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Abstract

Dyslipidemia in obesity results from excessive production and impaired clearance of triglyceride (TG)-rich lipoproteins, which is particularly pronounced in the postprandial state. Here, we investigated the impact of Roux-en-Y gastric bypass (RYGB) surgery on the postprandial VLDL1 and VLDL2 apoB and TG kinetics and their relationship with insulin responsiveness indices. 24 morbidly obese non-diabetic RYGB surgery patients underwent a lipoprotein kinetics study during a mixed meal test and a hyperinsulinemic-euglycemic clamp study before the surgery, and one year later. A physiologically based computational model was developed to investigate the impact of RYGB surgery and plasma insulin on postprandial VLDL kinetics. After the surgery, VLDL1 apoB and TG production rates were significantly decreased, whereas VLDL2 apoB and TG production rates remained unchanged. TG catabolic rate was increased in both VLDL1 and VLDL2 fractions, but only the VLDL2 apoB catabolic rate tended to increase. Furthermore, post-surgery VLDL1 apoB and TG production rates, but not VLDL2, were positively correlated with insulin resistance. Insulin-mediated stimulation of peripheral lipoprotein lipolysis was also improved after the surgery. In summary, RYGB resulted in a reduced hepatic VLDL1 production that correlated with reduced insulin resistance, an elevated VLDL2 clearance, and improved insulin sensitivity in lipoprotein lipolysis pathways.
INTRODUCTION
The prevalence of obesity has increased alarmingly over the past decades (1). Its associations with cardiovascular disease (CVD) and insulin resistance make obesity a major public health problem. Increased fasting and postprandial plasma triglyceride (TG) concentrations, reduced high density lipoprotein (HDL) cholesterol levels, and an increased number of low-density lipoprotein (LDL) particles are some key characteristics of the dyslipidemia observed in patients with obesity. These lipid abnormalities result from an imbalanced lipoprotein metabolism caused by increased TG-rich lipoproteins (TRL) production and their reduced clearance (2, 3). Moreover, the interplay between lipid abnormalities and insulin resistance gives rise to a significantly increased risk of developing CVD and premature mortality in patients with obesity (4–6).

The pathophysiology of dyslipidemia in obesity is attributed to the hepatic overproduction and secretion of apolipoprotein B (apoB)-containing, TG-rich lipoproteins (TRLs) and their impaired clearance from the circulation (2, 7–9). TRLs transport hydrophobic TG and cholesterol from the intestine and liver to peripheral tissues. The intestine produces and secretes chylomicrons (CM), whereas liver produces and secretes very low-density lipoproteins (VLDL). VLDLs are categorized into two subfractions based on their sizes as VLDL1 and VLDL2, where VLDL1 is the larger one and transports a greater amount of TG per particle. Although, other than their sizes and TG load, there is virtually no difference between VLDL1 and VLDL2, these VLDL subtypes exhibit different profiles under certain metabolic conditions. In patients with insulin resistance and/or hepatic steatosis, there is VLDL1 overproduction but not VLDL2 (10, 11). Moreover, hyperinsulinemia suppresses hepatic production of VLDL1 but not VLDL2 in both insulin sensitive and insulin resistant humans with low liver fat (12–14).

In target tissue capillaries, TGs in TRLs are hydrolyzed to glycerol and free fatty acids (FFA) by lipoprotein lipase (LPL), and the products are taken up by tissue cells. As a result, circulating TRLs are transformed into smaller and denser, cholesterol-enriched lipoprotein particles. Since these small lipoprotein particles are enriched in cholesterol, they are highly proatherogenic, and their accumulation in plasma poses a significant risk for developing CVD (15). Many studies have shown a close association between dyslipidemia and the occurrence of insulin resistance and type 2 diabetes (16–19). A plethora of hypotheses have been proposed in the literature to explain this relationship (20), but the molecular mechanism explaining how dyslipidemia per se may induce insulin resistance is not fully understood; more importantly, the chicken or egg question has not yet been answered.

Bariatric surgery is an effective treatment for patients with severe obesity, inducing sustained weight loss and improving both overall health and quality of life (21, 22). In addition to weight loss, bariatric surgery improves blood glucose regulation, insulin sensitivity, plasma lipid profiles and blood pressure (23, 24). Due to its high success rate, Roux-en-Y gastric bypass surgery (RYGB) is one of the most commonly performed bariatric procedures (25). Notably, RYGB results in dramatic improvements in insulin resistance and dyslipidemia that are not necessarily related to the extent of the weight loss (26–28). Such weight loss-independent effects of bariatric surgery often occur within a few days to weeks after the surgery and may be related to (acute) changes in caloric intake, the microbiome and/or gastrointestinal hormones (27, 29). However, the number of concurrent changes that take place after the surgery make it difficult to unravel the line of events that ultimately leads to improved glucose and lipoprotein metabolism.

Computational modeling has proven to be effective for analyzing complex biological systems data and understanding hidden cause-and-effect relations (30, 31). In this regard, several computational models of human lipoprotein metabolism have been developed to analyze stable isotope-labeled tracer data (32–34). In human lipoprotein metabolism studies, tracer techniques are usually employed under steady-state (fasting) conditions, where the influx and outflux of the tracee are equal and all regulatory elements are assumed to be fixed (35). However, humans spend the majority of their time under non-steady-state (postprandial) conditions. Moreover, postprandial metabolism is more strongly related to
metabolic and cardiovascular disorders (36, 37). The tracer methodology and computational modeling have been employed to study the non-steady-state kinetics of simpler metabolic networks, including plasma FFAs (38) and glucose metabolism (39). However, current modeling approaches fail in utilizing isotope-labelled tracer data to accurately describe the dynamics of human lipoprotein metabolism under postprandial conditions without using unnatural feeding regimens, such as continuous feeding of small amounts during kinetics studies (40, 41). These methods can give valuable insights, but they do not reflect the natural eating habits of humans and they also fail to comprehensively describe hepatic and intestinal lipoprotein dynamics. Recently, a physiologically relevant computational model was developed to describe the non-steady-state dynamics of the hepatic and intestinal lipoprotein metabolism on the basis of stable isotope-labelled tracer data collected during a mixed meal test (42). Although this model provides valuable insights about the postprandial kinetics of hepatic and intestinal lipoproteins, it does not include a physiologically relevant gastrointestinal module. Furthermore, this model does not account for the postprandial effects of insulin on lipoprotein metabolism. Therefore, it cannot be employed to investigate the effects of the anatomical changes caused by bariatric surgery and/or insulin-mediated processes on lipoprotein metabolism.

