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Effects of Gastric Bypass and Gastric Banding on Lipid Absorption and Their Influence on Glucose Metabolism

by

Andrey Vizhul

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Examining Committee

Dr. Thomas A. Churchill

Dr. Daniel W. Birch

Dr. Richard Lehner

Dr. Arya M. Sharma

Abstract

Roux-en-Y gastric bypass (RYGB) leads to sustainable weight loss and resolution of hyperlipidemia and type 2 diabetes in obese patients. Its hypolipidemic effect is presumed to be based on fat malabsorption. Comparison of lipid absorption and insulin resistance following RYGB with adjustable gastric banding (AGB) and medical treatment clarifies the importance of the bypassing aspect of RYGB. The presented work is a cross-sectional study of 48 matched obese patients following RYGB, AGB and medical treatment, whose plasma lipids, glucose and insulin were analyzed after a lipid meal. The results of the study demonstrate that postprandial triglycerides, apolipoprotein B48 and nonesterified fatty acids are lower following RYGB compared with AGB and medical treatment. The accelerated disposal of plasma triglycerides and chylomicrons is associated with normalized insulin sensitivity and this can be a key mechanism in the improvement of lipid metabolism after RYGB, which supports its indications in obese hyperlipidemic and diabetic patients.

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Acyl-CoA	Acyl coenzyme A
AGB	Adjustable gastric banding
ALT	Alanine aminotransferase
ApoB48	Apolipoprotein B48
ApoB100	Apolipoprotein B100
AUC	Area under the curve
BMI	Body Mass Index
BPD	Biliopancreatic diversion
BPDDS	Biliopancreatic diversion with duodenal switch
CE	Cholesteryl esters
CM	Chylomicrons
CON	Control
CRP	C-reactive protein
CVD	Cardiovascular disease
EDTA	Ethylenediaminetetraacetic acid
ELISA	Enzyme-linked immunosorbent assay
EWL	Excess weight loss
FA	Fatty acids
FC	Free cholesterol
GLP	Glucagon-like peptide
HbA1c	Hemoglobin A1c (Glycated hemoglobin)
HDL	High-density lipoprotein

HDL-C	High-density lipoprotein cholesterol
HOMA	Homeostatic model assessment
HPLC	High performance liquid chromatography
HSL	Hormone sensitive lipase
LDL	Low-density lipoprotein
LDL-C	Low-density lipoprotein cholesterol
LPL	Lipoprotein lipase
MG	Monoacylglycerol
NEFA	Nonesterified fatty acids
NIH	National Institute of Health
NPC1L1	Niemann-Pick C1-like 1 protein
PBS	Phosphate buffered saline
PC	Phosphatidylcholine
PDME	Phosphatidyl-N,N-dimethylethanolamine
PL	Phospholipids
POSCH	Program on the Surgical Control of Hyperlipidemia
PYY	Peptide YY
RYGB	Roux-en-Y gastric bypass mellitus
SD	Standard deviation
SEM	Standard error of means
T2DM	Type 2 diabetes
TC	Total cholesterol
TG	Triglycerides

VLDL	Very low-density lipoprotein
VLDL-C	Very low-density lipoprotein cholesterol

Chapter 1: Introduction

1.1. Thesis Overview

Laparoscopic Roux-en-Y gastric bypass (RYGB) leads to sustainable weight loss in patients with severe obesity (1). Moreover, RYGB has been shown to produce clinically important resolution of hyperlipidemia and type 2 diabetes (T2DM) (2). Fat malabsorption is presumed to be central to the weight loss induced by RYGB, along with gastric restriction (3). The malabsorptive component of RYGB may be responsible for greater improvements in lipid profile when compared to laparoscopic adjustable gastric banding (AGB), a purely restrictive procedure (4). RYGB is also associated with improvement in the metabolism of nonesterified fatty acids (NEFA) (5), which has been shown to be elevated in obese patients (6). NEFA are linked to insulin resistance, one of the main disturbances in obesity. RYGB has also been shown to lead to resolution of insulin resistance and type 2 diabetes, an effect that seems to precede postoperative weight loss (7). AGB has not been reported to improve insulin resistance and plasma lipids as fast as RYGB (8). As RYGB, when compared to AGB, improves plasma lipids and normalizes insulin resistance faster, it is reasonable to assume that the malabsorption of fats following RYGB is the mechanism responsible for this difference. However, direct evidence that fat malabsorption is responsible for dramatic improvements in plasma lipids, glucose and insulin following RYGB is lacking.

Apolipoprotein B48, or apoB48, is a specific apolipoprotein found on chylomicrons (CM), the intestine-generated particles carrying absorbed dietary lipids through the blood. The plasma concentration of apoB48 is a good measurement of circulating CM, which are major carriers of triglycerides (TG) absorbed after a meal (9). Measurement of early postprandial plasma TG and CM can reflect the rates of fat

absorption in surgically bypassed (RYGB) and non-bypassed (AGB) patients. Another factor that influences the level of postprandial triglycerides and chylomicrons is the rate of their clearance. TG from CM are mostly cleared by lipoprotein lipase (LPL), an enzyme stimulated by insulin. TG levels are elevated in obese patients, suggesting a non-responsiveness of LPL to increased circulating insulin (10). Furthermore, insulin resistance in obese patients will lead to increased plasma levels of NEFA (6). NEFA are mostly cleaved from TG deposited in the adipose tissue; their release is suppressed by insulin in the postprandial state (11). High postprandial levels of NEFA indicate resistance of the adipocyte lipolytic enzymes to the inhibitory action of insulin (6). Insulin resistance can be estimated by the homeostatic model assessment (HOMA), calculated based on fasting glucose and insulin levels (12). Taken together, by analyzing postprandial changes in plasma TG, apoB48, NEFA and calculating HOMA, it is possible to detect the relationship between changes in lipids and insulin resistance after a standardized lipid meal. By comparing patients following RYGB and AGB with matched medically treated (control) obese patients, the influence of the malabsorptive component of gastric bypass can be clarified.

The results of this study indicate that following RYGB, the accelerated clearance of postprandial TG and CM and decreased postprandial NEFA are related to the restoration of insulin sensitivity. Insulin resistance appears to be associated with elevated postprandial lipids and NEFA in the AGB and medically treated obese patients. These results suggest that the beneficial changes in lipid metabolism induced by RYGB may make it the preferred surgical option in patients with dyslipidemia, atherosclerotic heart disease, insulin resistance or T2DM.

1.2. Obesity as a Growing Problem

1.2.1. Epidemiology, Comorbidities and Mortality Rates

Obesity is a chronic disease defined as abnormal or excessive fat accumulation that adversely affects a person's health. The extent of overweight and obesity is measured by the Body Mass Index (BMI). BMI measures the weight of the patient in relation to his or her height. It is calculated by dividing patient's weight in kilograms by the square of the height in meters (kg/m^2). Based on the World Health Organization classification of obesity (13), the 1998 National Institute of Health (NIH) Clinical Guidelines on the Identification, Evaluation, and Treatment of Overweight and Obesity in Adults defined 3 classes of obesity and related risk of comorbidities (Table 1-1) (14). The risk of comorbid disease increases from overweight to Class III Obesity. Obesity of Class III (BMI $\geq$ 40 kg/m^2) is considered severe or morbid.

Obesity has currently reached epidemic proportions in North America and globally. It is associated with multiple clinically important co-morbid diseases, such as T2DM and impaired glucose tolerance, hypertension, dyslipidemia, cardiac and peripheral vascular disease, sleep apnea, osteoarthritis, certain types of cancers, female reproductive disorders and depression.

	BMI	Risk for Comorbidities
Normal	18.5-24.9	Average
Overweight:		
Preobese	25.0-29.9	Increased
Obesity Class I	30.0-34.9	Moderate
Obesity Class II	35.0-39.9	Severe
Obesity Class III	40 or more	Very severe

Table 1-1. BMI, classes of obesity and risk for comorbidities. Adapted from: Hutcher N. Incidence, Prevalence, and Demography of Obesity. In: Buchwald H (ed.). *Surgical Management of Obesity*. Saunders Elsevier: Philadelphia 2007; 10-17.

A Canadian parliamentary report of 2005 by Sheena Starky called "The Obesity Epidemic in Canada" cited the national prevalence of obesity among adults in 2004 as 23.4%, which was an increase from 15% in 2000. The report presented the economic burden of the problem as $4.3 billion annually (15). The prevalence of obesity in Alberta was 25%. Severe, or class III obesity affects 2.7% of Canadians, as reported by the 2004 Canadian Community Health Survey, based on directly measured height and weight of respondents (16).

As obesity rates are rising, its impact on the health of the population is deepening. One third of severely obese patients has type 2 diabetes (1). A recent epidemiological analysis done by Susan Stewart and colleagues from Harvard University suggests that as a result of increasing obesity rates, life expectancy will rise less rapidly in the next 10 years. The researches forecasted future rates of obesity and smoking and estimated their effects on length and quality of life based on data from the National (USA) Health Interview Survey from 1978 through 2006. The negative effect of increasing obesity overran the positive effect of declining smoking and would result in the reduction in life expectancy of 0.71 years and a reduction in quality-adjusted life expectancy of 0.91 years between 2005 and 2020. The estimations expect that, if the trend of the past three decades continues, obesity rates will increase from current 30-35% to 45%, or nearly half of the U.S. population by 2020 (17).

1.2.2. Obesity, Dyslipidemia and Cardiovascular Disease

The American Heart Association considers obesity as an independent risk factor for cardiovascular disease (CVD) (18). The risk of CVD in obesity is believed to be

associated with dyslipidemia. Dyslipidemia is a very common finding in obesity and worsens in morbidly obese subjects. Typically, it is defined as elevated plasma total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), triglycerides (TG) and lower high-density lipoprotein cholesterol (HDL-C). Dyslipidemia is recognized as a lipid profile that leads to the development of atherosclerosis and CVD. Dyslipidemia, glucose intolerance, hypertension and abdominal obesity constitute the metabolic syndrome and identify individuals at high risk of developing coronary heart disease and T2DM (19).

In a prospective study of 1462 women with a 12-year follow up, Bengtsson et al. found that obesity markers, such as waist:hip ratio and elevated serum TG were significantly associated with total mortality and death from myocardial infarction (20). Another study found no difference in TC between 572 obese and 1989 non-obese subjects, even as TG and insulin resistance index rose with an increase in BMI (21).

As recommended by the Third Report of the National Cholesterol Education Program Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol In Adults, (Adult Treatment Panel III, or ATP III), and the 2004 update, optimal LDL-C level should be <100 mg/dL (2.59 mmol/L), TC <200 mg/dL (5.17 mmol/L), HDL-C ≥ 60 mg/dL (1.55 mmol/L) (19). The desirable level of fasted plasma TG should be <200 mg/dL (2.26 mmol/L). Recommended approaches for correction of elevated plasma lipids include life-style change, diet, physical exercises, medical and surgical treatment for weight loss. Dietary changes should include decrease in calories, saturated fats and cholesterol-rich products. The most effective LDL-C-lowering medications are statins, but bile acid-binding sequestrants, fibrates, niacin and ezetimibe

are also used frequently (19). The benefits of weight loss for plasma lipids are well known. In a systematic review of medical and surgical prospective trials, Poobalan et al. have shown that weigh loss has long-term beneficial effects on plasma lipids, especially on TC and LDL-C (22). The goal of any treatment for obesity is to decrease the energy intake below the energy expenditure and thus intensify the use of endogenous fat deposits as fuel. Non-surgical modalities of treatment are considered mostly ineffective in morbidly obese patients, where it is important to achieve a substantial reduction in weight.

Bariatric surgery has been successful in improving plasma lipids. The earliest example of a surgical intervention for cholesterol lowering is the Program on the Surgical Control of Hyperlipidemia (POSCH). Buchwald and colleagues recently reported the results of this program (23), with a 25 year follow up of hyperlipidemic survivors of myocardial infarction randomized to partial ileal bypass surgery plus diet versus diet alone. Partial ileal bypass procedure connects the proximal ileum to colon, to induce malabsorption of cholesterol with bypassing the distal ileum. Approximately one third (31.5%) of the diet-assigned participants were treated later with cholesterol-lowering medication. Surgery-induced decrease in LDL and TC resulted in a significant increase in survival and reduction in total and cardiovascular mortality. The procedure is not in use any more due to its metabolic side effects, such as diarrhea, gallstones and kidney stones.

1.2.3. Obesity, Insulin Resistance and T2DM

Insulin-resistant, or T2DM is closely related to obesity. Ninety percent of all patients with this type of diabetes are obese. After prospectively following almost 85,000

women (1980-1996) in the Nurses' Health Study, obesity was discovered to be the single most important predictor of diabetes (24). The relative risk of diabetes increased almost forty times as the BMI index increased from 23 to above 35, the level considered as obesity.

1.2.4. Medical Weight Loss Leads to Improvement in Insulin Resistance and Obesity-Related Mortality

Intentional non-surgical weight loss has been shown to be associated with a 25% reduction in total mortality and 28% reduction in cardiovascular and diabetes related mortality in a 12-year follow-up of 4,970 overweight subjects with diabetes enrolled in the American Cancer Society Cancer Prevention Study (25). The highest reduction in mortality (33%) was associated with loss of 20-29 lbs of body weight. Paradoxically, major weight loss of more than 70 lbs was associated with a small increase in mortality. Kelley et al. (26) demonstrated that insulin resistance, hepatic glucose production and blood glucose significantly decreased after 7 days of a very low calorie diet in obese non-diabetic subjects, and that half of the overall improvement in glucose metabolism was due to calorie restriction with a weight loss of 12.7 ± 2 kg on average. In 1998, the NIH summarized available data from randomized trials and produced The Clinical Guidelines on the Identification, Evaluation, and Treatment of Overweight and Obesity in Adults (27). Based on review of 49 randomized trials on the effect of weight loss on fasting plasma glucose and insulin, the expert panel concluded that weight loss had an important positive impact on glucose tolerance.

1.3. Bariatric Surgery for Obesity and Related Comorbidities

1.3.1. Bariatric Surgery Induces Weight Loss and Corrects Related Comorbidities

In a systematic review, Buchwald and colleagues (1) were able to prove that bariatric surgery was an effective treatment to achieve substantial and durable weight loss and resolution of comorbidities in obese patients. In the prospective Swedish Obese Subjects Study (28), at 10-year follow-up, surgical treatment led to weight loss of 16.1% of baseline weight (compared to 1.6% increase in the medical treatment group), remission of diabetes (36% in the surgical vs. 13% in the medical group) and hypertriglyceridemia (46% in the surgical vs. 24% in the medical group), as well as decrease in the incidence of new cases of diabetes (7% in the surgical vs. 24% in the medical group), hypertriglyceridemia (17% in the surgical vs. 27 in the medical group) and hypertension (41% in the surgical vs. 49% in the medical group), as compared to conventional therapy. Recently a Cochrane review assessed the effects of bariatric surgery for obesity (29). Twenty six randomized controlled trials and prospective cohort studies comparing different surgical procedures and comparing surgical with non-surgical management for obesity were analyzed. The authors concluded that surgery resulted in a greater weight loss and reduction in comorbidities (diabetes and hypertension) than conventional treatment. The reviewers also concluded that gastric bypass was associated with a larger postoperative weight loss than adjustable gastric banding.

Improvement in the risk factors was shown to decrease predicted 10-year cardiovascular risk by 1.3% as early as 12 months after gastric bypass surgery (30). Weight loss surgery decreases overall mortality rate and reduces risk for development of cardiovascular, cancer, endocrine, infectious, psychiatric disorders (31). Overall, after all

types of bariatric procedures, type 2 diabetes resolved in 77% and improved or resolved in 85% of operated diabetics, based on meta-analysis of 1846 patients in 63 treatment groups (1).

1.3.2. Types of Bariatric Procedures and History of Gastric Bypass and Adjustable Gastric Banding

Bariatric surgery is indicated for morbidly obese individuals when other treatments are deemed to be unsuccessful or other serious obesity-related health problems are present. The current selection criteria for bariatric surgery are based on the NIH Consensus Development Conference Statement on Gastrointestinal Surgery for Severe Obesity (1991) (32) (Table 1-2).

The first bariatric operations were performed in the 1950s, they were jejunoileal and ileocolic bypass (partial ileal bypass used in the POSCH study (23)), purely malabsorptive and maldigestive procedures. They were abandoned later due to excessively high rate of nutritional deficiencies and side effects. In the 1960s gastric bypass procedures were developed, by combining intestinal malabsorptive component with restriction of gastric volume, bringing to existence the group of malabsorptive-restrictive operations. Gastric bypasses have less perioperative complications, their nutritional deficiencies are less severe and can be controlled medically.

Bariatric surgery is indicated for adult patients with:
1. BMI above 40
2. BMI 35 – 40, with related medical comorbidities
3. BMI 35 – 40, with functional limitations due to body size or joint disease
If, after evaluation by a multidisciplinary team, the patient is judged to:
1. Have a low probability of success with nonoperative weight-loss measures
2. Be well informed about the long- and short-term risks and benefits of surgery
3. Be highly motivated to lose weight through surgery
4. Have an acceptable operative risk
5. Be willing to undergo lifelong medical surveillance

Table 1-2. Patient selection criteria for bariatric surgery. 1991 NIH Consensus Statement. Adapted from: Santry et al. Patient Selection for Bariatric Surgery. In: Buchwald H (ed.). *Surgical Management of Obesity*. Saunders Elsevier: Philadelphia 2007; 93-101 (33)

In the 1970s and 1980s purely restrictive gastric procedures were developed, including gastric partitioning, gastroplasties and gastric banding. These operations have a low rate of perioperative complication but they may be less effective in inducing weight loss. Gastroplasties, with construction of a small upper stomach pouch to restrict the volume of ingested food, are out of favor now due to their high failure and late complications rates.

Original malabsorptive surgeries, such as jejunoileal bypass were abandoned because of their side effect and complications. The modern malabsorptive procedures, which include biliopancreatic diversion (BPD) and its combination with restriction – biliopancreatic diversion with duodenal switch (BPDDS) are performed currently in some centres. These operations are more complex, with higher rates of complications and mortality, but may be useful in extremely obese or non-compliant patients, due to a pronounced weight loss. These procedures involve gastric partitioning and resection of some degree, creation of a biliopancreatic limb to drain bile and pancreatic secretions from the excluded duodenum to the alimentary limb along with a short (50-100 cm) common channel in the distal ileum. In BPDDS (Figure 1-1), a restrictive component is added by performing a vertical gastrectomy. This later addition more recently gave origin to another bariatric procedure, the sleeve gastrectomy. BPD and BPDDS are known to induce the greatest weight reduction, up to 80% of excess weight loss. But they are also known to produce protein deficiency. Their mechanisms are believed to be based on the reduction of lipid, protein and starch digestion and absorption. These malabsorptive procedures represent currently only a small part of all performed bariatric procedures.

Gastric bypass, or laparoscopic RYGB (Figure 1-2), a hybrid malabsorptive-restrictive procedure, is considered currently the “gold standard” of weight loss surgery.

In this procedure, first described by Mason and Ito in 1967 (3), the stomach is divided with stapling, leaving a small gastric pouch of 15-30 mL connected to a jejunal limb, and the large lower gastric segment excluded from the contact with food. The jejunum is divided 40-50 cm from the duodenojejunal junction, its distal limb is connected to the created gastric pouch and the proximal end is connected to the jejunum at approximately 100 cm distance from the gastric pouch, reestablishing thus the flow of gastric, pancreatic and biliary secretions to the rest of the bowel. In this way, the consumed nutrients are excluded from contact with bile and pancreatic enzymes for the first 100 cm of the small bowel, precluding their digestion. Weight loss is believed to be achieved by restricting ingested volume of food with a small gastric pouch, and by inducing malabsorption of dietary fats with diversion of bile and pancreatic secretion more distally, so the digestion of nutrients will take place later, be decreased, and thus preclude complete absorption of fats by the shorter available small bowel. This type of bariatric procedure currently has a major acceptance in North America. Usually, the patient is expected to achieve over 60% of excess weight loss (%EWL) within the first 1-2 years (1). The rates of resolution or improvement of obesity-related comorbid diseases are very high after gastric bypass.

