Obesity Surgery (2019) 29:2571–2583
https://doi.org/10.1007/s11695-019-03885-6

ORIGINAL CONTRIBUTIONS

Longitudinal Impacts of Gastric Bypass Surgery on Pharmacodynamics and Pharmacokinetics of Statins

Asma El-Zailik[1] · Lily K. Cheung[2] · Yang Wang[1] · Vadim Sherman[3] · Diana S.-L. Chow[1]

Published online: 19 April 2019

Abstract

Purpose Undergoing Roux-en-Y gastric bypass (RYGB) is expected to affect orally administered drug absorption. Statins are commonly prescribed to patients with obesity for the prevention of atherosclerotic cardiovascular diseases by lowering cholesterol. This is the first longitudinal prospective study on impacts of RYGB on weight loss, pharmacodynamics, and pharmacokinetics of atorvastatin, rosuvastatin, and simvastatin, and their active metabolites, up to 1-year post-surgery.

Methods Forty-six patients were recruited, five patients on atorvastatin, twelve on rosuvastatin, nine on simvastatin, and twenty on no statin. The concentrations of atorvastatin, rosuvastatin, and simvastatin with their active metabolites were monitored.

Results Mean plasma concentrations of atorvastatin and metabolites and rosuvastatin normalized by the unit dose [(nM)/(mg/kg)] decreased by 3- to 6-month post-surgery. Conversely, simvastatin and its metabolite concentrations increased up to 6-month post-surgery, then declined to preoperative levels by 1-year post-surgery. The metabolisms of atorvastatin to hydroxyl-metabolites and simvastatin to simvastatin acid were decreased after RYGB. The weight loss and PD outcomes were comparable between statin and non-statin groups suggesting the key impacts were from RYGB. The discontinuation or reduction of dose of atorvastatin or rosuvastatin post-RYGB exhibited rebounds of LDL levels in some subjects, but the rebound was not apparent with patients on simvastatin pre-surgery.

Conclusion Discontinuations of statin dosing post-RYGB require LDL monitoring and reducing the dose to half seems to have better results. Patients on statin treatment post-RYGB should be followed-up closely based on our pharmacokinetic findings, to ensure therapeutic effects of the treatment with minimal adverse effects.

Keywords Roux en-Y gastric bypass · Statins · Active metabolites · Pharmacokinetics · Pharmacodynamics

✉ Asma El-Zailik
amel-zailik@uh.edu

Lily K. Cheung
lily.cheung@tsu.edu

Yang Wang
ywang36@central.uh.edu

Vadim Sherman
vsherman@houstonmethodist.org

Diana S.-L. Chow
dchow@uh.edu

[1] Department of Pharmacological and Pharmaceutical Sciences, College of Pharmacy, University of Houston, 4849 Calhoun Rd., Houston, TX 77204, USA

[2] Department of Pharmacy Practice and Clinical Health Sciences, College of Pharmacy and Health Sciences, Texas Southern University, 3100 Cleburne St., Houston, TX 77004, USA

[3] Department of Surgery, Houston Methodist Hospital, 6550 Fannin St., Houston, TX 77030, USA

Introduction

Due to the high and rising prevalence of obesity over the last decade, reaching in 2015–2016 nearly to 39.8% of the US adults, the disease is getting more and more attention in the health field. In 2017, the only available statistics are state-by-state data based on self-reporting weights and heights, with a range of 22.6–38.1% of adult obesity rate [1, 2]. People with obesity have a higher risk of multiple diseases than people with normal body weight [3]. Comorbidities of obesity include dyslipidemia, type 2 diabetes, hypertension, cardiovascular diseases, sleep apnea, certain cancers, and depression. In addition, obesity is associated with a higher mortality rate, especially from cardiovascular diseases [4].

Roux-en-Y gastric bypass (RYGB) has been a popular treatment option to reduce body weight in people with severe obesity in the USA, with approximately 40,500 patients (17.80%) in 2017. Sleeve gastrectomy is the most popular procedure in 2017 with 59.39%, due to the less invasive

nature of the procedure [5]. Nevertheless, RYGB provides the most lasting effect on weight loss [6–8]. The RYGB involves creating a small gastric pouch and bypassing the remnant stomach, the duodenum, and a short portion of the jejunum. There are physiological and anatomical changes associated with the surgery that are expected to influence the pharmacokinetics (absorption, distribution, metabolism, and excretion) of medications. The anatomical changes lead to a decrease in the gastric volume, available intestinal surface area for absorption, and intestinal transit time. There are also reports on the increase in gastric pH and changes in the emptying of the gastric pouch post-surgery [9–11]. Other drug specific effects include impacts on the enterohepatic circulation and exposures of intestinal transporters and metabolic enzymes [10].

Statins are considered the drugs of choice to treat dyslipidemia and reduce the risk of atherosclerotic cardiovascular diseases [12]. In patients with obesity, these conditions are common with a significantly higher annual prevalence and mortality rate than people with normal body weight [3]. The percentage of patients with obesity using cholesterol-lowering medications is about 60% [13], with statins being the most widely prescribed cholesterol-lowering medication. Statins use has increased in the US adults over the age of 40 by 79.8% from 2002–2003 to 2012–2013. Among all cholesterol-lowering medications, simvastatin has the highest usage percentage (41.4%), followed by atorvastatin, pravastatin, rosuvastatin, and lovastatin (28.3%, 16.2%, 11.2%, and 7.0%, respectively) in 2012–2013 [14].

Sparse information is in the literature on the effects of undergoing RYGB on the pharmacokinetics (PK) and pharmacodynamics (PD) of medications, especially those most widely used in patients with severe obesity. Only four publications are available with relevant information for PK of statins in RYGB patients. Two of them specifically discussed the short-term (3–8 weeks) and long-term (21–45 months) changes in atorvastatin systemic exposure post-RYGB in the same cohort of patients [15, 16]. The study reported that atorvastatin AUC_{0-8} (ng*hr/ml), with large inter- and intra-individual variability, varied significantly over time post-RYGB, with initial increases in 3–8 weeks and decreases in 21–45-month post-surgery as compared with preoperative values. The other two publications reviewed the effect of bariatric surgery on the dose usage and PK of some major drug classes most frequently used in patients with obesity, including lipid-lowering drugs with a focus on statins [17, 18]. The study revealed a decrease in lipid-lowering drug usage, in addition to a reduction in dose intensity at 12-month post-surgery. The study again confirmed the scarcity of information on the effect of bariatric surgery on statins PK and stressed the need for more clinical studies to find the most appropriate dosing regimen post-surgery.

The purpose of this study was to investigate the impacts of RYGB on the PD and PK of three of the most used cholesterol-lowering medications, atorvastatin, and simvastatin with their active metabolites, and rosuvastatin. This prospective study fills the gap in knowledge regarding the longitudinal changes in statin PD and PK following RYGB.

Material and Methods

Chemicals and Materials

Atorvastatin (ATV) standard, simvastatin (SMV) standard, and fluvastatin standard (used as the internal standard, IS) were purchased from Sigma-Aldrich Corp. (St. Louis, MO, USA). LC-MS grade acetonitrile, water, methanol, ammonium formate (≥ 99.0%), and formic acid (~ 98%) were also purchased from Sigma-Aldrich Corp. (St. Louis, MO, USA). Rosuvastatin (RSV) standard, simvastatin acid (SMV-A) standard, 2-hydroxy atorvastatin (2-OH-ATV) standard, and 4-hydroxy atorvastatin (4-OH-ATV) standard were purchased from Toronto Research Chemicals Inc. (Toronto, Ontario, Canada). Blank human plasma was purchased from Equitech-Bio, Inc. (Kerrville, TX, USA). Ethyl acetate (spectrometric grade, > 99.5%) was purchased from Alfa Aesar (Ward Hill, MA, USA).

Study Design

Adult patients, who met the Houston Methodist Hospital's policy which follows the guideline for bariatric surgery defined by NIH [19], decided to undergo RYGB after discussion with the bariatric surgeon and agreed to participate in the study (IRB approved protocol ID Pro00007036). The patient treatment regimen was the existing regimen prior to the surgery, prescribed by their treating physicians. After the patients were recruited for the study, they were divided into statin and non-statin groups based on their treatment regimen. The statin group was further divided into groups of ATV, SMV, and RSV, based on the statin used in preoperative therapies. The non-statin group was used as a control for the surgical effects without the influence of statins.