In the current study, we employ a detailed novel multicompartmental model of hepatic and intestinal lipoprotein metabolism in order to investigate the postprandial interactions between glucose and lipid pathways in patients with severe obesity and after bariatric surgery-induced weight loss. We recruited 24 patients and performed detailed postprandial lipoprotein kinetics experiments at baseline and at one year after RYGB. We also performed hyperinsulinemic euglycemic clamps (HEC) in order to assess the interactions between lipoprotein and glucose/insulin kinetics. Taken together our model provides deep insight into the postprandial lipoprotein metabolism of patients with severe obesity as well the changes induced by bariatric surgery.
RESULTS

Patient characteristics
The study population comprised 24 patients with severe obesity (Table 1). At one-year follow-up, RYGB resulted in significant weight loss in all patients. In addition, RYGB was associated with important improvements in metabolic health, including reduced intrahepatic TG (IHTG) content, plasma lipid concentrations, insulin sensitivity and glycemia (Table 1).

Postprandial VLDL₁ TG and apoB as well as VLDL₂ TG concentrations are reduced after RYGB
All subjects underwent a lipoprotein kinetics study that included a mixed meal test at baseline and one year after the surgery. During the study, plasma VLDL₁ and VLDL₂ apoB (Figure 1AB) and TG (Figure 1CD), chylomicron TG (CM TG) (Figure 1E) and total plasma TG (Figure 1F) were measured up to 10 hours. At T=2h, subjects were asked to consume a mixed meal and the postprandial phase started. In each panel in Figure 1, box plots show the pre-surgery (red) and post-surgery (blue) area under the concentration curves (AUC) calculated for the postprandial state. One of the key differences between pre- and post-surgery apoB and TG profiles was the response to the mixed meal. Before surgery, meal ingestion was followed by pronounced VLDL₁ and VLDL₂ apoB (Figure 1AB, red) and TG (Figure 1CD, red) accumulation in the plasma. However, after the surgery, VLDL₁ and VLDL₂ apoB (Figure 1AB, blue) and TG (Figure 1CD, blue) concentrations were only moderately increased following the meal. After the surgery, VLDL₁ apoB AUC was significantly reduced (Figure 1A), whereas VLDL₂ apoB AUC remained unchanged (Figure 1B). On the other hand, both VLDL₁ and VLDL₂ TG AUC were significantly reduced after the surgery (Figure 1CD). The average TG/apoB ratio, which is calculated as the ratio of the TG and apoB AUCs, did not change in the VLDL₁ fraction (31.1±7.1 vs 28.8±8.2 mg.TG/mg.apoB) but it was reduced in the VLDL₂ fraction (8.7±2.3 vs 6.3±1.9 mg.TG/mg.apoB, p<0.001) after the surgery. This indicates a decline in average VLDL₂ particle size after the surgery. CM TG AUC (Figure 1E) and plasma TG AUC (Figure 1F) were also significantly reduced after the surgery. Our modeling results show that post-surgery reduction in the postprandial CM TG concentration resulted from reduced lipid absorption from intestine and enhanced CM TG clearance rate. After the surgery, estimated CM TG clearance rate was increased by 72% on average (p<0.001).

Figure 1 also shows that after the surgery, postprandial plasma VLDL apoB and TG exhibit faster dynamics. After the surgery, plasma VLDL₁ and VLDL₂ apoB and TG peaked earlier, compared to the pre surgery case. After the surgery, postprandial plasma VLDL₁ apoB peak time was decreased from 4.8±1.5 to 3.3±0.7 hours (p<0.0005), and plasma VLDL₂ apoB peak time was reduced from 6.0 ±1.6 to 4.1 ±1.2 hours (p<0.0001) after the surgery. Similar peak time reductions were observed for plasma VLDL₁ TG (4.8±1.5 vs 3.3±0.6 h, p<0.0005) and VLDL₂ TG (5.7±1.6 vs 3.7±0.8 h, p<0.0001) after the surgery. Moreover, post-surgery plasma VLDL₁ and VLDL₂ apoB and TG returned to their respective baselines within the observation timeframe; this was not the case before the surgery. A similar pattern was evident for CM TG and plasma TG time courses. Before the surgery, meal intake was followed by a significant increase in CM TG (Figure 1E, red) and plasma TG (Figure 1F, red). After the surgery, postprandial CM TG and plasma TG elevation was lower as compared to pre-surgery status (Figure 1EF, blue). Our results show that after the surgery, CM TG peak time does not change much (4.9±0.4 vs 4.6±0.6 h). The lack of reduction in CM TG peak time may in part be due to the slower meal consumption after the surgery (10 vs 30 min., see Methods and Supplemental Material for details). However, total plasma TG peak time is reduced after the surgery (5.9±0.9 vs 5.1±0.8 h, p<0.01). Post-surgery CM TG and plasma TG also returned to their baselines within the observation timeframe (Figure 1EF, blue).

RYGB is associated with reduced postprandial VLDL₁ TG and apoB production as well as increased postprandial VLDL₂ TG clearance
To gain more insight into the mechanisms underlying the observed changes in lipoprotein kinetics, isotopic enrichment data from different pools were assessed and analyzed using the computational model described in Methods section. In Figure 2, pre- and post-surgery isotopic enrichment data from
VL DL₂ and VLDL₂ apoB (Figure 2AB) and TG pools (Figure 2CD) as well as plasma leucine (Figure 2E) and glycerol (Figure 2F) pools are shown along with the model simulations. The results in Figure 1 and Figure 2 show that the dynamics of the plasma concentrations and enriched materials in different pools are accurately captured by the computational model allowing the calculation of the parameters that describe VLDL₁ and VLDL₂ apoB and TG kinetics in detail. Pre-surgery and post-surgery kinetic parameters were estimated for each patient and their averages are given in Table 2.

