BILE ACIDS AND FGF19 IN THE REMISSION OF TYPE 2 DIABETES AFTER SLEEVE GASTRECTOMY AND GASTRIC BYPASS

REZA NEMATI

School of Science

Faculty of Health and Environmental Sciences,

2017

Thesis Submitted to the Auckland University of Technology in fulfillment of the requirements for the degree of PhD

Abstract

Prevalence of type 2 diabetes (T2DM) is increasing sharply. There is a direct relationship between obesity and T2DM. Currently, T2DM has no effective cure. However, there are several diabetes managements such as pharmacotherapy, diet and bariatric surgery considered as an effective treatment, and bariatric surgery is considered to be the most effective. There are four types of bariatric surgeries including adjustable gastric banding, biliopancreatic diversion with/without duodenal switch, gastric bypass (GB) and sleeve gastrectomy (SG). GB and SG are the most common bariatric surgery procedures for the treatment of T2DM and obesity. To evaluate the effect of an intervention on the treatment of chronic diseases such as obesity and T2DM, clinical follow-up studies are essential. Biomarkers are currently used in basic and clinical research. Their roles as essential endpoints in clinical trials are accepted universally. In this study two candidate biomarkers, fibroblast growth factor19 (FGF19) and bile acids (BAs) were assumed to play contributory roles in the remission of T2DM after bariatric surgery. Therefore, the main aim of this study was to quantify fasting and postprandial BAs and their computed compositions, and fasting and postprandial FGF19, on the remission of T2DM a year after SG and GB. Also, body composition and diabetes indices were measured a year after SG and GB. SG and GB were compared in this study to investigate which one is superior regarding diabetes alleviation. Due to the very low concentration of BAs in human plasma, liquid chromatography tandem-mass spectrometry (LC-MS/MS) has been used as a reliable method to measure BAs. BAs in this study were measured by using LC-MS/MS method, and FGF19 were quantified by using sandwich ELISA assay. It was found that fasting and postprandial BAs and FGF19 significantly increased a year after either SG or GB. However, fasting and postprandial BAs and FGF19 were not significantly differed between SG and GB. According to the definition

of diabetes remission, one-year after SG and GB, the remissions of diabetes were scored. In this study, 38% and 40% of patients who underwent SG and GB, respectively, achieved complete remission of T2DM. A year after bariatric surgery, patients were divided into two groups, remitted and non-remitted. Then, a comparison between the actual values and changes in different BA fractions (fasting and $AUC_{0\text{-}60min}$) and FGF19 (fasting and prandial) were performed to see whether they significantly differed between remitted and non-remitted. Despite the increased level of FGF19 and BAs within the remitted group, they were not significantly different from those of the non-remitted group. Therefore, it is concluded that firstly, both SG and GB are equally effective on the remission of T2DM. Secondly, the increased level of BAs and FGF19 a year after SG and GB play a contributory role for the remission of T2DM, but they are not the main reason of diabetes remission after bariatric surgery.

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List of abbreviations

ABCG5/ABCG8	ATP-cassette binding proteins G5/G8
ACC2	Acetyl CoA carboxylase 2
ADA	American Diabetes Association
AGB	Adjustable gastric banding
APCI	Atmospheric pressure chemical ionization
ASBT	Apical sodium-dependent bile transporter
ASMBS	American Society for Metabolic and Bariatric Surgery
AUC	Area under the curve
BAAT	Bile acid coenzyme amino acid N-acyltransferase
BAs	Bile acids
BAT	Brown adipose tissue
BCF	Beta-cell function
BCM	Body cell mass
BIA	Bioelectrical impedance analysis
BMC	Bone mineral content
BMD	Bone mineral density
BMI	Body mass index
BPD	Biliopancreatic diversion
BPD-DS	Biliopancreatic diversion-with duodenal switch
BS	Bariatric surgery
BSEP or ABCB11	ATP-binding cassette B11
CA	Cholic acid
CAD	Collisionally activated dissociation
CCK	Cholecystokinin
CDCA	Chenodeoxycholic acid
CNS	Central nervous system
CPT1	Carnitine palmitoyltransferase I
CREB PGC-1 α	cAMP regulatory element binding protein peroxisome proliferator-activated receptor gamma coactivator 1-alpha

CRFs	Chronic renal failures
CVDs	Cardiovascular diseases
CYP7A1	Cholesterol 7 alpha-hydroxylase
CYP7B1	Oxysterol 7-alpha-hydroxylase
DCA	Deoxycholic acid
DCCT	Diabetes Control and Complications Trial
DEXA	Dual-energy X-ray absorptiometry
DPA	Dual photon absorptiometry
DPP	Diabetes Prevention Program
DSS-II	Second Diabetes Surgery Summit
DXA	Dual-energy X-ray absorptiometry
EE	Energy expenditure
EIA	Enzyme immunoassay
ESI	Electrospray ionisation
ESRD	End-stage renal disease
EWL	Excess weight lost
FFM	Fat-free mass
FGF19	Fibroblast growth factor
FGFR4	Fibroblast Growth Factor Receptor 4
FGP	Fasting glucose plasma
FM	Fat mass
FPI	Fasting plasma insulin
FXR	Farnesoid X receptor
GB	Gastric bypass
GBP	Gastric banding procedure
GC/MS	Gas chromatography/mass spectrometry
GCA	Glycolic acid
GCA	Glycocholic acid
GCDCA	Glycochenodeoxy cholic acid
GDCA	Glycodeoxy cholic acid
GI	Gastrointestinal
GIP	Gastric inhibitory polypeptide
GLCA	Glycolithocholic acid

GLM	General linear model
GLP-1	Glucagon like peptide-1
GPBAR1	G protein-coupled bile acid receptor
HDL-c	High-density lipoprotein-cholesterol
HEC	Hyperinsulinemic-euglycemic clamp
HOMA-B	homeostatic model assessment-beta cell pancreas
HOMA-IR	homeostatic model assessment-insulin resistance
HRP	Horseradish peroxidase
HSD3B7	3β-hydroxy-Δ5-C27-steroid oxidoreductase
HSGAGs	Heparan sulfate glycosaminoglycans
IBABP	Ileal bile acid-binding protein
IBS	Inflammatory bowel disease
IDF	International Diabetes Federation
IFG	Impaired fasting glucose
IGI	Insulinogenic index
IGT	Impaired glucose tolerance
IR	Insulin resistance
ISI	Insulin sensitivity index
IV	Intravenous
JNK	c-JUN N-terminal kinase
LAGB	Laparoscopic adjustable gastric banding
LBM	Lean body mass
LC	Liquid chromatography
LCA	Lithocholic acid
LDL-c	Low-density lipoprotein-cholesterol
LGBP	Laparoscopic gastric bypass
LOD	Limit of detection
LOQ	Limit of quantification
LPS	Lipopolysaccharides
LSG	Laparoscopic sleeve gastrectomy
M/Z	Mass/ratio
MAPK	Mitogen-activated protein kinase
MDR3	Multi-drug resistance protein 3

MRM	Multiple reaction monitoring
NAFLD	Non-alcoholic fatty liver disease
NKHS	Non-ketotic hyperosmolar syndrome
NGSP	National Glycohemoglobin Standardization Program
NTCP/SLC10A1	Sodium (Na+)-taurocholate co-transporting polypeptide
OATP	Organic anion transporters
OGTT	Oral glucose tolerance test
OSTα/OSTβ	Heterodimeric organic solute transporter α/β
PHIH	Postprandial hyperinsulinemia hypoglycaemia
PXR	Pregnane X receptor
PYY	Peptide YY
QUICKI	Quantitative insulin sensitivity check index
R	Resistance
RCT	Randomised clinical trial
REE	Resting energy expenditure
RP	Reverse-phase
RQ	Respiratory quotient
RYGB	Roux-en-Y Gastric Bypass
SCAT	Subcutaneous adipose tissue
SCID	Stearoyl-CoA desaturase-1
SG	Sleeve gastrectomy
SHP	Small heterodimer partner
SOS	The Swedish Obese Subjects
SREBP1c	Sterol regulatory element binding protein 1-c
T2DM	Type 2 diabetes mellitus
TBA	Total bile acid
TBB	Total body bone
TBF	Total body fat
TC	Total cholesterol
TCA	Taurocholic acid
TCDCA	Taurochenodeoxy cholic acid
TGR5	G protein-coupled bile acid receptor

TGs	Triglycerides
TLCA	Taurolithocholic acid
TQMS	Triple quadruple mass spectrometry
TSH	Thyroid-stimulating hormone
TUDCA	tauroursodeoxycholic acid
UDCA	Ursodeoxycholic acid
VAT	Visceral adipose tissue
VDR	Vitamin D receptor
VLCD	Very low-calorie diet
VLDL	Very low-density lipoprotein
WHO	World Health Organisation
WHR	Waist-hip ratio
Xc	Reactance
2hpp	2 hours postprandial

Attestation of Authorship

"I hereby declare that this submission is my own work and that, to the best of my knowledge and belief, it contains no material previously published or written by another person (except where explicitly define in the acknowledgements), no material which to a substantial extent has been submitted for the award of any other degree or diploma of a university or other institution of higher learning".

Dedications

In the name of God. This thesis is dedicated to my beloved parents, particularly my father, Morteza Nemati and my mother, who supported and motivated me to have a higher education, to my lovely sisters, Nazanin and Nooshin for their extreme encouragement for me to accomplish my study, to My supervisor Professor Jun Lu and all my teachers who made me the way to reach this level of education and finally to my both grandmothers' spirit who were sisters and I missed them both over my PhD period and no chance to meet them before they left me forever.

Acknowledgment

I would like to express my deepest gratitude to my kind supervisor, Associate Professor Dr. Jun Lu who accepted me as a student, for his knowledge, generous guidance, encouragement, and his huge support that help me prepare this thesis. I would also like to show my sincere acknowledgment to my great co-supervisors, Associated Prof. Dr. Rinki Murphy and Associate Professor Dr. Lindsay Plank from the University of Auckland. Leading researchers who supported me in the best way with their comprehensive knowledge.

Also, I would like to have special thanks to AUT Vice Chancellor, Derek McCormack for being so generous to provide me a Vice-chancellor Scholarship that helped me to be so relaxed financially during my study.

I would like to thank Dr. Sonya Popoff, AUT Lab Manager, for all her support during my lab experiment. Also, thank you Dr. Ashveen Nand for your advice and support for doing my LC-MS/MS experiment. I would also like to thank my lovely friends, Kelvin Wang, who is my first friend in New Zealand and Dech Dokpuang, Ehsan Amiri, Najmeh Vatankhah and all my lab and office mates at WN level 5.

I would like to thank all participants who participated in this research study. I am grateful to my parents, and my sisters Nazanin and Nooshin for their love, support and extreme encouragement throughout my study.

Ethics Approval

Ethical Approval was received from New Zealand regional ethics committee (NZ93405). This study was prospectively registered at ANZCTR (ACTRN12611000751976) and retrospectively registered at https://clinicaltrials.gov/ (NCT01486680).

CHAPTER ONE

INTRODUCTION

1.1 Study background

Diabetes is "the epidemic of the new millennium" (Jovanovic & Gondos, 1999). Type 2 diabetes mellitus (T2DM) is a kind of multifactorial disease. It means both environmental and genetic factors contribute to the development of diabetes. In T2DM increased level of insulin secretion, decreased glucose use and elevated glucose production are seen. T2DM2 plays a major role in the development of end-stage renal disease (ESRD). In T2DM there are two critical pathways to develop abnormal glucose production. The first is impaired fasting glucose (IFG) and the second is impaired glucose tolerance (IGT) (A. Association, 2011). Glucose is the key regulator of insulin secretion. However, other substances such as ketones, amino acid nutrients, gastrointestinal peptides and neurotransmitters influence insulin secretion. There are several life-threating conditions considered to be obesity comorbidities, such as cancer, cardiovascular diseases (CVDs) and T2DM (Bloomgarden, 2000; Calle & Thun, 2004; Poirier & Eckel, 2002). Obesity and T2DM are also states of chronic inflammation associated with immune cells (T cells) (Feuerer et al., 2009; S. Nishimura et al., 2009). Obesity is the result of an imbalance in energy homeostasis whereby adipose tissue is increased. In obesity, a low-grade inflammatory state, the endothelial cells become dysfunctional. Elevated levels of proinflammatory factors lead to the development of insulin resistance (IR) (Hotamisligil, 2006; Olefsky & Glass, 2010; Plomgaard et al., 2007). Obesity not only plays a major role in the development of T2DM, but it has also been shown that the rate of dysglycaemia, or prediabetes, is higher among obese subjects (Cosson et al., 2010). T2DM is introduced as an insulin resistance disorder (R. DeFronzo, 1992; Vella et al., 2004) and the effect of obesity on the development of T2DM is completely clear (Panel, 1998). Therefore, the increased risk of obesity is seen as a greater risk of diabetes (Gregg et al., 2007).

There is no confirmed cure for T2DM mellitus. Bariatric surgery (BS) is currently utilised as the best treatment for diabetes. There are two common types of bariatric surgery, laparoscopic sleeve gastrectomy (LSG) and laparoscopic gastric bypass (LGBP). Although

there are several side effects to consider for bariatric surgery as an intervention for the treatment of diabetes. One of the most significant drawbacks for its consideration is the cost of the surgery. Bariatric surgery is responsible for changes in those who undergo the surgery which causes a remission of diabetes. Most of these causes are still unknown. For instance, insulin sensitivity has been shown to result in an acute improvement after Roux-en-Y gastric bypass (RYGB) or gastric bypass (GB) (Curry et al., 2011). Although bariatric surgery currently is the best treatment for the remission of T2DM and obesity (Melissas, 2008), insulin sensitivity can remain abnormal in muscles over a few weeks or months following bariatric surgery (R. Taylor, 2008).

For the first time, in 1994, the association between bile acids (BAs) and glucose metabolism was introduced (Garg & Grundy, 1994), and several studies, either in humans or animals, confirmed the impact of BAs on glucose metabolism (Claudel et al., 2005; Fonseca et al., 2008; Gerhard et al., 2013; Ryan et al., 2014). BA sequestrant administration has been shown to be effective for the improvement of diabetes either in fasting or postprandial conditions. Also, individual BAs (colesevelam is a prime example) have been indicated to enhance glucagon-like peptide-1 (GLP-1), improve IR, and reduce low-density lipoprotein-cholesterol (LDL-C) (Beysen et al., 2012). Furthermore, administration of individual BAs stimulates circulating fibroblast growth factor 19 (FGF19) after meals, decreases lipid metabolism and increases gastric inhibitory polypeptide (GIP) through activation of enteroendocrine L cell (Katsuma et al., 2005). This BA change has already been proposed as a crucial factor in the remission of diabetes. The most notable impact of BAs in the remission of diabetes is related to its role in glucose metabolism and its suppressing effect in liver lipogenesis via activation of the farnesoid x receptor (FXR) pathway (Yang et al., 2010). BA and FGF19 are related to each other. It has been shown that the level of FGF19 is increased after bariatric surgery, and there is a positive correlation between diabetes resolution and increased plasma level of FGF19 (Gerhard et al., 2013). A recent study has shown the further importance of BAs in demonstrating that binding FXR to BAs works as a signalling molecule, with subsequent improvement of diabetes just a few days after bariatric surgery, and without significant weight loss (Ryan et al., 2014). Treatment by BAs has been shown to have a blunting effect on T2DM (Bays et al., 2008; Claudel et al., 2005; Fonseca et al., 2008; Garg & Grundy, 1994; Goldberg et al., 2008). Moreover, T2DM has been confirmed

to be influenced by BAs (Gerhard et al., 2013; Haeusler et al., 2013; Vincent et al., 2013b; Wewalka et al., 2014b). Also, total bile acid (TBA) values among obese people with diabetes have been reported to be higher compared with obese non-diabetes subjects (Gerhard et al., 2013; Vincent et al., 2013b; Wewalka et al., 2014b). There are several proposed mechanisms for this effect, such as an interruption of enterohepatic circulation of BAs through the function of molecular signalling via FXR (Guzelian & Boyer, 1974). Also, the effect of BAs on an active cell membrane G protein-coupled receptor (TGR5) has been reported to induce incretin (GLP-1) without FXR effect (Kreymann et al., 1987). It has been shown that there is an increase in the level of BAs among subjects who underwent bariatric surgery (Nakatani et al., 2009). Increased serum levels of BAs after bariatric surgery (RYGB) is assumed to play an important role in the improvement of metabolic complications such as diabetes. This can be explained by their role as participants in a signalling pathway, which occurs as BAs are recognised as ligands for FXR, where they are essential for the normal absorption of lipids (Cipriani et al., 2009; Parks et al., 1999; Haibo Wang et al., 1999). Regardless of the type of surgery, increased plasma level of BAs may play an important role in the improvement of diabetes (Ahmad et al., 2013). Although there is scant information about the effect of BAs in the remission of diabetes in humans, recently it has been shown that the greater serum level of BAs could be introduced as a potent factor in the remission of diabetes after RYGB (Kohli & Seeley, 2013). Thus, the manipulation of BAs may be a new target for the remission of diabetes without any types of bariatric surgery (Kohli, Setchell, et al., 2013; Mencarelli et al., 2013).

FGF19 is a novel and important biomarker (Buhmeida et al., 2014). FGF19, which works as an endocrine hormone, also shows promise as a treatment of T2DM (Rimoldi et al., 2014; F. G. Schaap, 2012). FGF19 is a hormone that is released from the ileum (small intestine) after a meal and has a very important role in the metabolism of glucose in parallel with insulin (Smoot & Smoot, 2008). Although the highest level of FGF19 is detectable at 2 hour post-prandial (2hpp), it is at its lowest baseline when measured at times of fasting (De Giorgi et al., 2014), and its overall measurement depends on physiological status (Gerhard et al., 2013; Krssak et al., 2004; Schreuder et al., 2010). FGF19 gene expression is under control of BAs, FXR, and pregnane X receptor (PXR) (Inagaki et al., 2005; Hongwei Wang et al., 2011). BAs play a vital role in regulating FGF19 function. For example, plasma levels of FGF19 can be increased markedly among subjects who are treated with chenodeoxy cholic acid

(CDCA) (Lundåsen et al., 2006) and lithocholic acid (LCA) (Kreymann et al., 1987). There are several important functions considered for FGF19. It has been reported that the level of FGF19 is lower in patients with metabolic syndrome than in healthy subjects (Inagaki et al., 2008). FGF19 has several physiological compensatory effects. For instance, it can inhibit the accumulation of triglycerides in the liver, increase rest energy expenditure (REE) and regulate glucose metabolism (Tomlinson et al., 2002). In subjects with T2DM, the level of FGF19 decreased while BAs increased (Gerhard et al., 2013). Its role in the prevention of diabetes in animals has also been reported (Halpern et al., 2014). FGF19 also has an impact on the resolution of obesity. It has been demonstrated that increased levels of FGF19 are positively correlated with weight loss (Jansen et al., 2011). Furthermore, FGF19 has been shown to have a negative correlation with BMI and a positive correlation with adiponectin (Mundi & Collazo-Clavell, 2014; Ruta et al., 2013; D. Wang et al., 2013). However, these correlations need more study, particularly within lower BMI (less than 30 kg/m^2) subjects (De Giorgi et al., 2014). Further studies are needed in the areas of increased metabolic capacity (Halpern et al., 2014), reduced adiposity (S. A. Jones, 2012), elevated glucose storage (as glycogen) and protein production (S Kir et al., 2011; A.-L. Wu et al., 2011) in order to better understand these correlations. However, and most importantly for current investigations, BAs upregulate the production of FGF19 and the FGF19, in turn, downregulates BA synthesis (Beenken & Mohammadi, 2009). This human study showed that patients with diabetes had lower FGF19 values and higher amounts of BAs before their operation when compared with nondiabetic subjects. Additionally, in diabetic patients, after RYGB, the reduced level of FGF19 was significantly related to the increased liver expression of the cholesterol 7-hydroxylase (*CYP7A1*) gene, which regulates the production of BA.

Bone densitometry and physical measurement are quantitative measurement techniques used to evaluate changes in metabolic pathways. The new technologies associated with densitometry are sophisticated and highly advanced, with the capability of showing extraordinary skeletal scans that are then used to find out more details about metabolic changes. Such densitometry techniques are in part, superior to standard radiology and its images. For example, the newer technologies are capable of combining both accuracy and precision. Although clinical densitometry is a relatively recent methodology, densitometry as a practice is very old (Price, 1901; Sheldrick, 1986). Today's techniques enable us to measure bone density in almost all parts of the body. For instance, dual energy x-ray

absorptiometry (DXA) is capable of measuring the density of the spine and the proximal tight. Recent DXA devices can perform a total body scan in just a few minutes.

Body composition measurements differ from the measurement of weight and height as standard markers of physical form, although the types of measurement are related to each other. The main aim of body composition is to calculate the percentage and distribution of fat and lean tissues in the body. Most studies show that weight and BMI play a major role in evaluating a diseased state, but measuring the fat percentage and its distribution along with lean tissue is even more crucial than total weight and BMI in assessing a variety of diseases. To understand how obesity may develop from chronic energy imbalance, energy expenditure measurement is a must (Durnin, 1973). There are several methods to measure energy expenditure. The most reliable method for this is to examine the continuous measurement of heat or direct calorimetry.

One of the most common, rapid, safe and non-invasive methods for evaluating body composition in humans is bioelectrical impedance analysis (BIA). Fat and fat-free mass (FFM) have dissimilar conductivity. BIA is a popular method to quantify the percentage of lean and fatty tissues in the body to know more about alterations in fat and FFM tissues and to help increase the understanding of metabolic diseases such as diabetes and obesity.

1.2 Problem statement

The prevalence of T2DM is growing sharply worldwide, and without an agenda to prevent and control the disease, its prevalence is most likely going to continue to increase (K. G. M. Alberti et al., 2007; Tuomilehto & Schwarz, 2010). In Europe, more than 55 million people aged 20 to 79 had T2DM in 2010, and this number will increase to 66 million by 2030 (Wild et al., 2004).It has also been shown that among people who have diabetes, the risk of CVDs and low life-expectancy is four times more than people who have no diabetes (Manuel & Schultz, 2004). The occurrence of diabetes is not restricted to an exact population, and the prevalence is distributed disproportionately. The prevalence of diabetes is higher among poor people or in brief to a lesser extent among lower-income individuals (Chaufan & Weitz, 2009). It has predicted that the prevalence of diabetes will be increased by 70% in 2025, particularly among children aged 5 and less (Patterson et al., 2009). Diabetes as a cause of death is underreported. Based on the latest World Health Organisation (WHO) report, the

eighth leading cause of death in both males and females (and fifth overall in females) is diabetes. Diabetes per se can be the cause of micro and macrovascular diseases; T2DM leads to disability and premature death. Currently, more than 400 million people live with diabetes, and because of diabetes, more than 1.5 million people died in 2012 (WHO, 2016). It has been documented that different ethnicities show different patterns of diabetes. For instance, among Asian people, body fat (abdominal fat) and hepatic liver fat are significantly higher compared with other Caucasians with the same BMI (Wulan et al., 2010). Those people with these higher fat levels are more at the risk of insulin resistance. It has also been suggested that decreased body weight not be the only way to reduce diabetes or insulin resistance, even though it has been shown that lower insulin sensitivity is mostly observed among people who have higher body fat (Wulan et al., 2010). Although lifestyle changes will play a major role in attenuating the prevalence of diabetes, its prevalence is still predicted to increase to more than 330 million people (Wild et al., 2004). Along with total prevalence increase, it is estimated that the rate of death will increase to 3.7 million shortly. This number includes 1.5 million diabetes-related deaths and another 2.2 million deaths due to comorbidities such as CVDs, chronic renal failures (CRFs) and tuberculosis related to hyperglycaemia. Of note, hyperglycaemia causes about 7% of diabetes-related mortalities among males aged between 20 and 70 and 8% within females at the same age (WHO, 2016). New Zealand is not an exception to these statistics. The prevalence of diabetes is increasing in New Zealand as with other developed countries, and this is happening in parallel with the increasing prevalence of obese and overweight individuals. Among all population groups in New Zealand, the incidence of diabetes is highest in Maori and Pacific groups compared to other ethnicities (Coppell et al., 2013; Ministry of Health, 2002).

According to American Society for Metabolic and Bariatric Surgery (ASMBS) the number of metabolic/bariatric surgeries increased significantly in 2013; 42% of those surgeries belongs to SG, 34% RYGB, 14% adjustable gastric bypass (AGB), 6% to “revisional surgery”, 1% to biliopancreatic diversion with duodenal switch (BPD-DS) and the rest were classified as “other surgeries” (www.asmbs.org). Unfortunately, despite the positive effect of bariatric surgery as a treatment of obesity and diabetes, it is important, to note that bariatric surgery is not an inexpensive therapy. Currently, its cost is one which is not easily affordable for the general population, especially if insurance does not cover it. This means one of the most important considerations for bariatric surgery (apart from its contraindications) is the

economic issue. Each year more than 35,000 surgeries have been performed which cost more than $5 billion (Buchwald & Oien, 2009). According to Auckland Weight Loss Surgery, the cost of a given surgery regardless of its possible side effect(s) is about NZD 20,000 (http://www.aucklandweightlosssurgery.co.nz/what-does-it-cost). Despite the effect of bariatric surgery on the resolution of diabetes, this cost estimate does not cover "biochemical remission," and about half of the subjects who underwent bariatric surgery need to use antidiabetic medicines after a given time (Buse et al., 2009). Diabetes remission is defined by the serum concentration of HbA1c being less than 6.5% or 48 mmol/mol. In discontinued diabetes drugs, non-remission is to have an HbA1c >7% or 53 mmol/mol with or without antidiabetic agents (D. J. Pournaras et al., 2010). More research is needed to introduce a less invasive diabetes management method in the place of bariatric surgery, thereby eliminating post-operative complications, as well as an adverse economic hardship for the patient.

Because there is no precise cure associated with the treatments of diabetes, either with pharmaceutical products or by dieting (Bray, 2008; Kraschnewski et al., 2010), T2DM is a major issue for the public health sector. The current population of New Zealand has an alarmingly high risk of diabetes, particularly among the working age groups (Coppell et al., 2013). This working age group should be considered the priority of the health sector's efforts. The actions needed are to consider both sides of the problem, namely the economic and health issues. Furthermore, development of a novel way to remit diabetes may help to save time spent accessing the health care sector and decrease money needed to care for the disease.

1.3 Significance of study

This study is a randomised clinical trial (RCT). The long-term prospective control trial was carried out to determine whether the underlying mechanism for diabetes remission is due to systemic malabsorption, GI hormones, BA alterations, restricted calorie dieting or other factors. Bariatric surgery is the most effective treatment for diabetes. The use of bariatric surgery (particularly RYGB) to remit T2DM dates back almost 25 years, and indicates that 98% of diabetic patients have dramatic resolution after ten years (Pories et al., 1987). However, diabetes remission is not stable for a long time. In a recent clinical trial data indicates that the rate of diabetes improvement is decreasing over time (Sjöström et al., 2004). The reason for this loss of remission is unknown (Laferrère, 2011). Thus, there is no definite pattern of diabetes remission after bariatric surgery. For instance, in a prospective clinical

trial performed within a normal weight population, results showed that about 91% of subjects achieved a goal of normal HbA1c less than 7% without any antidiabetic medicines after bariatric surgery (De Paula et al., 2010a). While in another clinical trial carried out over a period of 11 years showed that the need for re-operation after SG is increased over time (Weiner et al., 2011). In addition, in a small postoperative longitudinal study, it was demonstrated that 31% of patients who underwent RYGB remitted (Eckhauser et al., 2007). Some researchers believe that the remission of diabetes after bariatric surgery is independent of weight loss (R. V. Cohen et al., 2012; Nemati et al., 2016), while others believe in the role of weight loss as a vital factor for the remission of diabetes (Pok & Lee, 2014; Pontiroli et al., 2009).

Bariatric surgery per se may not be the main reason for diabetes remission. Several results are likely to happen after bariatric surgery that can constrain diabetes. It has been shown that without any significant weight loss after bariatric surgery, blood glucose is decreased sharply. The higher remission rates of T2DM is mostly observed among people who were less obese preoperative (R. Cohen et al., 2006; W.-J. Lee et al., 2008), which may reflect the impact of other factors on the amelioration of diabetes mortalities than bariatric surgery on its own (Thaler & Cummings, 2009). Bariatric surgery aims to reduce weight, while the impact of bariatric surgery on the remission of T2DM is independent of weight loss (Pories et al., 1995; Schauer et al., 2003a). It has been reported that within a few days after bariatric surgery the glucose levels of diabetic subjects achieve a normal range, and patients are freed from taking antidiabetic medicines (Wickremesekera et al., 2005). This circumstance is not the same for all types of bariatric surgery (D. E. Cummings & Flum, 2008; Dixon et al., 2008), and so the question remains, why are there different patterns of diabetes remission after various bariatric surgeries, and further, what are the roles of FGF19 and BAs in the remission of diabetes after bariatric surgery.

FGF19 and BAs play a role in diabetes remission after bariatric surgery (Gerhard et al., 2013). Reduced levels of FGF19 are significantly related to the increased liver expression of the *CYP7A1* gene, which regulates the production of BA. FGF19 and BA levels, especially cholic acid (CA) and deoxycholic acid (DCA) are increased after bariatric surgery, and CDCA is the potent trigger of FGF19 (Lundåsen et al., 2006). FGF19 has two receptors that are proposed for the signalling function of FGF19: the FGF receptor 4 (FGFR4) and beta

klotho to suppress the expression of *CYP7A1* (Inagaki et al., 2008). Therefore, FGR19 works as a signalling molecule to regulate the conversion of cholesterol to BA through *CYP7A1* (Russell, 2003). In this process, FXR plays a major role in inducing FGF19 secretion through BAs to regulate glucose metabolism (F. Lee et al., 2006). Therefore, FGF19 and BAs need to be studied together.

The success of diabetes remission is not guaranteed after surgery (Jüllig et al., 2014). There are several studies which have been carried out in humans as well as in animals to find an appropriate remission mechanism for diabetes. However, it is still difficult to say why there is a remission after bariatric surgery. BAs and FGF19 may be novel and relevant biomarkers to reveal new areas of research in the remission of T2DM. In the current study, the effort of the researcher is to first understand the underlying pathway of physiological changes after bariatric surgery and second to suggest a non-invasive solution for the improvement of diabetes. The mechanism of BAs and FGF19 biomarkers on the remission of diabetes is the main question under consideration. There are several hypotheses behind the role of these biomarkers. For instance, enterohepatic circulation along with gut microbial populations play a crucial role in producing secondary BAs (Sjövall et al., 2010). The role of gut microbial flora has been documented in the remission of diabetes (Liou et al., 2013; K. R. Sharma, 2012). Still, it is not clear whether the level of BAs is associated with FGF19 (Weiner et al., 2011). Whether or not the administration of FGF19 and BAs may assist resolution of T2DM in humans needs more research. FGF19 and BAs may have dual properties in the remission of diabetes. Interestingly, some BAs have an intestinal permeability (Raimondi et al., 2008) while others do not (Bernardes-Silva et al., 2004). Interestingly, there are situations where, after bariatric surgery, some individuals' BAs are decreased, and some individuals' BAs are increased (De Giorgi et al., 2014; Myronovych et al., 2014). We need to study more about these important biomarkers as a means of identifying new targets for the treatment of diabetes.

1.4 Hypothesis

Individual BAs with their fractions and FGF19 are biomarker candidates that contribute to T2DM remission after either RYGB or SG.

1.5 Overall aim

To determine the association between BAs and FGF19 with diabetes remission, and the effect of these biomarkers on the aetiology of T2DM.

1.6 Specific objectives

To determine individual fasting plasma levels of BAs before surgery, and a year after SG or RYGB.

To determine the AUC of BA fractions before and a year after SG or RYGB.

To determine the plasma level of FGF19 at either baseline or postprandial levels before surgery and a year after SG or RYGB.

To determine body composition and physical measurements before and a year after SG or RYGB.

To compare the change in BA and FGF19 levels over a year after bariatric surgery.

To compare the change in body composition and physical measurements over a year after bariatric surgery.

To calculate, determine and compare different fractions of BAs and glucose indices within and between baseline and a year after RYGB and SG.

To find associations between changes in fasting and/or prandial FGF19 and BA fractions, and evaluate the effect of gender on changes of FGF19 and BA fractions.

To calculate diabetes remission after both bariatric surgeries.

CHAPTER TWO

LITERATURE REVIEW

2.1 Diabetes Definition

T2DM or simply diabetes in the human is characterized by a high level of blood glucose that results from the inability of the human body to produce a sufficient amount of insulin. According to American Diabetes Associating (ADA), T2DM (previously defined as "non-insulin-dependent diabetes" or "adult-onset diabetes") constitutes about 90-95% of all kind of diabetes (ADA, 2015). Currently, T2DM is mostly diagnosed based on HbA1c, measuring fasting plasma glucose (FPG) or 2hPP after a 75 g glucose during an OGTT (ADA, 2014; Committee, 2009). Table 2.1 shows clinical features to diagnose diabetes.

Table 2.1 Clinical features to diagnose T2DM

HbA1c ≥ 6.5% using an accepted method based on NGSP and certified by DCCT	OR	FPG≥ 7.0mmol/l after 8 hours fasting
2hpp ≥11.1 mmol/l after ingesting 75 gr glucose in water during 2 hour-time (OGTT)	OR	Random plasma glucose ≥ 11.1 mmol/l for those patients with traditional symptoms of hyperglycaemia

Adapted from ADA (A. D. Association, 2015)

Glucose homeostasis plays a crucial role in the pathophysiologic presentation of T2DM. The balance between liver glucose production and peripheral glucose uptake is called glucose homeostasis. Insulin plays an axial role to regulate glucose homeostasis. Insulin is an anabolic hormone. It means insulin causes the storage of fat, amino acids, and carbohydrates. The highest portion of ingested glucose found in the food after skeletal muscle utilises the meal. There are several pathologic states involved in the development of diabetes. The most important ones are beta cells in the pancreas becoming dysfunctional, along with insulin deficiency resulting in IR. Due to the disability of insulin secretion, several abnormalities occur for carbohydrate, fat and protein metabolism (Assal & Groop, 1999). There are two ways for insulin deficiency to arise. First, the given tissue is deficient in its response to insulin. Second, there is an inadequate secretion of insulin to the given tissue. Glucagon has

a negative feedback property on insulin. Glucagon is secreted by pancreatic alpha cells. Glucagon is released when the level of insulin or glucose is low. It stimulates glycogenosis and gluconeogenesis. Glucose production via the liver is increased through gluconeogenesis to reduce glucose uptake in skeletal muscle fat tissues (insulin sensitive tissue). The link between T2DM and obesity is inevitable. Diabetes is a major sequela of obesity (Haslam, 2007). Among obese subjects, who have a high incidence of fatty liver, the accumulation of fat near the pancreas (pancreatic fat) causes impaired insulin secretion. It has been shown that 30% of obese people have fatty liver disease. Since insulin is an anabolic hormone, more triglycerides (TGs) and fatty acids are stored in cases of hyperinsulinemia. During this phase, stable hyperinsulinemia and enhanced level of plasma glucose, more fatty acids are produced from glucose (Sidossis et al., 1996); de novo lipogenesis (Schwarz et al., 2003), which is a complex pathway to convert carbohydrates into fatty acids, is altered in obese people. It results in production and storage of more TGs in the liver. Those newly synthesized TGs can be either transferred as very low-density lipoproteins (VLDL) kept as liver TGs or oxidized (McGarry et al., 1977). The link between obesity, accumulation of fat in the liver, and insulin dysfunction together increase the risk of IR and T2DM (Beysen et al., 2012; W. Ma et al., 2015).

2.2 Diabetes and its complications

People with diabetes are more at risk of developing several disabling and life-threatening diseases compared with other individuals. T2DM has several serious complications. The long-term effect of T2DM is in the various organs. It leads to damage, dysfunction, and failure of almost all vital parts of the body. Figure 2.1 shows the impact of T2DM from head to toe in the human body. Diabetes has long-term complications including nephropathy, kidney failure neuropathy, foot ulcer, Charcot joints, gastrointestinal, genitourinary and cardiovascular diseases, sexual dysfunction, and limb amputation (ADA, 2010). The complications of diabetes are often classified into two groups, microvascular and macrovascular complications. Eyes, kidneys and the brain are just a few examples of human organs affected by diabetes. Retinopathy, nephropathy, and neuropathy are common microvascular complications, and CVDs and atherosclerosis are two traditionally macrovascular complications in T2DM. Diabetes is mostly characterised by symptoms such as thirst, polyuria, blurry vision, weight loss and insatiable appetite (polyphagia). Diabetes,

in the case of hyperglycaemia, may lead to coma and death regarding severe forms namely, ketoacidosis or nonketotic hyperosmolar syndrome (NKHS) (Kitabchi & Nyenwe, 2006; Kitabchi et al., 2009). T2DM sometimes is diagnosed in the presence of its complications. There is a direct relationship between metabolic control and long-term complications of T2DM (Cahill Jr et al., 1976; Ingelfinger, 1977; Siperstein, 1983; Siperstein et al., 1977; Stern & Haffner, 1988). One of the most significant limitations in the aetiology of diabetes is knowing about either microvascular or macrovascular complication of the disease. Also, the exact underlying mechanism of diabetes is unknown, and alterations in underlying mechanisms before or during diabetes are unclear. Unfortunately, good glycaemic control is not always achievable in all diabetic subjects. It has been shown that intensive therapy using insulin can lead to microvascular complications. Even with good glycaemic control, the chance of damaging vascular cells due to the intermittent hyperglycaemia is still present (Risso et al., 2001). Although good glycaemic management is possible using traditional methods still common for diabetes care, the development of novel therapies that target the cause of diabetic complications is likely to be crucial in long-term efforts to control the disease.

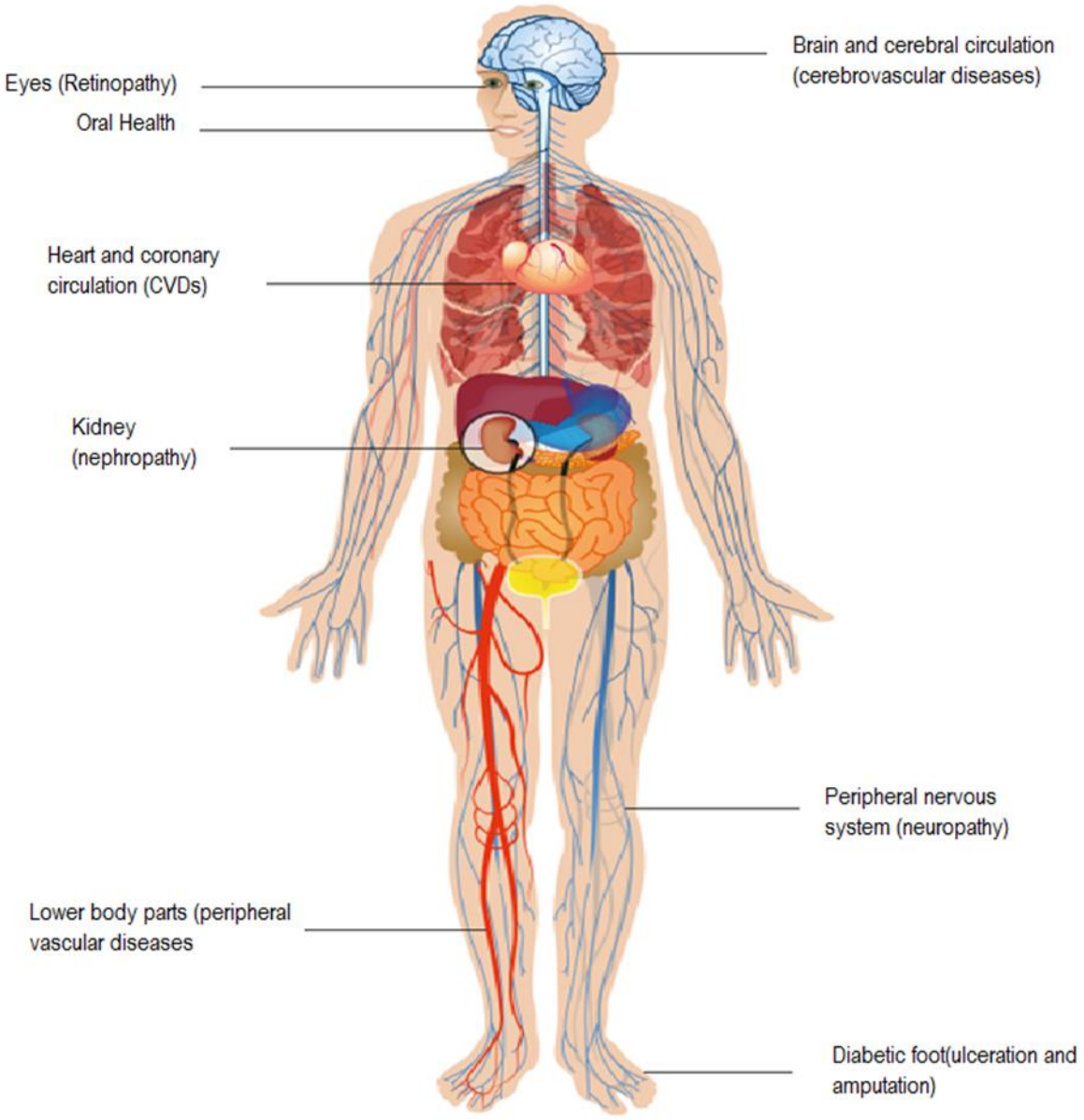


Figure 2.1 Adverse effects of T2DM on the human body

The adverse effect of T2DM on different organs from head to toe**.** Adapted from IDF 6th edition (Aguiree et al., 2013).

2.3 Worldwide Prevalence of Diabetes

Diabetes has no cure. Diabetes has four common types, type1, type 2, gestational diabetes, and "additional types." About 90-95% of all kinds of diabetes are T2DM. T2DM does not belong only to adult populations (Farsani et al., 2013). In 2005 diabetes was introduced as

the fifth leading cause of death in the world. Every day more than 4000 people are diagnosed with diabetes and more than 650 people die from diabetes. It has been shown that the rate of death increased by 45% from 1987. In the US more than $132 billion is spent on diabetes and its morbidities each year, and diabetes is responsible for 11% of public health costs (www.diabetes.org). It has been predicted that the prevalence of diabetes will be increased to more than 330 million people by 2030 (Wild et al., 2004). Nevertheless, good glycaemic control either by changing lifestyle or using another effective treatment may decrease this prediction significantly. For example, in a study from a randomised clinical trial called the Diabetes Prevention Program (DPP), it has been shown that the incidence of T2DM decreased by 58% with changing lifestyle and 31% of pharmacologic agents (metformin) (DPP, 2009). According to the latest IDF report, more than 415 million people currently live with T2DM, and it has been predicted this number will reach 642 million by 2040 (IDF, 2015). Diabetes is an economical, global burden. Most people who are affected by diabetes are in the working ages (40-59 years old). The Western Pacific, where New Zealand is located, has the highest prevalence of diabetes. According to the IDF report, every six seconds a person dies due to diabetes. As recently as 2013, about $548 billion was spent on this mysterious disease, with the amount of money projected to reach $627 billion by 2035. In the Western Pacific, 138 million people suffer from T2DM while 54% of these people are still undiagnosed. Almost 16% of deaths in the Western Pacific is due to diabetes and its complications. Unfortunately, half of those who died from diabetes were aged less than 40 years (IDF, 2013). It is therefore important to find inexpensive and user-friendly interventions which can reduce the higher expenditures that are predicted for diabetes treatments. Figure 2.2 shows the global outbreak of T2DM.

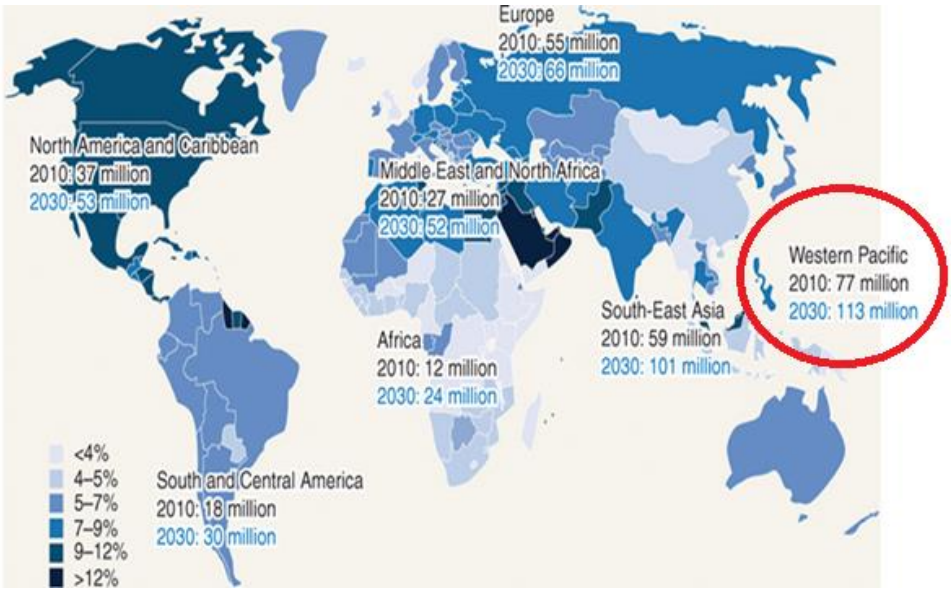


Figure 2.2 worldwide prevalence of T2DM.

The figure shows current and the future outbreak of diabetes in different part of the world. Western Pacific where New Zealand is included predicted to have 113 million diabetic patients by 2030. Adapted from (Longo et al., 2012).

2.3.1 National Prevalence of Diabetes

The exact prevalence of diabetes in New Zealand is unclear. Since the prevalence of undiagnosed diabetes is expected to be very high, it limits the accurate identification of the disease. The prevalence of diabetes in New Zealand is increasing sharply. The prevalence of diabetes is 7% worldwide, while the prevalence of prediabetes is 25.5% in NZ. There is a significant difference in diabetes prevalence between males and females in New Zealand. The prevalence of diabetes is higher among males compared to females (8.3% vs. 5.8%) (Coppell et al., 2013). According to Coppell et al. (2013), the prevalence of undiagnosed diabetes is higher among Pacific people, and this also varies among other ethnicities. The current population of New Zealand faces an alarmingly high risk of diabetes. A recent study showed that the working age population (35-44) are most at risk of diabetes (Coppell et al., 2013). Despite the available information on diabetes treatment in New Zealand (Sundborn et al., 2007; Tipene-Leach et al., 2004), finding an appropriate diabetes management program is still unsuccessful. The role of changing the lifestyle is highlighted more than other interventions (DPP, 2002) to prevent or at least control diabetes. It is important for public

health sectors and researchers to make an appropriate plan for people who are more at risk of diabetes and its complications; namely, people who are obese or overweight (Ackermann et al., 2011). Obesity plays a major role in the development of diabetes in New Zealand. Although in comparison with the US the incidence of extreme obesity is significantly lower among New Zealand people (4% vs. 2.7%, respectively), there is a significant difference based on different ethnicities among New Zealand people. It has been shown that Europeans have a lower prevalence of diabetes (0.8%), with moderate prevalence in Maori with 5.1%, and a higher prevalence within Pacific Islander ethnicities with 10.9% (Goulding et al., 2007). Diabetes is a "silent killer"; the study of diabetes remission is a major issue for New Zealanders. In 2009, 869 people died due to diabetes in New Zealand (Ministry of Health, 2009).

2.3.2 Diabetes and Ethnicity

It is entirely clear that different ethnicities have a different proportion of diabetes. For example, people from Native American, Polynesian or Micronesian, Asian-Indian Hispanic, or African-American descent are at higher risk of diabetes compared with people of European ancestry (King & Rewers, 1993). In New Zealand, the prevalence of diabetes is greater among Pacific people, and the lowest prevalence belongs to Europeans (Ministry of Health, 1997b). The highest and largest proportion of new or known diagnosed diabetes belongs to Pacific men and women aged 45-64. Also, in regards to IGT, Pacific and Maori in the working age <45 have the highest prevalence of IGT (Sundborn et al., 2007). Pacific people constitute almost 8% of the entire New Zealand population (Pacific Report, 2011). To diagnose diabetes is very simple, yet more than half of all diabetes cases remain undiagnosed, especially among Pacific people (Ministry of Health, 1997b, 2012). Being Maori or Pacific plays an important role in the increased risk of diabetes in New Zealand (Sundborn et al., 2007). It is now clear that the prevalence of diabetes is very high within the Maori population (Catherine & Zinman, 2007; Lawrenson et al., 2009; Tipene-Leach et al., 2004). Ethnicity also influences the complications of diabetes. For instance, among Maori, renal complications and deaths from renal diseases are significantly higher than those of European descent (Joshy et al., 2009). Accordingly, the highest risk populations should be considered as a priority to try any successful treatment for diabetes.

2.4 Most Common Diabetes Risk Factors

Over the last half-century, lifestyle changes have led to a significant increase in the outbreak of diabetes all around the world. Physical inactivity increased food consumption, and aging is the most important risk factors for diabetes. Nevertheless, genetic predisposition and socioeconomic status are not to be ignored. This is the most readily obvious among Maori and Pacific populations. The family history of diabetes has also been introduced as an independent risk factor for diabetes (Consortium, 2013). T2DM is no longer accepted as only a disease of adult people. As diabetes prevalence increases, the age of diabetes onset is also reducing. In New Zealand, one out of six people over 60 years old has diabetes. Also, the lower income has a negative correlation with the development of diabetes (Ministry of Health, 1997a). Uncontrolled glycaemia is another important issue as a risk factor for diabetes. Control of glycaemia is critical to the reduction of future hazards. It has been shown that even a single event of reducing blood glucose levels to a normal range reduces the future of diabetes risk factors significantly (Perreault et al., 2012). Perreault et al. (2012) reported that each time reduction of glucose within a diabetic group results in decreasing the risk of diabetes. For instance, a single one-time, second-time and third-time reduction of glucose are equal to 56%, 61% and 67% reduction of diabetes complications respectively. Any effort to reduce blood glucose to the normal level may be caused to increase life expectancy and healthy living for diabetic patients. Perreault's study also showed that age plays a major role in the diabetes risk factors. Young age participants had a higher risk of diabetes in the future (age< 45 years). Also, taking some medicines such as large doses of exogenous steroids may cause of diabetes (A. D. Association, 2006). Other common risk factors for diabetes include hypertension, hyperlipidaemia and gestational diabetes (IDF, 2015). In New Zealand, as with other countries, obesity, gaining weight, no exercise, and low consumption of fiber-containing foods are the most important risk factors for T2DM (Hu et al., 2001; Jenkin et al., 2011; Swinburn et al., 1999; Uusitupa, 2002). There is no doubt about obesity as a major risk factor for diabetes in all communities, regardless of their ethnicities. There is a myriad of evidence indicating obesity in the development of diabetes. Now, the impact of weight loss to improve diabetes is accepted universally (Feinglos & Bethel, 2008; IDF, 2015). Due to the significant role of obesity in the development of diabetes, it will be explained in more detail in the next section. Diabetes is a cocktail of several risk factors. This is why the exact underlying mechanism of diabetes is still questionable. Research efforts, therefore, should be

focused on finding the best way to control and prevent diabetes. Despite the enormous consensus about the crucial role of diabetes prevention, there is still no effective approach to the prevention of the disease (D. P. P. R. Group, 2009).

2.4.1 Obesity

Obesity is a significant issue in public health that is increasing rapidly, particularly in industrialized nations (Olson, 2016). The most widely accepted classification of obesity is based on BMI. In this classification, obesity has three grades, Grade 1 or overweight with BMI of 25-29.9 kg/m^2, Grade 2 or obesity with BMI of 30-39.9 kg/m^2 and Grade 3 or morbid obesity (severe obesity) with BMI $\geq$40 kg/m^2 (http://apps.who.int/bmi/) obesity is also categorized according to the body fat percentage. That is percentage of body fat >25%, with 21-25% being borderline and percentage of body fat >33%, with 31-33% being borderline for men and women respectively (Aronne, 2002). There are several kinds of measurements to evaluate obesity, such as BMI, waist circumference, waist/hip ratio calculation, DXA, and BIA. The link between diabetes and obesity goes back many years. Elliott P. Joslin introduced obesity as the risk factor of T2DM for the first time in 1921 when he mentioned that the rate of diabetes is higher among obese people (Joslin, 1921). Obesity is the sixth greatest risk regarding leading burden of disease globally (Guh et al., 2009). It was shown that only a 1 kg loss of weight resulted in a decrease in the risk of diabetes by 20%. In addition, it has been documented that 5% or a 5 kg weight loss resulted in decreasing the incidence of diabetes by 58% (Motala et al., 2008). In another study, researchers showed that the risk of diabetes increases by four-fold in people who are aged 20 to 44 years old (Vanitallie, 1992). In a cohort study of more than 50,000 people, it was reported that the risk of diabetes is strongly related to BMI. The higher an individual's BMI, the greater risk of diabetes. Therefore, BMI is considered to be the dominant risk factor for T2DM (Carey et al., 1997). Although the exact mechanism of obesity in the development of IR and T2DM is unclear, adipose tissue likely plays a vital role through acting on fat, liver or skeletal muscles to impair insulin activity. It has been shown that obesity is associated with hyperinsulinemia and increased levels of insulin, and it may stimulate IR via downregulation of insulin receptors. The potential candidate factors to contribute to IR are mostly adipocyte-based substances such as resistin and adiponectin and inflammatory factors such as interleukin-6. Also, free fatty acids are capable of inhibiting insulin activities (Lyon et al., 2003). Despite the increasing pace of gains in health-related knowledge, there is still no accurate treatment

for obesity. Every day we hear and read information about new findings for obesity treatments. As a population, we are still victims of too many deaths due to the misunderstandings of lifestyle changes. The role of obesity research must be to find an accurate and precise solution to remit obesity. Obesity management at first is usually followed by an intensive and a comprehensive lifestyle change such as diet, pharmacotherapy and later, for those who have continually unsuccessful treatment, bariatric surgery is chosen as the final decision (Aziret et al., 2016; Ioannides-Demos et al., 2005; Mun et al., 2001; Wadden et al., 2012).

2.4.2 Insulin Resistance

The exact mechanism of insulin resistance has not been discovered yet. However, the role of free fatty acids in the development of IR is leading consideration (Delarue & Magnan, 2007). IR is a complex metabolic issue. There are several indices to calculate IR in the human, but the "gold standard" for evaluation of IR is to calculate hyperinsulinemic euglycemic clamp (HEC). The complexity of measuring HEC led to the development of a simpler calculation to quantify IR and insulin sensitivity (IS). Most of those indices simply are calculated from OGTT (R. A. DeFronzo et al., 1979). It means indices are calculated based on FPG, fasting plasma insulin (FPI) or during the time of 0 to 120 min after drinking glucose. Most popular measurement methods include homeostasis model assessment‑insulin resistance (HOMA-IR), quantitative insulin sensitivity check (QUICKI) index, Matsuda and Belfiore index, area under the curve (AUC) and the Stumvoll index. These indices are utilised in both clinical and epidemiological studies. The calculation formula for each one is presented in Chapter three. IR is one the significant predictors of T2DM (ADA, 2015). Therefore it is crucial to calculate its risk using the above-mentioned quantification indices (Guenther Boden, 2001).

The homeostasis model assessment was introduced to quantify IR and BCF from fasting glucose, insulin, and C-peptide readings. HOMA measurement works as a bridge between glucose and insulin levels to predict basal steady-state glucose and insulin amounts for IR and BCF. The concentration of insulin depends on pancreatic beta cells, and blood glucose depends on insulin. Therefore, any alteration of beta cell function (BCF) will affect insulin secretion and the metabolism of glucose (D. Matthews et al., 1985). Each population has its range of HOMA-IR, but HOMA-IR<2.5 is considered as normal (Gutch et al., 2015). QUICKI was introduced by Quon (Quon, 2002). QUICKI is an empirical mathematic

formula derived from FPG and FPI to predict IS. It shows a better correlation with glucose determination in obese people (Vanhala et al., 2002). The proper range for non-obese, obese and diabetic subjects is 0.38, 0.33 and 0.30 respectively (H. Chen et al., 2003). The Matsuda Index was introduced by Matsuda and De Fronzo (Matsuda & DeFronzo, 1999), with the main aim of the measurement to calculate whole body insulin. The use of the Matsuda index is controversial. Sometimes it is considered as being equal to HOMA-IR for the estimation of IS (Kuo et al., 2002), and other times it is not (Chiu et al., 2002).