Dosage regimens, demographic data, body weights, and heights were recorded as baseline data pre-surgery. Body weights were monitored at each follow-up visit of 1-week, and 1-, 3-, 6-, and 12-month post-surgery. Blood samples were collected at baseline and 3-, 6-, and 12-month follow-up visits. Baseline samples served as the individuals' own controls. Concentrations of ATV and its two active metabolites, 2-OH-ATV and 4-OH-ATV, SMV, and its active metabolite, SMV-A, and RSV were quantified by our published LC-MS/MS method for statins in lipemic plasma samples [20]. Briefly, the blood samples were extracted by liquid-liquid extraction using ethyl acetate. ACQUITY UPLC BEH C18 column (2.1 × 100 mm I.D., 1.7 μm) was utilized to separate and

quantify the statins on ABI SCIEX API 5500 QTRAP mass spectrometer. The ion pairs that were monitored were: m/z 559.2 → 440.3 for ATV, m/z 575.4 → 440.3 for 2-OH-ATV and 4-OH-ATV, m/z 436.3 → 285.2 for SMV, m/z 437.2 → 303.2 for SMV-A, m/z 482.3 → 258.1 for RSV, and m/z 412.3 → 224.2 for fluvastatin (IS). Mobile phase consisted of: (a) 10 mM ammonium formate and 0.04% formic acid in water and (b) acetonitrile, in a gradient elution with a flow rate of 0.4 mL/min. Retention times were 3.2 min for ATV, 3.1 min for 2-OH-ATV, 2.8 min for 4-OH-ATV, 3.8 min for SMV, 3.6 min for SMV-A, 2.9 min for RSV, and 3.3 min for the IS. The linearity range for all analytes was 0.25 (LLOQ) to 200 ng/ml with $r^2 > 0.98$. Lipid panel analysis of total cholesterol (TC), triglyceride (TG), low-density lipoprotein (LDL), and high-density lipoprotein (HDL) was performed on the blood samples at baseline, and at 3-, 6-, and 12-month post-surgery.

Data Analysis

Weight Loss Outcomes

Two indicators of weight loss outcomes were utilized in this study, the BMI reduction from baseline and percentage excess weight loss (%EWL), which were calculated at 1-week, and 1-, 3-, 6-, and 12-month post-surgery. BMI at baseline was compared among the groups (statins vs non-statin, and among statin groups). In addition, BMI reduction from baseline was calculated as (BMI at baseline − post-surgery BMI) for various time points post-surgery.

The %EWL is one of the standard measurements in assessing the weight loss after bariatric surgery. It is a measure of the amount of excess weight lost as a percentage of the total excess weight at baseline. The surgery is considered successful if the patient has > 50% EWL by 12-month post-surgery. The %EWL is calculated as [(weight at baseline − post-surgery weight)/(weight at baseline − ideal body weight)] × 100, for various time points post-surgery. The ideal body weight in the calculation of %EWL was defined as the weight corresponding to a BMI of 25 kg/m^2 [21].

PK Concentration Normalization

The molar concentrations of ATV, 2-OH-ATV, 4-OH-ATV, SMV, SMV-A, and RSV (nM) were normalized by dose/body weight (mg/kg). This was essential to compare concentrations pre-and post-surgery, since four patients had a decrease in the prescribed dose post-surgery (two on ATV, one on SMV, and one on RSV). In addition, the significant and progressive weight losses after the surgery necessitate the concentration normalization, because the dose/body weight will be different even though the daily doses remained the same.

Preliminary PK/PD Correlation of ATV and Active Metabolites

An attempt to establish a preliminary PK/PD correlation between ATV, 2-OH-ATV, and 4-OH-ATV concentrations vs. LDL was made. It is known that the in vitro HMG-Co A reductase inhibition activities of both 2-OH-ATV and 4-OH-ATV are equivalent to that of ATV. In addition, approximately 70% of HMG-CoA reductase circulating inhibitory activity in vivo is attributed to the two active metabolites [22]. Based on these understandings, the PK/PD correlation was performed using the summation of the molar concentrations of ATV, 2-OH-ATV, and 4-OH-ATV vs. LDL.

Statistical Analysis

Comparisons at baseline among ATV, SMV, RSV, and non-statin groups of the demographic data in age, BMI, and excess weight were performed using ANOVA followed by Tukey post-hoc test. The comparison between all statin ($n = 26$) and non-statin ($n = 20$) groups was performed using Student's t test for other demographic data, except sex and race where Fisher's Exact test was employed. To compare weight loss and PD (TC, TG, LDL, and HDL) outcomes among the groups of ATV, SMV, RSV, and non-statin, either ANOVA followed by Tukey post-hoc test, or Kruskal-Wallis followed by Dunn's test were performed, depending on the distribution of data and the fulfillment of parametric test assumptions [23]. The comparison between statin and non-statin groups of weight loss and PD outcomes was performed using Student's t test or Mann-Whitney U test, based on the same criteria. Within each group, the longitudinal comparison among pre- and various time points post-surgery was performed with non-parametric Friedman's test with Bonferroni correction, mostly because the data failed the assumption of sphericity and thus repeated measure ANOVA could not be performed. All the statistical analyses were performed using SPSS® Version 25. The significant level of all statistical analyses was set at $p < 0.05$.

Missing data percentage was 33.7% in PD measurements and 13.4% in weight loss outcomes. Missing data of more than 10% is likely to cause bias in the statistical analysis. Thus, the missing data can be handled using two potential approaches, ignoring the missing values by listwise or pairwise deletion, or by imputation. The multiple imputation approach was selected in the current study to deal with the missing data. The rationale of the selection of multiple imputation over deletion is that the deletion of the cases that have missing data is plausible only for large data sets, but not for the limited size in the current study, due to the consequential loss of power for the statistical analysis [24]. Multiple imputation has the advantage over single imputation of minimizing bias by accounting for the uncertainty that accompanies each imputed value [25]. Multiple imputation is conducted in three

steps: (1) imputation of the missing data points where the values are drawn from a distribution several (m) times and generate m completed datasets, (2) analysis of the m completed datasets as necessary for the study using standard techniques and generating m sets of results, and (3) pooling of the m sets of results together to consolidate into one result by averaging. Multiple imputation with five imputations [26] was selected in the reported data set, and the required statistical analysis procedures were performed on each data set, and the results were pooled together within the group [27, 28].

Results

Patient Demographics

Forty-six patients were recruited for the study, with twenty-six patients in the statin group and twenty patients in the non-statin group. In the statin group, five patients were on ATV, nine on SMV, and twelve on RSV. Patient demographics are represented in Table 1. The mean age in combined statin group is 10 years older than that of non-statin group. When the different statin groups were compared to non-statin group individually, only RSV group was significantly older in mean age. Mean ages of subjects in ATV and SMV groups appeared to be older compared to non-statin group (51.4 and 56.4 vs. 43.8 years), but statistically insignificant. The excess weight and BMI at baseline were comparable among the groups. The racial distribution was also comparable among groups, with more than 40% Caucasian (2–17 subjects), and less than five African Americans, two Hispanics and four other ethnic subjects in each group. Sex composition showed 50–90% of female patients in individual groups, similar to those reported by Jakobsen [16] and Yska [17, 18]. The number of male subjects was significantly higher in statin group compared to the non-statin group (11 vs 2), while females had comparable subject counts (15 and 18, respectively).

Weight Loss Outcomes

BMI Reduction

The BMI reduction exhibited similar patterns post-surgery between combined statin and non- statin groups (Fig. 1), with a significant reduction of 6–7.5 units by 3 months, a further decrease to 9–10 units by 6 months, and to 11.5–13 units by 12-month post-surgery. The BMI reduction among individual statin groups did not show statistical significance by 3 months, probably due to the lack of power with small sample sizes of 5, 9, and 12 for ATV, SMV, and RSV, respectively. However, this difference between individual statin groups and combined statin group was resolved at 6- and 12-month follow-up visits. The similar time course of BMI reduction post-surgery in statin and non-statin groups suggests that the weight loss was the result of RYGB and apparently not affected by the statin treatments.

