Laparoscopic Roux-en-Y gastric bypass surgery influenced pharmacokinetics of several drugs given as a cocktail with the highest impact observed for CYP1A2, CYP2C8 and CYP2E1 substrates

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ABSTRACT

There is a lack of information about the changes in drug pharmacokinetics and cytochrome P450 (CYP) metabolism after bariatric surgery. Here, we investigated the effects of laparoscopic Roux-en-Y gastric bypass (LRYGB) surgery on pharmacokinetics of nine drugs given simultaneously which may reveal changes in the activities of the main CYPs. Eight obese subjects undergoing LRYGB received an oral cocktail containing nine drugs, substrates of various CYPs: melatonin (CYP1A2), nicotine (CYP2A6), bupropion (CYP2B6), repaglinide (CYP2C8), losartan (CYP2C9), omeprazole (CYP2C19/CYP3A4), dextromethorphan (CYP2D6), chlorzoxazone (CYP2E1), midazolam (CYP3A). The 6-hr pharmacokinetic profiles in serum and urine of each drug or corresponding metabolite as well as their metabolic ratios were compared before surgery with those at a median one year later. LRYGB exerted variable effects on the pharmacokinetics of these drugs. The geometric mean $AUC_{0\text{-}6}$ (90% confidence interval) of melatonin, bupropion, repaglinide, chlorzoxazone and midazolam after LRYGB were 27 (19-41)%, 54 (43-67)%, 44 (29-66)%, 160 (129-197)% and 74(62-90)% of the pre-surgery values, respectively. The pharmacokinetics of losartan, omeprazole and dextromethorphan did not change in response to surgery. Nicotine was not detected in serum, while geometric mean of $AUC_{0\text{-}6}$ of its metabolite, cotinine, increased by 1.7 times after surgery. There were 3.6- and 1.3-fold increases in the AUC ratios of 6-hydroxymelatonin/melatonin and hydroxybupropion/bupropion, respectively. The cocktail revealed multiple pharmacokinetic changes occurring after LRYGB with the greatest effects observed for CYP1A2, CYP2C8 and CYP2E1 substrates. Future studies should be focused on CYP1A2, CYP2A6, CYP2C8 and CYP2B6 to clarify the changes in activities of these enzymes after LRYGB.

INTRODUCTION AND BACKGROUND

The prevalence of obesity is increasing, with estimates of more than 1.9 billion overweight or obese people in the world [1]. Obesity is associated with a high mortality and comorbidity rate from conditions such as hypertension, type 2 diabetes mellitus, cardiovascular disease, non-alcoholic fatty liver disease, asthma and cancers [2]. Long-term drug treatment is often needed, and obese patients are provided with a significantly higher number of drug prescriptions than the normal-weight population.

Currently, bariatric surgery is considered as the most effective treatment for morbid obesity [3]. The Roux-en-Y gastric bypass (RYGB) remains the gold standard and one of the most commonly performed bariatric surgeries along with sleeve gastrectomy and gastric banding [4, 5]. Moreover, the laparoscopic RYGB (LRYGB) has demonstrated significant advantages

over laparoscopic gastric banding in terms of weight loss and a reduction in comorbidities after surgery [6].

LRYGB involves connecting an approximately 15–30 mL-stomach pouch to the jejunum, bypassing the stomach and a large portion of the proximal small intestine. As a result, the anatomical and physiological changes after LRYGB may affect post-operative drug pharmacokinetics which may cause adverse effects or alter their responses in patients thereby requiring dose adjustment in these patients [7]. For instance, the decreased gastric volume leads to an increase in the stomach pH (4-6) and a modified gastric emptying time that can result in an altered rate and extent of oral absorption [8]. Bypassing of the duodenum contributes to a reduction in the absorption surface and can modify intestinal transport and first-pass metabolism mediated by intestinal CYP enzymes [9]. Finally, the loss of body weight can lead to a normalization of the liver fat content, improved hepatic insulin sensitivity and reduced low-grade inflammation which may alter hepatic clearance and change the distribution of drugs [10, 11].

The previously published studies have revealed reduced bioavailability in around 46% of investigated drugs and increased bioavailability in 23% of drugs after RYGB [7, 12]. The majority of the studies have been investigations of one or two individual drugs [9, 13]. Fewer studies have investigated RYGB-associated changes in activities of intestinal/hepatic CYP enzymes [13, 14]. Tandra *et al.* assessed the effect of RYGB on the activity of four enzymes (CYP1A2, CYP2C9, CYP2C19, CYP3A4) using a cocktail approach by comparing a group of RYGB patients with healthy control subjects [15]. As CYP enzyme activity may vary significantly between subjects, the investigation of the effects of RYGB on CYP-mediated elimination in the same patients before and after the surgery can eliminate inter-individual variability. Moreover, there is a lack of knowledge about the impact of RYGB on the activities of other important CYP enzymes [16].