The Matsuda index covers both hepatic and peripheral tissue insulin sensitivity. Matsuda index values less than 4.3 predict T2DM (Gutch et al., 2015). The Belfiore index is a formula which compares measured glucose and insulin levels. Belfiore index values >1.27 predict pathologic IR (Belfiore et al., 1998). The Stumvoll index is a type of calculation to measure IS, insulin release and BCF from demographic parameters such as age, sex, and BMI, along with glucose and insulin levels during an OGTT to predict BCF (Stumvoll & Gerich, 2001). Calculation of the AUC gives more information on the concentrations of insulin and glucose against time (Allison et al., 1995). Technically speaking, the glucose or insulin amount is measured at certain time points, and the trapezoidal rule is applied to calculate the area under the curve (hence the name, AUC).

2.5 Traditionally Diabetes Management

T2DM includes a cluster of dysfunctions which lead to hyperglycaemia and result in IR, poor insulin secretion and increased or inapplicable glucagon secretion. Figure 2.3 shows the pathophysiology of T2DM.

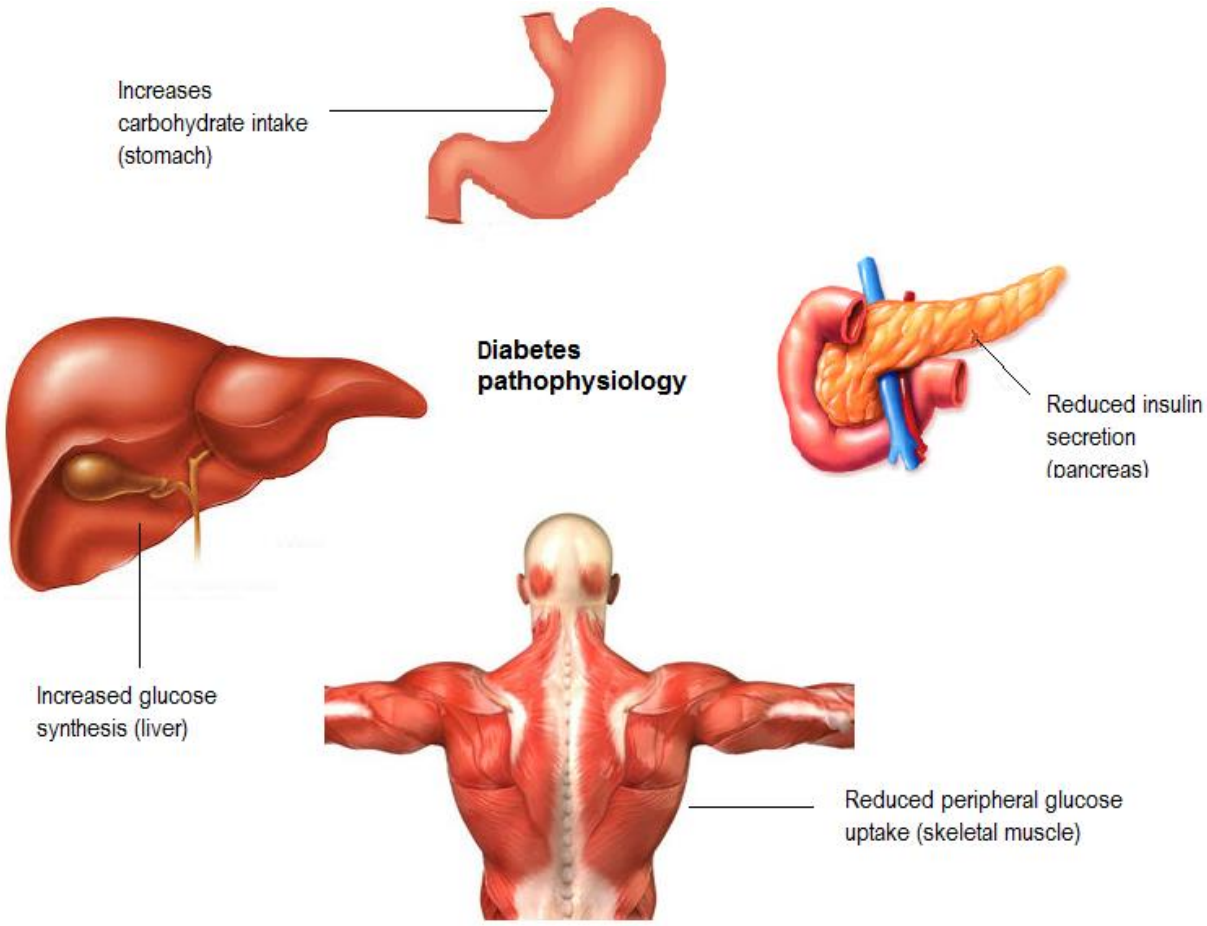


Figure 2.3 Pathophysiology of T2DM.

Four organs, pancreas, liver, stomach and skeletal muscle play most important roles to the development of T2DM.

The management of T2DM follows three important concepts. First, risk reduction of microvascular complications such as eye and kidney diseases via glycaemic control and blood pressure. Second, risk mitigation of macrovascular complications such as CVDs and peripheral vascular diseases via controlling lipids and high blood pressure (hypertension), as well as smoking cessation. Third, risk reduction of metabolic and neurologic diseases thorough glycaemic control (Inzucchi et al., 2012a; Inzucchi et al., 2012b; D. Keller, 2012). Chronic illness such as diabetes and obesity need self-management along with medical treatment to be successful. Stages of lifestyle changes associated with management of illness include pre-contemplation, contemplation, preparation, action, and maintenance (Bazata et

al., 2008). However, there is no strong signal over extended periods of time which prevent individuals from developing metabolic diseases such as obesity and diabetes (Bazata et al., 2008). Even with all of its associated data over an extended period of time, the concept of normalisation of blood glucose has not been universally accepted as a prevention of diabetes or its complications (Ingelfinger, 1977; Mannucci et al., 2013). Even through an intensive glucose lowering management program, the risk of diabetes complications (i.e., retinopathy) in a short period was shown to increase (Assessment, 1984). It is of vital importance to find a solution which at least controls diabetes for a longer period.

2.5.1 Diet and Exercise

There is a direct correlation between diabetes and obesity (C. f. D. Control & Prevention, 2002; M. I. Harris et al., 1998; Moakdad et al., 2001). Therefore, weight loss significantly reduces the incidence of diabetes (Lu et al., 2003). The first step to reducing weight is to make and keep a lifestyle change and to exercise (Acheson, 2010). It has been shown that a mean weight loss of 11 kg reduced blood glucose from 10.4 to 7 mmol/l (Wilson et al., 1980). Exercise and weight loss are two important factors to reduce the destructive effects of diabetes. Very low-caloric diet (VLCD) is a well-known intervention for the remission of T2DM (Kelley et al., 1993). This practice is very similar to bariatric surgery (Isbell et al., 2010; Jackness et al., 2013). It has also been shown that weight loss outcomes with an intensive calorie restriction or VLCD, about 420 kcal/d, is similar to a hypocaloric diet (1200 kcal/d) intervention (Baik, 2013). The main aim of the lifestyle change for diabetic people is intended to improve IR and loss of weight. Four to five kg weight loss is associated with significantly improving IR and hyperlipidaemia along with hypertension (Amatruda et al., 1988; Greco et al., 2002). Lifestyle modification is advantageous for people who have T2DM. The reduction of energy consumption to 1,100 kcal/d has been shown to have a superior effect to reduce FPG in obese individuals with T2DM people in comparison to individuals who do not change their lifestyle. Decreased levels of glucose are due to reduced hepatic glucose synthesis. Insulin sensitivity and FPG decreased less than a month after the beginning of calorie restriction with a weight loss of about 6 kg. However, the weight loss caused by intensive diet restriction has no positive impact on pancreatic beta cells' capacity to release insulin (Maggio & Pi-Sunyer, 1997). Thus, using energy restriction interventions such as VLCD not only does not guarantee a sustainable weight loss solution, but it can lead to another health problem when used as a long-term consumption option (Henry &

Gumbiner, 1991). Long-term lifestyle changes such as more exercise and/or less food has no promising effect on the remissions of diabetes and obesity (Ackermann et al., 2008; Colquitt et al., 2009; Laferrère, 2011). For people who have diabetes, the management of the disease must involve a careful nutritional evaluation and replace harmful eating with a realistic dietary plan. The aim of diet therapy in T2DM is to control blood glucose, normalise lipid profiles and maintain an ideal body weight. The scenario for people who are obese with diabetes is slightly different. These individuals may require special weight loss plans along with changing some other habits to maintain their body weight (DCCT, 1995; Group, 1998). Unfortunately, for obese individuals, it is not always successful to stick to a strict plan for a long-term (Bazata et al., 2008). In most cases, diabetic people prefer to adhere to medications rather than exercise or diet (M. I. Harris, 2001). Exercise plays a crucial role in the management of diabetes. Physical activities can reduce blood glucose and maintain body weight. However, care must be taken for people who have diabetes to prevent hypoglycaemia caused by working out. The role of exercise to improve insulin sensitivity has been documented (Cunningham-Myrie et al., 2015; D.-W. Kang et al., 2016), and even has a few drawbacks. The primary drawbacks are that exercise is time-consuming and exercise by itself has only a modest influence on losing weight. Although it is clear that diet and exercise cause improved weight management (A. D. Association, 2000), the outcome of these interventions is not always promising after a distinct time (Gilis-Januszewska et al., 2011).

2.5.2 Pharmacotherapy

Some obese people with diabetes prefer to take pharmaceutical agents to reduce their weight even though there is no exact cure for the treatment of diabetes either by medicine or diet (Bray, 2008; Kraschnewski et al., 2010). Some medications have been introduced to reduce weight between 5 and 10 percent in a year while maintaining good glycaemic control (Jacob et al., 2009; Lloret-Linares et al., 2008; O'neil et al., 2012). Despite the ease of use of pharmacotherapy to treat diabetes and obesity, it is not always a reliable intervention for diabetes over a long-term, when it compared with diet and bariatric surgery. Additionally, using any pharmaceutical agent has its side-effects, and weight loss alone is not always associated with an improvement in glucose metabolism (Manning et al., 1998; Van der Merwe et al., 1996). Further, there are unknown and several side effects for diabetes pharmacological agents apart from their cost. At the same time of developing diabetes, more medications are needed to be prescribed. Most people who are in higher demand of more

medications are overweight or obese (C. f. D. Control et al., 2005). For example, a study showed that about 70% of diabetic patients who take medications are overweight or obese (Wylie et al., 2002). If they are not treated by an appropriate method, they are likely to gain more weight, increased waist circumference, and increase blood pressure and the risk of CVDs. IR will likely worsen, and as a result, there is an increased risk of diabetes microvascular-related mortality. Although several studies show lifestyle intervention may be critical to reducing the prevalence of diabetes, still it is not confirmed for whole and different populations (Stumvoll et al., 2005). A few important criteria to have for finding and introducing a procedure for the remission of diabetes must be reliable, user-friendly, inexpensive and safe as treatments.

2.5.3 Bariatric surgery

Currently, bariatric surgery is the best and the only effective method to control and treat obesity and diabetes. It can provide a significant sustained weight loss among obese people along with an improvement in obesity-related diseases such as diabetes. Bariatric surgery is certainly a reasonable substitute in wisely selected subjects if an experienced team is available. Since 2011 (Dixon et al., 2011), bariatric surgery has been accepted as an appropriate treatment for obese people with T2DM who are disabled, and need to achieve a good glycaemic control after other medical therapies have failed (DPP, 2002). Alternatively, there is an elegant prospective clinical trial, the Swedish Obese Study (SOS) which involved 4047 obese subjects. This study evaluated the progress of obesity and diabetes over a period of 14.7 years. In this study bariatric surgery is associated with a lower rate of diabetes and obesity-related comorbidities when compared with other interventions such diet and exercise (Sjöström et al., 2012). Recently, according to a report from the Ministry of Health in 2014, bariatric surgery has been a popular intervention in New Zealand, where the prevalence of obesity is about 31% of adults and 10% in children (www.health.govt.nz/publication/annual-report). In New Zealand and Australia together, It has been reported that about 12,000 bariatric surgeries were performed by 2013 (Buchwald & Oien, 2013). Bariatric surgery is a panacea for the remission of diabetes. However, traditional therapies such as diet, exercise, and pharmacotherapy, despite their temporary effects on diabetes (Avenell et al., 2004), can be used with fewer limitations than bariatric surgery. For instance, bariatric surgery is mostly applied in obese people with grade 2 obesity or above (Buchwald et al., 2004; Buchwald et al., 2009). Although, according to the latest guideline released by the Diabetes Surgery

Summit (DSS-II), bariatric surgery should be applied among diabetic people with grade 1 obesity (R. V. Cohen et al., 2016; Rubino et al., 2016). Sometimes, the effect of bariatric surgery on the remission of diabetes happens in just a few hours or days (Abbas, 2006; Wickremesekera et al., 2005) which is entirely independent of weight loss (K. T. Nguyen & Korner, 2014). Therefore, it is not clear if the remission of diabetes after bariatric surgery is due to losing weight or some other combination of factors. The most aspirational treatment criteria for T2DM is to change abnormal glucose levels to normal levels, make the treatment an affordable treatment, available to everyone, as well as making it safe and non-invasive in a long-term and a sustainable method (Pinkney, 2011). Despite the appreciable effect of bariatric surgery on the remission of T2DM, it does not fulfill all the criteria cited. Thus, knowing about the underlying mechanisms that appear after bariatric surgery in the remission of diabetes, may help to find a novel way for therapeutic diabetes intervention independent of bariatric surgery.

2.6 Mechanism of Bariatric Surgery

The prevalence of obesity has continued to increase and become a universally important health issue. Chronic diseases such as obesity and diabetes account for 84% of health care costs (Moses et al., 2013). Lifestyle modification is the first choice to reduce weight. Since it is not an appropriate intervention for all individuals -- after 5 years, regaining weight is very common (Albrecht & Pories, 1999; Leibbrand & Fichter, 2002; Mingrone et al., 2012) -- there is a need to replace voluntary lifestyle changes with another intervention such as bariatric surgery (Sjöström et al., 2007).T2DM is considered to be a gastrointestinal disease (Pok & Lee, 2014). Bariatric surgery is currently the best treatment for obesity and its morbidities such as diabetes (Buchwald et al., 2009; Mahawar et al., 2016), CVDs (Lupoli et al., 2015; Pontiroli & Morabito, 2011) and cancer (Ashrafian et al., 2012; Upala & Sanguankeo, 2015). Using bariatric surgery is not a new intervention for weight loss (Kremen et al., 1954; Pories et al., 1987). For more than a half-century, this method has been used by a large number of people for weight loss, while the mechanism arising from the surgery which results in a resolution of diabetes is still not clear (Ferchak & Meneghini, 2004).The first kind of bariatric surgery was performed on a human in 1954. In this procedure, 91 cm of jejunum was anastomosed to 46 cm of ileum in an end-to-end form. Then, the bypassed segment of the gut was drained into the colon (Kremen et al., 1954). Bariatric surgery or the

“metabolic procedure” was defined for the first time by Henry Buchwald and colleagues as “the operative manipulation of a normal organ or organ system to achieve a biological result for a potential health gain” (Buchwald et al., 2009; Buchwald & Oien, 2013). There are several types of bariatric surgeries such as gastric bypass or RYGB, SG, biliopancreatic diversion (BPD) with or without duodenal switch, and adjustable gastric band. Figure 2.4 shows four different kinds of bariatric surgeries.

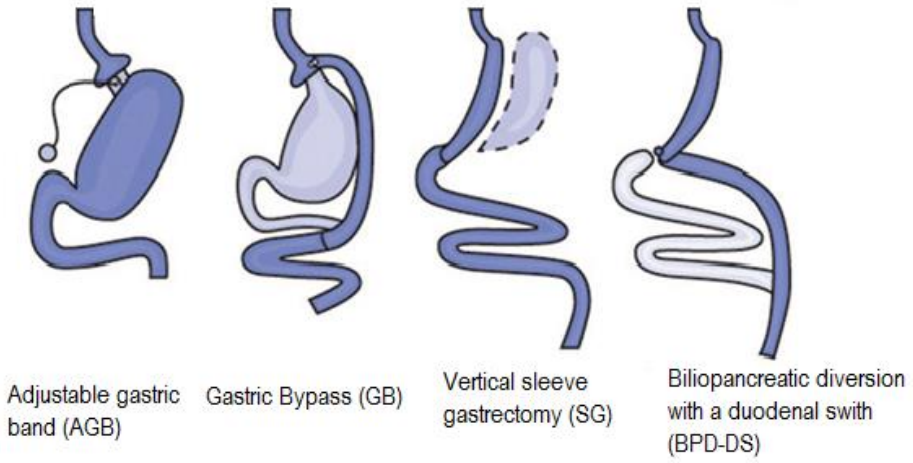


Figure 2.4 Four common different types of bariatric surgeries.

Adapted from (Vetter et al., 2012).

Most common types of bariatric surgeries for the remission of diabetes are RYGB and SG, while the rest of them are not popular (Caiazzo et al., 2010). Laparoscopic adjustable gastric band (LAGB) is the most common type of surgery after RYGB in the US. Although the rate of mortality in this kind of surgery is lower than RYGB, the increased weight loss is significantly less compared with RYGB (Parikh et al., 2005). Obviously, there is no bypass of digestive hormones after LAGB. So, this procedure is purely restrictive. This surgery is a good example of how different bariatric surgeries have different impacts on diabetes remission (Herron & Tong, 2009).

Another type of bariatric surgery is biliopancreatic diversion with a duodenal switch (BPD-DS), which is the most malabsorptive procedure (Herron & Tong, 2009). BPD-DS is a combination of a SG and intensive malabsorptive intestinal bypass. In this method, the first

part of the duodenum is separated and attached to the last 250 cm of the ileum whereas the rest of intestine remains attached for transport of bile and pancreatic secretions. This procedure is less restrictive than RYGB or gastric band, but it is a more malabsorptive approach (Herron, 2004). This surgery is one of the most efficient ones used to reduce weight, even after 13 years (Buchwald et al., 2004).

Each bariatric surgery has a common aspect with other types of bariatric surgeries, that is weight loss, restricted calorie intake, intestinal malabsorption, enzymes and hormonal alterations and structural differences in the digestive system. It is also why weight loss may not be the exact mechanism for the remission of diabetes (Balsiger et al., 2000). All bariatric surgeries follow malabsorption, restriction or a mixture of both. It has been documented that bariatric surgery in the improvement of T2DM acts through modulation of BAs, gut microbiota and alteration of incretin hormones. For example, gut microbiota may play a pathophysiological role in obesity. The microbiota's role has been proposed to act as a source of energy in the body (K. T. Nguyen & Korner, 2014). Bariatric surgery due to its mechanism may stimulate some changes in the structure and function of the intestinal flora (Furet et al., 2010; Liou et al., 2013; Husen Zhang et al., 2009). The function of bariatric surgery is generally based on two hypotheses, termed the foregut and hindgut hypotheses (Goh et al., 2016). Both theories are clearly supported by animal studies (Patriti et al., 2007; Preitner et al., 2004). The foregut hypothesis postulates that digested food is bypassing the duodenum results in a decrease in undetermined anti-incretin hormones, causing improved insulin sensitivity, (Hansen et al., 2011; W.-J. Lee, Chong, Ser, et al., 2011; Rubino et al., 2006). However, the hindgut hypothesis claims that early contact of undigested food to the hindgut results in an increase in the release of gut hormones followed by an improvement in glycaemic control, (Bose et al., 2009; Laferrere, 2011; W.-J. Lee, Chong, Ser, et al., 2011). There is a need to study more about the mechanism of bariatric surgeries in humans. Nevertheless, when comparing the scant information on human studies, there are conclusions available to be used for the improvement of diabetes which is gained from studies following bariatric surgeries in animals.

2.6.1 Laparoscopic Gastric Bypass or Roux-en-Y gastric bypass

The first type of gastric bypass or RYGB was performed in 1960 (Mason & Ito, 1967). RYGB was initially applied to the treatment of peptic ulcers caused by *Helicobacter pylori*

(Friedman et al., 1955; Seeley et al., 2015) and is the most common type of bariatric surgery (Buchwald & Oien, 2009, 2013; Santry et al., 2005). Nowadays, RYGB is done by laparoscopy. Laparoscopy results in a shorter period of post-operation recovery, fewer wounds, infections, hernias and wound discharges (Higa et al., 2001; Rausa et al., 2016). To perform RYGB, the surgeon first cuts the abdominal wall with five to 6 small incisions. Then, using multiple shootings of a surgical stapler the stomach is divided into two compartments, the upper stomach pouch, which is about 15 to 30 ml volume, and the lower, bypassed gastric residue. After that, the jejunum is surgically divided 50 to 100 cm beyond its origin at the ligament of Treitz and reconnected in a Y-shaped fashion such that one arm of the Y (the Roux limb or alimentary limb) drains the small gastric pouch, whereas other remaining parts provide a pathway for the gut secretions, bile, and pancreatic juices created from the gastric remnant and duodenum (Herron, 2004). Figure 2.5 illustrates the schematic of RYGB surgery. It is suggested that RYGB has a little more malabsorptive property than other methods due to the separation of ingested food from digestive enzymes (Herron & Tong, 2009). In other words, RYGB potentially facilitates gastric acid, bile, amylase, and lipase during the passage of food from the digestive system. That is why it is postulated that hormones may play a crucial role in weight loss after RYGB (D. E. Cummings et al., 2002). RYGB results in the decrease in need of antidiabetic medicine in a range of 80 to 98% (Herron & Tong, 2009). Previously, the malabsorption and restrictive implications of RYGB were considered to be the reasons for the remission of diabetes. However, more recently, the effect of hormonal changes is highlighted over the restrictive mechanism (Kashyap et al., 2010).

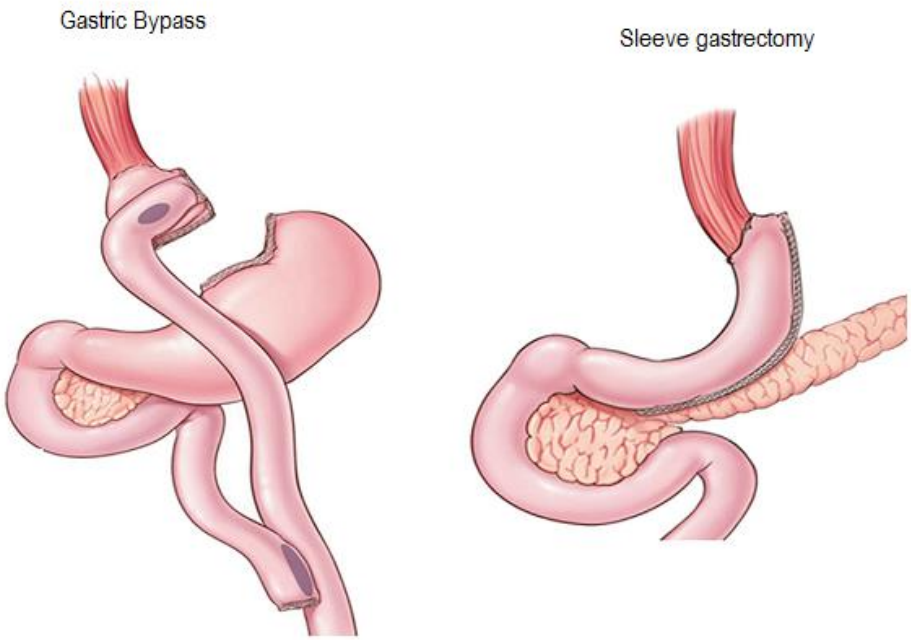


Figure 2.5 Most common type of bariatric surgery.

The figure shows most common bariatric surgeries for the treatment of T2DM. Briefly, in RYGB a small pouch is created just below the esophagus which is not in contact with the rest of the gut. The jejunum is anastomosed to this small pouch thus consumed food "bypasses" the remaining of the gut and upper small intestine and directs to the jejunum. In SG, almost 80% of the stomach is removed along with fundus, where gut hormones are produced and released. Then turning of the stomach into a "sleeve." Adopted from (Seeley et al., 2015).

RYGB Successes and Limitations

RYGB shows the greatest impact on obesity and diabetes improvement in comparison with SG and AGB (Gill et al., 2016). However, the exact underlying mechanism for the remission of diabetes after RYGB is yet to be elucidated. There are two types of RYGB, biliopancreatic or standard RYGB, and mini gastric bypass or single anastomosis (W.-J. Lee et al., 2005). Therefore, it may be helpful to interpret glycaemic control based on the type of RYGB. Figure 2.6 shows different anatomical changes after RYGB. Some assumptions are considered for the remission of diabetes after RYGB, namely in the form of hormonal changes. There are three hormones which associated with changed levels after RYGB: ghrelin, peptide YY (PYY), and glucagon-like peptide-1 (GLP-1). GLP-1 is sometimes considered to be the most important factor to remit T2DM after RYGB (Mitchell et al., 2013).

Also, Gut microbiota plays an important role in metabolic complications**.** Lipopolysaccharides (LPS), which are structural parts of intestinal microbiota work as important inducers of low-grade inflammatory response and IR (K. Harris et al., 2012)**.** It has already been documented that obesity is associated with IR (Bigornia et al., 2012) and it is considered to be an inflammatory disorder (Harman-Boehm et al., 2007; Schipper et al., 2012)**.** RYGB is a potent procedure performed to reduce LPS and inflammatory markers (Monte et al., 2012), and together with hormonal changes may be considered as an alternative procedure to reduce metabolic complications. RYGB has other advantages over the long-term, such as significant and sustained loss weight and excessive weight loss (EWL) (N. Shah et al., 2016; Valezi et al., 2011) thereby improving different medical health issues (Buchwald et al., 2004).

Although it has been shown that RYGB has a high rate of improvement for weight loss and metabolic complications like T2DM, it has been confirmed that small pouch and/or any other type of energy restriction cannot be responsible on its own for either weight loss or resolution of T2DM (De Paula et al., 2010b).

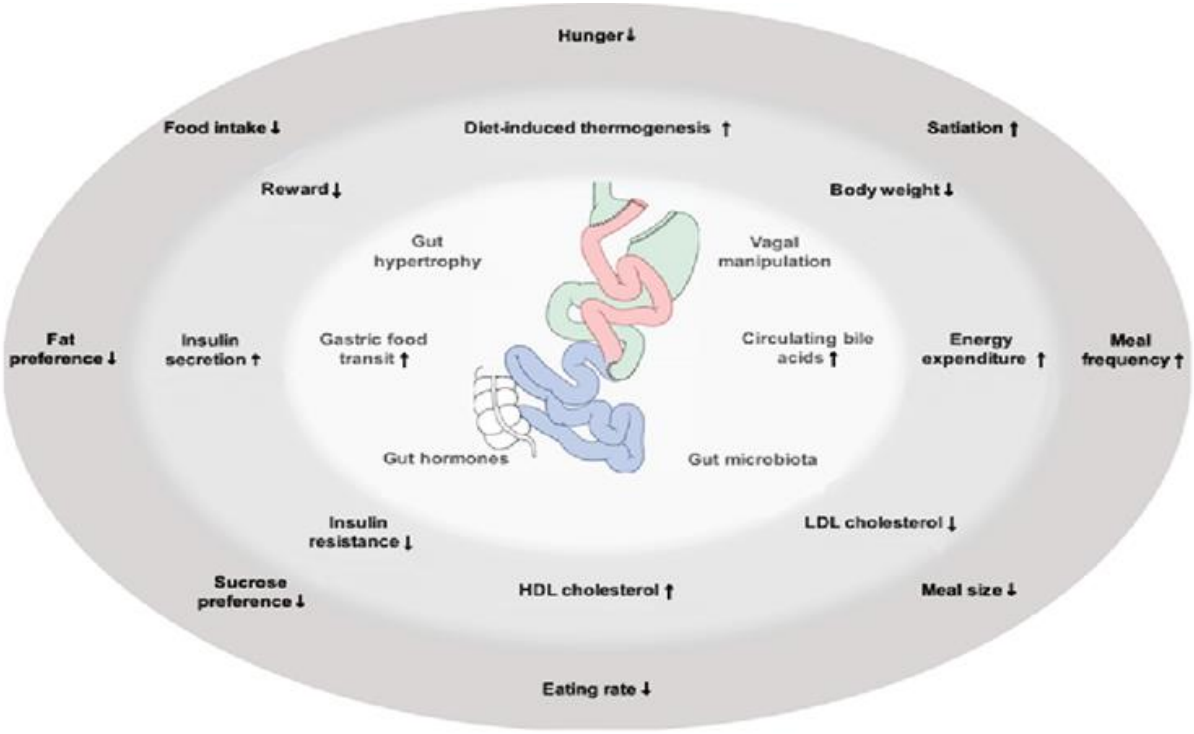


Figure 2.6 Gastric bypass is the most effective metabolic surgery.

Schematic illustration of the physiologic effect of most effective bariatric surgery, namely RYGB, on the remission of T2DM. There are several underlying mechanisms occurred after RYGB for the development of T2DM. Those changes are divided to GI effect (inner circle, white colour), systematic effects (middle circle, light gray) and behavioral effects (outer circle, dark gray). Adapted with modifications from (Lutz & Bueter, 2014b).

RYGB has several complications. Dumping syndrome is prevalent after RYGB. Dumping syndrome is a state of rapid movement of foods, particularly sugars from the gut to the small colon. Although the symptoms in different people are not the same, it is usually associated with early satiety, crampy belly pain, nausea, vomiting and dizziness (Beek et al., 2017; Mallory et al., 1996). Malabsorption of some nutrients like iron, vitamin B1, B12, and folate is commonly observed after RYGB. Also, in some patients, there is a distinct change in medicine absorption due to the shift in pH after RYGB (A. D. Miller & Smith, 2006; R Padwal et al., 2010). Also, a common syndrome of RYGB is postprandial hyperinsulinemia hypoglycaemia (PHIH) with neuroglycopenia (Service et al., 2005). Although this syndrome is treatable, some subjects need to undergo another surgery to remove the pancreas (Mala, 2014). Another consideration before doing bariatric surgery is to know that if after surgery

there is a significant reduction in GI hormones, especially after RYGB (Berthoud, 2008). It should be noted that most of those hormones are found in the CNS. Therefore, any alteration in levels of those peptides (hormones) after surgery may directly affect the neural system. Furthermore, other issues in RYGB are requiring more hospitalization, IV treatment for dehydration, reoperation transfusion, haemoglobin reduction to less than 5 g/dl, transient kidney insufficiency or kidney failure, cholelithiasis, ketoacidosis, infection, pneumonia, hernia, anaemia, hypokalemia and anastomotic ulcer (Schauer et al., 2012). Last but not least, the compensatory effect of RYGB towards a remission of T2DM (Thaler & Cummings, 2009; Vetter et al., 2009), is not always available in all patients (Deitel, 2011).

2.6.2 Laparoscopic Sleeve Gastrectomy

SG compared with other types of bariatric surgeries is a new procedure. It was originally derived from BPD-duodenal switch procedure by Doug Hess in 1988 (Jossart & Anthone, 2010). In SG about 80% of the stomach is removed to improve obesity-related complications (Pories, 2008). SG was introduced as an option for treating to achieve the remission of diabetes in 2003 as a part of BPD-DS, but because of the good result of this technique as a "first stage" in BPD-DS, it was used as an independent technique for losing weight (Regan et al., 2003). Current rates of using sleeve gastrostomy are increasing above those seen for AGB. Similarly, SG is mostly done by laparoscopy procedure. To do SG, the greater part of the stomach or left side is resected. The gut is then stapled over a sizing tube ranging from 11 to 20 mm diameter. It creates a banana-shaped stomach. By decreasing the volume of the gut, the SG forms a substantial restrictive effect. Most parts of the fundus, where ghrelin (an appetite stimulator hormone) is secreted, is removed. This mechanism may partly result in the remission of diabetes (D. E. Cummings et al., 2002; Herron & Tong, 2009; Shi et al., 2010). In SG, the volume of the stomach is decreased to 150 to 200 ml (Herron & Tong, 2009). Figure 2.5 is a schematic illustration of SG.

SG Success and Limitations

SG has been suggested as the first step for the treatment of severe morbidly obese patients or patients who are at high risk for contraindications of other operations such as BPD or RYGB (Langer et al., 2006). The size of the stomach is necessary to have more benefits from the procedure. Follow-up studies of SG showed that the greater remission is associated with, the

smaller size of the stomach (Johnston et al., 2003; C. M. Lee et al., 2007). Also, SG is safer, faster and compatible with laparoscopy in comparison with other surgeries (Jossart & Anthone, 2010). Nevertheless, the smaller size of the stomach causes an increased risk of bleeding and dehiscence, particularly close to the gastroesophageal junction (Jossart & Anthone, 2010). There is an animal study shows that 40% of obese people with T2DM show improvement in disease symptoms a year after SG (Schauer et al., 2012).

SG, like RYGB, has several complications such as pulmonary, cardiovascular and wound issues (Jossart & Anthone, 2010). In a study which was done for 11 years, the need for re-operation after SG increased (Weiner et al., 2011). The exact mechanism of SG and its alterations on the remission of diabetes is a big question. Thus, more research is needed to find out any correlation between SG and the remission of diabetes.

2.7 RYGB and SG a Comparison

The foregut theory, or duodenum and upper jejunum bypass surgery, showed the greatest impact in remission of T2DM, with about 93% resolution compared with 47% resolution of T2DM after SG (Karamanakos et al., 2008; W.-J. Lee, Chong, Ser, et al., 2011). There are several observational studies which indicate the promising results of RYGB to improve metabolic complications disorders (Buchwald et al., 2009; Pories et al., 1995; Schauer et al., 2003a; Scopinaro et al., 2005; Sjöström et al., 2004). For example, In Schauer et al. (2012) research indicated that there is a greater weight loss after RYGB than SG. Also, there was a significant difference regarding BMI observed between RYGB and SG. On the other hand, changes in BMI were greater among patients who underwent RYGB instead of SG. Although both RYGB and SG were superior to medical therapy for weight loss, 88% of patients who underwent RYGB showed greater weight loss in comparison with SG (81%) (Schauer et al., 2012). On the contrary, it has been reported that RYGB has a higher preventive effect on IGT in comparison with gastric banding procedure (GBP), namely 99-100% vs. 50-60% respectively (Ferchak & Meneghini, 2004), while the effect of SG on IGT has not clearly reported yet. In one of the recent study, the safety of RYGB, SG, and AGB has been compared to two years (Gill et al., 2016). In this study, it was also shown that RYGB yielded a better change in BMI than either SG or AGB. Nevertheless, the obesity-related comorbidities seem to be better in RYGB than the other two surgeries (Gill et al., 2016). However, it seems SG is safer and easier than RYGB with fewer rates of leakage related to

the surgery (Jossart & Anthone, 2010) although, sometimes after SG there is a need for RYGB (W.-J. Lee, Chong, Ser, et al., 2011).

It has been shown that long-term complications of SG are less than complications from RYGB; namely, SG is not often associated with dumping syndrome, peptic ulcer, hernia and less malabsorptive (Moy et al., 2008). The EWL after SG is between AGB and RYGB (Arroyo et al., 2010). A 66% EWL 3 years after SG (Armstrong & O'Malley, 2010) with a significant reduction in the fat mass has been reported (Schauer et al., 2003a). Also, there was a significant resolution reported after SG. On the contrary, in some populations, there was no difference observed concerning hormonal changes between RYGB and SG (Peterli et al., 2009).

2.8 Bariatric Surgery Disputes

Although bariatric surgery has been introduced to the improvement of metabolic complications (A. D. Association, 2016; Holzbach, 1977; Moxley III et al., 1974; Hongwei Zhang et al., 2017), several factors need to be considered before deciding to do bariatric surgery. The first and maybe the most important consideration is that the remission of diabetes happens just in a few days after bariatric surgery without a significant weight loss (Wickremesekera et al., 2005). It means the compensatory effect of bariatric surgery is independent of weight loss (Polyzogopoulou et al., 2003; Pories et al., 1992; Pories et al., 1995; Schauer et al., 2003a; Wickremesekera et al., 2005); since after bariatric surgery, blood glucose is reduced before changing the weight (Rubino et al., 2004; Wickremesekera et al., 2005) while the role BCF is highlighted as well (Nannipieri et al., 2011). Also, the higher remission of T2DM after bariatric surgery is mostly observed among people who were less obese (R. Cohen et al., 2006; W.-J. Lee et al., 2008; Thaler & Cummings, 2009).

Other concerns are the complications of bariatric surgery such as arthritis, skin disorders, liver failure (Mason & Ito, 1996), and malabsorption, particularly lactose intolerance (K. Miller & Hell, 2003; Stocker, 2003). Other complications include protein malnutrition (Strohmayer et al., 2010). This phenomenon is varied among different types of surgery with approximately 1-5% occurrence in RYGB and approximately 4-18% in BPD (Brolin et al., 2002; Faintuch et al., 2004). Of note, in restrictive surgery, the rate of protein malnutrition is negligible (Malinowski, 2006). However, protein malnutrition is significant as with any

leakage of protein, which is mostly seen one to two years after RYGB and BPD and results in low levels of albumin muscle atrophy and oedema (Malinowski, 2006). In addition, bariatric surgery is mostly an intervention for obesity. It is labour intensive, invasive and cost-effective. The rate of success in the surgery is varied by changing the procedure (Buchwald et al., 2004; Stefater et al., 2012). Moreover, genetic discrepancies are likely to have a role for the remission of diabetes after bariatric surgery. In other words, the effect of bariatric surgery on different people is not the same. Ethical history of a subject is directly related to the promising effect of surgery on to diabetes resolution (W.-J. Lee, Chong, Ser, et al., 2011).

Remission of diabetes after bariatric surgery may not be sustained for a long time. For instance, in a study which was conducted among 177 patients during a 5-16 year follow-up, it showed 43% of patients who had initial remission of diabetes after one year, again experienced a recurrence of T2DM and regaining weight (Chikunguwo et al., 2010). Regaining weight may be a cause for committing suicide in people who underwent bariatric surgery. It has been shown that elevated levels of BMI are linked with enhanced levels of depression (Mitchell et al., 2013). Suicide is mostly observed among subjects who underwent bariatric surgery while the suicide risk is mostly reduced among obese subjects who did not undergo bariatric surgery. RYGB seems to have a greater potential of patients committing suicide than other surgeries (Goldfeder et al., 2006; Mitchell et al., 2001; Pories et al., 1995; Powers et al., 1997). Thirty percent of suicides among bariatric surgery patients happen during the first two years after bariatric surgery, while the rest occurred three years after surgery (Kohli, Setchell, et al., 2013). Also, it has been shown that within people who underwent bariatric surgery, there is an increased level of alcohol abuse and death due to cirrhosis. Nevertheless, the acute effect of bariatric surgery to reduce blood glucose is not always present (Jüllig et al., 2014; Lettieri et al., 2008; Schauer et al., 2003b) and there is no similar mechanism for different bariatric surgery on the remission of T2DM (D. E. Cummings & Flum, 2008; Dixon et al., 2008; Stefater et al., 2012).

Taken together, finding more about the underlying mechanisms which occur after bariatric surgery may assist in the search for an independent surgery treatment for diabetes.

2.9 Randomised Clinical Trial

The Randomised Clinical Trial (RCT) is defined as a prospective intervention study in human subjects to evaluate experimental drugs, a new therapy, medical instruments or clinical methods. Research design in clinical trials is particular, and it plays a vital role in biomedical research. Clinical trials according to their criteria are classified into two general groups, uncontrolled and controlled clinical trials. In a clinical trial that is not involving a comparison between case and control group regarding a specific treatment or intervention, is called an uncontrolled clinical trial. However, controlled clinical trials involve a group of control subjects to compare with the case group. The validity of randomized, double-blind (which means neither patients nor researchers know the identity of the intervention or treatment), has the highest validity and accuracy (Yin, 2013). If a clinical trial is well-designed and appropriately conducted it is a powerful way to make a conclusion based on a given intervention. Whenever subjects meet the criteria, they enter into the study on different calendar days. All clinical trials have a study protocol. This protocol generally includes information about the background of the disease, rationale for the study, which methods will evaluate an intervention's efficacy and safety, and statistical design along with methodology ("statistical consideration"). In this design, the potential risks and advantages of the intervention, the size of sample, should be described. Also, all clinical trials have a general objective and several specific targets. All objectives must be practicable and clinically significant. Bias is a systematic error in any clinical trial. One of the best methods to reduce bias is to conduct a blind clinical trial. Another one is randomisation which effectively monitors patients within different interventions. In an RCT, confounding effects can be reduced significantly (Yin, 2013). Randomisation can be simply done by a computer. Therefore, using RCT can be a powerful tool to investigate for a new treatment or intervention of chronic diseases such as T2DM and obesity.

2.10 Biomarker Study

Biomarkers are currently used in basic and clinical research. Biomarkers are "biological molecules that show health and disease states" which are also called signature molecules and molecular marker (Lyons & Basu, 2012). Their role as essential endpoints in clinical trials is accepted universally. Some types of biomarkers are specific. It means they have been well characterised and consequently are confirmed to predict given clinical outcomes across a

diversity of treatments and populations. Nevertheless, the validity of a biomarker must be evaluated and re-evaluated (Strimbu & Tavel, 2010). There are a couple of different biomarker definitions by WHO (Organization, 1993, 2015). Both definitions overlap a concept; namely a link between biomarker and predicting the incidence of the outcome of a disease, and reproducibly at the same time for different populations. It is, however, important to know that biomarkers are always "provisional." Risk factors sometimes increase the chance of getting a disease. The most important effect of biomarkers is to detect a risk factor before appearing in the clinical pattern of a given disease. Biomarkers should be confirmed, at least, by two independent populations (Lyons & Basu, 2012). For example, HbA1c is a well-known biomarker to evaluate glycaemic control for diabetic patients who undergo a given intervention (Robb et al., 2016). Taken together, biomarkers play a crucial role to pave a way to improve drug discovery and biomedical research. Understanding a relationship between quantifiable biological substances and clinical outcomes is essential to expand our knowledge for treatment and knowing the pathophysiology of a disease. This goal is impossible to achieve without doing a retrospective analysis of biomarker through clinical trial studies.

2.11 Bile Acids

The end product of cholesterol is BA. BAs are steroid acids found in the gallbladder. They are produced by the liver and stored in the gallbladder. BAs are mostly classified in two forms, primary and secondary. BAs form bile salt and need to be conjugated by glycine and taurine (Lefebvre et al., 2009; Russell, 2003).

2.11.1 Biochemistry of Bile Acids

BAs are steroid-related substances that are created from cholesterol within hepatocytes (parenchymal) in the liver. BAs are released by the liver into the gallbladder after taking a meal (Lefebvre et al., 2009). There are several enzymes involved in the synthesis of BAs in different cells, but the liver is the main organ to have all complete sets of enzymes to produce BAs (Russell, 2003). There are several roles considered for BAs apart from their roles as biological detergents to clean up lipids. Roles such as solubilisation of fat-soluble vitamins (A, E, K and D) and participating in glucose metabolism (D. R. Schmidt et al., 2010; Smushkin et al., 2013) are among these roles. There are at least 17 enzymes involved in BA synthesis. The synthesis and metabolism of BAs are under very precise controlling. The main

reason of this is the cytotoxic property of BAs (Russell, 2003). It is why a little alteration in the amount of BAs results in serious consequences (Stiles et al., 2009). BA synthesis occurs via two common ways, "classical and alternative pathway" synthesis.

The main pathway for synthesis of BAs is through the "classical or natural pathway." This pathway is initiated by hydroxylation of cholesterol at the seven position through the activation of cholesterol 7α-hydroxylase (CYP7A1). CYP7A1, which is a member of cytochrome P450 family, originates from the endothelial reticulum. CYP7A1 is a rate-limiting enzyme, and its expression occurs only in the liver (Lefebvre et al., 2009). Figure 2.7 illustrates the classical pathway in brief.

The second mechanism to synthesise BAs is the "alternative pathway." In this pathway, the synthesis begins by hydroxylation of cholesterol at the 27 position via mitochondrial enzyme sterol 27-hydroxylase (CYP27A1). Those BAs produced by the action of CYP27A1 are eventually hydroxylated via oxysterol 7α-hydroxylase (CYP7B1) on the seven position. The alternative pathway is also called the "acidic pathway." In comparison with the classical pathway, only 6% of BAs are synthesized by the alternative pathway (Lefebvre et al., 2009). However, it does not mean that this pathway is not necessary. Any alteration in this pathway can lead to life-threating disease. For example, obesity, T2DM, hyperlipidaemia and CVDs can be influenced by alteration of the BA alternative pathway (Thomas, Pellicciari, et al., 2008). Figure 2.7 shows the alternative pathway. To complete the BA synthesis, the hydroxyl group at position 3 is in the β-orientation, it must be epimerised into the alpha orientation. The epimerisation is begun through conversion of the 3β-hydroxyl to a 3-oxo group catalysed by 3β-hydroxy-Δ5-C27-steroid oxidoreductase (HSD3B7). This is a critical conversion during the synthesis of BAs (Ferdinandusse & Houten, 2006). After this action, two end products of these pathways are released, chenodeoxycholic acid (CDCA) and cholic acid (CA). CDCA and CA are called primary BAs. The spreading primary BAs is under the activation of sterol 12α-hydroxylase (CYP8B1). The synthesis of CA is under intensive control of CYB8B1. Thus, the action of the CYP8B1 can determine the ratio of CA to CDCA (Lefebvre et al., 2009; Russell, 2003).

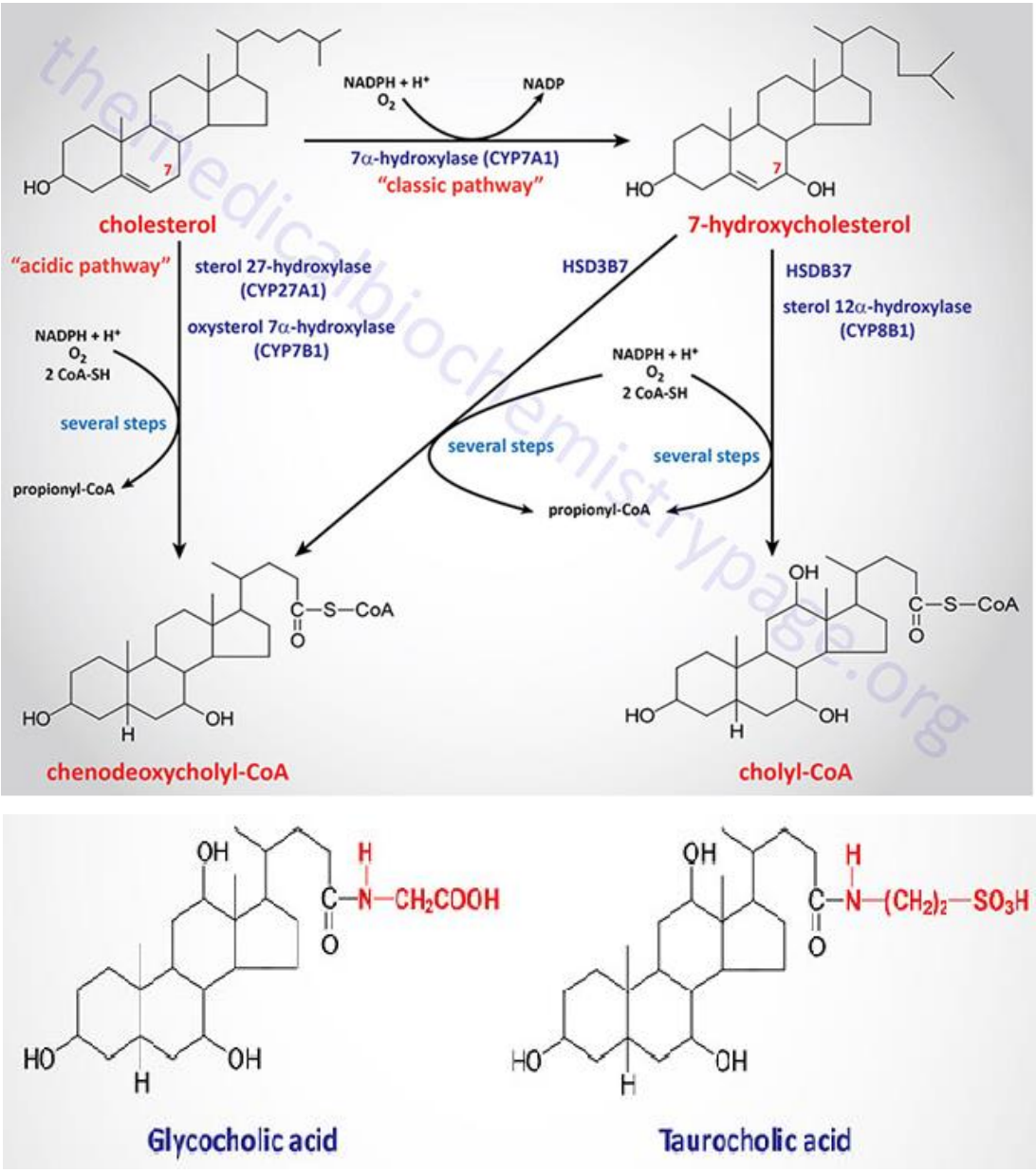


Figure 2.7 Classic and alternative pathway of BA synthesis.

The figure illustrates two different pathways of BA synthesis. BAs are produced from cholesterol. The main pathway of BA synthesis which is called "classic" or "natural' pathway is initiated through hydroxylation of cholesterol at the seven position in relation to CYP7A1. In another pathway, "alternative" or "acidic" pathway, cholesterol is hydrolysed at the 27-hydroxylase (CYP27A1). About 6% of BA synthesised by "alternative" pathway in the human. However, this pathway is essential for the BA metabolism. Primary BAs, CA and CDCA, is produced by "classic and acidic" pathway. A kay enzyme for the synthesis of BAs is CYP7A1 which is expressed in the liver only. FXR regulates BAs. BAs then conjugated in the intestine by two amino acids glycine and taurine. More information is provided in the text. The illustration is adapted from The Medical Biochemistry Page, http://themedicalbiochemistrypage.org/bileacids.php.

To convert primary BAs to secondary ones, anaerobic bacteria from the colon are required. Secondary BAs include deoxycholic acid (DCA), ursodeoxycholic acid (UDCA) from CA and lithocholic acid (LCA) from CDCA. Secondary BAs are passively absorbed from the colon or excreted in the stool. Those primary and secondary absorbed BAs and bile salts are carried out into the liver again via sodium (Na+)-taurocholate co-transporting polypeptide (NTCP/SLC10A1) and organic anion transporters (OATP) to uptake bile salts and BAs respectively (Denson et al., 2001; Hagenbuch & Meier, 2004). Again, in the liver, those BAs re-conjugated and re-secreted along with newly produced bile salts. This is called "enterohepatic circulation." Returned LCA in the liver undergoes a sulfation and is consequently excreted into the stool. The BA pool includes 2-4 g BAs and is recycled 6-10 times a day by enterohepatic circulation. Only 0.2-0.6 g of BAs are excreted in the stool every day. De novo hepatic BA synthesis from cholesterol acts to compensate for this amount that is lost (Lefebvre et al., 2009). Figure 2.8 gives more details on enterohepatic circulation.

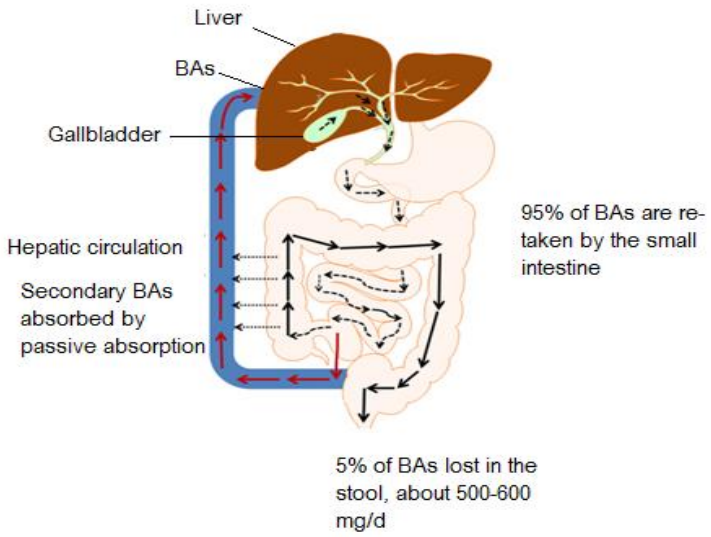


Figure 2.8 Enterohepatic circulation of BAs.

BAs firstly are produced in the liver and move to the gallbladder for the storage during the fasting state. BAs are mostly conjugated by glycine and taurine. Upon eating, BAs move into the duodenum through the bile duct. Almost 95% of BAs that delivered to the duodenum reabsorbed by the hepatic portal vein from the ilium (enterohepatic circulation). The rest,

5%, wasted in the stool. The figure adapted with modifications from (H. Zhou & Hylemon, 2014).

2.11.2 Conjugated Bile Acid

CDCA and CA are the most plentiful BAs in the human body. Primary BAs need to be conjugated through an amide bond at the terminal carboxyl group before releasing into the canalicular lumen. The conjugated forms are called glyconjugated BAs after conjugation with amino acid glycine and tauroconjugated BAs after conjugation with taurine amino acid. The different types of BAs stem from their hydroxylation and conjugation properties (Sjövall et al., 2010).The conjugation process plays a crucial role in the metabolism of BA synthesis. BAs are naturally very toxic to cells. The conjugation increases the amphipathic property of BAs, leading them to be less toxic (Lefebvre et al., 2009). Figure 2.7 shows both conjugated forms of BAs. Synthesised BAs in the liver via the action of the bile salt export protein or ATP-binding cassette B11 (BSEP or, ABCB11) are released into the bile canaliculi (Trauner & Boyer, 2003). Transportation of phospholipids into the canaliculi needs ABCB4. ABCB4 is also identified as multi-drug resistance protein 3 (MDR3), which is a member of the P-glycoprotein family of transporters. In addition, free cholesterol is also moved out of hepatocytes and released into canaliculi through the action of ATP-cassette binding proteins G5/G8 (ABCG5/ABCG8). This complicated transport needs ABCB4. Each of which is critical for the normal hepato-biliary function (Elferink & Groen, 2002). So that, any alteration in this process leads to a severe impact on the human body. Nevertheless, the accumulation of toxic bile salts in the liver leads to liver failure (Russell, 2003).

The complex of bile salts, phospholipids and cholesterol is carried out via canaliculi into the gallbladder. The gallbladder is the organ where the digestive mixture is concentrated to form bile. Bile constitutes of 85% water, 67% bile salts, 22% phospholipids and 4% cholesterol along with electrolytes, minerals, tiny amounts of proteins, bilirubin and biliverdin pigments (de Aguiar Vallim et al., 2013). The main role of bile is to solubilise cholesterol to stop cholesterol crystallisation and prevent gallstone formation (Maldonado-Valderrama et al., 2011).

2.11.3 Bile Acid Function

Cholesterol metabolism plays an axial role in producing BAs in humans and other animals. In the human body, 400-800 mg cholesterol is converted to BAs daily. Bile salts are released

into the duodenum. About 95% of bile salts are reabsorbed into the terminal ileum. The reabsorption process is performed through the apical sodium-dependent bile transporter (ASBT) of the enterocytes. Fatty acid-binding protein subclass 6 (FABP6), or ileal bile acid-binding protein (IBABP), is introduced as a molecule to transport bile salts from enterocytes to the basolateral membrane. Bile salts from the basolateral membrane are effluxed into the blood via the heterodimeric organic solute transporter α/β (OSTα/OSTβ) (K. R. Sharma, 2012). A small portion of the bile salts is not reabsorbed. They become deconjugated through gut microbiota before being absorbed or transformed into secondary BAs. BA metabolism is significant to degrade cholesterol (Cook, 2015). There are five common types of human BAs, CA, CDCA, DCA, LCA and UDCA (Sjövall et al., 2010). Two amphipathic bile salts, namely glycolic acid (GCA) and taurocholic acid (TCA) are considered as biological detergents. Through them, dietary lipids are converted to a mixture of BAs and TGs (K. R. Sharma, 2012).

The gastrointestinal (GI) system plays an important role in BA metabolism. There is a link between the GI system and T2DM (Wolosin & Edelman, 2000). Normal microbes of the large intestine influence the metabolism of BAs. In turn, BAs and the gallbladder result in the suppression of bacterial colonization in the small intestine (Begley et al., 2005; Ridlon et al., 2006). Colonization is necessary to prevent the loss of BAs from uptake in the ileum. In the large intestine, several events occur such as oxidation, deconjugation, oxidation of hydroxyl groups at C3, C7, C12, and 7α/7β dihydroxylation. Various types of bacteria possess hydroxysteroid dehydrogenase which is essential for the oxidation of C3, C7 and C17 in the steroid ring framework (Setchell et al., 1983). Also, just after taking a fatty diet, L cells in the duodenum release cholecystokinin (CCK) hormone into the blood circulation. This action stimulates contraction of smooth muscle cells within the gallbladder, along with the release of bile into the duodenum. The duodenum is a place where fat soluble vitamins and dietary fats are absorbed and digested (Koop, 1990). The conversion of cholesterol into BAs possesses biological detergent properties in the intestinal system that is vital for liver BA formation and absorption of meal lipids and lipid-soluble vitamins from the small intestine. The terminal ileum plays a major role in the reabsorption of BAs. As a result, helping to accumulate some mass of BAs in the body (i.e., BA pool), as well as to circulate BAs between the intestine and liver through enterohepatic circulation. This precise and complex mechanism is necessary to have sufficient concentrations of BAs for proper

digestion (Lefebvre et al., 2009; Russell, 2003). Enzymatic modifications by BA coenzyme amino acid N-acyltransferase (BAAT) are responsible for conjugation of BAs to either taurine or glycine before the secretion of BA in the gallbladder (Falany et al., 1994). From the study of familial hypercholanemia, characterized by increased levels of BAs in the blood, it is known that BAAT is merely responsible for the conjugation reaction (Carlton et al., 2003).

