ORIGINAL RESEARCH ARTICLE

Neither Gastric Bypass Surgery Nor Diet-Induced Weight-Loss Affect OATP1B1 Activity as Measured by Rosuvastatin Oral Clearance

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Abstract

Introduction Rosuvastatin pharmacokinetics is mainly dependent on the activity of hepatic uptake transporter OATP1B1. In this study, we aimed to investigate and disentangle the effect of Roux-en-Y gastric bypass (RYGB) and weight loss on oral clearance (CL/F) of rosuvastatin as a measure of OATP1B1-activity.

Methods Patients with severe obesity preparing for RYGB ($n = 40$) or diet-induced weight loss ($n = 40$) were included and followed for 2 years, with four 24-hour pharmacokinetic investigations. Both groups underwent a 3-week low-energy diet (LED; < 1200 kcal/day), followed by RYGB or a 6-week very-low-energy diet (VLED; < 800 kcal/day).

Results A total of 80 patients were included in the RYGB group (40 patients) and diet-group (40 patients). The weight loss was similar between the groups following LED and RYGB. The LED induced a similar (mean [95% CI]) decrease in CL/F in both intervention groups (RYGB: 16% [0, 31], diet: 23% [8, 38]), but neither induced VLED resulted in any further changes in CL/F. At Year 2, CL/F had increased by 21% from baseline in the RYGB group, while it was unaltered in the diet group. Patients expressing the reduced function *SLCO1B1* variants (c.521TC/CC) showed similar changes in CL/F over time compared with patients expressing the wild-type variant.

Conclusions Neither body weight, weight loss nor RYGB per se seem to affect OATP1B1 activity to a clinically relevant degree. Overall, the observed changes in rosuvastatin pharmacokinetics were minor, and unlikely to be of clinical relevance.

Key Points

Both surgery and diet intervention led to a matched weight loss between the study groups.

Roux-en-Y gastric bypass surgery does not affect OATP1B1 activity in patients with severe obesity.

No dose adjustments of rosuvastatin appear to be necessary following weight loss induced by bariatric surgery and/or low-calorie diet.

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1 Introduction

Obesity represents a global epidemic [1], and is associated with an increased risk of comorbidities including type 2 diabetes, cardiovascular disease and cancer that often requires pharmacological treatment [2, 3]. Severe obesity is classified as a body mass index (BMI) between 35.0 and 39.9 kg/m^2 in combination with comorbidity, or $\geq$ 40 kg/m^2, for which weight loss is the primary treatment [4]. Bariatric surgery has shown superior effects with respect to achieving long-lasting weight loss and improvement of comorbidities compared to non-surgical interventions in patients with severe obesity [5, 6]. The Roux-en-Y gastric bypass (RYGB) procedure reduces the proximal stomach, and the duodenum and proximal intestine are bypassed [7, 8]. Due to this gastrointestinal rearrangement, drug absorption and disposition may be altered. Altered gastric pH, reduced transit-time, and decreased surface area for passive and active absorption, as well as the bypassing of intestinal segments rich in

drug-metabolising enzymes and transporters, are among the suggested mechanisms leading to variable drug disposition following RYGB [9].

The 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase inhibitor, rosuvastatin, is a commonly used lipid-lowering agent. Rosuvastatin is more hydrophilic compared with most other statins, and it displays a low degree of passive diffusion into tissues [10]. The hepatic uptake transporters organic-anion transporting polypeptides (OATP) 1B1 (*SLCO1B1*), OATP1B3 (*SLCO1B3*), OATP2B1 (*SLCO2B1*), as well as the sodium taurocholate co-transporting polypeptide (NTCP; *SLC10A1*) transport rosuvastatin into the hepatocytes. Organic-anion transporting polypeptides 1B1 contributes to 49–86% of rosuvastatin hepatic uptake, and rosuvastatin is thus considered an established and preferred probe drug to study OATP1B1 activity [11–13]. Rosuvastatin is sparsely metabolised, and the majority of the absorbed dose is excreted unchanged in faeces (~ 76%). Hepatic elimination accounts for approximately 70% of total clearance of rosuvastatin, with biliary excretion, mediated by canalicular transporters such as breast cancer resistance protein (BCRP; *ABCG2*) and permeability-glycoprotein (P-gp; *ABCB1*), as the main clearance mechanism [14, 15]. Genetic polymorphisms of the key hepatic uptake transporter OATP1B1 have been shown to influence rosuvastatin disposition. The single-nucleotide polymorphism (SNP) c.521T>C in *SLCO1B1* is associated with decreased membrane expression of OATP1B1 in vivo [16]. Consequently, systemic exposure of rosuvastatin is reported to be 19–68% higher in patients with c.521TC or c.521CC [17].

In patients with a wide range of body weight, using midazolam as a probe drug, both the activity and protein expression of the quantitatively most important drug metabolising enzyme, cytochrome P450 (CYP) 3A, have been shown to be inversely associated with body weight. This was hypothesised to be due to low-grade inflammation and/or non-alcoholic fatty liver disease (NAFLD) [18–20]. Inflammatory cytokines such as interleukin (IL) 6 and tumour necrosis factor alpha (TNF-α), as well as NAFLD have been associated with lower expression and activity of several CYP enzymes [18, 21]. However, there are limited data regarding the effect of weight loss, inflammation, and NAFLD on drug transporters. The primary aim of this work was to study the short- (6 weeks) and long-term (2 years) effects of weight loss induced by strict diet or RYGB on OATP1B1 activity by investigating the pharmacokinetics of rosuvastatin, and to secondary compare OATP1B1 activity in a control group of normal-to-overweight individuals with patients with severe obesity.