In the present study, we investigated the impact of LRYGB on the pharmacokinetics of a cocktail of nine drugs [17, 18] that are substrates of the main CYP enzymes. The changes in plasma and urinary pharmacokinetic profiles and metabolic ratios of drugs were assessed in the same subjects before and one year after surgery. This is the first extensive study investigating the impact of LRYGB on the drug pharmacokinetics utilizing a cocktail approach, where each patient served as his/her own control.

METHODS

Study Design and Population

The study was conducted in accordance with the Basic & Clinical Pharmacology & Toxicology policy for experimental and clinical studies [19]. Eight patients were recruited into this non-randomized single-group study (NCT03563287) as a part of a clinical trial (Kuopio Obesity Surgery Study, KOBS) investigating metabolic effects of bariatric surgery in Kuopio University Hospital [20-22]. The recruitment period of the study was from January 2012 to November 2013. The inclusion criteria for the study were as follows: (1) age 18–60 years; (2) body mass index (BMI) > 40 $kg \cdot m^{-2}$ or (3) BMI 35–40 $kg \cdot m^{-2}$ and a comorbidity or its risk factor, such as type 2 diabetes, hypertension, sleep apnea, osteoarthritis of weight bearing joints or polycystic ovarian syndrome; (4) previous conservative treatment for obesity had been proven to be ineffective; (5) patients were assigned to undergo LRYGB according to the standard protocol [7]. The selection of patients for the surgery was in line with European guidelines [23]. The nature and potential risks of the study were explained to all subjects. Written informed consent was obtained from all individual participants included in the study before embarking on any study procedures. Only non-smoking individuals consuming alcohol <20 g per day were included in the study. All subjects were interviewed about their disease histories and their current drug treatment. Subjects were excluded if they were taking medications known to change CYP activity or had a history of hypersensitivity to the drugs used in the cocktail. Study participants were instructed not to consume ethanol within 48 hr and to avoid exercise and caffeine use for 24 hr before the visits for the pharmacokinetic study. Medications were not allowed on the morning of these visits. The first pharmacokinetic study visit was conducted at a median 20 days (range 6 – 46 days) before the surgery. The second pharmacokinetic study visit occurred at a median 354 days (range 261 – 412 days) after LRYGB, when both significant and stable weight loss had been achieved.

The analysis of the additional reference group consisting of healthy subjects (age 18-60 years, BMI 18-25 kg/m^2, non-smokers) are described in Supporting information Methods.

Ethical approval

The study protocol was approved by the Ethics Committee of the Northern Savo Hospital District (27/2010). All procedures performed 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.

Cocktail administration and pharmacokinetic study

During study visits 1 and 2, the oral cocktail of drugs was administered to the patients at the doses mentioned below. The selection of drugs used in the cocktail was based on their previous use as CYP probe drugs (Table S1). However, their interaction potential in the present cocktail has not been fully investigated. To avoid the adverse effects, the doses of probe drugs, except for melatonin and dextromethorphan, were at least halved in comparison to marketed doses. The cocktail was given as two equally loaded cellulose capsules. The cocktail was compounded in a local hospital pharmacy from crushed tablets and consisted of 2 mg melatonin (prolonged-release tablet, RAD Neurim Pharmaceuticals EEC Limited, UK), 1 mg nicotine (tablet, McNeil Products Limited, UK), 37.5 mg bupropion hydrochloride (tablet, GlaxoSmithKline UK), 0.25 mg repaglinide (tablet, Novo Nordisk A/S, Denmark), 12.5 mg losartan potassium (tablet, Merck Sharp & Dohme Limited, UK), 10 mg omeprazole (gastro-resistant tablet, AstraZeneca UK Limited), 62.5 mg chlorzoxazone (tablet, BioPhausia, Sweden), 1.85 mg midazolam (tablets, Roche Oy). Dextromethorphan hydrobromide 30 mg (oral solution, Orion Pharma, Finland) was administered separately as an oral solution directly after the capsules. Venous blood samples (5 mL) were taken into non-heparinized tubes while urine samples were collected into 50 mL Falcon tubes at the following time points: at baseline, 1, 2, 4 and 6 hr after cocktail administration. Blood samples were left undisturbed for 30 min. at room temperature and centrifuged at 15000 × *g* for 20 min. The supernatant was used as a serum sample. Urine and serum samples were frozen at −80°C until subsequent analysis.