After the surgery, VLDL₁ apoB production rate was decreased (Table 2), whereas VLDL₂ apoB direct production rate, which is the rate of apoB that is directly secreted in the form of VLDL₂ from liver, did not change. As a consequence, the relative fraction of apoB that was directly secreted as VLDL₂ from liver was increased from 32±14% to 43±16% (p<0.05) after the surgery. On the other hand, fractional transfer rate (FTR) of apoB from VLDL₁ pool to the VLDL₂ pool tended to increase. Consequently, the total VLDL₂ apoB production rate, which is the sum of the VLDL₂ apoB that is directly secreted from liver and the VLDL₂ apoB that is derived from the VLDL₁ pool in circulation, is slightly increased but this change did not reach statistical significance. VLDL₁ TG production rate was significantly reduced and VLDL₂ TG direct production rate was slightly decreased. This indicates a noticeable shift in the distribution of hepatic TG secretion towards VLDL₂ (18±12% vs %23±11%, p<0.05) after the surgery. Together with the increase observed in the VLDL₁ TG FTR, the total VLDL₂ TG production rate remained the same after the surgery.

Following the surgery, VLDL₁ apoB fractional catabolic rate (FCR) did not change (Table 2), whereas VLDL₂ apoB FCR tended to increase. In line, the VLDL₁ apoB fractional direct catabolic rate (FDC), which is the rate of VLDL₁ apoB that is directly removed from the circulation, did not change. After the surgery, a remarkable increase took place in VLDL TG catabolic rates, where VLDL₁ TG FCR tended to increase from 27.7±23.4 to 34.0±17 pools/day (p=0.1) and VLDL₂ TG FCR was significantly increased from 10.8±6.1 to 17.0±10.1 (p=0.002). However, VLDL₁ TG FDC did not change after the surgery.

**Insulin-mediated stimulation of lipoprotein lipolysis is enhanced after the surgery**

After the surgery, homeostatic model assessment insulin resistance index (HOMA-IR) was significantly reduced (4.4±2.5 vs 1.2±0.7, p<0.005), and it was positively related to VLDL₁ apoB and TG production. After the surgery, there was a significant positive correlation between HOMA-IR and VLDL₁ apoB (r=0.61, p<0.005, Figure 3A blue) and TG (r=0.65, p<0.005, Figure 3B blue) production rates. In the pre-surgery condition, trends between VLDL₁ apoB and TG production and HOMA-IR were the same but the correlations were not statistically significant. When pre- and post-surgery data were combined and analyzed together, the correlations between HOMA-IR and VLDL₁ apoB production (r=0.39, p<0.01, Figure 3A black) and VLDL₁ TG production (r=0.53, p<0.005, Figure 3B black) remained significant. On the other hand, VLDL₂ apoB or TG production was not correlated with HOMA-IR before or after the surgery.

Since insulin regulates both glucose and lipid homeostasis, we aimed to quantify the contribution of insulin to the regulation of postprandial lipoprotein metabolism by incorporating an insulin-mediated stimulation of lipoprotein lipolysis pathway into the computational model. The responsiveness of the lipoprotein lipolysis pathway to circulating insulin can be expressed as the lipoprotein lipolysis insulin sensitivity index (lipoprotein lipolysis ISI), which was estimated by model from the individual patient experimental data as described in the Supplemental Material. In 5 patients, the model detected no insulin effect on lipoprotein lipolysis before surgery, but a detectable effect at 1-year follow-up. In all patients, post-surgery lipoprotein lipolysis ISI was significantly increased (Figure 4A, 0.2±0.15 vs 0.42±0.21, p<0.001). Furthermore, parameters of insulin sensitivity from the clamp studies were directly correlated to the model-derived lipolysis ISI in post-surgery subjects (Figure 4B-D).
DISCUSSION

Data from this study demonstrates that bariatric surgery is not only associated with significant weight loss and improved metabolic health, but also with decreased postprandial VLDL$_1$ production, increased postprandial VLDL$_2$ clearance and improved insulin sensitivity of the lipolysis pathway. Using physiology-based kinetic modelling, we provide deeper insight into the complexity of human lipoprotein homeostasis. We show that the reduction in plasma VLDL-apoB after bariatric surgery is a consequence of seemingly opposing effects on VLDL$_1$ vs VLDL$_2$ apoB kinetics: VLDL$_1$ apoB production was reduced, with an unaltered VLDL$_1$ apoB catabolic rate, whereas the VLDL$_2$ apoB direct production rate was unaltered, but its clearance rate tended to be increased. Together, this implies a shift from the hepatic secretion of large VLDL$_1$ particles before surgery towards the secretion of smaller VLDL$_2$ particles after surgery. Consistent with such a shift, we found that the relative fraction of hepatic apoB that was secreted in the form of VLDL$_2$ increased by more than 33% after surgery. Moreover, bariatric surgery-induced weight loss was associated with decreased VLDL-TG concentrations in both fractions, and our computational modeling indicated that this reduction was due to decreased VLDL$_1$ TG production as well as increased VLDL$_1$ and VLDL$_2$ TG turnover.

Previously, Padilla et al. studied the impact of gastric bypass and sleeve gastrectomy on hepatic apoB kinetics under constant feeding conditions (41), where they showed a significant decrease in plasma VLDL apoB following both gastric bypass and sleeve gastrectomy surgeries due to reduced hepatic production and increased fractional catabolic rate. For the gastric bypass, their report showed a trend toward a decrease in hepatic apoB production but apoB fractional catabolic rate remained the same. However, this study did not account for the different VLDL subfractions and, more importantly, their data was collected under a constant feeding regime, which does not reflect the natural eating habits. Several other studies investigated postprandial plasma lipid profiles after bariatric surgery. In patients with obesity and/or type 2 diabetes, it was shown that postprandial plasma TG and plasma cholesterol concentrations were significantly reduced during a mixed meal test that was performed two weeks after the sleeve gastrectomy or gastric bypass (43). In a follow up study, a similar postprandial reduction in plasma TG and cholesterol concentrations were reported two years after the surgery (44).

In a third study, a standard oral fat load test was performed three months after sleeve gastrectomy, where a significant reduction in postprandial VLDL/chylomicron remnant TG concentration was reported (45). Although, the reported post-surgery changes in plasma TG concentrations in these studies are consistent with our findings, the design of these studies did not allow to investigate the production or the turnover kinetics of lipoproteins. Hence, they couldn’t provide mechanistic insights of reduced plasma concentrations.