Adjustable gastric banding or laparoscopic AGB, described first by Kuzmak in 1986 (34) consists of a hollow silicone inflatable band placed over the upper stomach and connected to a tube attached to a subcutaneous reservoir in the upper abdomen on the anterior rectus sheath (Figure 1-3).

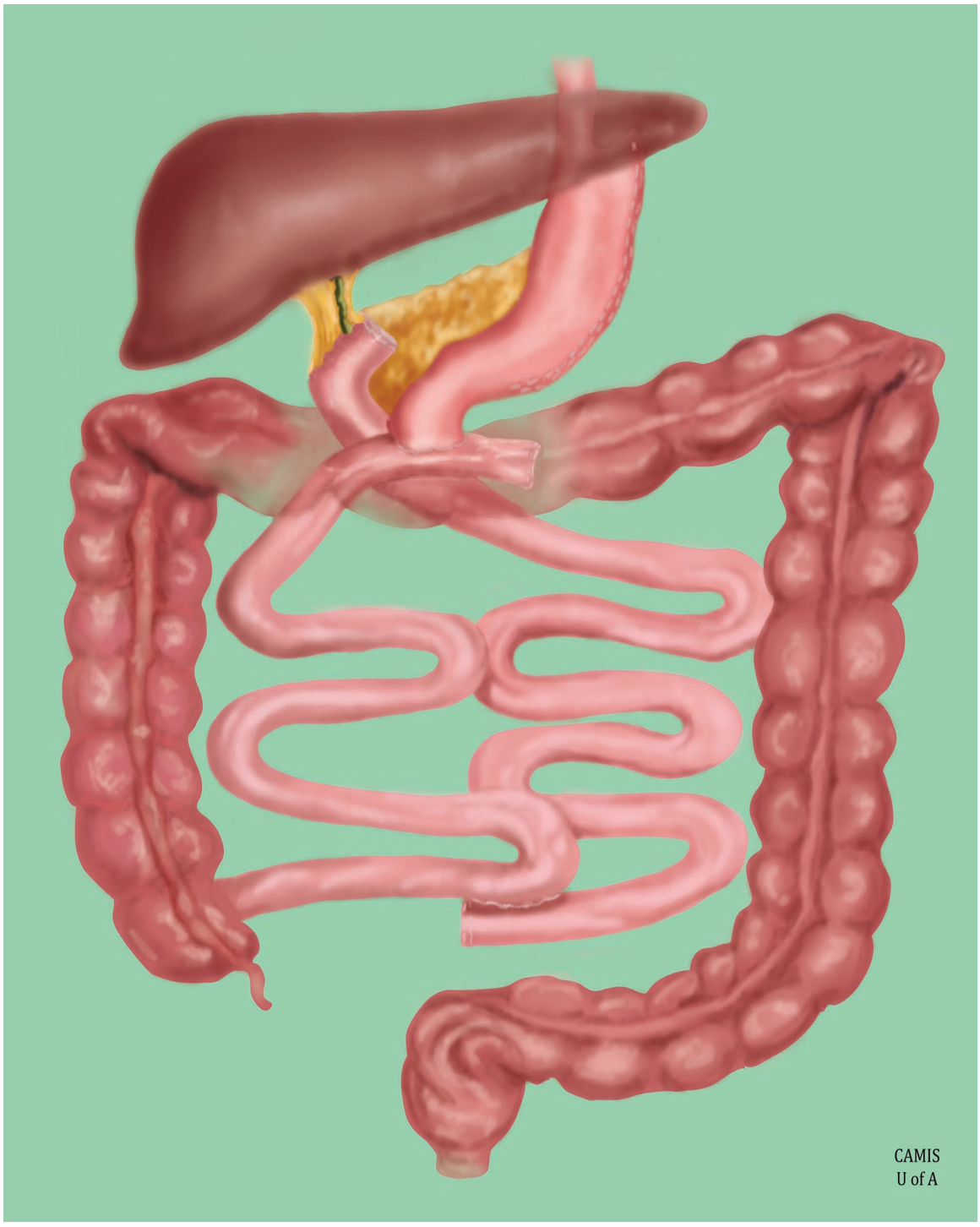

Figure 1-1. Biliopancreatic diversion with duodenal switch (BPDDS). Adapted from the collection of images of the Centre for the Advancement of Minimally Invasive Surgery, University of Alberta.

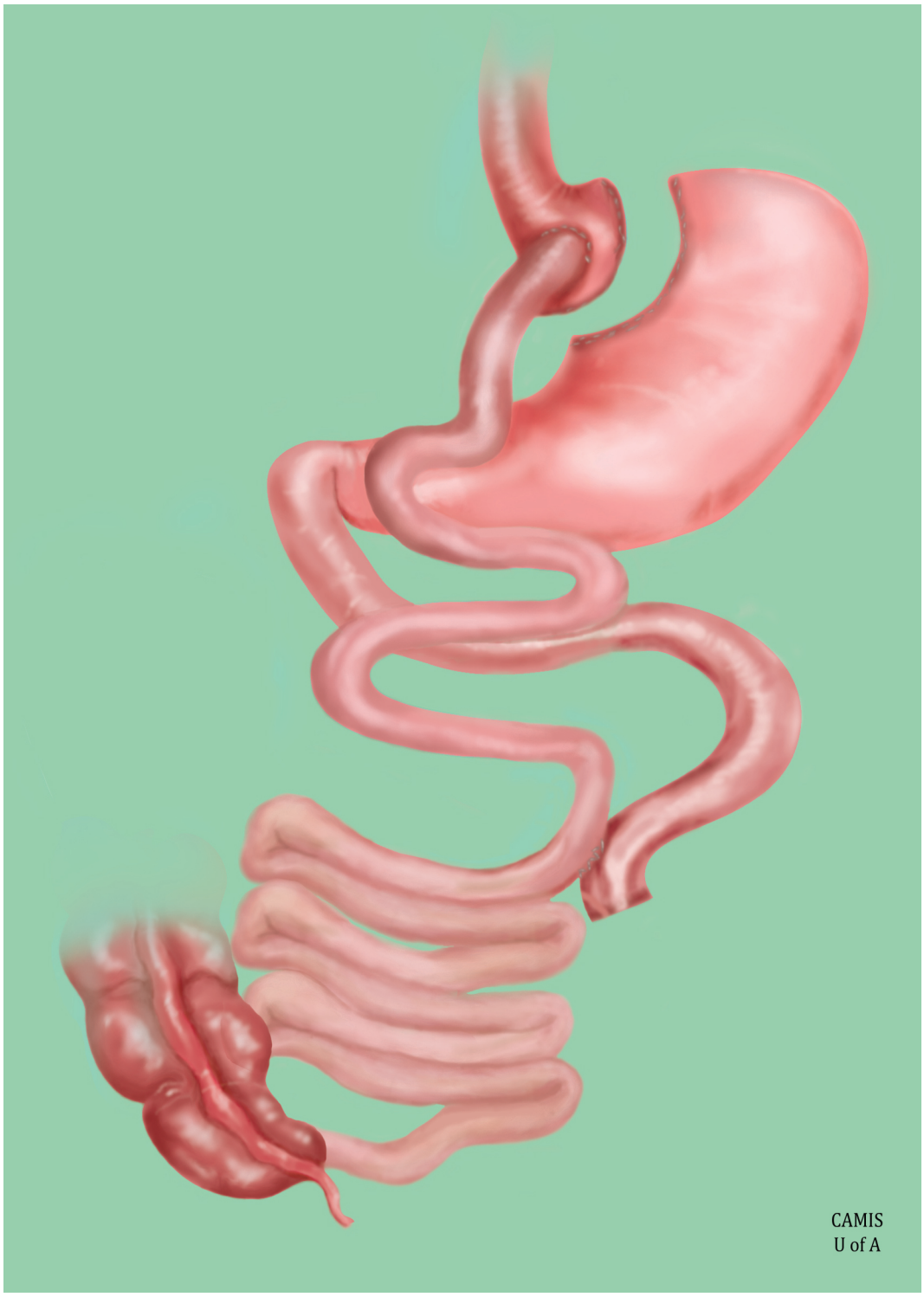

Figure 1-2. Laparoscopic Roux-en-Y gastric bypass (RYGB). Adapted from the collection of images of the Centre for the Advancement of Minimally Invasive Surgery, University of Alberta.

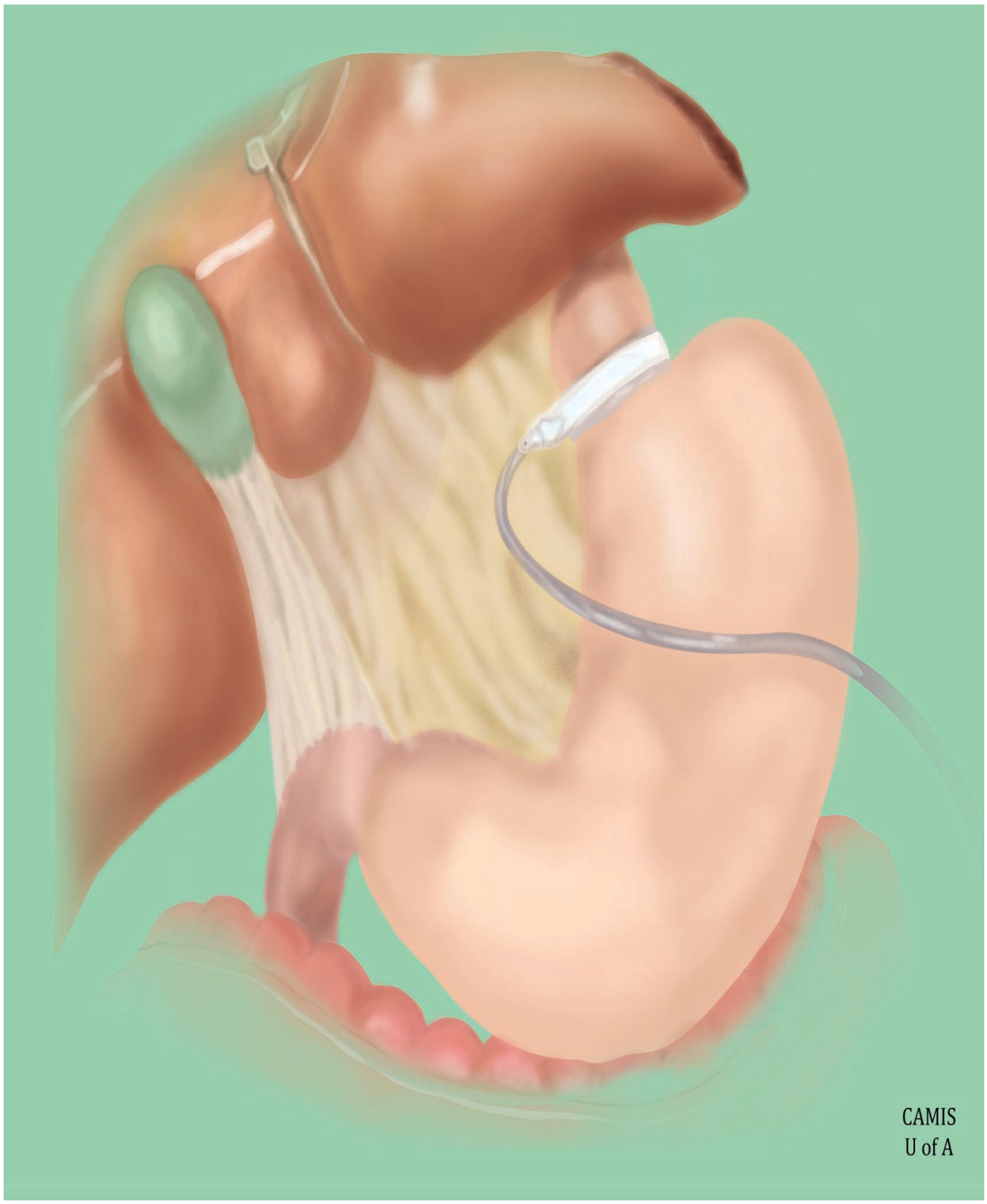

Figure 1-3. Laparoscopic adjustable gastric banding (AGB). Adapted from the collection of images of the Centre for the Advancement of Minimally Invasive Surgery, University of Alberta.

The subcutaneous reservoir permits adjustment of the gastric restriction by injection or withdrawal of saline from the reservoir and regulating thus the distal opening of the created stomach pouch. AGB is a purely restrictive procedure. This bariatric operation is one of the most frequently performed in the world. Its popularity is based on its very low morbidity and mortality rates, technical simplicity and low complication rate. Problems may arise when a patient is non-compliant leading to excessive caloric intake and less than expected weight loss. In many institutions this procedure is done on a day surgery basis. Patients are expected to achieve a 45% excess weight loss within the first 1-2 years (1). Furthermore, AGB has been reported to produce an improvement in obesity-related co-morbidities, such as T2DB (35) and dyslipidemia (8). This procedure is not indicated in extremely obese patients due to its moderate excess weight loss, but can be a very good option for moderately obese compliant patients, particularly when a specialized obesity clinic is available.

1.3.3. Comparative Effectiveness of RYGB and AGB for Weight Loss, Improvements in Plasma Lipids and Resolution of T2DM

The two most frequently used surgical procedures for weight loss, AGB and RYGB vary in their average %EWL. This may contribute to a disparity in their impact on comorbid disease. A meta-analysis of 91 patient population studies (22094 patients) by Buchwald and colleagues (1) summarized that, as a result of bariatric surgery, a substantial weight reduction was achieved with an excess weight loss of 61.2% for all patients, 47.5% for gastric banding, 61.6% for gastric bypass and 70.1% for biliopancreatic diversion or duodenal switch.

In a systematic review comparing gastric banding versus bypass, Tice et al. (4) summarized clinical outcomes from 14 comparative studies. They reported that weight loss was by 26% greater following RYGB 1 year after surgery, with the excess weight loss of 76% after bypass versus 48% after banding in the highest-quality study. Resolution of diabetes was 78% and 50% following RYGB and AGB, respectively. Both procedures resulted in a very low postoperative mortality (<0.5%) but perioperative complications were more frequent after RYGB (9%) than after AGB (5%). The long-term reoperation rate favored gastric bypass, i.e. 16% vs. 24%.

RYGB has been shown by multiple authors to improve lipid markers of CVD risk. Nguyen et al. reported an improvement in lipid profile at 3 months postoperatively. At 12 months TG levels dropped 63%, total cholesterol 16%, LDL-C 31%, very low-density cholesterol (VLDL-C) 74% and HDL-C levels increased by 39% compared to the preoperative values. Lipid-lowering medication was discontinued in 82% of patients (36). Interestingly, atherogenic LDL fraction reduction correlated with a decrease in plasma TG but not with the magnitude of weight loss or diet-induced fatty acid (FA) modifications 12 months after AGB, as shown by Zambon and colleagues (37).

Ballantyne and colleagues demonstrated that greater caloric restriction led to significant improvements in insulin sensitivity, with lower postoperative insulin resistance following RYGB comparing with AGB (38). Insulin resistance was calculated with the HOMA index method (12). The resolution of T2DM after RYGB in 83% of diabetic patients, with reduction in use of oral antidiabetic medication and insulin in 80%, and a loss of 60% of excess weight was reported by Schauer et al (39). Similar improvements in diabetes outcomes were reported by many other authors, as summarized

in a meta-analysis of bariatric surgery by Buchwald et al (1). More recently, Dixon and colleagues reported remission of T2DM in 73% of AGB patients versus 13% of conventionally treated diabetics in a randomized controlled trial with a 2 year follow-up (35). These improvements in glucose metabolism in addition to the correction of other risk factors translated into reduced mortality of operated obese patients. In the Swedish Obese Subjects Study, a large prospective controlled interventional trial involving 4047 morbidly obese subjects, with half of them treated surgically and half treated conventionally, after a 10-year follow-up the investigators reported the reduction of unadjusted overall mortality by 23.7%, and adjusted by gender, age and risk factors by 30.7%. In this study the improvement in mortality rates was correlated with the reduction of such contributing factors as cardiovascular, diabetes and cancer (40).

1.4. Metabolism of Dietary Fats

1.4.1. Digestion and Absorption of Dietary Fats

Dietary fat is comprised mostly of TG and small amounts of cholesterol, cholesteryl esters (CE) and phospholipids (PL). Digestion of fats starts with the action of salivary and gastric lipases that mostly work in the stomach, but this accounts for less than 20% of all fat digestion in an adult. The stomach also grossly emulsifies the dietary fats with its continued peristalsis. Emulsification continues in the duodenum where bile is added to the food bolus. Bile acids and phosphatidylcholine (PC) from the bile blend with the ingested food in the duodenum and emulsify water-insoluble fat particles into fine lipid droplets to facilitate their contact with water-soluble digestive enzymes. PC molecules intercalate through their fat-soluble ends into the fat globules and project their

water-soluble head groups externally making the fat easier to be fragmented by agitation in the small bowel. This also facilitates the action of water-soluble pancreatic lipase over the fat surface. Pancreatic lipase in the presence of colipase and Ca^{2+} hydrolyzes emulsified TG into free FA and 2-monoacylglycerol (2-MG) in the duodenum and upper jejunum. Cholesterol ester hydrolase from the pancreatic secretion acts on CE, TG and esters of lipid-soluble vitamins A, D and E. Phospholipase A_2 from the pancreatic secretion hydrolyzes PL to release FA and lysophospholipids. Short- and medium-chain fatty acids can be absorbed directly into the portal blood in small amounts without being converted into TG. Long-chain FA, 2-MG and other lipids will form small micelles with the help of bile acids. Micelles, due to their small size and relatively large surface area, facilitate contact of apolar hydrophobic lipids contained in them with the enterocytes for absorption. Lipids from the micelles are completely absorbed in the jejunum but bile salts are absorbed in the distal ileum. Bile salts are taken by the enterohepatic circulation back to the liver where they are recycled and excreted again into the bile (41).

The products of lipolysis are absorbed through the apical membrane located at the luminal border of the enterocyte and transported to the endoplasmic reticulum. FA are believed to be absorbed into the enterocytes through simple diffusion in normal physiological conditions. FA transport proteins, such as the membrane transporter CD36 and FATP4 play a role when luminal intestinal concentration of lipids is very low (42). MG are also absorbed into the enterocytes. FA and MG absorption is very efficient, close to 100%. Cholesterol absorption through the apical membrane of the enterocyte is facilitated by Niemann-Pick C1-like 1 protein (NPC1L1). More than two thirds of the cholesterol in the intestinal lumen originate from bile and the rest from diet. Nearly 50%

of luminal cholesterol is absorbed. Cholesterol absorption is increased in patients with type 1 and 2 diabetes, and coronary artery disease (10). Reabsorption of bile acids in the distal ileum is facilitated by apical Na^+-dependent bile acid transporter and Na^+-independent organic anion transporting peptides (10).