Sample analysis and pharmacokinetic parameters

The concentrations of probe drugs and their corresponding metabolites (Table S1) were measured in serum and urine samples by a recently developed and validated liquid-chromatography tandem mass-spectrometric method [17]. Pharmacokinetic parameters in serum - area under the serum concentration – time curve from 0 to 6 hr post dose ($AUC_{0\text{-}6}$), maximum serum concentration (C_{max}), time to reach C_{max} (t_{max}), the apparent volume of distribution at the terminal phase to bioavailability per body weight (Vd/F/BW), oral clearance per body weight (CL/F/BW) were calculated for each drug using non-compartmental analysis with Phoenix WinNonlin software (version 6.3, Certara, USA). The validated CYP phenotypic metrics such as urinary excretion ratio losartan/E3174 (CYP2C9) or metabolic ratio of metabolite to parent drug ($AUC_{0\text{-}6\ metabolite}$ / $AUC_{0\text{-}6\ parent\ drug}$) were used to assess CYP activity. The information about phenotypic metric is presented in Table S1. The percentage of dose excreted into urine as a parent drug, the corresponding metabolite and the sum of both as well as urinary excretion ratio (parent drug/metabolite) were calculated. A

validated phenotypic metric was not available for the CYP2C8 probe repaglinide, therefore repaglinide metabolites were not quantified.

Data and statistical analyses

Paired analyses between preoperative and postoperative periods were conducted for AUC_{0-6}, C_{max} and phenotypic metrics with the bioequivalence module of Phoenix WinNonlin using the study period as a fixed effect (preoperative as reference) and subject as a random effect. The ratio of geometric means as a percentage and its 90% confidence interval (CI) are reported. The study periods were considered equivalent when the 90% CI included 100% and non-equivalent otherwise. The primary outcome measure was AUC_{0-6} of the parent drug since AUC (extrapolated to infinity) is inversely related to oral clearance (Cl/F) that will determine the average steady-state concentration of the drug in the serum, and thereby indicate whether the dose needs to be changed in multiple dosing to keep the same total drug exposure.

The statistical comparison of urinary excretion data, the comparison between healthy volunteers and obese patients before surgery are described in Supporting information Methods.

RESULTS

Study population

Eight obese patients (2 men and 6 women) from the KOBS prospective cohort were enrolled into the present pharmacokinetic study. The demographic data of the subjects are presented in Table 1. Hepatic histological characteristics (as described in Supporting information Methods) are presented in Table S2. None of the subjects had liver cirrhosis and none were using drugs which could interact with the cocktail drugs. One recruited patient considered as a possible smoker or being on nicotine substitution therapy was excluded from the analysis of nicotine, melatonin, bupropion, chlorzoxazone, omeprazole and midazolam pharmacokinetics, as these can be affected by nicotine consumption [24, 25].

The characteristics and results of the pharmacokinetic study of the reference group which consisted of six healthy volunteers are presented in Supporting information Results and Table S3.

Pharmacokinetic study

Parent drugs

LRYGB exerted a variable impact on the pharmacokinetic parameters of the parent drugs (Table 2, Fig. 1). The geometric mean AUC_{0-6} of melatonin, bupropion, repaglinide and midazolam reduced after LRYGB by 3.7-, 1.8-, 2.3- and 1.3-fold, respectively. The geometric mean C_{max} of melatonin, bupropion and repaglinide also decreased. The AUC_{0-6} and C_{max} of chlorzoxazone increased after LRYGB by 60% and 89%, respectively, whereas C_{max} of omeprazole increased by 68%. The AUC_{0-6} and C_{max} of losartan and dextromethorphan were equivalent in the preoperative and postoperative periods, as was the AUC_{0-6} of omeprazole.

Metabolites and phenotypic metrics

The geometric mean AUC_{0-6} of 5-hydroxyomeprazole and dextrorphan increased by 1.9- and 1.6-fold after LRYGB, respectively, while for cotinine demonstrated 1.7-fold increase in the geometric mean AUC_{0-6} (Table 2). The geometric mean C_{max} of these metabolites showed similar changes. In addition, the geometric mean C_{max} of 5-*O*-desmethylomeprazole increased 2 times after surgery. The serum concentration curves of the metabolites are shown in Fig.1. The phenotypic metrics for assessment of CYP1A2, CYP2B6, CYP2C9, CYP2D6, CYP3A activities were calculated (Table 3, Fig. 2). The major change observed in the phenotypic metrics was the 3.6-fold increase in 6-hydroxymelatonin/melatonin AUC ratio (CYP1A2) (Table 3). The ratio of hydroxybupropion/bupropion AUC_{0-6} increased by 32%. The changes in phenotypic metrics for assessment of activities of other enzymes showed high interindividual variation, which might partly result from the polymorphic nature of some CYPs (CYP2C9, CYP2C19, CYP2D6). While nicotine was not detected in patients' serum, the geometric mean of AUC of its main metabolite, cotinine, increased by 1.7-fold after surgery (from 28.8 h·ng/mL, range 19.6-61.4 h·ng/mL before LRYGB to 49.8 h·ng/mL, range 17.6-222 h·ng/mL after LRYGB).