An advantage of our computational modeling-based approach was that the detailed insight could be obtained in pre-surgery and post-surgery dynamic regulation of lipoprotein metabolism during the postprandial state. Our data show that in the pre-surgery phase, there is a profound VLDL accumulation in plasma during the postprandial state. In line with earlier reports, we hypothesize that this is primarily due to the competition for LPL-mediated lipoprotein lipolysis (46–50). The competition between different lipoprotein species for lipolysis pathways becomes particularly evident in the postprandial state as the digested lipids enter the circulation in the form of chylomicrons. Studies show a positive relation between particle size and the lipolysis rate (51–53), which gives chylomicrons the priority for lipolysis by LPL. Hence, in the postprandial state, increased chylomicron size in the circulation promotes competition and results in a significant reduction in VLDL apoB and TG fractional catabolic and transfer rates. Our results show that, after the surgery, postprandial plasma VLDL apoB and TG concentrations do not increase much (Figure 1 blue), compared to the pre-surgery condition (Figure 1 red) for three reasons. First, after the surgery, hepatic apoB and TG production are significantly reduced. Second, a reduced post-surgery intestinal lipid absorption and increased CM TG clearance rate, results in lower plasma CM TG (Figure 1E, blue). Although, reduced intestinal lipid absorption is a model estimate and it has not been verified by measuring lipids in stool samples, it is consistent with the previous studies showing a significant reduction in intestinal lipid absorption after
RYGB surgery (54–58). Hence, in the postprandial state, the competition between hepatic and intestinal lipoproteins for the lipolysis pathways remains weak. Third, after the surgery, postprandial stimulation of lipoprotein lipolysis by insulin is greatly improved (Figure 4A). As a consequence of these factors, after the surgery, postprandial VLDL apoB and TG accumulation in plasma remains modest. Furthermore, elevated plasma TG and apoB concentrations return to their baselines within the time course of the study (8 hours), whereas postprandial lipids remain elevated for more than 8 hours on average in the pre-surgery condition. This implies that, following the surgery, after consuming a meal, the plasma lipid profiles are more likely to return to their baselines before the next meal is consumed, and this effectively prevents residual lipids from the previous meal to further increase plasma lipid concentrations.

The current study also demonstrated significant improvements in post-surgery insulin responsiveness indices, and a positive correlation between insulin resistance and hepatic VLDL₁ production after the surgery. Insulin reduces hepatic VLDL secretion by reducing apoB lipidation and promoting apoB degradation in the hepatocyte (59–61). Homeostatic model assessment insulin resistance index (HOMA-IR) was significantly reduced after the surgery. We showed that HOMA-IR was positively correlated with post-surgery VLDL₁ TG and apoB production (Figure 3AB) but not with VLDL₂ apoB or TG production. This finding emphasizes the role that insulin plays on the regulation of hepatic lipoprotein production, where patients with high insulin resistance tend to produce greater amounts of large hepatic lipoproteins. The lack of association between VLDL₂ apoB or TG production and HOMA-IR might be explained by independent regulation of hepatic VLDL₁ and VLDL₂ apoB and TG production as suggested before (10, 11). This indicates that the production of larger hepatic lipoprotein particles is increased with insulin resistance but the production of smaller lipoprotein particles is not affected. The lack of a statistically significant association between pre-surgery insulin resistance indices and estimated kinetic parameters might be due to the relatively high insulin resistance and large inter-patient variability in comparison to the relatively small population size. Nevertheless, when pre- and post-surgery data were analyzed together, the correlations between HOMA-IR and VLDL₁ apoB and TG production (Figure 3AB black) remained significant. This may indicate that, after the surgery, the associations between HOMA-IR and VLDL₁ apoB and TG production do not change, but become more pronounced.

The current study also shows a post-surgery improvement in insulin mediated stimulation of lipoprotein lipolysis pathway. Insulin is known to stimulate lipoprotein lipolysis by its impact on lipoprotein lipase at both transcriptional and posttranslational levels (62, 63). To quantify the responsiveness of the lipoprotein lipolysis pathway to circulating insulin, we have introduced the lipoprotein lipolysis insulin sensitivity index (lipoprotein lipolysis ISI), which was estimated for each subject by utilizing individual experimental data with the model. Our results indeed show that insulin mediated stimulation of lipoprotein lipolysis was improved after the surgery, reflected in the significantly increased lipoprotein lipolysis ISI (0.20±0.15 vs 0.42±0.20, p<0.001, Figure 4A). Moreover, calculated post-surgery lipoprotein lipolysis ISI values were strongly correlated with measured insulin mediated adipose tissue lipolysis suppression (Figure 4B), EGP suppression (Figure 4C) and glucose R₇ stimulation (Figure 4D) indices. Since lipoprotein lipolysis ISI is defined as the sensitivity of lipoprotein lipolysis pathway to the fractional increase in insulin over the baseline, we compared lipoprotein lipolysis ISI against the clamp derived sensitivity indices normalized over the fractional increase in insulin during the clamp studies. However, the associations between lipoprotein lipolysis ISI and clamp derived insulin sensitivity parameters were not present for the pre-surgery data (Figure S5). This may in part be due to the fact that model could not detect an insulin mediated lipoprotein lipolysis stimulation in 5 out of 24 pre-surgery patients’ data. Nevertheless, a linear trend between lipoprotein lipolysis ISI and insulin mediated lipolysis suppression was evident in the combined pre- and post-surgery data (Figure S5B), which was not the case for other clamp derived insulin sensitivity parameters (Figure SSCD). The dynamics of the insulin-mediated suppression of the hepatic apoB production was also incorporated into the model as a delayed forcing signal generated by the portal vein insulin, where
the portal vein insulin signal was derived from plasma insulin data. However, insulin-mediated effects on the hepatic apoB production pathway remained undetectable due to the uncertainty associated with portal vein insulin concentration and resulting parameter estimates. Therefore, insulin mediated suppression of the hepatic apoB production was removed from the final version of the model.