Other novel roles are predicted for BAs. BAs control their metabolism as well as transportation through enterohepatic circulation and regulate fat and glucose metabolism (Mazidi et al., 2017). They have also been viewed as signalling molecules that regulate energy expenditure and liver regeneration (Fan et al., 2015; Watanabe et al., 2006).

2.11.4 Bile acids and Cell Signalling

There are two important receptors for BAs, farnesoid X receptor (FXR) (Tu et al., 2000) and G protein-coupled bile acid receptor (GPBAR1) or TGR5 (Kawamata et al., 2003).

The FXR was firstly cloned as an orphan nuclear receptor (Maloney et al., 2000). Two genes encode FXR, *FXRα,* and *FXRβ.* FXR is dominantly expressed in the liver, intestine, kidney and adrenal glands and small expression levels are found in adipose tissue and the heart. However, the exact mechanism of the expression of FXR is unclear. The FXR protein belongs to the nuclear receptor superfamily (Tu et al., 2000). The main target of FXR is called the small heterodimer partner (SHP). SHP suppresses CYP7A1 and results in the inhibition of BA synthesis. FXR can stimulate or inhibit gene expression in BA synthesis. For example, *NTCP*, which is a gene to uptake BAs via the basolateral membrane in the liver, is induced by FXR. Inhibition of this gene by FXR results in protection of the liver from toxicity due to the accumulation of BAs (Frank G. Schaap et al., 2014). FXR's levels in the liver are increased in the fasting state (Duran-Sandoval et al., 2005). The liver is the most important part of the body to control cholesterol homeostasis. Any extra production of cholesterol is converted to BA. A pivotal role in this conversion is proposed for FXR. In an animal study of FXR null mice that underwent different diets, there was no recorded obesity. It was also shown that with only a 1% administration of CA for five days in the diet of FXR- knockout, there was a loss of fat tissue and rapid body weight loss. The FXR-null experiments show high levels of BAs in serum and low levels in stool samples (Chawla et al., 2000).

Furthermore, FXR knockout mice show a 20-fold reduction in BSEP, which is the member of ABC family which carries and exports bile salts from hepatic cells to the gallbladder for release into the intestinal lumen. In addition, FXR-null mice show higher levels of ileal BA binding protein (I-BABP), which has been proposed to be involved in facilitating intracellular transport of bile salts and keeping enterocytes from damage due to the detergent properties of BAs (Bahar & Stolz, 1999). BAs are natural ligands for FXR (Chawla et al., 2000). FXRs were first recognised for binding to farnesol metabolites, but after more research, it has been shown that FXRs are unique receptors for BAs and their mechanism is negatively regulated by BAs (Neimark et al., 2004; Haibo Wang et al., 1999). Different BAs have different impacts on the activation of FXR. For instance, CDCA is the most potent trigger followed by LCA, DCA and CA (Bhowmik et al., 2014; Hylemon et al., 2009). Also, BAs are considered to be metabolic integrators participating in the control of glucose, lipid, and energy expenditure via dependent and/ or independent FXR signalling pathways (Lefebvre et al., 2009). Studying the activities of FXR serves an important role to find out more about lipid and glucose metabolism. Because FXR is a potent receptor for BAs, it causes the downregulation of expression of several genes in the metabolism of BAs. In the liver, for example, FXR action is identified as controlling the expression of genes in lipid and glucose metabolism. The other contribution of BAs in signalling processes is to participate through the c-JUN N-terminal kinase (JNK) and mitogen-activated protein kinase (MAPK) pathways. There are other important receptors for BAs such as pregnane X receptor (PXR), which is from nuclear receptor superfamily which detoxifies exogenous toxins from the body, and the vitamin D receptor (VDR) (Frank G. Schaap et al., 2014). Figure 2.9 is the schematic illustration of FXR action.

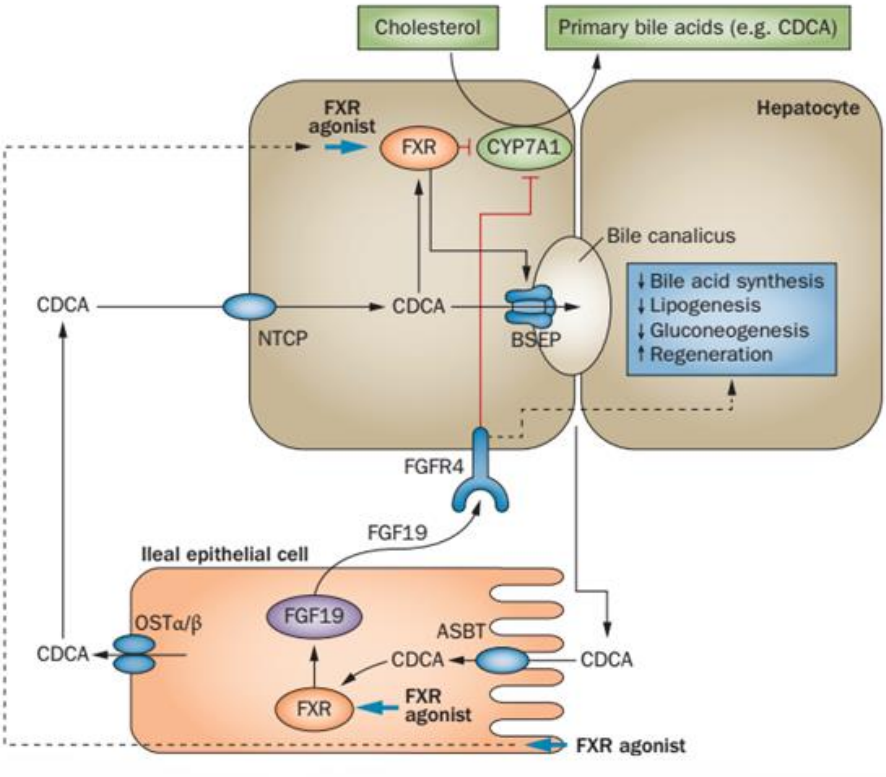


Figure 2.9 Schematic effect of FXR on BA metabolism.

CDCA is a primary BA which is produced from cholesterol in the liver. BAs are released through BSEP into the canicular lumen. Then BAs re-uptake by ASBT in the terminal ilium enterocytes. After that, FXR with activation of different cell signaling molecules (e.g., OSTα/β-NTCP) is involved in BA metabolism. FXR has a property to repress BA synthesis. In addition, FXR is strongly trigged by CDCA. More details are provided in the text. The figure is adapted from (Frank G Schaap et al., 2014).

G protein-coupled receptor (GPCR), TGR5, which is also called BG37, is produced in response to BAs (Kawamata et al., 2003; Keitel et al., 2007; Maruyama et al., 2002). TGR5 is a protein of interest in recent studies regarding the metabolism of BAs. TGR5 is highly expressed in the gallbladder, although other organs such as the ileum, colon, brown and white adipose tissue, skeletal muscle, liver, and immune cells also express TGR5 (Prawitt et al., 2011). LCA, TLCA, CA, DCA, and CDCA, in order of highest to the lowest affinity for the receptor, can activate TGR5 (Prawitt & Staels, 2010). The role of TGR5 in glucose and energy metabolism has been documented (Thomas, Pellicciari, et al., 2008). It has been postulated that the increased levels of BAs after RYGB may be a result of the effect of TGR5

(Jansen et al., 2011). The activation of TGR5 leads to the relaxation of the gallbladder (Tingting Li et al., 2011), as a result protecting the bile duct from detergent effects of BAs (Beuers et al., 2010).

2.11.5 Bile acid Fractions

BAs are very complicated molecules. A minor change in the amount of BAs in the body may link to the pathophysiology of a particular disease. BAs are varied regarding their composition through development, between the fetal, neonatal and adulthood. Additionally, in terms of BA classifications, there are several differences between healthy and people with particular diseases (Sjövall et al., 2010). The normal taurine/glycine conjugated BA ratio in humans is 3:1 (Lefebvre et al., 2009). Nevertheless, this ratio is changed in several diseases. For example, the ratio is increased in cholestatic hepatic disease and decreased in gallbladder drainage (Lefebvre et al., 2009). In parenchymal liver disorders, the CA production is lower than CDCA due to the reduced level of activity of 12-α hydroxylase (K. R. Sharma, 2012). Predominantly the amount of conjugated CA is highly increased in acute and chronic bile disorders (Greim et al., 1972). Also, it was shown that the level of taurine was grown in cholestasis (Hardison, 1978). Also, it has been reported that taurine and glycine-conjugated BAs are increased during cirrhosis (Okuda et al., 1984), whereas CDCA remained unchanged, while in adrenocortical and hepatobiliary diseases BAs metabolism is often abnormal (K. R. Sharma, 2012). Measuring the levels of serum BAs is a major marker assay for diagnosing hepatic diseases (Linnet, 1982). In adrenocortical and hepatobiliary diseases, BAs metabolism is also often abnormal (Fausa & Gjone, 1975).

Several kinds of BA ratios can be calculated to show BA physiobiological function. The calculation of the ratio is based on the site of BA synthesis. Namely, there are primary/secondary BA ratios, conjugated/unconjugated BA ratios, taurine/glycine conjugated BA ratios, 12α-hydroxylation/non12α-hydroxylation BA ratios (12α-OH/12α-OH) and total BA ratios. The formula to calculate different BA ratios is provided in Chapter 3. The calculation of the proportion of BAs may be helpful to understand more about pathophysiological pathways during a chronic metabolic disease such as obesity and T2DM.

2.11.6 Bile Acids and Diabetes in Animals and Humans

Several animal studies have reported the most important role of BAs to be affiliated with the remission of diabetes (Myronovych et al., 2014; Ryan et al., 2014; Stefater et al., 2011; Watanabe et al., 2004), while there is no clear information about why BAs have an impact on the remission of T2DM. The administration of BAs inhibits the production of cytokines and TGR5 in macrophages (Kawamata et al., 2003), while CA enhances energy expenditure in brown adipose tissue (BAT), and inhibits the development of obesity and insulin resistance (IR) (Chawla et al., 2000; Lefebvre et al., 2009). Nevertheless, administration of BAs is not always a safe intervention (Lefebvre et al., 2009), in fact, it can be life-threatening (Sinal et al., 2000). DCA results in the stimulation of intestinal inflammation (Bernstein et al., 2006), and tauroursodeoxycholic acid (TUDCA) has a protecting impact onto the progression of IR (Özcan et al., 2006). An animal study showed that treatment with FXR has a strong function on the improvement of blood glucose and insulin sensitivity (Cariou et al., 2006; Y. Zhang et al., 2006). The action of BA as a potent metabolic regulator is mostly related to FXR, TGR5, and thyroid hormone deiodinase (Ryan et al., 2014; Watanabe et al., 2006). Studies in induced diabetic mice indicated that FXR expression is decreased (Duran-Sandoval et al., 2004; Y. Zhang et al., 2006). Therefore, any manipulation of BAs may have a great potential to impact on the improvement of diabetes (Cipriani et al., 2009; Parks et al., 1999).

The production of BAs is regulated in very precise ways (Russell, 2003). A human study showed intracellular cAMP is changeable by BA manipulation (Maruyama et al., 2002), while the role of cAMP on glucose metabolism has been well documented (Dermot & Valentina, 2014). It has also been shown that both unsaturated free fatty acid, LCA, and DCA, can induce the production of GLP-1 (Katsuma et al., 2005; T. Wu et al., 2013). Currently, the best intervention to lose weight is bariatric surgery (Sjöström et al., 2007). Bariatric surgery has a potential role in attenuating obesity complications like as T2DM (Falkén et al., 2011). BAs are proposed in this context to have a remission property for diabetes after bariatric surgery. Gerhard et al. (2013) indicated that greater serum levels of BAs and ilial-derived hormone after bariatric surgery may have an important role in the remission of T2DM in humans. It was also demonstrated that the levels of some types of BAs alter only after RYGB, not in AGB. (CA is an example of this kind of occurrence.) Also, it has been indicated that postprandial levels of BAs are increased after RYGB (Kohli, Bradley, et al., 2013b; D. J. Pournaras et al., 2012) and glycine-conjugated BAs are also increased

significantly after RYGB (Werling et al., 2013). Werling et al. (2013) suggested that there is an upregulation of BAs after RYGB. Where the exact mechanism of how bariatric surgery, e.g., RYGB, resolves T2DM is not clear, some studies suggest the role of BAs in the remission of diabetes (Gerhard et al., 2013; D. Pournaras et al., 2012; Ryan et al., 2014; Werling et al., 2013). As there is a difference between humans and rodents BAs (Lefebvre et al., 2009), it is hard to merge these two models to get the same result from the impact of BAs on the remission of T2DM. The level of BAs pre-surgery has shown to be lower preoperative. Thus, whether or not BAs play a role in the remission of diabetes requires more studies.

2.12 Bariatric Surgery, Bile acid, and Diabetes

There are more than 20 different BAs in the BA pool (Steiner et al., 2010). It has been shown that plasma levels of 7α hydroxycholesterol and 7α-hydroxy-4-cholesten-3-one (C4) reflect synthesis of BAs in the body (Björkhem et al., 1987; Steiner et al., 2010). There are two important roles assumed for BAs, first, as signalling molecules that influence FXR activity. Animal studies have shown that the level of FPG was higher among FXR deficient mice (knockout) than normal mice (wild-type) (Ryan et al., 2014). Second, a role for BAs is proposed through interaction with TGR5 (Houten et al., 2006). It has been shown that TGR5 in the liver is decreased after the administration of BAs through FXR-related processes (Watanabe et al., 2004) and TGR5-null mice had lower levels of glucose tolerance (Thomas, Auwerx, et al., 2008).

Bariatric surgery has already been introduced as a “metabolic procedure” (Buchwald et al., 2004). It has been shown that there is an increase in the level of BAs among subjects who underwent bariatric surgery (Nakatani et al., 2009). The mechanism of RYGB is to shorten the distance between the stomach and ileum through bypassing the proximal intestine and inserting a Roux limb (this describes the accelerated delivery hypothesis) may be responsible for the increased levels of concentrations of BAs after bariatric operations. Although it was suggested that anatomical changes may not be considered as potent factors to change BA secretion after surgery (Ahmad et al., 2013), more studies are needed to confirm this hypothesis (Ikramuddin et al., 2013). BAs may play a vital role in the remission of diabetes after bariatric surgery due to the stimulation of L cells, and as a result elevate the level of GLP-1 just after surgery (Ballantyne et al., 1989b; D. J. Pournaras et al., 2012; D. J. Pournaras et al., 2010). Nevertheless, reduced level of IR are correlated with elevated levels

of BAs in fasting situations after bariatric surgery (Jansen et al., 2011; Patti et al., 2009). BAs are essential to the role of glucose metabolism in gluconeogenesis (De Fabiani et al., 2003; Thomas, Pellicciari, et al., 2008). Also, their role in the activation of thyroid hormone (Watanabe et al., 2006) through phosphatidylinositol three kinase/serine to activate and stimulate insulin signalling and increase glucose storage (glycogenesis) is documented (S. I. Han et al., 2004).

Bariatric surgery per se may not be the reason for diabetes remission. In animal studies, if the weight is the same at the time of measurement there are other underlying mechanisms on the improvement of diabetes rather than bariatric surgery (Ryan et al., 2014). Also, manipulation of BAs without doing bariatric surgery gives the same result of diabetes resolution as bariatric surgery (Kohli, Bradley, et al., 2013b). Increased serum levels of BAs are assumed to play a major role in the improvement of metabolic complications such as diabetes. This is likely explained because of the role of BAs in the signalling pathway (X. Chen et al., 2011; Jansen et al., 2011; Laferrère, 2012). Furthermore, an increased level of BAs after SG has been reported in animals (Myronovych et al., 2014). In this study, it showed that the level of BAs is higher among mice who underwent SG. Moreover, Myronovych et al. found that the level of some specific kinds of BAs was elevated significantly after the surgery while several genes were downregulated. They concluded that bariatric surgery *per se* could not be the main reason of the remission.

The role of BA as a metabolic regulator in humans has already been documented (Lefebvre et al., 2009). Previously, BAs were considered as an important mediator in the metabolism of lipids. Recently, however, BAs' roles have also been suggested to involve glycaemic metabolism (Schipper et al., 2012). BAs activate TGR5, which is a potent stimulator of incretin, GLP-1 independently of FXR (Kreymann et al., 1987). GLP-1 induces insulin secretion and compromises glucagon secretion from the pancreas after taking a meal (Holst, 2007). Also, GLP-1 works as an important factor to suppress appetite. Thus, it helps to prevent diabetes (R. Steinert et al., 2013). It has been shown that administration of TCA increases plasma levels of GLP-1 and PYY, and as a result, insulin sensitivity increases in humans, and blood glucose is reduced (T. E. Adrian et al., 2012). In patients who underwent bariatric surgery there is a higher level of fasting plasma BAs two to four years after bariatric surgery compared with obese patients without the surgery; in the latter group, the level of

BAs is negatively correlated with 2hpp and positively associated with GLP-1 (K. Ma et al., 2006). Also, in a human study, levels of BAs, particularly glycine-conjugated BAs, increased significantly after bariatric surgery (Werling et al., 2013). Although the exact mechanism of how bariatric surgery resolves diabetes is not clear, some studies suggest that it may be due to the alteration of individual BAs (Gerhard et al., 2013; Ryan et al., 2014).

Ratios of BAs are also altered after bariatric surgery. Predominantly the levels of glycine-conjugated BAs are significantly higher than other individual BAs except for GLCA, which is lower in comparison with other BAs after bariatric surgery. It has also been found that there is no significant change regarding the primary BAs, CA and CDCA, 15 months after the surgery (Werling et al., 2013). Also, Werling et al. observed an increase in the level of the non-fasting and fasting secondary BA, DCA, after RYGB. Furthermore, in another study patients who underwent RYGB, showed total BAs having an inverse correlation with 2hpp. Moreover, the levels of individual BAs namely, TCDCA, TDCA, GCA, and GCDCA are significantly higher in post-surgery subjects compared to subjects without surgery or pre-operative subjects (Patti et al., 2009). Regardless of bariatric surgery, increased levels of BAs may play a major role in the improvement of diabetes (Clifton, 2011).

The role of BAs has already been documented in compromising liver lipogenesis via activation of an FXR circuit (Yang et al., 2010). Nevertheless, all bariatric surgeries do not have the same function and mechanism. For instance, the elevation of BAs after AGB has not reported (Kohli, Bradley, et al., 2013b). This fact may indicate that the underlying mechanism of RYGB may play a vital role in the effect on the production of BAs. Investigating the differences observed after bariatric surgery may open a new therapeutic door which mimics biomarker interventions instead of surgery to remit T2DM. For instance, primary BAs and their conjugated forms as therapeutic activists are effective in the remission of diabetes (Kohli et al., 2010).

Taken together, BAs may be considered as scalable and non-invasive pharmaceutical facilitators for diabetes remission in the near future. However, more studies are needed to understand more about the underlying mechanisms of the remission of diabetes. Such studies are most required in humans rather than animals due to differences between animals and humans (Kuipers & Groen, 2014).

2.13 Fibroblast Growth Factor 19

Fibroblast growth factor 19 (FGF19) is a member of fibroblast growth family, and its role is to be involved in the synthesis of BAs (S. A. Jones, 2012).

FGF19 is a novel and important biomarker (Buhmeida et al., 2014), which works as an endocrine hormone, and has promise as a potential treatment of T2DM (Frank G. Schaap et al., 2014).

2.13.1 FGF19 Biochemistry and Mechanism

FGFs are polypeptide growth factors. The FGF family is only seen in metazoans, and not in unicellular organisms. FGFs consist of about 300 amino acids with a conserved core of about 120 amino acids, sharing approximately 30 to 60% sequence similarity between family members (Itoh & Ornitz, 2004), and play vital roles in proliferation, differentiation, foetal development and organogenesis (Beenken & Mohammadi, 2009). FGFs in mice and humans are the same with an exception, mice have FGF15 instead of FGF19 (Itoh & Ornitz, 2008). There are three types of functions for FGF family proteins, namely paracrine (canonical), endocrine (hormone-like) and intracrine (intracellular) functions. Intracrine functions were likely the initial roles of ancestors of the FGF family (Itoh & Ornitz, 2011). In the family, 18 members release proteins, and four members have intracellular signalling protein properties. FGF19 works as an endocrine hormone (Beenken & Mohammadi, 2009). FGF19 is highly expressed by small intestinal cells where FXR is activated by BAs (Jansen et al., 2011; Samuel et al., 2006; F. G. Schaap, 2012), and the receptor can also be found in brain, kidney gallbladder, retina, skin and intestinal tissues (Beenken & Mohammadi, 2009). FGF19 is mostly expressed in tissues where β klotho and FGFR4 are released. The liver is the place where the highest amount of β-klotho and FGFR4 are expressed. The liver is a target organ for FGF19 action (Lundåsen et al., 2006; Tomlinson et al., 2002) where the protein is a kind of hormone which is released by the intestinal system after a meal. Activation of FGFR4 through FGF19 can be in the presence or absence of β klotho (X. Wu et al., 2009). In the absence of medications, the level of FGF19 fluctuates, but FGF19 also has a diurnal rhythm in the human body parallel to increasing levels of BAs. It has been reported that there are two peaks of FGF19 in a day, 3 and 9 pm (Lundåsen et al., 2006), and the highest level of FGF19 is mostly seen 2-3 hours after a meal (T. Nishimura et al., 1999)

FGF19 has two important roles. First, it is a regulator of the biliary tract. Second, FGF19 inhibits CYP7A1 (Cicione et al., 2012). Administration of FGF19 results in increasing gallbladder volume in animals. Thus, gallbladder filling and emptying play a major role in regulating the flow of BAs into the small intestine. During the fasting status, the gallbladder stores and concentrates bile salts. When necessary, the gallbladder release bile salts into the small intestine for proper digestion. FGF19 stimulates gallbladder filling through cAMP-dependent activation of gallbladder smooth muscle (Choi et al., 2006).

Figure 2.10 shows the mechanism of FGF19.

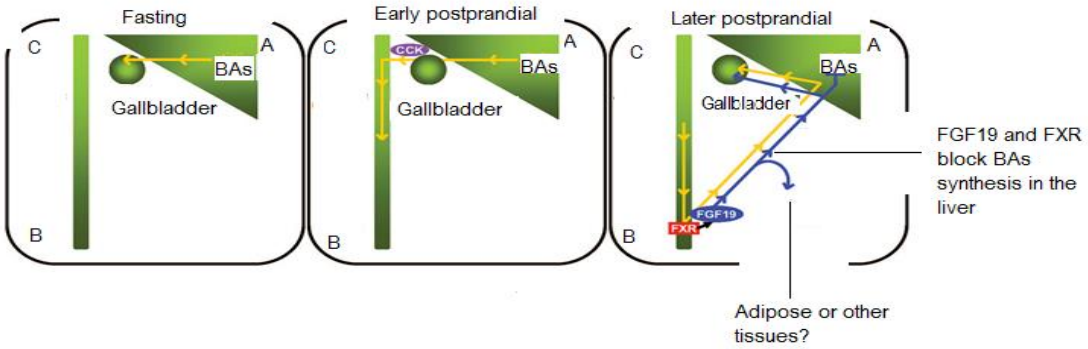


Figure 2.10 Effect of FGF19 on BA and gallbladder

In the fasting state, BAs are produced in the liver and transferred to the gallbladder for storage. Upon ingestion the meal (postprandial), CCK signals to the gallbladder to constrict, releasing bile into the duodenum. If BAs reach to the ilium, FXR receptor is activated to transcribe FGF19. Then, FGF19 is secreted from enterocytes and signals to the liver to stop BAs synthesis and induce fat metabolism along with stimulation of the gallbladder to refill. Abbreviations in the figure: A, liver; B, ilium; C, duodenum. Adapted and modified from (S. Jones, 2008).

2.13.2 FGF19 Function

FGF19 has been introduced as a novel prognostic marker in different diseases related to T2DM (Buhmeida et al., 2014; Sahebkar et al., 2017). There are several important functions considered for FGF19. It has been found that the level of FGF19 is lower in people with metabolic syndrome in comparison with healthy subjects (Inagaki et al., 2008). FGF19 has several physiological compensatory effects. For instance, it can inhibit the accumulation of triglycerides in the liver (Jansen et al., 2011), as well as increase energy expenditure and

regulate glucose metabolism (Tomlinson et al., 2002). Other prominent roles include regulation of bile salt metabolism through inhibition of CYP7A1 (Inagaki et al., 2008) and gallbladder filling (Choi et al., 2006), decreasing gluconeogenesis (S Kir et al., 2011), increasing glucose storage (glycogenesis), protein synthesis (S Kir et al., 2011; A.-L. Wu et al., 2011), regulation of metabolic capacity (L. Fu et al., 2004) and decreasing adiposity (S. A. Jones, 2012).

FGF19 may cause an increase in BAT (Russell, 2003; J. ZHANG et al., 2014). The level of FGF19 may also be affected by mutation status (Mráz et al., 2011). Additionally, FGF19 induces production of proteins and glycogen in the liver without lipogenesis (Serkan Kir et al., 2011). Therefore, this property of FGF19 may play a crucial role to the homeostasis of glucose metabolism. Although it has been already mentioned, FGF19 regulation is dependent to FXR (Holt et al., 2003; Inagaki et al., 2005), and this mechanism needs to be more fully elucidated (Morton et al., 2013). Albumin production in the liver, for example, is increased by 40% after administration of FGF19. Also, administration of FGF19 results in the inhibition of glycogen synthesis kinase 3 α/β (GSK 3 α/β), which are both important to inhibit gluconeogenesis (Serkan Kir et al., 2011).

FGF19 also has an impact on the resolution of obesity. It has been demonstrated that increased levels of FGF19 are positively correlated with weight loss (Jansen et al., 2011; Tomlinson et al., 2002). Also, it has been shown that there is no significant change in a number of FGF19 within malnourished patients in comparison with non-obese people (Dostálová et al., 2008). Furthermore, FGF19 has been shown to have a negative correlation with BMI and a positive correlation with adiponectin (Mundi & Collazo-Clavell, 2014; Ruta et al., 2013; D. Wang et al., 2013). However, these correlations need more studies on people with different BMI (De Giorgi et al., 2014). FGF19 downregulates acetyl CoA carboxylase 2 (ACC2), a converter of acetyl CoA to malonyl CoA. ACC2 is a repressor of carnitine palmitoyltransferase I (CPT1), a potent factor in fatty acid oxidation. Another property of FGF19 in lipid homeostasis is to downregulate stearoyl-CoA desaturase-1 (SCID), a lipogenic enzyme (L. Fu et al., 2004; Holt et al., 2003).

FGF19 has a role in the remission of T2DM (L. Fu et al., 2004). It may be related to the similarity between FGF19 and insulin (S Kir et al., 2011). For example, FGF19 carries out a

similar function to insulin, acting to reduce gluconeogenesis (Potthoff et al., 2011). However, there are some differences between insulin and FGF19. Table 2.2 shows more information about FGF19 and insulin similarities.

Table 2.2 Comparing FGF19 with insulin in different metabolism regulations

Functions	FGF19	Insulin
BA synthesis	Down	-
Protein synthesis	Up	Up
Glycogen synthesis	Up	Up
Gluconeogenesis	Down	Down
Lipogenesis	-	Up
Triglycerides	Down	Up
Cholesterol	Down	-

Adapted from (S Kir et al., 2011)

2.13.3 FGF19 a Signalling Molecule

Some FGF family members have signal peptides (Beenken & Mohammadi, 2009). Different physiological patterns of FGF originated from amino- and carboxyl- termini tail sequences of FGFs (Mohammadi et al., 2005). In the FGF family, 18 members release proteins, and four members have intracellular signalling properties. Those 18 members have a higher affinity to heparin sulfate glycosaminoglycans (HSGAGs) while they bind to cell-surface tyrosine kinase FGF receptors (FGFRs). Whereas FGF15/19 has a markedly lower affinity to HSGAGs. Klotho proteins (α klotho and β klotho protein) are potent single-pass transmembrane glycoproteins which bind FGF for the activation of FGF-signalling properties (Cicione et al., 2012).

2.13.4 FGF19 and Diabetes in Animals and Humans

Administration of FGF19 in mice showed the promising effects of FGF19 on the prohibition of obesity and diabetes or “Diabesity” (L. Fu et al., 2004; Serkan Kir et al., 2011; Russell, 2003; Tomlinson et al., 2002; Walters, 2014). The observed prohibition may be due to the

alteration of FGF19 nutritional status (Morton et al., 2013; Ruta et al., 2013) because CYP7A1 repression reduces fat-burning food absorption (S. Jones, 2008). Therefore, FGF19 can decrease fat production and elevate metabolic rate or energy expenditure in animals. Also, in mice, there is a direct correlation between increasing levels of leptin and FGF19. Moreover, in transgenic FGF19 mice, elevation of metabolic rate, reduction of adiposity, increased food consumption and insulin sensitivity are observable. Transgenic FGF19 mice are resistant to obesity even after consuming high-fat diets with an increase in BAT (L. Fu et al., 2004). However, administration of FGF19 is not always safe. One of the most significant concerns about the administration of FGF19 is its effect as a carcinogen, and it can be potentially life-threatening (Miura et al., 2012a; Nicholes et al., 2002; X. Wu & Li, 2011). Contrary, FGF19 manipulation may reduce the risk of cancer via avoiding proliferation and mutagenesis (X. Wu et al., 2010).

In a study among a Chinese population, the fasting levels of FGF19 are inversely related to FPG levels, and FGF19 levels are decreased significantly among the Chinese with impaired fasting glucose (Fang et al., 2013). Fang et al. showed a significant association between fasting FGF19 levels, FPG, and age, without significant correlations between FGF19 and 2hpp, HbA1c, and insulin sensitivity.

The obese state influences FGF19. In normal lean people, the level of FGF19 is higher than subjects who are obese and/ or diabetic. Also, the levels of FGF19 have a negative correlation with BMI and a positive correlation with adiponectin (Mráz et al., 2011). Also, it was reported that the level of FGF19 is lower in metabolic syndrome's patients than healthy subjects (Stejskal et al., 2008).

Although the highest level of FGF19 is detectable at 2hpp (De Giorgi et al., 2014), measurement values depend on physiological issues (Gerhard et al., 2013; Krssak et al., 2004; Schreuder et al., 2010). Also, it has been indicated that just after intervention (3 hours) the levels of FGF19 are decreasing, although not significantly. From this, it is believed that being diabetic has a major role in altering FGF19 concentrations (Mráz et al., 2011).

FGF19 also influences insulin resistance. Coffee has been shown to have a fundamental role in the improvement of insulin resistance (Bhupathiraju et al., 2012; Van Dam et al., 2006; Wedick et al., 2011) and is a potent stimulator of FGF19 (Styer et al., 2014). In a human

study, the response of hepatic cells to increased plasma levels of FGF19 is dysfunctional and declined in IR and non-alcoholic fatty liver disease (NAFLD) (Schreuder et al., 2010). NAFLD is also usually observed along with T2DM (Sasaki, Nitta, et al., 2014). In brief, gluconeogenesis is prevented by FGF19 through the inhibition of the cAMP regulatory element binding protein peroxisome proliferator-activated receptor gamma coactivator 1-alpha (CREB PGC-1 α) (Potthoff et al., 2011; Samuel et al., 2006). FGF19 may work as a blood glucose regulator after activation of insulin (Potthoff et al., 2011). Therefore, it is likely that the remission of diabetes partially depends on FGF19.Studies in mice can help to find out more about the functions of FGF19. However, recognising the differences between humans and mice is crucial. For example, mice do not have FGF19. Also, In humans, BAs are reported to peak twice a day while this is not observed in mice (Cecilia Gälman et al., 2005; Mitchell et al., 2013). Therefore, there is a need for researchers to perform more studies on humans rather than animals to reveal the interesting effects of FGF19 on T2DM.

2.14 FGF19 and Bile Acid Relationship

FGF19 gene expression is under the control of BAs, FXR, and PXR (Inagaki et al., 2005; Hongwei Wang et al., 2011). The levels of FGF19 concentrations vary from 27 to 1314 pg/ml (Lundåsen et al., 2006; Schreuder et al., 2010) and 250% markedly increases them among subjects who are treated with CDCA. The increased levels of C4, a marker of CYP71, has an inverse correlation with CDCA administration levels (Lundåsen et al., 2006). FGF19 plays important roles in the body such as downregulation of de novo BA production in liver cells through decreasing the expression of CYP7A1, as well as sterol regulatory functions by binding to protein 1c. Also, lipid production and gluconeogenesis are activated by FGF19 and phosphoenolpyruvate carboxy kinase (Bhatnagar et al., 2009; Shin & Osborne, 2009). Although some studies suggest that the increase in levels of circulating BAs has a direct correlation with increased levels of FGF19 (Frank G Schaap et al., 2009), other studies are inconsistent with this finding (Brufau, Stellaard, et al., 2010; Schreuder et al., 2010). FGFR4 is the receptor for FGF19, which works as a signalling molecule, and has a major role in the metabolism of BAs, lipids and glucose metabolism. This process is a crucial factor to regulate meal ingestion and BA production afterward (Jansen et al., 2011). Just after taking a meal, the gallbladder is contracted and releases bile salts to activate FXR. As a result, FGF19 is

produced and activated from the distal ileum to the bloodstream (Inagaki et al., 2005). BAs after that induce FGF19 expression in the intestine (Stejskal et al., 2008).

The type of diet plays an axial role in the expression of FGF19 and BAs. One hour after drinking beverages there is a temporary reduction in the levels of FGF19. Also, consumption of carbohydrates results in an increase of FGF19 plasma levels and return back to the basal level five hours after taking a meal. Also, proteins have higher effects onto the increased levels of FGF while there is no change found in lipid consumption. Interestingly, the level of bile is increased only after lipid consumption. The FGF19 levels after consumption of a carbohydrate beverage peaks 160 minutes after being taken. However, despite the increase in levels of FGF19 after consuming a carbohydrate beverage, there is no increase in levels of BAs after consuming carbohydrates (Morton et al., 2013).

In humans, the level of FGF19 is elevated after meals through CDCA and is reduced by the actions of other individual BAs (Lundåsen et al., 2006). CDCA and LCA both stimulate FGF19 expression (Wistuba et al., 2007). FGF19 is produced by the ileum and signals in liver cells by two receptors, FGFR4 and beta klotho, prevent CYP7A1 expression (Inagaki et al., 2008). The effect of unconjugated BAs on the stimulation of FGF19 is higher than conjugated BAs (GCA, GDCA, GCDCA) (Styer et al., 2014).

In healthy people, the level of FGF19 is directly related to an increase in the levels of BAs postprandial (Holt et al., 2003; Lundåsen et al., 2006). In BA malabsorption, the level of FGF19 is decreased while the level of primary BAs is increased along with diarrhea (Walters, 2014). This situation has also been reported in patients without distal ileum with inflammatory bowel disease (IBS) (Lenicek et al., 2011).

It has been shown that BAs upregulate the production of FGF19 and FGF19 downregulates BA synthesis (Beenken & Mohammadi, 2009). This action has an impact on the improvement of IR through the increase of mitochondrial activity by FGF19 (S Kir et al., 2011). In subjects with T2DM, the level of FGF19 decreased while BAs increased (Gerhard et al., 2013). Whether or not FXR activation is the only pathway to activate FGF19 secretion needs to be elucidated (Morton et al., 2013). In figure 2.11, a relationship between BAs, FGF19, TGR5and FXR has been illustrated.

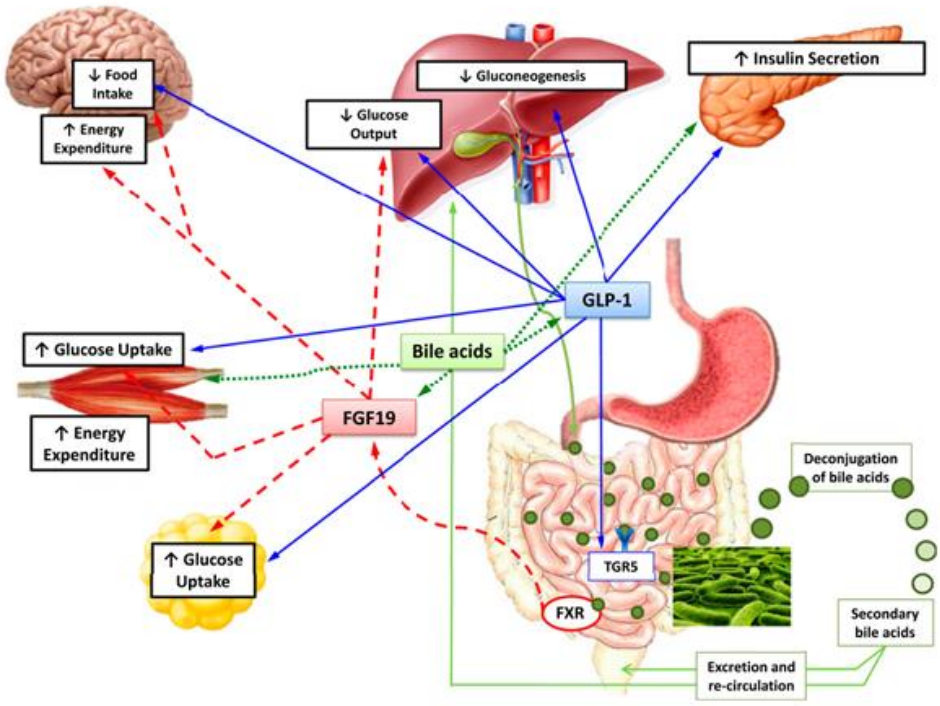


Figure 2.11 Relationship between BAs, FGF19, and TGR5 in metabolic homeostasis.

BA receptors, FXR and TGR5, are likely to play crucial roles in metabolic homeostasis through BAs and FGF19. Red circle shows how TGR5 and FXR respectively play a role in metabolic homeostasis. For instance, in the ilium, BAs are reabsorbed by ASBT in the terminal ilium enterocytes. Then FXR is activated and this induces the transcription of FGF19. In the liver, FGF19 binds to its receptor, FGFR4. FGFR4 actives a signalling pathway to suppress CYP7A1 to downregulate BA synthesis. Orally administrated FXR has been shown to strongly downregulate CYP7A1 by FGF19 or independent of FGF19 (Frank G Schaap et al., 2014). The figure is adapted with modification from (Batterham & Cummings, 2016).

2.15 Diabetes, Bariatric surgery, FGF19 and Bile Acid

Either in animals or humans, it is widely accepted that increased level of BAs by RYGB and SG leads to improved glucose metabolism and diabetes. FGF19 needs more studies to confirm its role in the remission of diabetes after bariatric surgery (Batterham & Cummings, 2016; Gerhard et al., 2013; Kuipers & Groen, 2014; Ryan et al., 2014; Tremaroli et al., 2015). This alteration cannot be seen after energy restriction or AGB (Sachdev et al., 2016). It has been shown that after VLCD, levels of total BAs (TBA) among non-diabetes subjects

reduced significantly while TBAs within people with T2DM had no change (Angelin et al., 2012). In the both groups, however, levels of FGF19 were unchanged during the follow-up. This study showed that improvement of diabetes after RYGB is not related to changes in FGF19 and TBA (Jørgensen et al., 2014).

One of the most important hypotheses to explain the increase in BAs after bariatric surgery is the anatomical rearrangement which takes place, particularly after RYGB. The rearrangement leads to delays in the mixture of BAs with undigested foods (Kohli et al., 2010). For instance, after RYGB, in the terminal ileum, BAs through TGR5 bind to L-cell enterocytes, and stimulate GLP-1, FXR, FGF19 production and BA secretion (Angelin et al., 2012; Knop, 2010). Bariatric surgery may not be the main reason for increasing BAs. In an animal study found that SG results in quick gastric emptying and ingestion of nutrients inside of the duodenum. Also, in mice, higher expression of ASBT causes the greater circulating BAs (Ding et al., 2015); hence other mechanisms are also considered to increase BAs rather than bariatric surgery alone.

People with T2DM mostly have lower amounts of circulating BAs and FGF19 in comparison with normoglycaemic people, and for those who remitted after RYGB, higher levels of FGF19 and BAs are observable (Gerhard et al., 2013). Furthermore, BA diversion is related to decreased hepatic glucose synthesis and enhanced intestinal gluconeogenesis (Batterham & Cummings, 2016). Taken together, these manifests provoke a correlation between T2DM, IR, FGF19 and BAs.

It is unclear why there is no consistency in the alteration of FGF19 and BAs after bariatric surgery in the context of the remission of diabetes. In a recent study BA and FGF19 were measured at baseline, a month, and two years after GBP among obese people with T2DM (Dutia et al., 2015). In this study, BAs increased two years after the surgery while AUCs of FGF-19 showed a non-significant rising trend two years after GBP. The study indicated that there is no significant correction between BAs and insulin sensitivity (Dutia et al., 2015) while altered BA metabolism has an important role in the development of T2DM (Prawitt et al., 2011). In addition, the time of increasing BAs is controversial (Patti et al., 2009; Werling et al., 2013). It is not clear when the levels of BAs increase after bariatric surgery. Some studies show that it can be between 1 and 3 months after RYGB (Ahmad et al., 2013; Jansen et al., 2011; D. J. Pournaras et al., 2012), while others suggest that BAs increase only one

year after bariatric surgery (R. E. Steinert et al., 2013). Furthermore, it is unclear which type or ratio of BAs are essential to the remission of diabetes. For instance, data from long-term follow-up has been shown that, either conjugated (Ahmad et al., 2013; Werling et al., 2013) or unconjugated BAs (Kohli, Bradley, et al., 2013b; Simonen et al., 2012) increase after RYGB, while only conjugated BAs have been reported to be blunted among obese people (Glicksman et al., 2010). In addition, total BAs among obese individuals with diabetes is reported to be higher compared with obese non-diabetes subjects, which may be linked to an increase in glycine/taurine-conjugated BAs (Gerhard et al., 2013; Vincent et al., 2013a; Wewalka et al., 2014a). Finally, different responses of patients to various bariatric surgeries can influence the effect of FGF19 and BAs on the remission of diabetes and obesity (Adams et al., 2012; Christou et al., 2006; Magro et al., 2008; Puzziferri et al., 2008; Sjöström et al., 2007). For example, after RYGB, hormonal alterations were shown to be superior over altered levels of FGF19 to reduce weight (de Hollanda et al., 2014). Taken together, the exact mechanism of FGF19 and BAs on the improvement of diabetes needs more studies.

2.16 Unknown Mechanism for Diabetes Remission

A long-term prospective RCT study is done to determine whether the underlying mechanism for diabetes remission is due to malabsorption, incretin hormones, restricted calorie diet and BA alterations, or any other causes. Some of these interventions are invasive while some of them are labour consuming or expensive. Bariatric surgery is not a certain intervention for the remission of diabetes. Remission is dependent on the type of surgery, and particularly the anatomical position affected by the surgery (D. J. Pournaras et al., 2012). The effect of bariatric surgery on to the remission of diabetes is limited to criteria such as BMI and the onset of diabetes (De Paula et al., 2010b).The role of the ilium in the remission of T2DM is another important issue to study.

Most studies regarding the effect of BAs on the remission of T2DM have been reported based on RYGB research, whereas SG can modulate the expression of liver genes and the metabolism of fat and BAs (Myronovych et al., 2014). Nevertheless, there is very scant information about the effect of BAs after SG.

Previous studies had limited inclusion criteria. For instance, single gender (De Giorgi et al., 2014), homogenous populations (Gerhard et al., 2013), and sample sizes are very narrow in

prevous research (Kohli, Bradley, et al., 2013b; D. J. Pournaras et al., 2010; R. E. Steinert et al., 2013).

Most of the studies used animal models to conclude the effect of BAs on the remission of T2DM. There are several differences between humans and animals in regards to BA metabolism. For instance, in humans, 27 hydroxylase deficiency is neurotoxic, while this deficiency in mice has a compensatory pathway (Rani et al., 2004).

The role of hormonal changes is another unknown issue. GLP-1 and PYY have a strong correlation with BAs (Ballantyne et al., 1989a; D. J. Pournaras et al., 2012; D. J. Pournaras et al., 2010). GLP-1 is directly related to insulin sensitivity (D. J. Pournaras et al., 2010) and reduced levels of IR is negatively correlated with elevated levels of fasting plasma BAs (Jansen et al., 2011; Patti et al., 2009). GLP-1 is increased one week after surgery without significant weight loss (T. Adrian et al., 2012; Holst, 2007; Patti et al., 2009; Reis et al., 2012). The correlation between BAs and GLP-1 by its production through TGR5 in the presence of intracellular cAMP (Katsuma et al., 2005) may convince us to find a solution for the remission of T2DM.

The relationship between FGF19 and BAs needs more studies. It is likely that the glycine/taurine ratio, which is considered as a marker for metabolic complications (K. R. Sharma, 2012), plays a role in remittance of T2DM. A recent study shows that there is no relationship between FGF19 and BAs, except in patients who are obese but not diabetic (Haluzíková et al., 2013; Patti et al., 2009), which is in contrast with the previous study (Jansen et al., 2011). In the latter study, subjects were obese with diabetes. The finding of this study is significant especially since the role of BAs is further highlighted relative to FGF19 and the remission of diabetes (Jansen et al., 2011). Furthermore, increased levels of BAs, particularly DCA, is associated with the inflammatory response (Baeuerle & Baltimore, 1988), while DCA is significantly increased in remission of diabetes after bariatric surgery (R. E. Steinert et al., 2013). Knowing about the impact of BAs on the inflammatory cascade may reveal new targets for the remission of diabetes. Lastly, there are undefined mechanisms of BA receptors with putative roles in the remission of diabetes. For instance, gluconeogenesis is suppressed with/without FXR (De Fabiani et al., 2003; Thomas, Pellicciari, et al., 2008). Taken together, FGF19 and BAs have several unknown mechanisms associated with the remission of diabetes waiting to be identified.

2.17 Filling the Gap

In lean people (without obesity), the level of FGF19 is higher than subjects who are obese with diabetes, therefore, being diabetic and obese plays a major role in changing the plasma levels of FGF19 (Ruta et al., 2013). It has been revealed that in NAFLD, FGF19 is dysfunctional (Schreuder et al., 2010) and FGF19 administration has a compensatory effect on the treatment of NAFLD (Wojcik et al., 2014). NAFLD is almost always observed along with T2DM (Sasaki, Wakabayashi, et al., 2014). Administration of FGF19 has a compensatory impact on the development of T2DM, and it can elevate gallbladder volume (Halpern et al., 2014). However, despite the significant correlation of FGF19 with age and FPG, Fang et al. (2013) claimed that there is no association between FGF19 and 2hpp, HbA1c and IS. Whereas the Hao et al. (2013) study found a significant association between FPG, age, and FGF19 in animal models. Also, in this study, there is a significant correlation between 2hpp and BMI, which is in contrast with previous studies (Fang et al., 2013; Russell, 2003). Therefore, more studies are needed to investigate the possible impact of FGF19 on the remission of diabetes.

There are several studies carried out to find out an appropriate remission for diabetes either in humans or animals. However, it is hard to say why there is a remission after bariatric surgery. BAs and FGF19 may be novel and relevant biomarkers to reveal new areas of research in the remission of T2DM. Thus, efforts of researchers are to first understand the underlying pathways of physiological changes after bariatric surgery and then to find a non-invasive solution for the improvement of diabetes. Still, the principal mechanism of BAs and FGF19 biomarkers on the remission of diabetes is a big question. There are several hypotheses. For instance, enterohepatic circulation along with gut microbial influence plays a crucial role in producing secondary BAs (Sjövall et al., 2010). The role of gut microbial flora has been documented in the remission of diabetes (Liou et al., 2013; K. R. Sharma, 2012). It is not clear whether the level of BAs is associated with FGF19 (Weiner et al., 2011). Whether or not the administration of FGF19 and BAs may have a resolution on T2DM in humans needs more research. FGF19 and BAs may have dual properties relative to the remission of diabetes. If so, we need to find out more about these properties. Interestingly, some BAs have an intestinal permeability (Raimondi et al., 2008) while some of them do not (Bernardes-Silva et al., 2004). We need to study more about them to find putative targets for

the treatment of diabetes. We need to know why in some situations after bariatric surgery some individual BAs are decreased and some of them are increased (De Giorgi et al., 2014; Myronovych et al., 2014).

Bariatric surgery has an antidiabetic property. However, it is now known that bariatric surgery engages a cluster of peripheral and central alterations that together help to improve glycaemic control. Further studies in different physiological conditions are necessary. Differences such as gender variations, newly diagnosed diabetes versus already diagnosed patients, and the grades of obesity are important areas of investigation for future work (Batterham & Cummings, 2016). Table 2.3 shows the effect of different bariatric surgeries on the alteration of BAs and their relationships with clinical variables such as weight, BMI, insulin resistance and FGF19.

Table 2.3 Different bariatric surgeries and their effect on BAs along with FGF19 and BAs associations with the clinical values

Sample Size/ Operation	Time Postop	Fasted/ Fed	BA changes	Refs / Obesity and diabetes	Fasted/Fed		Feeding assay	Correlation			
					BA	FGF19		BAs		FGF	
								Fast	Fed	Fast	Fed
10 VSG	Pre-op,1, 3 mo.	Fasting, Feeding	Increased fasting and augmented feeding response Both 1 and three months increased from baseline	(Khan et al., 2016) No diabetes only obese	Fast, fed	Fast, fed	Meal test	N/A	N/A	N/A	No correlation with weight loss, positive correlation with feeding BAs Changes in feeding BAs
12 VSG 13 RYGB 15 Hypocaloric	7 days	Fasting	Increase total, conjugated, relative to hypocaloric diet	(Jahansouz et al., 2016) Obese with T2DM and prediabetes	N/A	N/A	N/A	N/A	N/A	N/A	N/A
19 VSG	Pre-op, 1, 3, 6,	Fasting	Minimal to no changes in BAs, but decreased	(Escalona et al., 2016)	Fast	Fast	N/A	N/A	N/A	N/A	N/A

	12 mo.		synthesis via surrogate marker. Increase but not significant after a year	Obese recruited, only 2 patients have T2DM (11%)							
24 RYGB	Pre-op, 1, 2, 6, 12, 24	Fasting	Bimodal increase in total, UDCA and conjugates early general increases by 1y not earlier time	(Albaugh et al., 2015) Obese with T2DM	Fast	Fast	N/A	Changes in conjugates, unconjugated, primary, secondary BAs checked. No significant correlation observed with them and changes in HbA1c, BMI, IR in 12 and 24 months after surgery	N/A	No correlation was seen with changes in BMI, IR, and changes in FGF19 at 12 and 24 month	N/A
12 NGT	1 wk., 13 wks.	Fasting, feeding	Fasting decreased at 1 week in NGT but	(Jørgensen et al., 2014)	Fast/	Fast/fed	Meal test	No correlation between changes in	No correlation was seen with	No correlation between Total BAs	Positive correlation between AUC of FGF19 and

12 T2DM	1 year After RYGB		unchanged in T2DM, increased thereafter to 1 year; AUC total BA decreased after MM at 1 week but increased thereafter in both T2DM and NGT	Obese with T2DM	fed			total BAs and fasting glucose and IR. No correlation of Total BA with LDL, HDL, and cholesterol Probably with the actual value of clinical variables not clear from their explanation.	glucose tolerance	and fasting glucose and IR.	AUC of total BAs No correlation was seen with glucose tolerance
18 VSG	Pre-op 6 mo.	Fasting	No total changes; decreased conjugated and increased UDCA 6 month after SG	(Belgaumkar et al., 2016) Only obese	Fast	Fast	N/A	No correlation between total BA and IR, inflammatory markers. Actual value measured	N/A	No correlation with total BA No correlation with IR and inflammatory markers;	N/A

										Actual value measured	
14 RYGB 14 Obese	11 wks.	fed	Increased in feeding, only a trend for increased fasting	(J. B. Schmidt et al., 2013) Only obese	Fed	Fed	Meal test	N/A	No correlation was seen between total BA and REE No correlation with glucose and c-peptide and total BAs No correlation with insulin, GLP-1, IR, and Matsuda index. Actual value measured	N/A	No correlation with REE No correlation with glucose and c-peptide No correlation with BAs. Actual value measured
22 RYGB 15 BPD	2-10wks (early) 1-2 year	Fasting	Both procedures	(Ferrannini et al., 2015)	Fast	N/A	HEC	Correlation of BAs did not see in RYGB with	N/A	N/A	N/A

			increased total BA	obese with T2DM				conjugated and unconjugated BAs; Actual value measured			
11 RYGB	33.8 mo.	fed	Increased fasting, earlier feeding rise	(De Giorgi et al., 2014) Only obese	N/A	Fed	Meal test	N/A	N/A	N/A	N/A
>30 RYGB >30 obese	>1 year	Fasting	Increased total with RYGB, larger % increase in diabetic» non-diabetic	(Gerhard et al., 2013) Obese with T2DM	Fast	N/A	N/A	Only total BAs reported but CA, DCA, and CDCA individually measured No correlation with total BA and weight loss Probably with the actual value of clinical variables not clear from their explanation.	N/A	No correlation with BAs No correlation with BMI Negative correlation with CYP7A1 in diabetic but not in nondiabetic No correlation with weight loss.	N/A

										Probably with the actual value of clinical variables not clear from their explanation.	
5 RYGB 7 Lean	1, 4, 40 wks.	Fed	Increased feeding rise by RYGB	(Ahmad et al., 2013) Only obese	Fast	N/A	Meal test	N/A	N/A	N/A	N/A
7 RYGB 7 VSG 6 Lean	1, 3, 12 mo.	Fasting, Feeding	Increased over one year, RYGB»VSG	(R. E. Steinert et al., 2013) Only obese	Fast, fed	N/A	Meal test	Correlations performed in each surgery group separately	No correlation between fasting and AUC of total BAs	N/A	N/A

								Negative correlation of total BA with BMI and improvement of glycaemia, Negative correlation between total BAs and BMI Positive correlation with GLP-1 1 year after SG, positive correlation with GLP-1 pre-op in RYGB Three month after SG positive correlation with PYY Fasting PYY with total BA 1 year after RYGB changes in BAs with	with GLP-1, PYY moreover, 1 year after SG with PYY positive correlation of total BAs with PYY 3 month after RYGB changes in BAs with clinical variables		

								clinical variables			
63 RYGB	Pre-op and at 15 mo.	Fasting, Feeding	increased fasting and feeding responses in RYGB	(Werling et al., 2013) Only obese	Fast, fed	N/A	Meal test Only obese	N/A	N/A	N/A	N/A
10 AGB 8 RYGB	Pre-op and 20% wt. loss	Fasting, feeding	Doubled fasting feeding in RYGB,	(Kohli, Bradley, et al., 2013a)	Fast, Fed	N/A	HEC	No correlation between	Total and conjugated BAs	N/A	N/A

			ABG No change or trend to decrease	Only obese			Meal test	total BAs and REE No correlation reported for primary, secondary taurine glycine with clinical variables changes in BAs with clinical variables	Positive correlation of Feeding BAs with GLP-1 No correlation between feeding total BAs and insulin secretion rate and insulin-stimulated glucose disposal Negative correlation of total Bas with TSH No correlation between total BAs and REE No correlation reported for primary, secondary taurine		

									glycine with clinical variables changes in BAs with clinical variables		

30 RYGB	Pre-op and 12 mo.	Fasting	BA 2-fold increase after RYGB	(Simonen et al., 2012)	Fast	N/A	N/A	No correlation between total BAs and weight loss and BMI No correlation between total BAs and REE and respiratory quotient (RQ) Positive correlation between conjugated BAs and RQ, glucose	N/A	N/A	N/A

				Only obese				oxidation and negative correlation with lipid oxidation No correlation observed between total BAs and conjugated BAs with weight loss, glucose, IR, HOMA-IS, FFA No correlation between DIO2 adipose tissue and total or conjugated BAs Negative correlation between taurine conjugated BAs with DIO2, DIO2 negatively correlated			

								with glucose oxidation, positively correlated with lipid oxidation, No correlation between DIO2 and REE, weight loss, TSH, IR, IS, FFA and glucose. Changes in BAs with clinical variables			
12 RYGB 6 AG	Day 4, 42	Fasting	Increased total in RYGB only, not AGB	(D. J. Pournaras et al., 2012) Only obese	Fast	Fast	N/A	N/A	N/A	N/A	N/A
35 RYGB	Pre-op, 3 mo.	Fasting	Total increased	(Jansen et al., 2011) Obese with insulin resistance	Fast	Fast	N/A	N/A	N/A	N/A	N/A

9 VSG 6 AGB 6 VSG/DS 13 RYGB	Pre-op, 1, 3 mo.	Fasting	Increased total and secondary by RYGB/DS, No increase in VSG, AGB	(Nakatani et al., 2009) Obese with T2DM	Fast	N/A	N/A	Total and primary BAs positively correlated with GIP, Primary BA positively correlated with GIP 1 and 3 months after surgery Primary BAs positively correlated with insulin 1 month after surgery but no correlation after 3 months, GIP positively correlated with insulin 1 month after surgery No correlation observed at 1 and 3 months between total and primary BAs	N/A	N/A	N/A

								and glucose HbA1c after surgery No correlation between total BA and primary BA with LDL and HDL in any intervention time point. Changes in BAs with changes in clinical variables			
9 RYGB 5 Obese Match	2-4 yr. post-op (cross-section)	Fasting	Increased total and	(Patti et al., 2009)	Fast	N/A	N/A	Total BAs negatively correlated	N/A	No correlation between	N/A

10 Lean Match			conjugated species	Only obese				with TG, 120 min glucose, positively with adiponectin and GLP-1. No correlation between total BAs and HDL, No correlation between total BAs and BMI, Negative correlation of total BAs with HbA1c and fasting glucose Negative correlation between total BAs and TSH; Actual values measured		FGF19 and total BAs. Actual values measured	

20 obese AGB	3,6,12 mo. (Observational study)	Fasting	Total, conjugated, and secondary BAs increased 3 months after AGB	(Thöni et al., 2017) Obese with T2DM	Fast	Fast	N/A	IR positively associated with total BAs, conjugated BAs, Insulin positively related to total BAs and conjugated BAs, CRP positively associated with primary BAs and conjugated BAs No correlation between IR and primary BAs, secondary BAs, unconjugated BAs, GLCA, No correlation between insulin and	N/A	No correlation between FGF19 and IR, insulin and CRP FGF19 positively correlated with total BAs, primary BAs, secondary BAs, unconjugated BAs and conjugated BAs, No correlation with GLCA. Actual value measured	N/A

								primary BAs, secondary BAs, unconjugated BAs, GLCA No correlation between CRP and total BAs, secondary BAs, unconjugated BAs, and GLCA. Actual value measured			

60 obese BPD RYGB	1,2, 5 years RCT	fasting	Total, glycine, taurine, unconjugated, secondary, increased in comparison with pre-op	(Risstad et al., 2017) Obese with 5 for RYGB and 6 for BPD at baseline	Fast	N/A	N/A	Total BAs negatively correlated with BMI after 5 years, negative correlation between total BAs and cholesterol, and weight loss, No correlation with actual value of LDL, HDL, TG, weight, glucose, HbA1c, c-peptide, insulin, IR, with actual value of total BAs 5 years after surgery Changes in total BAs negatively correlated with changes in weight, after 5 years,	N/A	N/A	N/A

								No correlation observed in changes in total BAs with cholesterol, LDL, HDL, TG, glucose, C-peptide, insulin, and IR. Actual value measured			
13 RYGB only women	Pre, 1 month and 2 years	Fasting, feeding	Increased 1 month and 2 years for all BAs	(Dutia et al., 2015) Obese with T2DM	Fast, fed	Fast, fed	OGTT	Body weight, not weight loss negatively correlated with actual value of fasting total BAs, secondary BAs, Insulin positively correlated with AUC total BAs, primary BAs,	No correlation between actual value of glucose and insulin with any feeding BAs AUC PYY positively correlated with feeding primary BAs, unconjugat	N/A	The actual value measured. Positive correlation between AUC total BAs and FGF19

								conjugated BAs, 12 OH BAs and non 12 OH. No correlation of IR and IS with any fasting BAs	ed BAs, non 12 OH, GIP and GLP no correlation with any BAs		

VSG, vertical sleeve gastrectomy; RYGB, Roux-en-Y gastric bypass; AGB, adjustable gastric banding; BPD, biliopancreatic diversion; DS, duodenal switch; BA, bile acid; UDCA, Ursodeoxycholic acid; N/A, not applicable; IR, insulin resistance; NGT, normal glucose tolerance; MM, medical management; REE, resting energy expenditure; GLP-1, Glucagon-Like Peptide-1; HEC, Hyperinsulinemic-euglycemic clamp; CA, Cholic acid; DCA, deoxycholic acid; CDCA, Chenodeoxycholic acid; TSH, Thyroid-stimulating hormone; FFA, free fatty acid; DIO2, Iodothyronine Deiodinase 2, IS, insulin sensitivity; GIP, Gastric inhibitory polypeptide; CRP, C-reactive protein; GLCA, Glycolithocholic acid, PYY, Peptide YY.