Urinary excretion data

Urinary excretion data on the parent drugs and their metabolites are shown in Table S4 and Fig. S1. There were statistically significant changes in the 6-hr urinary excretion of chlorzoxazone and midazolam between the preoperative and postoperative periods, while the recovery of both parent drugs was less than 1%.

Healthy volunteers

The pharmacokinetic parameters of healthy volunteers were compared with the obese patients before surgery in Table S3 and Fig. 1. There were 3-fold lower AUC_{0-6} of omeprazole and 2.5-fold higher AUC_{0-6} of chlorzoxazone in the healthy volunteers in comparison to obese patients before surgery. Similar changes were observed in the corresponding C_{max} values.
The changes in CL/F/BW and Vd/F/BW are presented in Tables S3 and discussed in Supporting information Results.

Safety

A slight sedative pharmacological effect of midazolam was observed, while no other adverse events were reported.

DISCUSSION

In the present study, we used for the first time a cocktail of nine drugs for the simultaneous investigation of changes in their pharmacokinetics after LRYGB, which may reveal changes in CYP activities in each individual patient. Theoretically, the relative exposure of the patient to the parent drug and its metabolite is best characterized by the metabolite/parent drug AUC ratio. A single time point metabolite/parent drug concentration ratio can be used as a

surrogate if there are no changes in the onset and rate of absorption (no changes in the dissolution, permeation, and transit times), e.g., when studying the effect of genotype or phenotype on the hepatic metabolism of drugs by various CYP enzymes. However, LRYGB may cause major changes in a drug's absorption, leading to a time shift of the whole serum concentration curve. Therefore, we preferred to assess the metabolite/parent AUC_{0-6} ratio rather than utilizing a single time point metabolite/parent drug concentration ratio. We also determined 6-hr urinary excretion of most CYP probes and their major metabolites.

LRYGB had the largest effect on the pharmacokinetics of melatonin, which was considered as a CYP1A2 probe [26]. The geometric mean AUC_{0-6} of melatonin decreased by approximately 3-fold after LRYGB (Table 2), whereas AUC_{0-6} of 6-hydroxymelatonin remained constant (Fig. 1) and 6-hydroxymelatonin and melatonin AUC_{0-6} ratio increased by 3.5-fold (Table 3), respectively. Additionally, 6-hr urinary excretion of melatonin and its metabolite was very low and did not change after LRYGB (Table S4). These findings do not provide conclusive evidence of whether these changes are attributable to alterations in melatonin's absorption or increased CYP1A2 activity. Additional blood sampling times before 1 hr would have been very valuable. Tandra *et al.* demonstrated no change in CYP1A2 activity measured as the AUC molar ratio of paraxanthine and caffeine in patients after RYGB compared to BMI-matched volunteers [15]. Therefore, more extensive studies will be required to investigate changes in melatonin pharmacokinetics and CYP1A2 activity after LRYGB.

The effect of LRYGB on the pharmacokinetics of CYP2C8 substrates has not been previously studied. The second largest change in pharmacokinetics was observed for the antidiabetic drug, repaglinide which has been recommended as a CYP2C8 probe by the European Medicines Agency (EMA)[27]. Thus, the AUC_{0-6} of repaglinide decreased to approximately 44% after LRYGB. Metabolite data were not available for this probe, and 6-hr urinary excretion was low and did not show a significant change after LRYGB. Several studies in rodent models of obesity have demonstrated decreased expression and activity of several CYPs including CYP2C [28, 29]. Similarly, the decrease in CL/F/BW of repaglinide in obese patients compared to reference group was observed. Interestingly, changes in pharmacokinetic parameters (AUC_{0-6}, C_{max}, Cl/F/BW and Vd/F/BW) of repaglinide were "reversed" by surgery to be comparable to those in reference group (Table S3). These findings may be explained by a weight loss associated normalization of lipid levels and other adipokines and cytokines that might lead to a normalization of enzymes and transporters [11]. Additional studies with validated CYP2C8 probes are recommended to investigate the effect of LRYGB on CYP2C8 activity.

The AUC_{0-6} of chlorzoxazone, a validated CYP2E1 probe [30], increased by 60% after LRYGB, and additionally, AUC_{0-6} before surgery was approximately one half of that in healthy volunteers (Table S3). The results are consistent with other studies revealing a

decrease in hepatic CYP2E1 protein expression after bariatric surgery [31] and a weight-dependent induction of hepatic CYP2E1 activity in morbidly obese subjects followed by a decrease in the oral clearance of chlorzoxazone after another form of obesity surgery – gastroplasty [32, 33].