%EWL

The pattern of weight loss represented by %EWL was similar between the combined statin and non-statin groups (Fig. 2), with a significant reduction at 3-month (40–41%) compared to 1-week follow-up visits (12–14%), followed by 54–65% EWL by 6-month and 65–75% by 12-month post-surgery. The time course of %EWL was similar to that of BMI reduction post-surgery. No significant difference in %EWL among all groups at any time point post-surgery, but the %EWL by 3 months among individual statin groups did not show statistical significance from 1-week follow-up, probably due to the small sample sizes in the groups.

Pharmacodynamics

Pharmacodynamic (PD) observations of TC, TG, LDL, and HDL are presented in Fig. 3. TC mean values in ATV, SMV,

Table 1 Patient demographics in the statin and non-statin groups

		ATV ($n = 5$)	SMV ($n = 9$)	RSV ($n = 12$)	Statins ($n = 26$)	Non-statin ($n = 20$)
Mean age (year) ± SD		56.40 ± 10.81	51.44 ± 8.44	55.75 ± 9.13 *	54.38 ± 9.11 *	43.80 ± 11.67
Excess weight (lb.) at baseline		102.13 ± 22.41	112.47 ± 38.15	111.95 ± 42.73	110.24 ± 36.95	125.19 ± 45.99
BMI (kg/m^2) at baseline		40.91 ± 4.28	42.86 ± 5.29	41.90 ± 6.16	42.04 ± 5.39	45.57 ± 6.97
Racial breakdown n (%)	Caucasian	2 (40.0)	5 (55.6)	10 (83.3)	17 (65.4)	9 (45.0)
	African American	2 (40.0)	2 (22.2)	1 (8.3)	5 (19.2)	5 (25.0)
	Hispanic	0 (0.0)	1 (11.1)	1 (8.3)	2 (7.7)	2 (10.0)
	Other	1 (20.0)	1 (11.1)	0 (0.0)	2 (7.7)	4 (20.0)
Sex n (%)	Male	2 (40.0)	3 (33.3)	6 (50.0)	11 † (42.3)	2 (10.0)
	Female	3 (60.0)	6 (66.7)	6 (50.0)	15 † (57.7)	18 (90.0)

*Mean age was significantly older in statin group compared to non-statin group ($p = 0.001$), and RSV group compared to non-statin group ($p = 0.015$)

† Statin group has a significantly different sex composition from non-statin group (Fisher's Exact test, $p = 0.022$)

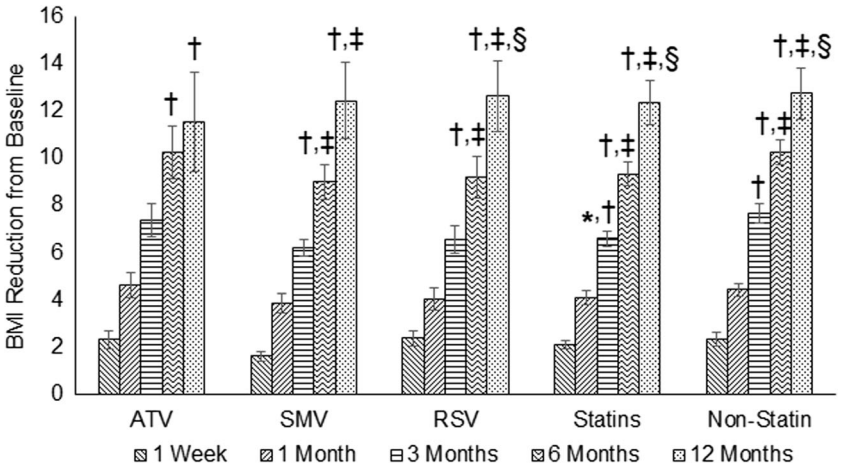

Fig. 1 Weight loss outcomes described as BMI reduction (mean ± SE) in time course of 1-week, and 1-, 3-, 6-, and 12-month post-surgery with multiple imputation data analysis. BMI reduction within each group became significant as compared with that of 1-week (†), 1-month (‡), and 3-month (§) post-surgery. BMI reduction significantly different (*) as compared to 3 months in the non-statin group, at $p < 0.05$

RSV, and combined statin groups showed a trend of decrease from 167 (161–173) mg/dl pre-surgery to 139 (137–142) mg/dl at 3-month, then increased gradually to 157 (156–159) mg/dl at 6-month and 170 (160–178) mg/dl at 12-month follow-up visits. A similar trend was observed in non-statin group with a decrease from baseline (182 mg/dl) to 3-month follow-up (153 mg/dl) but stabilized at 150 mg/dl at 6-month then increased to 171 mg/dl at 12-month visit. Mean TC levels were not significantly different between statin and non-statin groups at baseline nor any time point post-surgery.

The mean TG value pre-surgery in ATV group of five patients (101 mg/dl) appeared to be under control, resembling those of non-statin group (118 mg/dl), but lower than those in SMV and RSV groups (165–183 mg/dl). Post-surgery, all individual statin groups and combined statin group had TG of 105–117 mg/dl at 3–12 months, except further decreases in ATV group to 99 mg/dl and 87 mg/dl at 6- and 12-month follow-up visits, respectively. Non-statin group had a trend of continuous decrease in mean TG levels from baseline of 118 to 115, 98, and 91 mg/dl at 3-, 6-, and 12-month post-surgery, respectively, but the decrease was not statistically significant. The high preoperative values of TG in SMV and RSV groups were corrected by 3-month post-RYGB with comparable values in the non-statin group.

Mean LDL values for ATV, SMV, and RSV groups were at 85–95 mg/dl at baseline and decreased to 74–80 mg/dl by 3 months, then increased gradually to 79–85 and 86–109 mg/dl at 6- and 12-month follow-up visits. Combined statin group had a time profile for mean LDL of 90, 77, 82, and 96 mg/dl at baseline, 3-, 6-, and 12-month post-surgery, respectively. Non-statin group had a significantly higher preoperative LDL (110 mg/dl), but decreased to 78, 81, and 91 mg/dl by 3-, 6-, and 12-month post-surgery, comparable to those in the statin group. The significantly higher LDL in non-medicated patients was corrected by RYGB.

Mean HDL level in individual statin and combined statin groups was 42–50 mg/dl at baseline and remained at similar levels or decreased slightly at 3-month visit (41–46 mg/dl) but increased at 6-month (47–50 mg/dl) then significantly at 12-month (58–64 mg/dl) follow-up visits. A similar time profile was observed in non-statin group with baseline mean HDL level of 47 mg/dl, decreased to 41 mg/dl at 3-month and

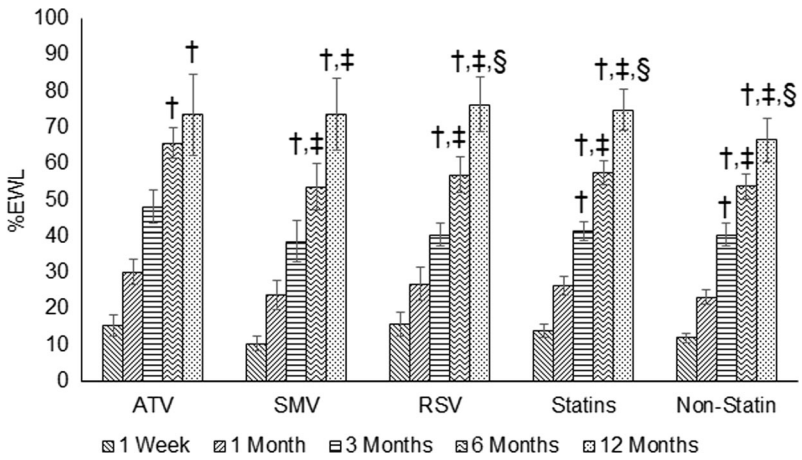

Fig. 2 Weight loss outcomes described as %EWL (mean ± SE) at 1-week and 1-, 3-, 6-, and 12-month post-surgery with multiple imputation data analysis. The %EWL within each group became significant as compared with that of 1-week (†), 1-month (‡), and 3-month (§) post-surgery, at $p < 0.05$