In the enterocytes, FA and MG are reesterified into new TG and packaged into CM (10). Absorbed FA and MG are esterified with the action of two key intracellular enzymes, acyl-CoA:monoacylglycerol acyltransferase and acyl-CoA:diacylglycerol acyltransferase. Absorbed cholesterol is esterified to CE by acyl-CoA:cholesterol acyltransferase-2. Newly formed TG and CE will be added, as a hydrophobic core, to nascent CM, a process mediated by microsomal triglyceride transfer protein. These newly formed CM particles also contain PL and free (nonesterified) cholesterol (FC) as a amphipathic peripheral shell. This process of gradual growth takes place in the endoplasmic reticulum where locally synthesized apolipoproteins associate with the surface of each lipid particle. Formed CM are secreted by exocytosis into the lymph. Lymph CM follow the thoracic duct and enter systemic circulation through the left subclavian vein, avoiding the liver. The steps of digestion and absorption of dietary fats are summarized in Table 1-3.

A similar process of formation of lipid-carrying particles takes place in the liver, where, instead of CM, VLDL are assembled. VLDL are converted in the circulation to LDL. ApoB plays an essential role in the assembly and secretion of CM and VLDL. Two isoforms of apoB exist in humans, apoB48 synthesized in the enterocytes, and apoB100 synthesized in the liver. ApoB48 is an incomplete form, missing the N-terminal 52% of apoB100. ApoB100 is a ligand for the LDL receptor mediating clearance of VLDL and

LDL particles from plasma. VLDL and LDL are particles that carry cholesterol and lipid energy (TG and CE) from the liver to the peripheral tissues, similarly to CM. Production of apoB reaches its peak in plasma 6 hours after meal. Only one apoB molecule can exist on each CM or VLDL/LDL particle, and thus it is possible to estimate the number of these particles by the apoB levels.

Serum levels of apoB were found to be better predictors of coronary artery disease than TC and LDL-C (9). Walldius and colleagues reported apoB to be a better predictor of fatal myocardial infarction than cholesterol in a prospective study of 175,553 participants followed for 5 or more years (43). Duez et al. demonstrated an increased production of apoB48 particles with a short-term elevation of plasma NEFA by intravenous lipid infusion (44), and with hyperinsulinemia of insulin-resistant subjects (45). Hyperinsulinemia, insulin resistance, elevated levels of NEFA and lipoproteins are features of obesity and T2DM.

Step	Acting factor or enzyme	Location	Effect
Initial digestion	Lingual and gastric lipases	Stomach	Hydrolysis of medium-chain TG
Initial emulsification of dietary fats	Gastric peristalsis	Stomach	Gross emulsification
Fine emulsification	Bile acids and phosphatidylcholine	Duodenum	Fine lipid droplets presented for lipase action
Hydrolysis of TG	Pancreatic lipase	Duodenum, jejunum	TG hydrolyzed to FA and MG, ready to be absorbed
Absorption and reesterification of FA and MG	Enterocyte	Jejunum, ileum	Reesterification of absorbed FA and MG into new TG
CM formation	Endoplasmic reticulum of the enterocyte	Jejunum, ileum	TG packaged into CM, secreted into lymph and circulation

Table 1-3. Steps of digestion and absorption of dietary fats.

T2DM subjects have an increased rate of production and a decreased catabolism of apoB48 and apoB100-containing lipoproteins, according to Hogue and colleagues (46).

Interestingly, Soriguer et al. reported low levels of the enterocyte TG and apoB48 in obese insulin resistant and T2DM patients with high fasting plasma levels of TG and apoB48 (47).

1.4.2. Metabolism of Lipoproteins and NEFA, and Insulin Resistance

TG in CM are hydrolyzed by LPL and the released FA are delivered to the adipose tissue, muscle and liver. This process occurs in the postprandial period when there is an excess of circulating energy carriers such as glucose and TG. FA are stored in the adipocytes and hepatocytes as resynthesized TG. LPL is localized on the microvascular endothelium. Insulin promotes TG storage in the adipocytes by stimulating LPL and inhibiting lipolysis (10). If the lipolytic activity of LPL is blunted with insulin resistance, more TG are left circulating. Thus, insulin-resistant subjects might have higher plasma TG and CM levels with an increase in the risk of cardiovascular events. When TG are removed from chylomicrons by LPL, CM are converted into TG-depleted CM remnants, which are taken up and catabolized by the liver.

When there is a need for energy, stored TG in adipose tissue are hydrolyzed by intracellular lipases called adipose triglyceride lipase and hormone sensitive lipase (HSL) (11). Released FA are effluxed from the adipocyte into the circulation where they immediately combine with plasma albumin forming a NEFA/albumin complex. In this form they can be transported through the blood. Adipose tissue HSL is normally

suppressed by insulin in the postprandial period. In insulin resistant states, HSL is not responsive to insulin, and the hydrolysis of the adipose TG will keep releasing FA despite their excess in the circulation from a recent meal. In obese patients, plasma NEFA levels are high, secondary to the increased rate of lipolysis in larger adipose stores, leading to dyslipidemia. Both acute and chronic increases in plasma NEFA can induce insulin resistance (6). High levels of NEFA lead to an accumulation of FA-derived metabolites in the muscle, liver and pancreas. The excessive uptake of FA into these tissues exacerbates hepatic and muscle insulin resistance and impairs insulin secretion from pancreatic beta cells, contributing to the development of glucose intolerance and T2DM (48). A higher risk of development of T2DM was found to be associated with increased levels of fasting plasma NEFA in prospective epidemiological studies. The Atherosclerosis Risk in Communities study reported a relative risk of 1.68 for diabetes in subjects with high NEFA levels (49). Improvement of insulin resistance in diabetic and non-diabetic patients was achieved by decreasing NEFA (6). T2DM in obese patients is frequently associated with a low grade inflammatory state, as indicated by elevated C-reactive protein (CRP) levels, adding a further risk factor for CVD (50).

1.5. Changes in Lipid Absorption and Metabolism Following RYGB and AGB

1.5.1. Lipid Malabsorption is Minimal or Not Present Following RYGB

The extent of fat malabsorption after a “malabsorptive” gastric bypass remains to be clarified. In theory, ingested nutrients, after delayed blending with bile and pancreatic enzymes, has less time and available jejunal surface to be absorbed. This should move non-digested fats to the colon with subsequent fat malabsorption and steatorrhea. In

practice, post-gastric bypass patients rarely present with bloating or steatorrhea. In a small study, Odstrcil et al. measured the intake and fecal output of macronutrients in 9 subjects at 5 and 14 months after long-limb (150 cm) RYGB (51). The authors specifically assessed the contribution of malabsorption and restricted intake on reduction of the total absorbed macronutrient energy. They found that 90% of post-surgical reduction in fat absorption was due to decreased fat intake, and only 10% was due to malabsorption. The authors also noticed a wide variety in fat malabsorption between patients. They found a significant post-operative decrease in fat absorption of 22% at 5 months and 26% at 14 months, although these percentages might be influenced by one participant with a bacterial overgrowth and very low fat absorption in their overall small group of participants. Other studies did not report persistent malabsorption and malnutrition after gastric bypass (52). Bradley and colleagues (53) in a study of malabsorption in Roux-en-Y reconstruction after total gastrectomy found moderate fat malabsorption and lower postprandial jejunal concentration of lipase and trypsin, but malabsorption was not clinically significant. Interestingly, a randomized clinical trial on dietary intake, body composition and energy expenditure reported by Olbers et al. (54) revealed that 30% of post-RYGB patients avoided fat meals despite a good tolerance of any food, and they lost more body fat than patients following vertical banded gastroplasty, a purely restrictive procedure, as demonstrated by x-ray absorptiometry and computed tomography.

1.5.2. Fasting Plasma Lipids Improve Following Bariatric Surgery

Improvements in plasma lipids and lipoproteins following bariatric surgery, which are also well-established cardiovascular risk factors, are well documented in numerous studies and reviews. Buchwald and colleagues in a meta-analysis (1) found hyperlipidemia improved in 93.6% of patients following gastric bypass and 71.1% following gastric banding, but the degree of improvement was higher in the post-bypass subjects. Zlabek and colleagues in their cohort of 168 patients, at 1 year after RYGB found a decrease in total cholesterol by 12.5%, LDL-C by 19.4%, triglycerides by 41.2%, and increase in HDL-C by 23.2% (55). It is evident that fasting plasma lipid markers change in different proportions following bariatric surgery. Typically, after gastric bypass, the TG decrease is very pronounced, the reduction in TC and LDL-C is moderate, and HDL-C increase is either modest or not significant (1, 56). Larger improvements in TG, LDL-C and TC are observed after procedures with a so-called malabsorptive component, such as gastric bypass or biliopancreatic diversion. Whether this is due to true malabsorption or a greater intake reduction remains unclear. Moreover, Brolin et al. reported that the reduction in plasma lipid, especially in TC, correlated with the degree of weight loss after standard RYGB and gastric restrictive operations (56). They also suggested that patients with the highest levels of preoperative TG and cholesterol would experience the greatest reduction in plasma lipids following both, gastric bypass and restrictive bariatric procedures, if significant weight loss was achieved.

Recently, Asztalos et al. reported significant improvements in HDL remodeling and profile, especially with high levels of cardio-protective α-1 particles and lower TG

levels in HDL as a result of a large loss of fat mass after RYGB. These beneficial improvements in HDL were greater than after hypolipidemic drug treatments (57).

RYGB but not AGB was reported to decrease cholesterol absorption by 26% in a study of Pihlajamaki and colleagues (58) who used serum plant sterol levels as surrogate markers of cholesterol absorption. This decrease in cholesterol absorption was not associated with clinically significant nutritional deficiencies. In their study, cholesterol synthesis decreased similarly after gastric banding and bypass, indicating that larger reduction in plasma TC and LDL-C and increase in HDL-C following RYGB were due to specific gastric bypass factors influencing cholesterol absorption.

More importantly, plasma lipids remained low even after regain of weight in operated patients. Brolin et al. followed a group of hyperlipidemic subjects following gastric bypass and gastroplasty for up to 5 years and found that even after regain of ≥15% of the lost weight or loss of less than 50% of excess weight, the low plasma lipids remains statistically unchanged when compared to the subjects without weight regain (56).

1.5.3. Improvements in ApoB Following Bariatric Surgery

One of the emerging cardiovascular risk factors, fasting apoB, has been reported to improve following RYGB, but not following AGB. Faraj and colleagues reported a greater reduction in total apoB than reduction in TC and LDL-C following RYGB (59). ApoB did not change 12 months after AGB with a pronounced weight loss (25% of the baseline body weight) in the study of Zambon and colleagues (37). Following biliopancreatic diversion, another malabsorptive procedure, the decrease in plasma apoB

was more pronounced (37%) than in TG (21%) (60). These reports suggest that there might be a relationship between gastrointestinal diversion of food and improvement of the levels of CM and VLDL.

1.5.4. Relationship of Plasma Lipids and the Degree of Weight Loss

A prospective comparison of the loss of the adipose mass with fasting biochemical markers at 1, 6 and 12 months following RYGB performed by Johansson and colleagues (61) revealed that the drop in plasma cholesterols but not TG was greatest in the 1st month after surgery, when no dramatic changes in weight were observed. NEFA and beta-hydroxybutyrate levels were much higher at the 1st month, when TG tended to increase as well, indicating enhanced lipid turnover. Weight decreased progressively by 12 months but the lipid markers did not improve much from the 1st postoperative month levels, demonstrating no clear correlation with weight loss. Insulin resistance was improving as weight was decreasing. The study of Johansson et al. also suggested that TG can be a poor indicator of improvements in cardiovascular factors, as it reflected instead higher levels of NEFA mobilized from stores. It was probably not due to increased intake but rather the rapid loss of fat deposits, especially in the early period after gastric bypass.

The decrease in plasma lipids after AGB is slower than after RYGB. Still, AGB leads to resolution or improvement of dyslipidemia in more than 70% of patients (1, 62). The pace of change in plasma lipids follows the degree of weight loss in AGB patients (62). With a longer follow up, the post-AGB values of plasma lipids became close or

similar to RYGB, and the improvements were long-lasting, at least up to 4 years after surgery (8).

1.5.5. Energy Intake and Expenditures Following RYGB

Total dietary intake and calorie intake decrease after both RYGB and AGB. In the study of Odstrcil et al. (51), the energy intake decreased from 3754 kcal/d preoperatively to 1556 kcal/d at 5 months (59% less) and 2241 kcal/d at 14 months (40% less) after a long-limb RYGB. Energy expenditure, both total and resting, decreased by 25% 14 months after gastric bypass, and this was predicted by the loss of fat and fat-free mass, as reported by Das and colleagues (63).

1.5.6. Changes in Lipolysis of Stored Lipids Following RYGB

Lipid metabolism in adipose tissue is affected by RYGB. Faraj and colleagues concluded that gastric bypass patients utilize and deposit diet-derived lipids more efficiently than lean never-obese control subjects, as they had less lipolyzed dietary TG in the plasma NEFA (5). In their stable isotope study, they also found no difference in resting energy expenditure, thermic effect of food, fat oxidation, apoB and fecal fat content (indicating no fat malabsorption) between RYGB patients and non-obese controls.

One year after RYGB, LPL activity, an adipose lipid storage enzyme, was normalized to the level of non-obese subjects, whereas HSL activity, responsible for lipolysis in the adipose tissue, remained similar to pre-operative high levels in obese patients despite weight loss, as reported by Pardina and colleagues (64). Fasting NEFA

remained high, indicating redirection of TG from storage sites to oxidation in muscles. All other plasma fasting lipids normalized at 12 months after bypass during that study. Thyfault et al. found that plasma NEFA oxidation in obese women remained lower after RYGB compared to non-obese participants and that severe obesity can be associated with a defect in plasma NEFA utilization (65). Resting energy expenditure, measured by indirect calorimetry, decreased with body weight and fat mass at 6 months after RYGB, in a study of Carrasco and colleagues (66).

Improvements in lipid metabolism following RYGB were associated with a significant reduction of hepatic steatosis (67), confirmed by biopsies, indicating a positive effect on non-alcoholic fatty liver disease.

1.5.7. Changes in Insulin, Insulin Resistance and Nonesterified Fatty Acids following RYGB

RYGB significantly improves insulin resistance and this change occurs as early as 1 week after surgery (68). Fasting and postprandial levels of insulin, proinsulin and glucose are significantly lower in RYGB patients than in morbidly obese controls, and close to levels of lean subjects (7). RYGB patients demonstrated faster appearance of exogenous glucose in plasma and shorter, but more pronounced, postprandial hyperglycemia, with high insulin response, comparing to AGB patients (69). Improvements in insulin resistance were followed by the improvements in type 2 diabetes. RYGB is well known for rapid remission of type 2 diabetes, with one-third of operated diabetic patients having normal blood glucose before discharge after their surgery and without any antidiabetic medication (70). In contrast, remission from T2DM

after AGB was shown to occur after 6 months postoperatively (35). Cummings and colleagues suggested (70) that RYGB might have a direct positive effect on pancreatic β-cell function. This stimulation of pancreatic β-cell insulin secretion, along with restored insulin sensitivity can cause a hyperinsulinemia leading sometimes to life-threatening hypoglycemia (71). The explanation for this specific and rapid antidiabetic effect of gastric bypass includes weight loss due to decreased caloric intake and changes in the enteric hormones. Intestinal hormone alterations can be induced either by accelerated arrival of food to the mid-gut, or by excluding the upper gut, as proposed by Rubino and colleagues (72). Changes in such intestinal hormones as ghrelin, glucagon-like peptide-1 (GLP-1) and peptide YY (PYY) were associated with improvement in diabetes following RYGB (2). In fact, the spectacular resolution of T2DM led the Diabetes Surgery Summit Consensus Conference (2010) to recommend bariatric surgery, and RYGB especially, as a treatment for this form of diabetes (73).

1.6. Rationale for the Study

Bariatric surgery is very effective not only for achieving weight loss but also for the resolution of atherogenic dyslipidemia, insulin resistance and T2DM. RYGB, which combines restrictive and intestinal bypass malabsorptive components, leads to faster and more pronounced changes in lipids, glucose and insulin than purely restrictive AGB. This suggests that mechanisms other than just restriction of gastric capacity are involved in the corrections of lipid and glucose metabolism. The malabsorptive component of RYGB might induce desirable changes in lipid absorption and metabolism, as it is known to be responsible for important improvement in insulin sensitivity. The varying impact of these

two most popular bariatric procedures on lipid metabolism can be clarified by comparing biochemical markers of the patients operated by RYGB and AGB, and additionally, by comparing them with matched non-operated obese patients. To characterize postprandial lipid changes, plasma lipoproteins and lipid fractions can be assessed before and after a standardized lipid meal rich in TG. Measurement of CM with a specific apoB48 test can give information about the changes of absorbed dietary lipids in plasma, as it is known that postprandial elevation in plasma TG after meal is 80% due to CM-carried TG. Changes in CM can be compared with measured major lipid fractions, such as TG, CE, FC and PC (major plasma PL), to determine if they vary with the surgically modified gastrointestinal anatomy. Plasma CE and PL can be elevated in hypertriglyceridemia and insulin resistant states, with the increased transfer of CE and PL between lipoproteins, remodeling of HDL (74)(75), participating in the cholesterol clearance, and thus, being involved in the atherogenesis. Insulin, glucose and NEFA can detect an association between changing insulin resistance and lipid metabolism in a very dynamic postprandial state. It has been shown that postprandial lipoprotein production reaches its peak at 4 hours after meal (44), and postprandial FA release reaches its peak at 90 minutes after meal (69). As dietary FA from the previous meal make important contribution to the plasma NEFA and lipoproteins after subsequent meal (44), it is important to give the same lipid meal twice, as the previous meal and the study meal.

To ensure the changes are only due to a different gastro-jejunal anatomy, all participants of the study should be similarly obese. It is presumed that lipid metabolism is different in obese persons compared to normal lean (5). RYGB patients can be matched to AGB patients and to non-operated, medically treated obese patients. The surgical

patients should be weight stable, which is typically reached 12 months after surgery (63). For the AGB patients, this weight stabilization should be without ongoing volume adjustment of implanted gastric band.

An improved understanding of postoperative changes in lipid metabolism could lead to refinement in the indications for specific bariatric procedures in obese patients with dyslipidemia, diabetes and CVD.

1.7. Hypotheses

Postprandial plasma lipids are lower following RYGB (restrictive-malabsorptive) than following AGB (restrictive) or non-surgical management in obese patients.

Reduced plasma lipids following RYGB are associated with improved insulin resistance.

To explore the proposed hypotheses, three specific aims were planned:

- To characterize changes in plasma lipid fractions and apoB48 in different times after a standardized lipid meal in three groups of obese patients treated with RYGB, AGB and non-surgical approach
- To assess the degree of drop in plasma NEFA levels in response to meal
- To investigate changes in postprandial glucose and insulin, and compare insulin resistance between the three study groups.

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Chapter 2: Materials and Methods

2.1. Study Design and Participant Selection

This study was approved by the University of Alberta Health Research Ethics Board. A matched-case comparison study on 48 obese patients was performed. Sixteen RYGB patients, 16 AGB patients, and 16 patients treated non-surgically were matched for sex, age, current BMI and diabetes. All participants were informed about the risks and benefits and all provided their written informed consent. Participants were recruited from the Weight Wise Program at the Royal Alexandra Hospital in Edmonton, Alberta. Surgical patients had been operated at the Royal Alexandra Hospital by three bariatric surgeons. All patients met the selection criteria for bariatric surgery defined by the NIH Consensus Development Conference Statement on Gastrointestinal Surgery for Severe Obesity, 1991 (1). All operated participants were preoperatively obese, either with BMI > 40 kg/m^2 (severely obese) or with BMI > 35 kg/m^2 and with related medical comorbidity, all of them had unsuccessful attempts of conservative treatment for their obesity before surgery. Only weight stable surgical patients were accepted. Weight stability was defined as the change of no more than 5% of weight in the previous 3 months. Typically, weight stability is expected to be achieved 12 months after surgery (2). Inclusion and exclusion criteria were defined as following:

Inclusion criteria

1) morbidly obese patients, male and female,

2) age 18-65,

3) with or without T2DM,

4) 12 or more months following RYGB or AGB, or patients waiting for mentioned procedures,

5) able to provide written informed consent.

