeys (Cui et al., 2021).

The present systematic review and meta-analysis of 13 randomised controlled trials has provided a more comprehensive understanding of the metabolic effects of GLP-1 receptor agonist treatment in patients with obesity and type 2 diabetes. The results have highlighted the potential use of GLP-1RA in reducing the risk of type 2 diabetes through its beneficial effects on metabolites, all of which have changed as expected based on previous research studies. The results suggest a multi-faceted effect of GLP-1RAs. The results indicate that GLP-1RA treatment led to a minor reduction in fasting insulin levels and a significant reduction in C-reactive protein and fructosamine. These findings show that GLP-1RA treatment may improve insulin sensitivity and glycaemic control, as well as reducing inflammation. The results also show that GLP-1RA treatment led to a reduction in ALT, AST, GGT, and Apolipoprotein B levels, indicating potential benefits for liver function and lipid metabolism. These findings indicate that GLP-1RA treatment may improve liver health and lipid profile, which are important factors in managing diabetes and its complications. GLP-1RA treatment also led to an increase in adiponectin and C-peptide levels, which may have positive effects on insulin sensitivity and glycaemic control. Additionally, the results show a reduction in leptin levels, which may help regulate appetite and body weight. The compiled analysis of the results can inform future study design by providing an insight into which variables and outcomes should be measured and provide guidance on appropriate follow up length.

Several limitations with this review should be acknowledged. Despite using a random effects model in the meta-analysis, heterogeneity was substantial for some variables. This could be due to inconsistent follow-up time between studies, which varied between 3 weeks and 68 weeks, potentially affecting results, and limiting comparability. While various systematic reviews are present which examine the effects of GLP-1RAs as a whole, this could be seen as a limitation due to the differences in magnitude of the effect of individual GLP-1RAs. Future research could consider subgroup analyses of different GLP-1RAs, although this was not possible for the present review due to paucity of research meeting inclusion criteria. Future metabolomic profiling of patients undergoing tier 3 weight management plans will reveal metabolomic markers and pathways involved in GLP-1 receptor agonist treatment. This review did not gather any data relating to adverse effects. While not in the scope of the present review, these should be considered when prescribing GLP-1RA as these remain an important limitation of GLP-1RA treatment.

The search strategy and subsequent data analysis were performed by an external researcher to avoid any potential bias in these processes. The scope of the review is clear and uses predefined inclusion/exclusion criteria for patient population, interventions, outcomes, and study design. Overall, the review was conducted following Cochrane guidance to ensure methodology was robust and systematic.

3.5 Conclusions

The current meta-analysis data concludes that treatment with glucagon-like peptide-1 receptor agonists (GLP-1RAs) have a positive effect on patients by altering the levels of the metabolic markers in patients with obesity and type 2 diabetes, including ALT, AST, GGT, fasting insulin, adiponectin, apolipoprotein B, leptin, c-reactive protein, fructosamine, creatinine and c-peptide. To the knowledge of the authors, this is the most comprehensive analysis of metabolic effects of GLP-1RA's on obesity and T2DM, as previously published research articles focus on the individual metabolite effects of obesity and T2DM. Due to metabolomics being an emerging biological technique, more trials need to be carried out to determine novel biomarkers of treatment effects and detect the mechanism of GLP-1RA action on molecular pathways.

Chapter 4 – Clinical and Metabolic Effects of Liraglutide on Patients with Overweight and Obesity

4.1 Introduction

Obesity constitutes a significant challenge to public health, arising from an imbalance between caloric intake and energy expenditure, leading to the accumulation of surplus adipose tissue in various locations, such as subcutaneous and visceral regions, as well as non-adipose tissues like skeletal muscles, liver, and pancreatic β cells (Galgani, Moro, & Ravussin, 2008). Under pathophysiological conditions, the metabolic function of lipids in adipose tissue becomes disrupted, leading to an elevated delivery of fatty acids to neighbouring peripheral tissues (Montgomery, De Nardo, & Watt, 2019). The proliferation of free fatty acids (FFAs) from adipose tissue and the secretion of hormones, cytokines, and pro-inflammatory markers, which are firmly associated with obesity, result in a decline in glucose uptake by muscle cells and an upsurge in hepatic glucose production. These metabolic disturbances lead to an excess of glucose in the bloodstream, ultimately resulting in glucose intolerance and the development of type 2 diabetes (Torretta, Barbacini, Al-Daghri, & Gelfi, 2019).

Evidence indicates that the process of hypothalamic lipid sensing is pivotal in regulating food intake, fat storage, and energy balance. It is increasingly understood that the disruption of this mechanism could contribute to the onset of obesity and type 2 diabetes (Picard et al., 2014). The hypothalamus, in particular, has garnered extensive recognition as a central orchestrator of systemic energy balance. Currently, it is widely acknowledged that the hypothalamus plays a crucial role in the regulation of several physiological processes, including body temperature, blood pressure, thirst, and hunger, serving as a fundamental integration point between the nervous and endocrine systems (Chari, Lam, & Lam, 2011). Recent research has suggested that the hypothalamic Sphingosine-1-phosphate (S1P) and Sphingosine 1-phosphate receptor 1 (S1PR1) is involved in the hypothalamic regulation of energy homeostasis (Silva et al., 2014). S1P is a bioactive lipid molecule produced by various tissues in the body, including the adipose tissue, and acts as a signalling molecule that modulates various physiological processes, such as inflammation, cell migration, and angiogenesis (formation of new blood vessels) (Nagahashi, Abe, Sakimura, Takabe, & Wakai, 2018). Silva (Silva et al., 2014) specifically shows that the S1P/S1PR1 signalling pathway in hypothalamic neurons regulates energy homeostasis in rodents. The research demonstrated that administering S1P through intracerebroventricular injections leads to a decrease in food consumption and an increase in energy expenditure in rats. This effect is achieved by the sustained activation of STAT3 and the melanocortin system. Conversely, when hypothalamic S1PR1 is specifically disrupted, it results in an increase in food intake and a reduction in the respiratory exchange ratio. There is

a positive correlation of S1P and body fat percentage (Kowalski, Carey, Selathurai, Kingwell, & Bruce, 2013), which shows an association of S1P and obesity, either negatively or positively. There are differing results of this, where S1P metabolism could play a positive role in insulin signalling in peripheral tissue, showing an adaptive role to counteract insulin resistance and regulation of glucose (S. Y. Lee et al., 2015; Ma et al., 2007; Osawa et al., 2011). Conversely, some studies argue that S1P could play a role in the causation of insulin resistance (Fayyaz et al., 2014; J. Wang, Badeanlou, Bielawski, Ciaraldi, & Samad, 2014).

Liraglutide is a glucagon-like peptide-1 (GLP-1) receptor agonist that has been widely studied and used for the management of type 2 diabetes and obesity. The administration of a once-daily subcutaneous dose of 3.0 milligrams of liraglutide, in conjunction with a caloric-restriction regimen and elevated levels of physical activity, has been sanctioned for the purpose of weight management in North America and Europe (C. W. Le Roux et al., 2017). Weight loss with Liraglutide is dose-dependent up to 3.0 mg once daily (Astrup et al., 2012). In clinical trials, liraglutide has been shown to be effective in reducing body weight in individuals with obesity and type 2 diabetes. In the SCALE study (Pi-Sunyer et al., 2015), at week 56, participants in the liraglutide group had lost a mean of 8.4±7.3 kg of body weight, and those in the placebo group had lost a mean of 2.8±6.5 kg. The results were found to be sustained for up to 56 weeks, with no significant difference in the incidence of adverse events between the liraglutide and placebo groups.

Additionally, liraglutide has been shown to improve glycaemic control in individuals with type 2 diabetes. In the LEADER trial (Zinman et al., 2018), individuals treated with liraglutide showed a significant reduction in HbA1c levels, a measure of long-term blood glucose control, compared to those receiving placebo. The drug also improved other markers of glucose metabolism, such as fasting plasma glucose and postprandial glucose levels (Matsumoto et al., 2013).

Although there are differing results regarding S1P and obesity, there have been no studies in which S1P levels were measured pre and post treatment with GLP-1 RA treatment. Therefore, we conducted a 24-week, open-label real-world study involving 62 participants with a BMI >30 kg/m^2 or >27 kg/m^2 if they had co-existing dyslipidaemia or hypertension. No participants had type 2 diabetes. Participants received once-daily subcutaneous liraglutide 3.0 mg, alongside NHS Tier-3 lifestyle advice. The reduced calorie diet was based on individual

estimated basic metabolic rate. The primary end point was change in body weight. Secondary outcomes included changes in anthropometrics and circulating biomarkers of metabolism. This study will be the first study providing real-life data on the effects of 3mg liraglutide on anthropometric parameters and specific markers of metabolism of participants in the UK.

4.2 Results

Baseline characteristics of the participants treated with Liraglutide (n=49) are shown in Table 4.1. Due to various reasons, such as dropout and inconsistent measurements, 13 participants data were not included in the final clinical analysis, leaving 49 participants in total. 43 participants (87.7%) were female, 6 (12.3%) were male. At the initiation of the trial, the average age of participants was 38.6±1.4 years. 41 participants (83.7%) were Caucasian, 7 were South Asian (14.3%) and 1 participant was Black (2%). Baseline weight was 117.5±3.5 kg, BMI was 41.3±1 kg/m^2. No participants were in the overweight weight category, 7 (14.3%) were obese class I (BMI 30-34.9 kg/m^2), 18 (36.7%) were obese class II (BMI 35-39.9 kg/m^2), and 24 (49%) were obese class III (BMI ≥ 40 kg/m^2).

Systolic blood pressure at baseline was 133.6±2.4 mmHg, diastolic blood pressure was 83.7±1.3. Body fat percentage was 49.9±0.9%, and fat mass was 59±2.3 kg. Fat-free mass was 58.4±1.7 kg. Glucose was 5.3±0.1 mmol/l. Baseline alanine aminotransferase (ALT) was 24.9±1.8 U/L, aspartate aminotransferase (AST) 20.4±0.8 U/L, γ-glutamyl transferase (GGT) 28.1±2 U/L, Amylase 48±2.5 U/L.

Table 4.1 – Baseline characteristics of patients. Plus-minus valued are observed means ± SEM

Baseline Characteristics of the Patients	
Characteristic	**Liraglutide (N = 49)**
Sex — no. (%)	
Female	43 (87.7)
Male	6 (12.3)
Age — yr	38.6±1.4
Race or ethnic group — no. (%)	
Caucasian	41 (83.7)
South Asian	7 (14.3)
Black	1 (2)
Height — cm	168.1±0.9
Weight — kg	117.5±3.5
Body-mass index	41.3±1
Body-mass index categories — no. (%)	
27-29.9: overweight	0 (0)
30-34.9 obese class I	7 (14.3)
35-39.9 obese class II	18 (36.7)
≥ 40: obese class III	24 (49)
Blood Pressure — mm Hg	
Systolic	133.6±2.4
Diastolic	83.7±1.3
Resting heart rate — bpm	78.7±1.7
Body fat — %	49.9±0.9
Fat mass— kg	59±2.3
Fat-free mass —kg	58.4±1.7
Glucose — mmol/l	5.3±0.1
Enzymes (U/L)	
ALT	24.9±1.8
AST	20.4±0.8
GGT	28.1±2
Amylase	48±2.5

Table 4.2 - Endpoint characteristics of patients. Plus-minus valued are observed means ± SEM

Changes in Physiological Parameters from Baseline to Treatment Termination			
End Point	**Liraglutide (N = 49)**	**Estimated difference (95% CI)**	**P Value**
Change in body weight			
% of body weight	-6.3±0.7	(-7.8 to -4.8)	<0.001
Kilograms of body weight	-7.9±1	(-9.9 to -5.8)	<0.001
Loss of ≥5% body weight — no. (%)	19 (38.8)		
Loss of ≥10% body weight — no. (%)	9 (18.4)		
Body weight-related endpoints			
Body-mass index	-6±0.8	(-7.6 to -4.5)	<0.001
% of body fat (%)	-5.3±1.1	(-7.6 to -3)	<0.001
% of fat mass	-10.7±1.6	(-13.8 to -7.6)	<0.001
% of Fat-free mass	-0.3±1.5	(-3.4 to 2.8)	0.853
% of glucose	-10.8±1.5	(-13.8 to -7.8)	<0.001
Change in Enzymes			
% of ALT	-10.5±5.8	(-22.2 to 1.1)	0.075
% of AST	-2.2±4.6	(-11.7 to 7.3)	0.643
% of GGT	-4.8±3.8	(-12.7 to 3.1)	0.225
% Amylase	8.8±2.8	(3.3 to 14.4)	0.002
Change in Blood Pressure			
% of Systolic	-3.8±1.5	(-6.8 to -0.7)	0.16
% of Diastolic	-2.8±1.4	(-5.6 to 0.01)	0.49
Resting heart rate — bpm	2.9±2.3	(-1.7 to 7.5)	0.217
Body-mass index categories — no. (change from baseline)			
27-29.9: overweight	2 (+2)		
30-34.9 obese class I	12 (+5)		
35-39.9 obese class II	18 (0)		
≥ 40: obese class III	17 (-7)		

Table 4.2 shows changes for participants at treatment termination. At week 24, participants had lost 7.9±1 kg or 6.3±0.7 % body weight ($p<0.001$). 55.1% of participants lost 5-10% and 18.4% lost >10% body weight ($P<0.001$). Body mass index reduced 6±0.8 kg/m^2 ($p<0.001$), with a reduction of 5.3±1.1% total body fat. Participants lost 10.7±1.6% fat mass ($p<0.001$), while fat free mass had a non-significant change of -0.3±1.5% ($p>0.05$). Fasting glucose levels changed by -10.8±1.5% after treatment ($p<0.001$). After 24 weeks Liraglutide treatment, ALT had a mean change of -10.5±5.8% ($p=0.075$), AST -2.2±4.6% ($p=0.643$), GGT -4.8±3.8% ($p=0.225$)

and amylase increased 8.8±2.8% (p=0.002). Resting blood pressure and heart rate saw no significant change after treatment (p>0.05).