In our study, geometric mean AUC_{0-6} values of bupropion and midazolam, CYP2B6 and CYP3A probes [27], decreased to 54% and 74% after the surgery, respectively. There were slight increases in the phenotypic metrics of CYP2B6 (1.3-fold) and CYP3A (1.4-fold) after LRYGB. However, these changes in the pharmacokinetics of bupropion and midazolam are unlikely to be clinically significant. Recently, Brill *et al.* [14, 34] studied the pharmacokinetics of oral and i.v. midazolam in morbidly obese patients before and one year after weight loss surgery. They used a semi-physiologically based pharmacokinetic model to interpret their data and concluded that the intrinsic hepatic clearance of midazolam increased slightly after the surgery.

In our study, LRYGB did not affect AUC_{0-6} values of losartan, omeprazole and dextromethorphan. However, omeprazole AUC_{0-6} in healthy volunteers was approximately one third of that in obese patients. These results are most likely attributable to the fact that the gastro-resistant omeprazole tablets were crushed in our study, leading to the increased degradation of omeprazole in the more acidic gastric fluid of the healthy volunteers [35].
There was a significant increase in exposure to the nicotine metabolite, cotinine, after the intervention. However, as nicotine was not detected in serum samples, it is evident that additional studies will be required to investigate changes in CYP2A6 activity after LRYGB.
The study provided valuable information for the improvement of the cocktail methodology in the future by revealing some limitations. Palmer *et al.* [36] found that AUC of oral midazolam increased by 82% when the drug was administered in a cocktail containing 250 mg chlorzoxazone. Even though we used a significantly lower dose of chlorzoxazone (62.5 mg), an interaction is still possible, and the composition of our cocktail should be changed, or the validity of the lower dose should be investigated by studying midazolam alone and in a cocktail. Moreover, the validation of all cocktail probes and investigation of the interaction potential between components in order to assess specific enzyme activity is required [37]. One should remember that although probe drugs are primarily metabolized by the corresponding CYP, other enzymes can be also involved in biotransformation. In addition, metabolites are often serially metabolized themselves by the same or different enzymes. Therefore, the changes in the parent drug pharmacokinetics are more important. In future studies, genotyping of the subjects with respect to CYP2D6, CYP2C19 and CYP2C9 would be valuable since LRYGB (or any other intervention under evaluation) may have variable effects in poor, extensive and ultra-rapid metabolizers. Although it would be desirable, collecting blood and urine samples for longer than 6 hr to cover the elimination phase of drugs is not feasible for practical reasons such as the duration of the patient's visit to the clinic. Inclusion of additional early sampling time points (before 1 hr) would provide more information about the absorption phase and the total drug exposure. Generally, the clinical

utility of our approach and the observations of the present study will need to be confirmed in a larger population.

In conclusion, our extensive study provided new insights into the complex impact of LRYGB on the pharmacokinetics of nine probe drugs after their simultaneous administration in obese patients. The study revealed significantly reduced exposure to melatonin and repaglinide and increased exposure to chlorzoxazone. Moreover, the observed changes in the pharmacokinetics of CYP1A2, CYP2A6, CYP2C8 and CYP2B6 probes and their metabolites after LRYGB will have to be confirmed in future studies to allow the clinical applicability of the findings to be demonstrated. The devised cocktail approach, with the adjustments proposed above, can be a valuable tool for assessing changes in the pharmacokinetics and CYP-mediated metabolism attributable to some intervention.

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Table 1. Patient demographic and clinical characteristics

Characteristics	baseline	1 year after surgery
Age – yr.		
Mean	46.4 ± 9.5	
median (range)	46.7 (27 - 60)	
Female sex – no. (%)	6 (75)	
Smokers – no. (%)	7 (87)	
Type 2 diabetes mellitus (%)	3 (37)	
BMI (kg/m^2)	41.0 ± 4.9*	34.2 ± 5.5
Alimentary limb – cm		
median (range)	115 (110 – 120)	
Biliopancreatic limb – cm		
median (range)	60 (40 – 70)	
Fasting glucose (mmol·L^{-1})	6.39 ± 1.6	5.58 ± 0.7
Fasting insulin (mU·L^{-1})	17.1 ± 7.3*	11.3 ± 9.1
ALT (U·L^{-1})	22.3 ± 4.2	23.1 ± 8.4
Total cholesterol (mmol·L^{-1})	3.84 ± 0.4	4.34 ± 0.8
LDL cholesterol (mmol·L^{-1})	2.07 ± 0.6	2.37 ± 0.8
HDL cholesterol (mmol·L^{-1})	1.21 ± 0.4*	1.53 ± 0.3
Total triglycerides (mmol·L^{-1})	1.21 ± 0.4	1.47 ± 0.3