Exclusion criteria

1) chronic liver disease,

2) maladaptive eating behavior,

3) current pharmacological treatment for obesity,

4) for patients following AGB – ongoing band volume adjustments,

5) hypothyroidism,

6) treatment with insulin,

7) undergone or undergoing revision of a previous bariatric procedure,

8) any major post-operative gastrointestinal complications, such as an anastomotic leak, outlet obstruction or persistent vomiting,

9) allergy to soy or any component of the study meal: Hormel Great Shake^{TM} Plus,

10) renal failure (glomerular filtration rate < 60 ml/min),

11) alcoholism,

12) acute illness,

13) pregnancy or nursing.

2.2. Operative techniques

Essential features of the gastric bypass included a 100 cm jejunal Roux-limb anastomosed by a hand-sewn or circular stapled gastrojejunostomy to a 30 ml gastric pouch, and a 40 cm biliopancreatic limb. The laparoscopic adjustable gastric banding was performed using the standard Swedish adjustable band pars flaccida technique (3).

2.3. Study Meal and Blood Sample Collection

All patients underwent preoperative and postoperative follow up with clinical examination and laboratory blood sampling for glucose, cholesterol, TG and other laboratory parameters. Their enrollment to the study and consent took place at the Weight Wise Clinic at the Royal Alexandra Hospital. The participants were admitted to the Clinical Investigation Unit at the University of Alberta Hospital, Edmonton, Alberta for the study phase, where the meal administration, patient monitoring and blood sampling were performed. To ensure plasma lipids with similar FA spectrum, the subjects were given the study lipid meal twice, at 7 PM night before, and after an overnight fast of 12 hours at 7 AM. As demonstrated by Heath and colleagues (4), FA from the previous meal compose substantial part of the TG in CM and VLDL released after the next meal. The study lipid meal consisted of 240 mL of Hormel Great Shake^{TM} Plus, an approved liquid nutritional supplement (490 Kcal, 49% calories from fat). This supplement was used in a study of postprandial lipids and NEFA and was well tolerated (5). The meal contained mostly TG of soybean and corn oil origin and its FA composition was determined by gas chromatography (Table 2-1). The study meals were donated by the manufacturer, Hormel Health Labs, Savannah, GA.

Blood samples were taken from an antecubital venous catheter before and at 30, 90, 240 and 360 minutes after the meal intake. Samples were collected into potassium fluoride treated tubes for glucose, and into tubes treated with sodium ethylenediaminetetraacetic acid (EDTA) for all other assays and processed immediately. The tubes for blood glucose and glycated hemoglobin were taken to the hospital laboratory and analyzed immediately. Other samples were centrifuged at 3000 rpm at room temperature for 15 minutes. Separated plasma samples were kept frozen at -80^{0}C until analysis.

2.4. Lipid analysis

To determine such lipids as TG, CE, FC and PL, plasma lipids were extracted using the modified Folch method (6) and subsequently analyzed using high performance liquid chromatography (HPLC). Stored plasma samples were thawed. To 100 μL of plasma sample in a glass 15 ml tube were added 30 μL of 1 mg/mL of phosphatidyl-N,N-dimethylethanolamine (PDME) as the internal standard and 870 μL of phosphate buffered saline (PBS). Four mL of chloroform: methanol (2:1) was added and the mixture was vortexed. The mixture was centrifuged 10 minutes at 2500 rpm to allow phase separation. The lower lipid phase was drawn and dehydrated by passage through a Pasteur pipette column filled with anhydrous Na_2SO_4. The effluent was evaporated under a stream of nitrogen and re-dissolved in 100 μL of chloroform: isooctane (1:1). The solution was transferred to Agilent HPLC vials and stored at -20^{0}C until the analysis.

Hormel Great Shake™ Plus (Hormel Health Labs, Savannah, GA) 240 mL

Nutrients	Fatty Acid	
Protein		16 g
Carbohydrate		46 g
Fat		26 g
	C16:0	12.4 %
	C18:0	1.9 %
	C18:1	28 %
	C18:2	55.3 %
	C18:3	0.9 %
Cholesterol		0 g
Calories		490 kcal

Table 2-1. Test meal composition.

Samples were analyzed by HPLC using an Agilent 1100 series (Agilent Technologies, Santa Clara, CA) equipped with a quaternary pump and an Alltech ELSD 2000 evaporative light-scattering detector (Grace, Deerfield, IL). The column used was an Onyx monolithic Si (Phenomenex, Torrance, CA) and the solvent system was based on the method of Graeve and Janssen (7). The separation of lipid classes was achieved with a gradient program of solvents. A combination of three solvent mixture was used. Solvent A consisted of isooctane: ethylacetate (99.8:0.2); solvent B was acetone: ethylacetate (2:1) with 0.02% acetic acid; solvent C was isopropanol: water (85:15) with acetic acid and ethanolamine each at 0.05%. Nitrogen flow was 3.0 L/min and drift tube temperature was set at 60^0C. Peaks were analyzed using Agilent Chemstation software and quantified using calibration curves prepared with commercial lipid standards (Sigma, Avanti Polar Lipids, Alabaster, AB).

2.5. NEFA

NEFA were determined using enzymatic colorimetric method assay with a commercially available kit HR Series NEFA-HR (2) (Wako, Japan), according to the manufacturer's instructions. The procedure consisted of two steps. First, the fatty acids in the samples were activated to acyl coenzyme A (acyl-CoA) by acyl-CoA synthetase. Acyl-CoA was then oxidized by acyl-CoA oxidase, producing hydrogen peroxide. Formed hydrogen peroxide, in the presence of peroxidase, allowed for the oxidative condensation of an aniline dye with 4-aminoantipyrine, leading to formation of a purple colored product. Optical density of the formed product was measured at 550 nm. Oleic

acid was used to generate a standard curve. The amount of NEFA in the sample was determined from the optical density of formed colored product.

2.6. ApoB48

ApoB48 concentration was measured using sandwich enzyme-linked immunosorbent assay (ELISA) with monoclonal antibodies against human apoB48, as described by Kinoshita and colleagues (8). These antibodies do not cross-react with apoB100. ApoB48 were quantified using commercially available Human ApoB-48 ELISA Kit (Shibayagi Co, Japan), by the manufacturer's instructions. Initially, to capture apoB48, samples were incubated in monoclonal antibody-coated wells. After that, biotin-conjugated anti-apoB48 antibodies were added. Captured and bound to the antibodies apoB48 were detected with horse radish peroxidase conjugated avidin, which reacted with added chromogenic substrate reagent. The absorbance of the formed yellow product was measured with a spectrophotometer at 450 nm. Plates with monoclonal antibody-coated wells and biotin-conjugated anti-apoB-48 antibodies were provided with the kit.

2.7. Glucose and HbA1c

Blood glucose was measured by glucose oxidase method on the Beckman Coulter DxC Chemistry analyzer. Glycated hemoglobin was determined using HPLC. Both glucose and HbA1c were determined at the clinical laboratory of the University of Alberta Hospital.

2.8. Insulin

Insulin levels were determined using a sandwich ELISA with a commercially available Human Insulin Assay Kit (Meso Scale Diagnostics, LLC, Gaithersburg, MD). The capture antibodies against insulin are pre-coated on electrodes in the wells of the plate provided by the manufacturer. The samples were added to the wells simultaneously with the anti-insulin detection antibodies labeled with an electrochemiluminescent compound. Immobilized on electrodes complexes of insulin, capture antibodies and detection antibodies were identified by electrochemiluminescence in the presence of a read buffer. Bound to insulin labeled detection antibodies emitted light when a voltage was applied to the electrodes. Emitted light was detected by MSD SECTOR instrument (Meso Scale Diagnostics, LLC, Gaithersburg, MD). The amount of insulin in the sample was calculated based on the measured emitted light. Both capture and detection antibodies were mouse monoclonal antibodies to human insulin.

2.8.1. Insulin resistance

HOMA index of insulin resistance was calculated using the formula described by Matthews and colleagues (9): [insulin (μU/mL) X glucose (mmol/L)] / 22.5. This simple method estimates baseline fasted insulin resistance based on a mathematical model. HOMA index has been validated in multiple studies against physiological methods, such as hyperinsulinemic-euglycemic clamp (10). HOMA index was found to be reliable in different patient populations including lean and obese, diabetics, both sexes and subjects of different ages (11). HOMA index above 2.5 indicates insulin resistance (12).

2.9. Standard Clinical Lipid Tests

Standard clinical plasma lipids, including total, LDL, HDL cholesterols, TG, CRP and alanine aminotransferase (ALT) were performed by the laboratory of Alberta Health Services using standardized hospital laboratory methods.

2.10. Statistics

Continuous data were presented as means ± standard error of means (SEM) and categorical variables were presented as frequency unless indicated otherwise. Demographic data were summarized with descriptive statistics. Biochemical variables were compared by analyzing the changes of means in different times and area under the curve (AUC). Continuous variable data were tested for normal distribution and statistically significant difference was determined using t-test if the data were normally distributed and Mann-Whitney test if the data were not distributed normally. For categorical variables, the data were compared using Fisher's test. P value < 0.05 was considered as a significant difference. Statistical analysis was carried out using STATA 10 software (StataCorp LP, College Station, TX).

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Chapter 3: Results

3.1. Demographic Characteristics

Consecutive patients coming for follow up appointments to the Weight Wise Clinic at the Royal Alexandra Hospital, Edmonton, Alberta, were enrolled into the study. All patients were interviewed and examined by the trainee investigator. Eligible patients were checked for the inclusion and exclusion criteria and matched between groups for sex, age, BMI and presence of T2DM. All patients signed informed consent to participate in the study.

All 48 participants completed the study protocol. The demographic characteristics of the 3 study groups are presented in Table 3-1. All three groups were similar in their weight, BMI, sex and age at the time of the study. T2DM was present in 3 patients of the RYGB, 2 patient in AGB group and 3 in the non-operated group. Women constituted 94% of participants in RYGB and medically managed group, and 75% in AGB group. There was no difference in waist circumference.

The clinical lipid profile was the most favorable in the RYGB group, with the lowest TG, total and LDL-C and the highest HDL-C (Table 3-2). In that group, when compared with the non-surgical control group, all markers except LDL were significantly different ($P < 0.05$), while when compared with the AGB group only LDL and TC were different ($P < 0.05$). Only HDL-C was significantly higher ($P < 0.05$) in the AGB versus medical treatment group, all other lipid markers were not different ($P > 0.05$).

Two participants had abnormally high TC, above 5.17 mmol/L, in the RYGB group, 7 participants in AGB group, and 7 in the non-surgical group.

Variable	RYGB n = 16	AGB n = 16	Control n = 16	P
Age (yr)	48 ± 10 (21-64)	45 ± 9 (31-60)	46 ± 9 (32-69)	> 0.05
BMI current (kg/m^2)	39.2 ± 5.6 (27.6-48.2)	37.8 ± 6.8 (27.9-49.6)	40.9 ± 7.3 (34.4-64.4)	> 0.05
Weight current (kg)	108.5 ± 21.1 (77-163)	108.7 ± 26.4 (69-147)	115.8 ± 17.1 (93-155)	> 0.05
Sex, female (n)	15	12	15	> 0.05
Type 2 diabetes (n)	3	2	3	> 0.05
Waist circumference (cm)	121 ± 16	118 ± 16	122 ± 8	> 0.05

Table 3-1. Patient demographic characteristics, mean ± SD, (range).

HDL-C below a recommended level of 1.55 mmol/L was observed in 9 RYGB, 12 AGB and 15 medically treated patients. All three groups demonstrated similar and normal ALT and CRP levels ($P > 0.05$). Glycated hemoglobin (HbA1c) was normal (< 6 %) and similar in all three groups ($P > 0.05$) (Table 3-2). Abnormal, or above 6 % HbA1c was found in only 4 patients treated medically, 2 patients after AGB and none after RYGB.

A minority of patients was taking medications for hyperlipidemia and hyperglycemia (Table 3-3). One patient in the RYGB group, 4 patients in the AGB group and 4 patients in the non-surgical groups were taking hypolipidemic medication. None of the patients received Orlistat, a pancreatic lipase inhibitor that could potentially influence the digestion of the study meal. As regarding oral hypoglycemic medication, 1 in the RYGB, 1 in the AGB and 3 in the non-surgical groups were taking them. None of the patients were receiving insulin therapy.

3.2. Clinical Results of Treatment

The two operated groups demonstrated considerably more weight loss than the medically treated group (Table 3-4). Weight and BMI at the time of admission into the obesity program (initial) were significantly different between the RYGB and non-surgical control group ($P < 0.001$). Initial BMI was also different between the RYGB and AGB groups ($P = 0.0092$). The difference between the AGB and control groups did not reach statistical significance in neither initial weight ($P = 0.0591$) nor BMI ($P = 0.0672$).

Variable	RYGB n = 16	AGB n = 16	Control n = 16	P RYGB vs. Control	P RYGB vs. AGB	P AGB vs. Control
Triglycerides (mmol/L)	1.10 ± 0.52 (0.5-2.7)	1.52 ± 0.97 (0.5-4.0)	1.61 ± 0.73 (0.5-3.1)	0.0478	0.3364	0.534
Cholesterol total (mmol/L)	4.35 ± 0.58 (3.7-5.6)	5.27 ± 1.31 (3.8-8.9)	4.85 ± 0.85 (3.4-6.2)	0.0437	0.0098	0.5465
LDL Cholesterol (mmol/L)	2.42 ± 0.55 (1.7-3.6)	3.2 ± 0.97 (2.0-5.6)	2.87 ± 0.85 (1.3-4.2)	0.0897	0.0053	0.4287
HDL Cholesterol (mmol/L)	1.43 ± 0.21 (1.1-1.7)	1.4 ± 0.35 (0.9-2.4)	1.18 ± 0.28 (0.9-2.0)	0.0037	0.3858	0.0237
HbA1c (%)	5.5 ± 0.4 (4.8-6.2)	5.6 ± 0.5 (5.8-0.7)	5.8 ± 0.7 (5.0-7.6)	0.073	0.5594	0.1997
CRP (mg/L)	9.4 ± 9.2 (1.5-24)	7.6 ± 1.5 (5.9-8.8)	6.4 ± 4.3 (2.3-16.7)	0.3623	0.756	0.647
ALT (U/L)	17.8 ± 5.7 (10-31)	21.1 ± 9 (11-41)	24.5 ± 10.7 (12-46)	0.0407	0.2149	0.3826

Table 3-2. Clinical biochemical markers, mean ± SD, (range).

Variable	RYGB n = 16	AGB n = 16	Control n = 16	P RYGB vs. Control	P RYGB vs. AGB	P AGB vs. Control
Hypolipidemic medication (n)	1	4	4	0.144	0.144	ns
Hypoglycemic medication (n)	1	1	3	0.285	ns	0.285

Table 3-3. Hypolipidemic and hypoglycemic medication taken by the patients at the moment of the study (n, number of patients; ns, non-significant).

Both RYGB and AGB led to a marked weight loss. The highest weight loss (32.8 ± 5.5 % of initial weight) was noted in the RYGB group, whereas the AGB patients lost 23.8 ± 9 % of initial weight which was significantly less than the RYGB group ($P < 0.001$). Medically treated patients also lost weight, 6.1 ± 7.5 % of initial, which was significantly less than in any of the surgical groups ($P < 0.0001$). When expressed as percent of excess weight loss (%EWL), the participants lost 58.7 ± 10.2 %, 49.2 ± 18.6 % and 13 ± 15.9 % in the RYGB, AGB and medical control groups, respectively, prior to enrollment in this study. Changes in the Body Mass Index of the participants are presented in Figure 3-1.

Variable	RYGB n = 16	AGB n = 16	Control n = 16	P RYGB vs. Control	P RYGB vs. AGB	P AGB vs. Control
BMI initial (kg/m^2)	58.6 ± 9.6 (41.2-76.8)	49.9 ± 8.1 (35.1-63.1)	43.9 ± 9.6 (34.1-75)	0.0002	0.0092	0.0672
Weight initial (kg)	162.2 ± 32 (109-227)	143.2 ± 32 (84-196)	124.3 ± 22 (93-180)	0.0005	0.1017	0.0591
Weight loss (kg)	53.7 ± 15.3 (24-91)	34.5 ± 14.5 (5-65)	8.5 ± 10.6 (-2-35)	<0.0001	0.001	<0.0001
Weight loss (% initial)	32.8 ± 5.5 (22-43)	23.8 ± 9.0 (6-43)	6.1 ± 7.5 (-2-26)	<0.0001	0.0018	<0.0001
% Excess Weight Loss	58.7 ± 10.2 (47-83)	49.2 ± 18.6 (20-83)	13 ± 15.9 (-4–52)	<0.0001	0.0365	<0.0001

Table 3-4. Clinical results of treatment, mean ± SD, (range).

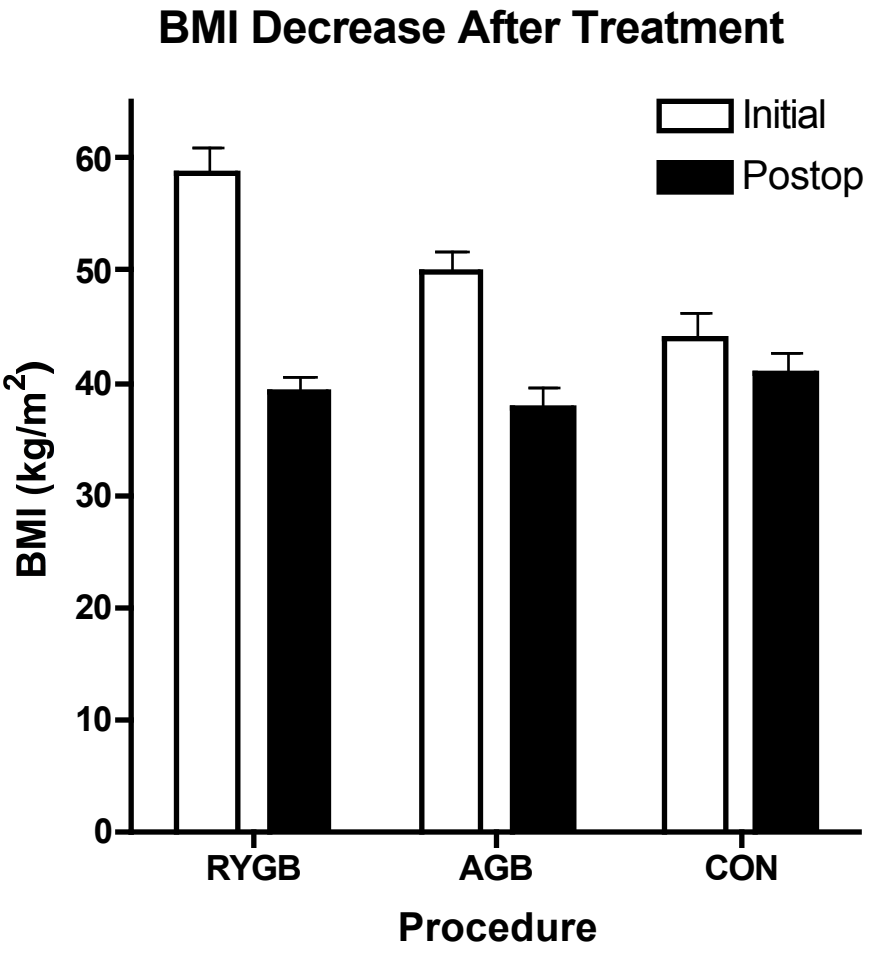

Figure 3-1. Changes in Body Mass Index (BMI) following different treatments.