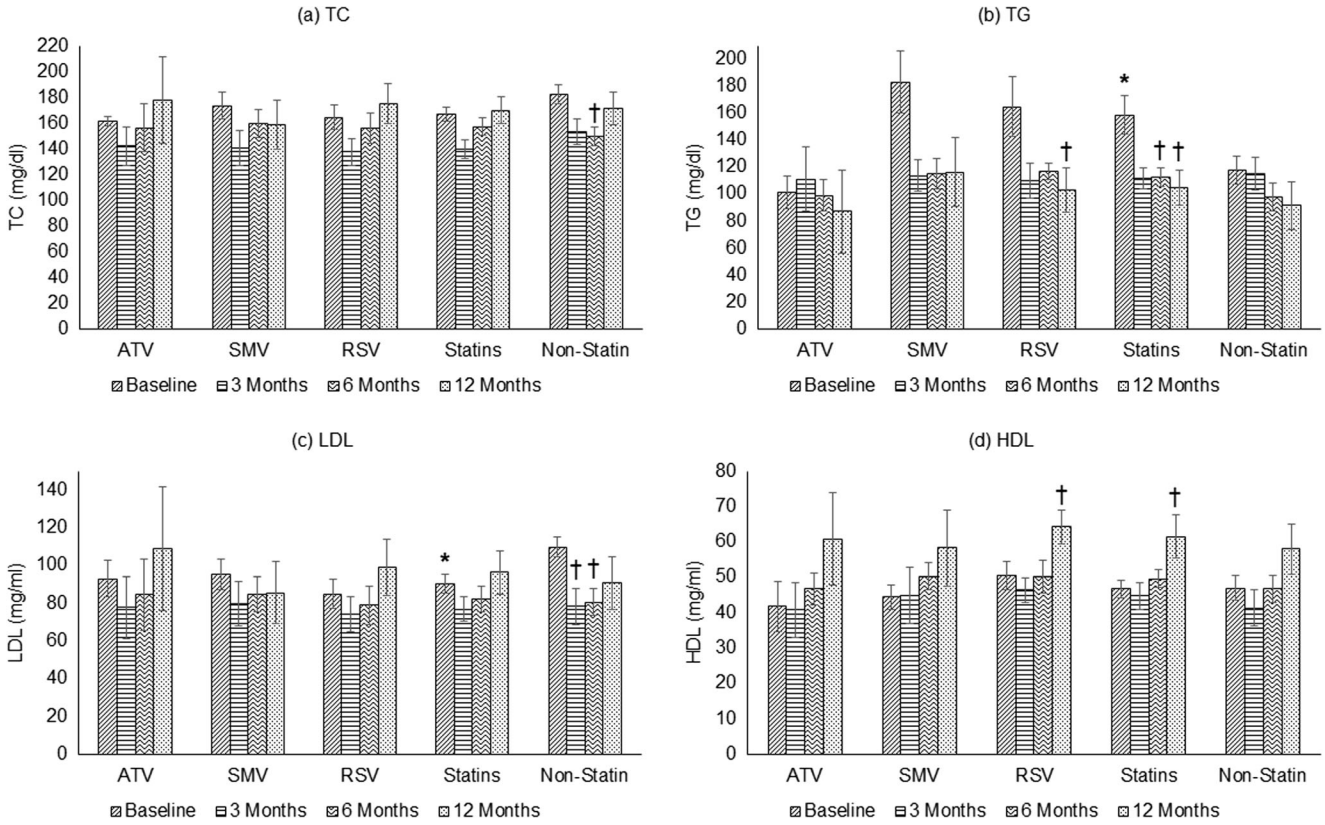

Fig. 3 PD outcomes described as measurements of **a** TC, **b** TG, **c** LDL, and **d** HDL (mean ± SE) in individual/combined statin and non-statin groups at baseline and 3-, 6-, and 12-month post-surgery with multiple imputation data analysis. Changes in PD outcomes within each group became significant (†) as compared with that of pre-operative values. PD pre-operative value significantly different (*) as compared to the non-statin group, at $p < 0.05$

increased to 47 and 58 mg/dl at 6- and 12-month visits, respectively. There was no significant difference in mean HDL levels between the statin and non-statin groups at baseline, nor any time point post-surgery, suggesting the changes in HDL were RYGB induced.

Relating LDL Levels to Statin Discontinuation and Dose Reduction Post-Surgery

Figure 4 represents the individual LDL levels (mg/dl) in patients on ATV, RSV, or SMV pre- and post-RYGB. Patients who discontinued using ATV ($n = 2$) and RSV ($n = 5$) have a rebound in their LDL levels higher than patients who continued using these two statins. Reducing the dose to half seems to have an effect on increasing LDL levels post-RYGB in patients on ATV ($n = 2$) and RSV ($n = 1$). Patients who discontinued SMV ($n = 2$) did not have available information about their LDL levels. One patient on SMV with a reduced dose to half after surgery with the LDL levels remain under the control post-RYGB.

Pharmacokinetics

ATV and Active Metabolites, 2-OH-ATV, and 4-OH-ATV

The individual concentrations normalized by dose/bodyweight, [(nM)/(mg/kg)], of ATV and its two active metabolites, 2-OH-ATV and 4-OH-ATV, showed a similar trend of significant decreases by 3 months, then at a slower rate of decline up to 6-month post-surgery (Fig. 5). The mean concentrations of the ATV and the two metabolites at each time point were tabulated (Table 2). The sampling time were different between subjects; however, the range of sampling time for individual patients pre- and post-surgery was similar as follows and made the trend of the profiles valid: patient 29 (13.5–19-h post-dose), patient 33 (12–18-h post-dose), and patient 40 (9.5-h post-dose; only baseline sample is available). Patient 43 had a sampling time of 11–11.5-h post-dose at baseline and 6-month follow-up, but the sampling time was 3 h at 3-month visit.

ATV pre-surgery was metabolized to 2-OH-ATV and 4-OH-ATV, with 63–78% vs 22–37% of the metabolites, respectively (Table 3). The metabolite ratios of 2-OH-ATV/ATV and