2 Methods

2.1 Study Design and Patients

The COCKTAIL study, a non-randomised, single-centre, 3-armed study, was carried out at the Morbid Obesity Centre, Vestfold Hospital Trust, Norway [22]. Patients with severe obesity (BMI ≥ 35 kg/m^2 in combination with comorbidity or BMI ≥ 40 kg/m^2) scheduled for elective weight-reducing intervention by either RYGB or strict diet were eligible for inclusion. The inclusion and exclusion criteria are fully described in the protocol paper [22]. Additionally, normal-to-overweight (BMI 18.5–29.9 kg/m^2) individuals scheduled for cholecystectomy were included as a cross-sectional control group. The study was approved by the Regional Committee for Medical and Health Research Ethics (2013/2379/REK) and performed in accordance with Good Clinical Practice and the Declaration of Helsinki (NCT02386917). Written informed consent was obtained prior to study participation.

2.2 Study Visits and Procedures

The patient flow in the study has been described previously [20], and is further detailed in Figure S1. Both the RYGB group and the diet group were prescribed an initial 3-week low-energy diet (LED; < 1200 kcal/day), followed by additional 6 weeks of strict caloric restriction (< 800 kcal/day) induced by surgery or a very-low energy diet (VLED), respectively, in order to obtain a similar weight loss between the two groups [22]. All patients were prospectively followed for 2 years. During the study period, four 24-h pharmacokinetic investigations were performed following single oral administration of 20 mg rosuvastatin. Blood samples were obtained via a peripheral venous catheter before and 0.25, 0.5, 1, 1.5, 2, 3, 4, 4.25, 4.5, 5, 5.5, 6, 8, 10, 12, 23, and 24 h post-dose. The investigations were performed at baseline (Week 0); after the 3-week LED (Week 3); after additional 6-week strict calorie restriction (Week 9), and after 2 years (Year 2). For the RYGB group, the investigation at Week 3 was performed the day before surgery. The cross-sectional control group of normal-to-overweight individuals underwent a single 24-h pharmacokinetic study the day before cholecystectomy (Week 0). Hepatic and jejunal biopsies were obtained at the day of surgery in the RYGB group, and hepatic biopsies were obtained in the normal-to-overweight control group at the day of surgery as previously described [23].

2.3 Outcomes

The primary outcomes were short- (Week 3 to Week 9) and long-term (Week 3 to Year 2) changes in oral clearance of rosuvastatin, as a measure of OATP1B1 activity. Secondary

outcomes included changes in rosuvastatin pharmacokinetics over time following RYGB or strict diet, assessed by oral clearance, maximum concentration (C_{max}) and time to maximum concentration (T_{max}), as well as hepatic concentration of OATP1B1.

2.4 Analytical Assay

Plasma concentrations of rosuvastatin were determined by Covance Laboratories (Madison, Wisconsin, USA), as previously described [24]. In brief, buffered plasma samples were extracted by supported liquid extraction. After evaporation, the residue was reconstituted and analysed with liquid chromatography tandem mass spectrometry (LC-MS/MS). The analyte was separated on a C18-column (Aquasil) with a gradient mobile phase of acetonitrile and 0.1% formic acid, using a LC system from Thermo Electron Corporation. Rosuvastatin was detected by MS/MS using a Sciex API 5500 with positive electrospray ionisation, monitoring the m/z 482.2–258.2 transition. The standard curve ranged from 0.04 to 40 ng/mL, using a human plasma sample volume of 0.1 mL. The assay variation coefficients of the rosuvastatin analysis were 7.1%, 4.4% and 4.5% at 0.12 ng/mL, 2 ng/mL, and 20 ng/mL ($n = 130$), respectively.

2.5 Clinical Chemistry and Genotyping

Clinical chemistry analyses were performed at the Department of Laboratory Medicine, Vestfold Hospital Trust, Tønsberg, Norway. Plasma concentrations of C-reactive protein (CRP) and high-sensitivity CRP (hsCRP) were measured using immunoturbidimetry (Advia Chemistry XPT systems, Siemens) at Fürst Medical Laboratory, Oslo, Norway. Genotyping of *SLCO1B1* and *ABCG2* variant alleles were performed using Taqman-based real-time polymerase chain reaction assays at the Center for Psychopharmacology, Diakonhjemmet Hospital, Oslo, Norway. The following variant alleles were assessed for *SLCO1B1*: c.521T>C (rs4149056); *ABCG2*: V12M variant (rs2231137) and reduced-function Q141K variant (rs2231142). Homozygote carriers of the V12M C/C and Q141K G/G were considered as the wild-type haplotype. A TaqMan assay for rs2231137 genotyping was not readily available, and as such an assay for rs4148150 was used, which is in complete disequilibrium ($R^2 = 1$ in Europeans) with rs2231137 [25].

2.6 Quantification of Hepatic and Intestinal Protein Concentration

Proteins were extracted from small intestinal and liver biopsies in an SDS-containing (2% w/v) lysis buffer and quantified as previously described [26, 27]. In short, samples were processed with the multi-enzyme digestion filter-aided

sample preparation protocol, using LysC and trypsin [28]. Proteomics analysis was performed with Q Exactive HF or Q Exactive HF-X MS in data-dependent mode. Mass spectrometry (MS) data were processed with MaxQuant (version 1.6.10.43) [29] where proteins were identified by searching MS and MS/MS data of peptides against the human UniProtKB (UP000005640). Spectral raw intensities were normalised with variance stabilisation [30] and were subsequently used to calculate the protein concentrations using the Total Protein Approach [31]. Batch effects were removed by geometric mean centring of proteins from samples analysed at different time points.