Patients were categorised into body mass index categories. 2 participants ended the treatment in the overweight (BMI 27-29.9 kg/m^2) category, while 0 participants started in this category. 12 participants finished in the obese class I category (BMI 30-34.9 kg/m^2), while 7 started in this category. There was no change in the obese class II category (BMI 35-39.9 kg/m^2), with 18 finishing in this class. The obese class 3 category (BMI ≥ 40 kg/m^2) had a reduction of 7 participants, finishing with 17 participants.

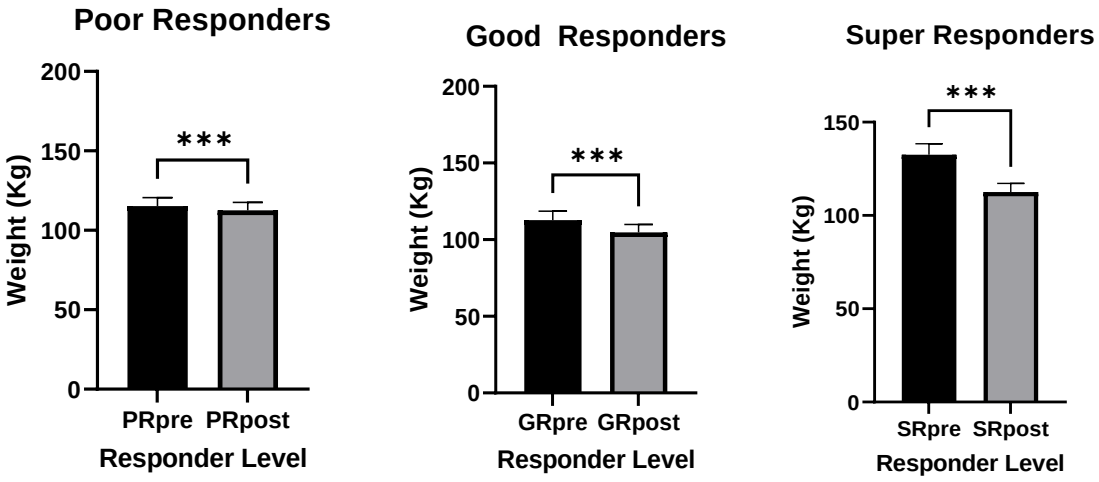


Figure 4.1 - Weight loss according to responder level in: A) Poor Responders, B) Good Responders and C) Super Responders

According to weight loss (WL) response, patients were divided into Poor responders (<5% WL, n=22), good responders (5-10% WL, n=18) and super-responders (>10% WL, n=9) (Figure 4.1). All groups showed significant weight loss. Poor responders had a baseline weight of 115.3±5.33kg, with final weight 112.6±5.03kg for a mean weight change of -2.73±0.62kg (p<0.001). Good responders had a baseline weight of 112.7±5.91kg, with final weight 104.7±5.23kg for a mean weight change of -8.022±0.71kg (p<0.001). Super responders had a baseline weight of 132.6±5.88kg, with final weight 112.5±4.71kg for a mean weight change of -20.02±1.81kg (p<0.001). It is important to note that weight loss tended to be lower in those patients who completed all 24 weeks of treatment when compared to those who only completed 16 weeks of treatment.

Table 4.3 - Biochemistry data of participants as a whole group (n=49). Plus-minus valued are observed means ± SEM. * denotes significance ($p<0.05$). AlbuminBCG denotes Albumin assay kit, AlkP Alkaline phosphatase, APOA1 Apolipoprotein AI, APOB Apolipoprotein B, Chol Cholesterol, CRP16 C-Reactive Protein, LDL Low Density Lipoprotein, Trig Triglycerides, HDL High Density Lipoprotein, Vit D25OH 25-hydroxy vitamin D test.

WHOLE GROUP		
	Pre	**Post**
Fatty Acid (nmol)	4.6±0.33	5.13±0.37
Fatty Acid (nmol/µL)	0.12±0.03	0.19±0.03
AlbuminBCG	43.45±0.75	44.68±1.19
AlkP	76.98±19.14	78.17±19.3
APOA1	1.54±0.05	1.52±0.05
APOB	1.11±0.04	1.08±0.05
Calcium	2.52±0.04	2.56±0.05
Chol	5.18±0.14	5.16±0.21
CRP16	9.22±1.84	7.1±1.01
Direct LDL	109.7±4.76	113.3±5.21
Trig	1.73±0.13	1.53±0.1
Ultra HDL	1.19±0.04	1.19±0.05
Vit D25OH	19.35±1.8	23.87±2.06*

When analysing data as a whole group, there were no significant changes between pre and post treatment of Liraglutide. There was an increase seen only on the variable of Vitamin D25OH from 19.35±1.8 to 23.87±2.06 ($p=0.031$).

Table 4.4 Biochemistry data of participants, categorised by responder level, pre and post Liraglutide treatment. A) Shows Non-Responders, B) Shows Good Responders, C) Shows Super Responders Plus-minus valued are observed means ± SEM. * denotes significance ($p<0.05$).

NON-RESPONDERS		
	Pre	**Post**
Fatty Acid (nmol)	4.08±0.58	3.9±0.42
Fatty Acid (nmol/µL)	0.33±0.05	0.31±0.03
AlbuminBCG	43.06±1.16	42.33±0.87
AlkP	76.14±3.79	78.27±4.07
APOA1	1.51±0.07	1.42±0.06
APOB	1.06±0.06	1±0.05
Calcium	2.49±0.06	2.45±0.04
Chol	4.95±0.22	4.76±0.18
CRP16	5.76±0.86	5.45±0.81
Direct LDL	106.61±7.69	105±6.26
Trig	1.73±0.17	1.58±0.13
Ultra HDL	1.16±0.07	1.11±0.05
Vit D25OH	22.59±2.57	24.24±2.3

GOOD RESPONDERS		
	Pre	**Post**
Fatty Acid (nmol)	5.44±0.44	5.78±0.37
Fatty Acid (nmol/µL)	0.43±0.03	0.46±0.03
AlbuminBCG	43.95±1.15	44.25±1.12
AlkP	78.87±4.69	80.48±4.76
APOA1	1.63±0.07	1.56±0.07
APOB	1.13±0.06	1.11±0.07
Calcium	2.55±0.05	2.57±0.06

Chol	5.31±0.23	5.18±0.26
CRP16	8.68±1.72	7.37±1.37
Direct LDL	110±8.05	111.5±7.55
Trig	1.81±0.26	1.49±0.18
Ultra HDL	1.23±0.06	1.2±0.04
Vit D25OH	18.53±2.9	20.82±3.48

SUPER RESPONDERS		
	Pre	**Post**
Fatty Acid (nmol)	3.34±0.41	5.39±0.87*
Fatty Acid (nmol/µL)	0.27±0.03	0.43±0.07*
AlbuminBCG	42.67±1.39	49.11±4.91
AlkP	70.75±6.99	72.88±6.39
APOA1	1.49±0.09	1.59±0.18
APOB	1.12±0.09	1.1±0.13
Calcium	2.46±0.06	2.73±0.21
Chol	5.15±0.23	5.61±0.79
CRP16	14.96±7.78	8.71±3.8
Direct LDL	111.67±7.58	124.67±15.96
Trig	1.64±0.13	1.48±0.15
Ultra HDL	1.17±0.08	1.32±0.18
Vit D25OH	15.1±2.74	32.03±4.28*

Biochemistry data were analysed for a number of variables. In both poor responders and good responders, no biochemistry data were significantly changed. In super responders, Fatty acid was significantly increased from 3.34±0.41 nmol to 5.39±0.87 nmol ($p<0.05$). Vitamin D25OH was increased from 15.1±2.74ng/mL to 32.03±4.28ng/mL ($p<0.05$).

Table 4.5. Insulin data of participants pre and post Liraglutide treatment. Plus-minus valued are observed means ± SEM. * denotes significance ($p<0.05$). HOMA-B denotes homeostasis model assessment of β-cell function, HOMA-S insulin sensitivity, HOMA-IR insulin resistance.

WHOLE GROUP		
	Pre	**Post**
Insulin	18.16±1.67	14.72±1.26*
Plasma Glucose	5.36±0.11	4.78±0.01*
HOMA-B	146.4±8.67	159.8±8.88
HOMA-S	53.86±4.04	67.26±5.05*
HOMA-IR	2.35±0.21	1.85±0.16*

When analysing data as a whole group, insulin levels decreased from 18.16±1.67 mmol/l to 14.72±1.26 mmol/l after treatment ($p<0.05$). Plasma glucose decreased from 5.36±0.11 mmol/l to 4.78±0.01 mmol/l. There were no significant changes in HOMA-B. Insulin sensitivity increased from 53.86±4.04% to 67.26±5.05%. Insulin resistance decreased from 2.35±0.21 to 1.85±0.16 ($p<0.05$).

Table 4.6. Insulin data of participants, categorised by responder level, pre and post Liraglutide treatment. A) Shows Non-Responders, B) Shows Good Responders, C) Shows Super Responders Plus-minus valued are observed means ± SEM. * denotes significance ($p<0.05$).

NON-RESPONDERS		
	Pre	**Post**
Insulin	18.72±3.4	15.66±1.85
Plasma Glucose	5.18±0.1	4.68±0.09*
HOMA-B	153.9±15.55	172±12.03
HOMA-S	57.05±9.12	59.56±6.72
HOMA-IR	2.4±0.41	1.97±0.23

GOOD RESPONDERS		
	Pre	**Post**
Insulin	19.34±2.7	16.65±2.3
Plasma Glucose	5.37±0.22	4.76±0.12*
HOMA-B	153.8±14.28	172.2±14.32
HOMA-S	49.96±5.05	31.31±7.9
HOMA-IR	2.5±0.35	2.09±0.23

SUPER RESPONDERS		
	Pre	**Post**
Insulin	15.26±1.94	9.91±1.36*
Plasma Glucose	5.61±0.2	5.02±3.34
HOMA-B	122.3±13.03	120.3±16.99
HOMA-S	56.19±6.95	88.96±10.74*
HOMA-IR	2±0.24	1.27±0.17*

In the super responder group, Insulin levels were decreased from 15.26±1.94 mmol/l to 9.9±1.36 mmol/l ($p<0.05$). Insulin sensitivity (HOMA-S) was increased from 56.19±6.95% to 88.96±10.74% ($p<0.05$). Conversely, insulin resistance (HOMA-IR) was decreased from 2±0.24 to 1.27±0.17 ($p<0.05$). Plasma glucose decreased significantly in both non-responders and responders, but no significant changes were seen in super responders.

Table 4.7. Proinflammatory cytokine data of participants pre and post Liraglutide treatment. Plus-minus valued are observed means ± SEM. * denotes significance ($p<0.05$). IL-1B denotes Interleukin-1 beta, IL-6 interleukin 6, IL-8 interleukin 8, TNF-α tumour necrosis factor alpha

	WHOLE GROUP	
	Pre	**Post**
IL-1b	0.84±0.27	0.37±0.03
IL-6	7.45±3.29	3.89±0.52
IL-8	6.24±0.95	5.87±0.62
TNF-α	9.31±0.68	9.2±0.66

Proinflammatory cytokines were analysed based on the data of 23 patients. There were no significant changes in IL-1B, IL-6, IL-8 or TNF- α after liraglutide treatment ($p>0.05$).

Table 4.8. Proinflammatory cytokine data of participants pre and post Liraglutide treatment. A) Shows Non-Responders, B) Shows Good Responders, C) Shows Super Responders. Plus-minus valued are observed means ± SEM. * denotes significance ($p<0.05$).

NON-RESPONDERS		
	Pre	**Post**
IL-1b	0.72±0.31	0.32±0
IL-6	2.71±0.25	3.57±0.73
IL-8	4.31±0.36	5.35±1.38
TNF-α	9.75±1.42	9.74±1.55

GOOD RESPONDERS		
	Pre	**Post**
IL-1b	1.31±0.6	0.43±0.1
IL-6	4.29±054	4.81±0.96
IL-8	8.6±2.05	6.36±1.24
TNF-α	9.74±1.32	9.07±1.2

SUPER RESPONDERS		
	Pre	**Post**
IL-1b	0.35±0.03	0.32±0
IL-6	3.23±0.23	1.96±0.3*
IL-8	4.85±0.74	5.7±0.45
TNF-α	8.39±0.7	8.91±0.76

The available patients have been broken down by weight loss level into Poor Responders (n=9), Good Responders (n=7) and Super Responders (n=7). IL-6 had significant positive correlation ($r=0.732$, $P<0.001$) with weight loss. In all super-responders, IL-6 concentration was significantly decreased from 3.23±0.23 to 1.96±0.3 ($p<0.05$), although there was no significant change in IL-6 in good responder and non-responder groups.