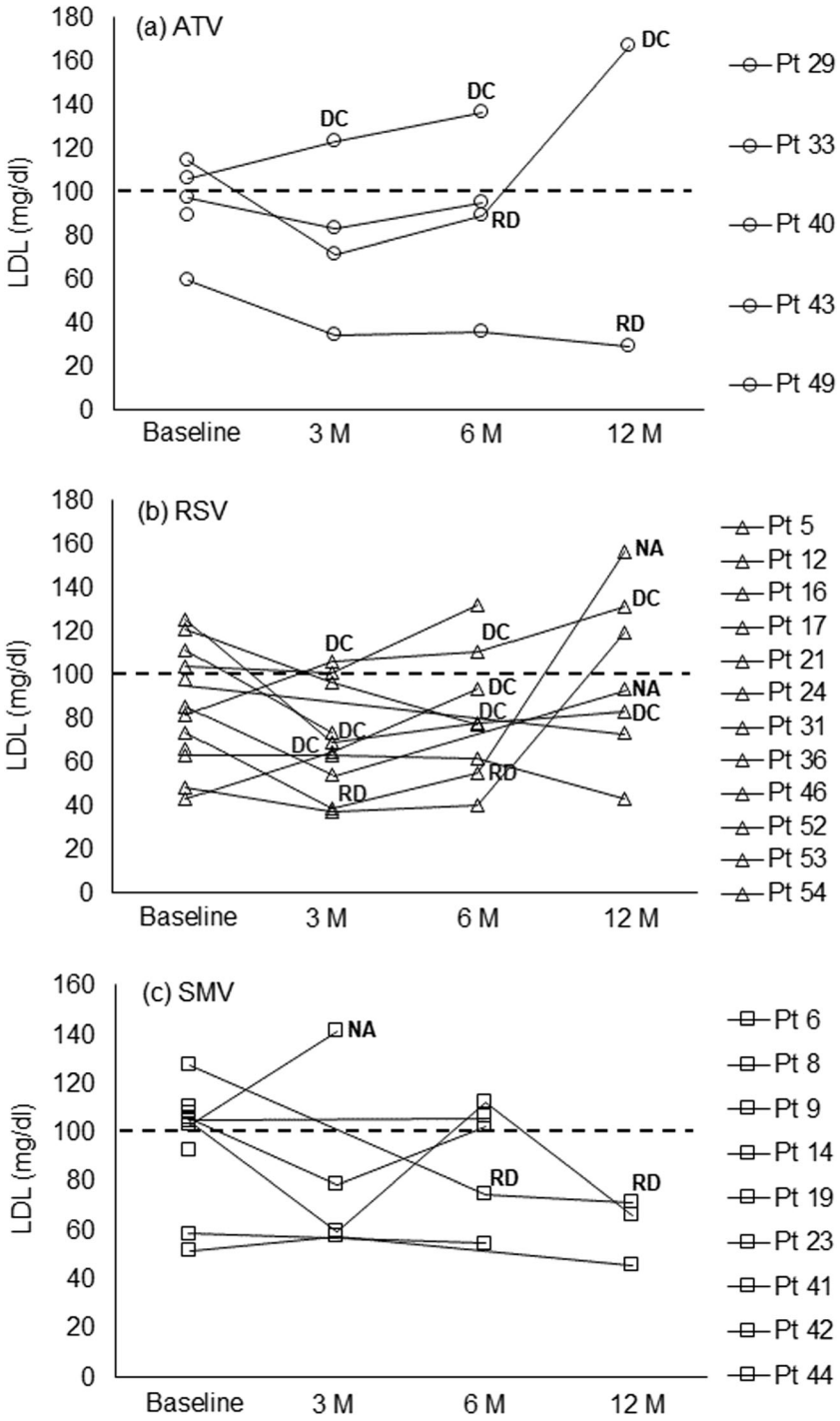

Fig. 4 Individual LDL levels in patients on **a** ATV, **b** RSV, and **c** SMV pre- and post-surgery. DC, discontinuation of statin; RD, reduced dose of statin to half; NA, no information of the dose is available. Dotted line represents 100 mg/dl (optimal level of LDL is < 100 mg/dl)

4-OH-ATV/ATV at baseline among the four subjects varied significantly, with 0.39–2.06 and 0.14–0.88, respectively. The metabolism of ATV to 2-OH-ATV and 4-OH-ATV was altered appreciably post-surgery, as expressed by the metabolite ratios of [2-OH-ATV/ATV] and [4-OH-ATV/ATV] (Table 3). Among the subjects who completed the follow-up visits, the ratios decreased by 3-month post-surgery, then remained relatively constant at the lower levels.

A preliminary PK/PD correlation was attempted by plotting the LDL values with the summation of the molar concentrations of ATV, 2-OH-ATV, and 4-OH-ATV at baseline, 3-month, and 6-month follow-ups, respectively (Fig. 6). It appeared that the threshold of effective ATV with active metabolites decreased from 40 nM pre-surgery to 20 nM at 3- and 6-month post-RYGB.

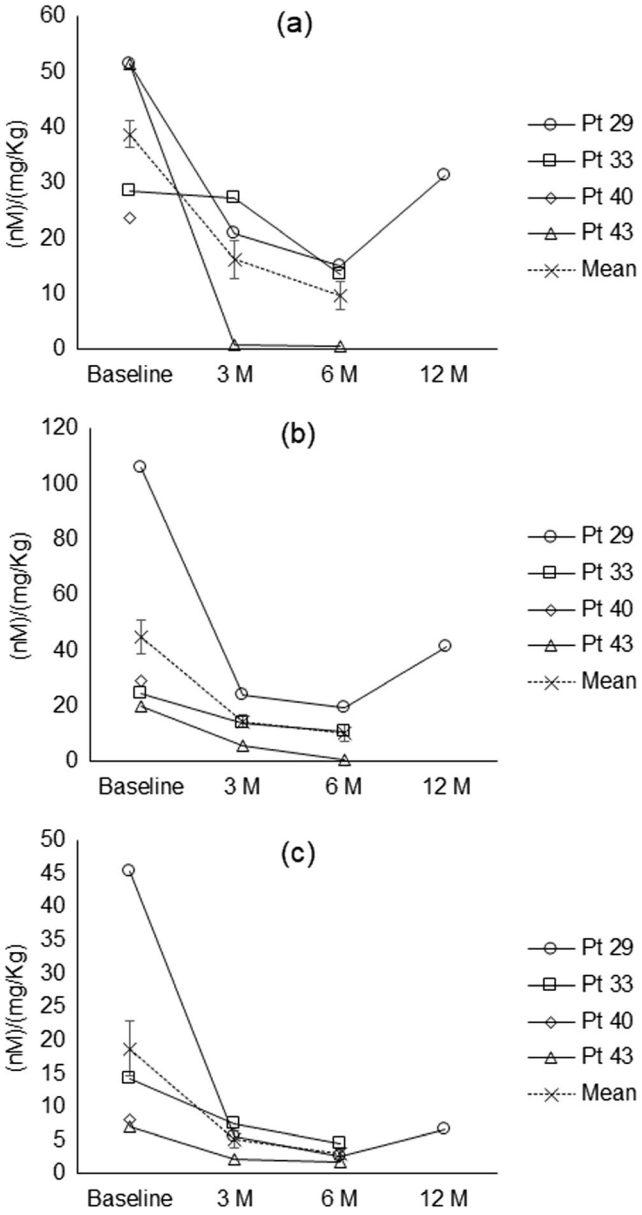

Fig. 5 Concentrations [(nM)/ (mg/kg)] of **a** ATV, **b** 2-OH-ATV, and **c** 4-OH-ATV in individual patients pre- and post- RYGB

RSV

The individual concentration profiles of RSV [(nM)/(mg/kg)] showed a similar trend of decrease as ATV post-surgery by 3 months, then at a slower rate of decline up to 6-month post-surgery (Fig. 7). Patient 53 had an obvious decrease in the normalized plasma concentrations at 3 and 6 months compared to that at baseline with similar sampling time pre- and post-surgery in the range of 4–4.5-h post-dose. Patient 46 also showed an apparent decline at 3 and 6 months compared to baseline with sampling times of 25-, 12.5-, and 9-h post-dose at baseline, 3, and 6-month post-surgery, respectively. Patient 17 had a slightly lower concentrations at 3 and 12 months compared to baseline with the sampling time in the range of 10–16-h post-dose. Patient 54 exhibited different profile, where the RSV concentration was higher at 3-month follow-up. However, this higher concentration might be due to the significantly different blood sampling time post the dose, where baseline sample was taken at 23 h while 3-month sample was at 4-h post-dose. The mean concentrations of RSV [(nM)/(mg/kg)] demonstrated the trend of decreased RSV concentration on the same dose basis post-surgery as compared to that of the preoperative therapy (Table 2).

SMV and SMV-A

The individual normalized concentration profiles of SMV and active metabolite, SMV-A, [(nM/ml)/(mg/kg)], are presented in Fig. 8. Three out of the six subjects with follow-up visits showed a trend of increase in SMV and SMV-A concentrations post-surgery with comparable sampling times (0.5–4 h for patient 9 and 14–16 h for patients 23 and 42). Two patients showed a trend of decrease in SMV and SMV-A concentrations at 6- and 12-month compared to 3-month visits (no baseline concentration was available). The sampling time range for these two patients (14 and 19) was 11–15 h. Patient 44 was the only patient with a complete profile at all time points pre- and post-surgery. The trend of the concentrations in this patient followed a zig-zag pattern, with a decrease at 3-month visit from baseline followed by an increase at 6-month visit and a decrease at 12-month visit. The sampling times post-dose for this patient were comparable with a range of 11.5–13 h post each dose. The mean profiles of SMV and SMV-A, showed a trend of increase after RYGB up to 6 months. At 12-month post-surgery, the mean plasma concentration levels were similar to the baseline levels (Table 2).

The hydrolysis and metabolism of SMV to SMV-A decreased appreciably post-surgery among five of the nine subjects, who had the follow-up visits. The metabolism decreased, as expressed by the ratios of [SMV-A/SMV], at 3-month or 6-month post-RYGB, then remained relatively constant at levels of lower ratios afterwards (Table 4). Only one subject (patient ID 9) showed an increase in the metabolism post-surgery compared to baseline, which could not be easily rationalized.