Statistical significance of differences in clinical parameters before and one year after surgery was tested by the Wilcoxon matched-pairs signed test, $P < 0.05$ (*). For mean values standard deviation provided

Table 2. Pharmacokinetic parameters for cocktail drugs and corresponding metabolites in serum of patients before and after LRYGB

Drug / dose (CYP)	Study day	n	AUC_{0-6}		C_{max}	
			Value (h·ng/mL)	vs before (%)	Value (ng/mL)	vs before (%)
Melatonin 2 mg	Before	7	7.93 (1.48-29.3)	-	3.74 (0.91-10.8)	-
(CYP1A2)	After	7	2.18 (0.31-7.07)	27 (19-41)	1.37 (0.18-4.64)	37 (23-59)
6-hydroxymelatonin	Before	7	1.12 (0.81-1.54)	-	0.33 (0.21-0.53)	-
	After	7	1.10 (0.94-1.28)	-	0.29 (0.22-0.39)	-
Nicotine	Before		-	-	-	-
(CYP2A6)[a]	After		-	-	-	-
Cotinine	Before	7	28.8 (20.2-41.2)	-	11.1 (7.90-15.5)	-
	After	7	49.8 (23.6-105)	-	18.9 (9.21-38.9)	-
Bupropion 37.5 mg	Before	7	67.9 (50.7-101)	-	21.0 (14.9-30.2)	-
(CYP2B6)	After	7	36.4 (20.3-76.9)	54 (43-67)	12.5 (8.03-22.1)	59 (44-79)
Hydroxybupropion	Before	7	247 (178-342)	-	62.2 (43.1-89.8)	-
	After	7	174 (106-285)	-	36.1 (21.8-59.8)	-
Repaglinide 0.25 mg	Before	8	5.58 (2.29-13.4)	-	2.77 (1.40-6.75)	-
(CYP2C8)[b]	After	8	2.43 (1.57-3.71)	44 (29-66)	1.39 (0.81-2.42)	50 (34-74)
Losartan 12.5 mg	Before	8	47.0 (13.5-86.2)	-	12.4 (4.09-29.5)	-
(CYP2C9)[c]	After	8	49.4 (24.9-88.9)	105 (82-136)	13.3 (7.12-24.3)	108 (87-133)
Omeprazole 10 mg	Before	7	228 (74.8-1740)	-	82.9 (31.2-551)	-
(CYP2C19/3A4)[c]	After	7	270 (149-976)	118 (87-160)	139 (69.6-235)	168 (124-227)
5-hydroxyomeprazole	Before	7	7.13 (3.45-14.8)	-	1.82 (1.06-3.15)	-
	After	7	13.7 (9.43-20.0)	-	6.13 (3.33-11.3)	-
5-*O*-desmethylomeprazole	Before	7	108 (60.7-191)	-	26.7 (16.1-54.5)	-
	After	7	158 (116-216)	-	54.9 (36.9-81.6)	-
Dextromethorphan 30 mg	Before	8	13.0 (4.97-68.6)	-	3.30 (0.06-9.92)	-
(CYP2D6)	After	8	13.9 (3.79-125)	107 (56-203)	3.74 (0.31-13.8)	113 (62-208)

Dextrorphan	Before	8	12.3 (3.05-49.5)	-	3.45 (0.78-15.1)	-
	After	8	20.6 (8.08-52.7)	-	5.88 (2.06-16.8)	-
Chlorzoxazone 62.5 mg	Before	7	1230 (960-2215)	-	412 (328-604)	-
(CYP2E1)[d]	After	7	1960 (1490-2359)	160 (129-197)	778 (510-1076)	189 (151-235)
Midazolam 1.85 mg	Before	7	20.3 (15.1-27.4)	-	6.76 (4.07-9.41)	-
(CYP3A)	After	7	15.1 (11.2-19.4)	74 (62-90)	5.42 (3.12-8.48)	80 (57-113)
1'-hydroxyomidazolam	Before	7	6.33 (4.51-8.89)	-	2.49 (1.47-4.23)	-
	After	7	6.76 (5.13-8.91)	-	2.91 (2.16-3.91)	-

Data are geometric mean (range of observed values) and the after surgery versus before surgery comparison for the parent drugs is the ratio of the geometric means as a percentage (90 % confidence interval)

[a] parameter was not calculated as compound was only detected in a limited number of samples

[b]repaglinide metabolites were not quantified due to lack of the validated phenotypic metric