Initial weight is at the first visit of the patient to the Weight Wise Clinic. Postoperative weight (Postop) is at the moment of the enrollment into the study. All data are expressed as mean ± SEM.

3.3. Meal Tolerance and Postprandial Hypoglycemia

For the interventional part of the study all participants were admitted and monitored in the Clinical Investigation Unit at the University of Alberta Hospital, Edmonton, Alberta. Tolerance of the standardized lipid meal was good, with only three cases of mild nausea (all or more than 90% of the meal was ingested). Blood pressure increase of 20 mm Hg from baseline was observed in one patient at one time point only without any clinical complaints and with normal glycemia.

Eleven of sixteen participants (69%) in the RYGB group had blood glucose level of 4 mmol/L or less, with two patients falling below 3 mmol/L at 90 min after meal (Figure 3-13). This hypoglycemia did not manifest itself with any clinical symptoms or changes in blood pressure and heart rate and normalized by the next measurement at 4 hours in all but one patient.

3.4. Plasma Lipids

3.4.1. TG

The plasma levels of TG varied between the study groups (Figure 3-2). The RYGB group had the lowest postprandial increase in TG levels beginning at 90 minutes and later, when compared to the AGB group ($P < 0.05$) and non-operated controls ($P < 0.01$). The baseline (fasting) level of TG in the RYGB group was also the lowest. In contrast, the postprandial increase in the AGB group showed a similar pattern as the medical controls but at a lower level, the differences did not reach statistical significance. The postprandial response of TG, measured by the total area under the curve (AUC), was the lowest in the bypass group (Figure 3-3).

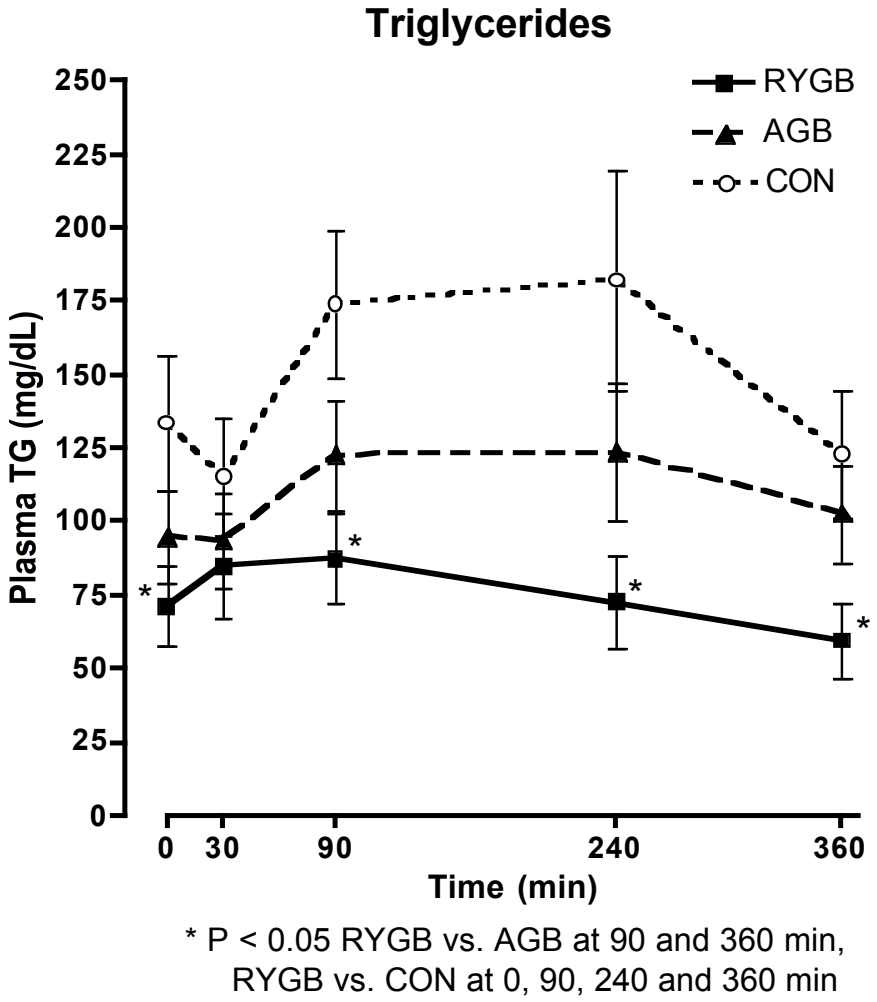

Figure 3-2. Postprandial changes in plasma triglycerides. All data are expressed as mean ± SEM.

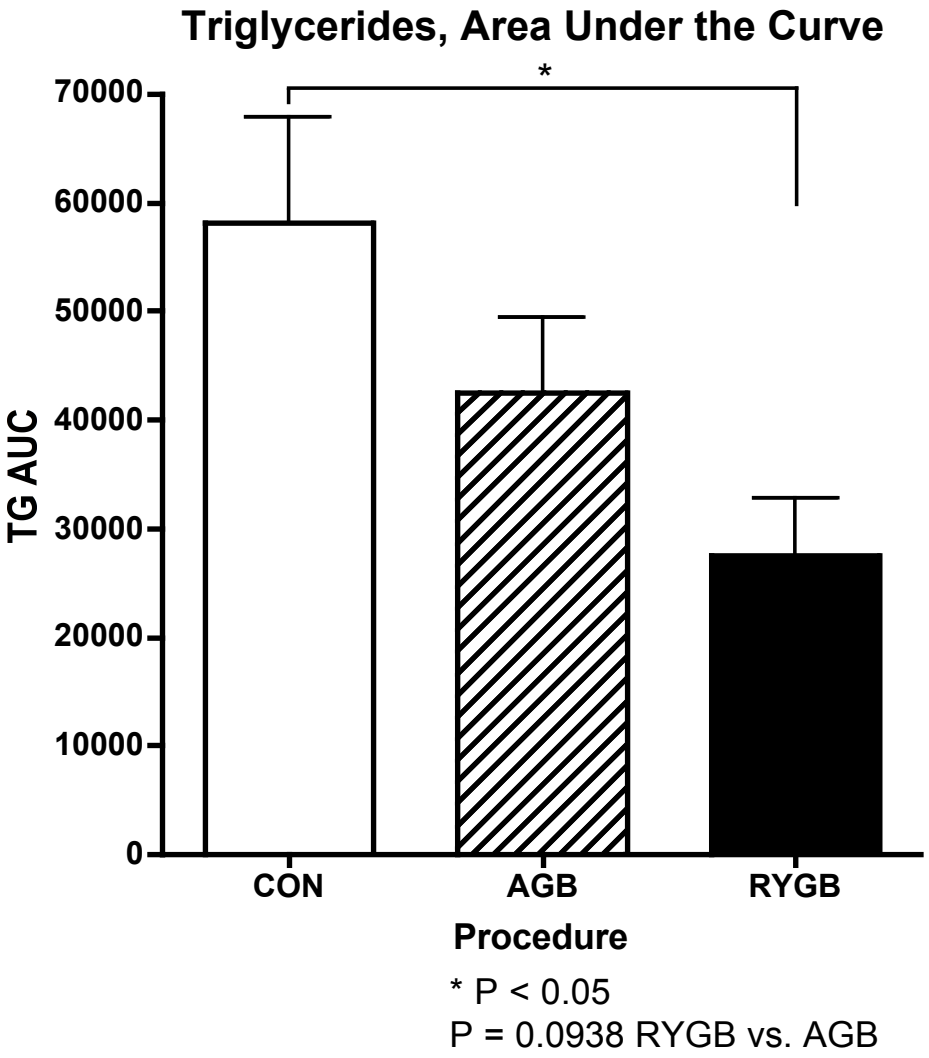

Figure 3-3. Triglycerides, area under the curve, between groups of treatment, mean ± SEM.

The AUC of TG was 27592 ± 21058 area units, 42574 ± 27484 area units and 58201 ± 37333 area units in the RYGB, AGB and medical control groups, respectively. The RYGB group AUC was statistically different (P = 0.0083) from the medical control group AUC, and did not reach significant difference (P = 0.0938) when compared to the AGB group AUC. The AUC of the AGB and medical control groups were not statistically different (P = 0.1928).

3.4.2. CE

Postprandial cholesterol esters levels were similar (P > 0.05) between the study groups at all time points (Figure 3-4). The AUC (Figure 3-5) were also similar between all three groups (P > 0.05). The values of AUC were 61637 ± 16482 area units, 69145 ± 31579 area units and 67102 ± 21568 area units in the RYGB, AGB and medical control groups, respectively.

3.4.3. FC

No differences (P > 0.05) were observed in the levels of plasma FC (Figure 3-6). The postprandial response measured by AUC (Figure 3-7) was also similar between the three study groups (P > 0.05). The values of FC AUC were 7256 ± 3360 area units, 7803 ± 3944 area units and 7115 ± 2989 area units in the RYGB, AGB and medical control groups, respectively.

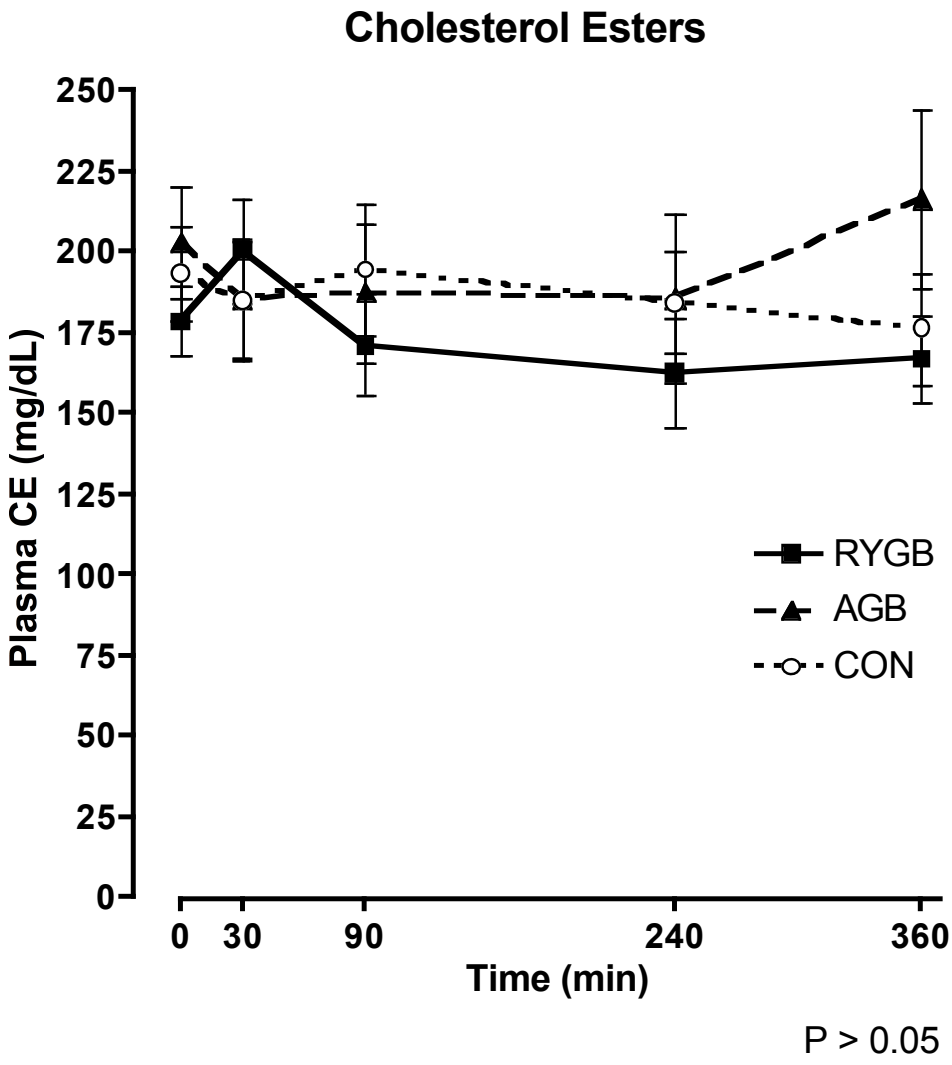

Figure 3-4. Postprandial changes in plasma cholesterol esters. All data are expressed as mean ± SEM.

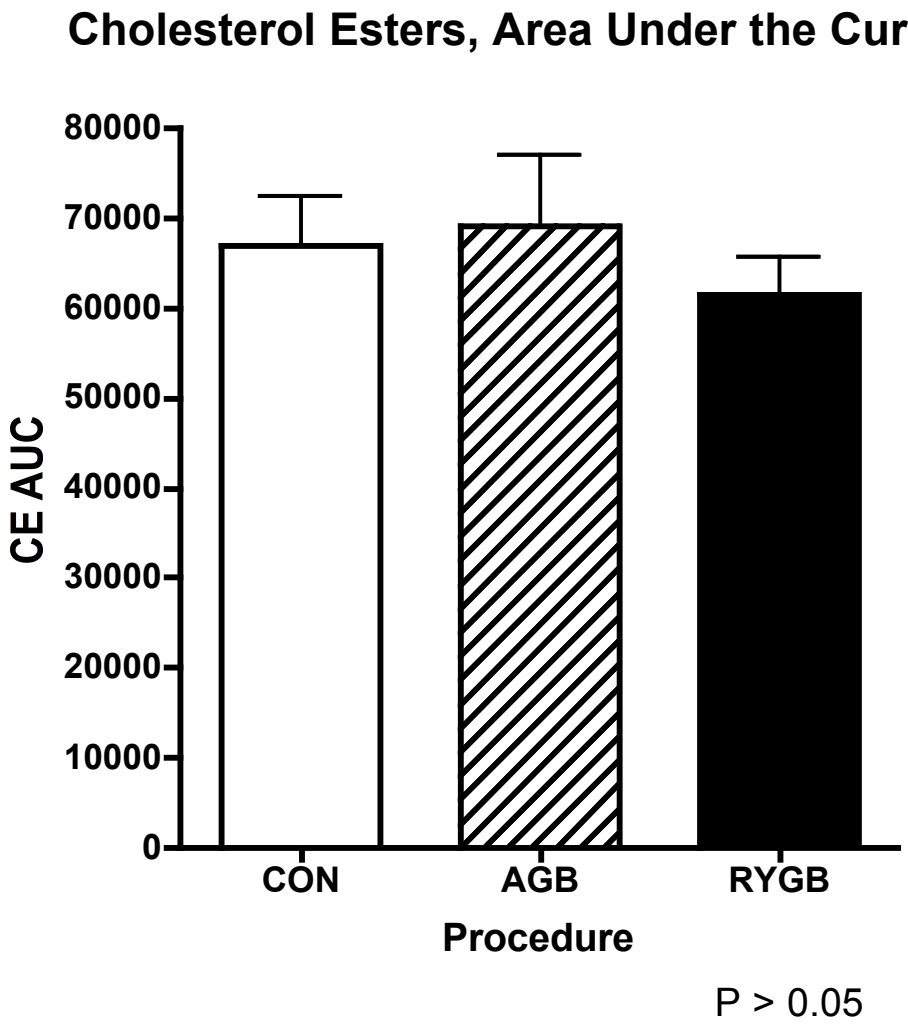

Figure 3-5. Cholesterol esters, area under the curve, between groups of treatment, mean ± SEM.

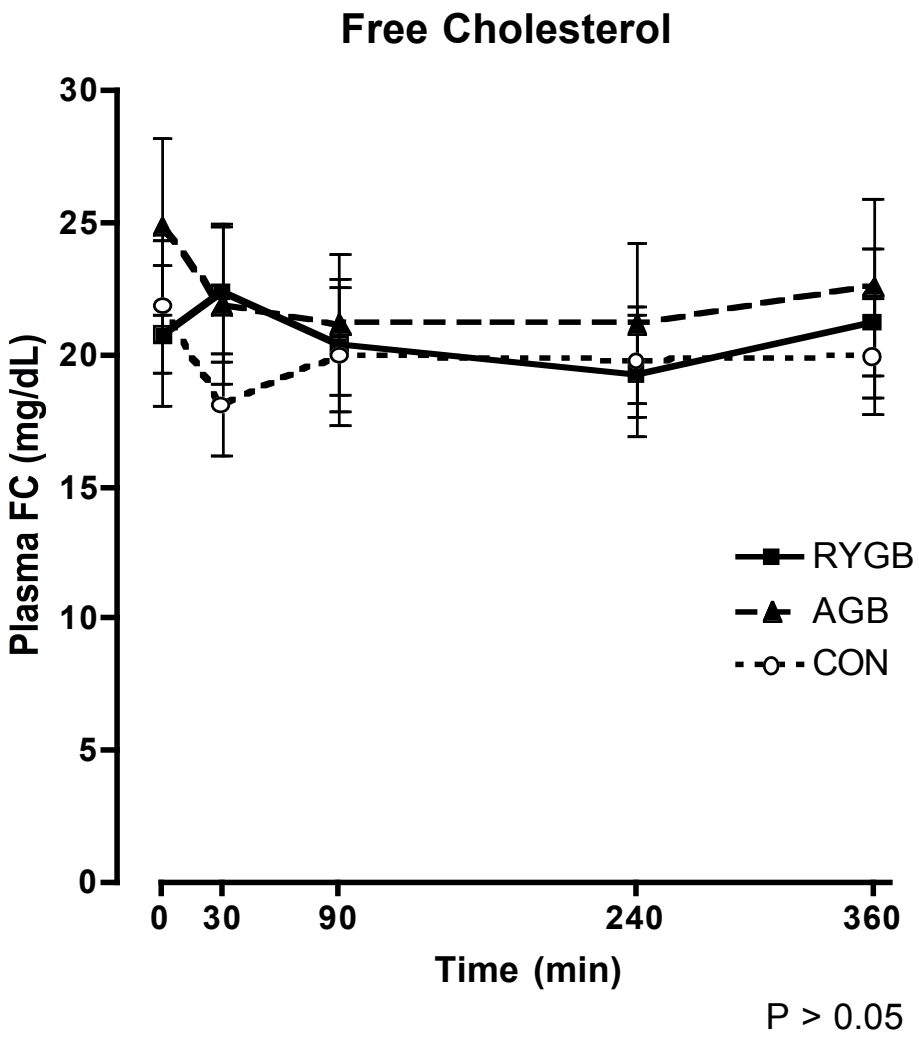

Figure 3-6. Postprandial changes in plasma free cholesterol (FC). All data are expressed as mean ± SEM.