2.7 Population Pharmacokinetic Modelling

A population pharmacokinetic model was developed to determine individual rosuvastatin pharmacokinetics at the different study visits. The purpose of the model was to provide accurate estimates of AUC, oral clearance, maximum concentration (C_{max}) and time to maximum concentration (T_{max}) in order to allow for a rational assessment of change over time and between groups. In short, a non-parametric adaptive grid approach implemented in a modified version of Pmetrics 1.9.4 [32] for R 3.6.0 was used. In total, 3630 rosuvastatin concentrations corresponding to 197 18-point and 111 9-point 24-h pharmacokinetic profiles from 98 patients were included. Additional model information and metrics are available in the Supplementary file (Figs S2, S3).

2.8 Pharmacokinetic Calculations

Posterior individual parameter values, as well as posterior individually predicted concentrations obtained from the final population pharmacokinetic model run with the complete dataset were used for all pharmacokinetic calculations. Predictions were made in 12-min intervals, and at each sample point. Area under the concentration-time curve from zero to infinity ($AUC_{0-\infty}$) was calculated with the trapezoidal approximation from individual posterior-predicted concentrations using the 'makeAUC'-function in the Pmetrics package for R. C_{max} and T_{max} were obtained directly from the individual predictions, while oral clearance was calculated by dividing the dose by the $AUC_{0-\infty}$.

2.9 Statistical Analyses and Calculations

Linear mixed effects models were used to evaluate changes over time with the parameter of choice as the dependent variable, while visit, intervention group, and their interaction were treated as fixed effects. Variant of OATP1B1 was added as a fixed effect when evaluating differences in change over time between genotypes. The unique patient identifier was used as a random effect. Model residuals were

evaluated for normality, and if appropriate, the dependent variable was transformed using the natural logarithm. Models with a logarithmically transformed dependent variable were adjusted for the introduced bias. Estimated marginal means and contrasts were used for factor combinations (of visit and intervention group) for all parameters of interest. The cross-sectional analysis at baseline was performed using Welch's two-sample t test comparing the control group with patients with severe obesity (RYGB group and diet group combined). Baseline rosuvastatin pharmacokinetics were used to assess differences between genotypes. To explore relationships between variables, Pearson's product-moment correlation was applied. Variables with non-normal distributions (assessed visually) were logarithmically transformed. Predictions of NAFLD and liver fat were performed using metabolic and genetic factors, with NAFLD Liver Fat Score values greater than -0.640 as a diagnostic of NAFLD [33]. Data are presented as mean $\pm$ standard deviation or mean [95% confidence interval (CI)] unless otherwise stated. With the predetermined $\alpha = 0.05$, 95% CIs not including zero and p values < 0.05 were considered statistically significant. Confidence intervals from linear mixed effects models were adjusted using Tukey method. All statistical analyses were performed using R 4.0.2 [34].

3 Results

3.1 Patient Characteristics

A total of 108 patients (44, 44 and 20 in the RYGB, diet, and control groups, respectively) were included in the study. In the present analyses, 98 patients (40, 40 and 18 in the RYGB,

diet, and control group, respectively) supplied at least one 24-h pharmacokinetic profile during the study period.

Mean total body weight at baseline was 132 ± 24 kg, 124 ± 23 kg, and 71 ± 11 kg in the RYGB, diet, and control groups, respectively (Table 1). There were no differences between the groups with respect to age, sex, or ethnicity. Routine clinical chemistry was similar in the three groups, except for higher value of ALT in the intervention groups compared with the control group (Table 1). At baseline, 71 of 98 patients had a NAFLD-score above -0.640, indicative of NAFLD, in the RYGB (90%), diet (82%) and control groups (11%).

3.2 Changes in Body Weight

The initial 3-week LED resulted in a mean weight loss of $5 \pm 2\%$ and $5 \pm 2\%$ in the RYGB and diet group, respectively. Total weight loss after the additional 6 weeks of VLED induced by RYGB or strict diet was $13 \pm 3\%$ and $11 \pm 4\%$, respectively (Fig. 1). At Year 2, the RYGB group demonstrated a total weight loss of $29 \pm 9\%$ from baseline. In contrast, several patients in the diet group had regained body weight at Year 2, resulting in a mean total weight loss from baseline of $3 \pm 6\%$ (Fig. 1). The hsCRP and predicted liver fat were similar between the RYGB and diet groups at Weeks 0, 3, and 9, but lower in the RYGB group at Year 2 (Fig. 1).