[c]parameters for E3174 and omeprazole sulfone were not calculated since the sampling period was too short to capture the whole curves of these metabolites

[d]6-hydroxychlorzoxazone was not detected in serum samples

Table 3. Phenotypic metrics for CYP probes before and after LRYGB

CYP	Phenotypic metric	Study day	n	Value	vs before (%)
CYP1A2	AUC_{0-6} 6-hydroxymelatonin/	Before	7	0.14 (0.05-0.59)	-
	AUC_{0-6} melatonin	After	7	0.51 (0.14-3.06)	358 (233-549)
CYP2B6	AUC_{0-6} hydroxybupropion/	Before	7	3.63 (2.44-6.21)	-
	AUC_{0-6} bupropion	After	7	4.78 (2.79-8.77)	132 (108-161)
CYP2C9	6 h urinary excretion ratio	Before	6	2.08 (1.37-2.72)	-
	losartan/E3174	After	6	2.25 (1.23-4.45)	98.1 (56.5-170)
CYP2D6	AUC_{0-6} dextrorphan/	Before	8	0.945 (0.003-6.16)	-
	AUC_{0-6} dextromethorphan	After	8	1.489 (0.01-5.19)	158 (76-325)
CYP3A	AUC_{0-6} 1'-hydroxyomidazolam/	Before	7	0.312 (0.20-0.61)	-
	AUC_{0-6} midazolam	After	7	0.448 (0.36-0.48)	144 (105-196)

Data are geometric mean (range of observed values) and the after surgery versus before surgery comparison is the ratio of the geometric means as a percentage (90 % confidence interval)
The phenotypic metric for CYP2E1 was not calculated as the metabolite, 6-hydroxychlorzoxazone, was not detected in serum samples. The phenotypic metric for CYP2C8 was not calculated as there is no available validated phenotypic metric.
The phenotypic metrics for CYP2C19 and CYP3A4 measured by probe drug omeprazole and its metabolites were not calculated since the sampling period was too short to capture the whole curves of the metabolites