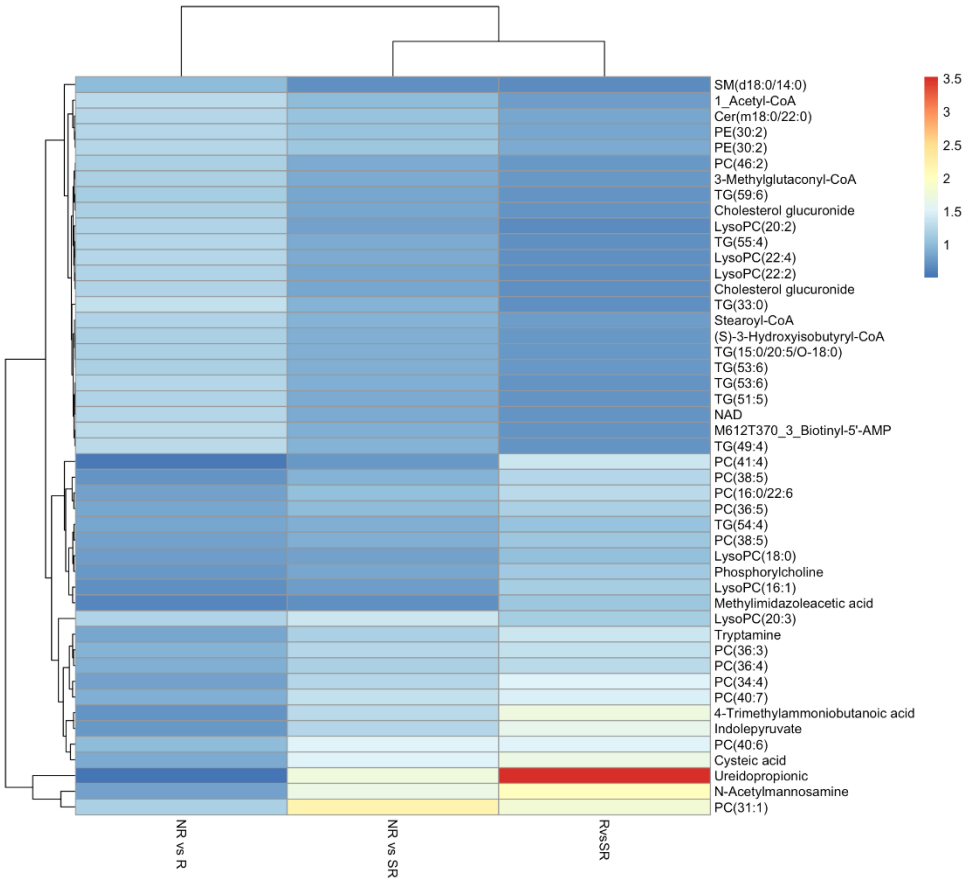


Figure 4.2 Heat Map analysis of Time Point 1. Each column shows, in order from left to right, Non Responders vs Responders, Non Responders vs Super Responders and Responders vs Super Responders. Time Point 1 = 2 months treatment, Time Point 2 = 4 months, Time Point 3 = 6 months.

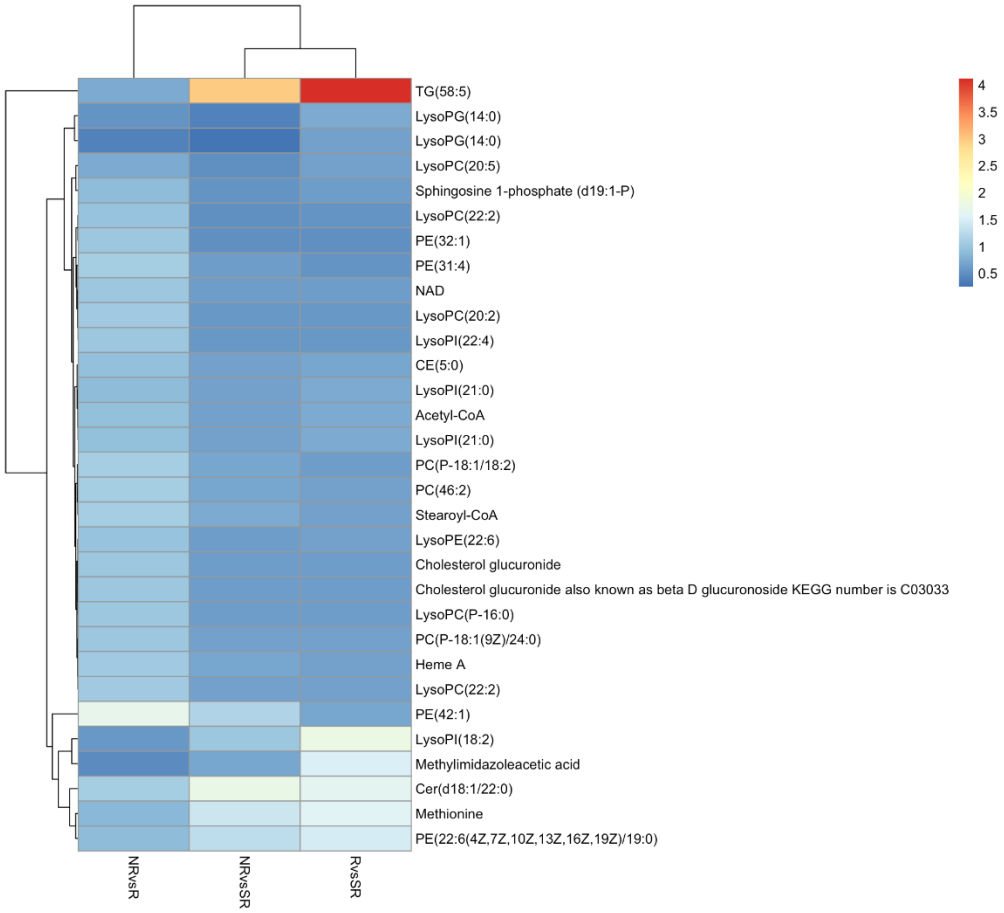


Figure 4.3 Heat Map analysis of Time Point 2. Each column shows, in order from left to right, Non Responders vs Responders, Non Responders vs Super Responders and Responders vs Super Responders. Time Point 1 = 2 months treatment, Time Point 2 = 4 months, Time Point 3 = 6 months.

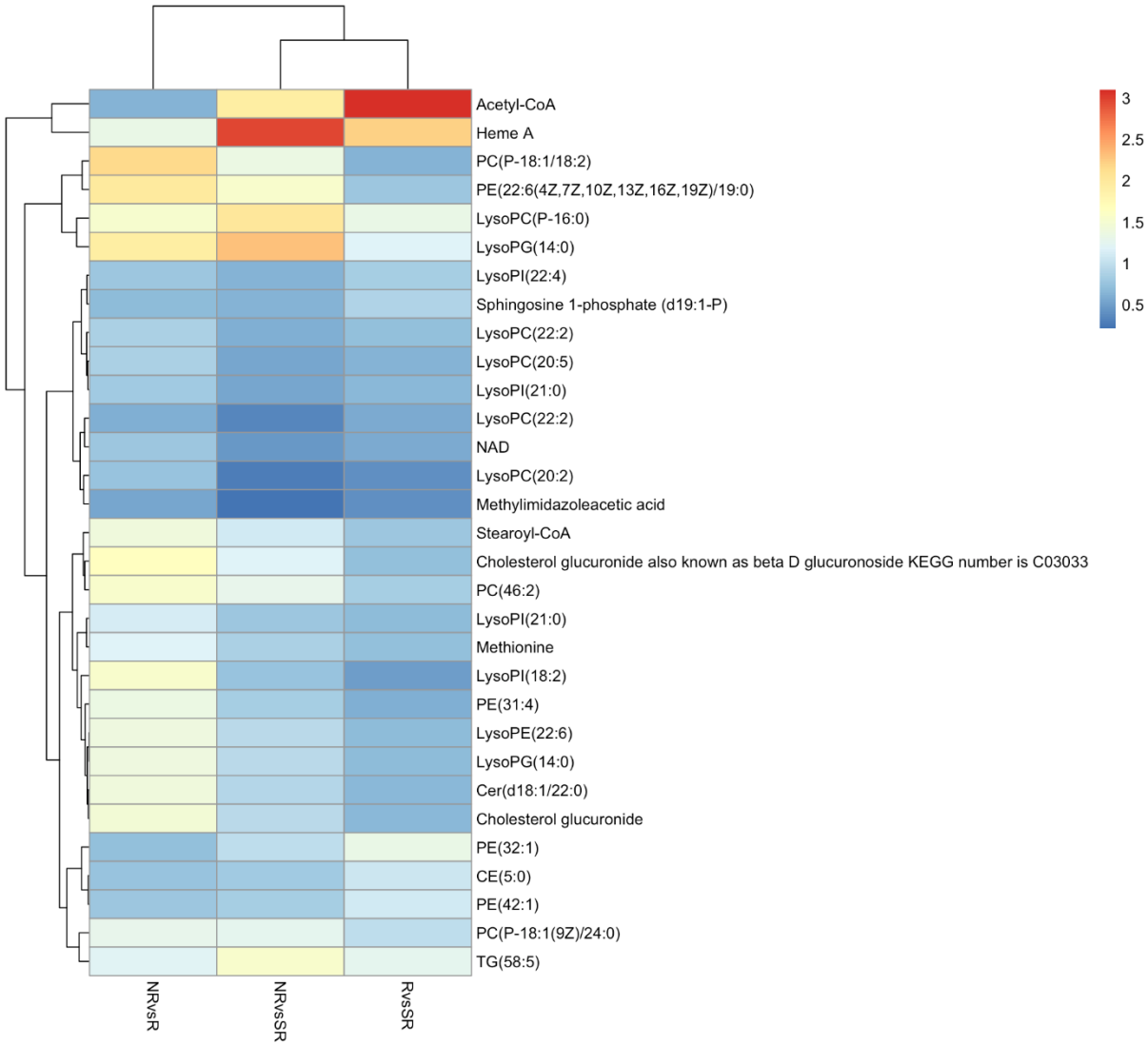


Figure 4.4 Heat Map analysis of Time Point 3. Each column shows, in order from left to right, Non Responders vs Responders, Non Responders vs Super Responders and Responders vs Super Responders. Time Point 1 = 2 months treatment, Time Point 2 = 4 months, Time Point 3 = 6 months.

A heat map analysis of each time point was carried out. Each column shows, in order from left to right, metabolite expression in groups of Non-Responders vs Good Responders, Non-Responders vs Super Responders and Good Responders vs Super Responders. Those metabolites which were highly expressed at each time point are coloured red, with the scale going from red at the highest expression, down to dark blue at the lowest. Time point 1 (Figure 4.2) shows data after 2 months treatment, time point 2 (figure 4.3) shows data after 4 months treatment, and time point 3 (figure 4.4) shows data after 6 months treatment.

Table 4.9. Major metabolite changes across Time Point 1 (2 months, TP1), Time Point 2 (4 months, TP2) and Time Point 3 (6 months, TP3) in Non-Responders vs Super Responders. Also shows regulation in super responders from baseline vs termination.

Metabolite	Pathway	TP1 NR/SR	TP2 NR/SR	TP3 NR/SR	Regulation in super responders (p<0.005)
Sphingosine-1-Phosphate	Sphingosine metabolism	0.685	0.878	0.868	Upregulated
Phosphatidylcholine	Glycerophospholipid metabolism	1.24	1.07	0.924	Downregulated
Phosphatidylethanolamine	Glycerophospholipid metabolism	1.07	1.086	2.29	Downregulated
Triacylglycerol	Glycerolipid metabolism	0.882	0.721	1.135	Initially upregulated, downregulated at TP3

Metabolomics analysis results revealed 4 major metabolite fold changes across the three time points in non-responders vs super responders. In the sphingosine metabolism pathway, the metabolite sphingosine-1-phosphate was significantly upregulated ($p<0.005$). In the glycerophospholipid metabolism pathway, the metabolites phosphatidylcholine (PC) and phosphatidylethanolamine (PE) were significantly downregulated ($p<0.005$). In the glycerolipid pathway, the metabolite triacylglycerol was initially upregulated, then downregulated at time point 3 ($p<0.005$).

4.3 Discussion

The administration of a daily dose of 3.0 mg of Liraglutide, in conjunction with standard NHS Tier 3 lifestyle advice and support for a period of six months, resulted in a significant increase in weight loss among overweight and obese adults without a diagnosis of diabetes in patients in the United Kingdom, which confirms the findings of another study which used 3.0 mg Liraglutide with patients in 27 other countries (Pi-Sunyer et al., 2015), as well as other studies using 3.0 mg Liraglutide in Europe (Astrup et al., 2012) and North America (Wadden et al., 2013).

The mean change in body weight with Liraglutide was -7.9±1 kg (-6.3±0.7%), with significant decreases in BMI., body fat percentage and fat mass. This is consistent with the results of the SCALE Obesity and Prediabetes trial, which included 3731 patients, which demonstrated that administering a daily subcutaneous dose of 3.0 mg of liraglutide in conjunction with a calorie-restricted diet and increased physical activity led to a notable decrease in weight among adults who were overweight or obese and did not have diabetes. No significant differences in response

to liraglutide were observed between subjects with prediabetes and those without, and the response was consistent across various baseline Body Mass Index (BMI) groups. The average weight loss associated with liraglutide treatment was 8 kg and remained relatively stable over the course of a one-year follow-up period (Pi-Sunyer et al., 2015). Liraglutide is also associated with significant BMI decreases in previous studies (Bensignor et al., 2021; Pi-Sunyer et al., 2015), which is consistent with the findings of the present study.

The results show a significant decrease in fat mass, with no significant change in fat free mass. A study by Ishii et al. (Ishii et al., 2019) found similar results, with Liraglutide (0.9 mg once daily for 24 weeks) reducing body fat, while having no significant effect on skeletal muscle. The results of the LEAD-3 trial, which involved administering a daily injection of either 1.2 mg or 1.8 mg of liraglutide for 52 weeks, also showed that there was no significant change in lean mass despite a noticeable decrease in body fat (Jendle et al., 2009).

In clinical trials, liraglutide has been shown to cause some changes in liver function tests, including AST (aspartate aminotransferase), ALT (alanine aminotransferase), and GGT (gamma-glutamyl transpeptidase). These enzymes are markers of liver function and are normally present in the blood at low levels. Elevated levels can indicate liver damage or disease. In studies, the most common liver-related side effect reported with liraglutide treatment was a mild, transient increase in ALT and AST levels. The increase in these enzymes tends to be small and typically returns to normal within a short period of time (Maor, Ergaz, Malnick, Melzer, & Neuman, 2021). In most cases, these elevations were not associated with symptoms of liver injury and did not lead to any serious side effects or discontinuation of treatment.