Discussion

Statins are prescribed to decrease the risk of atherosclerotic cardiovascular diseases. A published study reports the age as an independent risk factor for cardiovascular disease [29]. In the current study, the older mean age of subjects on statins could be attributed to the increased risk of cardiovascular disease with age, which causes an increased use of statins in older

Table 2 Mean concentrations [(nM)/ (mg/kg)] of statins and their active metabolites pre- and at various time points post-RYGB (data are presented as mean ± SE)

	ATV	2-OH-ATV	4-OH-ATV	RSV	SMV	SMV-A
Baseline	38.81 ± 2.36 (n = 4)	44.82 ± 6.11 (n = 4)	18.75 ± 4.18 (n = 4)	213.07 ± 22.87 (n = 10)	8.52 ± 3.04 (n = 6)	9.96 ± 3.99 (n = 6)
3 months	16.30 ± 3.41 (n = 3)	14.62 ± 2.40 (n = 3)	5.12 ± 1.20 (n = 3)	122.56 ± 9.67 (n = 4)	11.29 ± 1.96 (n = 5)	24.89 ± 6.90 (n = 5)
6 months	9.75 ± 2.52* (n = 3)	10.26 ± 2.95* (n = 3)	2.94 ± 0.80* (n = 3)	83.28 ± 6.39 (n = 3)	28.45 ± 3.54 (n = 4)	39.32 ± 7.95 (n = 4)
12 months	31.31 (n = 1)	41.09 (n = 1)	6.55 (n = 1)	195.94 (66.24, 325.65)	7.09 ± 0.42 (n = 3)	10.45 ± 2.39 (n = 3)

*Significantly lower than baseline concentration at $p < 0.05$ using Friedman test with Bonferroni correction

subjects. The racial distribution was comparable in statin and non-statin groups, with most subjects being Caucasians in both groups. Sex distribution between the two groups was significantly different, with majority of males being treated with one of the statins. This higher number of male subjects on statins might reflect their greater preventive medical need, as a higher incidence of cardiovascular diseases was reported in males compared to females in a published study [30].

The BMI at baseline had a range of 33–62 kg/m^2; however, all patients had a BMI of ≥ 35 kg/m^2 when they decided to undergo RYGB. The BMI at baseline and the pattern of BMI reduction post-surgery were similar in statin and non-statin groups. The similar patterns of BMI reduction post-surgery between statin and non-statin groups, with a significant reduction of 6–7.5 units by 3 months, a further decrease to 9–10 units by 6 months, and to 11.5–13 units by 12-month post-surgery, suggest that the weight loss was RYGB induced and apparently not affected by the statin treatments. At 12-month post-surgery, 86% (25/29) of the patients who completed the 12-month follow-up visit in our study had a satisfactory %EWL of > 50%. The time course of change in %EWL post-surgery had the same pattern as BMI reduction, and was very similar in statin and non-statin groups. This observation confirms the conclusion that weight loss was surgery induced and not correlated with statin treatments. These observations agree with the results from a published study, that concludes the weight loss between statin (atorvastatin, simvastatin, rosuvastatin, pravastatin, lovastatin, and fluvastatin) and non-statin groups is comparable in an average follow-up time of 5.6 months (range 1–36 months) [31]. On the other hand, our results disagree with that from another published study that reports patients on statins lose more weight 12-month post-surgery than patients who do not take any statin. However, the type of statin was not specified in that study [32]. Both studies did not examine the longitudinal weight loss pattern in individual statins as in this study.

The RYGB has an obvious effect on the pharmacodynamic outcomes as well. TG mean levels at baseline were higher in combined statin and individual SMV and RSV groups compared to the non-statin group. The TG levels in all these groups became comparable post-RYGB, which might be explained by the predominant surgical effect on TG levels in statin treatment groups. The RYGB effectively reduced TG levels by 3-month post-surgery in statin group from 159 mg/dl at baseline to 111 mg/dl. On the other hand, the LDL mean value at baseline for non-statin group was at 110 mg/dl, higher than those in statin groups. However, the significantly higher LDL in non-medicated patients was corrected by RYGB. HDL levels appeared to increase significantly from 47 mg/dl in both groups at baseline to 58–61 mg/dl at 12-month post-surgery with a similar pattern in both groups. This change in HDL levels could also be attributed to the effect of surgery.

Table 3 Ratios of [2-OH-ATV/ATV] and [4-OH-ATV/ATV] pre- and post-surgery in individual patients

	Pt ID							
	29		33		40		43*	
	2-OH-ATV/ATV	4-OH-ATV/ATV	2-OH-ATV/ATV	4-OH-ATV/ATV	2-OH-ATV/ATV	4-OH-ATV/ATV	2-OH-ATV/ATV	4-OH-ATV/ATV
Baseline	2.06 (70%)**	0.88 (30%)	0.86 (63%)	0.50 (37%)	1.22 (78%)	0.34 (22%)	0.39 (74%)	0.14 (26%)
3 months	1.16 (81%)	0.27 (19%)	0.50 (64%)	0.28 (36%)				
6 months	1.29 (88%)	0.17 (12%)	0.81 (71%)	0.33 (29%)				
12 months	1.31 (86%)	0.21 (14%)						

*Patient 43 had ATV levels below LLOQ of 0.25 ng/ml at 3- and 6-month follow-up visits, so the ratios could not be reliably derived

**(%) of metabolites between 2-OH-ATV and 4-OH-ATV

Individual LDL levels were analyzed in relation to statin discontinuation and dose reduction post-RYGB. The discontinuation or reduction of the dose of ATV or RSV post-RYGB exhibited rebounds of LDL levels in some subjects, but the rebound was not apparent with patients on SMV pre-surgery. Discontinuation of statin treatment post-surgery led to increases in LDL values higher than that at baseline (2 patients on ATV and 2 patients on RSV). Reduction of dose to half seemed to have better results in controlling LDL than discontinuation of therapy with ATV or RSV. The surgery itself seemed to have an immediate lowering effect on LDL, but the effect of surgery may be reversed at later time points (12 months) post-RYGB in some patients even if the dose remained the same (3 patients on RSV and 2 patients on SMV).

This is the first longitudinal prospective pharmacodynamics and pharmacokinetic study on three statins, with individual patients serving as their own controls pre- and post-RYGB. Two observations are unique in our study regarding atorvastatin: (a) decreases in atorvastatin metabolism to active 2-hydroxy and 4-hydroxy metabolites and (b) decline of plasma concentration exposures on the same dose basis, by 3-month post-RYGB. Atorvastatin, a lipophilic statin, is administered as the acid form, and it is metabolized by CYP 3A4 to two active metabolites, 2-hydroxy (63–78%) and 4-hydroxy (22–37%) atorvastatin. The decrease in metabolism of atorvastatin to the metabolites (ratios of metabolites to atorvastatin) 3-month post-surgery and leveled off is consistent with the known reduction of available intestinal CYP 3A4 after RYGB, in which the proximal small intestine (duodenum and part of the jejunum) is bypassed, where most of the intestinal CYP3A4 enzyme is expressed [33]. The mean concentrations of atorvastatin and the two active metabolites, normalized by dose/bodyweight, [(nM)/(mg/kg)], showed a significant decrease at 3 months and maintain up to 6-month post-surgery. This decline may be explained by decreased absorption after RYGB, due to the increased gastric pH to > 4 [34] and the decreased intestinal surface after the surgery. The equilibrium of ionized: unionized form of the atorvastatin acid favors the ionized form that is more soluble but less permeable at a higher pH post-surgery, and may lead to a decrease in the absorption of atorvastatin through passive diffusion. The decrease in the intestinal surface area post-RYGB will also decrease the absorption of atorvastatin.