2.18 Body Composition Measurement

The significant impact of body composition is to assess body situations such as physical activity and nutrition. Nevertheless, there is no doubt about the effect of excess fat on the onset of chronic diseases (Raj Padwal et al., 2016). There are several ranges of methods available for the assessment of body composition. They are categorised according to the number of compartments that they measure. A two-compartment method quantifies fat, and fat-free mass and three-compartment methods measure fat mass and two compartments of fat-free mass. Each method needs to be evaluated according to costs, validity or reliability, applications, risks and availability (Kyle et al., 2004). There are three types of classifications for body fat; fat mass (FM), which includes total extractable fats; fat-free mass (FFM), which includes all chemicals, water (≈73%), bones (≈7%) and internal organs connective tissues (≈20%); and lean body mass (LBM), which is small amounts of essential fats (Lohman, 1992). Body composition assessment is an accurate method to standardise classification of body fitness. Therefore, the application of body composition includes determination of health risks associated with low or high levels of fat, monitoring impacts of a given nutrition, exercise and intervention and measuring the body weight to assess growth, development, and maturation.

2.18.1 Ordinary Anthropometric Measurement

Obesity is defined as an excessive amount of body fat in association with body weight (Hubbard, 2000). Obesity can be a result of excessive energy consumption or alterations in the body energy expenditure that lead to a positive energy equilibrium (Flatt, 2007). The study of evaluating the human body based on the dimension of bones, muscles and fat tissues (adipose tissues) is called anthropometry. Anthropometric or body composition measurements are critical due to their potential to predict or proceed with monitoring the onset of metabolic disorders such as obesity and diabetes. Anthropometry refers to measuring the size of the body based on values and ratios. They are included but not limited to height to weight (BMI) or waist to hip ratio (WHR), as well as neck and femur circumferences. Circumferences are vital to record during anthropometry measurement. All different kinds of circumferences require the use of a tape measurement, and to increase validity and reliability of the measures, the position of the measuring tape must be consistently in the same position for all subjects.

Although BMI measurement is accepted as a universal indicator of obesity, it is not the best indicator of obesity. For instance, older people have more body fat at any given BMI compared with younger ones. BMI is not always equal to less body fat (Baumgartner et al., 1995). Therefore, it is crucial to measure other indices for body composition. For example, LBM is increased by obesity (Garby et al., 1988) and neck circumference measurements reflect upper-body fat indirectly (Ben-Noun et al., 2001). High proportions of waist-hip ratio (WHR) is a common calculation result used to predict the risk of T2DM (Vazquez et al., 2007). It works as a crude scale of fat distribution. Also, waist circumference has a direct relationship with abdominal content, musculature, subcutaneous adipose tissue (SCAT) and visceral adipose tissue (VAT) (Borruel et al., 2014). A recent clinical trial study of obese patients has shown that there is a direct correlation between neck circumference and metabolic diseases, and neck circumference information is superior to waist circumference data (Assyov et al., 2016). The waist circumference measurements are also considered to be the surrogate markers used to predict metabolic syndromes (J. Kaur, 2014). Of note, it is accepted that neck circumference cut-off values of 36, and 39 cm or greater in males and females respectively is associated with increased HOMA-IR and HbA1c (Assyov et al., 2016).

2.18.2 Dual Energy X-ray Absorption

Dual-energy X-ray absorptiometry (DXA, previously DEXA) is a 3-compartment method for the evaluation of body composition due to its abilities to measure bone mineral, fat and fat-free mass. DXA is also able to measure total body bone (TBB), total body fat (TBF), bone mineral content (BMC) and bone mineral density (BMD). DXA is replaced by dual photon absorptiometry (DPA). Instead of a radioisotope, X-rays are used in this method because X-rays are more accurate than DPA (Mazess et al., 1990). DXA is a straightforward and non-invasive procedure for the assessment of body composition. In DXA only 1 to 3 milliradians of x-rays is used. The principal of DXA procedures is based on the reduction of X-rays with high and low photons. Chemical components and the density and thickness of tissues are measurable from pixels created by the instrument (Carlson-Newberry & Costello, 1997). Nowadays, bone densitometry is a favourable method to diagnose osteoporosis. In practice, the best method to evaluate bone density functions is measuring BMD. Using DXA on spine and femur samples is the most reliable method to measure BMD (V. H. Heyward & Gibson, 2014). DXA is also a very precise method to assess body composition (Kiebzak et al., 2000).

Using DXA in biomedical research is quite new. Body composition plays a major role in the assessment and management of different diseases (Albanese et al., 2003). However, an increased prevalence of obesity and its comorbidities have accelerated curiosity in the utility of straightforward and accurate technologies such as DXA for the evaluation of body composition (Bazzocchi & Diano, 2014).

2.18.3 Bioelectrical Impedance Analyser

Bioelectrical impedance analysis (BIA) is a 2-compartment method. BIA is a non-invasive procedure which is performed by attaching electrical wires to the body to measure resistance (R) and reactance (Xc). Both R and Xc are used to quantify impedance (Z), phase angle, total body water (TBW), FFM, FM and body cell mass (BCM) values (Lukaski et al., 1985). In BIA, resistance to an electrical current is negatively related to the distribution of TBW and electrolytes (Kyle et al., 2004). The principal of BIA is based on the fact that different body components have different resistance to the passage of electrical current (V. Heyward, 2001). An electrical current is used to measure resistance as opposition the electrical current through the body, and reactance, which is measuring values originating from cell membranes. Resistance and reactance are based on electrical current and are capable of measuring FFM (Mialich et al., 2014). Adipose tissue and bone minerals have greater resistance to current flow than FFM, as FFM includes less water (V. Heyward, 2001). About 73% of the body's FFM is water. Hence total body water is estimated, and FFM is calculated based on water (Cornier et al., 2011). The higher conductivity of lean tissues is due to a large amount of water and electrolytes (low resistance). However, bone, skin and fat, due to their lower amounts of water and electrolytes have low conductivity (high resistance) (Mialich et al., 2014). Normally four electrodes are used for BIA measurements. In a horizontal position, two distal wires for the wrist and hand, and another two proximal wires for the ankle and foot are used with low electrical current (500 to 800 μA) in BIA (Kyle et al., 2004). Since BIA is an accurate procedure to measure body fat, it can be used instead of DXA (Leahy et al., 2012).

2.18.4 Energy Expenditure

Energy expenditure (EE) is defined as the number of calories consumed during a distinct time, often 24 hrs. The resting energy expenditure (REE) is a common method to represent EE. The proper range of REE is 1800-2200 kcal/24 hr (Haugen et al., 2007). Daily energy or

calorie consumption is quantified by energy expenditure in a resting position. Several factors such as disease states, age, and being obese, influence the calculation of EE (McClave & Snider, 1992). Measuring EE during a chronic disease sate is important. It can be helpful to prevent overfeeding in subjects during an intervention screening. REE directly aims to manage patients' care. It has been shown that obese females have higher EE in comparison with females with normal weight. This fact may be related to the higher amount of FFM in women (Horie et al., 2009). Also, there is a significant and positive correlation between EE and body size (Garby et al., 1988). Indirect calorimetry is used when EE is measured from VO_2 production because heat cannot be measured directly (Jequier, 1981). Indirect energy expenditure analyses respired gases, namely oxygen uptake and carbon dioxide expression from the lungs. The respiratory quotient (RQ) in a person is calculated as the ratio of exhaled CO_2 to consumed O_2. RQ is a popular index of EE (Feurer & Mullen, 1986). RQ values indicate consumed fats, carbohydrates and proteins being used for energy. The range of RQ is from 0.65 to 1.25; values below or greater than this range is considered to be a health issue, and any value inside of this range defines a stable-state condition. The RQ value also represents carbohydrate metabolism (RQ=1.0), lipid metabolism (RQ=0.71) and protein metabolism (0.80) (Haugen et al., 2007). Once the rate of metabolism is slow, a person has difficulty managing weight loss. A significant benefit of REE is that it monitors metabolic rate to avoid further unnecessary analysis. In other words, a given ratio and value from REE aims to evaluate the success of strategies for losing weight (S. M. Cummings et al., 1997). Also, monitoring REE and RQ is an easy way to check whether or not a person is following a given intervention appropriately (Turner et al., 2014).

2.19 Enzyme-Linked Immunosorbent Assay

ELISA or enzyme immunoassay (EIA) is a plate-based method planned for determining and measuring peptides, proteins, antibodies, and hormones. The principal of ELISA is binding an antigen which is already immobilised to a solid surface and then combining the assay with an antibody that is linked to an enzyme. Conjugated enzyme activity is the next step to detect a substance after an exact incubation time. It is crucial that the bond between antigen and antibody must be specific, which is ensured by washing away nonspecific binding proteins. ELISA is usually achieved in a 96-well polystyrene plate (Stevens et al., 1995). The washing step makes the ELISA procedure easier to separate bound from unbound substances during

the test, and it makes ELISA a powerful tool to detect specific analytes from various matrices at the same time. ELISA needs a detection enzyme which can be linked to the primary antibody of interest and a protein such as streptavidin when the primary antibody is biotin labelled. The most popular enzyme is horseradish peroxidase (HRP) due to its low binding affinity for different substrates (Gan & Patel, 2013). There are several modifications in an ELISA assay. For instance, immobilisation of a given antigen can be done by direct adoption or indirect through capturing an antibody that is then attached to a plate. The most popular, sensitive, powerful and robust assay format in ELISA is the "sandwich assay," which means the analyte to be detected is bound to two primary antibodies (a capture antibody and a detection antibody) (Natarajan & Remick, 2008).

Direct ELISA measurement is not very common. However, indirect methods using a labelled secondary antibody is mostly used as an ELISA format (Crowther, 1995). In a sandwich method, it is vital to use a specific secondary antibody to detect the primary antibody. The secondary antibody is chosen based on its potential to remove all antibodies which have an affinity to capture the primary antibody. There are several advantages to using an indirect ELISA assay. For example, a wide range of labelled secondary antibodies is present commercially. Also, its sensitivity is higher than direct ELISA. FGF19 is a hormone can be easily quantified using "sandwich ELISA" with high accuracy and sensitivity (Gerhard et al., 2013).

It is important to make sure that the method which is used for ELISA is validated. For validation of ELISA, both inter- and intra-assay interactions need to be calculated (Hanneman et al., 2011). CV is defined as standard deviation (SD) a set of calculations divided by the mean of the set and then reported in percentage. In large sample sizes such as longitudinal studies, it is compulsory that specimens be run on different (multiple) assay plate. That is each plate is assessed by its calibration curve and at least an internal control (if possible), with known concentration. The inter-assay CV comes from plate-to-plate consistency and is calculated from the mean values of the control on each plate. For instance, control is run on five different plates then after calculation of its mean, SD and CV are measured. Therefore, inter-assay %CV= (SD of plate means/ mean of plate means) x 100.

Since testing each specimen with greater values of replicates can produce statistically better outcomes, It is recommended in ELISA to measure any analytes in duplicate (Andreasson et

al., 2015). The intra-assay CV comes from the duplicated measurement. Therefore, intra-assay CV is presented as an average value measured from individual CVs for each duplicate. Calculation of intera-assay %CV= (calculation of SD for each duplicated/ mean of each duplicated) x100.

2.20 Liquid Chromatography Mass Spectrometry

Liquid chromatography (LC) was introduced to physiological and biological studies about 50 years ago (Cuatrecasas et al., 1968). Previously gas chromatography mass spectrometry (GC/MS) used to be most popular method to detect human body analytes but thanks to the advanced technology such as atomic pressure chemical ionisation (APCI) and electrospray ionisation (ESI), measuring different types of analytes with any concentration becomes easier than before (D. W. Johnson, 2005). ESI/MS can quantify polar molecules, and APCI is usually utilised for natural or less polar molecules (Pitt, 2009). For example, ESI has a property to separate isomeric forms of substances such as BAs (Yousef et al., 2003). Generally speaking, all MS measurements are due to three parts of machinery: the ion source, which is the part to vaporise and ionise; the mass analyser, which separates ions based on their charges; and the detector system along with a computer for data processing and a vacuum pump to control the pressure. The sensitivity of LC is based on a signal to noise (S/N) ratio at specific concentrations. A S/N ratio of 10 is acceptable, but it depends on the assay requirements. Thus, it is important to know the concentration of molecules of interest. MS works under atmospheric pressure. Low pressure is needed to constrain the number of ion collisions, which result in producing unwanted background and loss of charge (De Hoffmann & Stroobant, 2007). The quadrupole analyser is a format of MS. It usually monitors a specific mass to charge ratio (m/z). Multiple reaction monitoring (MRM) is also another part of MS, and MRM is a very delicate and selective procedure to quantify substances in complex matrices. Tandem MS or MS/MS is a technique to break down precursor ions into product ions or fragments. Fragments show the chemical structure of selected ions (precursor ions). When MS/MS is used, it is possible to inject samples into the ion source directly. This method is appropriate for samples that can be diluted readily, such as urine, but it is not suitable for high protein samples like as plasma or serum (Pitt et al., 2002). During method development, it is necessary to examine the impact of the matrix on the ionisation process. It is important to validate a method before applying it to the real

sample. Most of the time, each sample is subjected to preparations before injecting it into the LC-MS/MS machine. Samples usually come from a complex matrix where the analyte of interest is often present in very low concertation. The blend of LC with MS allows a high capability to differentiate between different compounds. Nevertheless, sometimes greater efforts are needed for better clean-up before using LC-MS to increase selectivity, sensitivity, and validity (Boyd et al., 2011). LC separation is mostly done by reverse-phase (RP) separation. RP works based on differences in hydrophobicity to complete partitioning between a non-polar stationary phase and a polar mobile phase. Mobile phase normally constitutes from polar organic solvents such as methanol or acetonitrile. Mobile phase facilitates the movement of the molecule of interest across the column. Also, RP along with an appropriate pH, temperature and flow rate increases the chance of getting good peaks and separations of the isobaric molecules.

Sample preparation and extraction is one of the most important steps in LC-MS. Sample preparation and extraction aim to remove any interferences during analysis of the analyte(s). There are several techniques to extract samples such as solvent extraction, solid phase extraction, dilution for simple matrices and protein precipitation for biological matrices. The latter is easier and an inexpensive procedure (Tagliacozzi et al., 2003), while the rest are usually expensive and time-consuming methods (Chang et al., 2007; Henion et al., 1998). In protein precipitation, often an organic solvent such as methanol or acetonitrile is added to a biological sample (e.g., plasma). Then, the mixture is vortexed, and supernatant separated to analyse by LC-MS/MS (Boyd et al., 2011). Another important part of measuring by LC-MS is to use an internal standard. Internal standards are known quantities of a well-characterized chosen compound which are differed from the analyte of interest. Internal standards can be used to rectify some issues such as drifting or as an internal calibrator, to optimise the instrument and its measurement parameters. Internal standards are isotopically labelled. It is important to know when internal standards should be added, although, most of the time they are added at the beginning of the analytical assay (Barwick, 2003).

LC-MS/MS has been introduced as a reliable method to measure BAs for many years (Burkard et al., 2005; Spinelli et al., 2016; Ye et al., 2007). LC-MS/MS is a profound and preferred method to determine micro-molecules, peptides, proteins and pharmaceutical compounds in an accurate and fast way within a broad spectrum of body fluids such as blood

(both serum and plasma) (Maurer, 2005), and other fluids (Drummer, 2006; W. Li & Tse, 2010). The principal of LC-MS/MS is to utilise ions, sort and determine them according to their m/z ratio. Triple quadrupole mass spectrometry (TQMS) is one of the most popular LC-MS methods. TQMS is comprised from three quadrupoles. Each quadrupole has four cubically shape rods. The first quadrupole (Q1) is utilised for choosing a primary ion. The next quadrupole (Q2) is where collision induced dissociation (CID) or collision cell occurs. The third quadrupole (Q3) produces a spectrum of the resulting ions. The role of Q1 is necessary to reduce the need for sample clean-up due to its property to discard nonanalyte ions. Although there are some methods to determine a different type of BAs, most of them are not sensitive, accurate and time-saving (S. Keller & Jahreis, 2004; Künnecke et al., 2007). LC-MS/MS needs to used for measuring BAs for several reasons; first, there are several types of individual BAs; second, the physiochemical properties such as the polarity gap between unconjugated and conjugated BAs; third, there is a very low concentration of BAs in the body's fluid such as blood and urine; fourth, there are tiny differences between the different types of BAs, while some of them are isomeric (isobaric) (Tagliacozzi et al., 2003).

Method validation is a necessary step to validate the method formally and is the last step to carry out in LC-MS. It is compulsory to assess a range of parameters which can affect the performance of the method. Parameters such as selectivity, which is the capability of the assay to quantify a molecule of interest (the analyte) without interference from other compounds in the sample; precision and reproducibility of the method; and limit of detection (LOD), which is the lowest concentration of the analyte which can be measured accurately (Thompson et al., 2002).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Design

The current study is a single-center, prospective, randomised, double-blind study. Both pre-surgery and post-surgical follow-up follow a similar protocol.

3.1.1 Ethical Approval and Ethical Consideration

Ethical Approval was received from New Zealand regional ethics committee (NZ93405). This study was prospectively registered at ANZCTR (ACTRN12611000751976) and retrospectively registered at https://clinicaltrials.gov/ (NCT01486680). The information sheet of this study was provided to the subject to decide to join in this study (Appendix A). For all the confirmed subjects for the bariatric surgery, a written informed consent was obtained (Appendix B).

3.1.2 Study Samples

Male and female subjects were recruited from the bariatric surgery outpatient clinic following a thorough assessment by a multidisciplinary team consisting of a bariatric surgeon, dietician, general physician endocrinologist and psychiatrist if required, to ensure that they were suitable for surgery. Participants were not being paid to join, nor were any medical costs paid additional to those incurred as part of current standard treatment. This heterogeneous study was carried out on obese New Zealander subjects who had been diagnosed for T2DM from different ethnic groups. Patients were free to withdraw from the study at any time.

3.1.3 Location of Sampling and Measuring

All samples were collected at Body Composition Unit, Auckland City Hospital. Although the anthropometric measurements were recorded and kept in the Body Composition Unit for further analysis, human plasma samples were aliquoted and transferred to the School of Science lab, AUT University for analysis.

3.1.4 Sample Size

The sample size was calculated to provide power to detect an expected diabetes remission rate of 88% in the RYGB group and 59% in the SG group (Murphy et al., 2016). This design showed a power of 80% and a risk of 0.05; assuming a patient drop-out rate of 20%, at least 34 patients per group were required to demonstrate a significant difference. For appropriate sample size, 37 and 36 subjects were recruited for SG and RYGB respectively. In this study, however, 29 and 32 of subjects who underwent SG and RYGB respectively were considered for statistical analysis both before and a year after bariatric surgery.

3.1.5 Randomisation

Participants were allocated to treatment groups by randomization with minimization based on age (grouped as 20-30, 30-40, 40-50); time since diagnosis of T2DM (grouped as <5yrs, 5-10yrs, >10yrs) and ethnicity (grouped as Māori, Pacific, European, or other). Computer with allocation concealment performed randomization.

3.1.6 Inclusion and Exclusion Criteria

The surgeon did the decision making for inclusion. All subjects were eligible to undergo either SG or RYGB. Table 3.1 Shows the inclusion and exclusion criteria in this study.

Table 3.1 Inclusion and exclusion criteria

Inclusion criteria
T2DM for at least 6 months (diagnosed by 2 hours 75g oral glucose tolerance test glucose level >11.1mmol/l or fasting glucose > 7mmol/l on at least 2 occasions or a random glucose >11.1mmol/l confirmed with either osmotic symptoms or a fasting glucose of > 7mmol/l, and/or HbA1c greater than 6.5%)
Previous failed attempts at weight loss through dieting and exercise
BMI ≥35 kg/m^2 for at least five years
Age between 20 and 50 years
Suitable for SG and RYGB
Able to give informed consent and willing to commit to follow-up
Exclusion criteria
BMI > 65
Pregnancy
Diagnosis of type 1 diabetes, diabetes resulting from pancreatic injury, or secondary forms of diabetes, e.g., acromegaly and Cushing's syndrome
Prior bariatric surgery of any kind, previous antrectomy or small bowel resection
History of chronic pancreatitis or idiopathic acute pancreatitis
Cardiovascular conditions, including significant coronary artery disease (CAD), peripheral vascular disease (PVD), congestive heart failure (CHF) or uncontrolled hypertension
Myocardial infarction (MI), coronary artery bypass surgery or stroke within the past six months
Chronic renal insufficiency with creatinine > 180mmol/L
Known history of chronic liver disease (except for non-alcoholic fatty liver disease (NAFLD) or non-alcoholic steatohepatitis (NASH)
Gastrointestinal disorders including portal hypertension, malabsorptive disorders or inflammatory bowel disease
Oral Steroid use
Psychiatric disorders including dementia, active psychosis, severe depression requiring medications, history of suicide attempts, alcohol or substance abuse within the past two years
Severe pulmonary disease defined as forced expiratory volume in 1 second (FEV1) < 50% predicted, or pulmonary hypertension
Malignancy (other than low-risk – basal cell skin cancer, in situ carcinoma of the cervix or in situ prostate cancer) within the past five years
Blindness

3.1.7 Intervention

A very low-calorie diet using Optifast® was prescribed for 2-4 weeks, in addition to an exercise program for 12 weeks pre-operatively. The very low-calorie diet included 2-3

sachets of dilute Optifast® (dilute one sachet in 400ml water) per day. A Pureed diet was then commenced for a further three weeks, with a solid diet introduced at six weeks post-operatively. All pre and post operation subjects were reviewed by the Dietician at outpatient clinic visits routinely. The role of consuming VLCD has been approved before bariatric surgery (Aills et al., 2008). Pre-surgery weight loss not only aims to facilitate the operation but also prepare subjects to adapt their eating behaviours after surgery, recognise amenable subjects, adjust the appropriate amount of vitamins and/or mineral supplements to avoid deficiency and improve IR (Schiavo et al., 2016). Also, consuming VLCD (Optifast) 3 to 4 weeks before bariatric surgery reduces the amount of lipids in the liver. Hence the liver becomes lighter to lift up by the surgeon during the operation (Lewis et al., 2006).

3.1.8 Record of Demographic Information

After receiving verbal consent, all the individuals were asked to fill in a survey form which includes the following items: age, height, weight, race, smoking, and alcohol drinking habits, bowel motion, the frequency of defecation and medical history. For the follow-up study, all subjects were asked to express their feeling after drinking the glucose syrup. An example of a demographic questionnaire is attached (Appendix C).

3.2 Anthropometric and Body Composition Measurements

Body composition obtained in this study include weight, height, circumference of waist, hip and neck. Weight was measured using an electronic measuring machine. To measure the height, the individuals were asked to take off their shoes, stand with their back against the wall with their feet together. The back on their feet, buttocks, shoulders, and the back of their head is adjusted by the researcher. They were asked to look straight ahead to ensure that their bodies were as straight as possible. Subsequently, their head level positions were marked with a pencil. A measuring tape was then used from the ground up to determine their height; BMI then was calculated using the formula: BMI = weight (kg) / Height^2 (m^2). Circumference measurements were taken at least twice; the measurement accepted if the difference between two measurements were not more than 2 cm. However, the third measurement was done if the difference was more than 2 cm. In this case, the two closest measurements were averaged and recorded for analysis. In this study, neck, hip, and waist circumference were measured as previously mentioned (V. H. Heyward & Wagner, 2004). To measure the waist circumference, the subject was asked to lift his/her clothes to put the tape in the appropriate

position. The subject was asked to stand erect with the belly relaxed. The tape was positioned in a horizontal position at the nearest part of the torso. For those subjects who were more obese, the area between the ribs and iliac crest was considered to measure the waist circumference. To measure the hip (buttocks) circumference, the subject was asked to stand erect with arms at the sides and feet closed together. The tape was positioned around the hip in a horizontal position without pushing the skin. Following waist and hip measurements, the WHR was calculated according to a guideline from WHO (Consultation, 2008).To measure neck circumference, the subject was asked to put off any clothes from his/her neck. The subject sat erect. The tape was positioned around the neck just inferior to Adam's apple with the least amount of pressure on the neck. The measurement was taken promptly to avoid any discomfort for the subject (Lou et al., 2012).

3.2.1 Bioelectrical Impedance Analysis

BIA, which is a rapid, non-invasive and inexpensive method, was used to measure body composition. Different procedures employed in BIA are dependent on various devices available. Fat is a poor conductor of electricity while a tissue which is free from fat (or FFM) is a good conductor of electricity. Therefore, the resistance to the electric flow is used to determine the proportion of body fat as a percentage. Measurement by BIA is a quick procedure, normally taking less than a minute. However, care should be taken during BIA. In lean people, the percentage of fat is overestimated, while in obese people it is underestimated. Since the result of BIA is directly related to the amount of water in the body, the hydration state of an individual can significantly affect the outcomes. In order to address hydration as a factor, before doing BIA, all subjects were asked to abstain from exercise and drinking alcohol for at least 8-12 hours. BFM and FFM were measured by BIA. The Body Stat Quadscan 4000, UK instrument was utilised in this study. The impedance (z) is measured through low-level electrical current pass through the patient's body using a BIA machine. Four electrodes were attached to the hand, wrist, foot, and ankle. An excitation current 200-800 uA at 50Hz was applied at the distal electrode on the hand and foot to detect impedance on the wrist and ankle. The patient was asked to lie supine on a non-conductive bed. Attachment of the electrodes began by first cleaning the attachment sites with alcohol. Proximal electrodes were placed on dorsal surfaces of the wrist and ankle. The distal electrode was placed close to the second or third metacarpal-phalangeal at hand and foot. The distance between proximal and distal electrode was at least 5 cm. Finally, lead wires were

attached to the electrodes. Red leads were connected to the wrist and ankle and black leads attached to hand and foot. Once leads were connected to the body, the device showed the amount of BFM and FFM.

3.2.2 Dual-Energy X-ray Absorptiometry

TBF, BMC, and BMD were measured by DXA (model iDXA, software V.15, GE-Lunar, Madison, Wisconsin, USA) according to the previous method (Peng et al., 2007). The DXA machine was calibrated according to the manufacturer's calibrator protocol. After measuring the patient's height and weight, patients were asked to lie in a supine position on the scanner bed for head-to-toe measurement. The scanning took approximately 20 to 40 minutes.

3.2.3 Rest Energy Expenditure

Resting energy expenditure (REE) was determined through an open circuit indirect calorimetry device (Deltatrac Metabolic Monitor MBM-100, Datex Instruments, Helsinki, Finland) as previously described (Plank et al., 2001). Each time 30 min prior to the start of measuring, the device was turned on to warm-up. According to the manufacturer's manual, a calibration test was performed to check the function of the instrument. To do REE, all subjects either before or after bariatric surgery were asked to lie in a supine position on the bed, and an oxygen mask was put on the subject to measure REE. This method took about 30 min to perform. REE is a straightforward and safe method to measure energy consumption. To calculate REE, the following formula was utilised as previously described (Plank et al., 2001).

$REE_{(kcal/d)} = 16.85 * FFM_{corr} + 725$, where FFM_{corr} is FFM in kg of the subject corrected for abnormal hydration.

3.3 Research Methods

3.3.1 Blood Samples

All subjects were asked to fast overnight (at least 12 hours). Blood was collected between 8 and 10 a.m. The blood sample was collected after the study subject had rested for 10 minutes. An intravenous cannula was inserted into the targeted vein to minimize the discomfort of repeated blood sampling, and 20 ml blood samples were drawn every 30 min up to the final

sample at 120 minutes. To perform the oral glucose tolerance test (OGTT), each patient was asked to drink a bottle of glucose drink (75g glucose in 300ml water). After 15 min of drinking the solution, the first sample was collected. Plasma separation would be carried out at 4500xg for 10 min at -4°C centrifuge and plasma was aliquotted into a clean tube for further analysis, before being put in -80°C for storage.

3.3.2 Biochemical Test

Lipid Profile testing included total cholesterol (TC), high-density lipoprotein cholesterol (HDL) and triglycerides (TG). Levels were measured by an auto analyser (Roche Diagnostics, Basel, Switzerland). To determine low-density lipoprotein (LDL) levels, the Friedewald equation was used (Friedewald et al., 1972; Fukuyama et al., 2008). The principle of this analysis was based on the enzymatic method. Insulin measurement was done by the same auto analyser as above. The principle of the test was according to the Elecsys assay using two monoclonal antibodies designed specifically to target human insulin protein. Human plasma glucose was measured by glucose oxidase assay. All time points from basal to 2hrs after glucose drinking were examined for each subject before and after each surgery. Further information about each test detail is available in Appendix D.

3.4 Biomarker Study

In this study fasting and postprandial FGF19 and individual BAs (either conjugated or unconjugated) are considered as biomarkers.

3.4.1 FGF19 Measurement

To measure FGF19, enzyme-linked immunosorbent assay (ELISA) was utilized.

3.4.1.1 Sample Preparation

For measuring FGF19, two-time points were considered, basal and 120 min after glucose drinking, before and after each type of the surgery. The ELISA test for all pre and post samples was done in duplicate, and the appropriate number of samples was calculated for each working day. The preoperative samples with “0” or “120” min were quantified first. To do this, samples were moved out of -80 °C storage and placed in a normal refrigerator (~4 °C) a day before running the ELISA to make sure that the samples were thawed gradually and appropriately. About one hour before performing the ELISA assay, all of the samples

were moved from the refrigerator, vortexed for about 2 min and left on the bench to reach room temperature.

3.4.1.2 FGF19 Measurement by ELISA

To measure FGF19, the RayBio ELISA kit (RayBiotech, Inc. GA, USA) was used. All solutions were prepared according to the manufacturer's manual.

For each ELISA run, a kit was removed from the refrigerator about 1 hour before starting and was left on the bench to reach to room temperature. Wash solution was prepared fresh on the day of each experiment. To prepare wash solution, the 20x wash stock solution was mixed gently to dissolve any crystals, and then 20ml of 20x wash was diluted with 380 ml double distilled water to yield 400ml of 1x wash buffer. Detection antibody was also prepared fresh on each day of experimentation. To prepare detection antibody, the vial was initially vortexed about 1 min. Then, 100 µl of 1x assay diluent was added to the vial. The solution was pipetted up and down several times to ensure its homogeneity. The HRP-Streptavidin vial was diluted 500-fold with assay diluent. To prepare a 500-fold dilution, 20 µl HRP-Streptavidin was added to a tube which contained 10 ml of assay diluent.

3.4.1.3 Standard Preparation and Calibration Curve

The vial containing the ELISA standard was vortexed for about 1 min. Four hundred µl of assay diluent was added to the standard vial to prepare a 50 ng/ml standard. Again, the standard was vortexed until all of the powder was completely dissolved in the vial. Eight tubes with 420 µl assay diluent were prepared by serial dilution. The highest concentration was 8,000 pg/ml, which was also used to produce the serial dilution. To prepare 8,000 pg/ml, 80 µl of a 50 ng/ml solution (as above) was added to 420 µl of assay diluent. 200 µl of each dilution was transferred to subsequent tubes and repeated until the lowest concentration was achieved. The assay procedure was followed according to the manufacturer's manual. The FGF19 concentration was calculated in pg/ml.

3.4.1.4 Method Validation for FGF19

Inter- and intra-assay evaluations were calculated to validate the ELISA method. Plasma level of FGF19 ranged from 31 to 286 pg/ml. The inter-assay percentage of coefficient of variation was 4.5, and inter-assay %CV was 6.5. Eight concentrations were used to generate a standard calibration curve.

3.4.2 Bile Acids Measurement by LC-MS/MS

BAs are present in human blood in tiny quantities but are extremely important. Currently, LC-MS is the most reliable method to measure them. Fasting and postprandial plasma levels of BAs were measured in both surgery groups by LC-MS/MS. The coefficient of determination or R values were calculated in a linear mode. R in all measurements was more than 0.99. Individual BA standard curves are provided in Appendix E.

3.4.2.1 Chemical and Solvent

Chemicals of the highest purity available were purchased for the measuring of human plasma BAs. Methanol and acetonitrile were purchased from VWR (New Zealand, Auckland), and formic acid and ammonium acetate salt were obtained from Sigma Aldrich (Germany). Glycine, taurine, amidated and unconjugated BA standards, including taurohyodeoxycholic acid (THDCA), glycoursodeoxy cholic acid (GUDCA), tauroursodeoxycholic acid (TUDCA), glycocholic acid (GCA), taurochenodeoxy cholic acid (TCDCA), taurodeoxy cholic acid (TDCA), glycodeoxy cholic acid (GDCA), glycochenodeoxy cholic acid (GCDCA), cholic acid (CA), taurolitho cholic acid (TLCA), chenodeoxy cholic acid (CDCA), deoxy cholic acid (DCA) and lithocholic acid (LCA) and deuterocholic acid-d4 (CA-D4) as an internal standard, were supplied by Steraloids.Inc (Newport, RI, USA). Highest Purity water was provided by Millipore water (EMD Millipore Milli-Q, USA). All chemical solvents were filtered by nylon membrane, 0.45um 47mm, non-sterile (MicroAnalytix, USA) before mobile phase used. For BAs separation a 2.1*150*2.7 C18 InfinityLab Poroshell 120 (Agilent Technologies, Inc., CA, USA) was purchased. The list of chemicals and solvents is attached in Appendix D.

3.4.2.2 Method Development

In this study BAs were measured using a well-known method which has already been developed by other researchers (Tagliacozzi et al., 2003), but with a slight modification. The gradient details are provided in Table 3.2.

Table 3.2 HPLC mobile phase gradient composition

Step	Time (min)	Mobile phase A (%)	Mobile phase B (%)	Flow rate (ml/min)	Max. pressure (bar)
0	0	40	60	0.3	550
1	3	40	60	-	-
2	10	5	95	-	-
3	14	5	95	-	-
4	16	40	60	-	-

Mobile phase A, 0.012%formic acid, 5mM ammonium acetate, and water; Mobile phase B, methanol, 0.012% formic acid and ammonium acetate at 5 mM.

3.4.2.3 High Performance Liquid Chromatography Mass Spectrometry

To make mobile phases A and B, 5 molar ammonium acetate was prepared by adding 7.7 g of ammonium acetate salt to 20 ml of water. After that, 1ml of ammonium acetate (5M) was added to 1 liter of water to make mobile phase A. A hundred and twenty microliters of formic acid were then added to mobile phase A. Mobile phase A contained 5 mM and 0.012% ammonium acetate and formic acid, respectively. Mobile phase B consisted from a liter of filtrated LC-MS grade methanol, the same amount of ammonium acetate and formic acid as mobile phase A.

BAs were analysed with an Agilent triple quadrupole mass spectrometer (6420 Triple Quadrupole LC/MS, Agilent Technologies Inc., CA, USA) operated in the ion evaporation mode with an ionspray ionization probe (sprayer voltage of –4500 V). Data were acquired and processed using MassHunter Workstation Qualitative Analysis Software B.06.00 (Agilent Technologies, Inc; CA, USA), for chromatographic and spectral interpretation, and MassHunter Workstation QQQ quantitative Analysis (Agilent Technologies, Inc., CA, USA) for the quantitative processing. Instrument optimisation was performed automatically using the “AutoTune” functionality included in the instrument’s protocol. All experiments were performed in negative ion mode and the orifice voltage set in the range of -61 to -74 V, according to the “AutoTune” option for each single compound. Tandem mass spectrometry

was performed through collisionally activated dissociation (CAD) along with a closed design collision cell operating with 8 mTorr pressure of nitrogen as the collision gas.

All quantitative data were acquired in the format of multiple reaction monitoring mode (MRM). In this setting, each specific precursor produces product ion parameters for each single analyte. With the "AutoTune" option for each BA, collision energy was adjusted with particular optimum values. 20 µM of each individual BA standard (v/v, in 50:50 methanol-water) was infused at 5 µl/min. To carry out chromatographic separation, an Agilent Technologies 1260 Quaternary Pump was used.

A reverse-phase C18 column, 2.1*150*2.7 C18 InfinityLab Poroshell 120 (Agilent Technologies, Inc., CA, USA) was used with the column flow rate initially set at 0.3 ml/min. The single run elution gradient was optimized to the coincident separation of either unconjugated or conjugated BAs. A Two-step gradient was performed to ensure complete separation of the samples, along with LCA, which was the final BA sample collected from the column. Table 3.3 tabulates each individual BA's information.

Table 3.3 Individual BAs details after LC-MS/MS

BAs	$[M-H]^-$ m/z	MRM	CAD (eV)	Retention time (min)	LOD (pg/ml)	LOQ (pg/ml)	R
THDCA	498.3	498.3>79.9	74	3.41	0.001	0.003	0.99
GUDCA	448.3	448.3>448.3	36	4.5	0.002	0.006	0.99
TUDCA	498.3	498.3>498.3	74	2.5	0.001	0.008	0.99
GCA	464.3	464.3>464.3	41	7.5	0.002	0.009	0.99
TCDCA	498.3	498.3>498.3	74	9.56	0.002	0.006	0.99
TDCA	498.3	498.3>79.9	74	5.65	0.002	0.006	0.99
GDCA	448.3	448.3>448.3	36	11.1	0.004	0.015	0.99
GCDCA	448.3	448.3>448.3	36	10.4	0.005	0.009	0.99
CA	407.3	407.3>407.3	54	10.58	0.003	0.006	0.99
TLCA	482.3	482.3>482.3	66	9.51	0.001	0.003	0.99
CDCA	391.3	391.3>391.3	22	11.56	0.006	0.012	0.99
DCA	391.3	391.3>391.3	22	13.1	0.004	0.012	0.99
LCA	375.3	375.3>375.3	28	14.3	0.002	0.006	0.99
CA-D4	411.3	411.3>411.3	54	10.58	0.003	0.005	0.99

THDCA, taurohyodeoxycholic acid; GUDCA,glycoursodeoxycholic acid; TUDCA, tauroursodeoxycholic acid; GCA, glycocholic acid; TCDCA; taurochenodeoxycholic acid; TDCA, taurodeoxycholic acid; GDCA, glycodeoxycholic acid; GCDCA, glycochenodeoxycholic acid; CA, cholic acid; TLCA; taurolithocholic acid; CDCA, chenodeoxycholic acid; DCA, deoxycholic acid; LCA, lithocholic acid; CA-D4, deuterated cholic acid.

3.4.2.4 Standard Preparation and Calibration Curve

A mixed stock solution of 10 g/l of BA standards was prepared in a 50:50 mixture of filtered methanol and water (50% v/v). A working solution of 10 µl of stock solution was transferred into 990 uL of 50% Methanol: water to yield 100 mg/l of standard solution. The working solution was serially diluted in concentrations of 50, 25, 12.5, 6.25, 3.125, 1.5625, 0.78, 0.39, 0.195, and 0.0975 mg/l. An internal standard of CA-D4 was also added into all calibration points using 10 mg/l final concentration. This amount was constant for all standards and samples.

For calibration of BAs, two calibration curves were generated for low and high concentration quantification. The first curve was prepared from duplicate injections of each calibration standard and then plotted in HPLC-MS/MS Mass Hunter Software (Agilent Technologies Inc.). For high concentration samples, six calibration points were utilized; 50, 25, 12.5, 6.25, 3.125, and 1.5625 mg/l. For the lower concentration of BAs, another calibration curve was prepared by using 6 calibration levels ranging from 3.125 to 0.0975 mg/L.

For quantification of BAs, the ratio of the area under the curve between internal standard and individual BA standards was utilised. All calibration curves showed a high correlation of the concentration increments, with R greater than 0.99 (Table 3.3).

3.4.2.5 Sample Preparation and Extraction

All samples were kept at -80°C prior to their measurement. A day before analysing the samples, they were placed in the normal refrigerator to thaw gently. Before starting the extraction, each sample was vortexed for about 1 min to make sure that all material was dissolved in the matrix. For sample extraction, all of the sample's proteins were precipitated. Precipitation was accomplished using 800 µl of acetonitrile added to 250 µl of the human plasma sample. One-minute of vortex-mixing was performed. After a 15 min spin at 13,000 g using an ultra-centrifuge, 900 µl of the clear supernatant layer was transferred to a new Eppendorf tube and blown to complete dryness by using a nitrogen flow evaporator. This particular step was quite time-consuming, and a new machine setup was required for each 20 samples processed. A photo of the machine is attached in appendix F. When all of the liquid inside of each tube was evaporated, 125 µl of both filtered methanol and water (50% V/V) was added to each tube. For quantification, the final dilution factor was equal to 1.17. All samples were centrifuged again at the same settings to ensure clearance of any undissolved materials. For the sample injection, 5 µl of this solution was injected. The sample extraction method was according to the previous method with slight modifications (Tagliacozzi et al., 2003).

3.4.2.6 Method Validation for LC-MS/MS

All chromatographic parameters were performed in this study using standard protocols. In brief, the linearity range was determined by measuring the highest and lowest concentration in a given series until the method could not detect or need extra dilution for the measurements. For the recovery test of the method, the BAs-free plasma method was used:

both low and high concentrations of mixed BAs were spiked into the plasma, followed by a normal extraction protocol. After finishing quantification of the values, the percent recovery was calculated. The mean of recovery was higher than 96%. To check for the precision and accuracy of the method, intra-day and inter-day run values had been established. By repeating 10 QC samples in a roll on the same day, intra-day precision was calculated by standard deviation techniques for obtaining a %CV. For inter-day precision, 10 QC samples were injected daily and then calculated as %CV as well. To check for the accuracy of the test, known values of BA QC samples had been used. For the limit of detection (LOD) and limit of quantification (LOQ), a signal-to-noise ratio of 3 and 10 had been used as standard protocols. Table 3.3 gives more details on BA method validation.

3.5 Ratio Calculations

3.5.1 Glucose and Insulin Indices Calculation

In this study, several glucose indices were calculated using the mathematical calculation from OGTT. The following measurements were performed for each sample:

For IR, Homeostatic model assessment (HOMA)-IR was calculated using the computer-based formula based on HOMA_IR = (fasting blood glucose [mmol/l] * fasting plasma insulin [µU / ml]) / 22.5 (www.dtu.ox.ac.uk).

Beta cell function (BCF) Homeostasis Model Assessment Beta Cell Function:

(HOMA-B) = (fasting plasma insulin [µU / ml] * 20) / (fasting blood glucose [mmol/l] – 3.5) (D. Matthews et al., 1985).

Quantitative Insulin Sensitivity Check Index (QUICKI)= 1/ [log (fasting plasma insulin [µU / ml] +log glucose [mg/dl])] (Katz et al., 2000).

To evaluate QUICKI, 0.382±0.007 for nonobese,0.331±0.010 for obese, and 0.304±0.007 for diabetic individuals were considered (Muniyappa et al., 2008a).

Insulin Sensitivity Index (ISI) or Matsuda index was calculated as below (Matsuda & DeFronzo, 1999):

$$\text{Matsuda index} = \frac{1000}{\sqrt{\text{G0 x I0 x Gmean x Imean}}}$$

Where G0 and I0 are fasting glucose and insulin respectively.

Belfiore-Index= /(((((0.5* fasting blood glucose [mmol/l]) + 60 min mean blood glucose [mmol/l] +(0.5*120 min blood glucose [mmol/l]) *(((0.5* fasting plasma insulin [µU / ml]) + 60min plasma insulin [µU / ml] + (0.5* 120 min plasma insulin [µU / ml])) / 638) + 1). Values above 1.27 indicate pathological IR (Belfiore et al., 1998).

Stumvoll Index= 0.226-(0.0032 * BMI [kg/m²])-(0.0000645 * 120 min plasma insulin [µU / ml]) - (0.00375 *90 min blood glucose [mmol/l]) (Stumvoll & Gerich, 2001).

Insulinogenic index (IGI)= (30 min plasma insulin – fasting plasma insulin) [µU/ml] / (30 min blood glucose – fasting blood glucose) [mmol/l] (Matsuda & DeFronzo, 1999).

The area under the curve (AUC) of glucose, insulin, and BAs were computed using trapezoidal integration at 0,15, 30, 60, 90 and120 min (J. Matthews et al., 1990).

In this study, insulin was initially measured in pmol/l. However, to calculate some of the above-mentioned ratios, it is compulsory to convert pmol/l to µU/ml and vice versa. So, the conversion was done as below:

µU/mL x 6.945 = pmol/l and pmol/l * 0.144 = µU/ml (Halperin et al., 2012).

3.5.2 Bile Acid Fraction Calculations

Different fasting ratios of BAs were calculated before surgeries and a year after both bariatric surgeries. Table 3.4 shows formulae for the calculation of fasting BA ratios. In this study, nine BAs, namely THDCA, GUDCA, TUDCA, GCA, TDCA, GDCA, and GCDCA were conjugated, and four Bas, namely CA, CDCA, DCA, and LCA were unconjugated. Primary BAs included CA and CDCA, and secondary BAs were DCA and LCA. All BA fasting ratios were computed for all the samples before and after a given bariatric surgery**.** Concentrations of individual Bas are presented in pg/ml.

Table 3.4 Computation of Fasting BA fractions

Fasting BA fractions	Calculation formula
Total bile acid	the sum of fasting BAs with conjugated forms
Primary BAs	CA+CDCA+GCA+GCDCA+TCDCA
Secondary BAs	LCA+TLCA+DCA+TDCA+GDCA+GUDCA+TUDCA
Primary/secondary BAs	CA+CDCA+GCA+GCDCA+TCDCA/ LCA+TLCA+DCA+TDCA+GDCA+GUDCA+TUDCA
12α-OH BAs	CA+GCA+TDCA+DCA+GDCA
Non 12α-OH BAs	CDCA+TCDCA+GCDCA+LCA+TLCA+GUDCA+TUDCA
12α-OH BAs/non 12α-OH BAs	CA+GCA+TDCA+DCA+GDCA/CDCA+TCDCA+GCDCA+LCA+TLCA+GUDCA+TUDCA/
Glycine conjugated BAs	GUDCA+GCA+GDCA+GCDCA
Taurine conjugated BAs	THDCA+TUDCA+TDCA
Glycine/taurine BAs	GUDCA+GCA+GDCA+GCDCA/ THDCA+TUDCA+TDCA
Primary conjugated BAs	GCA+GCDCA+TCDCA
Primary unconjugated BAs	CA+CDCA
Primary conjugated/primary unconjugated	GCA+GCDCA+TCDCA/ CA+CDCA
Secondary conjugated BAs	GDCA+TDCA+GUDCA+TUDCA+TLCA
Secondary unconjugated BAs	LCA+DCA
Secondary conjugated/secondary unconjugated	GDCA+TDCA+GUDCA+TUDCA+TLCA/ LCA+DCA

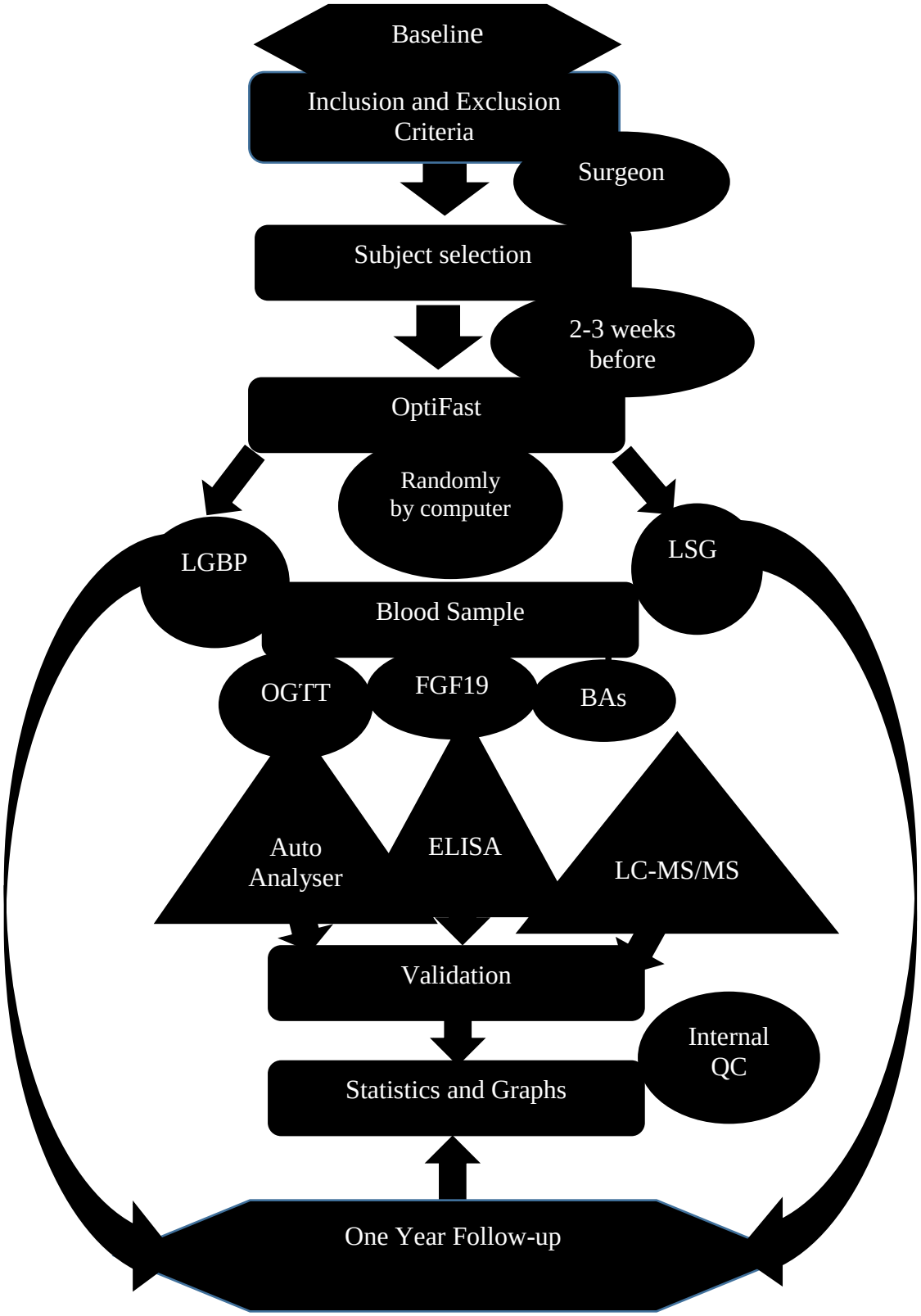
THDCA, taurohyodeoxycholic acid; GUDCA,glycoursodeoxycholic acid; TUDCA, tauroursodeoxycholic acid; GCA, glycocholic acid; TCDCA; taurochenodeoxycholic acid; TDCA, taurodeoxycholic acid; GDCA, glycodeoxycholic acid; GCDCA, glycochenodeoxycholic acid; CA, cholic acid; TLCA; taurolithocholic acid; CDCA, chenodeoxycholic acid; DCA, deoxycholic acid; LCA, lithocholic acid. BA compositions are presented in pg/ml.

3.6 Statistical Analysis

In this study, two software were utilised. Before doing statistical tests, the normality of all data was checked using Shapiro-Wilk, Kolmogorov–Smirnov and D'Agostino's K2 tests (Pearson & Bowman, 1977). For normal data, the results were presented as a mean± standard

error (SE). For those data points that were not normally distributed, logarithmic transformation was applied, and all the results were presented in logarithmic format. If the results were normally distributed after logarithmic transformation (log10), ordinary tests as paired t-tests were performed. However, for those results which were non-normally distributed a non-parametric t-test (Mann–Whitney U test) with median and interquartile values were presented. Also, the Chi-square test was used to compare two categorical variables. Also, unpaired t-test was utilised to the comparison between SG and RYGB. Additionally, the general linear model (GLM) was utilised to test the effect of gender and bariatric surgery on different variables. OGTT data were compared to one year between SG and RYGB using mixed model analysis. Further, a correlation test was examined to see if there was any correlation between different variables after both bariatric surgeries. Correlations of normally distributed data used Pearson's correlation coefficient (r), and for non-parametric results, Spearman's ρ (rho) test applied. Statistical Package for the Social Sciences (SPSS) version 22 was the software used for statistical analysis. GraphPad version 5.04 was used to draw graphs, perform additional statistical analysis including correlations and comparisons of glucose indices and the ratio of conjugated to unconjugated BAs for inter and/or intragroup baseline and follow-up data. Any value with p less than 0.05 was considered as a significant value.