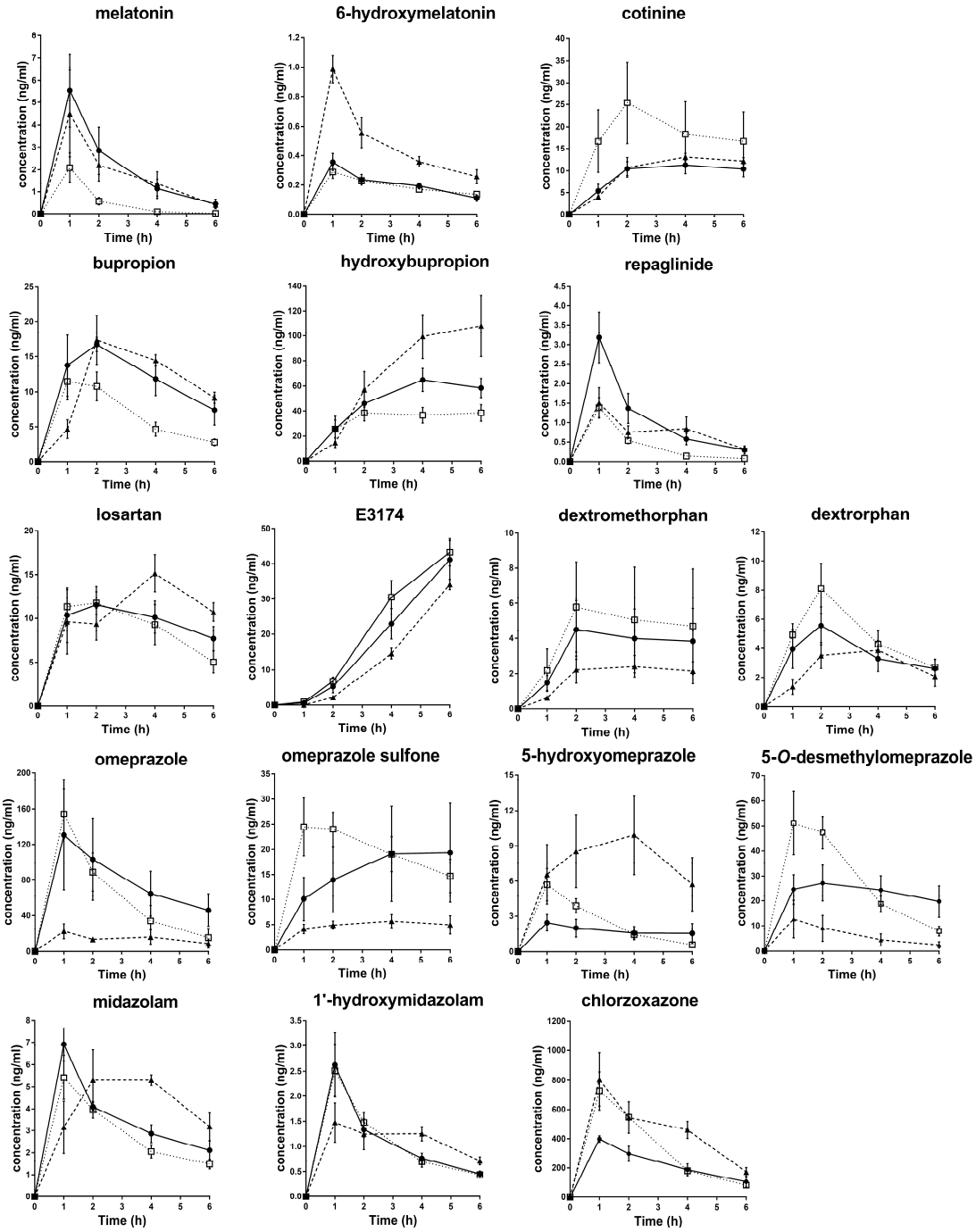

Fig. 1 Concentration–time profile of nine cocktail drugs and their corresponding metabolites in venous serum samples of patients (n = 8 for repaglinide, losartan, dextromethorphan and their metabolites) and (n = 7 for melatonin, bupropion, omeprazole, chlorzoxazone, midazolam and their metabolites including cotinine) before (●, solid line), after (□, dotted line) the surgery and healthy volunteers (▲, dashed line), n=6. Nicotine and 6-hydroxychlorzoxazone data are not presented. The data are presented as mean ± SEM at each time point.

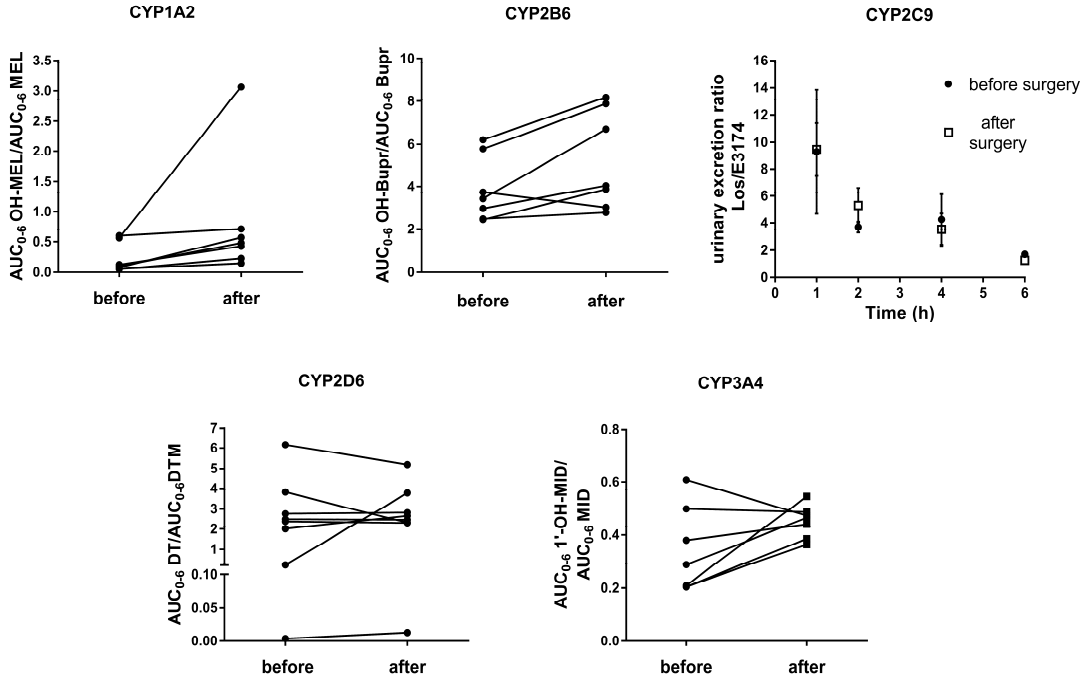

Fig. 2 Phenotyping of CYP enzymes before and after the surgery. The data presented individually (n = 8 for the assessment of CYP1A2, CYP2C9, CYP2D6 and n = 7 for the assessment of CYP2B6, CYP3A) or as mean ± SEM (losartan). MEL – melatonin, OH-MEL - 6-hydroxymelatonin, Bupr – bupropion, OH-Bupr – hydroxybupropion, Los – losartan, DT – dextromethorphan, DTM – dextrorphan, 1'-OH-MID – 1'-hydroxymidazolam, MID – midazolam.