The study shows that Liraglutide 3 mg daily increased Amylase by 8.8±2.8% ($p=0.002$). This is similar to the SCALE Clinical Development Program findings (Steinberg et al., 2017), which show that Liraglutide 3 mg daily increased mean levels of Amylase by 7%. Amylase is a common biomarker of pancreatic inflammation, research has indicated that hepatic amylase may play a role in the metabolism of glycogen (Hariri & Thibault, 2010), as a result, regulating the activity of amylase may be a crucial strategy for identifying and addressing high blood sugar levels (McCue, Kwon, & Shetty, 2005).

There were non-significant decreases in blood pressure with a minor, non-significant increase in heart rate. This is consistent with findings of other studies (Liakos et al., 2019), finding modest changes in blood pressure and heart rate.

Significant increases in VitD25OH were seen in both the whole group data, and in the super responders group. When compared with liraglutide or vitamin D treatments individually, liraglutide combined with vitamin D treatment was more effective in treating overweight mice (F. Wang & Zhang, 2020). It is important to note that vitamin D deficiency is common in people with type 2 diabetes, which could be due to various factors such as lack of sun exposure, unhealthy diet and being overweight, among others. Obesity is commonly associated with a high incidence of vitamin D deficiency. This is likely due to the fact that the larger volume of fat, muscle, liver, and blood in obese individuals dilutes the amount of vitamin D present, although other factors may also play a role (Vranić, Mikolašević, & Milić, 2019).

The study found significant reductions in fatty acids in the super responder group, and downregulation of triglycerides at time point 3 in the same group. Triglycerides are a type of fat that circulates in the bloodstream and are stored in adipose tissue, while fatty acids are the building blocks of triglycerides (Alves-Bezerra & Cohen, 2017). Elevated levels of these lipids are associated with an increased risk of cardiovascular disease, so reducing their levels is an important goal in the management of diabetes and obesity. Liraglutide treatment decreases triglycerides (TGs) and free fatty acids (FFAs) (Z. Li et al., 2019). Liraglutide has been shown to improve lipid metabolism by increasing the activity of lipoprotein lipase (H. Wang & Eckel, 2009), which helps to break down triglycerides in the blood and convert them into energy.

Liraglutide has been shown to reduce the levels of fasting plasma glucose (Wajcberg & Amarah, 2010), which was seen in both the whole group analysis, as well as the non-responder and responder groups. The same study has also found Liraglutide to lower fasting plasma insulin, which was seen in the super responder group. The results also suggest that Liraglutide treatment improves β-cell function, as indicated by increased insulin sensitivity (HOMA-S) and reduced insulin resistance (HOMA-IR), which is consistent with previous research findings (Harder, Nielsen, Thi, & Astrup, 2004).

IL-6 is a pro-inflammatory cytokine that has been linked to the development of insulin resistance and is often elevated in individuals with type 2 diabetes. Studies have shown that

treatment with Liraglutide has been associated with a decrease in IL-6 levels (Brock et al., 2019). It appears that Liraglutide might act by decreasing the inflammation in adipose tissue and in that way decrease the IL-6 production (Gutierrez et al., 2022). This reduction in inflammation is one of the factors that might help in the improvement of insulin sensitivity and glucose control. However, it is important to note that the effect of Liraglutide on IL-6 may vary among patients, and further research is needed to fully understand the underlying mechanisms and confirm these findings.

Metabolomics analysis revealed significant upregulation of Sphingosine-1-Phosphate in super responders, along with downregulation of phosphatidylcholine and phosphatidylethanolamine in the same group ($p<0.005$). At termination of treatment, triglycerides were also downregulated, although they were initially upregulated. Previous research has also evidenced the changes in PC, PE and triglycerides, where 26 weeks of Liraglutide treatment significantly reduced all three metabolites (Emilie H Zobel et al., 2021). Phospholipids such as PC, PE and triglycerides are lipids identified as biomarkers and potential causes for cardiovascular disease, atherosclerosis and type 2 diabetes mellitus (Mah, Febbraio, & Turpin-Nolan, 2021). Another study with a large cohort (n=3779) has demonstrated that higher levels of phospholipids are associated with higher risk of morbidity and mortality (Alshehry et al., 2016). There is little research surrounding the effect of GLP-1 RA treatment on S1P levels. However, various research suggests that GLP-1 RA treatment can reduce levels of ceramide (Emilie H Zobel et al., 2021), which has been shown to be metabolised to sphingosine through the action of sphingosine kinase and ceramidase (Ilona Juchnicka, Mariusz Kuźmicki, & Jacek Szamatowicz, 2021), therefore indicating an inverse relationship between ceramide and S1P levels.

4.4 Conclusion

In this study, the administration of liraglutide was found to have several notable effects in individuals who responded exceptionally well to the treatment. Specifically, the use of liraglutide led to an increase in the expression of S1P (sphingosine-1-phosphate) and a reduction in IL-6 (interleukin-6) levels in these super-responders. This suggests that the signalling pathway involving S1P may play a significant role in determining the response to liraglutide treatment. The inclusion of 3.0 mg daily Liraglutide as part of a comprehensive weight management program offered within a UK NHS Tier-3/4 service has demonstrated positive outcomes. When combined with a reduced calorie diet and increased physical activity,

this treatment approach has been associated with significant reductions in body weight and improvements in metabolic control. The observed results are consistent with findings from regulatory trials, indicating that the use of 3.0 mg daily Liraglutide in this context can yield similar benefits in terms of weight reduction and metabolic improvements. These findings highlight the potential of Liraglutide as a valuable tool for healthcare professionals in their efforts to support individuals with weight management and metabolic control. By combining the medication with appropriate dietary modifications and increased physical activity, significant improvements can be achieved, contributing to better overall health outcomes. However, the clinical implications of these findings in regard to the lipid regulating effect of liraglutide requires further investigations.

Chapter 5 – In Vitro validation of hits obtained from metabolomics data.

5.1 Introduction

Obesity is a multifactorial disorder characterised by excessive accumulation of adipose tissue, leading to an increased risk of developing various metabolic diseases such as type 2 diabetes, cardiovascular diseases, and non-alcoholic fatty liver disease (NAFLD) (Longo et al., 2019). Glycerolipids, a class of lipids composed of glycerol backbone and fatty acid chains (J. Park et al., 2021), play a crucial role in the development and progression of obesity (Prentki & Madiraju, 2008). Glycerolipids are involved in energy storage (primarily in the form of triacylglycerol) (P. Zhang & Reue, 2017), insulin signalling (C. Zhang, Klett, & Coleman, 2013) and inflammation (Glass & Olefsky, 2012). Alterations in their metabolism have been linked to the development of obesity-associated complications. The dysregulation of glycerolipid metabolism in obesity is characterised by increased synthesis, decreased turnover, and altered composition of glycerolipids in adipose tissue, liver, and other tissues (Chavez & Summers, 2010).

Glycerophospholipids, another class of lipids that contain a glycerol backbone, are also involved in the pathogenesis of obesity. In lipid metabolism, glycerophospholipids serve as structural components of cell membranes (Farooqui, 2014) and are involved in cell signalling pathways (Hishikawa, Hashidate, Shimizu, & Shindou, 2014). They also play a role in the transport and storage of lipids. An important function of glycerophospholipids is their involvement in the biosynthesis of other lipids, such as triacylglycerols (TAGs) and phosphatidylcholine (PC) (Pan, Hu, & Yu, 2018). The glycerol backbone of glycerophospholipids can be used to synthesise TAGs (Allen, DiRusso, & Black, 2015), which are important energy storage molecules in the body. Additionally, the head group of glycerophospholipids can be used to synthesise PC. Glycerophospholipids are also involved in lipid signalling pathways, where they serve as precursors for the synthesis of signalling molecules such as diacylglycerol (DAG) and inositol triphosphate (IP3) (Ayala, Muñoz, & Argüelles, 2014). DAG and IP3 play important roles in intracellular signalling pathways and regulate a variety of cellular processes, including cell growth and differentiation. Alterations in the metabolism of glycerophospholipids have been linked to insulin resistance, inflammation, and other obesity-related complications. The dysregulation of glycerophospholipid metabolism in obesity is characterised by changes in the abundance and composition of different glycerophospholipid species in adipose tissue, liver, and other tissues. In addition, altered expression and activity of enzymes and cytokines involved in glycerophospholipid metabolism have been observed in obesity (Mendes-Frias et al., 2020).

Sphingolipids, a diverse group of lipids that contain a sphingosine backbone, have also emerged as important players in the pathogenesis of obesity. Sphingosine metabolites such as ceramides and sphingosine-1-phosphate (S1P) have been implicated in the regulation of adipocyte differentiation, insulin sensitivity, inflammation, and cell survival (I. Juchnicka, M. Kuźmicki, & J. Szamatowicz, 2021). Dysregulation of sphingolipid metabolism has been linked to the development of insulin resistance, NAFLD, and other obesity-related complications (Boini, Xia, Koka, Gehr, & Li, 2017). In particular, increased ceramide levels have been observed in adipose tissue and liver of obese individuals and animal models of obesity (Shah et al., 2008). Ceramides have been shown to impair insulin signalling and promote lipotoxicity in adipocytes and hepatocytes (Chaurasia & Summers, 2021). On the other hand, S1P has been shown to have insulin-sensitizing and anti-inflammatory effects in adipose tissue and other organs (Guitton et al., 2020). Understanding the mechanisms underlying the dysregulation of glycerolipid, glycerophospholipid and sphingolipid metabolism in obesity may provide new insights into the development of effective treatments for obesity and its associated metabolic diseases.

Due to their dysregulation in metabolic diseases, various circulating miRNA's were measured in the same patient samples as chapter 4 by this research group. The findings showed that good responders (via weight loss) had downregulation of miR-424 ($P<0.001$) whilst poor responders had upregulation of miR-424 ($P<0.01$) (Dimitriadis, 2021). There were no changes in other miRNAs.

Overall, understanding the molecular mechanisms by which GLP-1 receptor agonists induce gene expression changes may provide insights into new targets for obesity treatment and improve understanding of the metabolic pathways involved in GLP-1 receptor signalling. The aim of this study was to validate the top hits from the metabolomics, where the metabolite S1P was upregulated in super-responders, phosphatidylcholine and phosphatidylethanolamine were downregulated in the same group. Finally, gene expression changes with liraglutide treatment were identified in comparison with non-treated cells.

5.2 Results

An in vitro validation study was conducted using Liraglutide 100nm treatment on human preadipocyte cells to confirm the findings of the metabolomics from the previous chapter.

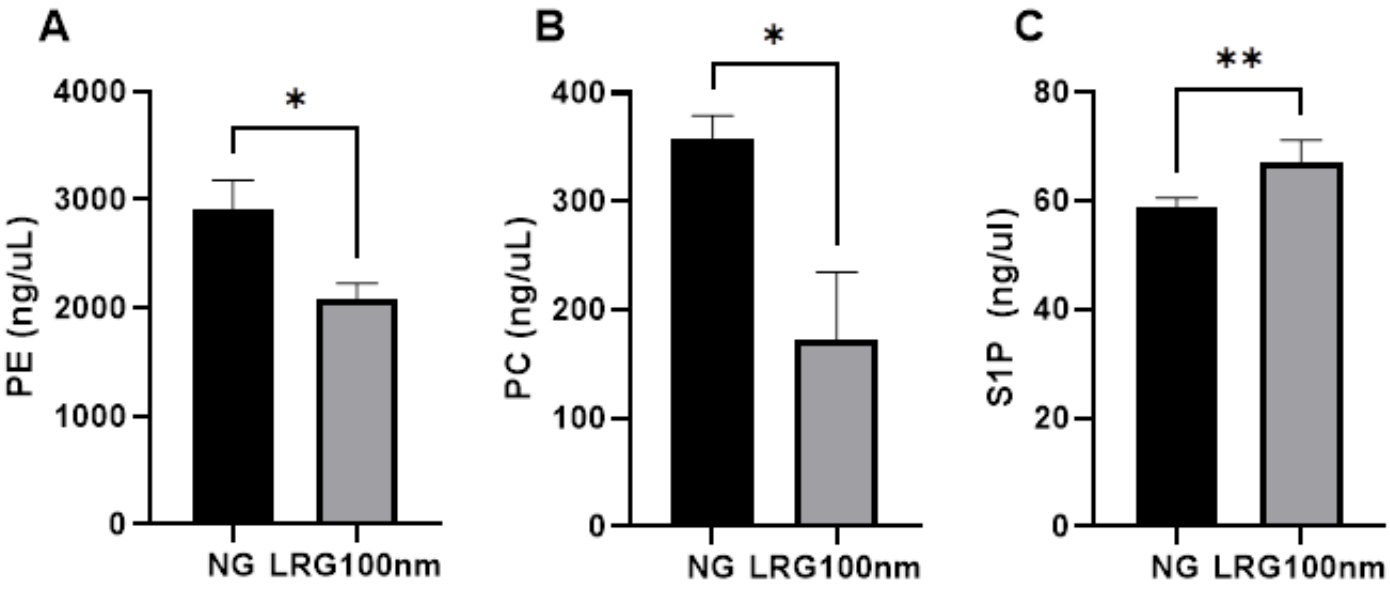


Figure 5.1 – Validation data for three most expressed metabolites in the human samples: A) Phosphatidylethanolamine, B) Phosphatidylcholine, C) Sphingosine-1-phosphate

After treating the preadipocytes with liraglutide, phosphatidylethanolamine was reduced by 828.2±321.1ng/uL, from 2908±863.7ng/uL to 2080±445.5ng/uL (p=0.019, n=5). Phosphatidylcholine was reduced by 187.2±66.39 ng/uL, from 358.6±45.86ng/uL to 171.4±141.2ng/uL (p=0.023, n=5). Sphingosine-1-phosphate was increased by 8.281±1.918 ng/uL, from 58.87±0.72 ng/uL to 67.15±1.78ng/uL (p=0.003, n=5).