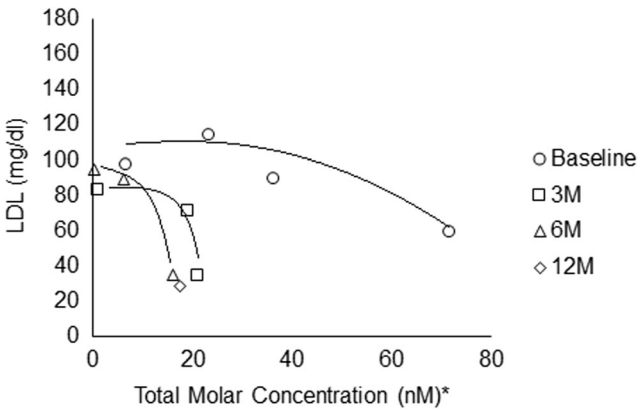

Fig. 6 LDL (mg/dl) correlation with summation of molar concentrations of ATV and two active metabolites, 2-OH-ATV and 4-OH-ATV. (*) Total molar concentration was calculated as [ATV + 2-OH-ATV + 4-OH-ATV] in nM

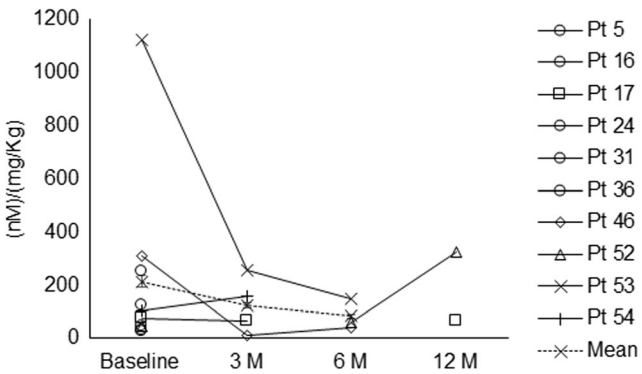

Fig. 7 Concentrations [(nM)/ (mg/kg)] of RSV in individual patients pre- and post-RYGB

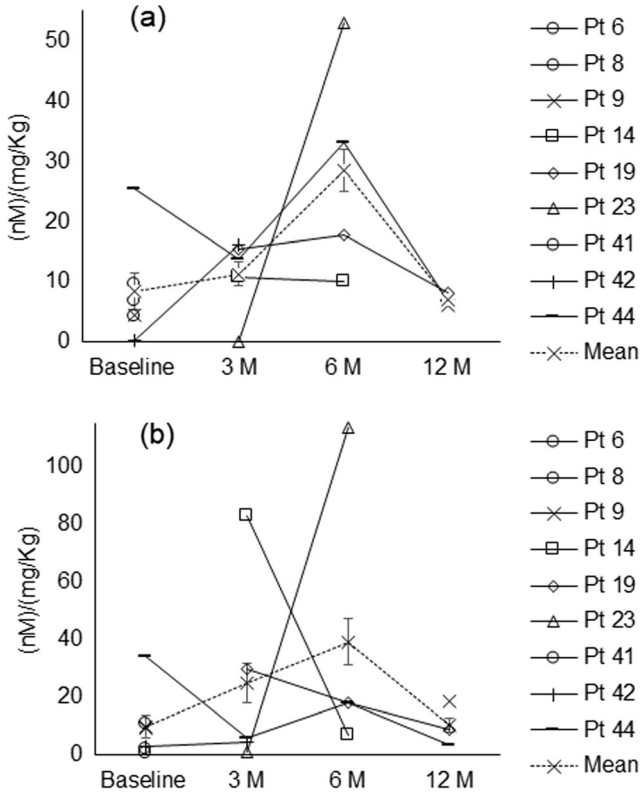

Fig. 8 Concentrations [(nM)/(mg/kg)] of **a** SMV and **b** SMV-A in individual patients pre- and post-RYGB

Rosuvastatin is considered a relatively hydrophilic statin compared to atorvastatin and simvastatin. It is administered in the acid form, and its metabolism is minimal, with most of the dose to be eliminated in bile. The mean and individual concentrations of rosuvastatin [(nM)/(mg/kg)] showed a trend of decrease by 3-month post-surgery, similar to that of atorvastatin and its two active metabolites. This may be explained by the fact that rosuvastatin absorption is facilitated by organic anion transporter (OAT2B1) [35], which is expressed along the small intestine at similar levels in all segments [36]. Since the proximal segment of the small intestine is bypassed after RYGB, the absorption of rosuvastatin is expected to decrease after the surgery.

Table 4 The ratios of [SMV-A/SMV] pre- and post-surgery in individual patients

	Pt ID								
	6	8	9	14	19	23	41	42	44
Baseline	0.10	0.43	2.13				1.12	7.85	1.34
3 months				7.67	1.92	17.14		0.28	0.42
6 months				0.72	1.02	2.15			0.55
12 months			3.09		1.09				0.51

Simvastatin mean concentrations showed a trend of increase up to 6-month post-RYGB. This may be partly explained by the increase in gastric pH after RYGB. Simvastatin is a prodrug, administered in the inactive lactone form. It converts in vivo into the active acid form through chemical and enzymatic hydrolyses. The interconversion of lactone to acid is minimal at pH 4–5 [37], which might lead to an increase in absorption and bioavailability due to the simvastatin lactone form. This explanation agrees with studies that demonstrated an increase in the bioavailability of simvastatin after administration of multiple doses of antacid [38, 39]. Another potential explanation is the bypass of the intestinal CYP3A4 enzyme that leads to an increase in the bioavailability of simvastatin. The return of plasma levels 1-year post-RYGB to the baseline levels suggests an adaptation of the gastrointestinal tract. The adaptation mechanism could be due to the increase in the gastric acid secreting cells in the small stomach pouch that was created during the surgery, or by an increase in the expression of CYP3A4 enzyme at the more distal segments of the small intestine. Simvastatin is considered a lipophilic statin; hence, its absorption is by passive diffusion. The bypass of the proximal small intestine leads to a decrease in the intestinal surface area available for passive diffusion, which is expected to decrease the levels of lipophilic drugs post-surgery. The interplay between all these factors leads to a large intra- and inter-individual variability in the pharmacokinetic results of simvastatin and simvastatin acid. Simvastatin shares many similar characteristics with atorvastatin, in lipophilicity, as a substrate for CYP3A4 enzyme and P-glycoprotein, with > 90% of plasma protein binding. However, the impacts of RYGB on pharmacokinetics of simvastatin and atorvastatin observed in this study are distinctively different that may lead to rationally differential approaches to modify their dosing regimen after RYGB.

A drawback of this study is the different sampling time post dose among individuals. This difference is expected to affect the interpretation of the results of simvastatin and its metabolite more than those of atorvastatin, its two metabolites, and rosuvastatin. Simvastatin and its metabolite have short half-lives of 2 and 1.9 h, respectively. The half-lives of atorvastatin and rosuvastatin are considerably longer at 14 and 19 h, respectively. However, each patient samples were taken at similar time points after each dose pre- and post-surgery. Another limitation for the study is the availability of one sample per patient per time point pre- or post-RYGB, which limits the possibility of deriving pharmacokinetic parameters such as elimination rate constant, and to characterize the post-RYGB changes as a function of time post-surgery.

Conclusion

This is the first longitudinal prospective study on the effects of Roux-en-Y gastric bypass on the weight loss performance, as well as pharmacodynamics and pharmacokinetics of atorvastatin, simvastatin, and rosuvastatin along with their active metabolites, for 1-week and 1-, 3-, 6- and 12-month post-surgery. The study showed a trend of decreased plasma concentration exposures for atorvastatin, its two active metabolites, and rosuvastatin, on the same dose basis, by 3-month and up to 6-month post-RYGB. However, an opposite trend was observed for simvastatin and simvastatin acid with an increase in the absorption post-surgery up to 6 months, then the effect normalized to baseline levels at 1-year post-surgery. In addition, by monitoring both parent compound and metabolites for atorvastatin and simvastatin using LC-MS/MS assay, the decreases in metabolisms of these statins post-RYGB are documented.

The comparison of weight loss outcomes (BMI reduction and %EWL) and pharmacodynamic measurements (TC, TG, LDL, and HDL) indicates the effect of surgery was predominant, as high LDL in non-statin group can be corrected post-surgery in un-medicated patients. However, discontinuations of statin dosing with ATV and RSV require the monitoring of LDL, as rebound LDL are often observed. Reducing the statin dose to half post-surgery with continuous monitoring of LDL semiannually, based on our study, seems to be a better approach. At the end, patients who are still on statin treatment post-RYGB should be followed up closely and modify the dose intensity rationally to ensure therapeutic effects of the treatment with no adverse effects.

Funding This work was supported by the Research Centers in Minority Institutions (RCMI) [grant number 5G12MD007605-23].

Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

Ethical Approval Statement All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed Consent Statement Informed consent was obtained from all individual participants included in the study.