3.7 Study Design Flow Chart



CHAPTER FOUR

RESULTS

4.1 Clinical Characteristics

Table 4.1 tabulates clinical characteristics of all subjects before and a year after a given surgery. Anti-diabetes medications treated subjects either in SG or RYGB at baseline (preoperative). Seventy-five percent and eighty-seven percent of the study population were off-medication12 months after SG and RYGB respectively but not significant between surgery group (p=0.3).

Table 4.1 Clinical characteristics comparison within surgeries

Clinical characteristics	Sleeve Gastrectomy				Gastric Bypass			
	Before (n=29)	One-year after (n=29)	Changes (%)	p-value	Before (n=32)	One-year after (n=32)	Changes (%)	p-value
Medication(s)	Met=14 Met+Pio=1 Met+Ins=5 Met+Ins+Gli=1 Met+Gli=5 Met+Gli+Pio=1 Ins=1 Pio=1	Met=4 Met+Gli=1 Met+Ins=1 Met+Sit=1 Nil=22	-	-	Met=12 Met+Ins=2 Met+Ins+Gli=2 Met+Gli=5 Met+Pio=1 Ins=2 Gli=2 Nil=6	Met=3 Ins=1 Nil=28	-	-
Clinical characteristics								
Gender (M/F)	18/11	18/11	-	-	12/20	12/20	-	-
Age	47±1.1	-	-	-	47±1.2	-	-	-
Weight (kg)	120.±4.1	92±2.8	-21	<0.0001*	116±3.7	84±3.2	-24.2	<0.0001*
BMI (kg/m^2)	40±1.1	31±1.2	-37	<0.0001*	40±1.2	29±1.0	-26.3	<0.0001*
Diabetes characteristics								
Fasting glucose (mmol/l)	6.7±0.45	6.1±0.2	-6.8	0.08	6.7±0.37	6.3±0.18	-2.3	0.07
Fasting insulin (µU/ml)	14±2.1	6.7±0.71	-40	<0.0001*	15±1.6	6.2±0.45	-31	<0.0001*

Glucose AUC_{0-120}	1464±60	1235±82	-9.3	0.02*	1567±64	1332±65	-6.9	0.01*
Insulin AUC_{0-120}	44796±3964	34215±2594	-1.0	0.03*	41352±3748	37388±2008	22.8	0.3
HbA1c (%)	7.9±0.1	6.2±0.2	-20.6	<0.0001*	8.2±0.2	6.2±0.1	-22.5	<0.0001*
Lipid profile								
Total cholesterol (mmol/l)	5.0±0.16	4.6±0.16	-18.7	0.04*	4.7±0.22	4.4±0.16	-3.4	0.2
Triglycerides (mmol/l	1.9±0.14	1.1±0.07	-23.3	<0.0001*	2.1±0.21	1.1±0.1	-31	<0.001*
HDL-c (mmol/l)	1.1±0.04	1.5±0.06	36.6	<0.0001*	1.1±0.04	1.4±0.05	36.7	<0.0001*
LDL-c (mmol/l)	3.1±0.15	2.6±0.14	-42	0.01*	2.7±0.18	2.5±0.16	15.9	0.4

Met, metformin; Pio, pioglitazone; Ins, insulin; Gli, gliclazide/glipizide; Sit; sitagliptin, HDL-c, high density lipoprotein-cholesterol; LDL-c, low density lipoprotein-cholesterol. Data presented in mean±SE.

There is no significant difference between the age of both groups, namely the mean age was 47 for SG and RYGB group. In this study, 29 subjects were recruited for baseline and a year after SG data. About 62% of subjects were males and 38% females. In the other arm of the study (RYGB group), 32 subjects participated for the baseline and a year after RYGB. About 63% of participants in the baseline and follow-up groups were females and 37% males.

In both groups that underwent bariatric surgery (regardless of the type of surgery), weight and BMI reduced significantly. In patients who underwent SG, the weight significantly decreased by 21 percent a year after SG. Also, the group's BMI which was 40 kg/m^2 before surgery showed a significant decreasing trend. A year after SG BMI reduced by 37% ($p<0.0001$). Similarly, this important reduction trend was observed in subjects who underwent RYGB (-24% and -26%, $p<0.0001$, for weight and BMI respectively).

Neither SG nor RYGB showed any statistically significant difference regarding fasting plasma glucose, despite the fact that in both groups the level of glucose was slightly reduced. Plasma levels of insulin in both surgery groups were significantly reduced a year after a given bariatric surgery. Fasting plasma levels of insulin were meaningfully reduced by 40% and 31% in SG and RYGB respectively.

After quantifying the AUC of glucose, it was found that not only the AUC of glucose was significantly higher at baseline in the SG group, but it was also substantially higher among the subject who underwent RYGB. Despite the decreased AUC of insulin after both surgeries, however, there was no statistically significant difference seen in the RYGB or SG groups.

HbA1c was also measured in this study. In both surgery groups, HbA_1C was significantly reduced 12 months after a given bariatric surgery.

The lipid profile in the fasting state was measured. Data from this profile determined that total cholesterol be significantly decreased by about 19% a year after SG. The plasma level of cholesterol also decreased one-year after RYGB, but not to the extent of being statistically significant. In both surgery groups, plasma level of triglycerides significantly reduced while in RYGB more change was seen (-23% vs. -31%, $p<0.0001$, SG vs. RYGB respectively). The effect of either SG or RYGB was almost the same on high-density lipoprotein-cholesterol (HDL-c). All

clinical characteristics in this study were compared to baseline in and between groups. No significant difference was observed (Appendix G).

4.1.1 Obesity Status Comparisons

Table 4.2 shows the switching status of obesity a year after a given bariatric surgery. Among patients who underwent SG, none of them were Grade One obese, while 51% and 49% were either Grade two or three respectively. Interestingly, a year after SG about 22% of those patients were classified in the normal weight range and 24% of them as Grade one obese, while a small proportion of subjects remained classified as Grade three of obese. The biggest percentage of subjects belonged to Grade two (~45%). Similarly, within subjects who were eligible for RYGB, none of them were Grade one. However, a year after RYGB, 22% of them were categorised in the normal weight range, while the biggest percentage still belonged to Grade two obesity. In this group, only one subject was still listed as Grade three obese after surgery.

Table 4.2 Obesity status before and after bariatric surgeries

	Sleeve Gastrectomy		Gastric Bypass	
Obesity status	Before	One-year after	Before	One-year after
	n (%)	n (%)	n (%)	n (%)
Normal weight	-	6 (21.7)	-	7 (21.8)
Grade I	-	7 (24.1)	-	13 (40.7)
Grade II	15 (51)	13 (44.8)	17 (53)	11 (34.4)
Grade III	14 (49)	3 (10.3)	15 (47)	1 (3.1)

4.1.2 Clinical Characteristics between Two Groups

Table 4.3 shows nonsignificant changes between a year after SG and GB.

Table 4.3 Clinical comparison between SG and RYGB

	Sleeve Gastrectomy	Gastric Bypass	
Clinical characteristics	One-year after (n=29)	One-year after (n=32)	p-value
Gender (M/F)	16/13	12/20	0.07
Weight (kg)	92±2.8	84±3.2	0.1
BMI (kg/m^2)	31±1.2	29±1.0	0.1
Fasting glucose (mmol/l)	6.1±0.2	6.3±0.18	0.6
Fasting insulin (µU/ml)	6.7±0.71	6.2±0.45	0.2
Glucose AUC_{0-120}	1235±82	1332±65	0.3
Insulin AUC_{0-120}	34215±2594	37388±2008	0.3
HbA1c	6.2±0.2	6.2±0.1	0.9
Total cholesterol (mmol/l)	4.6±0.16	4.4±0.16	0.7
Triglycerides (mmol/l	1.1±0.07	1.1±0.1	0.6
HDL-c(mmol/l)	1.5±0.06	1.4±0.05	0.9
LDL-c(mmol/l)	2.6±0.14	2.5±0.16	0.7

4.1.3 Change of Glucose and Insulin after OGTT

The mean values of glucose and insulin were analysed regarding different time points after OGTT based on the type of bariatric surgery. Plasma levels of glucose, regardless of the time point, decreased after SG. However, levels were significant only 15, 90 and 120 minutes after drinking glucose. In this group also, the plasma level of glucose peaked at 90 minutes at baseline, and the

lowest amount of glucose was seen 120 minutes after glucose drinking among subjects a year after SG. Similar levels were observed in the RYGB group, where plasma levels of glucose showed a downward trend a year after the surgery. Although plasma levels of glucose were significantly increased 15 and 30 minutes after OGTT, it then sharply reduced at 120 minutes after drinking glucose. The mean difference of glucose after performing OGTT a year after RYGB was significantly different at 15, 30, 90 and 120 minutes from baseline. For the SG group, plasma levels of glucose peaked at 30 minutes after drinking glucose (Figure 4.1).

Plasma levels of insulin were also evaluated according to different time points in both SG and RYGB groups. Figure 4.1 illustrates the highest and lowest levels of insulin after OGTT. At baseline, there was an uptrend of insulin seen, while a year after SG the level of insulin reduced sharply. It was found that there was a significant difference between baseline and a year after SG at fasting time points 15, 30, 90 and 120 minutes. Also, the highest amount of insulin recorded was 30 minutes after drinking, while the lowest was seen at the 120 minute time point, a year after SG. In the RYGB group, as with the SG group, the plasma levels of insulin followed a downward trend. Furthermore, there were significant differences seen at fasting time points of 15, 30, 90 and 120 minutes when baseline numbers were compared with those from a year after RYGB. Of note, in both surgeries, insulin levels peaked at 30 min when determined a year after surgery.

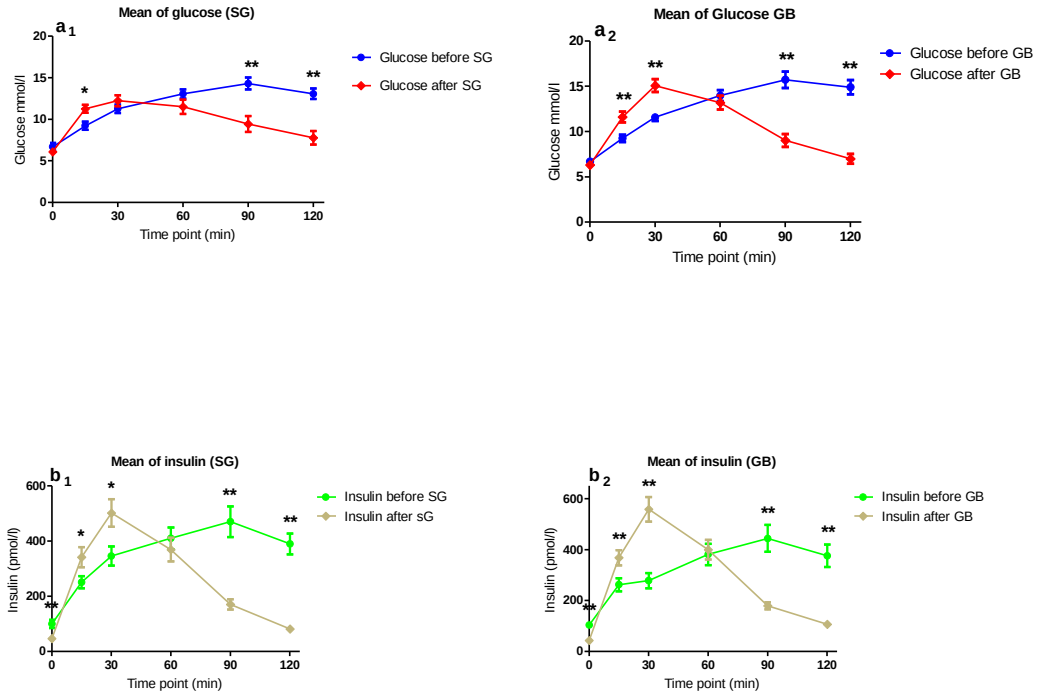


Figure 4.1 Glucose and insulin after OGTT.

The figure shows a comparison of blood glucose (a_1 and a_2) and plasma insulin (b_1 and b_2) level before and a year after SG and RYGB. Different means of glucose and insulin were calculated at fasting and postprandial after OGTT. Data presented in mean ±SE. * $0.05<p<0.01$; ** $p<0.01$(n=29, 32 after SG and RYGB respectively).

To evaluate if any given surgery had a better impact on the plasma levels of insulin and/or glucose, a comparison was performed. According to the levels of glucose, both surgeries had a similar downward trend, and no significant differences were observed except at the 30-minute time point after RYGB. This point was slightly, but significantly, higher than SG. Similarly, regarding insulin levels, both curves followed the same pattern of reduction. There was, however, a significant difference between SG and RYGB insulin data 120 minutes after OGTT. Figure 4.2 illustrates the trends of glucose and insulin between the two different bariatric surgeries.

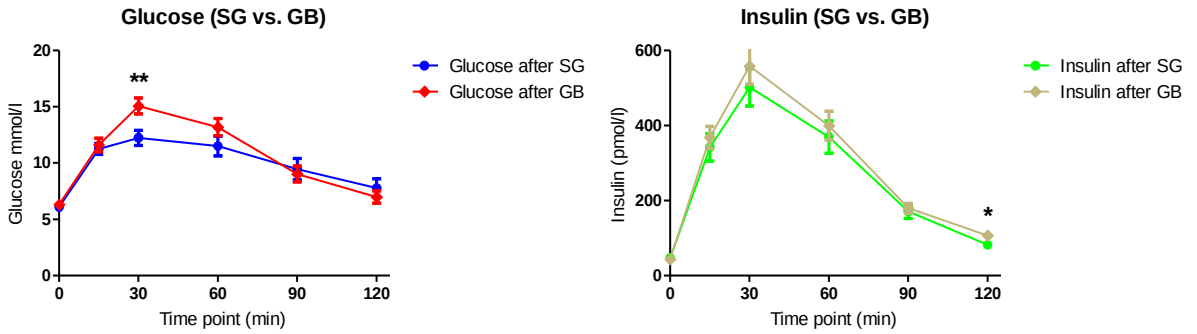


Figure 4.2 Comparison of glucose and insulin a year after SG and RYGB.

Data presented in mean ±SE (n=29, 32 after SG and RYGB respectively). * 0.05<p<0.01; ** p<0.01.

4.2 Bile Acids Measurement

In this study 13 individual BAs (THDCA, GUDCA, TUDCA, GCA, TCDCA, TDCA, GDCA, GCDCA, CA, TLCA, CDCA, DCA and LCA) were measured.

4.2.1 Fasting Individual Bile Acids Changes after Bariatric Surgery

Fasting plasma levels of all 13 BAs were measured before and a year after each type of bariatric surgery.

Nine out of thirteen fasting individual BAs significantly increased a year after SG, except GDCA which was not significantly increased. Three of the BAs (GCDCA, CA and TLCA) had quantities of less than the limit of detection a year after SG and were not considered in statistical analyses to compare before and one-year after SG changes. Table 4.4 shows changes of individual BAs after SG.

Fasting individual BAs were measured before and one-year after RYGB. Similar to SG levels, fasting levels of TDCA reduced a year significantly after RYGB. In this group, the quantity of TLCA was also less than the LOD and was not involved in statistical analyses. Table 4.4 tabulates the differences between baseline and a year after RYGB. Also, all fasting individual BAs from this

study were compared to baseline in and between groups. No significant differences were observed (Appendix G).

Table 4.4 Fasting individual BAs within surgeries

	Sleeve Gastrectomy			Gastric Bypass			Bariatric surgery p-value baseline vs. one-year		One-year p-value SG vs. RYGB	Δ p-value SG vs. RYGB
	Before (n=29)	One-year after (n=29)	Differences (Δ)	Before (n=32)	One-year after (n=32)	Differences (Δ)	SG	RYGB		
Individual BAs[a]										
Primary BAs										
CA	0.01 (0.00-0.02)	<LOD	-	0.01 (0.0-0.02)	0.01 (0.0-00)	-0.02 (0.0-0.0)	-	0.06	-	-
CDCA	0.07 (0.04-0.8)	0.18 (0.00-0.1)	0.1 (-0.5-0.1)	0.07 (0.04-0.1)	0.1 (0.1-0.3)	0.1 (0.0-0.2)	0.01	<0.0001	0.01	0.007
Primary Conjugated BAs										
GCA	0.1 (0.05-0.1)	0.2 (0.1-0.2)	0.1 (0.02-0.1)	0.1 (0.04-0.2)	0.2 (0.1-0.3)	0.09 (-0.01-0.1)	<0.0001	<0.0001	0.03	0.5
GCDCA	0.007 (0.00-0.03)	<LOD	-	0.02 (0.0-0.03)	0.01 (0.0-0.0)	-0.02 (0.0-0.0)	-	<0.0001	-	-
TCDCA	0.9 (0.00-3.0)	2.2 (2.2-5.7)	1.3 (-0.8-4.6)	2.1 (0.9-3.4)	3.9 (2.2-6.4)	1.4 (0.01-3.4)	<0.003	0.01	0.09	0.6
Secondary BAs										
DCA	0.07 (0.04-0.1)	3.3 (3.1-3.4)	3.3 (3.2-3.4)	0.09 (0.04-0.1)	3.4 (3.1-3.4)	3.3 (3.1-3.4)	<0.0001	<0.0001	0.2	0.6
LCA	0.09 (0.04-0.1)	0.5 (0.4-0.5)	0.4 (0.4-0.4)	0.06 (0.04-0.09)	3.1 (3.0-3.3)	3.4 (3.1-3.4)	<0.0001	<0.0001	<0.0001	<0.0001
Secondary conjugated BAs										
GDCA	0.007 (0.00-0.02)	0.01 (0.01-0.02)	0.01 (-0.02-0.0)	0.02 (0.0-0.03)	0.02 (0.01-0.03)	-0.01 (-0.0-0.0)	0.4	0.06	0.05	0.7
TDCA	0.5 (0.4-1.1)	0.03 (0.03-0.2)	-0.5 (-1.4-0.3)	0.5 (0.4-1.1)	0.1 (0.03-0.5)	-0.4 (-1.1-3.4)	<0.0001	<0.0001	0.06	0.08
TLCA	0.001 (0.00-0.00)	<LOD	-	0.001 (0.0-0.0)	<LOD	-	-	-	-	-
THDCA	0.004 (0.00-0.09)	3.1 (2.9-3.2)	3.2 (3.0-3.2)	0.004 (0.00-0.1)	3.1 (3.0-3.3)	3.2 (3.0-3.4)	<0.0001	<0.0001	0.8	0.5
GUDCA	0.01 (0.00-0.1)	2.1 (1.7-2.5)	2.0 (1.6-2.2)	0.01 (0.00-0.2)	1.8 (0.6-2.1)	1.6 (0.5-2.2)	<0.0001	<0.0001	0.06	0.1
TUDCA	0.001 (0.00-0.00)	0.03 (0.01-0.04)	0.03 (0.01-.0.3)	0.001 (0.0-0.0)	0.03 (0.0-0.04)	0.02 (0.03-0.05)	<0.0001	<0.0001	0.01	0.02

[a] Fasting bile acids presented in pg/ml; CA, cholic acid; GCA, glycocholic acid; CDCA, chenodeoxycholic acid; GCDCA, glycochenodeoxycholic acid; TCDCA; taurochenodeoxycholic acid; DCA, deoxycholic acid; GDCA, glycodeoxycholic acid; TDCA, taurodeoxycholic acid; LCA, lithocholic acid; TLCA, taurolithocholic acid; THDCA, taurohyodeoxycholic acid; GUDCA,glycoursodeoxycholic acid; TUDCA, tauroursodeoxycholic acid; LOD, limit of detection; Δ is the difference between baseline and a year after a given surgery. All data present in median (IQR, 25^{th}-75^{th}).

4.2.2 Fasting Individual Bile Acids Changes between SG and RYGB

To compare fasting levels of individual BAs between SG and RYGB, 29 and 32 subjects from SG and RYGB respectively, were involved in the statistical analysis. THDCA, GUDCA, TCDCA, TDCA, and DCA were not statistically comparable between a year after SG and RYGB. One year after the surgeries, there were 5 individual BAs whose levels were meaningfully changed by the type of bariatric surgery. Since fasting level of GCDCA, CA and TLCA were less than the limit of detection either in one surgery group or both, no comparisons of these acids were performed. Table 4.4 shows differences of fasting individual BAs between groups a year after SG and RYGB.

4.3 FGF19 Measurement

In this study fasting and 2hpp levels of FGF19 were measured by using ELISA kit.

4.3.1 Effect of Bariatric Surgery on FGF19

There was a significant difference of fasting and 2hpp levels of FGF19 in response to OGTT seen between baseline data and that taken a year after SG. FGF19 increased by about 50% and 47% ($p<0.0001$) at fasting and 2hpp respectively, one-year after SG. For the people who underwent RYGB, the levels of FGF19 either at fasting or 2hpp were also increased significantly. Table 4.5 shows the differences of FGF19 before and one-year after a given bariatric surgery

Table 4.5 Comparison of FGF19 before and a year after bariatric surgery

	Sleeve Gastrectomy				Gastric Bypass			
	Before (n=29)	One-year after (n=29)	Changes (%)	p-value	Before (n=32)	One-year after (n=32)	Changes (%)	p-value
FGF19 (pg/ml)								
Fasting (min)	62±6.8	142±9.9	51.3	<0.0001*	73.7±7.8	143.2±8.8	41.0	<0.0001*
2hpp (min)	70±8.2	139±5.7	46.5	<0.0001*	98.3±10.8	143.7±8.4	18.6	0.002*

Data presented in mean±SE

4.3.2 FGF19 Comparison between SG and RYGB

To observe if there were any significant changes between a year after SG and RYGB, 29 and 32 subjects of postoperative SG and RYGB respectively were compared. It was found that fasting levels of FGF19 were not statistically significant between SG and RYGB. Similarly, no significant difference was observed 2hpp for FGF19 levels between groups a year after SG and RYGB. Figure 4.5 illustrates the means of postoperative differences between SG and RYGB.

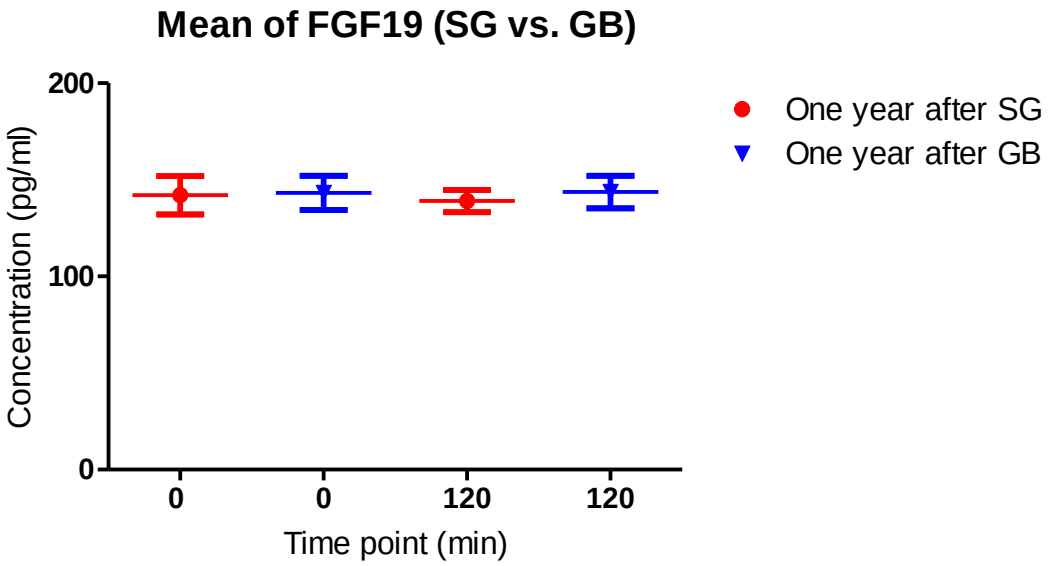


Figure 4.3 Postprandial FGF19 a year after bariatric surgery.

The figure illustrates 2hpp effect glucose ingestion on FGF19 one-year after SG and RYGB. No significant difference observed between SG and RYGB. Data presented in mean± SE (n=29, 32 after SG and RYGB respectively).

4.4 Body Composition Assessment

Different variables of body composition and anthropometry measurements were evaluated before and a year after SG and RYGB.

4.4.1 Body Composition and Bariatric surgery

Twenty-nine subjects were involved in the statistical analysis of body composition before and a year after SG. Resting energy expenditure, despite its small percentage of change, significantly

reduced a year after SG compared to baseline values. Likewise, RQ significantly increased a year after SG.

In BIA examination, TBF significantly decreased while the other variables measured by BIA did not show any statistically significant changes before and one-year after SG. Although BMC, FFM, and BMD slightly reduced a year after SG in comparison with baseline, measurements from DXA did not show any meaningful significance between them. Whereas, for the rest of variables, namely android fat, visceral fat, abdominal fat, leg and arm fat a significant reduction was seen one-year after SG. Anthropometric examinations for neck circumference did not achieve significant changes a year after SG, while circumferences of waist and hip readings, along with its waist-to-hip ratio, decreased comparably a year after SG.

Among subjects who underwent RYGB, REE reduced by 13% while RQ reduced over the course of the year after RYGB, but not significantly. Resistance (R) and reactance (X) did not show any statistically significant changes. However, TBF and LBM meaningfully reduced a year after RYGB by -47% and -8.3 % respectively. Findings from DXA did not show any significant changes of BMC, FFM, and BMD a year after RYGB while the rest of the variables which were measured by DXA were found to be significant. A year after RYGB, waist and hip circumference values reduced significantly by -14.3% and -12.7% respectively, but were not significant when analysed as their ratio (-2.0 ± 2.1, $p > 0.05$). Also, a year after RYGB no significant change was observed for neck circumference readings, despite the -7.5% reduction seen. Table 4.6 shows body composition assessments after a given bariatric surgery. All body composition variables from this research were compared at baseline in and between groups. No significant differences were observed (Appendix G).

Table 4.6 Body composition assessment after bariatric surgery

	Sleeve Gastrectomy				Gastric Bypass			
Variables	Before (n=29)	One-year after (n=29)	Changes (%)	p-value	Before (n=32)	One-year after (n=32)	Changes (%)	p-value
Energy expenditure								
REE (kcal/d)	1905±62.3	1562±40.9	-14.2	0.0002*	1671±77.2	1444±57.7	-13.0	0.03*
RQ	0.72±0.00	0.77±0.01	6.5	0.0004*	1.6±0.9	0.7±0.01	2.9	0.4
BIA								
Resistance (Ω)	426.8±12.1	454.6±16.0	10.0	0.1	447.9±13.5	475.7±16.7	12.2	NS
Reactance (Ω)	41.1±1.16	40.9±1.5	3.5	0.9	42.8±1.9	40.2±1.6	3.6	NS
TBF (kg)	53.6±2.4	31.8±1.9	-38.1	<0.0001*	53.4±2.5	26.2±2.1	-47.0	<0.0001*
LBM (kg)	63.6±2.3	57.8±2.6	-3.8	0.1	59.6±1.9	53.8±2.2	-8.3	0.05*
DXA								
BMC (kg)	3.2±0.09	3.1±0.1	-1.4	0.4	3.2±0.1	3.0±0.1	-4.5	0.1
FFM (kg)	66.9±2.4	61.0±2.7	-3.7	0.1	62.8±2.0	56.8±2.4	-8.2	0.1
BMD (g/cm^2)	1.3±0.01	1.2±0.02	-3.0	0.1	1.3±0.02	1.2±0.03	-4.2	0.1
Android fat (kg)	5.6±0.2	3.1±0.2	-42.0	<0.0001*	5.4±0.2	2.3±0.2	-51.4	<0.0001*
Visceral fat (kg)	2.5±0.1	1.1±0.08	-48.0	<0.0001*	2.6±0.2	0.9±0.09	-51.9	<0.0001*
Abdominal fat (kg)	31.9±1.4	18.0±1.0	-40.7	<0.0001*	32.2±1.5	14.3±1.2	-51.9	<0.0001*
Leg fat (kg)	15.0±0.8	9.4±0.7	-32.3	<0.0001*	14.1±0.9	7.6±0.6	-38.6	<0.0001*
Arm fat (kg)	5.3±0.2	3.3±0.1	-35.3	<0.0001*	5.8±0.3	2.9±0.2	-44.8	<0.0001*
Anthropometry measurement								

Waist circumference (cm)		124±4.7	101±3.8	-16.3	0.001*	119.4±3.3	102.1±4.0	-14.3	0.002*
Hip circumference (cm)		126.7±3.5	112.8±2.8	-10.3	0.001*	127.0±3.5	111.7±4.1	-12.7	0.008*
W/H ratio		0.97±0.02	0.9±0.02	-6.7	0.05*	0.94±0.02	0.91±0.02	-2.0	0.3
Neck circumference (cm)		43.0±1.5	40.6±1.0	-4.2	0.2	41.6±1.0	38.8±1.1	-7.5	0.07

4.4.2 Body composition Comparison between SG and RYGB

Whether or not body composition assessments had any meaningful differences between a year after SG and RYGB, 29 and 32 subjects were compared for SG and RYGB respectively. There were no significant differences in body composition seen a year after SG and RYGB apart from android and abdominal fat differences. Both were significantly higher among the subjects who underwent SG (Table 4.7).

Table 4.7 Body composition comparison between SG and RYGB

Variables	Sleeve Gastrectomy One-year after (n=29)	Gastric Bypass One-year after (n=32)	p-value
Energy expenditure			
REE (kcal/d)	1562±40.9	1444±57.7	0.1
RQ	0.77±0.01	0.7±0.01	0.3
BIA			
Resistance (Ω)	454.6±16.0	475.7±16.7	0.2
Reactance (Ω)	40.9±1.5	40.2±1.6	0.8
TBF (kg)	31.8±1.9	26.2±2.1	0.1
LBM (kg)	57.8±2.6	53.8±2.2	0.1
DXA			
BMC (kg)	3.1±0.1	3.0±0.1	0.4
FFM (kg)	61.0±2.7	56.8±2.4	0.1
BMD (g/cm^2)	1.2±0.02	1.2±0.03	0.6
Android fat (kg)	3.1±0.2	2.3±0.2	0.02*
Visceral fat (kg)	1.1±0.08	0.9±0.09	0.1
Abdominal fat (kg)	18.0±1.0	14.3±1.2	0.03*
Leg fat (kg)	9.4±0.7	7.6±0.6	0.1
Arm fat (kg)	3.3±0.1	2.9±0.2	0.2
Anthropometry measurement			
Waist circumference (cm)	101±3.8	102.1±4.0	0.2
Hip circumference (cm)	112.8±2.8	111.7±4.1	0.9
W/H ratio	0.9±0.02	0.91±0.02	0.4
Neck circumference (cm)	40.6±1.0	38.8±1.1	0.2

4.5 Ratio Calculations

4.5.1 Glucose Ratio's and Bariatric surgery

Different kinds of insulin and glucose metabolism were calculated and analysed before and a year after both bariatric surgeries.

HOMA-IR was significantly reduced by -42.5% ($p<0.002$) a year after SG. At the same time, ISI or Matsuda index, QUICKI, Stumvoll index and IGI all meaningfully went up a year after SG and HOMA-B were significantly reduced a year after SG. There was no significant change of the Belfiore index seen a year after SG. In the year after SG, IGI showed the highest percentage of change (174±68).

Within the group of patients who underwent RYGB, HOMA-IR and HOMA-B were significantly reduced a year after RYGB. However, Matsuda index, QUICKI, Stumvoll index and IGI were meaningfully increased one-year after RYGB. In this group, the highest percentage of change also belonged to IGI and was observed a year after RYGB. There was no significant change of the Belfiore index observed one-year after RYGB. Table 4.8 shows changes in insulin and glucose indices a year after a given surgery. All diabetes indices in this study were compared to baseline in and between groups. No significant differences were observed (Appendix G).

Table 4.8 Glucose indices after bariatric surgery

	Sleeve Gastrectomy				Gastric Bypass			
Variables	Before (n=29)	One-year after (n=29)	Changes (%)	p-value	Before (n=32)	One-year after (n=32)	Changes (%)	p-value
HOMA-IR	7.0±1.4	2.0±0.2	-42.5	0.002*	4.6±0.5	1.8±0.1	-28.9	<0.0001*
ISI (Matsuda index)	2.7±0.2	5.7±0.6	169	<0.0001*	2.5±0.3	4.3±0.4	122	<0.0001*
QUICKI	0.51±0.01	0.65±0.02	30.8	<0.0001*	0.54±0.01	0.66±0.01	25.9	<0.0001*
Belfiore index	0.13±0.00	0.15±0.00	13.7	0.8	0.13±0.00	0.13±0.00	3.0	0.9
Stumvoll index ($\mu mol\ min^{-1}kg^{-1}$)	0.09±0.00	0.05±0.00	63	<0.0001*	0.03±0.00	0.11±0.00	155	<0.0001*
IGI	0.54±0.08	0.84±0.1	174	0.04*	0.23±0.07	0.49±0.09	211	0.001*
HOMA-B	2.0±0.05[a]	1.7±0.08[a]	-23.7	0.0002*	1.9±0.06[a]	1.7±0.03[a]	-13.8	0.0007

ISI, insulin sensitivity index; QUICKI, quantitative insulin sensitivity check index; IGI, insulinogenic index; [a] data is transformed (log^{10}). Data presented in mean±SE.

4.5.2 Glucose Ratio's Comparison between SG and RYGB

Twenty-nine and thirty-two subjects were compared a year after SG and RYGB respectively. There was no significant difference between a year after SG and RYGB surgeries, except that observed for the Belfiore and Stumvoll indices. Table 4.91 tabulates the one-year's comparison between SG and RYGB.

Table 4.9 Glucose indices comparison between SG and RYGB

	Sleeve Gastrectomy	Gastric Bypass	
Variables	One-year after (n=29)	One-year after (n=32)	p-value
HOMA-IR	2.0±0.2	1.8±0.1	0.4
ISI (Matsuda index)	4.6±0.6	4.3±0.4	0.3
QUICKI	0.65±0.02	0.66±0.01	0.8
Belfiore index	0.15±0.00	0.13±0.00	0.04*
Stumvoll index (μmol $min^{-1}kg^{-1}$)	0.05±0.00	0.11±0.00	<0.0001*
IGI	0.84±0.1	0.62±0.09	0.1
HOMA-B	61.5±6.2	49.7±3.9	0.1

ISI, insulin sensitivity index; QUICKI, quantitative insulin sensitivity check index; IGI, insulinogenic index; Data presented in median (IQR, 25^{th}-75^{th}).

4.5.3 Fasting Bile Acid Fractions and Bariatric Surgery

Different ratios of fasting BAs were calculated according to their classification. All the ratios were significantly altered a year after SG. In the one-year after SG group, primary/secondary, 12α-OH BAs/non 12α-OH BAs, and conjugated/unconjugated BAs were meaningfully reduced. The rest of the BA fractions were significantly lower before SG compared to the levels taken a year after.

The ratio of fasting FGF19 to fasting total BA was comparably lower in a year after SG compared to baseline (9.8±0.9 vs. 21.2±3.5, $p<0.0001$ respectively). In a year after RYGB group, all different ratios significantly changed except the glycine to taurine ratio, which slightly but not significantly changed, a year after RYGB. One-year after RYGB, primary/secondary BAs, 12α-OH BAs/non 12α-OH BAs and conjugated/unconjugated BAs, similar to values observed with SG, were significantly reduced a year after RYGB. However, the rest of the computed BA fractions meaningfully increased a year after RYGB. Table 4.10 shows the differences before and a year after each bariatric surgery. The ratio of fasting FGF19 to total BA meaningfully reduced a year after RYGB in comparison with baseline values (30.9±4.8 vs. 5.6±0.7, $p<0.0001$ respectively).

Table 4.10 Different fasting ratio of BAs before and after bariatric surgery

	Before (n=29)	One-year after (n=29)	Differences (Δ)	Before (n=32)	One-year after (n=32)	Differences (Δ)	p-value baseline vs. one-year		p-value SG vs. RYGB	p-value SG vs. RYGB
BA fractions										
Total BAs	2.8 (1.8-7.3)	12.0 (12.0-12.9)	9.3 (4.7-12)	3.2 (1.7-5.7)	16.0 (15-86)	13.0 (12-83)	<0.0001	<0.0001	<0.0001	0.0002
Primary BAs	1.1 (1.1-5.6)	2.6 (2.6-6.0)	1.5 (-1.3-2.4)	2.3 (1.1-4.1)	5.0 (3-57)	2.9 (0.7-54)	0.002	<0.0001	0.09	0.03
Secondary BAs	1.2 (0.6-1.4)	6.2 (6.0-6.5)	5.1 (2.3-7.5)	1.0 (0.6-1.4)	9.0 (9-28)	8.6 (7.9-27)	<0.0001	<0.0001	0.04	<0.0001
Primary/Secondary BAs	1.8 (1.2-2.2)	0.4 (0.4-2.2)	-1.2 (-1.7-0.3)	1.9 (1.6-2.9)	0.4 (0.3-2)	-1.1 (-1.7-0.08)	0.004	0.006	0.1	0.6
Primary conjugated BAs	1.09 (1.0-3.4)	2.4 (2.4-5.9)	1.4 (-0.3-3.5)	2.2 (1.0-3.7)	2 (2-7)	1.4 (0.1-3.5)	0.006	0.02	0.7	0.5
Primary unconjugated BAs	0.08 (0.06-0.8)	0.1 (0.05-0.2)	0.1 (-0.2-0.1)	0.09 (0.06-0.2)	0.2 (0.2-51)	0.1 (0.09-51)	0.01	<0.0001	0.01	0.01
Secondary conjugated BAs	0.9 (0.4-1.2)	2.2 (1.8-2.4)	1.2 (-0.3-1.7)	0.8 (0.4-1.2)	2.0 (2-21)	1.8 (1.1-21)	<0.001	<0.0001	0.02	0.01
Secondary unconjugated BAs	0.15 (0.1-0.2)	4.0 (3.7-4.0)	3.8 (3.6-3.9)	0.1(0.09-0.2)	7.0 (6-7)	6.7 (6.3-6.8)	<0.0001	<0.0001	<0.0001	<0.0001
Glycine BAs	0.2 (0.1-0.4)	2.3 (1.9-2.3)	1.9 (1.3-2.3)	0.2 (0.1-0.4)	2.0 (0.9-2.0)	1.5 (0.5-2.2)	0.002	<0.0001	0.06	0.07
Taurine BAs	2.4 (1.4-5.4)	5.5 (5.4-9.5)	3.9 (0.6-6.1)	2.6 (1.4-5.1)	9 (5-27)	5.9 (3.0-23.0)	<0.0001	<0.0001	0.09	0.04
Glycine/Taurine BAs	0.08 (0.07-0.1)	0.4 (0.08-0.4)	0.3 (0.0-0.3)	0.08 (0.07-0.1)	0.09 (0.03-0.4)	0.01 (-0.07-0.2)	0.001	0.7	0.08	0.01
Conjugated BAs	2.5 (1.5-5.9)	7.9 (7.8-10.5)	6.1 (2.5-7.3)	3.0 (1.5-5.3)	10 (8-28)	6.9 (5.1-24)	<0.0001	<0.0001	0.4	0.09
Unconjugated BAs	0.2 (0.1-1.0)	4.1 (3.8-4.3)	4.0 (1.5-6.9)	0.2 (0.1-0.5)	7.0 (5-58)	7.0 (6.8-58)	<0.0001	<0.0001	0.03	<0.0001
Con/Unconj BAs	8.2 (3.7-12.7)	1.8 (1.8-2.0)	-5.4 (-11-0.0)	8.8 (5.8-25.5)	1.0 (0.5-1.0)	-8.7 (-27.0-6.6)	<0.0001	<0.0001	0.05	0.01
12α-OH	0.9 (0.6-1.7)	3.7 (3.3-3.9)	2.6 (0.5-3.2)	0.9 (0.6-1.4)	3.6 (3.8-4.0)	3.0 (2.3-3.2)	<0.0001	<0.0001	0.1	0.1
Non 12α-OH	1.2 (1.07-5.6)	5.1 (5.1-6.5)	4.0 (1.1-6.0)	2.5 (1.0-4.0)	9.0 (8-79)	7.3 (6.3-76)	<0.0001	<0.0001	<0.0001	0.0002
12α-OH/ Non 12α-OH	1.0 (1.0-1.0)	0.7 (0.1-0.7)	-0.2 (-0.8-0.1)	0.5 (0.3-0.6)	0.4 (0.05-0.5)	-0.2 (-0.5-0.02)	<0.0001	0.01	0.01	0.01
FGF19/Total BA	14.7 (7.2-26.4)	9.3 (7.2-12.9)	-2.8 (-16-2.1)	24.7 (6.6-51.9)	5.9 (1.6-14.5)	-20 (-49-2.5)	0.002	<0.0001	<0.0001	0.04

All data measured by nonparametric test; data presented in mean± SE.

4.5.4 Fasting Bile Acid Fractions between SG and RYGB

Fasting fractions of different BA classifications were compared a year after SG and RYGB. Total BAs, secondary BAs, non 12α-OH BAs, unconjugated BAs, primary unconjugated BAs, secondary conjugated and unconjugated BAs, all significantly increased a year after RYGB compared with one-year after SG. While the ratio of 12α-OH BAs/ non 12α-OH and conjugated/unconjugated BAs were significantly higher one year after SG in comparison with a year after RYGB. There were no statistically significant changes observed for the rest of the ratios between one year after SG and RYGB surgeries. According to the ratio of fasting FGF19/fasting total BAs, the ratio reduced a year after RYGB significantly. Table 4.10 shows different categories of computed BA fractions a year after SG and RYGB.

4.5.5 AUCs of BA fraction in SG and RYGB

Due to the several missing time point from 90 min to 120 min, all AUCs calculated over 60-minute time. All $AUCs_{0\text{-}60min}$ of BA fractions significantly increased a year after both SG and RYGB (all $p<0.01$) except $AUC_{0\text{-}60min}$ of glycine which did not significantly increase after RYGB ($p=0.1$) (Figure 4.4).

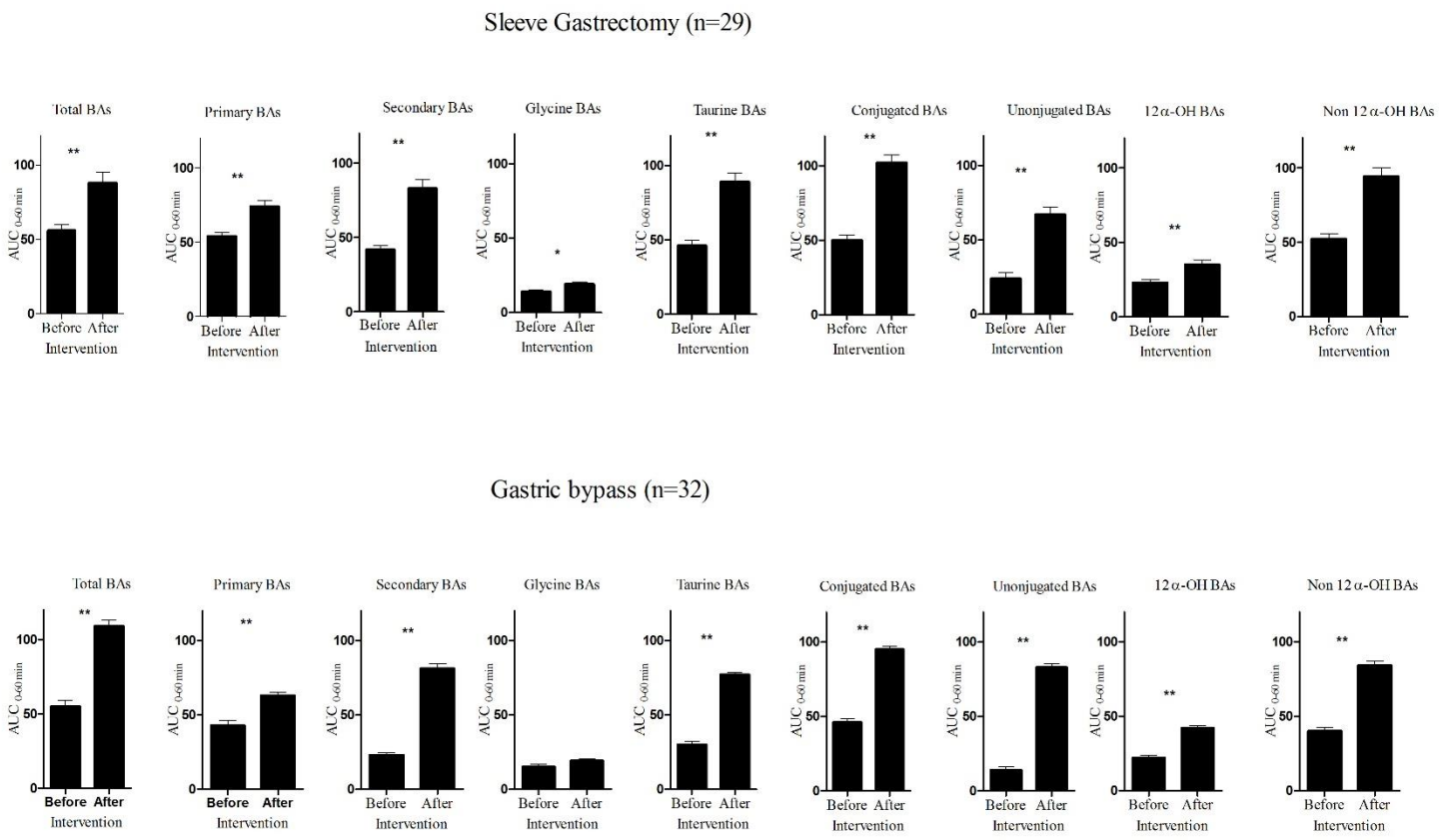


Figure 4.4 AUCs of BA fractions before and one year after bariatric surgeries

Different $AUC_{0\text{-}60min}$ BA fractions in a given bariatric surgery compared using pairwise t-test between pre-and post-surgery. As shown in the figure, all AUCs of BA fractions increased a year significantly after SG and RYGB except AUC of glycine which was not significantly increased after gastric bypass. All data are presented as mean ±SE (n=29, 32 for SG and RYGB respectively). * $p<0.05$, ** $p<0.01$.

4.5.6 Changes in Bile Acid Fractions and AUCs after OGTT

The changes in fasting BAs 1 year after SG and RYGB (relative to baseline values) are shown in table 4.10. Total BAs (both primary and secondary) increased after both types of surgery, but to a greater extent after RYGB ($p<0.0001$). Both secondary unconjugated BAs (DCA and LCA) increased after both types of surgery, but LCA increased significantly more after RYGB ($p<0.001$). Increases in secondary conjugated BAs (greatest increases in THDCA>GUDCA>TUDCA) occurred to a similar extent after both types of surgery. There was a greater increase in taurine BAs than glycine BAs after both types of surgery, but the increase in taurine BAs was higher after RYGB ($p=0.04$). There was a greater increase in non-12alpha-hydroxylated species than 12 alpha-hydroxylated species after both types of surgery, but the increase in non-12alpha-hydroxylated species was greater after RYGB ($p=0.0002$).

The changes of post-glucose load BA profiles over 60 min, relative to baseline BA profiles, with their corresponding change in AUCs at 1 year are shown in Figure 4.5. The increases in post-OGTT $AUC_{0\text{-}60min}$ for total BAs, secondary BAs, and unconjugated BAs at 1 year were greater after RYGB compared to SG (Figure 4.5).

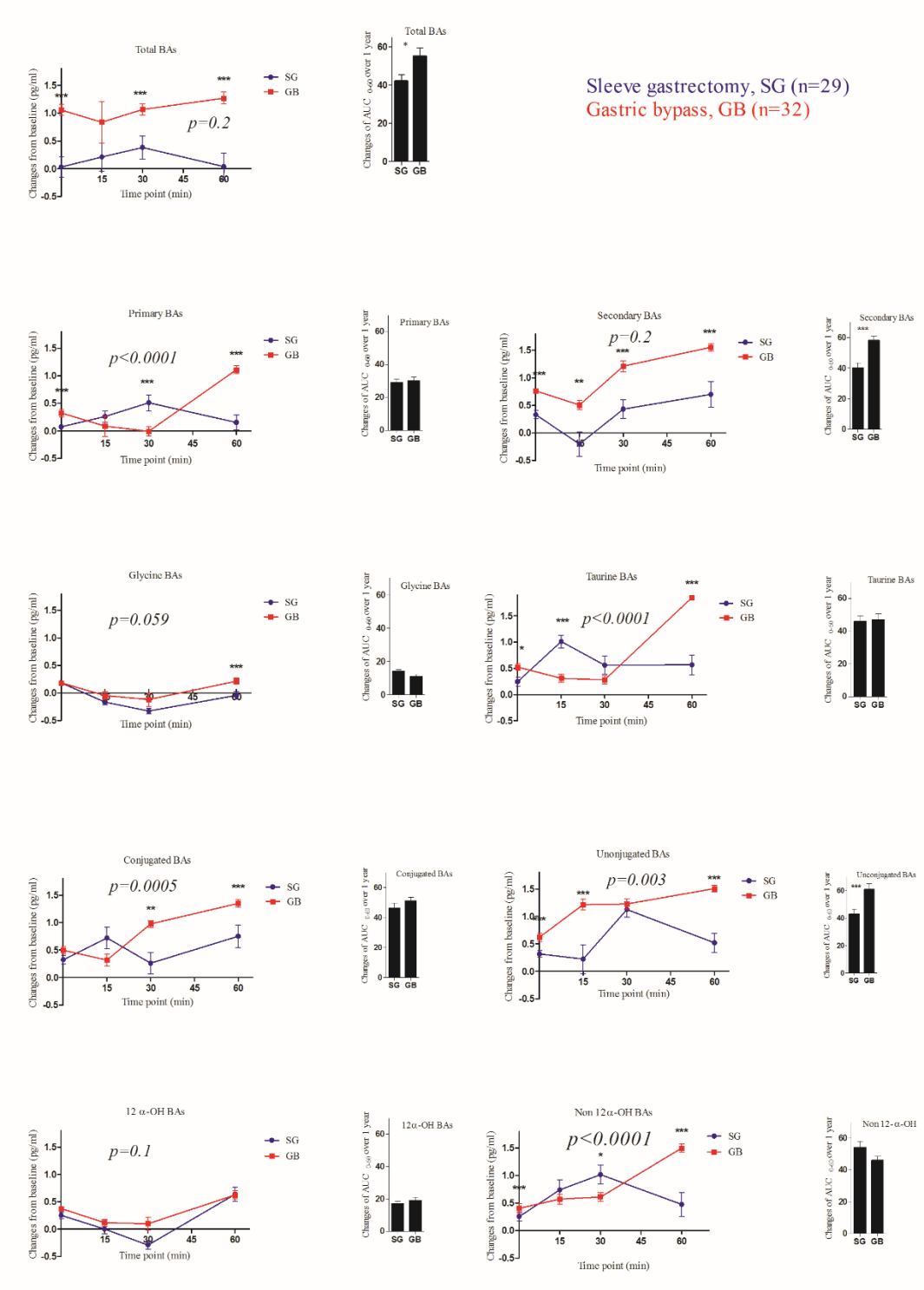


Figure 4.5 Changes in Fasting and postprandial BA fractions with their AUC changes.

Changes in BAs fractions (log-transformed data) over one year in patients who underwent SG or RYGB. Data are mean ±SE (SG=29, RYGB=32). P values show the group*time interaction effect. * p<0.05, ** p<0.01, ***p<0.0001 between-surgery comparisons at each time point.

4.6 Association Findings

There were weak but significant associations between BAs and a variety of variables in this study which are described below.

4.6.1 Associations between Clinical Characteristics and AUC of BAs

The correlations between different clinical features and AUC of individual BAs were assessed a year after bariatric surgery. Table 4.11 shows only those variables that were associated with GUDCA, TUDCA, GCA, GDCA, GCDCA, CA and DCA. The rest of the clinical characteristics or AUCs of individual BAs were not significantly correlated together (Appendix G). One-year after bariatric surgery, there were significant and negative correlations between BMI and GCA, GDCA and GCDCA. The AUC of GUDCA increased significantly relative to increased levels of fasting insulin while there was a negative association between CA and fasting insulin. Another positive correlation was observed between the AUC of TUDCA and the AUC of glucose, while the AUC of CA was the only individual BA that was associated negatively but significantly with the AUC of insulin. There was a significant correlation seen between HOMA-IR and the AUCs of GUDCA and CA. Also, a decreased AUC of CA was significantly associated with IGI. The Belfiore index was inversely associated with TUDCA, GCA, GDCA, and DCA.

Table 4.11 Correlation of clinical characteristics with fasting FGF19 and AUC of individual BAs

	GUDCA		TUDCA		GCA		GDCA		GCDCA		CA		DCA		FGF19	
Variables	r	p	r	p	r	p	r	p	r	p	r	p	r	p	r	p
BMI (kg/m^2)	-0.0	NS	-0.0	NS	-0.09	0.3*	-0.2	0.03*	-0.2	0.04*	-0.0	NS	-0.0	NS	-0.1	NS
Weight (kg)	0.0	NS	0.0	NS	0.0	NS	0.0	NS	0.0	NS	0.0	NS	0.0	NS	-0.3	0.01*
Fasting insulin (μU/ml)	0.2	0.04*	-0.0	NS	-0.0	NS	0.0	NS	-0.0	NS	-0.2	0.04*	-0.0	NS	0.0	NS
Glucose AUC $_{(0-120)}$	-0.0	NS	0.2	0.04*	-0.0	NS	0.0	NS	-0.0	NS	0.0	NS	0.0	NS	-0.0	NS
Insulin AUC $_{(0-120)}$	-0.0	NS		NS	-0.0	NS	0.0	NS	-0.0	NS	-0.3	0.01*	-0.0	NS	0.0	NS
HOMA-IR	0.2	0.04*	0.0	NS	0.0	NS	0.0	NS	0.0	NS	-0.2	0.04*	0.0	NS	-0.1	NS
IGI	-0.0	NS	-0.0	NS	0.0	NS	0.0	NS	0.0	NS	-0.2	0.02*	-0.0	NS	0.2	0.04*
Belfiore index	0.0	NS	-0.2	0.02*	-0.2	0.02*	-0.2	0.04*	-0.2	0.04*	-0.0	NS	-0.2	0.02	0.2	0.04*

IGI, insulinogenic index.

Table 4.12 Associations of body composition with fasting FGF19 and AUC of individual BAs

	THDCA		GDCA		GCDCA		CDCA		TUDCA		CA		LCA		FGF19	
Variables	r	p	r	p	r	p	r	p	r	p	r	p	r	p	r	p
REE (kcal/d)	0.3	0.01*	0.0	NS	0.0	NS	0.0	NS	NS	0.0	NS	0.0	NS	0.0	0.0	NS
RQ	NS	0.0	-0.3	0.02*	-0.3	0.01	NS	0.0	NS	0.0	NS	0.0	NS	0.0	-0.2	0.04*
Resistance (Ω)	NS	0.0	NS	0.0	NS	0.0	0.2	0.04*	NS	0.0	NS	0.0	NS	0.0	0.0	NS
Visceral fat (kg)	0.0	NS	0.0	NS	0.0	NS	-0.3	0.01*	-0.3	0.03*	0.0	NS	0.0	NS	-0.0	NS
Hip circumference (cm)	0.0	NS	0.0	NS	0.0	NS	0.4	0.01*	0.0	NS	0.0	NS	0.4	0.01*	-0.0	NS

4.6.2 Associations between Body Composition and AUC of BAs

Table 4.12 tabulates the correlation between body composition variables and different AUCs of individual BAs. There was no significant association between reactance, TBF, LBM, BMC, FFM, BMD, android, abdominal, leg and arm fat and AUCs of individual BAs (data not shown in the table, appendix G). However, increased AUC of THDCA was significantly correlated with REE. Also, a positive and significant association was seen between CDCA and resistance and hip circumference. Hip circumference was also significantly correlated with the AUC of LCA.

4.6.3 Association between Body Composition and Fasting BA Fractions

A Spearman's correlation (rho) was performed to assess associations between body composition and fasting BA fractions. Total, secondary, non 12α-OH, 12α-OH/non 12α-OH ratio, unconjugated, conjugated, conjugated/unconjugated ratio, secondary conjugated, and secondary unconjugated BAs were significantly correlated with some but not all body composition variables. Table 4.13 indicates those significant correlations. The rest of the body composition variables and fasting BA fractions that are not in the table did not show any statistical significant.

There were direct and significant associations between total, secondary, non 12α-OH, and unconjugated BAs with resistance. Moreover, the relationship between lean fat, BMC, BMD and FFM was negatively associated with total, secondary and non 12-α OH BAs.