3.3 Baseline Rosuvastatin Pharmacokinetics, Pharmacogenetics, and Proteomics

Cross-sectional comparisons of rosuvastatin pharmacokinetics in patients with severe obesity compared with the control group revealed a 0.9 [95% CI 0.4, 1.4] hour shorter $T_{\max}$ in the normal-to-overweight individuals, with no differences

Table 1 Demographic overview of patients at baseline

	RYGB ($n = 40$)	Diet ($n = 40$)	Controls ($n = 18$)
Male/female (n)	13/27	14/26	3/15
Age (years)	46 ± 9	49 ± 10	42 ± 15
Weight (kg)	132 ± 24	124 ± 23	71 ± 11
BMI (kg/m^2)	45 ± 6	42 ± 5	25 ± 3
hsCRP (mg/L)	8.2 ± 6.2	7.1 ± 6.6	2.5 ± 3.8
ALT (IU/L)	34 ± 17	32 ± 19	22 ± 15
AST (IU/L)	28 ± 10	28 ± 15	25 ± 11
Creatinine (μmol/L)	58 ± 11	59 ± 14	60 ± 12
Ethnicity (Caucasian/other)	40 / 0	39/1	17/1
SLCO1B1 c.521			
T/T | T/C | C/C	27 | 12 | 1	25 | 14 | 1	13 | 4 | 0[a]

Values are presented as mean ($\pm$ standard deviation) or count

ALT alanine aminotransferase, *AST* aspartate aminotransferase, *BMI* body mass index, *hsCRP* high-sensitive C-reactive protein, *RYGB* Roux-en-Y gastric bypass

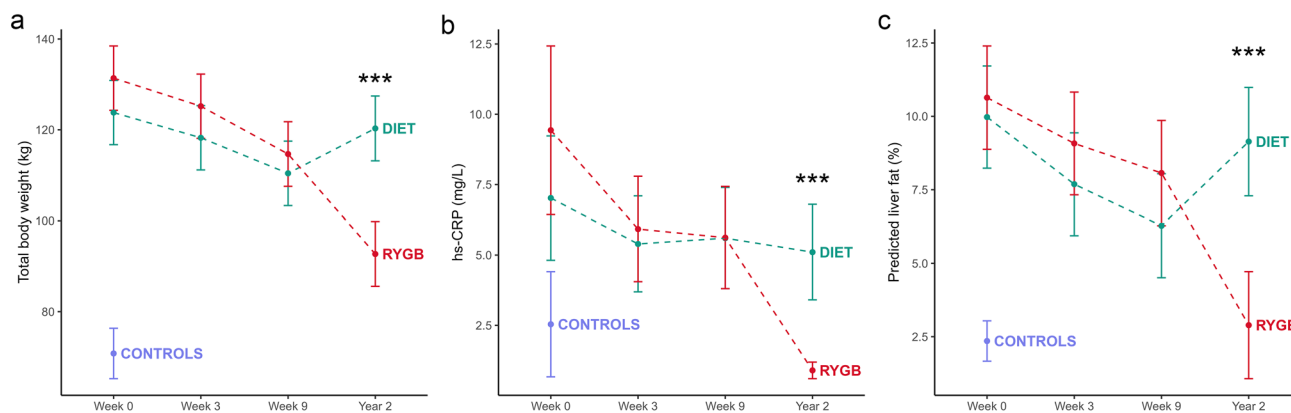[a]For one individual in the control group, genotype could not be determined

Fig. 1 Groupwise longitudinal overview of change in **a** total body weight, **b** high-sensitive C-reactive protein (hsCRP) and **c** predicted liver fat percentage. For the Roux-en-Y gastric bypass (RYGB) and diet groups, linear mixed model predicted marginal mean and 95% confidence intervals are presented. For the normal-to-overweight con- trol group, observed mean and 95% confidence interval is presented. Comparisons are made between the RYGB and diet groups at each visit, and significant differences are denoted with asterisks. Non-significant differences are not shown *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$

in C_{max} or oral clearance (Fig. 2). At baseline, total body weight was not associated with oral clearance ($r = 0.17$ [95% CI − 0.03, 0.36]). No difference in rosuvastatin oral clearance was observed in patients with NAFLD ($n = 69$) compared with those without ($n = 27$), and hsCRP was not associated with oral clearance of rosuvastatin ($r = − 0.13$ [95% CI − 0.32, 0.07]).

The frequency distribution of *SLCO1B1* genetic variants is presented in Table 1, and there were no differences in frequency distribution between the sexes ($p = 0.0867$). Frequency distribution for the *ABCG2* variants are presented in Table S1. Allele frequencies for all sequence variants investigated did not deviate from the Hardy–Weinberg equilibrium. At baseline, patients with *SLCO1B1* variant c.521TC ($n = 30$) or c.521CC ($n = 2$) demonstrated

30% ($p < 0.001$) lower oral clearance compared to those with c.521TT. Absolute differences in rosuvastatin oral clearance and systemic exposure within the genetic variants of *SLCO1B1* at baseline are presented in Supplementary Tables S2 and S3, respectively.