Glucagon-like peptide-1 (GLP-1) is best-known for its role on glucose homeostasis and insulinotropic effects (64–66). However, GLP-1 also plays a direct role in lipid and lipoprotein metabolism (67), and thus the GLP-1 receptor pathway has been the focus of pharmacological lipid research (68). GLP-1 reduces intestinal chylomicron production and secretion (69, 70) and activation of GLP-1 receptors reduces hepatic VLDL production (71, 72). Moreover, GLP-1 also triggers a signal through intrinsic gut-liver axis and ameliorates diet induced hepatic VLDL overproduction (73). These studies suggest that, other than being a potent insulin secretagogue, GLP-1 regulates lipoprotein metabolism in an insulin independent manner. Our results show that, after the surgery, fasting GLP-1 levels do not change (Fig. S4, 3.5 ± 3.9 vs 4.1 ± 3.6 pmol/l, p=0.26). However, post-prandial GLP-1 levels are significantly increased (Fig. S4, AUC: 2139.2 ± 1093.5 vs 7204.3 ± 4106.4 pmol.min/l, p<0.005), which is consistent with earlier reports showing a significant increase in GLP-1 levels following RYGB surgery (74–79). It is suggested that elevated GLP-1 levels play an important role in several metabolic improvements and diabetes remission following RYGB surgery (27, 78–81). Taking all together, post-surgery reduction in VLDL production and improved insulin mediated processes may in part be a consequence of elevated GLP-1 levels.

TG homeostasis directly influences hepatic steatosis, where our data indicates a significant reduction in IHTG after the surgery (9.9±9% vs 4±1.7%, p<0.05) as reported before (82–84). Adiels et al. have previously proposed that increased hepatic fat content, as a consequence of increased FFA flux to the liver due to the insulin resistance, results in overproduction of larger VLDL1 particles (10). However, it is not clear whether this association was a direct impact of hepatic insulin resistance or increased IHTG that was secondary to insulin resistance, since insulin resistance and hepatic steatosis are common comorbidities (85, 86). Indeed, we showed a strong association between post-surgery HOMA-IR and VLDL1 apoB and TG production but not VLDL2, which is consistent with earlier reports (10, 11).

The current study utilizes a physiologically based large computational model and a comprehensive dataset to investigate the impact of RYGB surgery on VLDL1 and VLDL2 apoB and TG kinetics under non-steady state postprandial condition during a mixed meal test. The study was designed to capture dynamic responses to naturel eating regimens as closely as possible. The complex and dynamic nature of lipoprotein metabolism together with multiple interactions occurring postprandially, made it necessary to make assumptions and simplifications during the model development. We acknowledge that, like all models that have been proposed in the literature and all the models that will follow, our model is not a complete account of the entire physiological processes involved. The developed model is not intended to capture all the biochemical or molecular details of the lipoprotein metabolism. The computational model was developed in a way to extract as much information possible from the available data, while preserving physiological relevance.

**Concluding remarks**

We conclude that physiologically based mathematical modeling of postprandial apoB and TG metabolism in different VLDL fractions in combination with gold standard measurements of insulin sensitivity provide deep insight in the effects of RYGB surgery on lipid handling and its interaction with glucose metabolism and insulin in the postprandial state. RYGB restores the homeostatic balance between insulin sensitivity and TG production and catabolism. Taken together, our experimental data combined with computational modeling show that RYGB in morbidly obese patients results in reduced postprandial VLDL TG due to reduced VLDL1 production and increased VLDL2 TG clearance rates, with improved responsiveness of lipoprotein homeostasis to circulating insulin levels.
METHODS

Design
This multicenter observational intervention study was part of RESOLVE, a European research program on the metabolic syndrome. The present study was designed to evaluate postprandial lipoprotein kinetics in humans before and after bariatric surgery-induced weight loss and their relation to insulin-mediated processes. For this purpose, we developed a physiologically based computational model of human lipoprotein metabolism, and used this model to analyze in-vivo data collected during the baseline (pre-surgery) and one year after the RYGB (post-surgery).

Study population
We recruited subjects with severe obesity from the outpatient clinic of two obesity centers in the Amsterdam metropolitan area. Subjects were eligible to participate in the present study if they: i) were aged >18 years; ii) met criteria for bariatric surgery in accordance with national guidelines (87); iii) were scheduled for elective RYGB; and iv) had stable weight for at least 3 months prior to surgery. Exclusion criteria were: i) the use of alcohol (>2 units day) or recreational drugs; ii) the use of lipid-lowering drugs, exogenous insulin, incretin mimetics, or psychoactive medication; iii) childhood-onset obesity; or iv) any somatic disorder except for common obesity-related conditions (for instance, dyslipidemia, hypertension, or obstructive sleep apnea).

Lipoprotein kinetics studies
Lipoprotein kinetics experiments were performed using \([5,5,5-\text{H}_3]\)-leucine and \([1,1,2,3,3-\text{H}_5]\)-glycerol in order to determine in vivo apoB and TG fluxes in VLDL\(_1\) and VLDL\(_2\) fractions (Supplemental Figure S1A). Experiments were performed shortly (<4 weeks) prior to the scheduled RYGB surgery and repeated one year after the operation. After an overnight fast, \([5,5,5-\text{H}_3]\)-leucine (7 mg/kg bodyweight; 99% enriched; Cambridge Isotopes, Andover, MA, USA) and \([1,1,2,3,3-\text{H}_5]\)-glycerol (500 mg; >99% enriched; Cambridge Isotopes, Andover, MA, USA) were infused via a venous catheter. Two hours after tracer infusion, subjects received a liquid mixed meal, which consisted of 2 bottles of Fresubin Protein Energy (Fresenius Kabi, Zeist, Netherlands), 40 ml of olive oil, 2 g of cacao powder, and 5 tablets of a non-caloric sweetener (Hermesetas, Hermes Sweeteners, Zurich, Switzerland). Patients were instructed to consume the meal within 10 (pre-operatively) or 30 (at 1-year follow-up) minutes. After the surgery, patients were given more time to consume the meal, because they experienced difficulty in completing the meal within 10 minutes. At -5, 15, 30, 45, 75, 90, 120, 150, 180, 240, 300, 360, 480, 600 and 1440 minutes after infusion, venous blood samples were drawn for the determination of \([5,5,5-\text{H}_3]\)-leucine and \([1,1,2,3,3-\text{H}_5]\)-glycerol enrichment in plasma and lipoprotein fractions.