Table 4.13 Correlations of body composition and physical measurement with fasting BA fractions

	TBA		Sec BAs		N12α-OH BAs		12α-OH/N12α-OH BAs		Unco BAs		Con/Unco BAs		Con BAs		Sec Con BAs		Sec BAs	Unco
Variables	rho	p	rho	p	rho	p	rho	p	rho	p	rho	p	rho	p	rho	p	rho	p
Resistance (Ω)	0.3	0.008*	0.2	0.03*	0.3	0.02*	-0.2	0.04	0.2	0.03*	-0.2	0.03*	0.1	NS	0.1	NS	0.1	NS
Lean fat (kg)	-0.3	0.005*	-0.3	0.08*	-0.3	0.01*	0.2	0.03*	-0.3	0.01*	0.3	0.02*	-0.1	NS	-0.2	0.04*	-0.1	NS
BMC(kg)	-0.2	0.03*	-0.3	0.02*	-0.3	0.02*	0.2	NS	-0.2	0.04*	0.2	NS	-0.1	NS	-0.2	NS	-0.1	NS
BMD(kg)	-0.2	0.03*	-0.2	0.03*	-0.3	0.02*	0.2	NS	-0.2	NS	0.2	NS	-0.0	NS	-0.1	NS	-0.2	0.05*
FFM(kg)	-0.3	0.006*	-0.3	0.008*	-0.3	0.01*	0.2	0.03*	-0.3	0.01*	0.3	0.02*	-0.1	NS	-0.2	0.04*	-0.1	NS
Neck circumference(cm)	0.1	NS	0.1	NS	0.3	0.01*	-0.4	0.008*	0.1	NS	-0.1	NS	0.1	NS	0.0	NS	-0.3	NS

BAs, bile acids; TBA, total bile acid; Sec, secondary; N12α-OH, non12α-OH; Unco, unconjugated; Con/Unco, conjugated/unconjugated; Con, conjugated, Sec Con, secondary conjugated;

4.6.4 Associations between Changes in Metabolic Characteristics and Changes in Fasting and Prandial BA Groups

Changes in BMI or HOMA-IR were not associated with variations in any BA groups either in fasting or prandial states. Only changes in HbA1c showed consistent correlations with changes in several BA groups in both fasting and prandial states. HbA1c was negatively associated with fasting and $AUC_{0\text{-}60min}$ of total BA, secondary BA and unconjugated BAs (table 4.14). HbA1c was also negatively associated with changes in fasting primary BAs and positively correlated with changes in fasting ratio of 12α-OH/non 12α-OH BAs.

Other clinical characteristics showed several correlations only with fasting BA groups: Changes in glucose AUC was positively associated with changes in 12α-OH/non 12α-OH while changes in IGI were negatively associated with changes in 12α-OH/non 12α-OH. Changes in visceral fat was negatively associated with changes in fasting total BAs and primary BAs. Changes in abdominal fat were positively associated with changes in fasting glycine BAs.

Other clinical characteristics showed correlations only with prandial BAs: Changes in glucose $AUC_{0\text{-}120min}$ were negatively associated with changes in $AUC_{0\text{-}60min}$ of total BAs, secondary BAs, and unconjugated BAs. Changes in triglycerides and HDL were negatively associated with changes in $AUC_{0\text{-}60min}$ non 12α-OH. Changes in REE were positively associated with changes in $AUC_{0\text{-}60min}$ taurine BAs.

There were no significant associations seen between changes in fasting FGF19 with changes in clinical characteristics (Table 4.14). However, changes in prandial $AUC_{0\&120min}$ of FGF19 were negatively associated with HbA1c and visceral fat.

Table 4.14 Correlations of Changes in fasting and $AUC_{0\text{-}60min}$ of BA fractions and $AUC_{0\&120min}$ of FGF19 with clinical outcomes

	AUC glucose $_{0\text{-}120min}$		HbA1c		HOMA-IR		IGI		Triglycerides		HDL		BMI		Visceral fat		Abdominal fat		REE	
	r	p	r	p	r	p	r	p	r	p	r	p	r	p	r	p	r	p	r	p
Fasting Total BAs	0.09	0.4	-0.3	0.01*	0.1	0.3	0.2	0.1	-0.0	0.8	-0.1	0.3	0.2	0.07	-0.3	0.03	-0.0	0.5	0.1	0.3
Prandial Total BAs	-0.3	0.02*	-0.2	0.04*	0.0	0.9	0.2	0.09	-0.0	0.7	-0.08	0.5	0.0	0.6	0.0	0.8	0.1	0.3	0.1	0.5
Fasting Primary BAs	0.1	0.4	-0.2	0.03*	0.2	0.06	0.2	0.1	-0.0	0.8	-0.0	0.9	-0.0	0.7	-0.3	0.01*	-0.0	0.5	0.1	0.4
Prandial Primary BAs	-0.0	0.7	-0.0	0.6	-0.0	0.8	-0.1	0.4	0..2	0.1	-0.2	0.07	-0.0	0.7	0.1	0.2	-0.0	0.8	0.0	0.8
Fasting Secondary BAs	0.07	0.5	-0.3	0.01*	0.1	0.3	0.1	0.1	0.0	0.8	0.03	0.8	0.01	0.9	-0.1	0.3	-0.1	0.2	0.2	0.1
Prandial Secondary BAs	-0.5	<0.01*	-0.4	0.01*	-0.0	0.6	0.2	0.04*	0.0	0.8	-0.2	0.08	-0.0	0.6	0.1	0.4	0.1	0.2	0.0	0.8
Fasting glycine BAs	-0.2	0.04*	0.2	0.07	0.2	0.1	0.0	0.6	-0.0	0.7	0.0	0.9	0.0	0.6	0.1	0.4	0.4	0.01*	0.07	0.6
Prandial glycine BAs	0.2	0.04*	0.2	0.07	0.0	0.6	-0.2	0.1	0.1	0.2	0.1	0.2	-0.0	0.7	-0.1	0.3	-0.1	0.3	0.0	0.6
Fasting 12α-OH BAs	0.0	0.6	0.1	0.2	0.2	0.1	-0.0	0.9	0.1	0.2	-0.0	0.5	0.0	0.5	0.0	0.9	-0.0	0.8	-0.0	0.9
Prandial 12α-OH BAs	-0.1	0.2	-0.1	0.2	0.0	0.5	0.2	0.1	-0.0	0.8	-0.0	0.6	0.0	0.9	-0.1	0.3	-0.1	0.4	0.1	0.3
Fasting Non 12α-OH BAs	0.0	0.5	-0.1	0.1	0.0	0.6	-0.0	0.8	0.1	0.2	-0.1	0.4	-0.0	0.5	0.0	0.9	0.0	0.9	0.0	0.7
Prandial Non 12α-OH BAs	-0.1	0.3	0.1	0.1	-0.1	0.3	-0.0	0.5	0.0	0.5	-0.2	0.03*	0.0	0.7	0.1	0.1	-0.0	0.9	-0.0	0.9
Fasting 12 α-OH/Non-12α-OH BAs	0.2	0.03*	0.3	0.01*	-0.1	0.6	-0.3	0.01*	-0.0	0.9	0.0	0.7	0.0	0.6	0.0	0.7	0.1	0.4	-0.09	0.5
Prandial 12α-OH / Non 12α-OH BAs	-0.0	0.8	-0.1	0.3	0.0	0.6	0.2	0.06	-0.2	0.05*	0.1	0.2	0.0	0.9	-0.1	0.3	-0.0	0.9	0.0	0.9
Fasting conjugated BAs	0.0	0.7	-0.2	0.08	0.0	0.7	-0.0	0.7	-0.1	0.1	0.0	0.9	-0.0	0.7	0.0	0.8	-0.0	0.5	0.0	0.9
Prandial Conjugated BAs	-0.2	0.1	-0.1	0.3	0.0	0.5	0.2	0.1	0.1	0.4	-0.0	0.8	0.0	0.7	0.0	0.7	0.0	0.9	0.2	0.1
Fasting unconjugated BAs	0.09	0.4	-0.3	0.01*	0.1	0.1	0.1	0.1	0.1	0.2	0.0	0.5	0.0	0.9	-0.3	0.06	-0.1	0.3	0.2	0.1
Prandial unconjugated BAs	-0.3	0.01*	-0.4	<0.01*	0.0	0.7	0.1	0.7	0.1	0.3	-0.1	0.4	0.0	0.8	0.0	0.5	0.0	0.8	-0.0	0.5
Fasting taurine BAs	-0.2	0.06	-0.1	0.1	-0.0	0.5	0.0	0.7	-0.0	0.6	0.0	0.7	0.0	0.5	-0.1	0.2	0.0	0.7	0.0	0.9
Prandial taurine BAs	-0.1	0.2	-0.0	0.6	-0.0	0.4	0.1	0.4	0.0	0.4	-0.2	0.1	0.2	0.09	-0.0	0.6	0.0	0.8	0.3	0.03*
Fasting FGF19	-0.1	0.09	-0.2	0.1	0.0	0.5	0.2	0.09	0.0	0.9	-0.2	0.1	0.0	0.7	-0.0	0.5	-0.0	0.9	0.2	0.1
Prandial FGF19	-0.2	0.06	-0.4	<0.01*	0.0	0.4	0.0	0.5	-0.0	0.5	0.1	0.1	0.1	0.1	-0.3	0.04	0.2	0.06	-0.1	0.4

4.6.5 General Linear Model Findings

To perform GLM, a statistical model was applied. In this model, all subjects before and a year after both bariatric surgeries were involved. Subjects were categorized based on gender, type (SG or RYGB) and time (before and one-year after) of bariatric surgery. Subjects were initially classified based on a given variable. Fasting FGF19, total, primary, secondary, 12α-OH, non 12α-OH, conjugated, unconjugated, glycine and taurine BAs were considered as the dependent variable for all comparisons. Weight, BMI, HbA1c, fasting glucose and fasting insulin were added to the model as covariates. Gender, type and time of bariatric surgery were fixed factors for all measurements. The effect of sex was considered as contrast (K matrix), and R^2 was reported for the predictor effect of fixed factors (gender). Finally, the mean of each of the dependent variables mentioned above was compared based on their classification. Table 4.15 indicates univariate analysis of each computed BA and fasting FGF19.

The Results from GLM showed that gender has no impact on any ratio of BAs and fasting FGF19 levels. However, as mentioned above, the type of surgery and time of intervention (preoperative or postoperative) were two important factors which influenced several fasting ratios of BAs as well as fasting plasma levels of FGF19.

Table 4.15 Effect of gender, bariatric surgery and time on fasting FGF19 and BAs

	Gender				Bariatric surgery				Time					
variables	Male n=58 mean (SE)	Female n=64 mean (SE)	F	p	SG n=58 mean (SE)	RYGB n=64 mean (SE)	F	p	Before n=61 mean (SE)	One-year after n=61 mean (SE)	F	p	p-value trend	R^2
Fasting FGF19	105 (6.8)	105 (6.4)	-	NS	102 (6.7)	108 (6.2)	0.3	NS	60.5 (8.9)	150 (7.7)	41.2	<0.01*	NS	0.36
Total BAs	17.2 (3.2)	22.9 (3.1)	1.4	NS	14.9 (3.1)	25.2 (3.0)	5.2	0.02*	7.4 (3.9)	32.7 (3.9)	14.9	<0.01*	NS	0.32
Primary BAs	8.8 (2.4)	14.3 (2.3)	2.3	NS	8.3 (2.4)	14.8 (2.3)	3.4	0.06	5.3 (3.0)	17.8 (3.0)	6.1	0.01*	NS	0.25
Secondary BAs	6.7 (0.8)	7.0 (0.8)	0.05	NS	4.9 (0.8)	8.7 (0.8)	9.5	0.03*	2.0 (1.0)	11.7 (1.0)	28.7	<0.01*	NS	0.49
12α-OH BAs	2.7 (0.1)	2.4 (0.1)	2.3	NS	2.4 (0.1)	2.6 (0.1)	1.6	NS	1.4 (0.1)	3.6 (0.1)	54.0	<0.01*	NS	0.51
Non 12α-OH BAs	12.8 (3.2)	18.9 (3.0)	1.7	NS	10.9 (3.1)	20.8 (3.0)	5.0	0.02*	5.8 (3.8)	25.8 (3.8)	9.4	<0.01*	NS	0.26
Con BAs	8.6 (1.0)	9.5 (0.9)	0.4	NS	7.8 (0.9)	10.3 (0.9)	3.2	0.07	4.8 (1.2)	13.3 (1.2)	17.8	<0.01*	NS	0.34
Unco BAs	8.6 (2.3)	13.3 (2.2)	1.9	NS	7.1 (2.3)	14.8 (2.2)	5.6	0.01*	2.5 (2.8)	19.3 (2.8)	12.3	<0.01*	NS	0.29
Glycine BAs	1.1 (0.08)	0.9 (0.08)	3.0	0.08	1.1 (0.08)	1.0 (0.07)	1.0	NS	0.3 (0.1)	1.8 (0.1)	79.0	<0.01*	NS	0.59
Taurine BAs	7.4 (1.0)	8.5 (0.9)	0.6	NS	6.7 (0.9)	9.3 (0.9)	3.4	0.06	4.5(1.2)	11.5 (1.2)	11.6	<0.01*	NS	0.28

4.7 Diabetes Remission after Bariatric Surgery

The proportions achieving diabetes remission (defined by HbA1c< 39 mmol/mol (5.7%) in the absence of glucose-lowering medications (A. D. Association, 2017) one year after SG and RYGB were 41% (12/29) and 41% (13/32) respectively, and not significantly different between types of surgery (p=0.9). Also, both bariatric surgeries showed a similar number of patients with improved insulin resistance. At one year, 20% and 25 % underwent SG and RYGB, respectively required antidiabetic medications (Table 4.16).

Table 4.16 Diabetes remission one-year after bariatric surgery

Type of surgery	One- year after Sleeve Gastrectomy N=29	One-year after Gastric Bypass N=32
Medications		
Non-remission, n (%)	6 (20)	8 (25)
Improved T2DM, n (%) (6%<HbA1c<7%)	12 (41)	13 (40)
Improved IR (HOMA-IR<2.5), n (%)	21(72)	21(65)
Completed remission (HbA1c<5.7%, off-medication for T2DM, n (%)	11 (38)	13 (40)

There were no any significant differences observed in either fasting or $AUC_{0\text{-}60\ min}$ BA fractions at baseline (before surgery). However, those who achieved diabetes remission, had higher increases in changes in non 12α-OH BAs (p=0.052), but no any significant differences in either actual $AUC_{0\text{-}60\ min}$ or changes in $AUC_{0\text{-}60\ min}$ BA fractions observed (figure 4.6).

Figure 4.7 shows FGF19 alterations a year after bariatric surgeries. Fasting and 2-hour prandial levels of FGF19 were similar at 1 year among those who achieved diabetes remission and those who did not. The changes in FGF19 between baseline and 1 year postoperatively were also similar among those who achieved diabetes remission and those who did not.

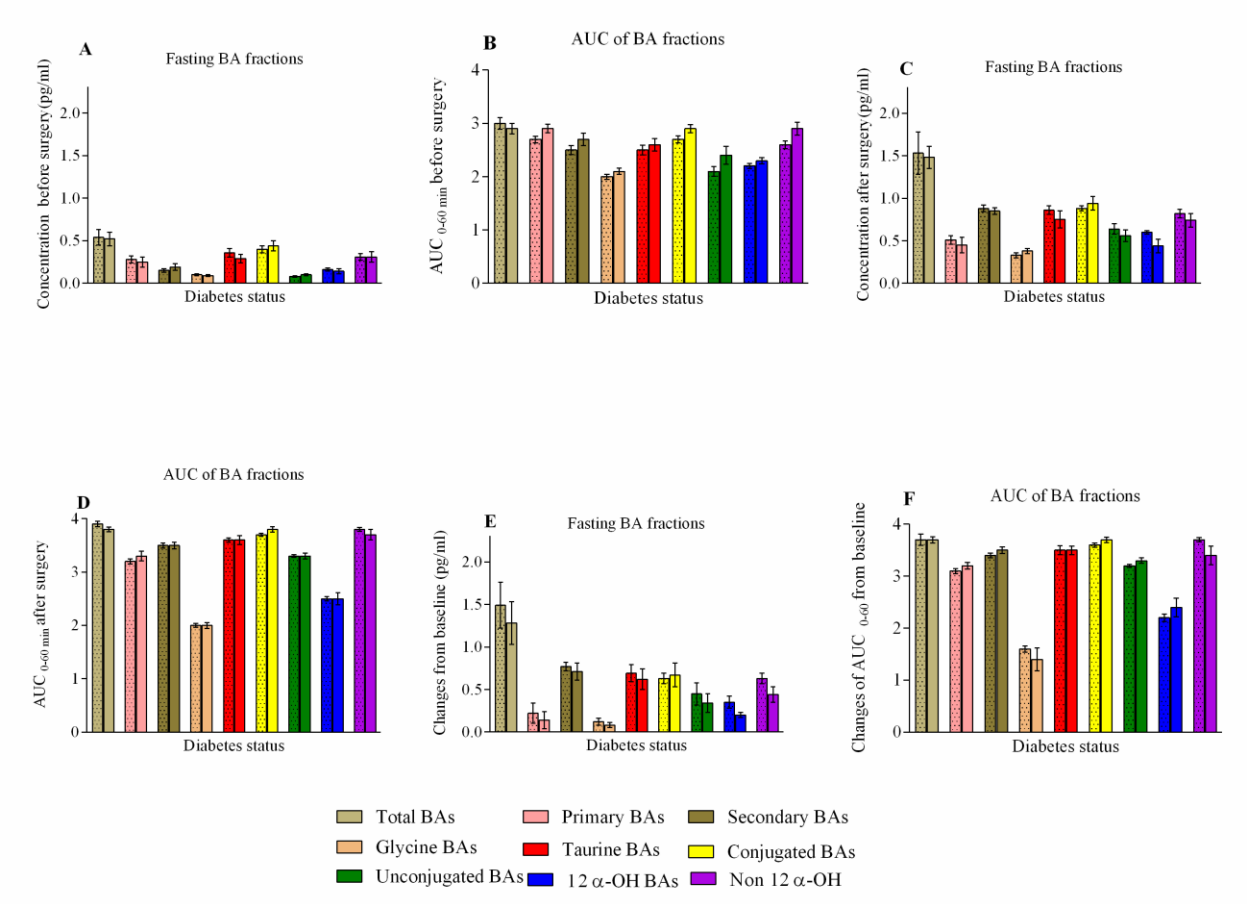


Figure 4.6 BA fraction comparisons between remitted and non-remitted

Concentrations of fasting bile acid (BA) fractions before surgery (A) were similar between diabetes remitted (RM, dotted pattern bar) and non-remitted (NR, non-dotted pattern bar) patients. $AUC_{0\text{-}60min}$ results at baseline (B) and fasting absolute values of BA fractions after surgery (C) did not reach significance between RM and NR. $AUC_{0\text{-}60min}$ results after surgery (D) did not reach significance between RM and NR. Similarly, change in fasting levels of BAs (E) and changes in $AUC_{0\text{-}60min}$ of BA fractions (F) from baseline were similar between RM and NR. All data are log-transformed and shown as mean ±SE.

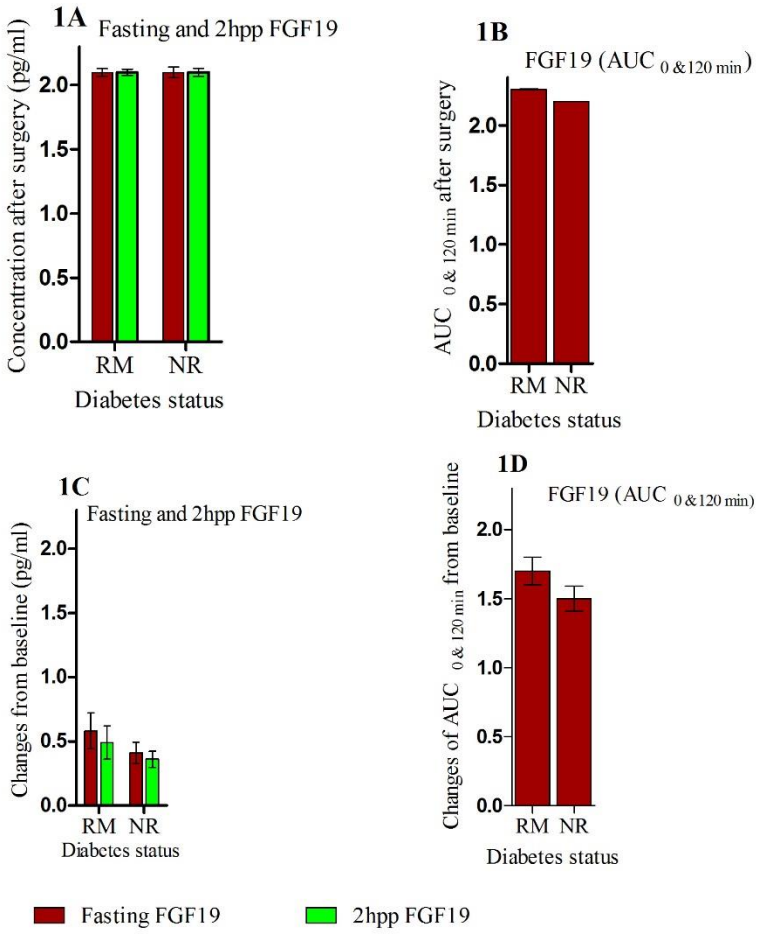


Figure 4.7 FGF19 between comparisons remitted and non-remitted

Actual fasting and 2 hour postprandial FGF19 (1A) and actual AUC $_{0\ \&\ 120min}$ of FGF19 (1B) did not reach significant between remitted (RM) and not-remitted (NR). Also, changes in fasting and 2 hour postprandial FGF19 (1C) and changes in AUC_0 & 120 min (1D) did not reach significant between RM and NR. All remitted (RM) in SG (n=11), RYGB (n=13) and non-remitted (NR) in SG (n=6), RYGB (n=8) pulled together as two groups of RM (n=24) and NR (n=14). Then, a Mann-Whitney U test comparing RM and NR. All data are log-transformed and present as mean ±SE. * $p<0.05$, ** $p<0.01$ between RM and NR.

CHAPTER FIVE

DISCUSSION

5.1 Clinical Characteristics

Prior to the surgeries performed in this study, all subjects underwent pharmacotherapy. Metformin was the predominant medication before bariatric surgery for all subjects regardless of the type of bariatric surgery. A year after SG, 22 out of 29 subjects were off-medication while the number of people who took medication a year after RYGB were 4 out of 32. This finding shows that bariatric surgery whether SG or RYGB had a considerable impact on improving T2DM. Consistent with pre-surgery statistics, a year after bariatric surgery, metformin is still the chosen medication for patients in non-remission of diabetes after bariatric surgery. This is also in agreement with a post-bariatric surgery study that identified metformin as the leading treatment (Cutolo et al., 2012).

The effect of weight loss on the remission of diabetes remains unclear after SG and RYGB. Although both weight and BMI significantly reduced a year after bariatric surgery, no significant change was seen between SG and RYGB. This finding is in agreement with the recent studies which indicate that there is no statistical significance of weight and BMI variables in a year after SG and RYGB (W.-J. Lee et al., 2017; Pekkarinen et al., 2016). This is also in agreement with previous studies that showed weight and BMI significantly reduce a year after SG (Cesana et al., 2014) or RYGB (Lager et al., 2017) in comparison with their baseline. It is postulated that the remission of T2DM mostly occurred early after bariatric surgery (Guidone et al., 2006). The reason of early remission of diabetes is mainly dependent on the change of incretin hormones resulting from anatomical alterations of the intestinal system (Laferrère et al., 2007). For example, the rapid delivery of consumed food to the terminal intestine causes a greater secretion of GLP-1, which is a potent hormone in metabolic functions (Steinert et al., 2017). It is important to note that all subjects before bariatric surgery in this study underwent an intensive low-calorie diet for two to three weeks. Preoperative weight loss has been postulated to be a strong predictor of successful weight loss after bariatric surgery. The larger preoperative weight loss, however, results in lower postoperative weight loss (Limbach et al., 2014; Pekkarinen et al., 2016). Taking this into consideration, VLCD before surgery for both surgery groups may be considered as a reason for

the observed non-significant statistical change of weight and BMI between a year after SG and RYGB.

When baseline values of fasting glucose were compared with one-year after either SG or RYGB, no significant change was seen. This was despite the fact that both surgery groups' levels of fasting glucose reduced slightly. Also, the effect of both bariatric surgeries on the levels of fasting glucose seems to be similar since no significant difference was seen between postoperative fasting glucose between SG and RYGB groups. This finding is supported by a recent study (W.-J. Lee et al., 2017) that shows both SG and RYGB surgeries are similar in their reduction of fasting levels of glucose.

Contrary to glucose levels, levels of fasting insulin significantly reduced a year after SG and RYGB. The percentage of its change in SG was greater than that observed in RYGB values but was not a significant difference between the two. Normal glucose homeostasis comes from a sufficient ability of pancreas β-cells to release insulin in response to glucose. Because of this, measurements of circulating insulin can be a potent marker of insulin sensitivity assessment (Bradley et al., 2012). One of the important risk factors of diabetes is increased body mass. When the body mass or BMI is increased, the pancreas has to release more insulin to facilitate the uptake of glucose into the body's cells. Thus, if the amount of insulin produced by the pancreas is not sufficient to remove glucose from plasma, the risk of T2DM is increased. Both SG and RYGB can reduce levels of insulin significantly. Taken together, decreased levels of fasting insulin in both SG and RYGB may be a major reason for the observed diabetes remission after bariatric surgery. Of note, and contrary to the significant reduction of insulin a year after bariatric surgery, glucose levels did not significantly reduce after bariatric surgery. Since plasma glucose is the main stimulator of insulin secretion, this phenomenon may be due to the effect of oral glucose ingestion on insulin rather than actual plasma levels of glucose (Perley & Kipnis, 1966). The "incretin effect" via GIP and GLP-1 are also important to increase insulin secretion and reduce insulin clearance (Meier et al., 2007). Significant reduction of insulin after SG and RYGB is in agreement with previous studies showing that there is a significant reduction of fasting insulin after SG (Casella et al., 2016) and RYGB (W.-J. Lee et al., 2016) with non-significant differences between the two surgeries (Kalinowski et al., 2016) . The results from OGTT showed that both glucose and insulin peaked at 30 minutes after ingestion of glucose one-year after SG and RYGB, which is in agreement with previous work which showed that regardless of the type of bariatric surgery (SG

or RYGB), the highest concentration of glucose and insulin is observable 30 minutes after OGTT (Falkén et al., 2011). In this study, glucose at 30 minutes was significantly higher a year after RYGB than SG. In accordance with a previous study (Keidar et al., 2013), in the point to point comparison it is found that the mean of glucose levels 2hpp was significantly higher at the baseline of SG and RYGB in comparison with 12 months after surgery accordingly.

Several studies measure glucose and insulin AUC after RYGB. However, there is scant information about the effect of SG on the AUC of glucose and insulin. In this study, we have shown that the AUC of glucose significantly reduced a year after both SG and RYGB while both bariatric surgeries have a similar effect on the AUC of glucose. AUC insulin only reduced meaningfully after SG and not after RYGB. One of the most common methods used to evaluate BCF is measuring the concentrations of insulin and glucose at specific time points. This approach allows an evaluation of the early and late response of insulin to glucose. Most studies indicate that the AUC of glucose significantly reduced after bariatric surgery (Bradley et al., 2012). However, insulin AUC has been shown to reduce significantly after SG (C.-Y. Chen et al., 2013; W.-J. Lee et al., 2010) but not significant after RYGB (Laferrère et al., 2008). Undoubtedly, anatomical alterations after bariatric surgery, which lead to hormonal alterations at the same time, play a crucial role to change glucose and insulin secretion (Laferrère et al., 2007). It is important to note, however, that in humans (Isbell et al., 2010) and animals (Chambers et al., 2011) it has been shown that reduced AUC of glucose and insulin are independent of weight loss. Taken together, decreased AUC of glucose and insulin after bariatric surgery suggests a contributory role in improved insulin secretion in response to OGTT which leads to the remission of diabetes after bariatric surgery. Nevertheless, due to a similar effect of both bariatric surgeries on the reduced AUCs of insulin and glucose, it is not possible to determine if one of the bariatric surgeries is superior to the other. The role of weight loss to reduce AUCs of insulin and glucose, however, is still a substantial possibility (Holter et al., 2017; Sjöholm et al., 2016).

Appropriate glycaemic control (HbA1c less than 6.5%) is achieved by patients who underwent SG and RYGB. One of the important factors to predict glycaemic control in T2DM is to assess HbA1c (Ensenauer et al., 2015). Reduced HbA1c markedly results in decreasing diabetes complications (D. Control & Group, 1993). HbA_1C significantly reduced a year after SG and RYGB, but there was not a significant change between SG and RYGB. Previous studies reported a significant

reduction in HbA1c after SG and RYGB, with no significant difference between SG and RYGB (Benaiges et al., 2013; Keidar et al., 2013). In a recent cohort study, however, a significant reduction of HbA_1C was identified after RYGB in comparison with levels found in the SG patients (W.-J. Lee et al., 2017). This finding is contrary to results of this study. There are at least two reasons for this inconsistency. First, Lee's study is a retrospective cohort study, not a prospective study. Second, patients in Lee's study entered their cohort with poorer glycaemic control (relatively higher HbA1c and HOMA-IR preoperatively) compared to subjects in this study.

The lipid profile including total cholesterol, triglycerides, HDL-c and LDL-c levels were completely improved for subjects after SG in this study. Despite the improved lipid profile one-year after RYGB, total cholesterol and LDL-c did not reach significant improvement levels. Since LDL-c is measured from cholesterol using the Friedewald equation (Friedewald et al., 1972), non-significant reduced of LDL-c may be related to the non-significant reduction of total cholesterol. The improved lipid profile after both SG and RYGB may be, in part, related to weight loss or the loss of fat mass to a considerable extent (Frige et al., 2009). Furthermore, reduced gastric volume (and as a result reduced production of gastric lipase and CCK, which physiologically induces secretion of lipase and protease), might be another reason of improved lipid profiles observed after bariatric surgery (Bays, 2004). There was a similar effect of SG and RYGB on total cholesterol, triglycerides, LDL and HDL, which is in agreement with a recent prospective study which compared lipid profiles between SG and RYGB subjects in a 12-month period (Menguer et al., 2017). Improved lipid profiles after bariatric surgery are a good indication of improving insulin resistance (Biddinger et al., 2008). Non-significant differences in lipid profiles between SG and RYGB in this study may result from relatively short-term follow-up (12 months) since it has been shown that lipid profiles are comparable between SG and RYGB for three years or more after the procedures (Schauer et al., 2014). Obesity is often associated with dyslipidaemia, hypercholesterolemia, and reduced HDL-c (Howard et al., 2003). Undoubtedly, hyperlipidaemia plays a pivotal role in the aetiology of T2DM (Janikiewicz et al., 2015). Thus, improved dyslipidaemia resulting from bariatric surgery may be considered as a factor indicating improved glucose metabolism and remission of diabetes (Carswell et al., 2016).

5.1.1 Obesity Classification

All subjects in this study, before and after a given bariatric surgery, were categorised for obesity according to WHO classification (http://apps.who.int/bmi/). Interestingly, a similar number of subjects (21%), 12 months after SG and RYGB were listed in the normal weight classification. This weight loss outcome from bariatric surgery is an important success, irrespective of the two different bariatric surgeries performed. Not surprisingly, before surgery, all patients were categorised as being grade two and three obese regardless of the type of surgery they were going to have performed. However, 12 months after surgery only four subjects were still classified as grade three obese. Only one out of the four belonged to the RYGB group, and the rest were from the SG intervention.

5.2 BAs, Bariatric Surgery, and Diabetes Remission

Thirteen fasting individual BAs were measured before and one-year after SG and RYGB. In the SG group, TDCA was significantly reduced and fasting GCDCA, CA, and TLCA were not detected by LC-MS/MS a year after SG. In addition, no significant change of GDCA was observed 12 months after SG. Similarly, fasting TDCA was reduced a year significantly after RYGB in comparison with baseline values while fasting TLCA was only detectable before RYGB. Fasting GDCA and CA were not changed a year after RYGB when compared with baseline. The rest of the fasting BAs were markedly higher a year after RYGB compared to their baseline values.

5.2.1 Effect of Bariatric surgery on Fasting Individual BAs

BAs are theorised to be involved in glucose metabolism. BAs are signalling molecules and act as ligands for FXR and TGR5 receptors, both of which influence lipid and glucose metabolism (Porez et al., 2012; Prawitt et al., 2011). For instance, TGR5 activation leads to induction of the secretion of GLP-1 (from L cells) and results in maintenance of normal glucose metabolism (normoglycaemia) (Thomas et al., 2009), and activation of FXR is crucial to maintaining normal lipid metabolism (normolipidaemia) (Y. Zhang et al., 2006), normoglycaemia and BA functions (K. Ma et al., 2006; Sinal et al., 2000). CDCA was initially proposed as a potent FXR ligand (Makishima et al., 1999), and intraduodenally injection of CDCA stimulates a rise in plasma concentration of BAs (Meyer-Gerspach et al., 2013). Secondary BAs (LCA and DCA) also have affinities to active FXR, but less affinity than CDCA (Lefebvre et al., 2009). BA derivatives (either

conjugated or unconjugated) display a tissue-specific activity, allowing the possibility for therapeutic abilities in their targeted organs (Chiang, 2009).

5.2.2 Effect of SG on Fasting Individual BAs

Human and animal studies suggest that BAs and the FXR receptor play a critical role in the improvement of T2DM, suggesting that improvement of diabetes after SG is achieved by more than just energy or calorie restriction (Ryan et al., 2014; Yamamoto et al., 2016). In this study, most individual BAs increased after SG. Although there are comparable differences between the number of studies designed based on SG in comparison with RYGB in humans, it has been shown that BAs increased significantly after SG (Escalona et al., 2016; Myronovych et al., 2014; Nakatani et al., 2009). Of note, the type of surgery plays an axial role to alter BAs metabolism. In other words, different patterns of BAs are directly dependent on the type of bariatric surgery (Ferrannini et al., 2015; Kohli, Bradley, et al., 2013b).

In this study fasting levels of LCA and DCA (secondary BAs) increased 12 months after SG. In the time of enterohepatic circulation, BAs move forward to the colon where they are deconjugated and dehydroxylation. These secondary BAs are only produced by dehydroxylation of primary BAs (CA and CDCA) in the large colon (Ridlon et al., 2006). LCA and DCA are passively absorbed from large bowel returning to the liver through the portal vein. Therefore, the portal vein has both primary and secondary BAs during each enterohepatic circulation. Next, primary and secondary BAs are actively transported to the liver. Then, biotransformed, conjugated (glycine and taurine) and again actively move to the bile. It is important to note the vital role of gut microbiota after bariatric surgery (Furet et al., 2010; Husen Zhang et al., 2009) for alteration of BAs because, during BAs circulation pathway, gut microbiota mediates conjugated and deconjugated BAs homeostasis. The result of fasting individual BAs confirms results in a previous study which showed a significant rise in fasting BAs a year after SG (R. E. Steinert et al., 2013).

Anatomical change alone cannot be a potent reason to alter BA metabolism because SG has no distinct anatomical modifications compared with other bariatric surgeries such as RYGB or BPD. Another cause of BA alterations after SG may rely on the change of gut hormone expression due to the restrictive effect of SG (Kamvissi-Lorenz et al., 2017). Furthermore, animal study data showed that increased level of individual BAs might be the result of the effect of TGR5 signalling

after SG (McGavigan et al., 2015). TGR5 has a potent role in improved insulin sensitivity. Administration of BAs in diabetic patients was found to be useful in improving glycaemic control, probably through FXR and TGR5 (Fonseca et al., 2008; Kamvissi-Lorenz et al., 2017). One of the possible mechanisms of improving glucose metabolism through FXR is the property of FXR to inhibit sterol regulatory element binding protein 1-c (SREBP1c) which interferes with liver lipogenesis, resulting in increased triglycerides clearance from blood circulation (Cariou et al., 2006). The exact mechanism of BA alteration after SG is still unclear.

5.2.3 Effect of RYGB on Fasting Individual BAs

Alteration in levels of BAs has been shown to be linked with T2DM, and this alteration may improve T2DM (Dutia et al., 2015). The result of this study shows that one-year after RYGB, fasting levels of most BAs increased. This result confirms what previous studies indicated, namely that BAs increased a year after RYGB among obese people with or without T2DM (Ahmad et al., 2013; Gerhard et al., 2013; R. E. Steinert et al., 2013). Nevertheless, it is unclear when exactly after surgery BAs increased. Increased levels of BAs are mostly observable over a long-term period (Patti et al., 2009; R. E. Steinert et al., 2013) which is of interest within the context of RYGB. RYGB results in a comprehensive anatomical change to the gastrointestinal system, particularly the upper gastrointestinal system, which could modify BA metabolism. Alterations such as changing pH, stimulating nutrient transportation time, the terminal mixing of unconsumed nutrients with gallbladder and pancreas discharges in the lower jejunum (Morínigo, Moizé, et al., 2006; N. Q. Nguyen et al., 2014; G. Wang et al., 2012), and changing of gut microbiota (Husen Zhang et al., 2009) are all additional influences in BA metabolism. Other contributing factors to alter BAs include changes of hepatic insulin sensitivity and enterohepatic circulation of BAs. It has been reported that one-year postoperative remission of diabetes after RYGB increases fasting BAs in humans (Gerhard et al., 2013), which is positively correlated with a postprandial peak of GLP-1 (Patti et al., 2009) and supports the proposal of BAs as new gut hormones (D. Pournaras & le Roux, 2013). The compensatory effect of BAs on glucose metabolism may result from TGR5, which is expressed on L-cells and causes increasing levels of GLP-1 (Madsbad et al., 2014). The contribution of increased postoperative BAs to the remission of diabetes remains poorly understood.

TUDCA and GCA increased after RYGB. It has already been shown that increased levels of TUDCA and GCA after RYGB had beneficial impacts on the remission of diabetes in comparison with a non-surgery group (Patti et al., 2009). Patti et al. suggested that increased BAs might be contributory to improving insulin sensitivity, gut hormone secretion, and glycaemic control. Although increased levels of BAs can occur early after bariatric surgery, significant changes in levels of BAs can only be observable between baseline and a year after bariatric surgery (R. E. Steinert et al., 2013). It is still unclear how BAs improve glucose metabolism after bariatric surgery. However, inhibition of gluconeogenesis is one mechanism proposed for the compensatory effect of BAs on remission of diabetes (D. J. Pournaras et al., 2012).

Fasting individual BAs were compared one year after SG and RYGB. Fasting TUDCA, GDCA, CDCA, and LCA were found to be significantly lower in SG than RYGB while fasting GCA increased a year after RYGB. Being hydrophobic or hydrophilic, BAs play a central role to improve insulin sensitivity. For instance, TUDCA, which was increased after SG and RYGB is a hydrophilic BA. Its role in improving insulin sensitivity through reducing inflammation and endoplasmic reticulum stress has been reported in mice (Özcan et al., 2006).

There is scant information about the comparisons of individual BAs between SG and RYGB data. Data from a one-year RCT study showed that SG and RYGB are equally effective on fasting individual BAs (R. E. Steinert et al., 2013), which is in contrast with this study. The inconsistency with Steinert et al.'s study relies on at least two reasons. First, only 14 obese subjects were recruited in their study, and of those, seven patients were assigned to each bariatric surgery, and their small sample size had no diabetic subjects. Secondly, only three individuals' BAs were evaluated in their study.

5.2.4 BA Fractions, Bariatric surgery, and Diabetes

In this study, several different fasting and prandial fractions of BAs before and a year after SG and RYGB were computed. These computed fractions were compared between SG and RYGB. Postprandial BA fractions were also calculated, and the results agreed with the previous study that evaluated BA fractions a year after RYGB among obese females with T2DM (Dutia et al., 2015). The fasting fractions of BAs was mostly addressed because of two main reasons. First, systemic

BAs, independent of enterohepatic circulation, influence metabolic effects. Second, systemic BAs exhibit overall changes in both metabolism and enterohepatic quantities (Cole et al., 2015).

Several fasting fractions were found to increase a year after RYGB in comparison with 12 months after SG. However, 12α-OH/non 12α-OH and conjugated/unconjugated BA fractions were comparably higher in samples from a year after SG compared to those one-year after RYGB.

TBA levels were increased significantly one year after SG and RYGB. It has been shown that TBA increased after RYGB in both obese individuals with or without T2DM (Dutia et al., 2015; R. E. Steinert et al., 2013). TBA have biphasic properties (Albaugh et al., 2015), although it is not exactly clear when TBA levels increase after bariatric surgery, it can be either early or late (Dutia et al., 2015; R. E. Steinert et al., 2013). An individual's metabolic status is an effective factor on the concentration of BAs. Obese people have less TBA compared with lean people (Ahmad et al., 2013) presumably due to the effect of glycine-conjugated BAs (Glicksman et al., 2010). Indeed, the relationship between BAs, diabetes, and obesity is complicated. For example, TBA in obese people with T2DM is higher in comparison with obese people without T2DM (Gerhard et al., 2013; Vincent et al., 2013b) and it may result from increasing conjugated BAs (Wewalka et al., 2014b). Nevertheless, both diabetes and obesity are independent contributors to BA metabolism (D. R. Taylor et al., 2014). Increased levels of TBA observed in this study a year after RYGB is in agreement with the previous study which showed a significant increase of TBA one-year after RYGB among obese people with T2DM (Ahmad et al., 2013). The exact mechanism of increased TBA after RYGB and SG is unknown. However, reduced enterohepatic circulation after malabsorptive surgeries such as RYGB or reduced cholesterol intake, as a result, increased BA secretion after restrictive surgery (SG) might be one of the possible mechanisms (Ionut et al., 2013). Limited data about the effect of SG on TBA indicated a significant increase of TBA one-year after SG in comparison with baseline data in humans (R. E. Steinert et al., 2013) and 5 months after SG in animals (B. P. Cummings et al., 2012). Also, human and animal studies showed an increase of TBA three months and a year after SG among obese subjects with and without diabetes (Escalona et al., 2016; Myronovych et al., 2014; Nakatani et al., 2009). Steinert et al. also compared TBA between time points one year after SG and RYGB. The researchers did not observe any significant difference between SG and RYGB, which is inconsistent with this study. This

difference may result from different sample sizes and/or incompatibility of the study designs. Additionally, they did not evaluate TBA for diabetic patients (R. E. Steinert et al., 2013).

TBA include several variations of structure and modification: total BAs consist of primary, secondary, conjugated and unconjugated types. TBA have been reported to elevate a few days after RYGB without significant weight loss (D. J. Pournaras et al., 2012). Although, the exact mechanism of this is unclear, undoubtedly, the manipulation of total BAs can affect glycaemic control, whole body weight and improvement of insulin sensitivity (H. Ma & Patti, 2014). For instance, among people with IGT, TBA levels are reported to be dysregulated (Shaham et al., 2008). Therefore, increased TBA after bariatric surgery may play a crucial role in the improvement of T2DM. It is worth noting that due to the anatomical modification after bariatric surgery, particularly after RYGB, it is likely that BAs' transit to the intestine has a relatively short time to mix with consumed food before going to the ileum. Thus, more free BAs remain in the gastrointestinal system for reuptake (L. Kaska et al., 2016).

Both conjugated and unconjugated BAs increase after bariatric surgery, while their ratio is significantly reduced a year after SG and RYGB compared with their baseline. It has been shown that conjugated BAs increase more than unconjugated BAs a year after RYGB (Ferrannini et al., 2015). Few studies have evaluated conjugated and unconjugated BAs with their ratio among obese people with diabetes a year after bariatric surgery. The finding from this study, however, is in agreement with previous studies which showed an increase of conjugated and unconjugated BAs and their ratios one-year after RYGB in humans (Ahmad et al., 2013; Albaugh et al., 2015; Simonen et al., 2012) and after SG in humans and animals (Ding et al., 2016; R. E. Steinert et al., 2013). Therefore, altered conjugated BAs after bariatric surgery is a potent metabolic mediator independent of TBA (Simonen et al., 2012).

Glycine-conjugated BAs increased after both bariatric surgeries. The role of glycine-conjugated BAs is more prominent in the literature compared to information regarding taurine-conjugated BAs. It has been reported that increasing postprandial levels of BAs are directly associated with glycine-conjugated BAs (Werling et al., 2013). Similarly, taurine-conjugated BAs also increased after both bariatric surgeries. It has been indicated that fasting taurine-conjugated BAs are higher in diabetes than non-diabetic people (Wewalka et al., 2014a) and rodents (Tsuboyama-Kasaoka et

al., 2006). Furthermore, administration of taurine-conjugated BAs has been reported to improve insulin sensitivity probably through the effect of FXR on reducing gene expression of gluconeogenesis (Cao et al., 2010).

Glycine- and taurine-conjugated BAs increased after both bariatric surgeries, but non-significant differences were observed between SG and RYGB. Increased glycine- and taurine-conjugated BA levels are associated with improved insulin sensitivity (Furet et al., 2010). This interesting finding may show a compensatory effect of glycine-conjugated BAs on taurine-conjugated BAs regardless of the type of bariatric surgery, and it may suggest both SG and RYGB have similar effects on two important mechanisms of insulin sensitivity.

Primary and secondary BAs increased a year after SG and RYGB. It has been shown that primary BAs mostly bind to FXR while secondary BAs have more affinity to TGR5. Glucose metabolism is impaired by FXR while TGR5 promotes glucose homeostasis (Kamvissi-Lorenz et al., 2017). Hence, the transformation of primary to secondary BAs plays a crucial role in glucose metabolism. Also, primary BAs are inversely associated with insulin sensitivity (Cariou et al., 2011) and positively correlated with GIP in severe obesity (Nakatani et al., 2009). The increased amount of primary and secondary BAs described in this study agrees with previous studies (Albaugh et al., 2015; Werling et al., 2013).

In this study primary unconjugated BAs increased a year after SG and RYGB, which is in agreement with the previous study which showed a significant rise of primary unconjugated BAs after RYGB (Sachdev et al., 2016). The concentration of secondary unconjugated BAs, however, increased after RYGB. Also, a recent cohort study indicated a non-significant increase of primary BAs while secondary BAs significantly increased a year after SG (Escalona et al., 2016). Inconsistencies between Escalona's study and this study may have resulted from a relatively higher number of females and a lack of diabetic subjects in their study. In addition, they did not observe a significant reduction of glucose a year after SG, while glucose due to its inverse correlation, is a potent stimulator of BA synthesis (Kohli et al., 2015; Tiangang Li et al., 2012). Secondary BAs also increased a year after SG and RYGB. Once primary BAs reach the intestine, they are transformed by gut microbiota into secondary BAs. Gut microbiota is extremely important to the metabolism of BAs for this reason (J.-L. Han & Lin, 2014). Additional importance of BAs is seen

in their anti-bacterial properties which can impact the number of bacteria in the intestine. As a result, alterations in the metabolism of BAs after bariatric surgery (Kurdi et al., 2006).

It is not understood why primary or secondary BAs and their ratios increased after SG or RYGB. However, information obtained from types of surgeries associated with the increases may be effective in adding to understanding the observation. For example, in SG analysis it has been shown that lack of some genes, increased gut hormone levels, and villi length may all be related to increased levels of BAs and its ratios after SG (Kohli et al., 2015). Another possibility may be seen in the effect of secondary BAs on the activation of TGR5 (Sinal et al., 2000) and this influence on glucose metabolism (Fiorucci & Distrutti, 2015).

In this study, despite the non-significant difference regarding 12α-OH/non 12α-OH BAs between SG and RYGB, a significant reduction of the ratio was seen a year after each bariatric surgery. This finding is in concordance with the studies which showed a decrease in the proportion of 12α-OH to non 12α-OH a year after RYGB among people with T2DM (Albaugh et al., 2015; Haeusler et al., 2013). *CYP8b1* gene, which is expressed in the liver and responsible for CA synthesis, determines this ratio. It has been shown that humans or animals with insulin resistance have higher 12α-OH to non 12α-OH ratios (Haeusler et al., 2013; Haeusler et al., 2012) and *CYP8b1* knockout results in better insulin secretion and glucose tolerance (A. Kaur et al., 2014). The role of TGR5 is also important on the 12α-OH to non 12α-OH ratio. It has been shown that TGR5 after SG induces a reduction of 12α-OH/non 12α-OH (McGavigan et al., 2015). Reduction of 12α-OH/non 12α-OH is equivalent to a reduction of BA hydrophobicity. It has been reported that hydrophobicity reduction of BAs causes a decrease in inflammation, and as a result improves insulin sensitivity (A. M. Johnson & Olefsky, 2013; Perez & Briz, 2009).

Apart from all underlying mechanism leading to the alteration of BAs, it is important to note that the type pharmacotherapy an individual receives also plays a vital role in the change of BAs. Most subjects either before or after a given bariatric surgery underwent metformin therapy, and the effect of metformin in altering BAs has been documented (Napolitano et al., 2014). In addition, short-term consumption of VLCD can increase fasting and postprandial BAs (van Nierop et al., 2016).

Our results from a large cohort of patients with T2DM pre-operatively add to the existing literature on the composition of plasma BA changes that occur with different types of bariatric surgery when

measured in the fasting and postprandial states. Most studies which have examined postprandial plasma BAs either after SG (Khan et al., 2016; R. E. Steinert et al., 2013) or after RYGB (Ahmad et al., 2013; De Giorgi et al., 2014; Kohli, Bradley, et al., 2013a; J. B. Schmidt et al., 2013; R. Steinert et al., 2013; R. E. Steinert et al., 2013; Werling et al., 2013) have reported similar increases in postprandial levels as in fasting levels. Only one small study investigated the pattern of change in fasting and prandial BA composition after RYGB compared with SG among 14 obese people without T2DM (R. E. Steinert et al., 2013), and reported that both fasting and postprandial total BAs increased after RYGB but only fasting total BA increased after SG at 1 year postoperatively, with all individual BA following the same pattern (R. E. Steinert et al., 2013). Most of these studies, except one (Dutia et al., 2015), have not included those with T2DM, who have higher BA (Gerhard et al., 2013; Vincent et al., 2013b; Wewalka et al., 2014a). In our larger study of 61 obese people with T2DM, we found a greater increment in both fasting and prandial levels of total, secondary and unconjugated BA measured 1 year after RYGB than after SG, while the composition of certain BA groups such as taurine BA and non-12 α-OH, which were higher after RYGB in the fasting state, was not higher after RYGB in the postprandial state. After synthesis from cholesterol in the liver, primary conjugated BAs are typically excreted into the duodenum to aid in lipid absorption. While most BAs are almost entirely reabsorbed in the distal ileum and recycled within the enterohepatic circulation, a small amount enters the peripheral blood and is thought to influence wider host physiology than lipid digestion. Given primary BA unconjugation and transformation to secondary BA occur by gut microbiota, it is likely that these specific gut microbiota reactions are enhanced by the presence of the biliopancreatic limb that receives only bile but not food and may result in discordant changes in BA metabolism during the fasting and postprandial state. Biliopancreatic diversion (BPD) have been reported to produce even greater increments in unconjugated BAs than RYGB (Ferrannini et al., 2015; Risstad et al., 2017), suggesting that the longer biliopancreatic limb in BPD leads to greater increases in primary BA unconjugation through such gut microbial reactions. However, no (Kohli, Bradley, et al., 2013a), or only a single BA species (GLCA) (Thöni et al., 2017), has been shown to be elevated after adjustable gastric banding, which produces the least impact on gut anatomy, and unlike SG does not permit more rapid delivery of bile to the distal small intestine.

Several early post-bariatric studies (mainly in patients without T2DM) report that total BAs do not rise as soon as 1 week after RYGB (Ahmad et al., 2013; D. J. Pournaras et al., 2012; R. E. Steinert

et al., 2013) and SG (R. E. Steinert et al., 2013), while others have found no increase at 1 month post RYGB (Dutia et al., 2015) or 1-6 months after SG (Belgaumkar et al., 2016; R. E. Steinert et al., 2013). In addition, weight loss itself does not result in significant BA changes given that in the presence of similar acute 20% weight loss achieved after RYGB and gastric banding, no increase in BAs was found to occur after gastric banding in contrast to a marked increase in BA observed after RYGB (Kohli, Bradley, et al., 2013a). It is unlikely that increases in BAs cause early glycemic benefits which are already observed at very early time points after both RYGB and SG types of surgery (Yip et al., 2014). However, BAs may contribute to sustaining these metabolic improvements which are superior after BPD, RYGB, and SG than after gastric banding (Buchwald & Oien, 2013). Taken together, understanding the underlying mechanism of bariatric surgery on alterations of BA levels and their ratios may open a new door to find alternative non- or semi-invasive therapies for T2DM.

5.3 FGF19, Bariatric Surgery and Diabetes

Fasting and postprandial FGF19 levels were measured before and a year after SG and RYGB. A comparison based on fasting and postprandial FGF19 was then performed between the time points one year after SG and RYGB. FGF19 significantly increased in fasting and postprandial states a year after both bariatric surgeries. Insufficient RCT information is available on FGF19 and its effect after bariatric surgery. There is only one RCT which examined FGF19 in baseline and one-year after RYGB time points and compared the values with intensive medical management (Sachdev et al., 2016). To this researcher's knowledge, this is the first study to compare FGF19 between baseline and a year after two common bariatric surgeries (SG and RYGB). Data from FGF19 levels in this study may be used as pilot information for future research.

5.3.1 Effect of SG on Fasting and Postprandial FGF19

Obesity is a cluster of a variety of metabolic abnormalities such as insulin resistance, dyslipidaemia, inflammation and diabetes (Reaven et al., 2004). Understanding the mechanism of action of novel biomarkers such as FGF19, which is produced in the ilium, may open a new door to finding novel therapies for the treatment of T2DM. Animal studies have shown that overexpression or exogenous administration of FGF19 helps to improve insulin sensitivity (L. Fu et al., 2004; Tomlinson et al., 2002). However, insufficient information is available on the effect

of FGF19 in humans relative to the improvement of diabetes. Due to different structure and mechanism of FGF15 in mice, FGF19 in humans, translating from animal results to humans is not an easy way nonetheless (M. Zhou et al., 2017).

This study determined that FGF19 increase by 51% one-year after SG in comparison with baseline in obese people with T2DM. Similarly, postprandial FGF19 also raised a year significantly after SG compared with baseline. In line with this study, a separate prospective study also showed that SG plays a major role in increasing FGF19 by 50% (Haluzíková et al., 2013). Haluzíková‘s study also found that FGF19 levels significantly increased and BA synthesis was reduced a year after SG. It is important to note that in Haluzíková’s study only 17 obese females (without diabetes) were recruited, and their results were compared with 15 lean females without diabetes. Postprandial FGF19 levels were not measured after either meal test or OGTT. In other words, the dynamic changes of gastrointestinal hormones related to food consumption were not examined. In contrast to these results, a recent human study indicated that baseline synthesis of BAs significantly decreased along with increased levels of FGF19 among obese people at a one-year time point after SG (Escalona et al., 2016). Interestingly, they did not observe a correlation between FGF19 and BAs a year after SG. Escalona and co-workers suggested gastric emptying may be the main reason of increased level of FGF19 after SG. Nevertheless, there is a negative link between obesity and FGF19 concentrations observed in humans and animals (L. Fu et al., 2004; Gallego-Escuredo et al., 2015). FGF19, through a complex signalling pathway (L. Fu et al., 2004) increases metabolic rate and improves dyslipidaemia, hyperinsulinemia and insulin sensitivity (Stejskal et al., 2008). Also, FGF19 intracerebroventricular injection in animals resulted in weight loss and improvement in insulin resistance (Ryan et al., 2012).

FGF19 and BAs have a mutual relationship. In liver cells, FGF19 binds to fibroblast growth factor receptor (FGFR4) to suppress CYP7A1 expression and BA synthesis through a signalling pathway. FGF19 has a negative feedback on BA synthesis (Holt et al., 2003). BAs are considered to be essential metabolic regulators through their receptors, FXR and TGR5, where they control the secretion of FGF19 (Haeusler et al., 2012; Song et al., 2009).The “BA-gut-liver axis” is influenced by FXR, and FXR controls BA uptake (Zwicker & Agellon, 2013). Once BAs stimulate FXR, FGF19 is released from the ilium to downregulate BA production in liver cells (Holt et al., 2003). Similar to insulin, FGF19 is released in response to feeding; however, FGF19 has a late-acting

effect in comparison with the immediate-acting effect of insulin on the feeding state (Potthoff et al., 2012). Therefore, FGF19 regulates glucose metabolism in parallel with insulin (Tomlinson et al., 2002).