In the patients undergoing RYGB, hepatic concentration of OATP1B1 was positively associated with oral clearance the day before surgery ($r = 0.36$ [95% CI 0.04, 0.61]), but this was not the case for the control group ($r = − 0.09$ [95% CI − 0.54, 0.39]) (Fig. S5). Furthermore, there were no differences in mean hepatic concentration of OATP1B1 between the RYGB (2.7 ± 0.9 fmol/μg) and control groups (2.6 ± 0.9 fmol/μg; $p = 0.630$), nor were there any differences between individuals in the combined RYGB and control groups with *SLCO1B1* variant c.521TT (2.8 ± 0.9 fmol/μg) or c.521TC/CC (2.5 ± 0.7 fmol/μg).
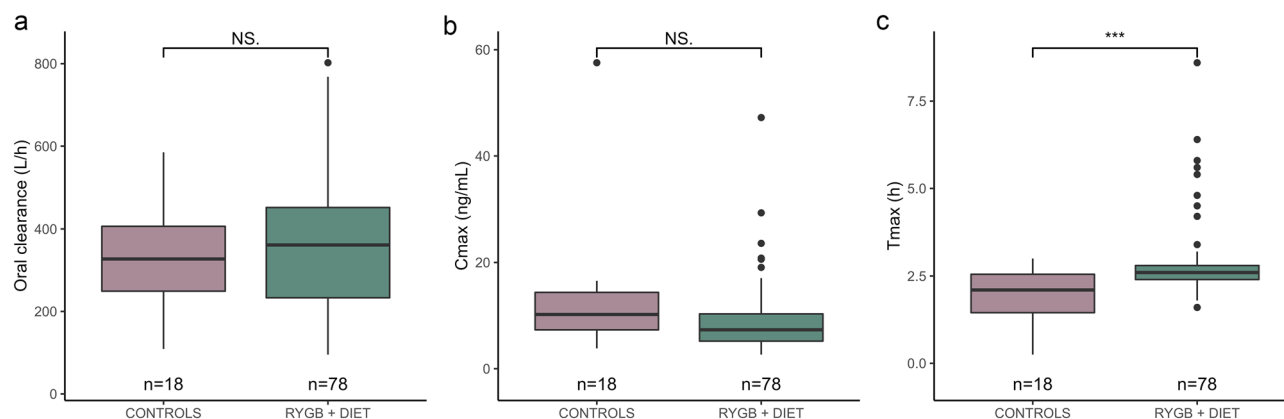

Fig. 2 Comparisons of rosuvastatin **a** oral clearance, **b** maximum concentration (C_{max}) and **c** time to maximum concentration (T_{max}) between patients with severe obesity and a normal- to overweight control group at baseline *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$

3.4 Short- and Long-Term Changes in Rosuvastatin Pharmacokinetics Following RYGB and Strict Diet

A total of 57 patients (RYGB = 29, diet = 28) participated in all four pharmacokinetic investigations. Mean predicted pharmacokinetic profiles of rosuvastatin are shown in supplementary Figure S6, and an overview of pharmacokinetic parameters for all groups across time is provided in Table 2. There were no differences in pharmacokinetic parameters of rosuvastatin between the two intervention groups at baseline (Table 3).

Following the initial 3-week LED, oral clearance decreased by 16% [95% CI 0, 31) and 23% [95% CI 8, 38] in the RYGB and diet groups, respectively (Table 3), and the change was not different between the groups (Fig. 3). Furthermore, no changes in C_{max} or T_{max} were observed (Table 3). In the RYGB and diet groups combined, the change in oral clearance was positively associated with the weight loss ($r = 0.25$ [95% CI 0.03, 0.45]) in the same period, but not associated with change in liver fat ($r = 0.05$ [95% CI − 0.18, 0.27]).

In the RYGB group, a more rapid rosuvastatin absorption, reflected by a 0.9 [95% CI 0.4, 1.7] hour reduction in T_{max} was observed at Week 9, compared with baseline (Table 3). There were no additional changes in oral clearance or C_{max} in either the RYGB or diet groups from Week 3 to Week 9. Still, the within group change in oral clearance during this time period was different between the two groups (Fig. 3).

At Year 2, the RYGB group demonstrated a total increase in oral clearance of 21% [95% CI 1, 41] and 57% [95% CI 31, 83] compared to baseline and Week 9, respectively. The change in oral clearance from Week 9 to Year 2 was 8-fold greater in the RYGB- compared with the diet group (Fig. 3). At Year 2, oral clearance of rosuvastatin was 22% (95% CI 0, 43) higher in the RYGB group than in the diet group (Table 3). Long-term change in oral clearance from baseline to Year 2 was not associated with change in hsCRP (RYGB: $r = 0.13$ [95% CI − 0.24, 0.46]; diet: $r = − 0.13$ [95% CI − 0.46, 0.25]) or estimated liver fat (RYGB: $r = 0.16$ [95% CI − 0.21, 0.48]; diet: $r = 0.28$ [95% CI − 0.09, 0.57]) in either group. The absorption was still faster at Year 2 in the RYGB group; T_{max} was reduced by 1.1 [95% CI 0.4, 1.9] hour(s) compared with baseline (Table 3), and was 0.8 [95% CI 0.2, 1.4] hour(s) shorter in the RYGB group compared with the diet group. At Year 2, C_{max} in the RYGB group was 27% [95% CI 3, 51] lower compared with Week 9, but not different from C_{max} at baseline or when compared with the diet group at Year 2. The diet group showed no difference from baseline in rosuvastatin pharmacokinetics at Year 2.

Neither short- nor long-term change in oral clearance was different in patients with the reduced-function variant (c.521TC or c.521CC) of *SLCO1B1* from those with the homozygote wild-type variant (c.521TT) in either group (Fig. 4). While a statistically significant difference in change in oral clearance between individuals with the c.521TT and c.521TT/CC variants were found in the diet group only during LED, no differences were found in the combined RYGB and diet groups in the same time period.