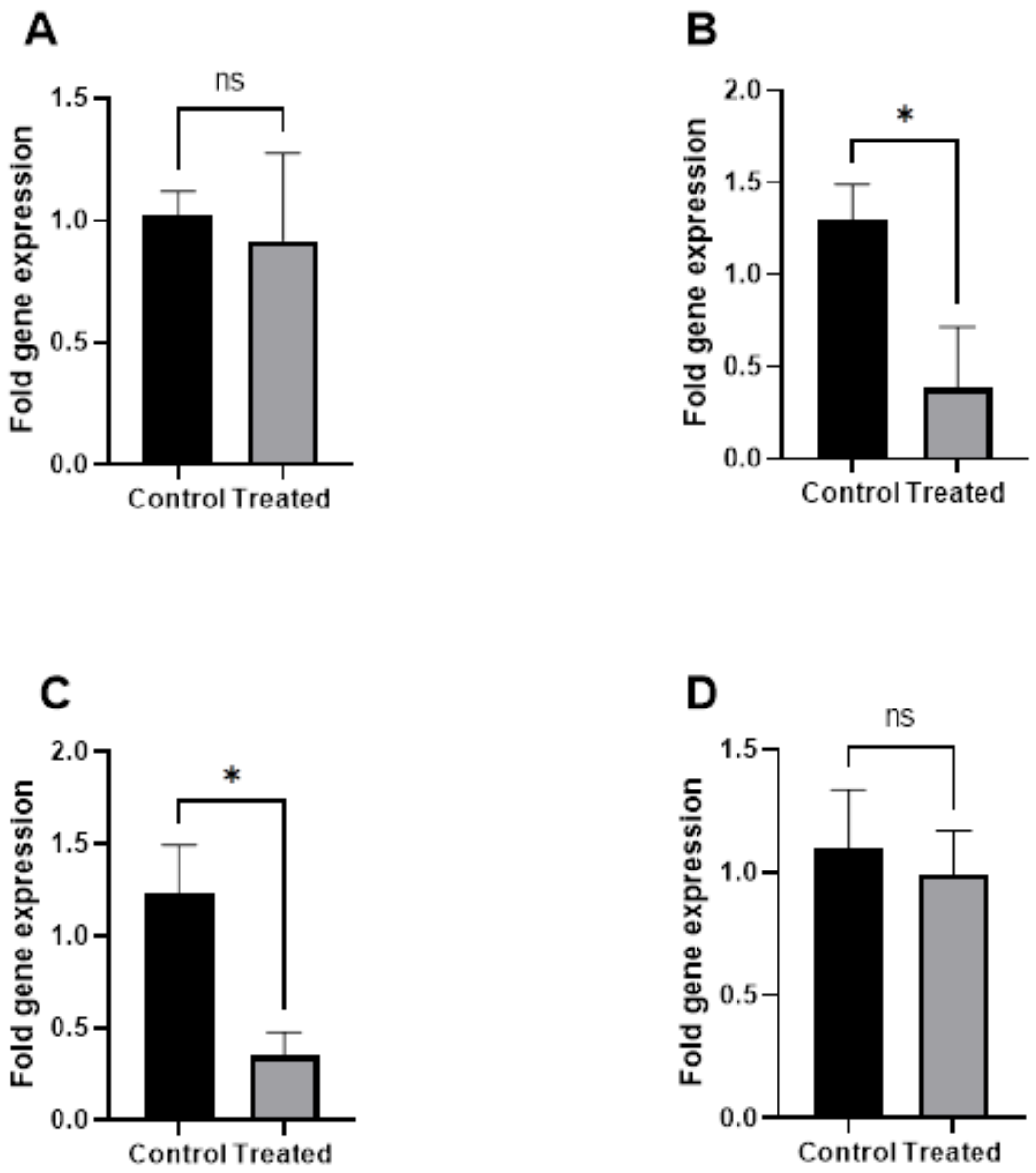


Figure 5.2 – Relative gene expression data for genes A) PLA2G2A, B) PLA2G4A, C) PLA2G6, D) PLA2G7

Relative gene expression data was measured using qPCR from cells treated with 100nm Liraglutide compared to non-treated samples, using housekeeping genes of GAPDH or Beta-actin. In PLA2G2A (Figure 5.2 A) no difference was seen between the control and treated

samples (-0.1050 ± 0.2729, p>0.05). There was also no discernible difference for PLA2G7 (Figure 5.2 D) between control and treated samples (-0.11 ± 0.34, p>0.05). PLA2G4A showed lower expression in the treated sample (Figure 5.2 B). Expression in the control sample was 1.3 ± 0.41, whereas the treated sample was 0.38 ± 0.58, a significant mean difference of -0.92 ± 0.35 (p=0.038). PLA2G6 also showed lower expression in the treated sample (Figure 5.2 C). Expression in the control sample was 1.23 ± 0.8, whereas the treated sample was 0.35 ± 0.25, a significant mean difference of - -0.88 ± 0.29 (p=0.013).

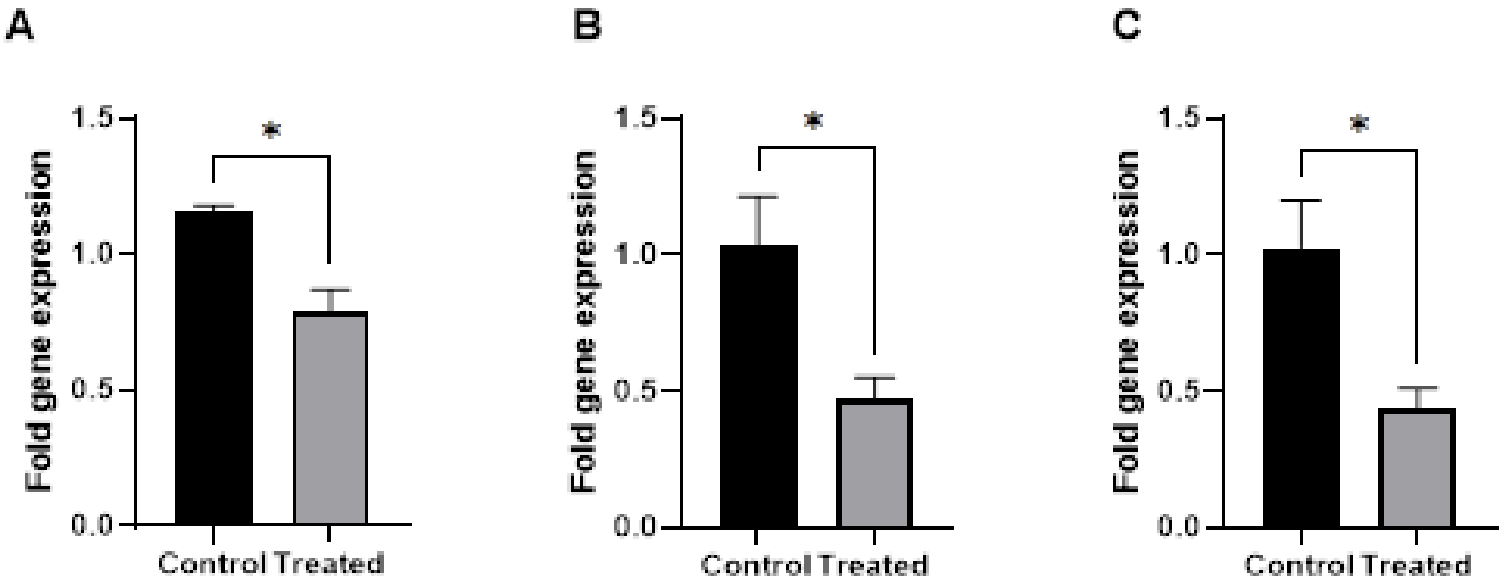


Figure 5.3 – Relative gene expression data for genes A) Ceramide, B) SPHK1, C) SPHK2

Ceramide showed a lower expression in the liraglutide treated samples when compared with the control samples (Figure 5.3A). In the control sample, expression was 1.16 ± 0.03, compared to 0.79 ± 0.14 in treated, for a mean difference of -0.37 ± 0.11 (p<0.05). Similarly, SPHK1 (Figure 5.3B) and SPHK2 (Figure 5.3C) both showed lower expression in the treated samples. SPHK1 control samples had expression of 1.03 ± 0.32, with liraglutide samples 0.47 ± 0.14 for a mean difference of -0.56 ± 0.2 (p<0.05). SPHK2 control samples had expression of 1.03 ± 0.3, with liraglutide samples 0.44 ± 0.12 for a mean difference of -0.59 ± 0.19 (p<0.05).

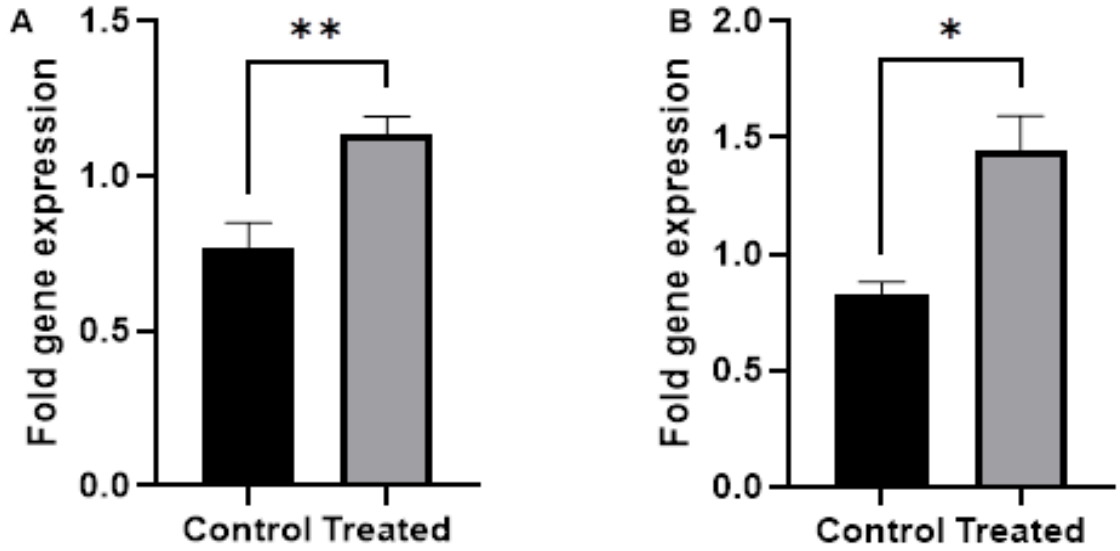


Figure 5.4 – Relative gene expression data for genes A) PTPN3, B) SGPP1

PTPN3 had higher expression in the liraglutide treated group, compared to the control samples (Figure 5.4 A). In the control sample, expression was 0.77 ± 0.16, compared to 1.14 ± 0.13 in treated, a mean difference of 0.37 ± 0.09 ($p=0.006$). SGPP1 (Figure 5.4 B) shows similar trends, with the liraglutide group having higher expression than the control group. In the control sample, expression was 0.83 ± 0.07, compared to the liraglutide group expression being 1.45 ± 0.25, a mean difference of 0.62 ±0.19 ($p<0.05$).

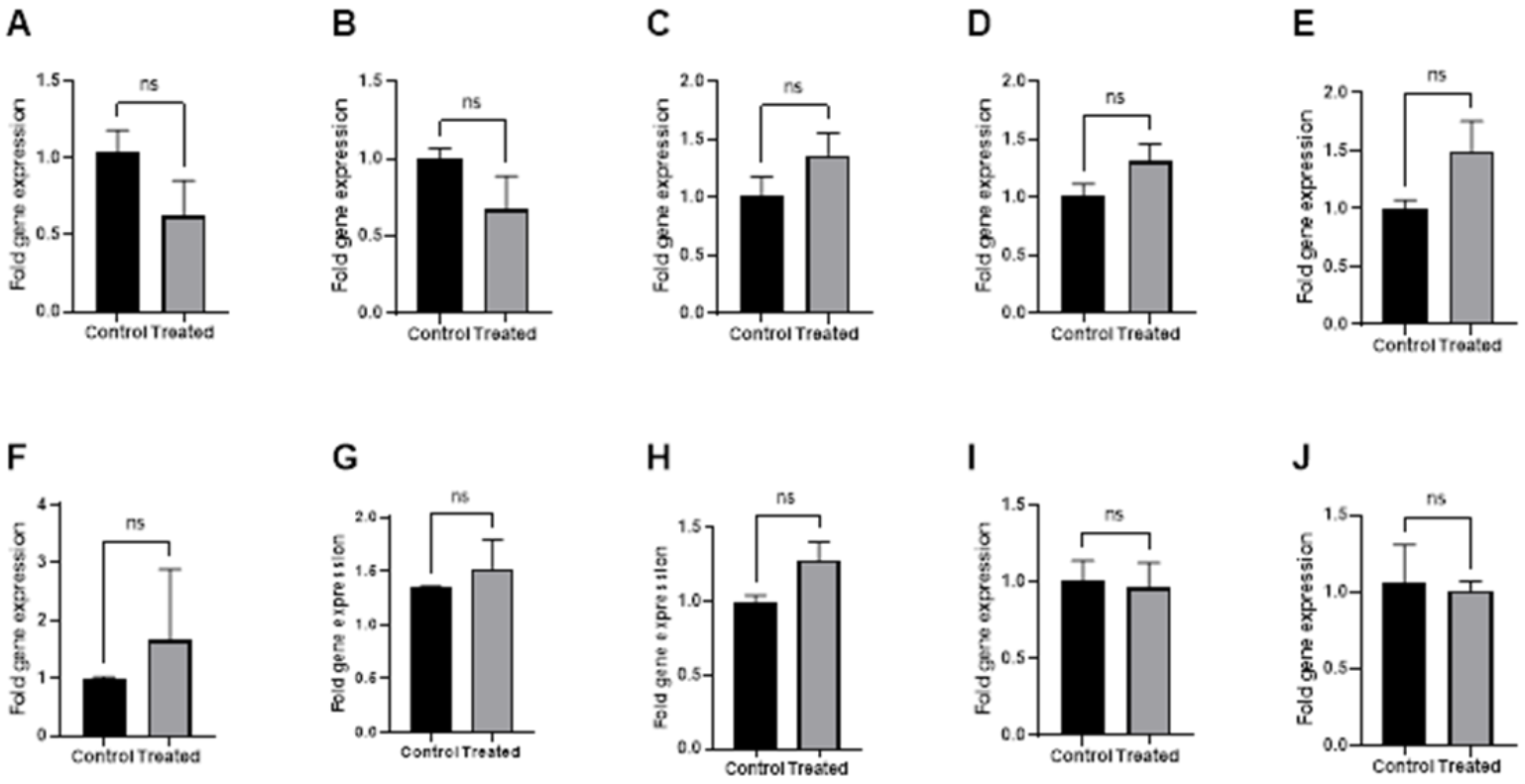


Figure 5.5 – Relative gene expression data for genes A) S1PR1, B) IGF1R, C) PLCD1, D) MAP2K1, E) RAF1, F) IRS1, G) AKT1, H) ZNF1, I) PDK1, J) SHMT

S1PR1 (Figure 5.5. A) had lower expression in the treated sample, although this figure was non-significant (p=0.141). The control sample had an expression of 1.04 ± 0.34 compared to 0.63 ± 0.39 in the treated, a mean difference of -0.42 ± 0.25.