Isolation of VLDL subfractions
VLDL\(_1\) and VLDL\(_2\) fractions were isolated from plasma by 3-step gradient ultracentrifugation using a SW41 rotor (Beckman, Indianapolis, IN, USA) in an Optima XPN-100 Beckman ultracentrifuge. In short, the density of 4 ml of plasma was adjusted to 1.1502 g/ml with NaCl. Then 0.5 ml NaBr/NaCl (d=1.182 g/ml) and 4 ml plasma (d=1.1502 g/ml) was transferred to an ultra-clear Beckman SW41 tube. The gradient was formed by layering the following salt solutions on the top of the plasma: 1) 2 ml 1.079 g/ml; 2) 2 ml 1.0722 g/ml; 3) 2 ml d=1.0641 g/ml; 4) 2 ml d=1.0588 g/ml. The different fractions were isolated using the following conditions: for chylomicrons (d<1.006 g/ml): 30 min, 18,000 rpm; for VLDL\(_1\): 51 min 39,000 rpm and for VLDL\(_2\) 16.36 h, 18,000 rpm. At each step, the upper 1 ml was aspirated and replaced by the appropriate density fraction. Isolated lipoprotein fractions were frozen at -80 °C until further analysis.

Determination of isotopic enrichments
In order to determine leucine enrichment in apoB, VLDL\(_1\) and VLDL\(_2\) fractions were precipitated with isopropanol, delipidated with ethanol-diethyl ether, dried and hydrolyzed with 6M HCl at 110 °C for 24h. The samples were then prepared for analysis of leucine enrichment as described (88, 89). Briefly,
leucine enrichment was determined on a GC-MS GC-MSD5975c (Agilent Technologies, Santa Clara, CA, USA) equipped with a VF17 ms column operated in SIM mode, using norleucine as internal standard. To calculate isotope enrichments, the average value of the \( m/z \) 161:158 ratio was determined using a calibration curve with known quantities of labeled and unlabeled leucine (90). The resulting \( m/z \) 161:158 was expressed as molar percentage ratio (MPE).

In order to determine glycerol enrichment in TG within VLDL\(_1\) and VLDL\(_2\), the isolated fractions were precipitated with isopropanol, delipidated with alcohol/di-ethyl ether and solubilized in isopropanol. The phospholipids were removed by adding 2 g activated zeolite (Merck, Darmstadt, Germany) to each tube. After centrifugation, the samples were evaporated under a 1.5 ml vial. The glycerol extracts were saponified with 2% KOH in ethanol, incubated for 2h at 60 °C and dried under \( \text{N}_2 \). Heptafluorobutyric acid (Sigma-Aldrich, Poole, UK)/ethanol acetate (1:3) and standards and controls were added and incubated for 10 min at 70°C. After evaporation under \( \text{N}_2 \), the samples were solved in ethyl acetate and analyzed by GC-MS as described before (89).

**Hyperinsulinemic euglycemic clamp**

We determined basal and insulin-mediated glucose fluxes as well as lipolysis rates during a two-step hyperinsulinemic euglycemic clamp (HEC) using \([6,6-\text{H}_2]\)-glucose and \([1,1,2,3,3-\text{H}_2]\)-glycerol (Supplemental Figure S1B), as described (91, 92). Briefly, primed continuous infusions of \([6,6-\text{H}_2]\)-glucose (prime: 11 µmol/kg continuous: 0.11 µmol/kg/min; >99% enriched, Cambridge Isotopes, Andover, MA, USA) and \([1,1,2,3,3-\text{H}_2]\)-glycerol (prime: 1.6 µmol/kg; continuous: 0.11 µmol/kg/min; >99% enriched, Cambridge Isotopes, Andover, MA, USA) were started and continued until the end of the study. Basal glucose [endogenous glucose production (EGP)] and glycerol (from lipolysis) production were determined after 2h of tracer equilibration. Next, insulin-mediated suppression of EGP and insulin-mediated suppression of lipolysis were determined after 2h of low-dose insulin infusion (step 1: Actrapid 20 mU/[m\(^2\) body surface area]/min, Novo Nordisk Farma, Alphen a/d Rijn, Netherlands). Finally, insulin-stimulated glucose disposal (Rd) was determined after an additional 2h of high-dose insulin infusion (step 2: 60 mU/m\(^2\)/min). During insulin infusion, blood glucose was held at 5 mmol/l by frequent bedside monitoring and variable exogenous glucose infusion (enriched with 1% \([6,6-\text{H}_2]\)-glucose in order to maintain stable enrichment in the plasma pool).

**Biochemical analyses**

Plasma glucose concentrations were determined with the glucose oxidation method using a Biosen C-Line glucose analyzer (EFK Diagnostics, Fullerton, Ca, USA). Insulin and cortisol were determined by immunoassay on an Immulite 2000 system (Diagnostic Products, Los Angeles, CA, USA) with intra-assay variation of 4-5% and 3-6%, respectively. Glucagon was determined by radioimmunoassay (Linco Research, St Charles, MO, USA) with intra-assay variation of 4-8%. Plasma free fatty acids (FFA) were analyzed by enzymatic colorimetric assay (NEFA-C kit, Wako Chemicals, Neuss, Germany). Plasma total cholesterol, LDL-cholesterol (LDL-C), HDL-cholesterol (HDL-C) and TG were analyzed on a Selektar autoanalyzer (Sopachem, Ede, The Netherlands). Plasma apoB was determined by immunoturbidimetric assay (Wako Chemicals) on a Selektar autoanalyzer.

**Calculations of basal and insulin-mediated fluxes**

We calculated glucose fluxes during HEC [EGP and the rate of disappearance (Ra)] using modified versions of Steele equations for the steady state (basal fluxes) or non-steady state (fluxes during the insulin infusion) (93, 94). Hepatic insulin sensitivity was defined as the percent suppression of EGP by step-1 hyperinsulinemia; peripheral or muscle insulin sensitivity as the percent stimulation of glucose Ra by step-2 hyperinsulinemia (95). Basal whole-body lipolysis was defined as the glycerol rate of appearance (Ra), where glycerol Ra was determined using the tracer dilution method (96). Adipose tissue insulin sensitivity was defined as the percent suppression of lipolysis by step-1 hyperinsulinemia.
Finally, given the inter-individual variation in insulin clearance, parameters of insulin sensitivity were normalized to insulin levels during the clamp.