4 Discussion

To the best of our knowledge, this is the first longitudinal, prospective pharmacokinetic study in patients with severe obesity, evaluating the effect of RYGB and strict diet on the activity of the hepatic uptake transporter OATP1B1, using rosuvastatin as a probe drug. The main finding was that neither body weight nor weight loss, induced by either RYGB or strict diet, seemed to substantially influence the activity of the hepatic uptake transporter OATP1B1. There were no systematic differences in short- or long-term change in oral clearance between patients with the reduced function variant of *SLCO1B1* or wildtype, providing the main evidence for unchanged OATP1B1 activity following RYGB and weight loss observed in the present study. Also, no statistically significant differences in rosuvastatin pharmacokinetics were shown between patients with severe obesity and the

Table 2 Summary of observed rosuvastatin pharmacokinetic parameters over time across groups

	RYGB				Diet				Controls
	Week 0 (n = 38)	Week 3 (n = 39)	Week 9 (n = 35)	Year 2 (n = 32)	Week 0 (n = 40)	Week 3 (n = 39)	Week 9 (n = 37)	Year 2 (n = 30)	Week 0 (n = 18)
Oral clearance (L/h)	374 ± 23	336 ± 23	306 ± 20	469 ± 18	360 ± 23	277 ± 22	329 ± 22	362 ± 27	337 ± 11
C_{max} (ng/mL)	9.1 ± 8.3	10.2 ± 8.3	14.1 ± 15.5	9.1 ± 9.1	9.0 ± 5.0	10.7 ± 6.6	9.5 ± 5.4	11.0 ± 8.3	12.9 ± 11.8
T_{max} (h)	3.0 ± 1.4	2.5 ± 0.8	2.3 ± 1.1	2.2 ± 1.5	2.7 ± 0.7	2.7 ± 0.9	2.6 ± 1.0	2.7 ± 0.8	2.0 ± 0.7

Values are presented as mean ± standard deviation, and from the final population pharmacokinetic model

C_{max} maximum concentration, *RYGB* Roux-en-Y gastric bypass, T_{max} time at maximum concentration

Table 3 Differences in rosuvastatin pharmacokinetics within groups over time, and differences between groups at each visit

	RYGB					Diet					Difference between groups			
	Week 0–Week 3	Week 0–Week 9	Week 0–Year 2	Week 3–Week 9	Week 9–Year 2	Week 0–Week 3	Week 0–Week 9	Week 0–Year 2	Week 3–Week 9	Week 9–Year 2	Week 0	Week 3	Week 9	Year 2
Oral clearance (L/h)	−60* [−119, 0]	−87* [−147, −27]	80* [3, 157]	−28 [−80, 25]	167* [91, 243]	−85* [−142, −28]	−37 [−96, 23]	−16 [−81, 50]	48 [−5, 102]	21 [−42, 85]	5 [−81, 91]	30 [−39, 100]	−45 [−119, 28]	101* [2, 200]
C_{max} (ng/mL)	1.1 [−1.3, 3.4]	2.3 [−0.3, 5.0]	−0.7 [−3, 1.5]	1.3 [−1.5, 4.0]	−3.0 [−5.8, −0.3]	1.9 [−0.7, 4.5]	0.7 [−1.8, 3.1]	0.7 [−1.9, 3.4]	−1.2 [−4.0, 1.5]	0.0 [−2.7, 2.8]	−0.6 [−3.3, 2.1]	−1.4 [−4.5, 1.7]	1.1 [−2.1, 4.3]	−2.0 [−4.9, 0.9]
T_{max} (h)	−0.6 [−1.4, 0.1]	−0.9* [−1.7, −0.4]	−1.1* [−1.9, −0.4]	−0.3 [−0.9, 0.3]	−0.2 [−0.8, 0.4]	−0.1 [−0.8, 0.7]	−0.3 [−1.0, 0.5]	−0.1 [−0.9, 0.7]	−0.2 [−0.9, 0.5]	0.1 [−0.6, 0.9]	0.2 [−0.4, 0.9]	−0.4 [−1.0, 0.2]	−0.5 [−1.0, 0.1]	−0.8* [−1.4, −0.2]

Values are presented as mean [95% confidence interval] and are obtained from linear mixed model estimated marginal means. Difference between groups is calculated with the RYGB group as reference (RYGB–DIET). Values in bold marked with * are statistically significant to $p < 0.05$

C_{max} maximum observed concentration, *RYGB* Roux-en-Y gastric bypass, T_{max} time at maximum concentration

normal-to-overweight control group at baseline, suggesting no effect of body weight per se on OATP1B1 activity or rosuvastatin pharmacokinetics. This is supported by similar findings using a semi-mechanistic proteomics-informed model in a subset of the same patients [27].

Given that the changes in rosuvastatin oral clearance were also present in patients with genetically decreased OATP1B1 transport ability, it seems plausible that physiological alterations associated with caloric restriction and/or RYGB induced the observed alterations in oral clearance in the present study. Following the 3-week LED with similar weight loss, oral clearance decreased by approximately 20% in both intervention groups. It is known that caloric restriction may affect both liver size and composition. In a systematic review of LEDs (800–1200 kcal/day) prior to bariatric surgery, liver size was found to be reduced by 12–27%, and approximately 80% of total reduction in liver size was described to occur in the initial two weeks of diet [35]. As rosuvastatin displays a high hepatic extraction ratio (0.63), clearance and bioavailability may be susceptible to changes in both portal and hepatic blood flow [14]. It is possible that hepatic blood flow could be altered following rapid reduction in liver size, as hepatic blood flow has been found to increase following bariatric surgery [36, 37]. A transient increase in portal vein blood flow could thus lead to an increased bioavailability of rosuvastatin, possibly explaining the reduced oral clearance following the initial LED. However, this hypothesis will require further investigation into hepatic haemodynamics following diet-induced weight loss. During the initial LED, change in oral clearance was not associated with change in predicted liver fat content, suggesting that liver composition may be less important with regard to rosuvastatin disposition. Additionally, oral clearance of rosuvastatin was not different in patients with NAFLD-score above the diagnostic cut-off value compared with those with normal scores. This agrees with findings from Tirona and colleagues, who reported no differences in rosuvastatin pharmacokinetics in patients with obesity with magnetic resonance imaging (MRI)-confirmed NAFLD compared to a control group [38]. Furthermore, RYGB or 6 weeks of additional VLED did not induce additional changes in oral clearance despite further reduction in body weight, which indicates that weight loss is not the sole explanation for the observed effects. Nonetheless, a statistically significant between-group difference in the change in oral clearance following RYGB or six weeks of additional VLED was found. However, considering that no significant changes within either group were observed in this period, the observed between-group difference was considered to be less important in the overall interpretation of the results.