References

1. Hales CM, Carroll MD, Fryar CD, et al. Prevalence of obesity among adults and youth: United States, 2015–2016. NCHS Data Brief. 2017;2017(288):1–8.
2. Warren M, Beck S, Rayburn J. The state of obesity 2018: better policies for a healthier America. Washington (DC): Trust for America's Health and The Robert Wood Johnson Foundation; 2018. p. 1–68.
3. Li Q, Blume SW, Huang JC, et al. Prevalence and healthcare costs of obesity-related comorbidities: evidence from an electronic medical records system in the United States. J Med Econ. 2015;18(12):1020–8.
4. Khaodhiar L, McCowen KC, Blackburn GL. Obesity and its comorbid conditions. Clin Cornerstone. 1999;2(3):17–31.
5. American Society for Metabolic and Bariatric Surgery. Estimate of Bariatric Surgery Numbers, 2011–2017 2018 June 2018 [cited 2018 October 23]. Available from: https://asmbs.org/resources/estimate-of-bariatric-surgery-numbers.
6. Golzarand M, Toolabi K, Farid R. The bariatric surgery and weight losing: a meta-analysis in the long- and very long-term effects of laparoscopic adjustable gastric banding, laparoscopic Roux-en-Y gastric bypass and laparoscopic sleeve gastrectomy on weight loss in adults. Surg Endosc. 2017;31(11):4331–45.
7. Lager CJ, Esfandiari NH, Subauste AR, et al. Roux-en-Y gastric bypass vs. sleeve gastrectomy: balancing the risks of surgery with the benefits of weight loss. Obes Surg. 2017;27(1):154–61.
8. Melissas J, Stavroulakis K, Tzikoulis V, et al. Sleeve Gastrectomy vs Roux-en-Y gastric bypass. Data from IFSO-European chapter Center of Excellence Program. Obes Surg. 2017;27(4):847–55.
9. Horowitz M, Cook DJ, Collins PJ, et al. Measurement of gastric emptying after gastric bypass surgery using radionuclides. Br J Surg. 1982;69(11):655–7.
10. De Smet J, Van Bocxlaer J, Boussery K. The influence of bypass procedures and other anatomical changes in the gastrointestinal tract on the oral bioavailability of drugs. J Clin Pharmacol. 2013;53(4):361–76.
11. Quercia I, Dutia R, Kotler DP, et al. Gastrointestinal changes after bariatric surgery. Diabetes Metab. 2014;40(2):87–94.
12. Stone NJ, Robinson JG, Lichtenstein AH, et al. 2013 ACC/AHA guideline on the treatment of blood cholesterol to reduce atherosclerotic cardiovascular risk in adults: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines. Circulation. 2014;129(25 Suppl 2):S1–45.
13. Mercado C, AK DS, Odom E, et al. Prevalence of cholesterol treatment eligibility and medication use among adults—United States, 2005–2012. MMWR Morb Mortal Wkly Rep. 2015;64(47):1305–11.
14. Salami JA, Warraich H, Valero-Elizondo J, et al. National Trends in statin use and expenditures in the US adult population from 2002 to 2013: insights from the medical expenditure panel survey. JAMA Cardiol. 2017;2(1):56–65.
15. Skottheim IB, Stormark K, Christensen H, et al. Significantly altered systemic exposure to atorvastatin acid following gastric bypass surgery in morbidly obese patients. Clin Pharmacol Ther. 2009;86(3):311–8.
16. Jakobsen GS, Skottheim IB, Sandbu R, et al. Long-term effects of gastric bypass and duodenal switch on systemic exposure of atorvastatin. Surg Endosc. 2013;27(6):2094–101.
17. Yska JP, van der Linde S, Tapper VV, et al. Influence of bariatric surgery on the use and pharmacokinetics of some major drug classes. Obes Surg. 2013;23(6):819–25.
18. Yska JP, van der Meer DH, Dreijer AR, et al. Influence of bariatric surgery on the use of medication. Eur J Clin Pharmacol. 2016;72(2):203–9.
19. NHLBI Obesity Education Initiative Expert Panel on the Identification, Evaluation, and Treatment of Obesity in Adults (US). Clinical guidelines on the identification, evaluation, and treatment of overweight and obesity in adults: the evidence report. Bethesda (MD): National Heart, Lung, and Blood Institute; 1998.
20. El-Zailik A, Cheung LK, Wang Y, et al. Simultaneous LC-MS/MS analysis of simvastatin, atorvastatin, rosuvastatin and their active metabolites for plasma samples of obese patients underwent gastric bypass surgery. J Pharm Biomed Anal. 2018;164:258–67.

21. Brethauer SA, Kim J, El Chaar M, et al. Standardized outcomes reporting in metabolic and bariatric surgery. Obes Surg. 2015;25(4):587–606.
22. Lipitor (atorvastatin calcium) [package insert on the internet]. Dublin, Ireland: Pfizer Ireland Pharmaceuticals. 2009 [cited 2019 Jan 19]. Available from: https://www.accessdata.fda.gov/drugsatfda_docs/label/2009/020702s057lbl.pdf.
23. Nayak BK, Hazra A. How to choose the right statistical test? Indian J Ophthalmol. 2011;59(2):85–6.
24. Bennett DA. How can I deal with missing data in my study? Aust N Z J Public Health. 2001;25(5):464–9.
25. Kang H. The prevention and handling of the missing data. Korean J Anesthesiol. 2013;64(5):402–6.
26. Graham JW, Olchowski AE, Gilreath TD. How many imputations are really needed? Some practical clarifications of multiple imputation theory. Prev Sci. 2007;8(3):206–13.
27. Dong Y, Peng CY. Principled missing data methods for researchers. Springerplus. 2013;2(1):222.
28. Biering K, Hjollund NH, Frydenberg M. Using multiple imputation to deal with missing data and attrition in longitudinal studies with repeated measures of patient-reported outcomes. Clin Epidemiol. 2015;7:91–106.
29. Dhingra R, Vasan R-S. Age as a cardiovascular risk factor. Med Clin N Am. 2012;96(1):87–91.
30. Vitale C, Fini M, Speziale G, et al. Gender differences in the cardiovascular effects of sex hormones. Fundam Clin Pharmacol. 2010;24(6):675–85.
31. Perna M et al. Statins and the bariatric patient: characterization and perioperative effects of statin therapy in the gastric bypass patient. Am Surg. 2011;77(1):44–7.
32. Taylor BA, Ng J, Stone A, et al. Effects of statin therapy on weight loss and diabetes in bariatric patients. Surg Obes Relat Dis. 2017;13(4):674–80.
33. Thorn M, Finnstrom N, Lundgren S, et al. Cytochromes P450 and MDR1 mRNA expression along the human gastrointestinal tract. Br J Clin Pharmacol. 2005;60(1):54–60.
34. Smith A, Henriksen B, Cohen A. Pharmacokinetic considerations in Roux-en-Y gastric bypass patients. Am J Health Syst Pharm. 2011;68(23):2241–7.
35. Johnson M, Patel D, Matheny C, et al. Inhibition of intestinal OATP2B1 by the calcium receptor antagonist ronacaleret results in a significant drug-drug interaction by causing a 2-fold decrease in exposure of rosuvastatin. Drug Metab Dispos. 2017;45(1):27–34.
36. Meier Y, Eloranta JJ, Darimont J, et al. Regional distribution of solute carrier mRNA expression along the human intestinal tract. Drug Metab Dispos. 2007;35(4):590–4.
37. Nigovic B, Mornar A, Sertic M. A review of current trends and advances in analytical methods for determination of statins: chromatography and capillary electrophoresis, chromatography - the most versatile method of chemical analysis. In: de Azevedo Calderon L (ed). IntechOpen; 2012. p. 385–428. https://doi.org/10.5772/48694. Available from: https://www.intechopen.com/books/chromatography-the-most-versatile-method-of-chemical-analysis/a-review-of-current-trends-and-advances-in-analytical-methods-for-determination-of-statins-chromatog.
38. AlAkhali K, Alavudeen S. Multiple dose of liquid antacid enhance simvastatin bioavailability in Malaysian male volunteers. Asian J Pharm. 2014;8(2):90–4.
39. Alakhali K, Vigneshwaran E, Shaik M. Effect of food and antacid on simvastatin bioavailability on healthy adult volunteers. J Health Res Rev. 2018;5(1):26–32.