**Determination of liver fat content and excess body weight**

Intrahepatic TG content (IHTG) was determined by proton magnetic resonance spectroscopy as described before (98). Excess weight was calculated as the weight above a body mass index (BMI) of 25 kg/m².

**Computational modeling**

We employed a computational modeling approach to investigate the effects of the RYGB surgery on postprandial lipoprotein kinetics and to explore the complex interactions between glucose and lipid fluxes. To achieve this, we translated the metabolic network of systemic lipoprotein metabolism into a physiologically based mathematical model as illustrated in Supplemental Figure S2. The model describes systemic lipoprotein kinetics using five interconnected modules: the gastrointestinal, plasma, liver, tracer injection and insulin modules. For computational simulations and analyses, the system dynamics were described with a system of ordinary differential equations. The mathematical model was then implemented into the MATLAB (MathWorks, R2018b) programing environment. The kinetic transfer rate parameters were estimated from the experimental isotopic enrichment and biochemical concentration data by employing MATLAB’s optimization toolbox. The details of the computational model are given in Supplemental Material.

**Statistical analysis**

All statistical analyses were performed using the MATLAB (MathWorks, R2018b) programing environment. Normally distributed data are presented as mean ± sd. We used median and interquartile range (IQR) to present non-normally distributed data. We used 1-tailed paired sample t-tests to compare baseline data to 1-year follow-up data. Bivariate correlations were evaluated using Pearson correlation coefficients. Findings were considered significant if $p < 0.05$.

**Study Approval**

The study was approved by the Amsterdam University Medical Center Medical Ethics Committee. All subjects provided written informed consent in accordance with the Declaration of Helsinki. The study was prospectively registered in the Netherlands Trial Registry (www.trialregister.nl: NL4531).

**Data availability**

Computer codes for the computational model and data files are publicly available in GitHub data repository at [https://github.com/vehpi/lipoprotein_kinetics.git](https://github.com/vehpi/lipoprotein_kinetics.git). If necessary, additional information will be provided by the corresponding author, VY, upon request.

**Author Contributions**

GMDT, NAWR, MJS, AKG and MN designed the study; KWH, PWG and MJS performed the human studies; DH, HS, HJ, AS and MTA performed the laboratory analysis; VY and NAWR developed the computational model; VY wrote the computer programs, created figures and tables; VY wrote the first draft; VY, KWH, GMDT, NAWR, MJS, AKG and MN revised the first draft, and finalized the manuscript.

**Acknowledgments**

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**Figure 1:** Pre-surgery (red) and post-surgery (blue) plasma VLDL₁ and VLDL₂ apoB (A, B) and TG (C, D), plasma chylomicron TG (E) and plasma TG (F) concentrations measured during the lipoprotein kinetics studies. Filled circles and error bars show data as mean ± sd. Solid curves show model simulations generated with model parameters estimated from average data (Supplemental Table S2). Dotted vertical lines mark mixed meal ingestion time point. In each panel, box plots show the pre-surgery (red) and post-surgery (blue) area under the concentration curves (AUC) calculated for the postprandial state (2-10 hours). Statistical significance was tested with paired sample t-test and significance levels were reported as, ****: p<0.0005, n.s.: Not significant. VLDL: very low-density lipoprotein, apoB: apolipoprotein B, TG: triglyceride, CM: chylomicron.
Figure 2: Pre-surgery (red) and post-surgery (blue) population averages for enrichment data (filled circles with error bars) and the model simulations (solid lines). A,B) VLDL₁ and VLDL₂ apoB [5,5,5-²H₃]-leucine enrichments. C,D) VLDL₁ and VLDL₂ TG [1,1,2,3,3-²H₅]-glycerol enrichments. E) Plasma [5,5,5-²H₃]-leucine enrichment. F) Plasma [1,1,2,3,3-²H₅]-glycerol enrichment. Data are presented as mean ± SD. VLDL: very low-density lipoprotein, apoB: apolipoprotein B, TG: triglyceride.
Figure 3: Relationship between pre- and post-surgery VLDL apoB (A) and TG (B) production rates and HOMA-IR. In each panel, the regression lines for pre-surgery, post-surgery and combined data are shown in red, blue and black, respectively. Correlation coefficients (r) and associated p-values are reported in each panel. VLDL: very low-density lipoprotein, apoB: apolipoprotein B, TG: triglyceride, HOMA-IR: homeostatic model assessment insulin resistance.
Figure 4: (A) Pre- and post-surgery lipoprotein lipolysis insulin sensitivity index (lipolysis ISI). (B) Post-surgery insulin mediated peripheral lipolysis suppression per insulin increased over basal level ($\Delta(\%)$insulin) from the clamp studies vs lipoprotein lipolysis ISI. (C) Post-surgery insulin mediated endogenous glucose production (EGP) suppression per $\Delta(\%)$insulin vs lipoprotein lipolysis ISI. (D) Post-surgery insulin mediated glucose rate of disappearance ($R_d$) stimulation per $\Delta(\%)$insulin vs lipolysis ISI. In each panel, the regression lines for the post-surgery data are shown in blue. Correlation coefficients ($r$) and associated p-values are reported in each panel.
Table 1: Clinical and biochemical characteristics of included subjects before and after surgery.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Pre-Surgery</th>
<th>Post-Surgery</th>
<th>P-Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex (women + men)</td>
<td>12 ± 12</td>
<td>12 ± 11</td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>46.1 ± 11</td>
<td>47.1 ± 11</td>
<td></td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>135.1 ± 23.4</td>
<td>98.6 ± 18.6</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>Body fat content (%)</td>
<td>45.6 ± 5.9</td>
<td>30.9 ± 10.6</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>Fat-free mass (kg)</td>
<td>73.3 ± 14.8</td>
<td>66.6 ± 12.9</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Excess weight (kg)</td>
<td>57.8 ± 20.0</td>
<td>21.3 ± 16.6</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>43.4 ± 6</td>
<td>31.7 ± 5.1</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>Body fat content (%)</td>
<td>45.6 ± 5.9</td>
<td>30.9 ± 10.6</td>
<td>&lt;0.005</td>
</tr>
<tr>
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<tr>
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<td>21.3 ± 16.6</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>43.4 ± 6</td>
<td>31.7 ± 5.1</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>IHTG content (%)</td>
<td>9.9 ± 9</td>
<td>4 ± 1.7</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Plasma cholesterol (mmol/L)</td>
<td>4.9 ± 0.8</td>
<td>4.1 ± 0.6</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>HDL cholesterol (mmol/L)</td>
<td>1.2 ± 0.2</td>
<td>1.5 ± 0.3</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>LDL cholesterol (mmol/L)</td>
<td>3.1 ± 0.7</td>
<td>2.2 ± 0.6</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>Plasma TG (mmol/L)</td>
<td>1.3 ± 0.6</td>
<td>0.8 ± 0.5</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>apoB (mg/dL)</td>
<td>81.5 ± 16.6</td>
<td>67.1 ± 19.7</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>Fasting FFA (mmol/L)</td>
<td>0.66 ± 0.18</td>
<td>0.63 ± 0.14</td>
<td>0.25</td>
</tr>
<tr>
<td>Fasting glucose (mmol/L)</td>
<td>5.0 ± 0.6</td>
<td>4.5 ± 0.3</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>Fasting insulin (pmol/L)</td>
<td>143.2 ± 77.1</td>
<td>41.5 ± 23.7</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>4.4 ± 2.5</td>
<td>1.2 ± 0.7</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>Basal EGP (µmol/kgFFM/min)</td>
<td>12.6 ± 1.5</td>
<td>12.2 ± 1.9</td>
<td>0.5</td>
</tr>
<tr>
<td>Basal lipolysis (µmol/kg/min)</td>
<td>2.5 ± 0.8</td>
<td>3.8 ± 1.7</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Insulin suppression of EGP during step 1 of clamp (% of basal)</td>
<td>75.2 ± 14.0</td>
<td>93.1 ± 17.5</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Insulin suppression of lipolysis during step 1 of clamp (% of basal)</td>
<td>52.6 ± 20.2</td>
<td>80.3 ± 9.4</td>
<td>&lt;0.005</td>
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<tr>
<td>Insulin stimulation of Rd during step 2 of clamp (% of basal)</td>
<td>404.5 ± 156.5</td>
<td>544.2 ± 111.7</td>
<td>&lt;0.005</td>
</tr>
</tbody>
</table>