FGF19 can induce gallbladder filling (Choi et al., 2006). This function of FGF19 is necessary to ensure that BAs produced in the liver is mixed well with reabsorbed BAs in the ilium. Taken together, an increased level of FGF19 after SG may result from mixing changes that occur after SG. Actually, after SG, indigested food reaches the ilium sooner due to resection of 80% of the stomach. As a result, BAs start digesting the ingested food, and the levels increase as a consequence of a negative feedback mechanism which is necessary to suppress over-production of BA synthesis. FGF19 has a negative feedback mechanism on BA synthesis. Therefore, FGF19 needs to be released in greater amounts to suppressing BA synthesis. It is for this reason that FGF19 is likely increased after SG.

5.3.2 Effect of RYGB on Fasting and Postprandial FGF19

Fasting and postprandial FGF19 were increased a year after RYGB in comparison with baseline. BA synthesis and transportation are negatively regulated by FGF19, which also inhibits lipid absorption and hyperlipidaemia (Chiang, 2009). A human study indicated that FGF19 concentration is inversely related to the levels of fasting and postprandial BAs (C Gälman et al., 2011). In addition, studies in animals showed that administration of BAs decreased plasma levels of FGF19 (Cecilia Gälman et al., 2008). In a separate human study, FGF19 was significantly lower in people with diabetes than without diabetes a year after RYGB (Gerhard et al., 2013). Based on the study by Gerhard et al., increased levels of FGF19 after RYGB were only seen among people with remission of diabetes, and diabetic patients had lower levels of FGF19. In another human study, a correlation between FGF19 and BAs observed before bariatric surgery (Haluzíková et al., 2013) suggests that a change of BAs may not be reflective of intestinal alteration of BAs after bariatric surgery.

A recent human study showed that FGF19 decreased a year after intensive medical management, but increased after RYGB compared with baseline. No correlation was seen between BAs and FGF19 (Sachdev et al., 2016). Likewise in a cross-sectional study, no relationship was observed between BAs and FGF19 a year after RYGB (de Hollanda et al., 2014). Further, despite a marked

increase in BA levels a year after BPD, a study showed that FGF19 decreased in comparison with baseline among obese people with T2DM (Ferrannini et al., 2015). Ferrannini et al. also suggested an early reduction of FGF19 after RYGB is due to the smaller area of ilium after bariatric surgery. However, they have reported a significant increase in FGF19 twelve months after RYGB. It should be noted that all (8 females and 1 males) subjects in the RYGB group were obese without diabetes while subjects in the BPD group (6 females and 9 males) were obese with diabetes. Moreover, they postulated that increased BAs after bariatric surgery may be independent of FGF19 signalling. Increased plasma levels of FGF19 a year after RYGB confirms the previous human prospective study which reported a significant rise in fasting plasma levels of FGF19 a year after RYGB among obese people with T2DM (Jørgensen et al., 2014).

Postprandial FGF19 compared between SG and RYGB and no significant difference observed between the time points at one year after SG and RYGB. Nevertheless, in this study postprandial FGF19 did not reach significantly higher levels of concentration after 2hpp. It has been shown that the highest level of FGF19 was observed 2-3 hours after a meal (Lundåsen et al., 2006). Although the exact reason for the non-significant difference between fasting and postprandial level of FGF19 is unclear, this may suggest two hypotheses. First, a diabetic state might influence 2hpp levels of FGF19, based on the peak of postprandial FGF19 reported among healthy people (Lundåsen et al., 2006). Second, increased level of postprandial BAs attenuates the level of postprandial FGF19 because it has been shown in human studies that FGF19 concentrations are changed by different concentrations of BAs (Jørgensen et al., 2014).

5.3.3 FGF19 to Total Bile Acid Ratio

For the first time, the fasting ratio of FGF19/total BAs was computed from findings in this study. The FGF19/total BA ratio was significantly reduced a year after both surgeries, but the fasting FGF19/fasting TBA ratio was significantly higher a year after SG compared to RYGB.

The ratio of fasting FGF19 to TBA maybe reveal a new biomarker to evaluate endoplasmic reticulum stress after bariatric surgery. Obviously, TBA are increased by bariatric surgery. Also, a direct correlation between FGF19 and increased endoplasmic reticulum stress has been reported in animals (Shimizu et al., 2013). Nevertheless, a link between obesity and diabetes and endoplasmic reticulum stress has been documented (Eizirik et al., 2008; Özcan et al., 2004). Taken

together, these associations inspired the researcher to compute and evaluate the ratios and their correlations with other variables in this study. Although reduction of the ratio of fasting FGF19 to fasting TBA may be considered as a novel biomarker to assess the progress of diabetes improvement, more studies in different populations are needed to confirm the ratio's utility.

FGF19 has a negative feedback that leads to suppression of hepatic synthesis of BAs (Shin & Osborne, 2009). In a human randomized, double-blind, placebo-controlled clinical trial, FGF19 injection reduced BA synthesis significantly (J. Luo et al., 2014). Also, in patients with Crohn's disease and ilium resection, FGF19 significantly reduced while BAs increased 5- to 20- fold (Lenicek et al., 2011). This study showed that FGF19 increased a year after both SG and RYGB. This trend is in agreement with previous studies indicating an increase of FGF19 after bariatric surgery (Jørgensen et al., 2014; Rysz et al., 2015). However, it is important to note a few differences between this current study and previous studies. Firstly, in most of these studies females were exclusively involved (Dutia et al., 2015; Haluzíková et al., 2013; Tremaroli et al., 2015) Secondly, human studies indicated a rise of FGF19 only seen in studies with higher remission rate (Barutcuoglu et al., 2011; Gerhard et al., 2013; Sonne et al., 2016) Thirdly, there is no RCT to evaluate the effects of FGF19 between SG and RYGB. Fourthly, previous studies mostly measured FGF19 on a homogenous population (e.g., Caucasians) (Gerhard et al., 2013; Sonne et al., 2016) while this study benefited from a heterogeneous population. Fifthly, previous studies on FGF19 performed in a relatively small sample size in comparison with this study (Haluzíková et al., 2013; Sonne et al., 2016). Finally, fasting and postprandial FGF19 measured in the both bariatric surgeries to see if fasting or feeding state has any determining effect on FGF19.

5.4 BAs, FGF19 and Diabetes

FGF19, which plays a major role in regulating BA metabolism through FXR (F. G. Schaap, 2012), cannot simply be used as a medication for the treatment of diabetes due to its carcinogenic concerns (Hyeon et al., 2013). Animal studies have indicated transgenic FGF19 mice had lower body weight (Tomlinson et al., 2002) while in another different study, treatment by FGF19 increased plasma level of triglycerides and cholesterol, and as a result induced weight gain (X. Wu et al., 2013). It may highlight that there is a dual mysterious effect of FGF19 under different conditions (F. Zhang et al., 2015). In humans, also, the role of FGF19 is controversial. For example, a human study showed that plasma levels of FGF19 are similar between diabetic and non-diabetic subjects

(Brufau, Stellaard, et al., 2010) while another observed that there are reduced levels of FGF19 among people with diabetes (F. G. Schaap, 2012). Taken together, the expression of FGF19 is regulated by a complex regulatory pathway and factors such as chemical, oxidative and endoplasmic stress play crucial roles to alter levels of FGF19 (F. Zhang et al., 2015). Thus, more studies are required to understand the FGF19 mechanism among obese people with diabetes after bariatric surgery.

Due to the direct effect of FGF19 on BA metabolism, adding FGF19 to studies is inevitable to study the pathophysiological effect of BAs on metabolic diseases. In fact, FGF19 is a main reason why fibroblast growth factors are mostly considered hormones rather than standard growth factors (Kohli & Seeley, 2013). FGF19 is synthesised in the ilium and performs its role in the liver and gallbladder where BAs are produced and stored, respectively. It has been shown that a regulatory effect of FGF19 occurs through FXR (Prawitt et al., 2011).

FGF19 is still viewed as a less popular option for treatment of T2DM compared to BAs. The administration of FGF19 has shown promise for the treatment of diabetes in animals through its ability to regulate BAs (Rysz et al., 2015). However, there are currently no studies in humans due to FGF19's effect of inducing cancer cells (Nicholes et al., 2002). One of the important issues which should be considered is the lack of FGFR4 in the animals studied, while the receptor is present in humans (Rysz et al., 2015). Therefore, merely translating the compensatory effect of FGF19 from animals to humans can be a source of serious concerns. Nevertheless, FGF19 has only ~51% similarity with its orthologue, FGF15, in animals (F. Zhang et al., 2015). Also, the role of FGF19 to improve insulin sensitivity in humans is controversial. For instance, it has been indicated that FGF19 is not related to nutritional status and insulin sensitivity (Dostálová et al., 2008). Furthermore, treatment using colesevelam in diabetic people showed that there was no relationship between FGF19 and diabetes improvement, while a strong negative correlation was observed between BA synthesis and FGF19 (Brufau, Stellaard, et al., 2010). In this study FGF19, either in fasting or postprandial contexts, was found to be higher at 12 months in comparison with baseline values; and the exact reason(s) for this remains to be investigated with further studies.

5.5 Bariatric Surgery and Diabetes Indices

In this study, seven diabetes indices include HOMA-IR, insulin sensitivity index or Matsuda index, QUICKI, Belfiore index, Stumvoll index, IGI, and HOMA-B were computed using OGTT results before surgery and a year after both SG and RYGB. A comparison between the time point one year after SG and RYGB was performed to observe if either of the given surgeries is superior to the other one.

In this study, OGTT was utilised to assess glucose tolerance because it is a reliable test (Bradley et al., 2012) and surrogate indices which are incorporated from OGTT were calculated to assess insulin resistance/sensitivity (Muniyappa et al., 2008b).

5.5.1 Effect of SG on Diabetes Indices

Insulin resistance obviously improved 12 months after SG in comparison with its baseline. HOMA-IR, a well-known clinical surrogate marker of hepatic insulin resistance (Tripathy et al., 2004), was found to be decreased by 42% a year after SG. Conversely, Matsuda index, Stumvoll index and IGI, which are markers of insulin sensitivity, showed a significant increase. In addition, HOMA-B, an epidemiological marker of insulin sensitivity, reduced a year after SG. Two less presented insulin resistance indices, QUICKI and Belfiore indices were also significantly increased a year after SG.

Prevalence of obesity and its comorbidities such as T2DM is increasing sharply (Sturm & Hattori, 2013) and bariatric surgery is the most trusted intervention for obese people with T2DM compared with other interventions (Schauer et al., 2017). SG is one of the common procedures to improve insulin resistance. In agreement with this study, a recent human study among obese people who underwent SG showed a significant reduction of HOMA-IR a year after SG and authors concluded reduced level of cholesterol results in improving insulin resistance (De Vuono et al., 2017). Also, in parallel to the study, it has been shown that weight loss significantly reduced HOMA-IR and improved insulin sensitivity one and two years after SG (Abbatini et al., 2010; Peterli et al., 2012). Although different populations have different ranges of HOMA-IR (Gayoso-Diz et al., 2013), in this study, postoperative HOMA-IR scores among patients who underwent SG were determined to be within the normal range in comparison with baseline values, which is in accordance with the previous studies (Cătoi et al., 2016). The exact reason of improving insulin resistance (reduced

HOMA-IR) after SG is yet to be found. However, the role of ghrelin along with postprandial rises in PYY and GLP-1 and hindgut theory (Peterli et al., 2012; Roslin et al., 2014) are highlighted to cause the reduction in HOMA-IR score after SG. In other words, ghrelin which is found in the gut fundus is capable of blocking insulin secretion. Removing gastric fundus in SG helps to improve insulin secretion (Papailiou et al., 2010). Additionally, PYY and GLP-1 are likely to be elevated after SG due to gastric emptying (hindgut theory) (Malin & Kashyap, 2016).

QUICKI significantly increased a year after SG. QUICKI is an approved, powerful, reliable and reproducible mathematical measurement of fasting glucose and insulin concentrations with very high positive correlation with insulin sensitivity (Muniyappa et al., 2008b). QUICKI is a valuable index to evaluate people with insulin resistance (Hanley et al., 2003). Thus, QUICKI is an appropriate index in clinical and longitudinal research to assess changes in insulin sensitivity in the absence of a "clamping method."

One of the important risk factors of diabetes is multi-organ insulin resistance and/or insufficient insulin secretion from pancreatic beta cells. In people with normoglycemia, there is an appropriate response to insulin to glucose after OGTT. In other words, once glucose is ingested an appropriate concentration of insulin is released to aid in the uptake of excessive glucose from the plasma (R. A. DeFronzo, 1999). Obviously, however, among people with diabetes, this pathway is dysfunctional (Bradley et al., 2012). Thus, finding a procedure to improve insulin sensitivity is the highest point of interest for the treatment of diabetes. Insulin plays an axial role to regulate numerous metabolic functions in the body, and abnormal insulin sensitivity represents several metabolic complications (C. R. Kahn, 1978). In this study BCF is evaluated after measuring fasting and postprandial insulin levels, using IGI and Matsuda index. Obviously, there is no definite clinical test to assess beta cell function with the highest accuracy and precision except by using the "clamping method" which is very time and labour consuming (Ferrannini & Mingrone, 2009). However, evaluating indices such as Matsuda index and IGI may be helpful to assess insulin secretory and glycaemic control in long-term and acute changes of BCF (Ferrannini & Mingrone, 2009). For example, calculation of the Matsuda index requires values for fasting glucose and insulin to reflect hepatic insulin sensitivity and meaning for glucose and insulin to assess skeletal insulin sensitivity (Abdul-Ghani et al., 2007). IGI and Matsuda index values were both increased after SG, which may be in part related to reduced body mass. IGI, which is calculated from insulin

and glucose, exhibits an index of early insulin secretion in relation to the change of glucose (Seltzer et al., 1967). IGI significantly increased a year after SG. SG and RYGB elevate the rate of delivery of consumed glucose into peripheral circulation which results in a peak of glucose in the plasma (Peterli et al., 2009; Roslin et al., 2012), while glucose is the main inducer of insulin secretion. Thus, insulin is released to uptake glucose. This is a potent factor to show how SG and RYGB can improve BCF.

Taken together, in line with recent studies that evaluated the effect of SG on insulin resistance/sensitivity in either short-term or long-term time points (Casella et al., 2016; Mingrone & Cummings, 2016; R. Sharma et al., 2016), this study suggests that SG is more than a restrictive procedure to remit T2DM.

5.5.2 Effect of RYGB on Diabetes Indices

All indices for SG mentioned above were calculated for the subjects before RYGB surgery and 12 months after RYGB. Insulin resistance improved a year after RYGB because HOMA-IR reduced by ~29% and it was in a normal range. Also, the Matsuda index and IGI improved a year significantly after RYGB. As reported for SG results, HOMA-B reduced a year after RYGB while QUICKI and Belfiore index values reached a significant rise 12 months after RYGB.

HOMA-IR scores were significantly reduced among people who underwent RYGB. Although in this study acute improvement of insulin resistance was not examined, it is likely that improved insulin resistance occurred early after RYGB due to the consumption of low-calorie diet after RYGB (Isbell et al., 2010; Lim et al., 2011). Undoubtedly, anatomical alterations, particularly in the upper gastrointestinal system, play a profound role in the remission of diabetes after RYGB (Buchwald et al., 2009). Findings from this study are in accordance with several previous studies which showed a significant reduction of HOMA-IR and improved insulin resistance a year after RYGB (Bradley et al., 2012; Cazzo et al., 2016; Trastulli et al., 2013; Vix et al., 2013). It should be noted that BMI is the most important predictor of insulin resistance (Camastra et al., 2011; Campos et al., 2010). It is why bariatric surgery is also called “metabolic surgery.” Thus, improved insulin resistance after bariatric surgery may partially be related to reduced weight and BMI. Although, it needs more studies to confirm due to the controversy (C.-Y. Chen et al., 2013). Another reason for improved insulin resistance after RYGB could be due to higher scores of

HOMA-IR preoperatively because it has been shown that people with normal or lower scores of HOMA-IR did not show a significant reduction of HOMA-IR after bariatric surgery (Vix et al., 2013)

Currently, RYGB is viewed as the superior and efficient bariatric surgery method for the treatment of diabetes in comparison to intensive diet and pharmacotherapy (Schauer et al., 2017). It has been suggested that remission of diabetes after RYGB is mainly due to the reduction of insulin resistance rather than an effect of incretin hormones in non-morbidly obese subjects (C.-Y. Chen et al., 2013; W.-J. Lee, Chong, Chen, et al., 2011). Although, in other studies, it has been indicated that enhanced insulin secretion and reduced HOMA-IR are associated with higher activity of GLP-1 in diabetic subjects who underwent RYGB (Ł. Kaska et al., 2015). In prospective human studies, results showed that IGI significantly increased a year after RYGB along with reduction of HOMA-IR scores which are in agreement with this study (C.-Y. Chen et al., 2013; W.-J. Lee, Chong, Chen, et al., 2011; D. J. Pournaras et al., 2010). Insulin has a biphasic pattern. The first phase of insulin secretion takes a few minutes while the second phase lasts for much longer. Among people who have impaired fasting glucose the first phase is reduced whereas first and second phases are reduced in T2DM (Bacha et al., 2009; Kanat et al., 2012). Due to the substantial dynamic effect of early insulin secretion in the pathogenesis of T2DM (Seino et al., 2011), in this study, IGI was measured to observe early changes of insulin secretion before and after bariatric surgery. Increased IGI after RYGB in comparison with preoperative values indicated improved early secretion of insulin for the uptake of glucose from the plasma and may somehow be related to the remission of diabetes after RYGB. Nevertheless, this trend is also observed a year after SG.

Matsuda index values increased by 122% a year after RYGB in this study. This is a consistent continuation of results from a study which showed that a rise of Matsuda index values is observed 6 months after RYGB (Navaneethan et al., 2010). In addition, this is in accordance with previous studies which showed a significant increase of IGI and Matsuda index a year after RYGB (García-Fuentes et al., 2006; Morínigo, Lacy, et al., 2006). This is also in accordance with the recent study which compared RYGB with AGB (Holter et al., 2017). Holter and colleagues concluded that increased Matsuda index a year after bariatric surgery is independent of the type surgery. In other words, losing weight is likely to be the main reason of insulin sensitivity after bariatric surgery. Of note, interpretation of insulin resistance/sensitivity after RYGB needs to be done carefully.

Contrary to SG outcomes, after RYGB a comprehensive anatomical alteration has occurred and leads to transferring glucose for ingestion sooner in the surgically modified gastrointestinal system compared to the non-surgically modified system. Also, increased QUICKI after RYGB is in agreement with the previous study which indicated a raised QUICKI score a year after RYGB (Salinari et al., 2013).

5.5.3 A Comparison between One-year after SG and RYGB

Apart from the Belfiore index results, which were slightly but significantly higher in SG than RYGB, no significant differences were observed after a year between SG and RYGB. It should be noted that all subjects, regardless of the type of surgery, underwent an intensive low-calorie diet (i.e., VLCD) for at least two weeks before bariatric surgery. It has been shown that calorie restriction plays a pivotal role in insulin sensitivity. For example, consumption of a low-calorie diet of about 1100 kcal/day for less than 2 days reduces intrahepatic storage of triglycerides and HOMA-IR. As a result improving insulin sensitivity (Kirk et al., 2009). Moreover, early consumption of VLCD may lead to a rapid synthesis reduction of glycogen and glucose in the liver and cause a rise of insulin sensitivity (Bradley et al., 2012). Therefore, similar changes of insulin resistance/sensitivity indices between a year after SG and RYGB may be, in part, because of taking VLCD at the baseline. Nevertheless, in line with HOMA-IR results from this study, several studies did not observe any difference between SG and RYGB (Benaiges et al., 2011; Boza et al., 2012; Chouillard et al., 2011; Griffo et al., 2016; Iannelli et al., 2011; Kehagias et al., 2011; Menguer et al., 2017; Peterli et al., 2017). No significant weight difference was observed between the studies' results a year after SG. The effect of weight loss on the long-term improvement of insulin sensitivity is highlighted. It has been shown that computed glycaemic indices from OGTT are positively related to weight loss (Nannipieri et al., 2011). In addition, bariatric surgery implies several changes such as alterations in adipokine secretions due to the reduced body fat, reduction of inflammatory responses, alterations in several gut hormones, molecular changes and aversion to sugary food (Arab et al., 2017; R. Rao et al., 2012). Finally, in this study, it was determined that total cholesterol, triglycerides, and low-density lipoprotein levels be reduced a year after both bariatric surgeries. This reduction in lipid profile could also partly be related to improved insulin sensitivity/resistance (Tewari et al., 2015).

The researcher is aware of conflicting findings. Previous studies have reported that insulin sensitivity improved more in RYGB than SG (Kashyap et al., 2013) and in another study, SG showed better improvement than RYGB (Abbatini et al., 2010). This conflict in study results may be in part related to the method of insulin sensitivity assessment. In the former study, Kashyap and colleagues used a meal test, while in the latter study Abbatini et al. used a hyperinsulinemic-euglycemic clamp to assess insulin sensitivity. Taken together, improved insulin resistance after bariatric surgery is a cluster of several known and unidentified mechanisms.

Data from this study showed improved insulin resistance/sensitivity and glycaemic control. Undoubtedly, bariatric surgery rather than pharmacotherapy is more efficient to improve insulin resistance (Schauer et al., 2017). However, there are several issues that should be considered when considering one method of treatment over the other. First and likely the most important consideration is the fact that T2DM is a multifactorial disease. It means that beta cell dysfunction and insulin resistance states can be varied in different patients with different ages, genders, and conditions (Færch et al., 2016; G.-F. Wang et al., 2015). Studies with lack numbers of subjects who were off-medications after bariatric surgery is another important confounding factor in providing an easy interpretation of results for the efficacy of bariatric surgery (Jiménez et al., 2012; Robert et al., 2013). Also, unfortunately, there is no universally accepted definition for the remission of diabetes yet (Blackstone et al., 2012; D. Pournaras et al., 2012). Another issue for consideration is computing HOMA-IR and B indices to observe the improvement of diabetes. HOMA-IR is calculated from fasting glucose and insulin, and the potentially dynamic effect of insulin and glucose which occurs post-prandially is missed (Purnell et al., 2016). For this reason, it is almost always necessary to perform OGTT, which is not a convenient test for the patients. Factors such as alteration in energy balance, lack of a standard method to measure insulin or beta-cell dysfunction (Lima et al., 2010; Muniyappa et al., 2008a; Wallace et al., 2004) can seriously impact on the interpretation of the result. Moreover, it is not always easy to compare data from different bariatric surgeries on the remission of diabetes due to a variety of differences such as different populations and different methods being used to stimulate insulin secretion. Furthermore, types of bariatric surgery can impact on the interpretation of diabetes indices. For instance, Matsuda index after RYGB can be difficult to use due to the alterations in glucose fluxes in comparison with non-surgically modified intestine (R. Rao et al., 2012). In this study, the effect of bariatric surgery was evaluated over the course of one year. The duration time of follow-up

beyond surgery, relative to diabetes might have an impact on interpreting the beneficial effect of bariatric surgery. This idea is supported by the fact that it has been shown that longer follow-up times with a shorter duration of diabetes gained more benefits among obese people with diabetes who had undergone bariatric surgery (Baskota et al., 2015). Finally, the "clamping method" which is the "gold standard" to evaluate insulin sensitivity was not performed for inclusion in this study's results due to its difficulties. Notably, the method has recently been shown to have comparable results with OGTT (Bojsen-Moller et al., 2017).

5.6 Energy Homeostasis and Bariatric Surgery

Using four different protocols include rest energy expenditure, BIA, DXA and traditional anthropometric measurements body composition were assessed at baseline and a year after SG and RYGB. One year after bariatric surgery in both surgery groups a meaningful reduction of several variables was observed. Thus, understanding the underlying mechanism of these reductions after bariatric surgery may be useful to find out more details on compensatory effects of bariatric surgery on diabetes remission.

Bariatric surgery is a procedure to reduce weight through a negative balance between consumed energy and energy expenditure. Bariatric surgery reduces energy expenditure while inducing weight loss. This reduction is sufficient to alter body frame and composition (Benedetti et al., 2000). Thus, reduced energy expenditure after bariatric surgery may cause weight loss. For instance, in a recent systematic review of assessing body composition using BIA and DXA in SG and RYGB subjects, results indicated that dramatic reduction of total body fat and lean body mass occurred in the first three months after bariatric surgery in parallel to weight loss (Ito et al., 2016). Also, a strong association between LBM and energy expenditure has been reported (Gomes et al., 2016). In addition, bariatric surgery affects central and humoral functions leading to reduced appetite and improved satiety (Buchwald et al., 2009; R. S. Rao, 2012). Furthermore, gut microbiota plays a contributory role in energy homeostasis (Kamvissi-Lorenz et al., 2017; Liou et al., 2013; Tremaroli et al., 2015).

5.6.1 Effect of SG on Body Composition Assessment

Obesity (along with its comorbidities such as T2DM) is a state of imbalance between input and output energy (S. E. Kahn et al., 2014). Obesity is associated with insulin resistance, and gaining weight increases the risk of insulin resistance (B. B. Kahn & Flier, 2000).

Although the exact underlying mechanism of reduction in REE after SG is still unclear, post-operative SG patients have less appetite than those patients who underwent RYGB (Miras & Le Roux, 2014), Thus, reduced REE may be related to a change of eating habits. In addition, an increased level of meal-stimulated hormones due to raised gastric emptying after SG could be another reason for a reduction in REE after SG (Haluzíková et al., 2013; Ionut et al., 2013; Karamanakos et al., 2008; Mells & Anania, 2013). In this study, results have determined that REE reduce a year after SG. This is in concordance with previous study results which indicated reduced REE a year after SG (Tam et al., 2016).

Respiratory Quotient (RQ) was significantly increased one-year after SG. RQ is a direct volumetric ratio of carbon dioxide and oxygen, V_{CO2}/V_{O2}, and provides a ratio to calculate substrate oxidation amounts for glucose and fats (Oshima et al., 2016). RQ is an important indicator to estimate energy utilisation in the body. The greater value of RQ, the lower rate of fat oxidation (Stylopoulos et al., 2009). Nowadays, it is accepted that stored triglycerides in the liver are hydrolysed to fatty acid and glycerol, and insulin inhibits this pathway (Himsworth, 1939). Obesity leads to rising levels of circulating free fatty acids, and this increase leads to inhibition of glucose oxidisation. It has been shown that obese people mostly oxidise fats as opposed to carbohydrates (Golay et al., 1984). Reduced free fatty acid levels after BPD leads to an increase in RQ, and this trend may result from increased gluconeogenesis (Benedetti et al., 2000). After removing the fundus in SG, some alterations occurred in gut hormones such as increased GLP-1 (Peterli et al., 2012) leading to improved carbohydrate metabolism. The value range of RQ is often between 0.7 and 1.0. The values nearer to 0.7 and 1.0 means fat and carbohydrate oxidation respectively (Haugen et al., 2007). There is a link between oxidisation of free fatty acid and T2DM (G Boden, 2003). Taken together, it is likely that increased RQ values are somehow related to the improvement of insulin resistance/sensitivity after SG.

TBF and LBM were significantly reduced a year after surgery. This finding is in agreement with a human non-randomised prospective study that compared the effect of SG on body composition at baseline and a year after SG (BuŽga et al., 2015). In the BuŽga study, the role of ghrelin is highlighted to explain altered anthropometric measurements.

There is scant information about the effect of SG on body composition among obese people with T2DM (Madsbad et al., 2014). Thus, more studies are needed to confirm these findings, particularly for DXA and BIA measurements.

5.6.2 Effect of RYGB on Body Composition Assessment

RYGB is a malabsorptive procedure. It has been shown that chronic calorie restriction leads to reduced REE (M Bueter & Le Roux, 2011). REE, which expresses the function of metabolically active tissues, is expected to reduce postoperative in parallel with the reduction of FFM, LBM, and TBF. REE reduced by 13% a year after RYGB in comparison with baseline. Also, a year after RYGB, TBF and LBM were significantly reduced. In a recent RCT with a 12-month period, it was determined that a significant reduction of TBF and LBF after RYGB occur (Coen et al., 2015). Body composition in this study was assessed at two-time points, baseline (preoperative) and 12 months (postoperative) after a given bariatric surgery. However, data from this short-term RCT may be linked to the fact that early improvement of energy metabolism occurs after RYGB. Presumably, the main reason for reductions in TBF and LBM is due to weight loss after RYGB. In addition, data from this study agree with previous research, showing a decline of TBF, REE, and FFM in obese people with or without diabetes one year after RYGB (Dirksen et al., 2013; Tam et al., 2016). Parallel to findings of this study, a recent prospective human study also showed that TBF and LBM were reduced one year after RYGB among obese people without T2DM (Magkos et al., 2016).

It has often been observed that a few days after RYGB, glycaemic control has been achieved, without significant weight loss, and there is no longer need to take anti-diabetic medications (Bradley et al., 2012). Early after RYGB, the main reason for this achievement is a noticeable decrease in energy intake, which is probably due in turn to the effect of VLCD (Isbell et al., 2010). However, data from this study showed that REE is still lower than baseline even a year after RYGB, and most patients are off-medication without consumption of VLCD at this point. Thus,

reduction of REE after bariatric surgery may not have resulted from only taking VLCD. Nevertheless, the exact reason of weight loss after RYGB is unclear, but postoperative changes in REE may play a major role in weight loss after RYGB (Dirksen et al., 2013). Reduction of TBF may be another strong contributor to reduce REE, because fat tissue plays a crucial role to the alteration of REE and as observed in this study, a prospective one year follow-up study among obese people without diabetes showed a significant reduction of LBM, TBF and REE after RYGB (Das et al., 2003). Also, a human prospective follow-up study showed TBF, LBM, REE, and waist and hip circumferences significantly reduced 6 months after RYGB (Carrasco et al., 2007). Carrasco and colleagues also introduced a predictor effect of HOMA-IR on changes of body composition. Most probably, reduced REE after RYGB is not related to more physical activity or raised body temperature (Marco Bueter et al., 2010). Factors such as alterations in gut hormones, brown adipose tissue activity, and upper gastrointestinal system may have contributory effects to modulation of REE after RYGB (Albaugh et al., 2016; Bächler et al., 2016).

Android, visceral or intra-abdominal, abdominal, leg and arm fat were measured before and a year after RYGB. All of them were significantly reduced after RYGB. One study showed that visceral fat was significantly reduced after RYGB, but the reduction is not correlated with insulin sensitivity (Fabbrini et al., 2010). However, in a recent prospective study among obese people with diabetes, it was demonstrated that not only visceral fat, TBF, and LBM decreased a year significantly after RYGB, but the reduction of visceral fat is also associated with improvement of insulin resistance (Tan et al., 2016). Tan et al. highlighted the role of reduced branched-chain amino acids in their finding. Branched-chain amino acids have been reported to modulate insulin resistance after RYGB (Lips et al., 2014). Although in this study amino acids were not measured, reduction of visceral and abdominal fat may, in part, be related to changes in amino acid levels.

5.6.3 SG vs. RYGB and Body Composition Assessment

In this study, no comparable variables were observed between time points taken one year after SG and RYGB, except for android and abdominal fat, which was significantly lower in RYGB compared to SG, There is a limited number of RCT information sources to compare a variety of body composition variables between one-year time points after SG and RYGB.

In a human case-control study which compared anthropometric and body composition between SG and RYGB in a 6-month period, it was shown that both bariatric surgeries had a similar effect to reduce TBF, FFM and waist circumference (Iannelli et al., 2011). Waist circumference, which is a potent marker of metabolic syndrome in morbidity obese people (K. G. M. M. Alberti et al., 2006), seemed to be non-significant between SG and RYGB. Thus, both bariatric surgeries had an equal effect on the improvement of the metabolic syndrome. Weight and BMI changes were similar after both bariatric surgeries. This may be associated with the non-significant difference between REE noted at the time point. In another prospective follow-up study which compared baseline and a year after SG and RYGB, and similar to this study, it was indicated that LBM and TBF were significantly reduced after SG and RYGB, with similar changes between both surgeries (Otto et al., 2016). Contrary to this finding, Strain, and colleagues found significant differences of LBM and TBF between SG and RYGB (Strain et al., 2009). This discrepancy may be due to the different size of samples and populations between the studies.

Both SG and RYGB have a propensity to reduce weight and treat obesity and diabetes (Buchwald & Oien, 2013). However, both SG and RYGB can be harmful to the skeleton, particularly regarding mineral metabolism, and undergoing these surgeries increases the risk of bone fracture (Ko et al., 2016). A different mechanism of action is postulated between SG and RYGB on bone metabolism. Due to the removal of the fundus in SG, ghrelin activity, which is known to stimulate osteoblasts, is altered while in RYGB, Also associated with this malabsorption procedure, by bypassing the small intestine , several hormonal and anatomical modifications occurred (Alexandrou et al., 2014; Vix et al., 2014). Data from this study shows that weight reduction was greater but not significant one-year after RYGB compared to SG individuals. In an animal study, it was reported that weight loss in RYGB is more than SG at the first year time point after surgery (Stemmer et al., 2013). In humans, however, it is unclear which bariatric surgery has a more detrimental effect on bone metabolism (Hsin et al., 2015; Nogués et al., 2010). In this study, BMD and BMC, visceral and abdominal fat were measured by DXA to evaluate the effect of SG and RYGB on bone metabolism. It is important to note that measuring by DXA to evaluate BMD is subject to artificial changes after extreme weight reduction (Javed et al., 2009). Therefore, the interpretation of the result after bariatric surgery needs to be carefully considered. BMD, BMC, and visceral and abdominal fat were significantly reduced 12 months after SG an RYGB, while no significant difference was observed between a year after SG and RYGB, except for abdominal fat

which was significantly higher in SG than RYGB. This finding is in accordance with recent cohort studies showing that a non-significant difference of BMD and BMC was observed between one year after SG and RYGB (Bredella et al., 2017; Ivaska et al., 2017). Changes of TBF also play a role in bone metabolism. It has been shown that there is an inverse correlation between TBF and BMD (Hsu et al., 2006). Additionally, visceral and abdominal fat has a negative and protective effect on bone metabolism, respectively (Stein & Silverberg, 2014). It should be noted that there are myriad of studies to evaluate the effect of RYGB on bone metabolism, but only a few studies have already done this in case of SG. Thus, more studies are required to understand more about the effect of SG on bone metabolism.

It has been shown that waist and hip circumference and their ratio are better risk indicators of diabetes than BMI (Consultation, 2008). In this study, waist and hip circumferences were significantly reduced a year after both SG and RYGB in comparison with their baseline measurements, but non-significant differences were observed between one- year post- SG and RYGB. Also, despite the reduction of waist to hip ratio, this ratio reached significance only a year after SG. Although the exact mechanism of this decrease remains to be elucidated, reduced visceral and android fat may have a contributory role to decrease waist and hip circumference and their ratio (T. Kang et al., 2012).

Taken together, several body composition and anthropometric measurements were reduced a year after both bariatric surgeries. It is important, however, to note that whether or not low energy metabolism supports a propensity to weight gain after bariatric surgery in humans is still unclear (Bächler et al., 2016; Flatt, 2007). This conflict may somehow be related to the method of measuring REE in different people with different body sizes. For example, two individuals with the same FFM and LBM have a different REE (Ravussin, 1993). Nevertheless, data from animal studies are more consistent (Bächler et al., 2016). The inconsistent findings in humans may be related to population discrepancies while animal studies are often characterised by homogeneous populations (Lutz & Bueter, 2014a). Increased RQ may also play a major role in the reduction of REE after bariatric surgery since the reduction of RQ results in increased REE (Lutz & Bueter, 2014a).

A balanced energy expenditure, therefore, can undoubtedly be a strong predictor of metabolic diseases and understanding the underlying mechanism(s) of alteration of body composition will certainly be helpful in attenuating metabolic diseases such as obesity and diabetes (Lam & Ravussin, 2016).

5.7 Association Findings

Regardless of the type of bariatric surgery, correlations between all available variables and FGF19, BAs and their ratios, were examined after the one-year follow-up. The main reason for presenting the results together, apart from sharing common mechanisms between SG and RYGB, was an equal effect of both bariatric surgeries on almost all of the different variables. For example, it has been shown that SG and RYGB often have a very similar impact on energy metabolism (Lutz & Bueter, 2014b). Another reason for presenting the results together is the lack of information on correlations of SG with body composition variables and diabetes indices. Also, instead of merely using correlations between fasting individual BAs, AUCs of BAs were selected to make sure that not only the fasting state was added for correlation, but dynamic (postprandial) effects of individual BAs are also tested.

Fasting FGF19 values and fasting FGF19 to TBA ratios, along with fasting BA fractions have also been selected for their association with examinations. No significant association was observed between FGF19 and the AUC of BAs and their ratios. This finding agrees with previous studies which indicated non-significant correlations between FGF19 and BAs after bariatric surgery (de Hollanda et al., 2014; Gerhard et al., 2013; Jørgensen et al., 2014). Although, in a recent non-randomised clinical study a vigorous and positive correlation was observed between TBA and FGF19 (Dutia et al., 2015). This positive correlation is contrary to the effect of FGF19 on BA metabolism (Chiang, 2009). Although Dutia and colleagues did not provide a reason for this discrepancy, it may be related to the design of their study, because only 13 females were recruited in their study and no men were involved. Other factors such as preoperative weight and higher values of HOMA-IR, which means a poor glycaemic control, may also help to explain this discrepancy. However, in a human study that used a BA (colesevelam) orally administrated to control and diabetes groups within an 8-week period, a strong negative, significant correlation was observed between FGF19 and BAs at baseline (before administration) within the control group while a non-significant correlation was seen in patients with diabetes (Brufau, Stellaard, et al.,

2010). Collectively, it seems that the association between BAs and FGF19 is not only controversial, but this association is also vulnerable to interpretation due to the different assays used to investigate their characteristics.

Non-significant associations were observed between TBA and glycaemic indices, which is in agreement with a recent cross-sectional study among obese people with diabetes (Wewalka et al., 2014a). In addition, regarding BA fractions, significant correlations were only observed between 3 ratios (primary, primary/secondary, and primary conjugated Bas) with diabetes markers and the rest did not reach significant levels of correlation. However, BA fractions were found to be more associated with body composition variables. Finally, FGF19 was examined for all variables, and significant correlations were only observed between weight and RQ with FGF19.

5.7.1 Bile Acid, FGF19, and Weight, BMI Correlations

The prevalence of obesity is increasing sharply, and it is predicted to reach 700 million people in 2015 (James, 2008). Currently, unfortunately, there is no exact treatment for obesity. However, several mechanisms have been suggested to consider for treatment of obesity. One of the proposed mechanisms is the effect of BA regulations in obesity (Glicksman et al., 2010). Thus, a correlation test performed to find any association between BAs and BMI, which is an acceptable index for obesity, is of use. In this study, BMI had weak but significant negative correlations with the AUC of three glycine-conjugated BAs; namely, GCA, GDCA, and GCDCA. In agreement with this study, in a cohort study of 11 healthy subjects, it was shown that the AUC of glycine-conjugated BAs were inversely associated with BMI (Suzuki et al., 2014). Furthermore, a recent non-randomised clinical study showed a negative correlation between secondary BAs and their conjugated forms with BMI, but non-significant association between TBA, 12α-OH, and conjugated/unconjugated BAs a year after (RYGB) bariatric surgery among obese subjects with diabetes (Dutia et al., 2015).

However, in a relatively small RCT with 7 obese individuals without diabetes, subjects indicated an inverse association between TBA and BMI one year after bariatric surgery (SG and RYGB) (R. E. Steinert et al., 2013). The inconsistency may be related to the different number of subjects and lack of diabetes in their study.

BAs receptors may be another reason for the association between BAs and BMI. TGR5 and FXR, through their ligands, probably play a fundamental role in being able to observe this negative correlation (Noel et al., 2016; Svensson et al., 2013). For example, GDCA, a glycine-conjugated form of DCA, is a potent endogenous ligand for FXR (Haibo Wang et al., 1999), and increased levels of GDCA are inversely associated with weight and BMI reduction (Yu et al., 2015). This negative correlation between BAs and BMI suggests that BAs in combination with other mechanisms may have a contributory role in altering obesity. Nevertheless, this hypothesis is controversial. As it has not been confirmed, the exact effect of BAs on changes of BMI after bariatric surgery is unknown (Jørgensen et al., 2014; Kohli, Bradley, et al., 2013b; R. E. Steinert et al., 2013). Nevertheless, the exact underlying mechanism of BAs on weight loss remains to be elucidated. Therefore, more studies are needed to be carried out to understand underlying mechanisms of BAs on obesity status.

Bariatric surgery is the best choice to reduce weight (Buchwald & Oien, 2013). Several mechanisms are postulated regarding weight loss after bariatric surgery, such as increased levels of BAs and FGF19 concentrations (Haluzíková et al., 2013; D. J. Pournaras et al., 2012). In this study, the correlation between BAs and FGF19 were assessed with weight. Despite weight loss after bariatric surgery, no significant correlation was observed between fasting ratios and AUCs of BAs with weight. It may suggest that BAs on their own do not influence weight reduction. This is in agreement with the pervious study indicating no correlation between weight and BAs (Gerhard et al., 2013).

Interestingly, however, increased fasting plasma levels of FGF19 are inversely and significantly associated with weight. It confirms the effect of FGF19 on nutritional status (Mráz et al., 2011). In animals, studies showed that overexpression and exogenous administration of FGF19 lead to reduced weight with antidiabetic and hypolipidemic effects via several gene interactions (L. Fu et al., 2004; Tomlinson et al., 2002). This suggests that in FGF19 transgenic mice, white adipose tissue decreased and brown adipose tissue increased at the same time, along with an increase in energy expenditure and reduction in triglycerides (Tomlinson et al., 2002). However, insufficient information is available for the effect of FGF19 on weight in humans (Haluzíková et al., 2013; Lundåsen et al., 2006; Schreuder et al., 2010). The negative correlation of FGF19 with weight is in agreement with a human prospective non-randomised study showing a negative correlation

between FGF19 and weight in 17 obese women a year after bariatric surgery (SG) (Haluzíková et al., 2013). Although the exact mechanism of this negative correlation is unclear, FGF19 is an interesting endocrine hormone with several compensatory effects on the body. The negative correlation between FGF19 and weight may in part depend on the effect of FGF19 on the reduction of triglycerides and cholesterol or increasing energy expenditure (S Kir et al., 2011). Obesity is an inflammatory disease (Saltiel & Olefsky, 2017) and FGF19 has an anti-inflammatory effect. It has been shown that reduced levels of FGF19 results in increased levels of cholesterol and inflammatory responses (Mutanen et al., 2015). Besides the reasons above, recent findings relative to the brain function show that in combination with FGF19, it has been suggested that weight reduction occurs through the signalling effect of FGF19 on the brain to regulate appetite (Stanley & Buettner, 2014). In a recent human clinical study, however, it was reported that a non-significant correlation exists between FGF19 and weight in a 12-month follow-up study (Sachdev et al., 2016), which is in disagreement with this study. Sachdev and co-workers designed a comparison between RYGB and intensive medical management in 15 subjects and assessed patients at two-time points, baseline and a year after RYGB. The discrepancy may result from the duration of diabetes onset since, in the RYGB group, diabetes duration was listed as 11 years. In addition, preoperative weight was relatively lower in their study among people who underwent RYGB in comparison with the weight of RYGB group in this study (105±3.3 vs. 116±3.7 kg, respectively). Finally, the number of subjects recruited in their study (n=15) was relatively lower than numbers used in this study.

Taken together, the increased level of FGF19 after bariatric surgery may play a contributory role in the remission of diabetes.

5.7.2 Bile Acids and Diabetes Correlations

BAs may have a contributory effect on insulin resistance/sensitivity after bariatric surgery through several mechanisms (Vítek & Haluzík, 2016). Presumably, alterations of gut hormones after bariatric surgery is the most important reason (R. E. Steinert et al., 2013). For instance, BAs enable the stimulated secretion of GLP-1 via TGR5 (Parker et al., 2012). GLP-1 has a property to improve diabetes (Baggio & Drucker, 2007; Docherty & Le Roux, 2015). Under normal situations, GLP-1 is released after eating to induce insulin secretion and inhibit glucagon hormone (Holst, 2007). Moreover, GLP-1 plays a vital role to delay gastric emptying and prevent appetite, hence

improving glycaemic control (Steinert et al., 2017). A link between BAs, TGR5 and glycaemic control has already been reported in humans and animals (T. Adrian et al., 2012; Thomas et al., 2009). For instance and interestingly, in a recent study, it was reported that TGR5 is expressed by pancreatic beta cells (Kumar et al., 2016). Also, anatomical alterations and modifications after bariatric surgery facilitate delivery of BAs to L-cells, where TGR5 is expressed (Peterli et al., 2012; D. J. Pournaras et al., 2012). Thus, TGR5 is likely to mediate glucose homeostasis through increasing GLP-1 and repressing hepatic glycogenolysis (Martinot et al., 2017). Furthermore, the inflammatory state is an important risk factor for insulin resistance (Ehses et al., 2010), and BAs have anti-inflammatory effects through TGR5 (Kawamata et al., 2003).

FGF19 was increased a year after bariatric surgery, and FXR plays a crucial role in regulating FGF19 metabolism (Keely & Walters, 2016). Within intestine cells, FXR activates the expression of FGF19. Another reason may be related to the combined effect of FXR and FGF19 since FXR knockout mice are at a higher risk of insulin resistance than wild type, and the effect of FGF19 to inhibit gluconeogenesis has been reported in animals (Serkan Kir et al., 2011; K. Ma et al., 2006). It was found that AUCs of conjugated BAs, namely, TUDCA, GCA, GDCA, and GCDCA are inversely correlated with the Belfiore index, a surrogate marker for insulin resistance. BAs and their conjugated forms are potent ligands for FXR (Martinot et al., 2017). Besides the role of FXR in BA homeostasis, FXR has also been proposed to regulate lipid metabolism, and due to the link between hypertriglyceridemia and diabetes (Sniderman et al., 2001), it plays an important role to regulate glucose metabolism (Duran-Sandoval et al., 2004). Likewise, FXR also has an anti-inflammatory role (Maran et al., 2009). The compensatory effects of FXR on glucose metabolism result from animal studies, and there is no definite study on humans yet. Nevertheless, there is a significant difference in BA homeostasis between humans and animals (Thomas, Pellicciari, et al., 2008). Cholic acid may have a major role in improving glycaemic control. A negative correlation was observed between AUCs of CA and fasting insulin, insulin AUCs, HOMA-IR and IGI values. It has been already shown that CA is inversely associated with insulin sensitivity (Cariou et al., 2011; Gerhard et al., 2013) and CA (natural FXR ligand) via either FXR-dependent or FXR-independent mechanisms can suppress gluconeogenesis (Arab et al., 2017).

Interestingly, a weak but significant negative association was only observed between primary, primary/secondary and primary conjugated BAs with HOMA-IR and fasting glucose, while a

positive correlation was found between primary, primary/secondary and primary conjugated BAs and QUICKI, which is a marker of insulin sensitivity. This suggests that gut microbiota in part may play a major role in the improvement of diabetes after bariatric surgery. Nevertheless, gut microbiota was not investigated in this study. Gut microbiota, however, may be another important contributory factor for the improvement of diabetes after bariatric surgery (J.-L. Han & Lin, 2014). Bariatric surgery alters gut microbiota, while gut microbiota plays an important role in the conversion of primary to secondary BAs (Martinot et al., 2017). Primary BAs (CA and CDCA) are synthesised from cholesterol in the liver. Once primary BAs have reached the intestine, gut microbiota converts them to secondary BAs (LCA and DCA). Therefore, more studies are needed to find out any relationship between FGF19, FXR and glycaemic control in humans.

5.7.3 BA and Body Composition Correlations

Body composition is changed in T2DM and obesity (Solanki et al., 2015). Although weight loss and its change were evaluated after bariatric surgery, body composition was also needed to be assessed because weight loss is different from fat loss (Malin & Kashyap, 2015). Consequently, FGF19, AUCs of BAs, and fasting ratios of BAs were tested to find any possible associations with different body composition variables after bariatric surgery. There is a strong relationship between the concentration of BAs and metabolic parameters such as anthropometric and body composition (H. Ma & Patti, 2014). Presumably, manipulation of BAs may have therapeutic effects for the treatment of metabolic diseases such as obesity and diabetes. For instance, adding CA to dietary intake enhances energy expenditure and reduces weight (Liaset et al., 2011). Although the exact mechanism of the action of BAs on energy homeostasis is unclear, it has been suggested that expression and activity of the type 2 iodothyronine deiodinase (D2) is increased by BAs through TGR5-cAMP pathway as a result of thyroid hormone (T3) activity, and is raised to regulate energy homeostasis (Russell, 2009). TGR5 is expressed in several tissues including the thyroid gland, brown adipose tissue, and skeletal muscle (Vítek & Haluzík, 2016). In skeletal muscle and brown adipose tissue in mice, for instance, TGR5 stimulates the conversion of T4 to T3 as a result increased energy expenditure (Watanabe et al., 2006). Similar to findings in mice, human skeletal muscle also plays a vital role in energy expenditure. For example, increased levels of BAs after bariatric surgery (RYGB) resulted in increased skeletal muscle TGR5 signalling (Kohli, Bradley, et al., 2013b).

A FXR-dependent mechanism can also be considered for the effect of BAs on energy metabolism after bariatric surgery. BAs through FXR activate a signalling pathway to regulate energy metabolism and decrease adiposity (Holt et al., 2003).

Upon taking food, levels of BAs increased sharply, and this increase is necessary to facilitate micelle formation, which is crucial to the fatty food and fat soluble vitamins' digestion, and energy metabolism. Since the energy metabolism rate is lower within obese individuals compared to lean people, it may highlight a role of BAs in energy metabolism because obese people have lower levels of postprandial BAs compared with healthy, lean people (Ahmad et al., 2013; Glicksman et al., 2010).

Data from this study shows a positive significant association between AUCs of CDCA and THDCA with REE, and a negative correlation between AUCs of GDCA and fasting FGF19 with RQ. This suggests a contributory role of FGF19 and BAs to regulate energy metabolism. Due to the effect of BAs to stimulate FGF19, GLP-1, and brown adipose tissue, it has been shown that the rise of BAs is strongly related to energy homeostasis (Ahmad et al., 2013; Beysen et al., 2012; Dutia et al., 2015; Patti et al., 2009). Although most human studies show a reduction in resting energy expenditure after bariatric surgery, the effect of bariatric surgery on energy expenditure is controversial (J. B. Schmidt et al., 2013).

Although there is no clear reason to describe the mechanisms of BAs on energy metabolism, it is important to note that changes in energy expenditure after bariatric surgery may in part be dependent on the effect of diet on BAs. This study assessed patients at baseline and a year after bariatric surgery, and it was almost impossible to record daily diet information during follow-up. It is known that the Western diet can stimulate taurine-conjugated BAs which results in important changes to gut microbiota (Devkota et al., 2012). Once taurine-conjugated BAs are increased, the rate of taurine deconjugation is reduced, and BA synthesis is impaired (B. V. Jones et al., 2008). Gut microbiota, which mediates conjugation mechanisms of BAs, have been shown to regulate energy metabolism in animals (Bäckhed et al., 2004). Another possibility of energy metabolism alterations after bariatric surgery may be related to the negative correlation between thyroid stimulating hormone (TSH) and BAs (Ockenga et al., 2011). In addition, anatomical changes and modifications of the gastrointestinal system after bariatric surgery may be involved in energy

metabolism (P. Luo et al., 2016). Bariatric surgery (RYGB) speeds up food delivery to the distal intestine and stimulates GLP-1 to attenuate satiety. Thus, it leads to regulated energy metabolism (Ionut et al., 2013).

In agreement with this study, one-year after RYGB in a human study of 30 obese subjects without diabetes, a negative correlation between RQ and conjugated BAs showed and no significant association between REE and BA fractions reported (Brufau, Bahr, et al., 2010; Simonen et al., 2012). Simonen and co-workers concluded from this result that altered conjugated BAs are superior to TBA in regulating energy metabolism after RYGB.

Most available information on the effect of BAs on energy metabolism is from studying animals, while data from animal studies are often consistent compared with humans (Lutz & Bueter, 2014b). Nevertheless, very limited information is available on the effect of BAs and FGF19 on body composition assessments after bariatric surgery among people who have diabetes, and this study may be used as a pilot study. Therefore, more studies are required to confirm findings from this study.

Several fasting BA fractions were negatively correlated with body composition variables. This may suggest that BAs via an unidentified mechanism(s) have effects on body composition. It is probable that the role of gut hormones, particularly GLP-1, is involved (Docherty & Le Roux, 2015; M. Shah & Vella, 2014; Sweeney & Morton, 2014) because GLP-1 is altered by both SG and RYGB (Meek et al., 2016). Also, BA receptors can regulate GLP-1 (Trabelsi et al., 2015).

Taken together, it seems that BAs through their receptors FXR and TGR5 enable the alteration of several metabolic profiles (Arab et al., 2017). Although in this study expression of FXR and TGR5 were not investigated, the study of FXR and TGR5 can have a major impact on clarifying underlying mechanisms of bariatric surgery on the remission of diabetes. Since not only FXR and TGR5 have effects on glucose and lipid metabolism, they may also play important roles in regulating whole-body energy homeostasis (Keely & Walters, 2016). It is also important to note that this study is relatively new in its approach to examining different variables and their correlations with FGF19 and Bas. Some correlations such as the Belfiore index are presented for the first time. Thus, more RCTs with larger populations are felt to confirm these findings.

5.7.4 BA Fractions, FGF19 and Clinical Characteristics

Besides the collective role of BAs in lipid absorption, the individual BAs are part of a broader signaling network in response to ingested nutrients that are thought to include glucose metabolism and body weight regulation. Our study is the first to examine the correlations between changes in several fasting and postprandial BAs after RYGB and SG with changes in clinical variables among those with T2DM. Most other reports have focused on correlations between the achieved fasting BA levels with the clinical state post-operatively among patients without T2DM (Ahmad et al., 2013; Belgaumkar et al., 2016; De Giorgi et al., 2014; Khan et al., 2016; Kohli, Bradley, et al., 2013a; Patti et al., 2009; D. J. Pournaras et al., 2012; J. B. Schmidt et al., 2013; Simonen et al., 2012; R. E. Steinert et al., 2013; Werling et al., 2013). Fasting total BA have generally shown no association with BMI (Albaugh et al., 2015; Patti et al., 2009; Simonen et al., 2012), glycaemia (Albaugh et al., 2017; Jørgensen et al., 2014; Nakatani et al., 2009), lipids (Jørgensen et al., 2014; Nakatani et al., 2009) or insulin resistance (Albaugh et al., 2017; Belgaumkar et al., 2016; Jørgensen et al., 2014), but a few studies have found a negative correlation between fasting total BA and BMI (Dutia et al., 2015; Risstad et al., 2017; R. E. Steinert et al., 2013), glycaemia (Patti et al., 2009; R. E. Steinert et al., 2013), and with lipids (Patti et al., 2009; Risstad et al., 2017). Only one study of RYGB and biliopancreatic diversion reported a positive correlation between the change in fasting total BA with weight loss (Risstad et al., 2017). However, we found no associations of either change in fasting or postprandial BA species with either weight loss or change in insulin resistance. This discrepancy may be due to under-representation of people with T2DM in the previous study (Risstad et al., 2017).

Greater increases in fasting and postprandial secondary and unconjugated BA were also associated with greater decreases in HbA1c, while greater increases in postprandial secondary and unconjugated BAs were associated with lower glucose AUC. This is consistent with secondary BA being potent activators of the TGR5 pathway which is thought to influence energy expenditure and glucose metabolism (Chiang, 2009; Fiorucci & Distrutti, 2015). Other studies have shown that higher 12α-OH/non 12α-OH BAs is associated with insulin resistance (Haeusler et al., 2013) however, we found that changes in fasting 12α-OH/non 12α-OH were negatively associated with changes in IGI and positively associated with glucose AUC. The association of changes in postprandial taurine conjugated BA with changes in REE and the changes in fasting glycine

conjugated BA with changes in abdominal fat are in line with previous studies (Simonen et al., 2012).

We found that only *changes* in prandial FGF19 were negatively correlated with *changes* in HbA1c and visceral fat. Most other studies have found no correlation between the fasting FGF19 level and any achieved clinical variable after bariatric surgery such as BMI (Albaugh et al., 2015; Gerhard et al., 2013), or insulin resistance (Albaugh et al., 2015; Belgaumkar et al., 2016; Jørgensen et al., 2014; Thöni et al., 2017), or inflammatory markers (Jørgensen et al., 2014). Few studies examining prandial FGF19 after bariatric surgery have also reported no correlations with any achieved clinical variable such as glucose tolerance (Jørgensen et al., 2014), fasting glucose or C-peptide (J. B. Schmidt et al., 2013). This suggests that changes in FGF19 after surgery, rather than absolute achieved values, may be more important in the link between FGF19 and improvements in metabolic outcomes.