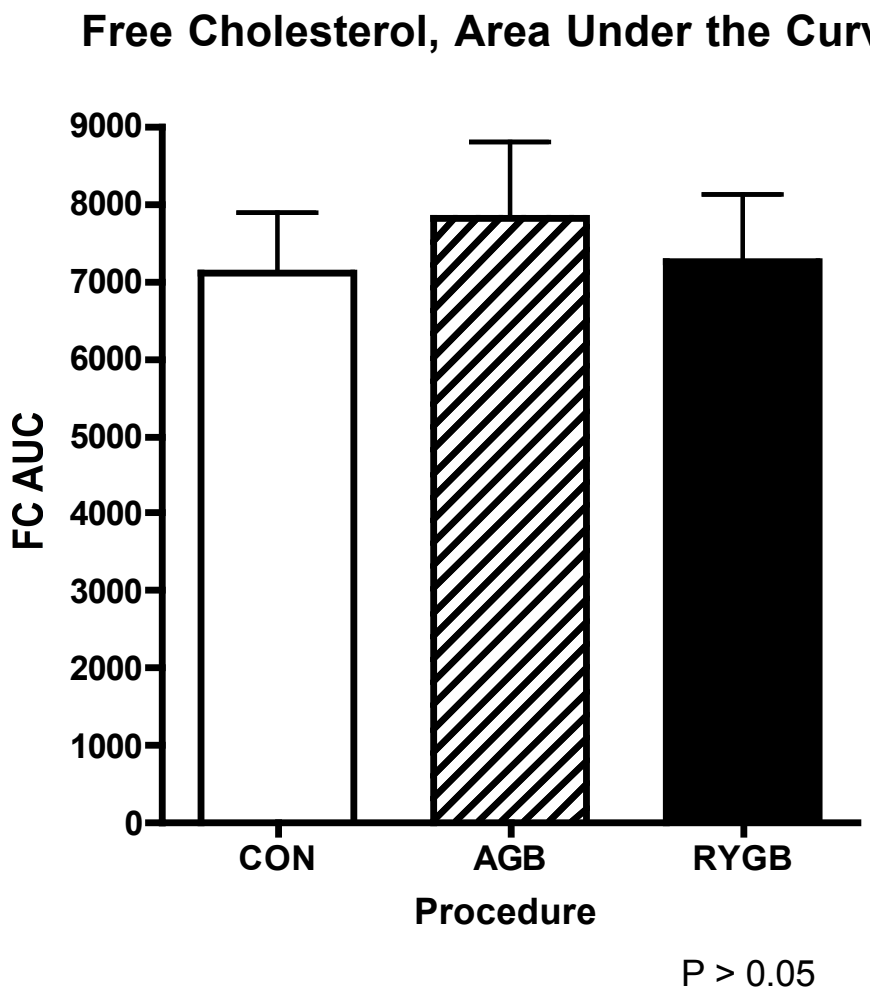

Figure 3-7. Free cholesterol, area under the curve, between groups of treatment, mean ± SEM.

3.4.4. PC

Postprandial PC levels were lower in the RYGB group beyond 90 minutes but the difference was not statistically significant ($P > 0.05$), except at 240 minutes when comparing the RYGB group and medical controls ($P = 0.021$) (Figure 3-8). The AUC were similar ($P > 0.05$) between all three groups (Figure 3-9). The values of phosphatidylcholine AUC were 25776 ± 6165 area units, 29384 ± 12691 area units and 32485 ± 12281 area units in the RYGB, AGB and medical control groups, respectively.

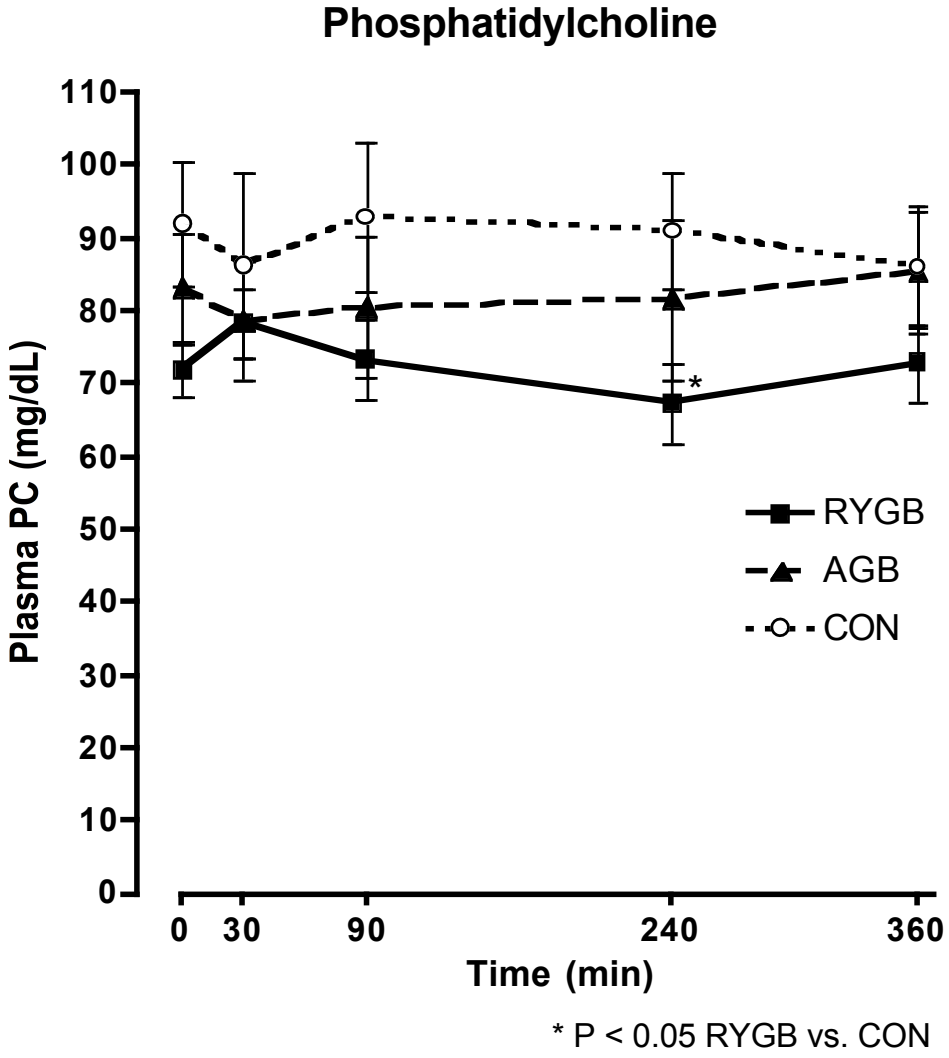

Figure 3-8. Postprandial changes in phosphatidylcholine. All data are expressed as mean ± SEM.

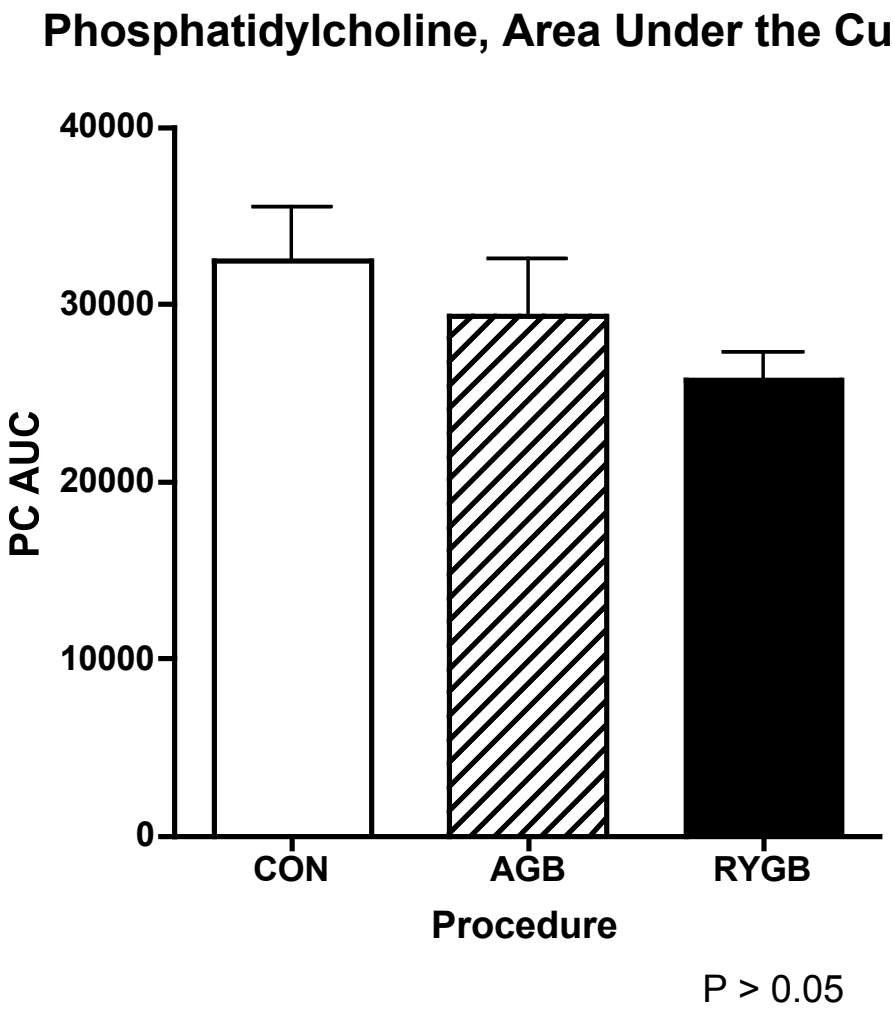

Figure 3-9. Phosphatidylcholine, area under the curve, between groups of treatment, mean ± SEM.

3.5. ApoB48

ApoB48, a specific measure of total CM particle number, was similar at baseline and increased to a peak level at 90 minutes in each of the three study groups, indicating a similar production rate and maximum concentration of formed chylomicrons (Figure 3-10). Following this peak, the apoB48 levels reduced significantly faster ($P < 0.05$) in the RYGB patients than in the AGB patients or medical controls. The postprandial response of apoB48 measured by AUC (Figure 3-11) was not different between the three study groups ($P > 0.05$). Specific values of apoB48 AUC were 2839 ± 1268 area units, 3353 ± 931 area units and 3531 ± 1233 area units in the RYGB, AGB and medical control groups, respectively.

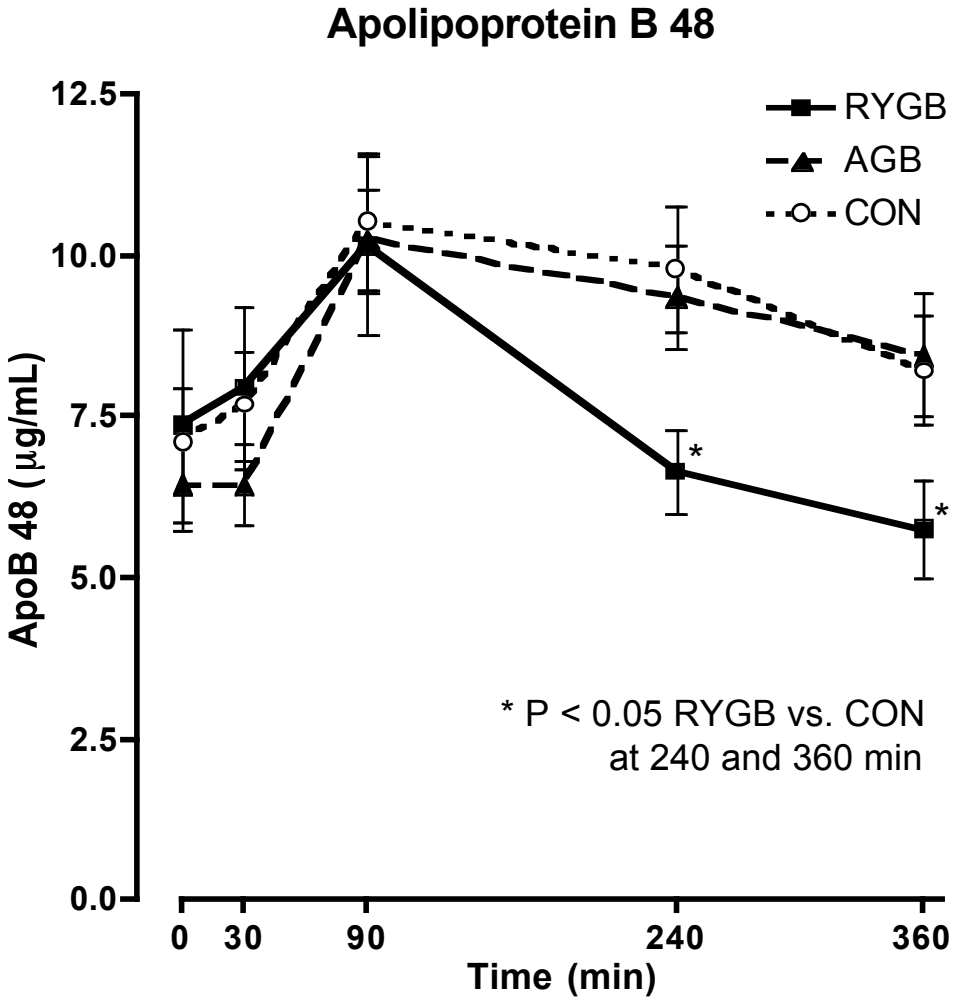

Fig. 3-10. Postprandial changes in apolipoprotein B48. All data are expressed as mean ± SEM.

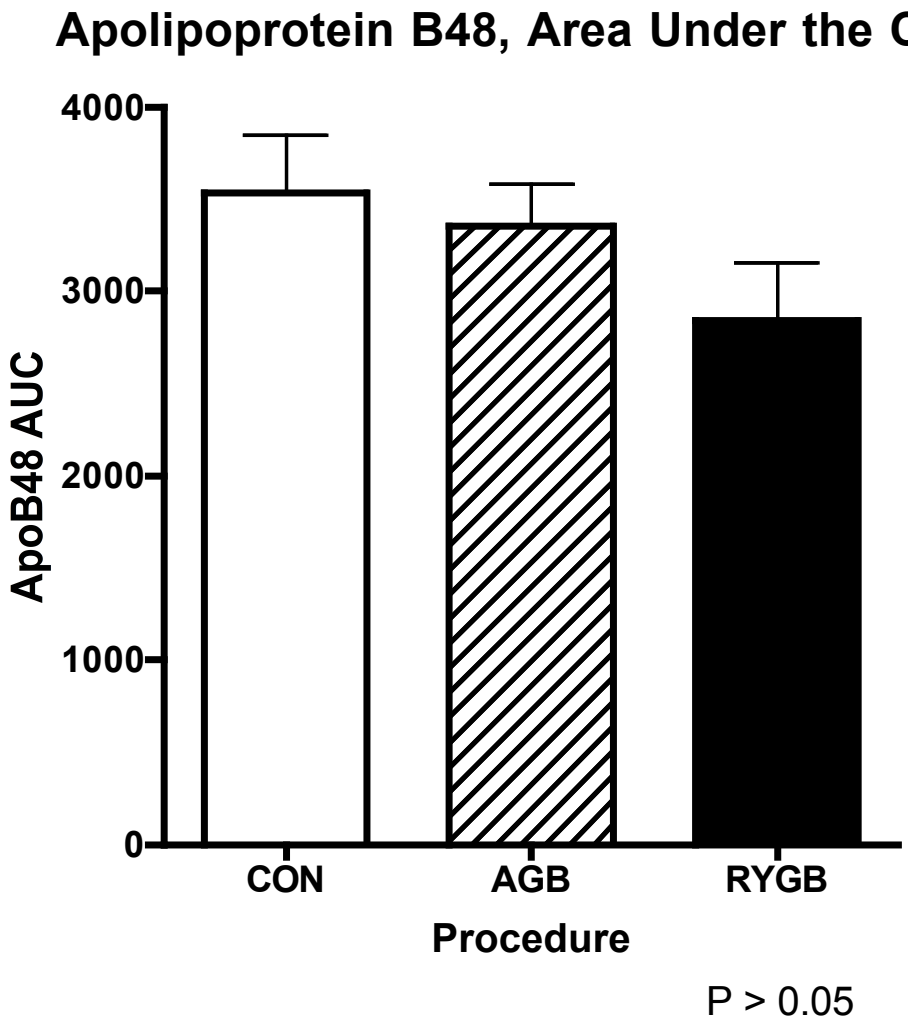

Figure 3-11. Apolipoprotein B48, area under the curve, between groups of treatment, mean ± SEM.

3.6. NEFA

NEFA levels were compared at the fasting state and at the peak levels of TG and apoB48 to evaluate the postprandial decrease after different procedures. NEFA were similar at the baseline between the three groups (Figure 3-12). At 90 min however, the greatest decrease was seen in the RYGB participants as compared with the AGB or medical controls ($P < 0.05$). NEFA levels in the AGB group were slightly lower than in the medical controls but the difference was not statistically significant ($P > 0.05$).

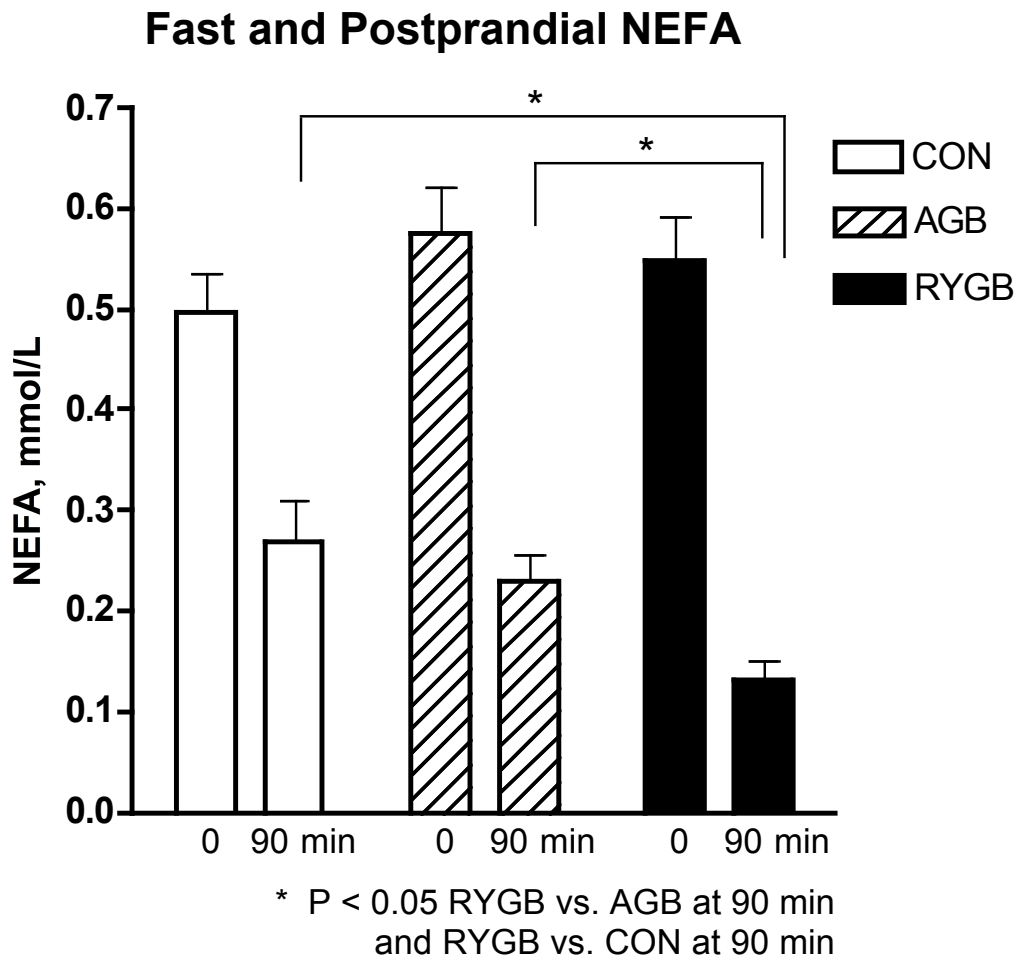

Figure 3-12. Postprandial decrease in plasma nonesterified fatty acids. All data are expressed as mean ± SEM.