Data is given as mean ± sd. Paired sample t-test was used to test the statistical significance of the differences between pre- and post-surgery values. IHTG: intrahepatic TG, FFA: free fatty acids, EGP: endogenous glucose production, HOMA-IR: homeostatic model assessment insulin resistance index, LDL: low density lipoprotein, HDL: high density lipoprotein, TG: triglycerides, apoB: apolipoprotein B.
Table 2: Pre-surgery and post-surgery kinetic parameters.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Pre-Surgery</th>
<th>Post-Surgery</th>
<th>P-Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>VLDL₁ apoB production rate (mg/day)</td>
<td>867.9±510</td>
<td>667.9±624.2</td>
<td>0.05</td>
</tr>
<tr>
<td>VLDL₁ apoB FTR (pools/day)</td>
<td>6.2±7.8</td>
<td>8.2±7.7</td>
<td>0.16</td>
</tr>
<tr>
<td>VLDL₁ apoB FCR (pools/day)</td>
<td>18.0±11.6</td>
<td>21.1±13.1</td>
<td>0.14</td>
</tr>
<tr>
<td>VLDL₁ apoB FDC (pools/day)</td>
<td>11.9±5.7</td>
<td>13.0±6.8</td>
<td>0.19</td>
</tr>
<tr>
<td>VLDL₁ TG production rate (gr/day)</td>
<td>34.2±18.2</td>
<td>23.4±15.2</td>
<td>0.005</td>
</tr>
<tr>
<td>VLDL₁ TG FTR (pools/day)</td>
<td>2.8±2.5</td>
<td>5.4±6.3</td>
<td>0.02</td>
</tr>
<tr>
<td>VLDL₁ TG FCR (pools/day)</td>
<td>27.7±23.4</td>
<td>34.0±17</td>
<td>0.10</td>
</tr>
<tr>
<td>VLDL₁ TG FDC (pools/day)</td>
<td>24.9±21.8</td>
<td>28.6±12.6</td>
<td>0.20</td>
</tr>
<tr>
<td>VLDL₂ apoB direct production rate (mg/day)</td>
<td>404.7±54.8</td>
<td>409.7±315.2</td>
<td>0.46</td>
</tr>
<tr>
<td>VLDL₂ apoB total production rate (mg/day)</td>
<td>611.3±328.2</td>
<td>647.6±439.3</td>
<td>0.31</td>
</tr>
<tr>
<td>VLDL₂ apoB FCR (pools/day)</td>
<td>5.4±2.9</td>
<td>6.7±4.1</td>
<td>0.06</td>
</tr>
<tr>
<td>VLDL₂ TG direct production rate (gr/day)</td>
<td>7.5±8.2</td>
<td>6.5±4.4</td>
<td>0.31</td>
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<tr>
<td>VLDL₂ TG total production rate (gr/day)</td>
<td>10.6±8.9</td>
<td>10.5±7.3</td>
<td>0.49</td>
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<tr>
<td>VLDL₂ TG FCR (pools/day)</td>
<td>10.8±6.1</td>
<td>17.0±10.1</td>
<td>0.002</td>
</tr>
</tbody>
</table>

Pre-surgery and post-surgery VLDL₁ and VLDL₂ apoB and TG kinetics. Data are presented as mean ± sd; statistical significance is tested by paired sample t-test. VLDL: very low-density lipoprotein, apoB: apolipoprotein B, TG: triglyceride, FCR: fractional catabolic rate, FTR: fractional transfer rate from VLDL₁ pool to VLDL₂ pool, FDC: fractional direct catabolic rate, (for VLDL₂ the total production includes direct hepatic production and transfer from VLDL₁ pool in circulation).