At Year 2, no changes in rosuvastatin pharmacokinetics from baseline were observed in the diet group, while the RYGB group demonstrated a net increase in oral clearance
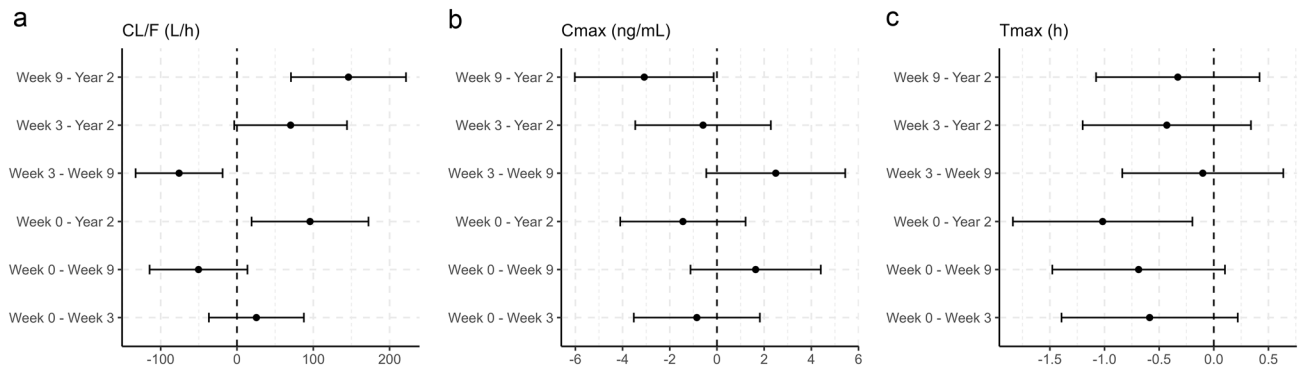
Fig. 3 Between-group differences in within-group change for the Roux-en-Y gastric bypass (RYGB) and diet groups for **a** oral clearance (CL/F), **b** maximum concentration (C_{max}) and **c** time to maximum concentration (T_{max}). Difference between groups are made with the RYGB group as reference (positive values indicate greater change in the RYGB group), and are presented as marginal mean with 95% confidence interval
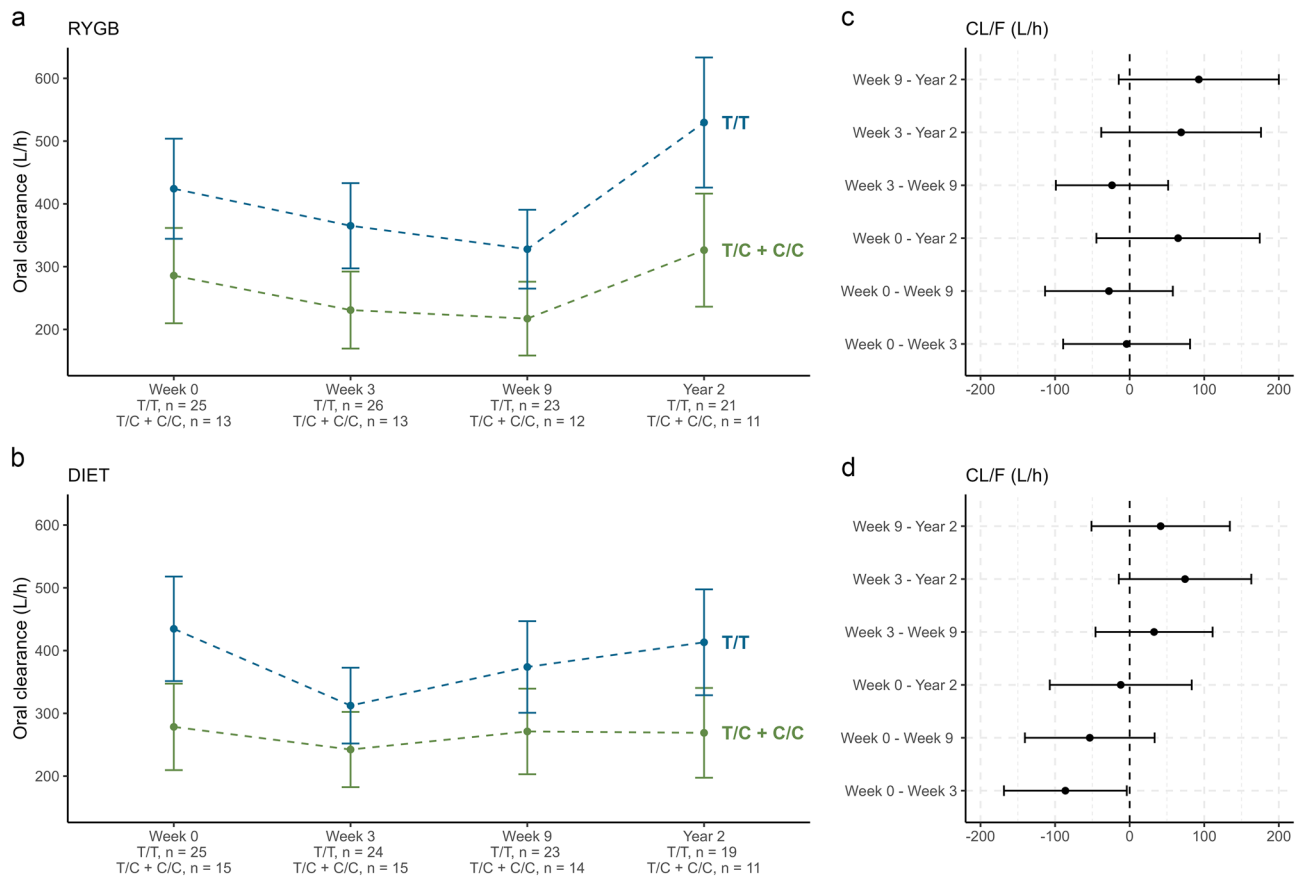