3.7. Glucose and Insulin

The RYGB group was markedly different in postprandial glucose and insulin levels and dynamics (Figures 3-13 and 3-14). Blood glucose and corresponding insulin levels increased early and sharply after the test meal ($P < 0.05$). Insulin levels at 30 minutes were higher in the RYGB group, as compared to the AGB and medical controls ($P < 0.0001$), which was associated with a subsequent sharp drop in blood glucose in the RYGB participants, without symptoms of hypoglycemia. The glucose levels subsequently normalized. In the non-surgical and AGB groups the postprandial glucose and insulin levels followed similar patterns however the medical controls always had higher values that was significant for the glucose ($P < 0.05$) and not statistically significant for the insulin levels. Both surgical groups had significantly lower fasting glycemia ($P < 0.01$) than the medically treated group.

3.8. Insulin Resistance

The HOMA index of basal insulin resistance was significantly lower in the RYGB group, when compared to the medical controls ($P < 0.05$), and it was lower but had not reach statistical significance ($P = 0.0517$) when compared to the AGB group (Figure 3-15). AGB group also demonstrated lower HOMA index than medical control group ($P < 0.05$). Moreover, the HOMA indexes of both surgical groups were below 2.5, a cut-off level for insulin resistance.

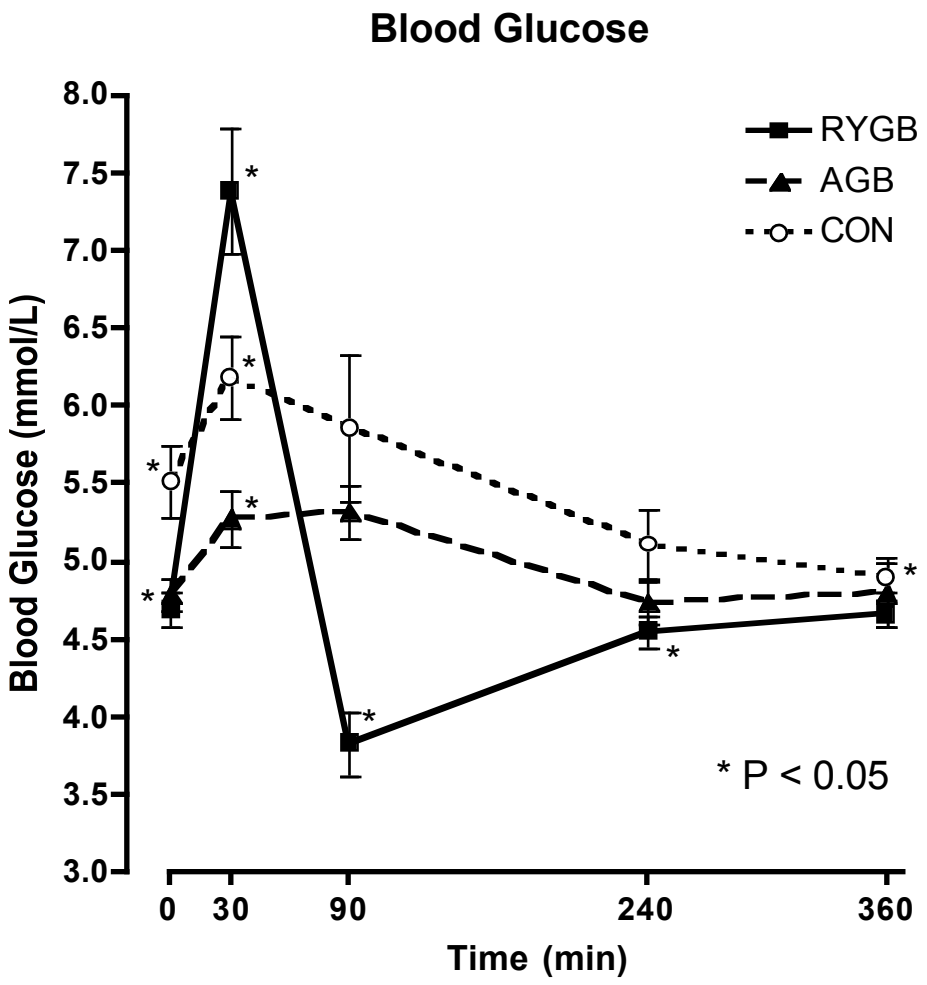

Figure 3-13. Postprandial blood glucose. All data are expressed as mean ± SEM.

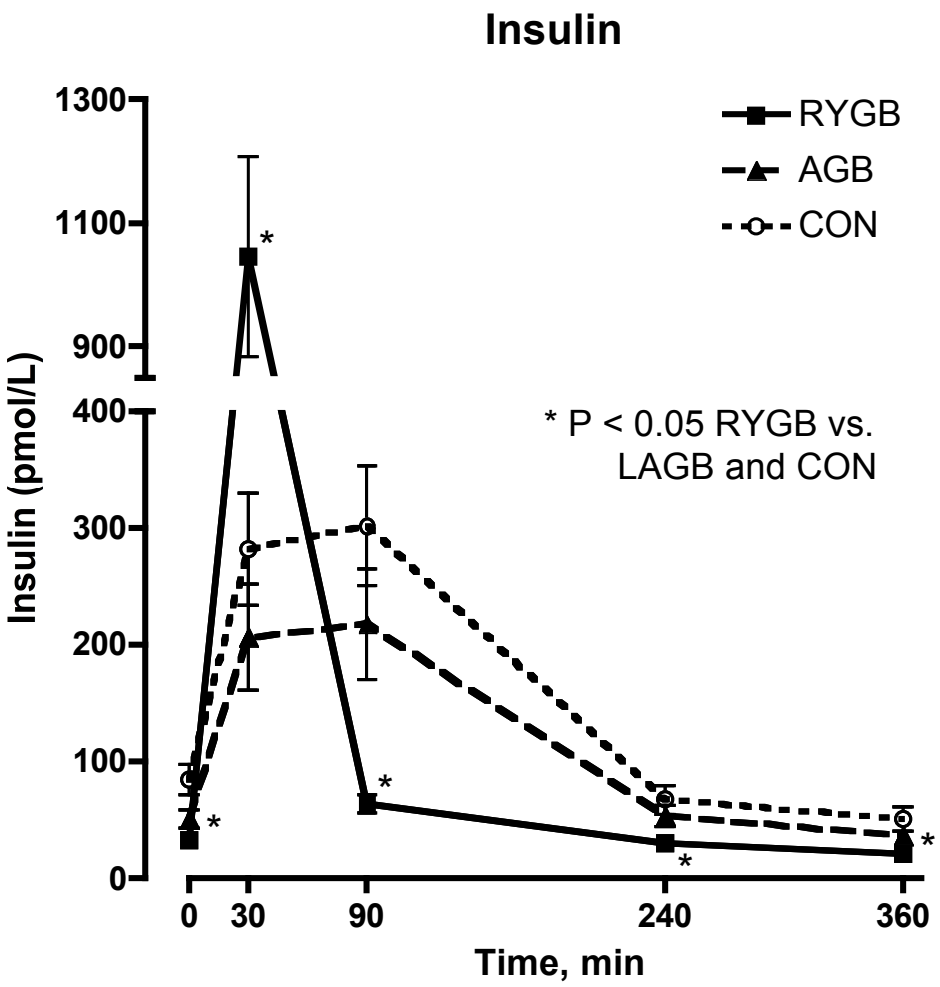

Figure 3-14. Postprandial changes in plasma insulin. All data are expressed as mean ± SEM.

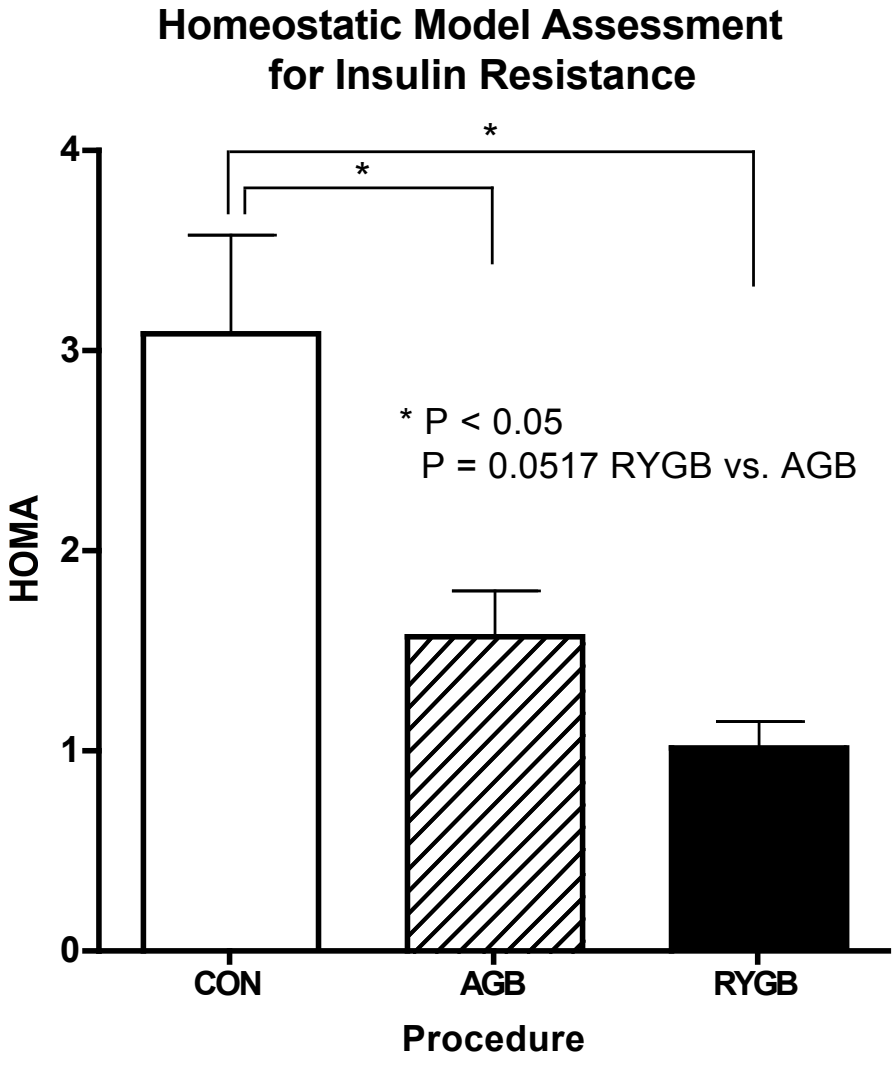

Figure 3-15. Homeostatic model assessment of insulin resistance. All data are expressed as mean ± SEM.

3.9. Insulin Resistance and Clearance of TG

Postprandial response of TG, expressed as total AUC, correlated significantly with the HOMA index of insulin resistance ($P = 0.0002$, $r^2 = 0.2619$). As shown in Figure 3-16, the decrease in insulin resistance was associated with a significant reduction in TG levels. In this way, the patients with higher HOMA index of insulin resistance were found to have higher levels of circulating TG. This correlation was accentuated in the RYGB group, as indicated by a steeper slope of the graph. Moreover, the RYGB graph is much shorter, since all the RYGB patient values are located in the area with low HOMA and triglycerides.

Contrarily, postprandial apoB48, expressed as AUC, did not correlate significantly with HOMA index of insulin resistance ($P = 0.1$, $r^2 = 0.059$), as shown in Figure 3-17. However, it can be noticed on the RYGB graph that individual patient values are concentrated in the area of lower insulin resistance and apoB48 levels, and the slope of the graph line is steeper than in the other groups, without reaching statistical significance.

3.10. Insulin Resistance and Postprandial Changes in NEFA Levels

The degree of postprandial decrease in NEFA, expressed as percent of baseline level, correlated significantly with insulin resistance expressed as the HOMA index ($P = 0.0205$, $r^2 = 0.1161$). When insulin resistance increases, plasma NEFA will decrease less, contributing thus to postprandial dyslipidemia. This correlation was more evident in the RYGB group, as indicated by the graph in Figure 3-18.

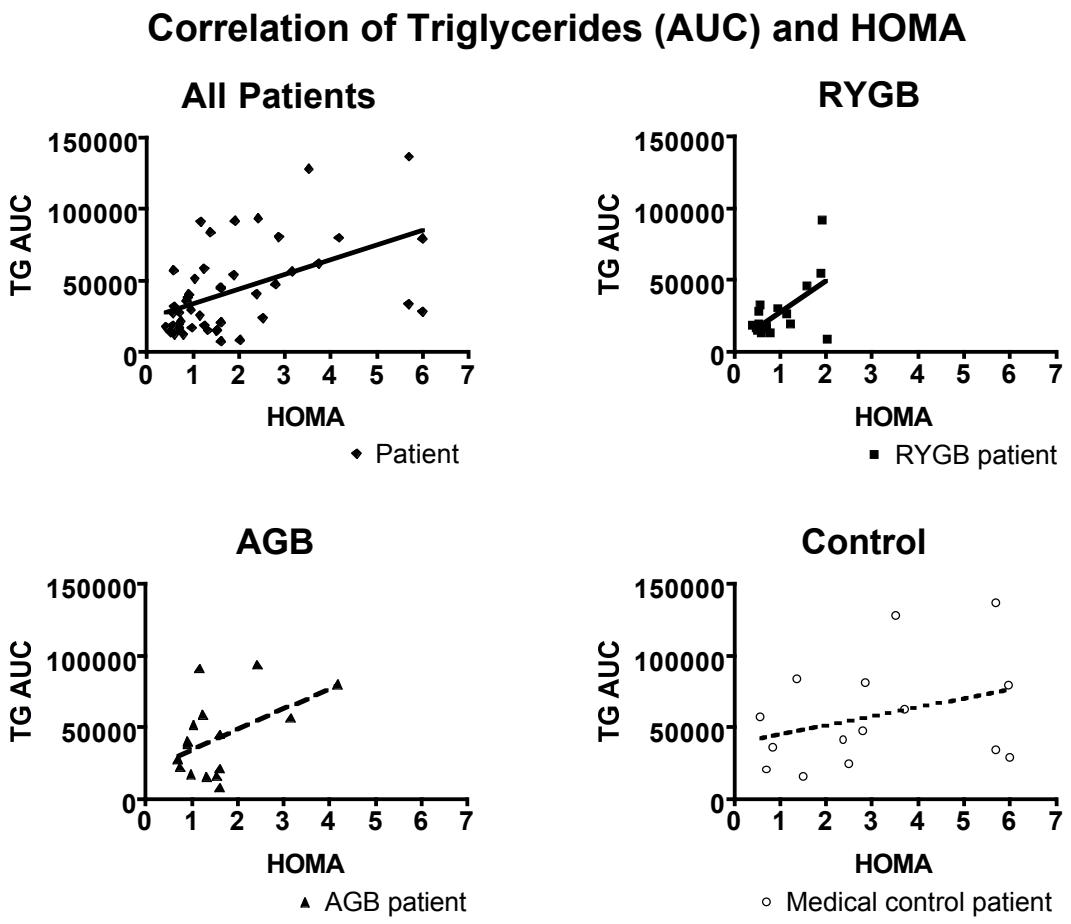

Figure 3-16. Correlation between insulin resistance (HOMA) and circulating triglycerides. AUC: area under the curve; HOMA: homeostatic model assessment.

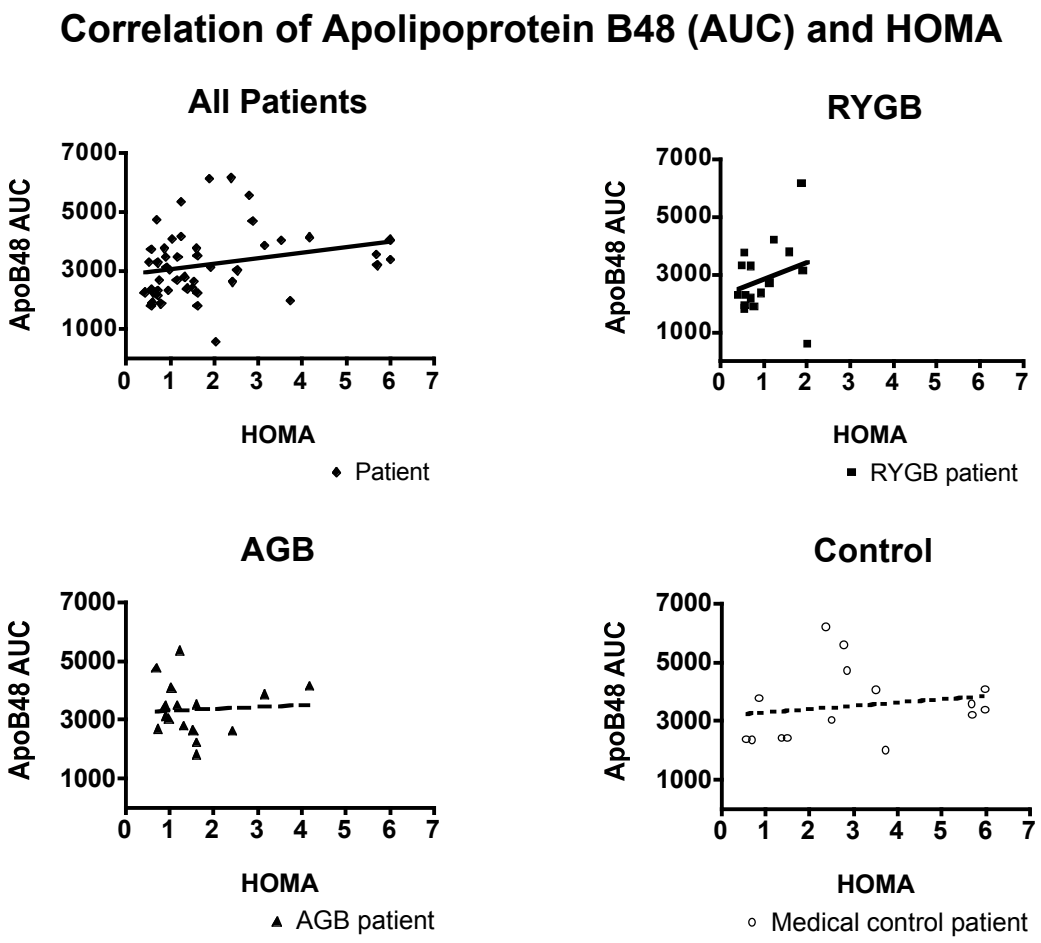

Figure 3-17. Correlation between insulin resistance (HOMA) and circulating apolipoproteins B48. AUC: area under the curve, HOMA: homeostatic model assessment.