IGF1R (Figure 5.5. B) had lower expression in the treated sample (p=0.07). The control sample had an expression of 1.05 ± 0.12 compared to 0.67 ± 0.37 in the treated, a mean difference of -0.3780 ± 0.1719. PLCD1 (Figure 5.5C) had higher expression in the treated sample (p=0.235). The control sample had an expression of 1.02 ± 0.26 compared to 1.36 ± 0.33 in the treated, a mean difference of 0.3400 ± 0.2434. MAP2K1 (Figure 5.5 D) had higher expression in the treated sample (p=0.169). The control sample had an expression of 1.01 ± 0.18 compared to 1.31 ± 0.26 in the treated, a mean difference of 0.3033 ± 0.1810. RAF1 (Figure 5.5E) had lower expression in the treated sample (p=0.134). The control sample had an expression of 1 ± 0.13 compared to 1.5 ± 0.44 in the treated, a mean difference of 0.4967 ± 0.2652. IRS1 (Figure 5.5 F) had higher expression in the treated sample (p=0.622). The control sample had an expression

of 1 ± 0.43 compared to 1.66 ± 2.13 in the treated, a mean difference of 0.6567 ± 1.232. AKT1 (Figure 5.5 G) had slightly higher expression in the treated sample (p=0.07). The control sample had an expression of 1.36 ± 0.01 compared to 0.53 ± 0.37 in the treated, a mean difference of 0.1700 ± 0.2650. ZNF1 (Figure 5.5 H) had higher expression in the treated sample (p=0.102). The control sample had an expression of 1 ± 0.08 compared to 1.28 ± 0.22 in the treated, a mean difference of 0.2800 ± 0.1324. PDK1 (Figure 5.5 I) had no change between control and treated samples (p=0.809). The control sample had an expression of 1.01 ± 0.22 compared to 0.96 ± 0.29 in the treated, a mean difference of -0.05333 ± 0.2064. SHMT (Figure 5.5 J) had no change between control and treated samples (p=0.866). The control sample had an expression of 1.06 ± 0.44 compared to 1.01 ± 0.12 in the treated, a mean difference of -0.04667 ± 0.2593.

From the results of the previous chapter, the metabolic pathways of Sphingosine, Glycerophospholipid and Glycerolipid were changed in the super responder group based on weight loss. Table 5.1 shows each gene along with their associated pathway of the three above, as well as showing if they are involved in insulin signalling pathways.

Table 5.1 – Genes analysed in qPCR, with full name and symbol, metabolic pathway, direction of expression change with liraglutide compared with control, and associated p value from t-test. *denotes significance.

GENE INFORMATION		METABOLIC PATHWAY				STATISTICS	
Gene symbol	**Gene Name**	**Sphingosine**	**Glycerolipid**	**Glycerophospholipid**	**Insulin Signalling**	**Expression change**	**p Value**
AKT1	AKT serine/threonine kinase 1	✓	✓	✓	✓	↑	0.587
CER	Ceramide synthase 2	✓	✓	✓		↓	0.041*
IGF1R	Insulin-like growth factor 1 receptor				✓	↓	0.07
IRS1	Insulin receptor substrate 1			✓	✓	↑	0.622
MAP2K1	Dual specificity mitogen-activated protein kinase kinase 1			✓		↑	0.169
PDK1	3-phosphoinositide dependent protein kinase-1				✓	↔	0.809
PLA2G2A	Phospholipase A2 group IIA		✓	✓		↓	0.712
PLA2G4A	Phospholipase A2 group IVA		✓	✓		↓	0.038*
PLA2G6	Phospholipase A2 group VI	✓		✓		↓	0.013*
PLA2G7	Phospholipase A2 group VII	✓	✓	✓		↓	0.764
PLCD1	Phospholipase C delta 1			✓		↑	0.235
PNPLA3	Patatin-like phospholipase domain containing 3		✓	✓		↓	0.434
PTPN3	Protein tyrosine phosphatase, non-receptor type 3				✓	↑	0.006*
RAF1	RAF proto-oncogene serine/threonine-protein kinase				✓	↑	0.134
S1PR1	Sphingosine-1-phosphate receptor 1	✓				↓	0.141
SGPP1	Sphingosine-1-phosphate phosphatase 1	✓				↑	0.047*
SHMT1	Serine hydroxy methyltransferase 1				✓	↔	0.866
SPHK1	Sphingosine kinase 1	✓				↓	0.048*
SPHK2	Sphingosine kinase 2	✓				↓	0.036*
ZNF1	Zinc finger protein 1					↑	0.102

5.3 Discussion

The aim of this study was to validate the metabolomics findings from the previous chapter, which identified changes in lipid metabolism associated with liraglutide treatment. The results of this in vitro validation study confirmed the metabolomics data and shed light on the molecular mechanisms underlying these changes.

The treatment of human preadipocyte cells with liraglutide resulted in a significant decrease in the levels of phosphatidylethanolamine and phosphatidylcholine, which are important components of cellular membranes (van der Veen et al., 2017). Liraglutide has been shown to promote weight loss in humans (Alruwaili, Dehestani, & le Roux, 2021), and it is possible that the decrease in PE and PC levels observed in preadipocyte cells treated with liraglutide may reflect a reduction in the size or number of adipocytes (van der Veen et al., 2017). This could occur if liraglutide is promoting the breakdown of triglycerides within the adipocytes, leading to a decrease in the size of the lipid droplets and ultimately the size of the adipocytes themselves. Therefore, the decrease in PE and PC levels may be an indicator of reduced fat storage in adipocytes, which could contribute to the weight loss effects of liraglutide. Sphingosine-1-phosphate, a lipid that is involved in cell proliferation and migration, was increased by liraglutide treatment, which could reflect the activation of signalling pathways that promote cell survival and growth. S1P is seen as a protective lipid molecule for T2D, which can work in the central nervous system or in the peripheral organs (He et al., 2021). In the central nervous system, S1P supresses appetite by inhibiting neurotransmitter function in the hypothalamus (Obici, Zhang, Karkanias, & Rossetti, 2002). S1P exerts various effects on peripheral organs including resistance to inflammation and oxidative stress, inhibition of insulin resistance progression, increase of GLP-1 secretion and efficacy, and kidney protection (He et al., 2021).

The study group also investigated the changes in microRNA expression associated with liraglutide treatment (Dimitriadis, 2021). MicroRNAs are short RNA molecules that regulate gene expression and have been implicated in the pathogenesis of obesity and diabetes (Williams & Mitchell, 2012). The results showed that miRNA 424 was significantly reduced in the responder group but increased in the non-responder group after 2 months of liraglutide treatment. This finding suggests that miRNA 424 may be a biomarker for predicting response to liraglutide treatment and could potentially be targeted for therapeutic intervention. Research

has suggested that miR-424 is upregulated in obese children when compared with non-obese children (Xiao, Zhu, Fu, Guo, & Chi, 2021).

The expression of miR-424 target genes were analysed, including PLA2G2A, PLA2G4A, PLA2G6, SGPP1, PTPN3, S1PR1, SPHK1, and SPHK2. The results showed that liraglutide treatment led to a significant decrease in the expression of PLA2G4A and PLA2G6, which are involved in the hydrolysis of phospholipids and the generation of inflammatory mediators (Deng et al., 2016; Hartiala, Gilliam, Vikman, Campos, & Allayee, 2012). This finding suggests that liraglutide may reduce adipose tissue inflammation by inhibiting the activity of these enzymes.

The results of the study demonstrated a significant reduction in the expression of ceramide in the samples treated with liraglutide. Ceramide is a type of sphingolipid, a class of lipids that plays a critical role in cellular signalling and metabolism. Ceramide is involved in a variety of cellular processes, including cell death, inflammation, and insulin resistance. (Mandal et al., 2021). In the context of metabolic disorders such as type 2 diabetes and obesity, increased ceramide levels have been shown to contribute to the development of insulin resistance and pancreatic beta-cell dysfunction, leading to impaired glucose homeostasis and hyperglycaemia (Mandal et al., 2021). The reduction in ceramide expression observed in the liraglutide-treated samples may be due to the drug's ability to enhance insulin sensitivity. Insulin resistance is known to increase ceramide levels by activating ceramide synthesis enzymes and inhibiting ceramide breakdown enzymes (Sokolowska & Blachnio-Zabielska, 2019). Liraglutide, by improving insulin sensitivity, may reduce the production of ceramide and promote its breakdown, leading to a decrease in ceramide expression.

In addition to its effects on insulin sensitivity, liraglutide has been shown to have anti-inflammatory effects (Meurot et al., 2022). Chronic low-grade inflammation is a characteristic feature of metabolic disorders such as type 2 diabetes and obesity (Oguntibeju, 2019) and has been linked to the development of insulin resistance and other complications. Ceramide has been shown to promote inflammation by activating pro-inflammatory signalling pathways and inducing the production of inflammatory cytokines (Maceyka & Spiegel, 2014). By reducing ceramide expression, liraglutide may help to attenuate this inflammatory response, leading to a reduction in inflammation and improved overall health.

In contrast, liraglutide treatment led to a significant increase in the expression of PTPN3 and SGPP1, which are involved in the regulation of insulin signalling and lipid metabolism (Elbein et al., 2011). PTPN3 and SGPP1 are two genes that play important roles in regulating insulin signalling and lipid metabolism. Specifically, PTPN3 is a protein tyrosine phosphatase that regulates insulin signalling by dephosphorylating the insulin receptor (Dubois et al., 2006), while SGPP1 is an enzyme involved in the breakdown of sphingolipids (Ogretmen, 2018), which are important components of cellular membranes and signalling pathways. This finding suggests that liraglutide may improve metabolic health by activating these pathways.

The sphingosine pathway is a complex network of lipid metabolites, enzymes, and signalling molecules that play crucial roles in several cellular processes, including cell growth, differentiation, and apoptosis (Pralhada Rao et al., 2013). Ceramide, sphingosine, and sphingosine-1-phosphate (S1P) are key metabolites in this pathway, and their levels are tightly regulated by various enzymes, including sphingosine kinase 1 (SphK1), sphingosine kinase 2 (SphK2), and sphingosine-1-phosphate phosphatase 1 (SGPP1). The findings have demonstrated a decrease in ceramide levels, an increase in S1P levels (from the previous chapter), a decrease in SphK1 levels, a decrease in SphK2 levels, and an increase in SGPP1 levels. These changes suggest an overall increase in the conversion of ceramide to S1P, which is primarily mediated by the decrease in SphK1 and SphK2 levels. Additionally, the increase in SGPP1 levels suggests an enhanced breakdown of S1P to sphingosine and eventually to ceramide (Hammerschmidt & Brüning, 2022).

5.4 Conclusion

In summary, the results of this study provide further evidence for the beneficial effects of liraglutide on lipid metabolism, inflammation, and insulin sensitivity. The findings also suggest that miRNA expression and gene expression profiling may be useful tools for predicting response to liraglutide treatment and identifying novel therapeutic targets for obesity and diabetes. However, further studies are needed to validate these findings and elucidate the molecular mechanisms underlying the observed effects of liraglutide on adipose tissue function.

Chapter 6 – Overall Discussion and Conclusions

6.1 Overall Discussion

This thesis researched the clinical and metabolic effects of Liraglutide on patients with overweight and type 2 diabetes. The study aimed to assess the effect of low dosage Liraglutide 3.0mg once daily in patients with overweight (BMI: ≥27Kg/m^2) or obesity (BMI: ≥30Kg/m^2) with regards to weight loss related to treatment. It also aimed to investigate the beneficial metabolic sequelae of Liraglutide in patients with obesity or overweight, including changes in vital signs, anthropometric characteristics (weight, body mass index and body composition), biochemical parameters, metabolomics, and miRNA molecules from blood tests. A comprehensive and methodical analysis, adhering to both the Cochrane and PRISMA guidelines, was undertaken in order to establish an evidence base regarding the current research on the efficacy of Liraglutide, specifically in relation to metabolic changes resulting from its administration. The application of these established guidelines was deemed necessary in order to ensure a robust and reliable review of the available literature. This review did not examine the efficacy of Liraglutide in regard to weight loss, as this is already widely researched and was not necessary. A prospective open label study was used to gather data from human participants treated with Liraglutide. Patients received standard lifestyle advice and support in addition to the medication and were followed for six months after treatment initiation. Patients who did not achieve at least a 5% weight loss after four months of treatment were withdrawn from the study. The trial included several visits where patients underwent physical examinations, blood tests, and body composition analyses using the BodPod. Patients were given education on using the medication and managing potential side effects. After this study, in vitro studies were used to examine further effects of Liraglutide. Standardised analytical laboratory techniques were employed to systematically research the role of Liraglutide in metabolic health. This body of research has yielded various novel findings, which offer valuable mechanistic insight into the therapeutic application of liraglutide for the treatment of overweight and obesity. Additionally, the research has identified promising therapeutic targets that may be utilised to enhance metabolic health.