5.7.5 Effect of Gender on FGF19 and BA Fractions

Apparently, one of the important strengths of this study is the recruitment of both males and females. Due to different percentages of males (47.5%) and females (52.5%), a general linear model was created to evaluate the effect of gender on fasting FGF19 and fasting BA fractions. Gender did not predict changes in FGF19 and BA fractions. In other words, being male or female has no impact on changing concentrations of fasting FGF19 and fasting BA fractions. However, as mentioned above, fasting FGF19 to fasting BA ratio are comparably different between preoperative and postoperative values, and most but not all fractions are significantly higher in RYGB group subjects of SG individuals. Although there is a human study indicating individual BAs are higher in men compared with women (C Gälman et al., 2011) an animal study indicated higher amounts of BAs in female rather than male mice (Turley et al., 1998), to the best of this researcher's knowledge this is the first study to evaluate the effect of gender on BA fractions within obese people with diabetes. Nevertheless, there are previously reported higher amounts of BA pool sizes in healthy men (Bennion et al., 1978) and higher differential ratios of BAs in female mice (Z. D. Fu et al., 2012). Despite the non-significant effect of gender on BA fractions, all ratios except taurine-conjugated and 12α-OH BAs were relatively higher in females. This may suggest that T2DM may have a contributory effect on the change of BA fractions in humans. Nevertheless, the small size of this sample does not permit definite conclusions. Larger sample sizes will allow

for greater accuracy to confirm this finding. The finding may also suggest that the increased ratio of BAs is equally effective on males and females. Therefore, any therapeutic applications or interpretations from BA fractions might be applicable in both genders.

FGF19 levels were not gender dependent. This is in agreement with a previous study which reported concentration changes of FGF19 is gender-independent, but in people with the hepatic disease (Wunsch et al., 2015).

It is important to note that, different findings in different studies on FGF19 and BAs after bariatric surgery can originate from various factors. Factors such as different gut hormonal responses, a different number of gut bacteria, various selection criteria and study design, different assays and of course, surgical procedures and population discrepancies potentially are important in the results of different findings. Taken together, it is most likely that the change of BAs and FGF19 play a vital role in the remission of diabetes after bariatric surgery. However, the exact mechanism(s) of these interesting biomarkers on diabetes remission remains to be elucidated in future and further studies.

5.8 Remission of Diabetes after Bariatric Surgery

At the end of this study, the remission of diabetes was scored. Due to the absence of a definite definition of diabetes remission or resolution (Blackstone et al., 2012; Buse et al., 2009; D. Pournaras et al., 2012; Rubino et al., 2010), diabetes remission was scored based on HbA1c, HOMA-IR and fasting glucose levels, and complete definition was reported when subjects were off-medication, HbA1c<6% and fasting plasma glucose levels <5.9 mmol/l.

Thus, data from this study shows that although SG and RYGB could not reach a complete remission of diabetes in all patients, both methods of surgery are capable of improving insulin resistance in a higher number of patients within a one-year period. Also, bariatric surgery can meaningfully reduce the number of preoperative patients who were required to take metformin and insulin, postoperatively. Due to the different diabetes remission definitions, different studies get different results. For example, diabetes remission has been defined as having a fasting plasma glucose less than 5.6 mmol/l and HbA1c<6.5% without taking antidiabetic medications, this according to a two-year RCT of obese individuals with T2DM (Mingrone et al., 2012). Mingrone

and colleagues reach 0%, 75% and 95% remission of diabetes after intensive diet, RYGB, and BPD respectively. In a one-year RCT, however, with a different diabetes definition given as HbA1c<6% with or without antidiabetic medications, the rate of diabetes remission changed to 32%,42% and 12% for SG, RYGB, and diet respectively (Schauer et al., 2012). Thus, different definition criteria have different results.

It is possible that longer-term follow-up evaluation of diabetes remission after bariatric surgery may favour RYGB as a surgery of choice. Taken together, however, all data from this study shows that it is worthwhile to conclude that both SG and RYGB are equally effective on the remission of diabetes over the postoperative period of one-year.

CHAPTER SIX

SUMMARY, GENERAL CONCLUSIONS, AND RECOMMENDATIONS FOR FUTURE RESEARCH

6.1 Summary

The current study was carried out to look into two different bariatric surgeries, SG and RYGB, and their effect on remission of T2DM through changing levels of FGF19 and BAs. LC-MS/MS and ELISA were used to quantify BAs and FGF19, respectively. This study demonstrates statistically significant changes of both selected candidate biomarkers in diabetes remission after bariatric surgery.

6.2 Conclusions

RYGB is currently the best treatment for obese people with diabetes. Nowadays, however, SG has gained interest as another treatment of T2DM, since it is also associated with improved glucose metabolism. Although the exact mechanism of bariatric surgery on the remission of diabetes is unclear, improved insulin sensitivity and BCF after bariatric surgery is likely to play a role. Still, some of the benefits of bariatric surgery on the resolution of diabetes can also be associated with weight loss. This study determined that a change in obesity status a year after SG and RYGB and the necessary effect of weight loss from the surgery are consistent with benefits highlighted in several different studies (Cole et al., 2015).

This study showed that several insulin sensitivity indices improved after either SG or RYGB. Also, it was found that SG and RYGB had an equal effect on improved insulin sensitivity. This is one of the important outcomes of this study because, despite instances of incomplete remission of diabetes after bariatric surgery, most subjects who underwent bariatric surgery experienced a distinct improvement in glycaemic control. This is a primary reason for why bariatric surgery should be considered as an effective treatment available for sufferers of T2DM.

This study also indicates that diabetes remission after bariatric surgery is through an insulin secretion-related mechanism. Both SG and RYGB are equally effective in remission of T2DM in

trial participants. Higher levels of BAs and FGF19 may be potent contributing factors to achieve remission of diabetes one year after bariatric surgery. Although it is impossible to draw a definite long-term conclusion from this relatively short-term study, it is clear that if diabetes is not in complete remission after either SG or RYGB, there is still improvement in the state of diabetes over the one-year period following surgery.

According to this study, changes of BAs and FGF19 are associated with the remission of T2DM after bariatric surgery. There are substantial alterations in BA metabolism after bariatric surgery; although, the exact mechanism is yet to be elucidated. Alterations in RYGB may be explainable by the effect of anatomical changes which occur in the upper gastrointestinal system, but that hypothesis cannot be applied to SG since the tissue is not removed. Also, the significant correlation of FGF19 and BAs with insulin resistance/sensitivity may be considered as another important finding of this study because these relationships, in part, may explain a reason for improved glucose metabolism after bariatric surgery. Thus, therapeutic manipulation of BA concentrations may have a considerable metabolic impact on T2DM. Also, increased level of FGF19 after bariatric surgery may play another important role in improving glycaemic control after bariatric surgery. Despite a few reports of increased levels of FGF19 after diabetes remission, using FGF19 as a therapeutic way to treat diabetes still, requires more studies. Assessment of FGF19 therapeutic levels is critical to understanding safe doses of the biomarker in humans. For example, administration of FGF19 leads to cancer (Hyeon et al., 2013) while reduction of FGF19 levels inhibits cancer via enhancing apoptosis (Miura et al., 2012b). It is important, however, to note that alterations in BAs and FGF19 per se may not be the main reason for improved glycaemic control after bariatric surgery. Levels of BAs are found to be different in one surgery compared to another, while remission of diabetes is equally observed after both bariatric surgeries. Several other underlying factors such as gut hormones, gut microbiota, and energy expenditure should also be considered to draw a proper conclusion on the remission of diabetes after bariatric surgery. However, this study indicates that BAs and FGF19 certainly have contributory roles in the remission of diabetes.

This study has indicated several differences with previous studies. Some of the important differences between this study and previous results include, but are not limited to, first of all, this is an RCT to compared two common bariatric surgeries, namely SG and RYGB. Secondly, this is

a well characterised and heterogeneous study sample with no differences between groups at preoperative (baseline) recruitment. Thirdly, this study took advantage of recruiting males and females. Fourthly, it has a low dropout rate of participants. Fifthly, a variety of risk factors related to diabetes were assessed in parallel, and several of these findings were presented for the first time through this study. Finally, this is the largest study so far to evaluate the effect of BAs and FGF19 on the remission of T2DM after bariatric surgery.

The strength of this study relies on comprehensive prospective evaluation of fasting and postprandial BAs along with their AUCs and several BA fractions, fasting and postprandial FGF19 levels, anthropometric and body composition measurements, and lipid and glycaemic metabolism within obese people with diabetes at baseline and 12 months after two common bariatric surgeries (SG and RYGB).

Taken together, it is accurate to say that there is an equal effect of SG and RYGB on the improvement of T2DM. It is also possible to argue that SG is not merely a restrictive procedure. Thus, SG has the potential to be used as a suitable replacement of RYGB among eligible people who are concerned about RYGB or other metabolic surgeries' related risks.

6.3 Study Limitations and Future Recommendations

The protocol of this study was based on two-time points, basal and one-year, in a randomization design. Thus, any changes outside of these two points were not measured. Several crucial changes may occur within just a few hours or days after bariatric surgery. Hence, it is strongly recommended to evaluate immediate changes of BAs and FGF19 levels after bariatric surgery. Another limitation of this study is the lack of measurements of BAs in urine and faecal samples. Furthermore, in this study, all recruited subjects were selected according to their BMI, and there was no chance to observe the effect of bariatric surgery on the remission of diabetes among people with normal BMI (normo-weight or over-weight) values. Thus, it is recommended to assess the effect of bariatric surgery on individuals who are not morbidly obese. In this study, all subjects underwent two weeks of VLCD before a given bariatric surgery. We do not know how much of an impact the time of this dieting relative to the surgery had on the outcomes of this study relative to levels of BAs and FGF19. Therefore, it is recommended that a study of similar design to this

RCT be carried out with new subjects who exclusively undergo VLCD and then BAs and FGF19 are evaluated over short-term time points rather than only at baseline and one-year time points.

Another limitation of this study is that there are multiple definitions of diabetes remission, which is sometimes subjective. Besides the universally accepted definition of diabetes resolution, there are other definitions based on switching from a class of anti-diabetes medication to another class or definitions which consider glucose indices (i.e., HOMA-IR). Therefore, scoring diabetes remission needs to be interpreted carefully. In this study, the gene expression of TGR5 and FXR before and a year after bariatric surgery were not evaluated, while both FXR and TGR5 play crucial roles in the improvement of glucose and energy metabolism.

In this study, a pool of different ethnicities was compared, and data is not presented as per a distinct population. European, Asians, Maori and Pacific populations constituted the pool sample size and are all presented together. It is recommended to evaluate FGF19 and BAs based on a homogenous population to see if there are any differences regarding FGF19 and BAs in this context.

Although RYGB is still considered to be the best procedure for treatment of diabetes, with a stable growth trend (Yamamoto et al., 2016), there must be additional methods of addressing treatment options of the disease. It is important to the public and private sectors to support more obese people with diabetes and develop ways which decrease the obstacles for all eligible people to choose bariatric surgery as a treatment.

Further understanding of the mechanism of diabetes remission after SG and RYGB will provide an opportunity to develop a safe and efficient medical cure for T2DM mellitus. Nowadays, investigation of the roles of BAs and FGF19 on obesity and diabetes is at the forefront of diabetes research. Most of the available data on BAs is based on data from RYGB-associated studies while insufficient information is available from SG data. This study provides a considerable and unique contribution to the literature regarding FGF19 and SG. It is the only study to present a comparison between SG and RYGB surgeries based on fasting and postprandial BAs and almost all possible ratio of BAs, fasting and postprandial FGF19, body composition assessment, and glucose homeostasis among obese people with T2DM in New Zealand. Despite the complex criteria used to select patients for bariatric surgery and the fact that there is no guarantee of a complete remission of diabetes after bariatric surgery, it is important to always consider bariatric surgery as an effective

alternative for the treatment of diabetes. As shown in this study, most patients experienced a marked improvement of glycaemic control.

A key issue which arises from the context of this study is the need to review eligibility criteria for bariatric surgery candidates. For example, bariatric surgery is mostly performed among people with a BMI of more than 35 kg/m^2, and this discrimination excludes many diabetic patients from access to an effective treatment for their condition. Still, by better understanding the underlying mechanisms of bariatric surgery on the remission of diabetes, researchers may find a non-invasive treatment for diabetes which is more favourable than bariatric surgery.

Findings in this study concerning the elevated values of FGF19 and BAs indicate that these biomarkers are not the main reason for the remission of diabetes following bariatric surgery. In other words, the remission of diabetes depends on other metabolic regulator factors as well.

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Appendices

APPENDIX A

(Information sheet)

Waitemata
District Health Board
Te Wai Awhina

SURGICAL SERVICE
North Shore Hospital
Shakespeare Road,
Takapuna
Private Bag 93-503
Auckland
Telephone: 09 486 1491

Gastric bypass versus sleeve gastrectomy for the management of type 2 diabetes in obese patients:
Gut hormone and gut bacteria substudy

You are being invited to take part in an additional part of the research project comparing the two commonly performed weight loss surgeries (gastric bypass and sleeve gastrectomy), which is designed to see if these surgeries affect the hormones your gut produces or the bacteria living in the gut, differently. Before you decide, it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully and discuss it with others if you wish. Ask us if there is anything that is not clear or if you would like more information. Take time to decide whether or not you wish to take part.

What is the purpose of the study?
Weight loss surgery is the most effective long-term treatment for obesity and type 2 diabetes, but we don't know exactly how they improve these conditions. The effects of both the gastric bypass and sleeve gastrectomy surgery in producing weight loss and diabetes reversal seems to be greater than what we would expect through simple reduction in stomach size and less food absorption. Other mechanisms such as changing the appetite and glucose controlling hormones produced by the gut seem to be important. Bacteria that live in the gut are also thought to play a role in obesity and type 2 diabetes, by influencing the amount of energy we can extract from our diet, the hormones we produce in response to food, and the amount of irritation and inflammation we get from certain gut bacterial parts entering our blood stream.

By studying the levels of gut hormones produced in response to a sugar test (oral glucose tolerance test), also used in the diagnosis of diabetes, we can compare the impact of the two types of surgery on gut hormone production. By collecting a urine and fecal sample before and after either of the two types of weight loss surgery, we can compare the types of gut bacteria and their function, in those who have had a gastric bypass operation to those who have had a sleeve gastrectomy.

Who can take part in the study?
Patients aged 20-50 years, with type 2 diabetes diagnosed at least 6 months ago, obesity (with a body mass index of 35-65 kg/m^2), no contraindications to surgery and committed to long term follow-up.

Who cannot take part in the study?
Those with a body mass index over 65 kg/m^2, type 1 diabetes, pregnancy, blindness, cancer, or severe lung, kidney, liver or heart disease that would make surgery dangerous.

Do you have to take part?
Your participation is entirely voluntary (your choice). You do not have to take part in this study and if you choose not to take part you will receive the standard treatment / care available. If you do agree to take part in the study, you are free to withdraw at any time without having to give a reason and this will in no way affect your future health care.

SubStudyPatientInfoSheet
(Version 1 – 23/11/11)

Participation in this study will be stopped should any harmful effects appear or if the doctor feels it is not in your best interest.

What are the benefits of taking part?
You will have regular contact with the health professionals involved in the study and thus may benefit from the extra monitoring you will receive. The oral glucose tolerance test is one of the tests use to diagnose diabetes, so this will be an extra measure of whether your diabetes has resolved.

What will happen to you if you take part?

Once you have successfully completed the screening visit, during the second pre-surgery visit when you are given a date for your DEXA scan, you will be asked to keep a 3 day food diary leading up to this date. You will also be given a fecal sample pack to collect and store a faecal sample one or two days before your DEXA scan date, and to bring this sample with you to the DEXA visit at Auckland City Hospital. The faecal sample pack will contain instructions, gloves, a small fecal sample tube with a scoop, 2 large square plastic containers and some blue tac)

During the DEXA visit an oral glucose tolerance test will be performed lasting 2 hours, so you should allow 3 hours in total for this visit, which will include the time for the DEXA scan.

5 day food diary: Please record everything you eat or drink for five days prior to your DEXA visit date as accurately as possible on the sheets provided. For example:

- the number of whole or half pieces of fruit
- state whether it is raw or cooked vegetables and how much eg: one and half cup of mashed potatoes, 1 cup of lettuce, avocado, tomato salad
- 1 serving of meat is about the size of your palm, eg: 2 servings of beef steak or 1 cup of lamb stew
- amount of liquids eg: 1 cup of tea with green top milk

Faecal sample: Please take this a couple of days before the visit and place in the freezer as soon as it is taken. Additional instructions provided in the kit.

- Using the plastic gloves, place the large square plastic container on top of the water in the toilet bowl before going to the toilet (preferably after urinating).
- Remove the plastic container with the faeces from the toilet bowl.
- Using the lid of the small faecal tube scoop the faecal sample into the tube. There needs to be enough of a sample to fill atleast half of the specimen tube. Dispose of the square plastic container after use (wrap in a plastic bag and place in rubbish bin)
- Ensure the lid is firmly on the specimen tube and clean up any spillage by rinsing the outside of the tube under running water. Please record the date of collection on the tube.
- Fill the larger tube with water almost to the top (80ml) then put the labelled specimen tube into the water. Close the larger tube with its own screw cap lid. Place this in the bio-hazard bag provided, seal it and put in the deep freezer as soon as possible.
- On the day of the DEXA visit, place the frozen package in the brown paper bag, ready to bring with you.

The types of bacteria present in your faecal sample will be analysed, along with short chain fatty acids they produce. It is important that the sample is frozen as soon as possible after collection to preserve all the bacteria in their original condition and prevent certain types of bacteria from overgrowing.

On the morning of the DEXA visit, remember not to eat or drink anything other than water from midnight the night before.

Oral glucose tolerance test: We will insert a small cannula (very fine plastic tube) into your vein, then withdraw blood before and after a glucose drink, at 30mins, 60mins, 90 mins, and 120min after the drink. You will only feel the slight discomfort of the first cannula, and all other blood samples will be taken from the same cannula. A total of 80mls of blood will be taken during this test (less than a quarter of what is collected in a single blood donation).

From your blood samples, we will measure glucose levels, insulin, c-peptide, GIP and GLP-1 (which are hormones from the intestines that help to control glucose levels), free fatty acids, glucagon and inflammatory markers. It is important that you do not have anything to eat or drink on the morning of the OGTT, as this can interfere with how much hormones your gut produces.

Urine sample: During your DEXA visit, you will also be asked to provide a urine sample, which will be frozen soon after collection. This sample will be used to analyse certain chemicals produced by the gut bacteria relative to your own.

Appetite Assessment: When you arrive for your DEXA visit, you will be asked four questions about your hunger and appetite. The study staff will explain that you need to answer these questions based on your current appetite sensations at that moment, since a spontaneous answer is required.

You will be asked to do these tests again at your next DEXA scan visit at 1 year and 5 years after your surgery.

Are there any risks?
No there are no significant risks to this component of the research.

Confidentiality?
No material that could personally identify you will be used in any reports on this study. All data collected in the study will be assigned coding to avoid the use of participant names or other identifying information. All records will stored in password protected computers and a locked storage facility. Records will be kept for 15 years after completion of the study for monitoring purposes and subsequently destroyed.

What will happen at the end of the study?
The study will last for 12 months from the date of your surgery. Once you have reached this time point, you will return to standard outpatient follow-up clinic visits at regular intervals, which is currently routine for all our bariatric patients. Once the data for all the patients included in the study has been analysed, you may receive a copy of the results upon written request. You will also be invited back for a 5 year follow up DEXA visit for the gut hormone and gut bacteria sub-study

Cultural support to participants?
Cultural support will be available to all ethnic groups as required. Mo Wai Te Ora Maori Health Services Waitemata DHB will provide cultural support to Maori participants.

What if something goes wrong?
In the unlikely event of a physical injury as a result of your participation in this study, you may be covered by ACC under the Injury Prevention, Rehabilitation, and Compensation Act 2001. ACC cover is not automatic, and your case will need to be assessed by ACC according

to the provisions of the Injury Prevention, Rehabilitation, and Compensation Act 2001. If your claim is accepted by ACC, you still might not get any compensation. This depends on a number of factors, such as whether you are an earner or non-earner. ACC usually provides only partial reimbursement of costs and expenses, and there may be no lump sum compensation payable. There is no cover for mental injury unless it is a result of physical injury. If you have ACC cover, generally this will affect your right to sue the investigators. If you have any questions about ACC, contact your nearest ACC office or the investigator. You are also advised to check whether participation in this study would affect any indemnity cover you have or are considering, such as medical insurance, life insurance and superannuation.

If you have any queries or concerns regarding your rights as a participant in this study, you may wish to contact an independent health and disability advocate:
Freephone: 0800 555 050
Free fax: 0800 2 SUPPORT (0800 27877678)
Email: advocacy@hdc.org.nz

Please feel free to contact the researcher below if you have any questions about this study.

Dr Michael Booth
Consultant Upper GI & Bariatric Surgeon
Department of Surgery, North Shore Hospital, Private Bag 93 503, Takapuna, Auckland

Tel 021867898

Dr Rinki Murphy
Consultant Diabetes Physician
Department of Medicine, University of Auckland, Private Bag 92019, Auckland

Tel 0211428470

APPENDIX B

(Consent Form)

THE UNIVERSITY OF AUCKLAND
NEW ZEALAND
Te Whare Wānanga o Tāmaki Makaurau

Faculty of Medical and Health Sciences
The University of Auckland
Private Bag 92019
Auckland 1142

Consent Form

Name of Study: Gut hormone and gut bacteria substudy of Gastric bypass versus sleeve gastrectomy for the management of type 2 diabetes in obese patients

REQUEST FOR INTERPRETER

		Circle one	
Deaf	I wish to have a NZ sign language interpreter	Yes	No
English	I wish to have an interpreter.	Yes	No
Maori	E hiahia ana ahau ki tetahi kaiwhakamaori/kaiwhaka pakeha korero.	Ae	Kao
Cook Island	Ka inangaro au i tetai tangata uri reo.	Ae	Kare
Fijian	Au gadreva me dua e vakadewa vosa vei au	Io	Sega
Niuean	Fia manako au ke fakaaoga e taha tagata fakahokohoko kupu.	E	Nakai
Samoan	Ou te mana'omia se tasi e auai e fa'amatalaina upu i le gagana Samoa	Ioe	Leai
Tokelaun	Ko au e fofou ki he tino ke fakaliliu te gagana Peletania ki na gagana o na motu o te Pahefika	Ioe	Leai
Tongan	Oku ou fiema'u ha fakatonulea.	Io	Ikai

- I have read and I understand the information sheet (dated 23/11/11 version 1) for volunteers taking part in the study: "Gut hormone and gut bacteria substudy"
- I have had the opportunity to discuss this study. I am satisfied with the answers I have been given.
- I have had the opportunity to use family/whanau support or a friend to help me ask questions and understand the study.
- I understand that taking part in this study is voluntary (my choice) and that I may withdraw from the study at any time and this will in no way affect my future health.
- I have had this project explained to me by principal investigator.
- I understand that my participation in this study is confidential and that no material which could identify me will be used in any reports on this study.
- I understand that the research procedures and tests will be stopped if it should appear harmful to me.
- I have had ample time to discuss with whanau/family and friends when a decision is required or when making a decision.

I consent to blood samples being collected for this study YES / NO

I consent to urine samples being collected for this study YES / NO

I consent to fecal samples being collected for this study YES / NO

I consent for data to be stored for future related studies YES / NO

I consent for blood, urine and fecal samples being stored for future related studies	YES / NO
I agree to my GP or other current provider being informed of my participation in this study/the results of my participation in this study	YES / NO

I _____ (full name) hereby consent to take part in this study.

Signature _____ Date _____

Project explained by _____

Project role _____

Signature _____ Date _____

Interpreter

I _____ translated the project to the participant

Signature _____ Date _____

APPENDIX C

Table 1. Sigstad's clinical diagnostic index: weighting factors allocated to postprandial symptoms and signs of the dumping syndrome[1]

Pre-shock, shock	+5
'Almost fainting', syncope, unconsciousness	+4
Desire to lie or sit down	+4
Breathlessness, dyspnea	+3
Weakness, exhaustion	+3
Sleepiness, drowsiness, yawning, apathy, falling asleep	+3
Palpitation	+3
Restlessness	+2
Dizziness	+2
Headache	+1
Feeling of warmth, sweating, pallor, clammy skin	+1
Nausea	+1
Fullness in the abdomen, meteorismus	+1
Borborygmia	+1
Eructation	−1
Vomiting	−4

[1]A clinical diagnostic index of +7 or above indicates dumping, indices of +4 or below, non-dumping. Eructation and vomiting were not included in calculating patient scores.

Presence of dumping = score >= +7 and non-dumping as a score <= +4. Sigstad weighted t symptoms negatively: eructation or belching or regurgitation (-1) and vomiting (-4) in order to distinguish dumping from the afferent loop and small stomach syndromes, however, these negative scores have unclear relevance for bariatric patients, and we hope to analyse if they have any relationship with incretin hormone levels obtained during the OGTT!

Participant ID		Waist circumference (cm)	
Patient name		Hip circumference (cm)	
Age		Waist to hip ratio	
Gender		BP	
Ethnicity		Pulse	
Height (cm)		Neck circumference	
Weight (kg)		BMI	

(Demographic Questionnaire)

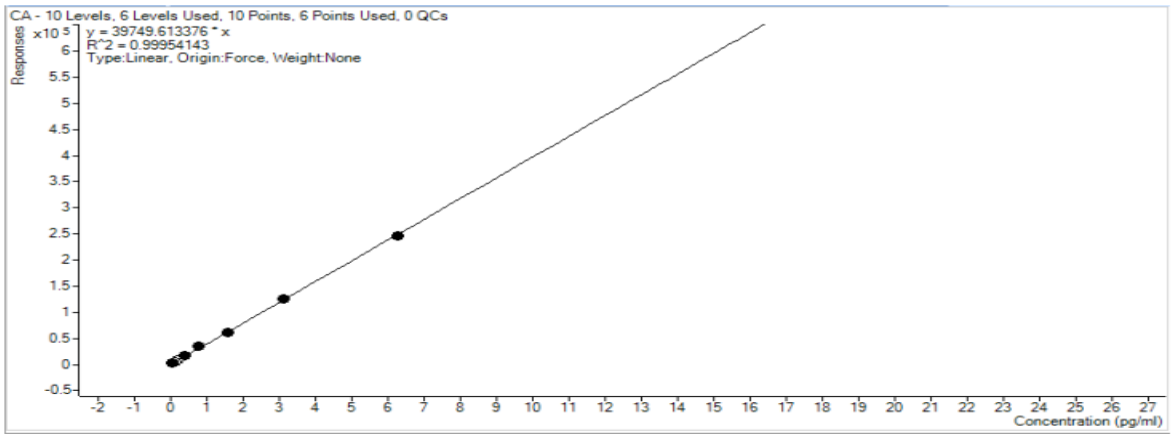
APPENDIX D

(Chemicals and Reagents)

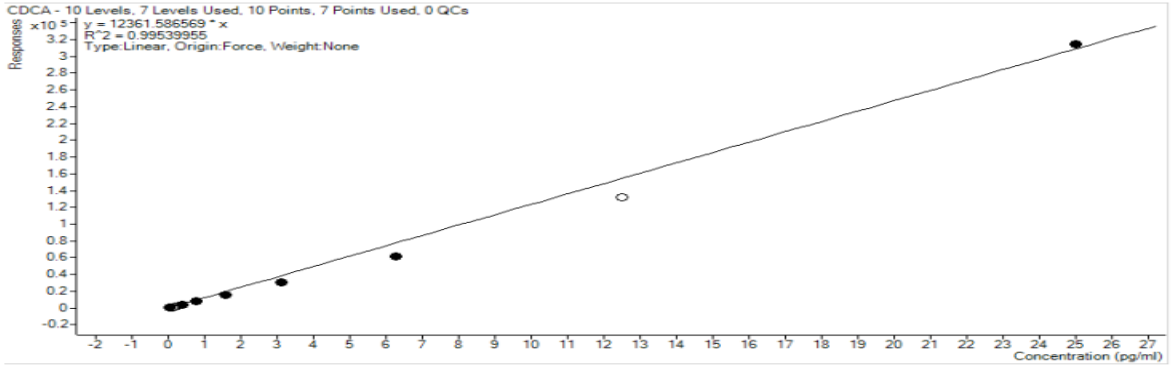
Materials	Catalogue number	Supplier
Consumable		
9mm short screw-thread vial, 1.5ml, wide opening with write-on spot, amber 11.6 x 32mm	V945	Interlab
15 x 45mm, Borosilicate Type 70	V1317	Interlab
white silicone / red PTFE septa 8.5mm centre hole	SC133131	Interlab
Insert 250uL glass tapered, 31 x 6mm	THC06090669	Interlab
vial rack micotube 1.5 ml 72 place	BAT18841.0000	Thermo Fisher
filter syringe millex GS 0.22uM 33mm with MF membrane for Mobile Phase filtration	MILSLGP033RS	Thermo Fisher
Nylon member filter,0.45um	R04SP04700	Thermo Fisher
Chemicals		
BA standards	QX173803	Steraloids, Inc
Methanol for HPLC LC-MS grade	VWRC83638.320	Thermo Fisher
Acetonitrile for HPLC LC-MS grade	VWRC83640.320	Thermo Fisher
Ammonium acetate	A1542 SIGMA	Sigma-Aldrich
Formic acid	F0507 Sigma-Aldrich	Sigma-Aldrich
Biochemical		
FGF19 ELISA kit	RB-ELH-FGF19-0	Huntingtree Associates
Glucose (Gluco-quant Glucose/HK)	11876899 216	Roche Diagnostics GmbH
Insulin	12017547 122	Roche Diagnostics GmbH
Cholesterol (Cholesterol CHOD-PAP)	11875540 216	Roche Diagnostics GmbH
Triglycerides	11730711 216	Roche Diagnostics GmbH
HDL	04713257 190	Roche Diagnostics GmbH

Appendix E

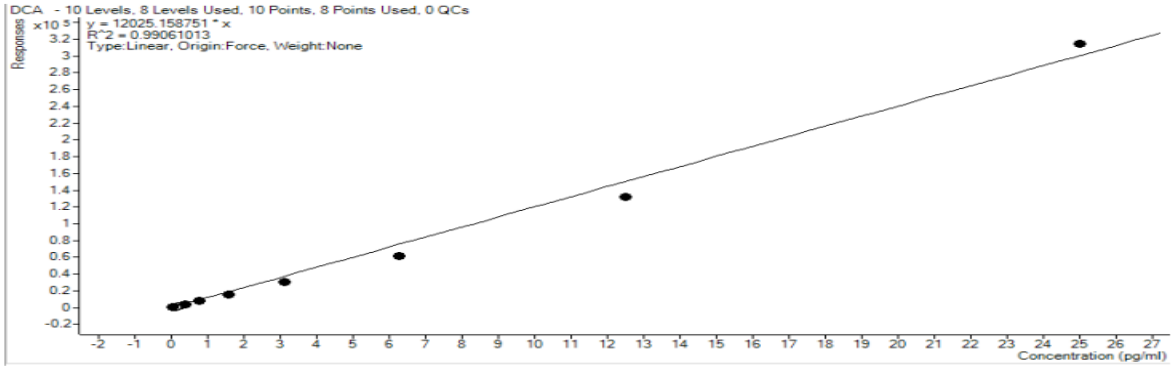
(calibration curve)



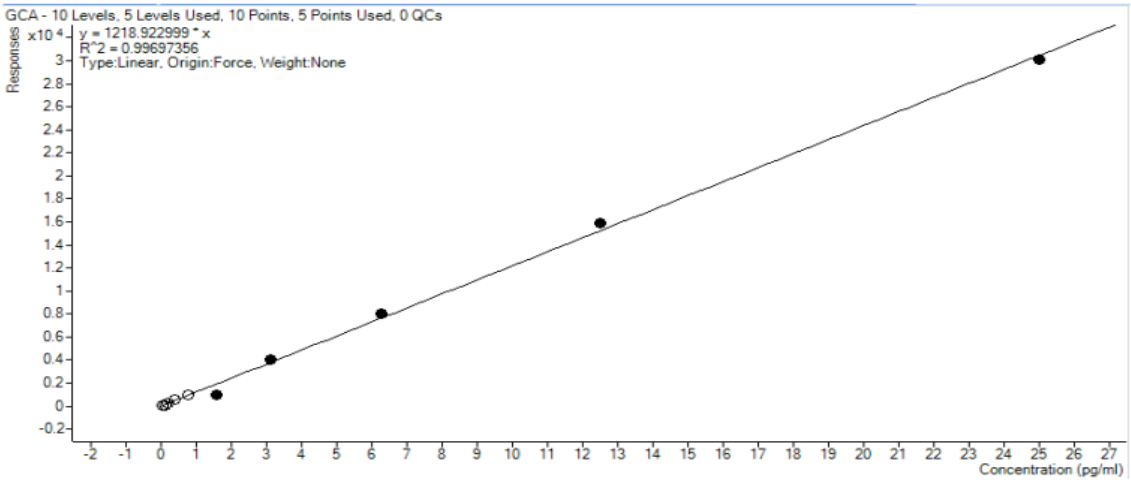
CA



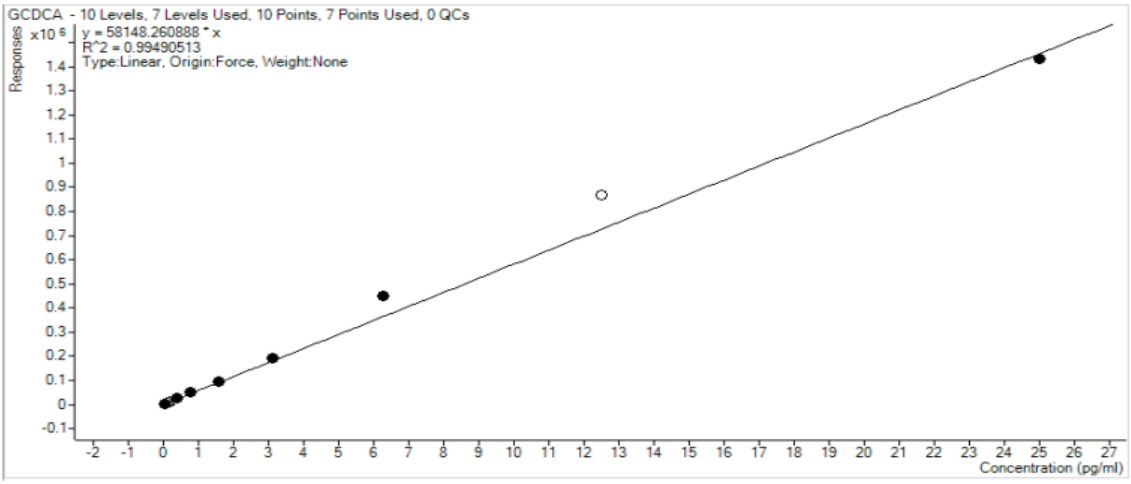
CDCA



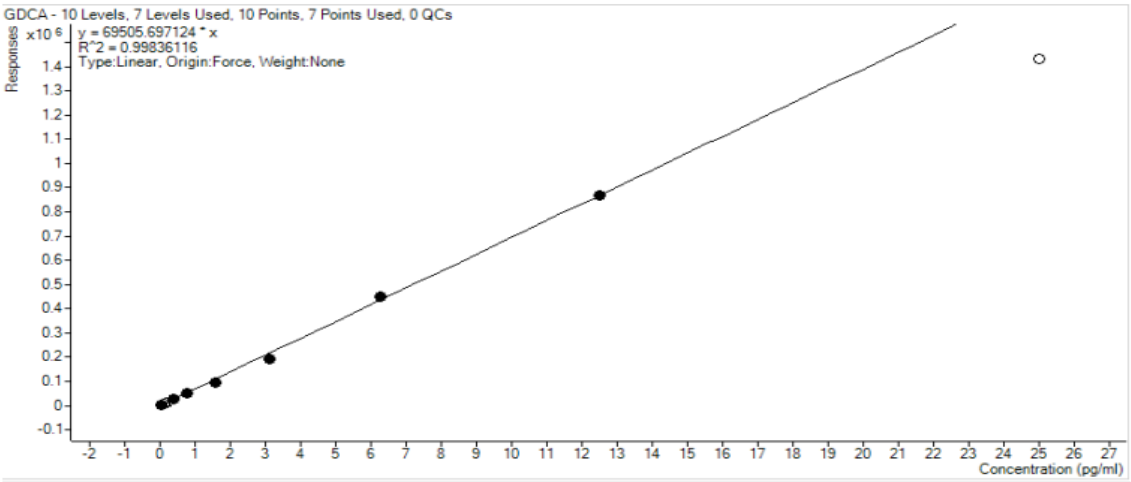
DCA



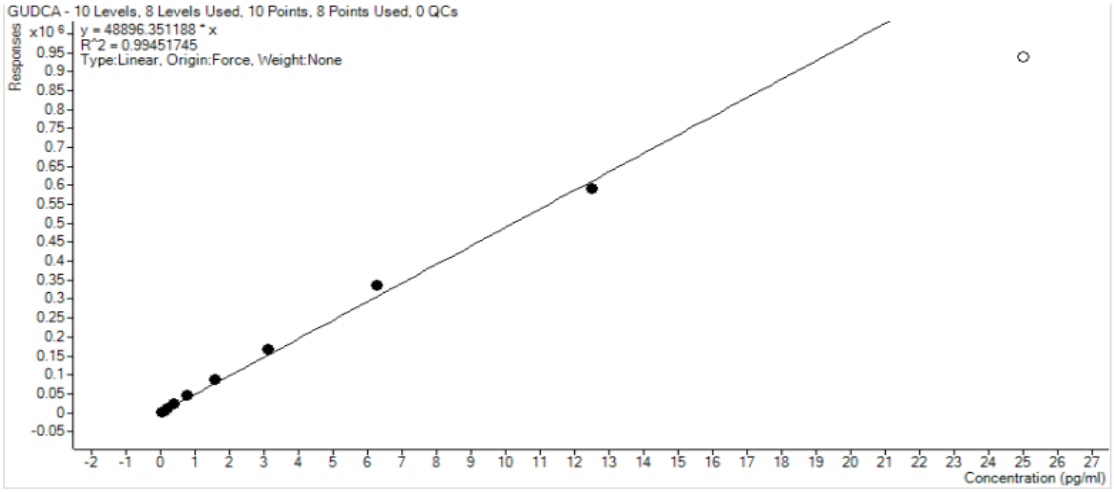
GCA



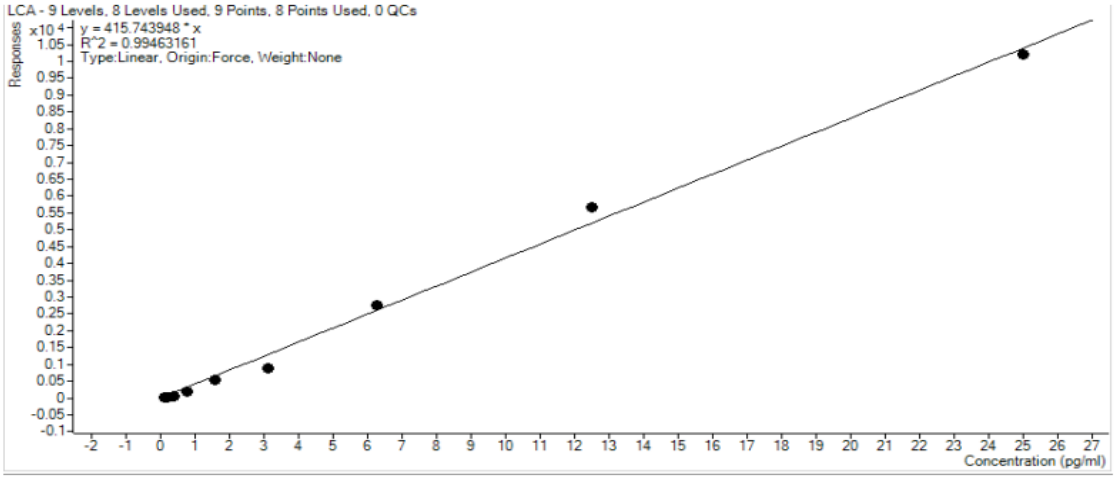
GCDCA



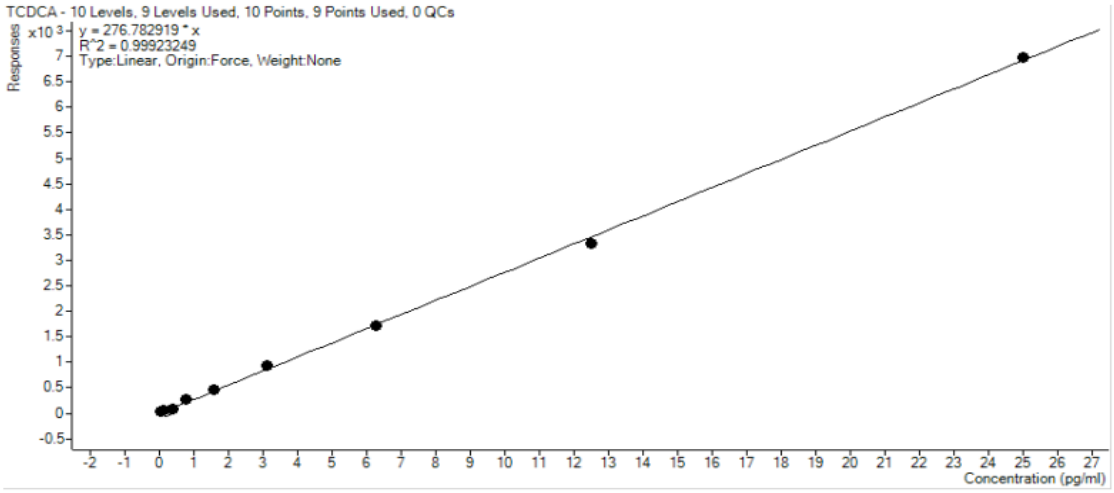
GDCA



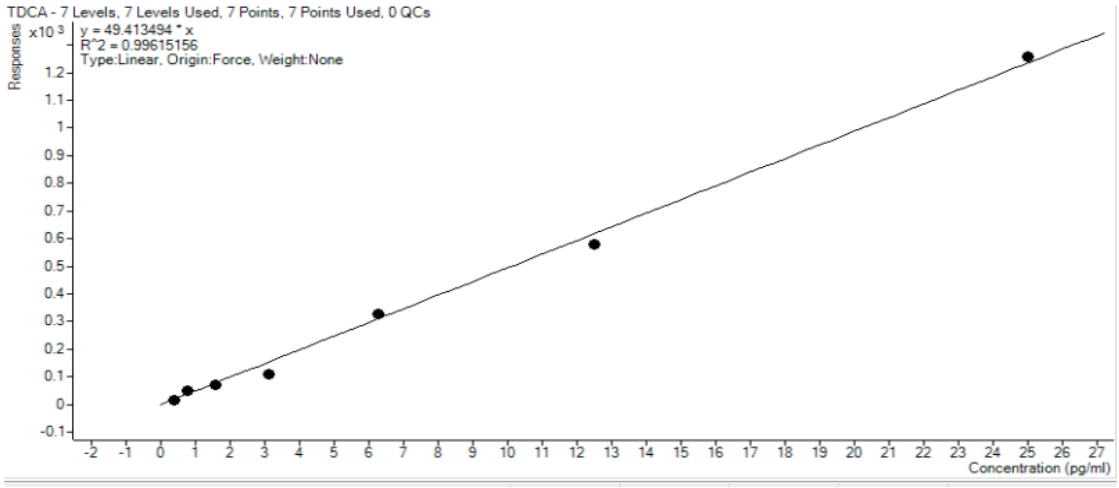
GUDCA



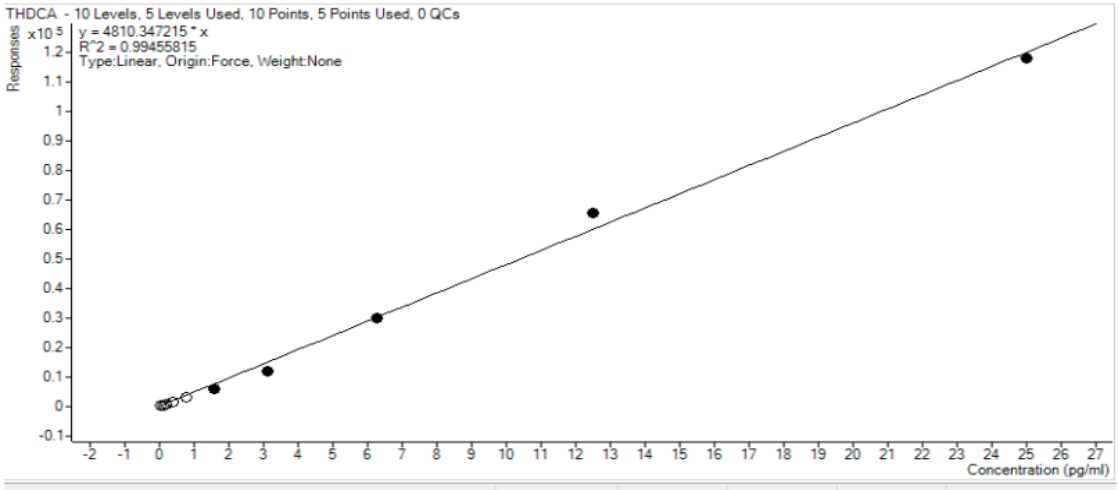
LCA



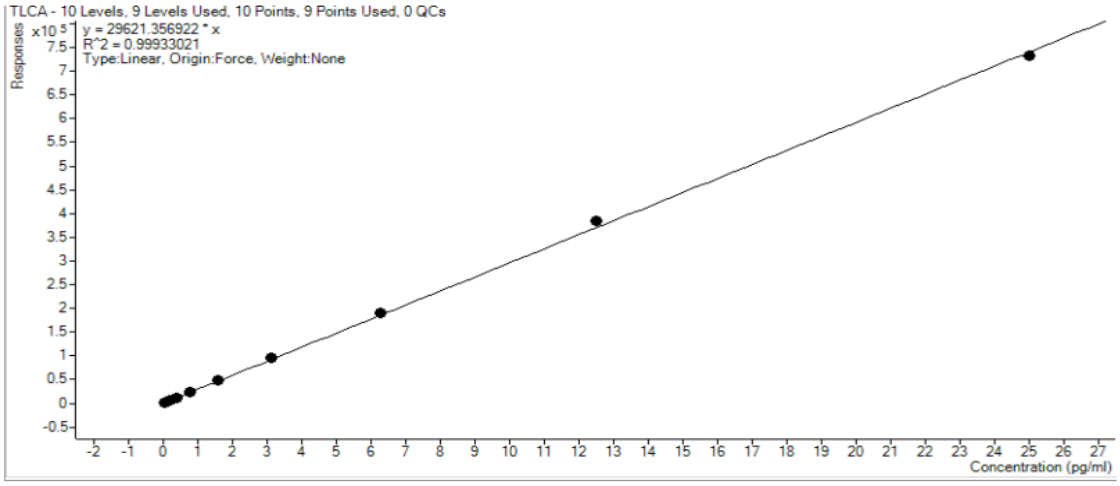
TCDCA



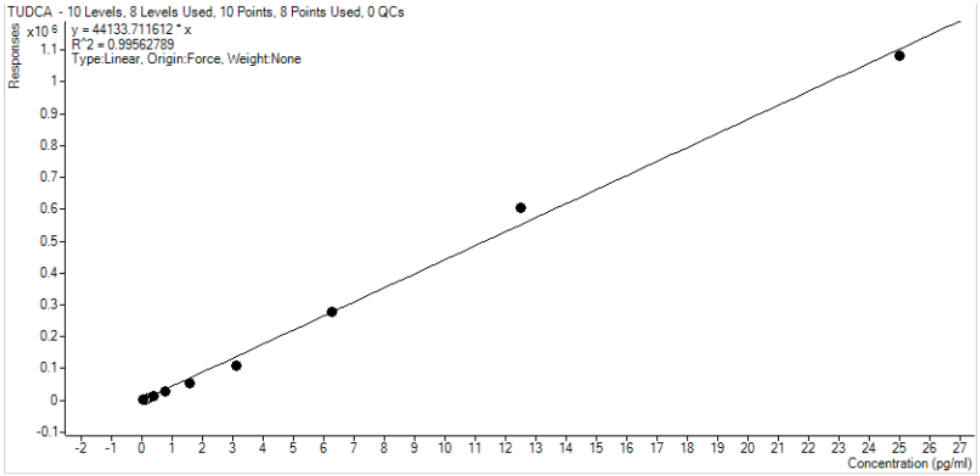
TDCA



THDCA



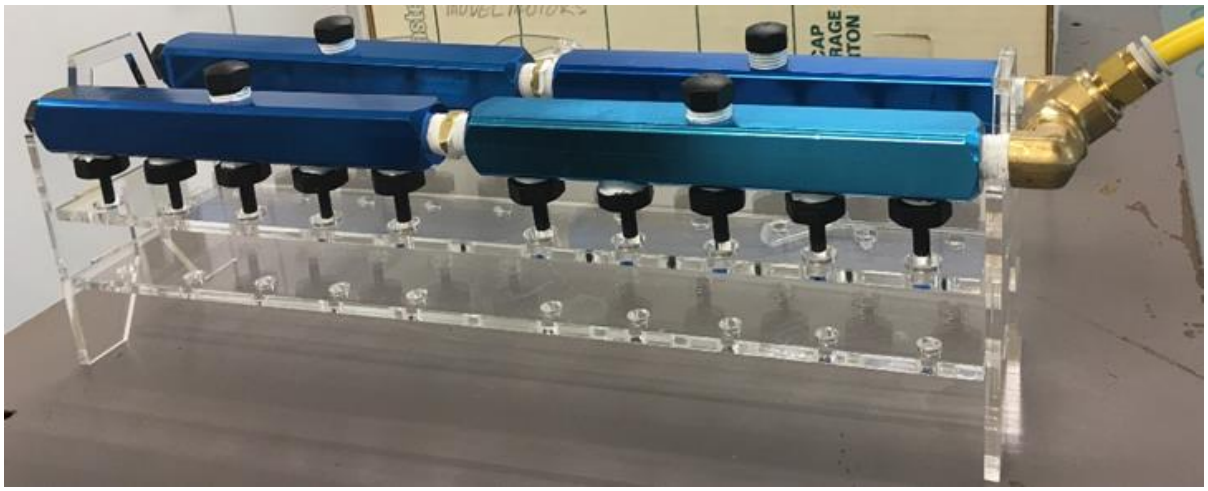
TLCA


TUDCA - 10 Levels, 8 Levels Used, 10 Points, 8 Points Used, 0 QCs
y = 44133.711612 * x
R^2 = 0.99562789
Type:Linear, Origin:Force, Weight:None
Responses
x10 6
Concentration (pg/ml)

TUDCA

Appendix F

(Nitrogen evaporators)



Appendix G

(preoperative analysis)

Table A.1 clinical characteristics, body composition and diabetes indices preoperative comparisons (SG vs GB)

Clinical characteristics	Bariatric surgery Sleeve Gastrectomy (n=29)	Gastric Bypass (n=32)	p-value
Weight(kg)	120.±4.1	116±3.7	NS
BMI(kg/m^2)	40±1.1	40±1.2	NS
Fasting glucose(mmol/l)	6.7±0.45	6.7±0.37	NS
Fasting insulin(µU/ml)	14±2.1	15±1.6	NS
Glucose AUC_{0-120}	1464±60	1567±64	NS
Insulin AUC_{0-120}	44796±3964	41352±3748	NS
HbA1c (%)	7.9±0.1	8.2±0.2	NS
Total cholesterol (mmol/l)	5.0±0.16	4.7±0.22	NS
Triglycerides (mmol/l	1.9±0.14	2.1±0.21	NS
HDL-c(mmol/l)	1.1±0.04	1.1±0.04	NS
LDL-c(mmol/l)	3.1±0.15	2.7±0.18	NS
Body composition			
Energy expenditure			
REE (kcal/d)	1905±62.3	1671±77.2	0.02
RQ	0.72±0.00	1.6±0.9	NS
BIA			
Resistance (Ω)	426.8±12.1	447.9±13.5	NS
Reactance (Ω)	41.1±1.16	42.8±1.9	NS
TBF (kg)	53.6±2.4	53.4±2.5	NS
LBM (kg)	63.6±2.3	59.6±1.9	NS
DXA			

BMC (kg)	3.2±0.09	3.2±0.1	NS
FFM (kg)	66.9±2.4	62.8±2.0	NS
BMD (g/cm^2)	1.3±0.01	1.3±0.02	NS
Android fat (kg)	5.6±0.2	5.4±0.2	NS
Visceral fat (kg)	2.5±0.1	2.6±0.2	NS
Abdominal fat (kg)	31.9±1.4	32.2±1.5	NS
Leg fat (kg)	15.0±0.8	14.1±0.9	NS
Arm fat (kg)	5.3±0.2	5.8±0.3	NS
Anthropometry measurement			
Waist circumference (cm)	124±4.7	119.4±3.3	NS
Hip circumference (cm)	126.7±3.5	127.0±3.5	NS
W/H ratio	0.97±0.02	0.94±0.02	NS
Neck circumference (cm)	43.0±1.5	41.6±1.0	NS
Diabetes indices			
HOMA-IR	7.0±1.4	4.6±0.5	NS
ISI (Matsuda index)	2.7±0.2	2.5±0.3	NS
QUICKI	0.51±0.01	0.54±0.01	NS
Belfiore index	0.13±0.00	0.13±0.00	NS
Stumvoll index (μmol min^{-1}kg^{-1})	0.09±0.00	0.03±0.00	NS
IGI	0.54±0.08	0.23±0.07	NS
HOMA-B	2.0±0.05[a]	1.9±0.06[a]	NS

HDL-c, high density lipoprotein-cholesterol; LDL-c, low density lipoprotein-cholesterol; REE, resting energy expenditure; RQ, respiratory quotient; TBF, total body fat; LBM, lean body mass; BMC, bone mineral content; BMD, bone mineral density; W/H ratio, waist to hip ratio. All data presented in mean±SE.

Table A.2 AUC of BAs and BA composition preoperative comparisons (SG vs.GB)

	Bariatric surgery		
BAs	Sleeve Gastrectomy (n=29)	Gastric Bypass (n=32)	p-value
AUC of BAs[a]			
THDCA	115(110-157)	99(59-142)	NS
GUDCA	72(26-107)	64(23-108)	NS
TUDCA	71(25-106)	259(12-658)	NS
GCA	178(131-213)	178(135-222)	NS
TCDCA	372(287-514)	362(227-507)	NS
TDCA	115(80-174)	114(93-190)	NS
GDCA	4(3-5)	4(3-5)	NS
GCDCA	4(3-4)	4(2-5)	NS
CA	18(6-239)	14(3-239)	NS
TLCA	7(4-9)	7(4-10)	NS
CDCA	413(12-927)	142(11-245)	NS
DCA	110(44-174)	111(40-159)	NS
LCA	102(38-134)	55(39-139)	NS
Composition of BAs[b]			
Total BAs	4.5±0.6	4.4±0.5	NS
Primary BAs	2.8±0.4	3.05±0.4	NS
Secondary BAs	1.5±0.2	1.4±0.1	NS
Primary/Secondary BAs	2.0±0.2	2.4±0.3	NS
12α-OH	1.5±0.2	1.3±0.1	NS
Non 12α-OH	2.9±0.4	3.1±0.4	NS
12α-OH/ Non 12α-OH	1.0±0	0.6±0.1	NS
Conjugated BAs	3.6±0.4	3.8±0.4	NS
Unconjugated Bas	0.8±0.2	0.6±0.1	NS
Con/Unconj BAs	11.6±2.0	15.8±2.6	NS
Glycine BAs	0.3±0.4	0.3±0.04	NS
Taurine BAs	3.3±0.4	3.5±0.4	NS
Glycine/Taurine BAs	0.2±0.07	0.2±0.1	NS
Primary conjugated BAs	2.1±0.3	2.5±0.3	NS

Primary unconjugated BAs	0.7±0.1	0.5±0.1	NS
Secondary conjugated BAs	1.4±0.2	1.2±0.1	NS
Secondary unconjugated BAs	0.15±0.01	0.14±0.01	NS
FGF19/Total BA	21.2±3.5	30.9±4.8	NS

[a] Fasting bile acids presented in pg/ml; [b] median and interquartile (IQR,25th-75th percentile). THDCA, taurohyodeoxycholic acid; GUDCA,glycoursodeoxycholic acid; TUDCA, tauroursodeoxycholic acid; GCA, glycocholic acid; TCDCA; taurochenodeoxycholic acid; TDCA, taurodeoxycholic acid; GDCA, glycodeoxycholic acid; GCDCA, glycochenodeoxycholic acid; CA, cholic acid; TLCA; taurolithocholic acid; CDCA, chenodeoxycholic acid; DCA, deoxycholic acid; LCA, lithocholic acid; All data measured by nonparametric test; [b] for the sake of simplicity data presented in mean±SE.