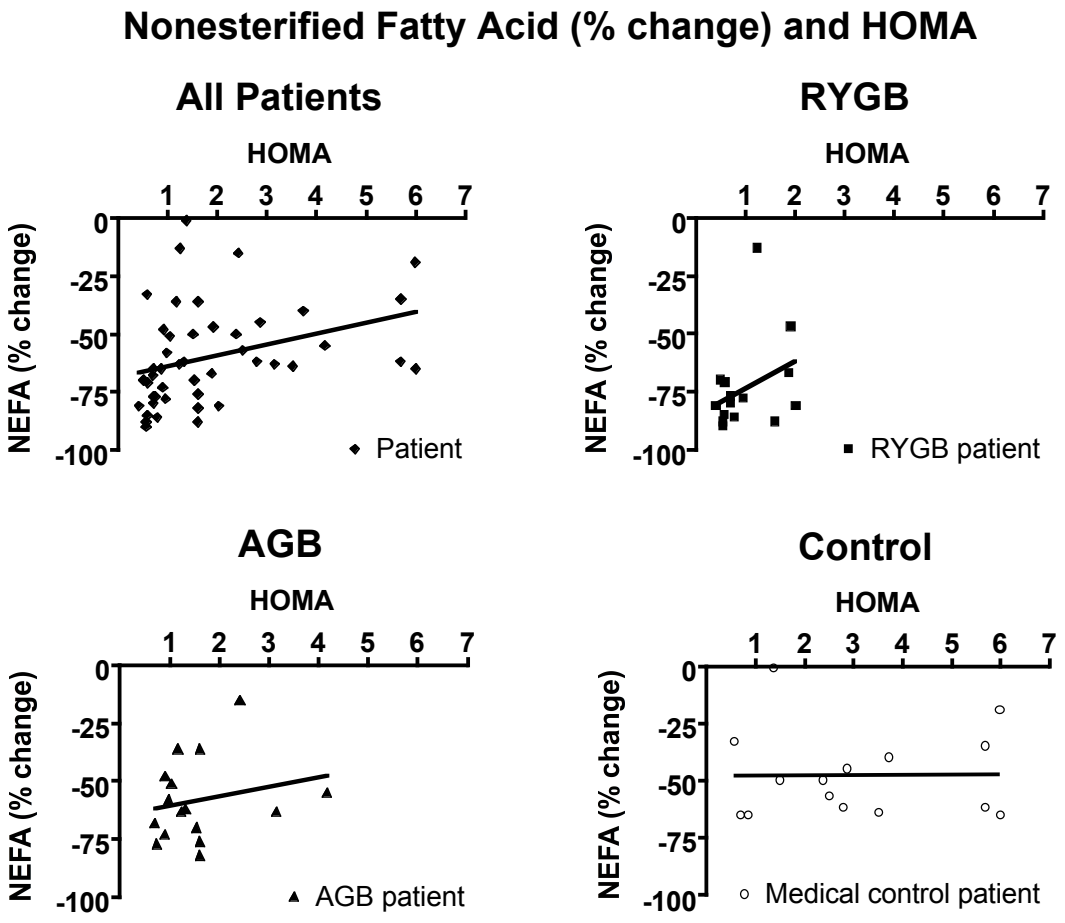

Figure 3-18. Correlation between insulin resistance (HOMA) and degree of postprandial decrease of nonesterified fatty acids. Postprandial change of nonesterified fatty acids expressed as percent baseline level. HOMA: homeostatic model assessment; NEFA: nonesterified fatty acids.

Chapter 4: Conclusion and Discussion

The results of this study strongly suggest that improvements in postprandial plasma lipids following RYGB are due to both accelerated clearance of plasma TG and CM and decreased release of NEFA from the adipose tissue due to restored insulin sensitivity.

In our study we compared similarly obese patients with different gastrointestinal anatomy, modified in one group by RYGB, in the second group by AGB, and the third group were not operated. We found that postprandial plasma TG and apoB48 were much lower and cleared faster following RYGB than following AGB and non-surgical treatment. Lower apoB48 levels reflect smaller numbers of circulating CM. A review of the literature suggests minor fat malabsorption following RYGB performed with the standard 100 cm Roux limb technique. Furthermore, there were no clinical signs of fat malabsorption in our study participants. This leads to an assumption that the lipids from the study meal were absorbed similarly in all three groups. Initial increase in CM concentration in the RYGB group was similar to the AGB and medical control group, suggesting similar initial CM production rate following all three treatments. However, the subsequent faster decrease of CM concentration in the RYGB group, together with low triglyceride levels, suggested faster clearance of chylomicron-bound triglycerides from plasma.

An advantage of our study design, a matched-case comparison study, was that we compared matched obese patients treated similarly in all other aspects except the type of procedure they were submitted. All patients, operated and not, went through a vigorous

process of medical treatment for weight loss at the same obesity clinic. As a result, there were no difference in the preoperative management of the operated patients and medical treatment of the control group. Medical control group demonstrated the same amount of weight loss (6.1 ± 7.5% of initial weight) that is expected from the surgical candidates in order to be considered for a bariatric surgery (Table 3-4). As a result of this weight loss and medical treatment, the non-operated patients had biochemical markers of diabetes, liver function and inflammation in the normal range. Normal ALT and CRP suggest normal liver function and absence of marked inflammation, indicating thus satisfactory control of obesity-related comorbidities in all three groups. HbA1c was normal, indicating good control of glycemia, even in the medically treated patients, as presented in section 3.1. Fasting cholesterol markers, including TC, LDL-C and HDL-C were in the recommended range in all three study groups (Table 3-2), indicating good control of dyslipidemia. All three study groups were comparable in weight, age, sex and presence of T2DM (Table 3-1).

4.1. Improved Clearance of Plasma TG Following RYGB

The results of our study indicated that the levels of CM were similar between three study groups at baseline and at the peak 90 minutes after a meal. Therefore, there was no delay in the beginning of fat absorption in the gastric bypass participants, as the peaks of the plasma CM occurred at the same time in all three groups. However, after the 90 minutes peak, the RYGB group demonstrated a marked decrease in apoB48 levels compared to the AGB and medical control groups (section 3.5). This can indicate an accelerated clearance of CM following gastric bypass. As CM carry the majority of food-derived TG in postprandial state, this finding suggests faster clearance of absorbed dietary lipids from plasma following RYGB. Therefore, the levels of circulating TG and CM are lower and this can explain lower fasting TG levels following RYGB reported by the majority of researchers (1). In our study, TG levels were lower in the RYGB group both at baseline and the postprandial measurements.

Improved TG clearance and storage following RYGB may be caused by improved insulin sensitivity of the target organs and tissues, such as adipose tissue, liver and muscles. Furthermore, insulin down regulates the release of FA from the storages in postprandial period in normal lean persons. This response to insulin is diminished in obesity and is known as insulin resistance, with persistent FA release into the blood following meals (2). In our study, RYGB patients demonstrated a sharp drop in NEFA after meal, reflecting thus a restoration of response to insulin (sensitivity) and a greater postprandial suppression of lipolysis in the peripheral organs and tissues. The medically treated patients did not demonstrate reduction in their NEFA levels in the same magnitude, possibly due to uncorrected insulin resistance. Insulin resistance, measured by

the HOMA index, was much lower following both surgical procedures than after medical treatment, indicating that insulin sensitivity improves with a greater degree of weight loss postoperatively. There was a trend of lower postprandial TG in the AGB patients, possibly related to better handling of lipids with weight loss induced by gastric banding.

Lower TG, apoB48 and NEFA, with a decrease in the HOMA index all indicate improved clearance of TG from plasma due to restored sensitivity to insulin in the storage tissues. Our observations agree with reports of other researchers who have observed lower fasted TG (3) and total apoB (4). Our study may be the first report on the use of specific apoB48 monoclonal antibodies to determine postprandial changes in chylomicrons following RYGB. ApoB has been shown recently by large prospective studies to be better predictor for increased risk of CVD and mortality (5). Moreover, accelerated clearance of apoB48-carrying particles might be responsible for the observed improvement in predicted cardiovascular risk following RYGB (6).

Additional findings in our study were postprandial hypoglycemia in response to a spike of insulin early after a meal in post-RYGB patients. This phenomenon is known to surgeons and researchers as hyperinsulinemic hypoglycemia (7), although it is not observed in all studies (8).

Studies on changes in fasting plasma lipids and lipoproteins following bariatric surgery frequently report greater reductions of TG, TC and LDL-C following procedures with malabsorptive component, such as gastric or intestinal bypasses than purely restrictive, or AGB (1). A smaller amount of TG in plasma after a meal can be either due to decreased lipid absorption and secretion of CM or due to accelerated clearance of TG from the circulation.

4.2. Fat Malabsorption is Not Significant Following Standard RYGB

The proposed mechanism for all types of bypasses was to delay contact of ingested nutrients with bile and pancreatic enzymes and to reduce available absorptive surface of the small bowel for digested lipids and bile. This would induce malabsorption of macronutrients and cholesterol with their subsequent excretion. Clinical practice and reports suggest that malabsorption is minimal to nonsignificant (9). RYGB is known as a relatively safe procedure with no marked macronutrient malabsorption and malnutrition. This applies to not only the standard 100 cm long Roux limb but also so called long limb, or 150 cm (10). This can be explained by adaptation of the small intestine. Intestinal crypt proliferation, dependent on increased glucagon-like peptide-2 (GLP-2), has been suggested recently by le Roux and colleagues (11) as one of the possible mediators of this adaptation. The investigators observed several mechanisms such as increased crypt cell proliferative activity and thickening of the small bowel associated with increased GLP-2 (a known stimulator of the gut mucosa proliferation) in post-RYGB rats. They also observed a rise in GLP-2 in RYGB patients with a maximum observed at 6 months after surgery. Intestinal adaptation of the common ileal channel with mucosal hypertrophy and increased lumen caliber was described following bilio-pancreatic diversion (BPD), a much more aggressive malabsorptive bariatric procedure (12). In one study on the small bowel adaptation to jejunoileal bypass in human obese subjects, Dudrick et al. (13) reported increased villous length and mucosal cell hyperplasia that was more expressed in the ileum than jejunum. Typically, intestinal adaptation is expected to be completed by 1 year after surgery. Even after biliopancreatic diversion, steatorrhea was observed to stop

after 4 months following surgery (14). The length of the Roux limb in RYGB may have a modest influence on postoperative weight loss in so-called superobese (BMI >50kg/m^2) subjects. MacLean et al. (15) found in their group of 96 superobese patients small but significant differences in postoperative BMI (32.7 kg/m^2 with 100 cm Roux and 100 cm biliopancreatic limb versus 35.8 kg/m^2 with 40 cm Roux limb and 10 cm biliopancreatic limb (P = 0.049)) after 5 years of follow up. Currently only 100 cm or longer Roux limb gastric bypasses are performed. Typically, Roux limbs of up to 180 cm are not associated with protein-calorie malnutrition. Diverting the food bolus from the duodenum seems to play an important role in weight loss. This importance of keeping duodenal digestive passage for weight preservation was shown with the Ulm pouch, connecting the esophagus with the duodenum after total gastrectomy, where patients had higher body weight, better regulation of gastrointestinal hormones and no glucose intolerance as compared to Roux-en-Y reconstruction (16).

4.3. RYGB Reduces Calorie Intake

Disrupted bowel continuity and distal bowel exposure to nutrients in RYGB has been found to induce larger changes in appetite, satiety and metabolism of lipids and glucose. RYGB is known to reduce total and caloric intake more profoundly than purely restrictive procedures such as gastroplasty, with high-calorie food and sweets consumption decreased more than the intake of proteins (17). Lower intake leads to negative energy balance with greater weight and fat mass loss. We agree with Cummings and colleagues (18) that weight loss following RYGB cannot be explained only by reduced gastric capacity and that there may be a role of the exclusion of the stomach,

duodenum and upper jejunum. We did not observe clinically significant symptoms of dumping syndrome in our patients even with episodes of asymptomatic hypoglycemia, so we believe this syndrome did not contribute to a decreased intake in our patients.

4.4. Improved Lipid Metabolism is Associated with Restored Insulin Sensitivity Following RYGB

Loss of body adipose mass and important changes in gut hormones following bariatric surgery can induce more efficient utilization of circulating and deposited lipids. A summative effect of these beneficial changes is additional weight loss and improved levels of plasma lipids, fasting and postprandial. As indicated in section 4.1, the RYGB group demonstrated the lowest TG levels and accelerated clearance of TG-carrying CM. Circulating postprandial TG (area under the curve) correlated significantly with insulin resistance (Figure 3-11). As insulin resistance decreases, there are less circulating TG in the blood. Circulating postprandial apoB48 (area under the curve) demonstrated the similar trend (Figure 3-12) in correlation with insulin resistance but they have not reached statistical significance ($P = 0.1$). Possible explanations for this can be a substantial spread of the data in all three groups, or the fact that the early increase in apoB48 levels was similar between the groups. The observed accelerated clearance of TG and CM can be explained by increased activity of LPL. Adipose tissue LPL is a key enzyme that hydrolyzes plasma TG and directs released FA to storage in adipocytes, especially after a meal. Insulin stimulates LPL, inducing the deposition of postprandial excess of circulating TG into adipose tissue, liver and muscles. In obesity-related insulin resistance this enzyme becomes non-responsive to insulin (19) with a subsequent

accumulation of TG in plasma, i.e. hypertriglyceridemia (20). LPL is known to increase its activity after substantial non-surgical weight loss in severely obese subjects (21). A recent study on AGB subjects showed a non-significant reduction in LPL activity 1 year after surgery with a significant decrease in plasma TG and 20% weight loss (22). RYGB patients in our study have significantly reduced insulin resistance with substantial weight loss, and their accelerated clearance of circulating TG can be explained by restored activity of adipose tissue LPL. This improvement in LPL activity may not be as dramatic in the AGB group, whereas the decrease in insulin resistance and weight loss were smaller, resulting in persistent high postprandial levels of TG. Furthermore, the non-operated obese group had the highest TG levels at all times suggesting the highest insulin resistance of lipoprotein lipase.

Another desirable effect of RYGB was postprandial decrease in NEFA. A greater decrease in postprandial NEFA in the bypass group suggested a greater suppression of postprandial lipolysis of the stored TG, largely dependant on insulin, and it was important to determine if this group was more insulin sensitive. The HOMA index of insulin resistance was lower in both surgical groups (Figure 3-10). Thus, both surgical procedures significantly improved control of glycemia and lipidemia but these beneficial changes were more pronounced following gastric bypass. The medically treated patients, despite some weight loss, still had elevated fasting insulin levels and basal insulin resistance as assessed by the HOMA index. This was correlated with a smaller decrease in postprandial NEFA levels in the medical control group (Figure 3-7). Furthermore, the degree of postprandial decrease in NEFA correlated significantly with insulin resistance

(Figure 3-13). Thus, insulin resistant patients (higher HOMA) were found to have smaller postprandial NEFA decrease.

Plasma levels of NEFA are more dependent on the lipolysis of the stored in adipocytes TG by adipose tissue lipases. Adipose tissue lipases, including HSL, are suppressed by insulin, however this suppression was shown to be impaired in obesity (20) with continuous release of FA after a meal leading to persistently high circulating NEFA levels. Pardina and colleagues noted that elevated fasting plasma NEFA persisted (at the same level as pre-operative) in obese subjects even 1 year following gastric bypass despite remarkable weight loss (BMI dropped from 49 to 31 kg/m^2) and improvements in plasma lipids (23). After observing continuously active adipose HSL following gastric bypass, with a decrease in lipid contents of the adipocytes, the authors concluded that post-surgical patients had diminished capacity to store triglycerides in addition to postoperative loss of subcutaneous fat. High activity of HSL might be related to its decreased inhibition by insulin. As insulin levels decrease postoperatively, there is less insulin-mediated suppression of HSL in fasting state, and more FA are removed from adipose tissue for their utilization elsewhere. Therefore, less fat is left in adipose tissue, leading to body fat mass and weight loss. In our study, all three groups had the same NEFA levels in fasting state, but to reach the same FA release they needed quite different amounts of insulin. Another study reported adipocyte lipolytic capacity and protein content of HSL decreased to normal with fat mass loss 2 years after AGB in women but not men (24). In our study, while the AGB and medical controls had much higher baseline insulin concentration, suggesting insulin-resistance in these groups, the RYGB patients needed much less insulin to keep releasing FA at the same level. As insulin

sensitivity is restored, the RYGB patients sharply dropped their NEFA levels after a meal, suggesting that adipose lipases became sensitive to the rise of postprandial insulin. This did not occur in the AGB patients and medical controls, where NEFA levels decreased in much less proportion in response to increased insulin levels after a meal, suggesting persistent insulin resistance. Elevated levels of NEFA aggravate insulin resistance, and thus the decreased lipolysis and release of NEFA will in turn lead to alleviating of insulin resistance.

Similarly to Faraj and colleagues (4), we found accelerated postprandial clearance of TG from plasma following gastric bypass, and we believe that this may be due to the RYGB-related restored insulin sensitivity of adipose LPL. Contrary to their findings, NEFA dropped sharply 90 minutes after a meal in our RYGB group and much more than in the AGB and medically treated participants. This may be due to the fact that all three groups of patients were still obese, so their fasting NEFA were higher at baseline, thus the postprandial drop following RYGB was more pronounced. By comparing obese bypassed with obese not bypassed patients, we can also conclude that increased efficiency in storage of dietary TG from plasma into peripheral tissues is due to surgical bypass of the upper small bowel and not to their obesity, as was concluded by Faraj and colleagues (4), who compared bypassed lean with lean non-obese participants. It should be recognized that adipocyte lipolysis is a complex process involving numerous enzymes and is influenced by several hormones in addition to insulin, which can mediate the effect of gastric bypass.

Overall, the results of our study suggest that the degree of beneficial changes in postprandial lipids is more pronounced following RYGB and it is associated with improvement in insulin sensitivity.

4.5. Other Potential Mechanisms of Weight Loss Following Roux-en-Y Gastric Bypass

As we discussed in sections 4.1 and 4.2, following RYGB, postprandial plasma lipids improve due to more efficient clearance rather than fat malabsorption. Improved TG and CM clearance and decrease in NEFA can be due to improved insulin sensitivity. In turn, insulin sensitivity improves with a greater weight loss following RYGB. If the weight loss plays a bigger role in the improvements of plasma lipids, it can be reasonable to enquire the cause of a higher loss of weight following RYGB comparing to AGB. It is known that gastric bypass induces profound reduction in food and energy intake, in some cases patients avoid fats and sweets (17). It might be that reduced intake is not only due to reduced gastric capacity, which can be similar in gastric bypass and banding, but some other factors, specifically related to gastrojejunal Roux-en-Y bypass. Potential factors might include gastrointestinal hormones ghrelin, PYY, and GLP-1, which are altered following RYGB (25), and might be involved in the reduction of appetite and accelerated satiety, leading to lower intake, negative energy balance, and as a result, desirable weight loss. Vagotomy, as a result of gastric partitioning, can also potentially decrease food intake. But these potential mechanisms are out of the scope of this study and will not be discussed.

As RYGB "malabsorptive" component does not produce significant malabsorption in a typical situation, it is proposed to change the procedure classification name to hybrid restrictive-bypassing procedure, as reflecting correctly multiple mechanisms related to gastro-intestinal bypass.

4.6. Study Limitations

The study has several limitations. It is not a prospective randomized trial, however, we were able to find differences in lipid metabolism of obese participants of similar weight, which might not be possible in a longitudinal comparison between bariatric surgical and non-surgical patients. The changes in chylomicrons were not compared with changes in other lipoproteins, such as VLDL/LDL and HDL, which were expected to occur later than in chylomicrons. Lipid assays did not include labeled techniques, which can improve the localization of the ingested lipids after meal. And correlation of plasma lipids with activities of LPL and HSL might shed more light on the fate of absorbed dietary fat.

4.7. Concluding Remarks

To summarize the results of our study, we believe that the improvements in plasma TG and CM following bariatric surgery may be due to accelerated clearance from circulation. These beneficial changes were especially compelling following RYGB when compared to AGB or medical management of severe obesity.

Additionally, we found faster clearance of TG and CM appeared to be correlated with an improvement in insulin resistance. Accordingly, the release of FA was greatly

reduced after a meal in more insulin-sensitive gastric bypass patients. Improved utilization of plasma lipids may be a key factor in the postoperative normalization of obesity-related dyslipidemia. These favorable changes were more explicit following RYGB then AGB.

Practically, our research would suggest the use of RYGB for weight loss in obese patients with hyperlipidemia, insulin resistance, T2DM and metabolic syndrome, and the use of AGB in the patients without the mentioned comorbidities. These may be the most appropriate and current indications for these two most popular bariatric procedures.

4.8. References

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