We conducted a study on the current evidence relating to obesity, type 2 diabetes, and the subsequent treatment options. The main outcome of the initial literature review found the following: the global incidence of obesity has reached epidemic proportions, with the prevalence of obesity having doubled since 1980. This phenomenon represents a significant issue among both children and adults.

Obesity is considered a crucial risk factor for the development of type 2 diabetes, primarily due to the accumulation of excess adipose tissue in the body, leading to insulin resistance and impairment of insulin utilisation. Consequently, this condition leads to elevated blood glucose levels, ultimately resulting in hyperglycaemia. In the United Kingdom alone, obesity-related costs have exceeded £6.1 billion in 2014/15, underscoring the magnitude of the problem. Given its multifactorial nature, obesity has a wide array of causes, including but not limited to genetics, biology, healthcare access, mental health, sociocultural factors, equity, ultra-processed foods, economics, commercial determinants, and environmental determinants, which interact in complex ways. Type 2 diabetes has emerged as a significant complication of obesity, as chronic inflammation arising from excessive adipose tissue contributes to insulin resistance and the eventual development of type 2 diabetes. Notably, a substantial proportion of individuals diagnosed with type 2 diabetes have a history of overweight or obesity. The biological mechanisms underlying type 2 diabetes mellitus include peripheral insulin resistance, impaired hepatic glucose production regulation, and declining β-cell function, culminating in β-cell failure.

Given the extensive body of research on the use of GLP-1 receptor agonists for overweight and obesity, a systematic review was deemed necessary to determine the current state of knowledge regarding the treatment's effects. Although numerous studies have examined the clinical outcomes of GLP-1 receptor agonist therapy, a significant portion of the research has focused on weight loss without elucidating the mechanisms underlying the observed effects. Therefore, the aim of the systematic review was to identify the metabolic alterations associated with GLP-1 receptor agonist therapy. The systematic review in chapter 3 evaluated the metabolic effects of GLP-1 receptor agonist treatment for patients with obesity and type 2 diabetes. The analysis included 13 randomised controlled trials with 713 participants, and the results showed that GLP-1 RA treatment led to reductions in ALT, AST, GGT, fasting insulin, adiponectin, apolipoprotein B, leptin, c-reactive protein and fructosamine. GLP-1RA treatment increased creatinine and c-peptide levels. Overall, the findings suggest that the effects of GLP-1RA treatment in reducing body fat and weight may be linked to the reduction of the liver enzymes AST, ALT, and GGT. Additionally, GLP-1 agonist treatment significantly decreased c-reactive protein and fructosamine, which are markers of inflammation and glycaemic control, respectively. This study offers novel data, as it is the most comprehensive analysis of the effects of GLP-1RA's on obesity and type 2 diabetes. The clinical implications of these findings are at present limited, due to heterogeneity of the included studies, as well as GLP-1RA's in this study

being analysed together, rather than as the individual compounds such as semaglutide and liraglutide. However, the study has provided more evidence that GLP-1 receptor agonist (RA) treatment can be a viable option for managing metabolic disorders such as obesity and type 2 diabetes, as it has shown to have beneficial effects on various metabolic parameters. The reduction in fasting insulin levels observed with GLP-1RA treatment could be due to weight loss and decreased insulin resistance (C. Guo et al., 2016). Therefore, GLP-1RA treatment may be especially beneficial for patients with hyperinsulinemia and obesity. The increase in creatinine levels seen in the meta-analysis could be an indirect reflection of skeletal muscle mass, which is associated with type 2 diabetes (Song, Hong, Sung, & Lee, 2022). Therefore, GLP-1RA treatment may have a positive effect on muscle mass in diabetic patients. While the meta-analysis overall found a slight decrease in adiponectin levels, the high heterogeneity observed in the studies may indicate that GLP-1RA treatment has a variable effect on adiponectin levels, with some studies showing an increase and some showing a decrease. However, an increase in adiponectin levels is generally considered beneficial for metabolic health (Achari & Jain, 2017). The significant decrease in c-reactive protein levels observed with GLP-1RA treatment may indicate a decrease in inflammation (S. Verma et al., 2023), which is associated with metabolic disorders such as obesity and type 2 diabetes. Finally, the decrease in leptin levels seen with GLP-1RA treatment may help control appetite and energy intake (Iepsen et al., 2015), making it a potential treatment option for obesity alone without the presence of type 2 diabetes.

The study in chapter 4 provides novel clinical findings, as it was, to our knowledge, the first of its kind in the UK. It found 3.0 mg daily Liraglutide as an adjunct to a reduced calorie diet and increased physical activity offered within a UK NHS Tier-3/4 weight management service is associated with reduced body weight, and improved metabolic control similar to that which has been reported by regulatory trials. In the SCALE (Pi-Sunyer et al., 2015) trial conducted in 27 countries across Europe, North America, South America, Asia, Africa and Australia, patients body weight changed -8.4±7.3 kg, which is a similar finding to the present study, with change of -7.9±1 kg. A systematic review of Liraglutide 3.0mg treatment also validates the findings of the study, as the review found that Liraglutide induces weight loss of 5.9-8.0% (A Christou, Katsiki, & N Kiortsis, 2016), the present study found a weight loss of 6.3% initial body weight.

The study also suggests that Liraglutide treatment induces an increase in insulin sensitivity. However, this result was only significant in the super responder group, indicating that this

effect is more pronounced with higher degrees of weight loss. Several studies have investigated the relationship between weight loss and insulin sensitivity. Takeshita (Takeshita, 1995) demonstrated that weight loss in obese hypertensive patients resulted in a significant increase in insulin sensitivity of 54.3%. Similarly, a 2004 study (Ryan & Nicklas, 2004) reported that weight loss was associated with a reduction in cytokine concentrations, which was linked to improvements in insulin sensitivity. Houmard (Houmard et al., 2002) revealed that weight loss led to a decrease in intramyocellular long-chain fatty acyl-CoAs, which might contribute to the enhanced insulin action observed in obese individuals after weight loss. Schenk (Schenk, Harber, Shrivastava, Burant, & Horowitz, 2009) further confirmed that decreased fatty acid mobilization and uptake is a primary mediator of improved insulin sensitivity following weight loss. A recent study (Vazquez Arreola, Knowler, & Hanson, 2022) found that weight loss decreased insulin secretory demand and increased compensatory insulin secretion, which was directly related to the degree of weight loss. Moreover, another study (Clamp, Hume, Lambert, & Kroff, 2017) found that individuals who maintained successful weight loss had improved insulin sensitivity compared to those with no history of weight loss, and weight loss itself was the strongest predictor of enhanced insulin sensitivity, while weight regain was associated with reduced insulin sensitivity. Overall, it has been shown that insulin sensitivity gradually increased with weight loss in obese patients (Kong, Xiao, Zhang, & Liu, 2020), a finding which has been corroborated with the study in chapter 4.

The findings of the study also indicate that IL-6 is significantly correlated with weight loss, and that the group with weight loss over 10% initial body weight saw a significant decrease in IL-6 concentrations. These results are consistent with current knowledge regarding the role of IL-6 in the metabolism of stored fat and its release into the bloodstream during calorie deficit, which is known to occur during weight loss (Kistner, Pedersen, & Lieberman, 2022). Thus, it is possible that changes in IL-6 levels may be associated with changes in body weight. The observed decrease in IL-6 concentrations in the group with weight loss over 10% initial body weight suggests that, as the body weight decreases, the need to mobilize stored fat decreases as well, resulting in lower levels of IL-6 (Trinh et al., 2021). These findings align with previous research that has reported a correlation between IL-6 levels and body mass index (BMI), with higher levels of IL-6 seen in individuals with obesity (Sindhu et al., 2015). Overall, this study provides additional evidence for the relationship between IL-6 and weight loss and highlights the potential use of IL-6 as a biomarker for weight loss success. However, further research is

needed to fully comprehend the underlying mechanisms and therapeutic implications of this relationship.

In the study in chapter 4, metabolomics analysis revealed a significant upregulation of Sphingosine-1-Phosphate (S1P) in super responders, accompanied by a downregulation of phosphatidylcholine (PC) and phosphatidylethanolamine (PE) in the same group. Additionally, triglycerides were downregulated at the end of treatment, despite being initially upregulated. Prior research has established that treatment with the GLP-1 receptor agonist Liraglutide can lead to a reduction in PC, PE, and triglycerides. These lipids are biomarkers and potential causes of cardiovascular disease, atherosclerosis, and type 2 diabetes mellitus. The LiraFlame (E. H. Zobel et al., 2021) study with a large cohort (n=3779) demonstrated that higher levels of phospholipids are associated with an increased risk of morbidity and mortality. The initial increase of triglycerides could be due to liraglutide slowing gastric emptying which can reduce the clearance of lipids from the bloodstream and increase triglyceride levels. Additionally, GLP-1 RAs have been shown to increase lipolysis, the breakdown of stored fats in adipose tissue. This can release triglycerides into the bloodstream and contribute to elevated levels. While an initial increase in triglycerides after starting GLP-1 RA treatment may be concerning, it's important to note that long-term treatment has been associated with improved lipid profiles and reduced cardiovascular risk. Although little research exists on the effect of GLP-1 RA treatment on S1P levels, studies suggest that GLP-1 RA treatment can reduce ceramide levels (Wretlind et al., 2023). Ceramide is metabolized into S1P through the action of sphingosine kinase and ceramidase, indicating an inverse relationship between ceramide and S1P levels. S1P is a bioactive lipid that has been shown to regulate immune function, inflammation, and vascular integrity.

The metabolomics findings listed above were confirmed at the start of the study in chapter 5, which confirmed the findings using an in vitro validation study. Human studies can provide valuable insights into the effectiveness of a treatment or drug in the human body, but their complexity and multiple variables can limit the depth of understanding of the underlying mechanisms. In vitro studies, on the other hand, are conducted in a laboratory setting outside of a living organism using isolated cells or tissues, offering a more controlled environment for experimentation. Validating the findings of a human study in vitro can provide a more targeted and refined analysis by isolating specific variables and controlling for other factors. In vitro studies can also offer insights into molecular and cellular targets, which may not be feasible in

human studies. The results from in vitro studies are more interpretable and provide a more robust foundation for informed decision-making. Thus, the in vitro study in chapter 5 confirmed the findings of chapter 4, as the treatment of human preadipocyte cells with liraglutide resulted in significant decrease of phosphatidylethanolamine and phosphatidylcholine, alongside a significant increase in sphingosine-1-phosphate.

Chapter 5 also included miRNA changes with Liraglutide treatment, as the data is pertinent to the findings of this thesis and was carried out by the same research group. The presented results suggest that the expression of miRNA 424 may be affected by weight loss treatment in a differential manner depending on individual response. Initially, when miRNA 424 was analysed as a whole group, a significant decrease in fold change was observed after 2 months of treatment, although some individuals showed an increase in expression. This indicates that the overall effect may be influenced by individual response to treatment. To further explore this possibility, the participants were stratified into responders and non-responders based on weight loss data. The results indicate that changes in miRNA 424 expression after 2 months of treatment differed significantly between these two groups. Specifically, miRNA 424 was significantly reduced in the responder group, while it significantly increased in the non-responder group. These findings suggest that miRNA 424 may serve as a promising biomarker for predicting individual response to weight loss treatment. The differential effects of treatment on miRNA 424 expression observed in responders and non-responders could provide valuable insights into the underlying mechanisms of weight loss and pave the way for more personalized weight loss interventions. However, it is important to acknowledge the limitations of the study, such as the relatively small sample size. Further research with larger cohorts is necessary to validate these findings. Additionally, it would be beneficial to investigate the functional implications of the observed changes in miRNA 424 expression and elucidate potential interactions with other molecular pathways involved in weight loss.

The relative gene expression data obtained through qPCR analysis indicated that liraglutide treatment at a concentration of 100nm did not significantly alter the expression of PLA2G2A and PLA2G7, which are genes involved in the regulation of lipid metabolism (M. Zhang, Xiang, Glorieux, & Huang, 2022). On the other hand, the expression of PLA2G4A and PLA2G6, which are also involved in lipid metabolism, was significantly reduced in the liraglutide-treated group compared to the control group. These findings suggest that liraglutide may potentially regulate lipid metabolism by downregulating the expression of specific genes

in this pathway. There could be several reasons why the expression of PLA2G4A and PLA2G6 was significantly reduced, while PLA2G2A and PLA2G7 were not affected by liraglutide treatment in this study. One possibility is that PLA2G4A and PLA2G6 are more sensitive to changes in the cellular environment, such as changes in intracellular signalling pathways or alterations in the expression of other genes involved in lipid metabolism. Another possibility is that liraglutide may affect the expression of these genes indirectly, through the regulation of other molecular pathways.

The expression of PTPN3 and SGPP1, two genes involved in inflammation, were significantly increased in the liraglutide-treated group compared to the control group. These findings suggest that liraglutide may have anti-inflammatory effects by upregulating the expression of specific genes involved in the regulation of inflammation. It is important to note that the present study was conducted in vitro and may not reflect the in vivo effects of liraglutide on gene expression.

The results of chapter 5 also showed a significant reduction in the expression of ceramide, SPHK1, and SPHK2 in the liraglutide treated group when compared to the control group. These are associated with the results in chapter 4, which found a significant upregulation in sphingosine-1-phosphate. These findings suggest that liraglutide may have an impact on the sphingolipid metabolism pathway.

The sphingosine pathway begins with the breakdown of cellular membranes, releasing sphingomyelin. Sphingomyelin can then be converted into ceramide by enzymes known as sphingomyelinases (Pralhada Rao et al., 2013). In the study, it was observed that the treatment of cells with Liraglutide led to a reduction in ceramide levels. This decrease in ceramide could be attributed to the decrease in sphingomyelinase activity or a shift in the balance between ceramide production and degradation.