Fig. 4 Longitudinal overview of linear mixed effects model predicted oral clearance for individuals expressing the reduced function variant *SLCO1B1* c.521CC and TC compared with c.521TT in the **a** Roux-en-Y gastric bypass (RYGB) and **b** diet groups, respectively. Data are presented as marginal mean with 95% confidence interval. The between-variant difference in within-variant change for the individuals with *SLCO1B1* c.521TT or TC genotype compared with c.521TT are shown for the **c** RYGB and **d** diet group, presented as marginal mean with 95% confidence interval, with c.521TT as the reference group (positive values indicate greater change in patients with c.521TT)

of approximately 21%. This increase in oral clearance long term seems to be a surgery-specific effect on rosuvastatin absorption, leading to a net reduced bioavailability that may not have been readily apparent or sufficiently developed six weeks post-surgery. Given the anatomical and physiological changes in the gastrointestinal tract following RYGB, and the fact that rosuvastatin is moderately absorbed (~ 50%) [39] and displays a complex, dissolution rate-limited absorption (BCS class II), a reduction in the absorption and thus oral bioavailability after surgery is not unexpected. Surprisingly, this effect was not observed shortly after surgery. Moreover, it cannot be ruled out that other surgery-specific effects, such as alteration in incretins, expression/activity of other drug transporters such as NTCP or gut microbiota, may have contributed to the observed change in oral clearance in the long term. To date, only one study has evaluated the effect of RYGB on rosuvastatin pharmacokinetics. However, with only one dose- and weight-normalised blood sample, the data were not sufficient to draw conclusions regarding the effect of RYGB on the pharmacokinetics of rosuvastatin [40].

At the time of surgery, hepatic expression of OATP1B1 was positively associated with oral clearance in the RYGB group, but no such associations were shown in the control group, despite similar mean hepatic expression of OATP1B1 in the two groups. In contrast to the controls, the RYGB group was subjected to an initial LED, and thus a subsequent weight loss prior to surgery in the present study. This may potentially explain some of these differences, as rosuvastatin oral clearance was already reduced in the RYGB group when biopsies for protein expression measurement were obtained.

A strength of the present study was that oral clearance of rosuvastatin was calculated using a precise measurement of $AUC_{0-\infty}$, obtained from extensive blood-sampling in combination with an internally validated population pharmacokinetic model. Additionally, by including a dietary control group with matched short-term weight loss, we were able to differentiate the effects of surgery and weight loss on rosuvastatin pharmacokinetics. However, it is also important to recognise the limitations of the presented work. Most importantly, without concomitant intravenous administration it is not possible to determine absolute bioavailability of rosuvastatin, which is likely to change following RYGB. As such, true clearance could provide additional mechanistic understanding of the underlying processes. Another weakness was that NAFLD was not confirmed with biopsy or MRI, but predicted based on metabolic factors. It is also important to recognise that rosuvastatin has a reported half-life of 20 h [14], but sampling was only performed up to 24 h. As such, population pharmacokinetic modelling was used for parameter estimation over non-compartmental methods, as the former is not as reliant on rich sampling in the elimination phase. While additional samples may have improved the accuracy of parameter estimation, we could not justify the added patient inconvenience and logistical challenges for this probe drug.

5 Conclusions

In conclusion, neither body weight, weight loss, nor RYGB per se seem to affect OATP1B1 activity. This is primarily substantiated by the fact that individuals with the reduced function variant of *SLCO1B1* showed similar change in oral clearance of rosuvastatin over time. The short-term decrease in oral clearance following LED is reversible, as indicated by the return to baseline values following regained weight in the diet group while the long-term increase in oral clearance observed in the RYGB group appears to be due to a surgery-specific alteration in rosuvastatin disposition leading to an increased oral clearance. Overall, the observed changes in rosuvastatin pharmacokinetics in the present study were small, and dose adjustments of rosuvastatin following RYGB- or diet-induced weight loss appears to be unnecessary.

Declarations

Data availability statement Access to data collected from this study, including anonymised individual participant data, may potentially be made available following publication on e-mail request to the corresponding author. After approval of a proposal, data will be shared with investigators whose proposed use of the data has been approved by the COCKTAIL steering committee, according to the consent given by the participants and Norwegian laws and legislations.

Ethics approval The study was approved by the Regional Committee for Medical and Health Research Ethics (2013/2379/REK).

Consent to participate Written informed consent was obtained from all patients prior to any study-specific procedures.

Consent for publication Not applicable.

Code availability Not applicable.

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