The observed decrease in ceramide levels following liraglutide treatment is consistent with previous findings that GLP-1 receptor agonists can decrease ceramide levels in cells (E. H. Zobel et al., 2021). Ceramide has been shown to have pro-inflammatory (Arana, Gangoiti, Ouro, Trueba, & Gómez-Muñoz, 2010) and pro-apoptotic (Moro, Nagahashi, Gabriel, Takabe, & Wakai, 2019) effects, and therefore, its reduction could have anti-inflammatory and anti-apoptotic effects. This may partially explain the beneficial effects of liraglutide in treating diseases associated with inflammation and cell death such as obesity and type 2 diabetes.

Ceramide can then be metabolized by ceramidase to generate sphingosine. Sphingosine can be phosphorylated by sphingosine kinases (SPHK1 and SPHK2) to form S1P. In the study, it was observed that the treatment of cells with Liraglutide led to a decrease in SPHK1 and SPHK2 expression. This decrease could be due to the downregulation of these enzymes or a shift in the balance between SPHK1/2 expression and degradation.

The decrease in SPHK1 and SPHK2 expression following liraglutide treatment is surprising, given their roles in S1P production. SPHK1 and SPHK2 are key enzymes responsible for the production of S1P, a signalling molecule with pleiotropic effects, including regulating inflammation and apoptosis.

S1P can be degraded by S1P lyase or dephosphorylated by S1P phosphatases. It was observed that the treatment of cells with Liraglutide led to an increase in SGPP1 expression, which could explain the increase in S1P levels despite the decrease in SPHK1/2 expression. This increase in SGPP1 expression could result in the dephosphorylation of S1P, leading to the accumulation of S1P in the cells. SGPP1 is a key enzyme responsible for the degradation of S1P. Therefore, the observed increase in SGPP1 expression could partially explain the increase in S1P levels despite decreased SPHK1 and SPHK2 expression. The increase in SGPP1 expression could also be a compensatory mechanism to maintain S1P homeostasis and prevent the accumulation of S1P, which can have pro-inflammatory effects.

One possible explanation for the increase in S1P levels despite decreased SPHK1 and SPHK2 expression is the existence of alternative pathways for S1P production, such as the salvage pathway (recycling of sphingosine) or dephosphorylation of sphingosine-1-phosphate by S1P phosphatases (Kitatani, Idkowiak-Baldys, & Hannun, 2008). In addition, S1P can be transported out of cells through specific transporter proteins (Nagahashi et al., 2014). This means that even if SPHK1 and SPHK2, the enzymes responsible for producing S1P, are reduced, S1P levels can still increase if there is increased activity of these transporters. These transporters are known to be expressed in various cell types, and their activity can be modulated by various factors such as cytokines and stress signals. Therefore, it is possible that the observed increase in S1P levels in response to liraglutide treatment is due to a compensatory increase in the activity of these transporters.

As mentioned previously, the study in chapter 5 also found PLA2G4A and PLA2G6 were significantly reduced. These can also be linked to the sphingosine pathway, giving further evidence that the sphingosine pathway could be an important pathway to target for future disease treatment. In the sphingosine pathway, phospholipase A2 (PLA2) enzymes are involved in the hydrolysis of phospholipids (Pralhada Rao et al., 2013), which generate arachidonic acid and lysophospholipids. These bioactive lipids act as signalling molecules and can activate downstream pathways, including the production of prostaglandins and leukotrienes. Prostaglandins and leukotrienes cause inflammation in the body (Jo-Watanabe, Okuno, & Yokomizo, 2019; Ricciotti & FitzGerald, 2011), which can contribute to diseases like obesity and type 2 diabetes. Inhibiting the enzymes that make prostaglandins and leukotrienes, like PLA2G6 and PLA2G4A, can thus inhibit the development of obesity and type 2 diabetes.

PLA2G6 and PLA2G4A specifically play a role in the production of ceramide, a key molecule in the sphingosine pathway. Ceramide can be generated by several pathways, including the de novo synthesis pathway and the sphingomyelinase pathway. PLA2G6 and PLA2G4A are involved in the sphingomyelinase pathway (Rodriguez-Cuenca, Pellegrinelli, Campbell, Oresic, & Vidal-Puig, 2017), which generates ceramide by the hydrolysis of sphingomyelin. Liraglutide treatment led to a significant reduction in PLA2G6 and PLA2G4A expression, which resulted in a reduction in ceramide production and potentially influenced downstream effects on inflammation and cell death.

Taken together, the observed results of this thesis suggest that Liraglutide treatment leads to a decrease in ceramide levels, a decrease in SPHK1/2 expression, an increase in S1P levels, and an increase in SGPP1 expression. These findings provide a target pathway in which further research should focus to treat obesity and type 2 diabetes. These changes in the sphingosine pathway could have important implications for cellular signalling and metabolism.

6.2 Research Strengths and Weaknesses

Chapter 6 has presented the results of a comprehensive research study on the metabolic effects of Liraglutide on obesity. In this section, the strengths and weaknesses of the research will be critically evaluated to assess the reliability and validity of the findings. The strengths of the research include the robustness of the research methodology, the consistency of findings with current knowledge, and the generation of new knowledge that can contribute to the development of obesity treatment and drug development. The weaknesses of the research

include the limitations of the research design, such as sample size and population demographics. These limitations and challenges will be discussed in detail to provide a comprehensive assessment of the research.

Furthermore, this section will also explore the broader implications of the research for the field. This will involve a discussion of how the findings contribute to existing knowledge, as well as the potential implications for practice. Additionally, this section will identify areas for future research that can build on the strengths of this study and address the limitations identified. Overall, this section provides a critical evaluation of the research that is essential for understanding the significance and potential impact of the study in the field.

One of the key strengths of the research is the robustness of the systematic review methodology. The systematic review methodology used in this study was designed to identify and analyse existing literature on GLP-1 receptor agonist treatment on obesity and type 2 diabetes. The methodology involved a comprehensive search strategy that included searches through MEDLINE, EMBASE, CENTRAL and SCOPUS, which ensured that the review was as comprehensive as possible. The protocol was developed in accordance with the Preferred Reporting Items for Systematic Review and Meta-Analysis Protocols (PRISMA-P), which provides a standardised and transparent approach to developing systematic review protocols. Additionally, the methodology followed the methods outlined in The Cochrane Handbook for Systematic Reviews of Interventions, which is considered the gold standard for conducting systematic reviews. The inclusion and exclusion criteria were developed to ensure that only studies of high quality and relevance were included in the analysis, and the data extraction process was rigorous and transparent. Additionally, the use of a structured approach to analysing the data ensured that the findings were reliable and valid. Overall, the systematic review methodology used in this study provides a strong foundation for the analysis and synthesis of the existing literature and enhances the reliability and validity of the findings.

The other studies presented in this thesis have several key strengths. Firstly, the study is novel and represents the first of its kind in the UK, providing unique insights into the effect of Liraglutide on overweight and obesity. Previous research has focused on European and American populations, whereas this study focuses on UK only. The novelty of the research is a strength as it offers a new perspective on the issue and brings fresh ideas to the field. As the first study of its kind in the UK, the findings offer a foundation for further research, which can

build on the insights provided by this study. Furthermore, this novelty is important as it has the potential to influence future policy and clinical practice.

Secondly, the study findings are consistent with existing research in terms of weight loss outcomes. This consistency is a strength as it adds validity and reliability to the study's findings. The fact that the results are consistent with other research suggests that the study has produced reliable data, which can be used to guide clinical practice. Moreover, consistency with other research demonstrates the robustness of the study methodology, which enhances the credibility of the findings.

Thirdly, the study identifies a novel pathway that can be targeted for future treatment. This is a strength as it provides new directions for further research and clinical interventions. By identifying a new pathway, the study offers a fresh perspective on the issue and presents a new area of focus for clinical interventions. This has the potential to lead to the development of new treatments and approaches to patient care, which can ultimately improve patient outcomes.

Overall, the strengths of this clinical study lie in its novelty, the consistency of the findings with existing research, and the identification of a new pathway for future research and treatment. These strengths highlight the importance of the study's findings and their potential to make a significant impact on clinical practice and patient care.

While the systematic review methodology used in this thesis offers many strengths, there are also several limitations to be considered. Firstly, the search strategy may not have captured all relevant studies, which could have resulted in important studies being missed. While efforts were made to ensure a comprehensive search, it is possible that some studies were not identified. This limitation could have impacted the validity of the study's findings. There is a possibility of publication bias, whereby studies with negative results may not have been published, potentially skewing the overall findings of the review. These limitations should be considered when interpreting the findings of this study and highlight the need for caution when drawing conclusions based on the review.

While the clinical study presented in this thesis has many strengths, there are also several weaknesses that need to be considered. The first weakness is the relatively small sample size of the study. Although efforts were made to recruit as many participants as possible, the sample

size was limited, which could have impacted the generalisability of the study's findings. Larger sample sizes may have provided a more representative picture of the population and strengthened the study's findings.

The second weakness of the study is the lack of diversity in the sample. The majority of participants were female, and most were white. This lack of diversity could have impacted the applicability of the study's findings to other populations, such as men or non-white individuals. Future studies should aim to recruit more diverse samples to ensure that the findings are applicable to a wider population.

The third weakness of the study is the lack of a placebo group to compare the results. While the study was able to demonstrate the effectiveness of the intervention, the absence of a placebo group makes it difficult to determine the true impact of the intervention. Future studies could include a placebo group to assess the efficacy of the intervention more accurately.

Overall, the weaknesses of the clinical study include the relatively small sample size, the lack of diversity in the sample, and the absence of a placebo group. These limitations should be considered when interpreting the study's findings and highlight areas for improvement in future research.

6.3 Implications of the Research and Future Perspectives

The results of this clinical study have important implications for future research and clinical practice. Firstly, the study provides support for the effectiveness of the intervention in promoting weight loss. The results showed similar weight loss for Liraglutide treatment with NHS Lifestyle advice when compared to research in other countries. This suggests that Liraglutide could be an effective tool for promoting weight loss in a clinical setting in the United Kingdom.

The findings of this thesis have important implications for future research and clinical practice. The observed decrease in ceramide levels, decrease in SPHK1/2 expression, increase in S1P levels, and increase in SGPP1 expression in response to Liraglutide treatment provides a target pathway for further research to explore potential treatments for obesity and type 2 diabetes.

These changes in the sphingosine pathway could have important implications for cellular signalling and metabolism. It may be possible to develop new treatments that target this pathway to improve insulin sensitivity and glucose homeostasis. Additionally, the results suggest that Liraglutide may have potential as a therapeutic agent for the treatment of metabolic disorders.

Looking towards the future, further research should aim to investigate the underlying mechanisms of the observed changes in the sphingosine pathway in response to Liraglutide treatment. This could involve exploring the effects of different doses or durations of Liraglutide treatment, as well as investigating the pathway in different populations.

Moreover, additional studies could examine the clinical effectiveness of Liraglutide treatment in reducing the risk of cardiovascular disease and other metabolic complications. The findings of this thesis have provided a foundation for future research to build upon, and the potential for further developments in the field of metabolic disorders is promising.

6.4 Conclusion

In conclusion, this thesis has researched the effect of Liraglutide treatment on overweight and obesity. This topic has been researched through a systematic review of existing literature, a clinical study and in-vitro studies examining the potential molecular mechanisms behind the clinical results.

The main outcomes from these studies are as follows. The systematic review provided evidence that treatment with GLP-1 RAs can have positive effects on metabolic markers in patients with overweight and obesity, including liver enzymes (ALT, AST, GGT), fasting insulin, adiponectin, apolipoprotein B, leptin, CRP, fructosamine, creatinine, c-peptide and fasting GLP-1. This led to the clinical and metabolomics study, which revealed that Liraglutide and NHS weight management services were associated with weight loss and improved metabolic control similar to other studies, and also provided evidence that S1P could be involved in determining response to treatment with Liraglutide. This chapter also provided evidence that PE and PC were significantly reduced after treatment. Finally, this led to the final study, which confirmed the results of the previous chapter through an in vitro validation study. Gene expressions were also measured here, providing evidence for Liraglutide in the reduction of various genes implicated in the sphingosine pathway.

Collectively, the findings of this thesis suggest that liraglutide treatment leads to changes in sphingolipid metabolism, including reductions in ceramide and SPHK1/2 expression, as well as increases in S1P and SGPP1 expression. Further studies are needed to understand the underlying mechanisms involved in these changes and their potential therapeutic implications.

The present thesis aimed to investigate the effects of a specific treatment on different aspects of metabolism, including changes in body composition, chemical processes, and biochemical parameters. To achieve this, we conducted a comprehensive study using a variety of methods, including body composition analysis, metabolomics, and biochemical assays. Our results demonstrated that the treatment led to significant changes in body composition, including a reduction in body fat and BMI. We also identified changes in chemical processes involving products of metabolism while on treatment, such as altered lipid metabolism. Furthermore, we observed changes in several biochemical parameters, including changes in glucose and insulin levels, which are important markers of metabolic health. Finally, gene analysis allowed us to identify novel metabolic pathways that could be potential targets for new drug discovery. Overall, this thesis has successfully achieved all its objectives and provided valuable insights into the metabolic effects of the treatment, which could have important clinical implications for the management of metabolic disorders.

In conclusion, the results of this thesis shed light on the impact of liraglutide treatment on sphingolipid metabolism, revealing significant changes in the expression of key enzymes and metabolites in this pathway. These findings may have important implications for the development of new treatments for metabolic disorders, such as type 2 diabetes and obesity. However, further research is needed to fully understand the mechanisms underlying these changes and to explore their potential therapeutic applications. The potential impact of these findings cannot be overstated, as they offer new insights into the complex interplay between metabolic pathways and disease and suggest new avenues for drug discovery and development. Ultimately, the findings of this thesis represent an important contribution to our understanding of sphingolipid metabolism and its role in metabolic health and have the potential to pave the way for new treatments and interventions that can improve patient outcomes and quality of life.

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