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CYP450 activities before and after Roux-en-Y gastric bypass: correlation with their intestinal and liver content.

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Running head: **CYP450 activities after RYBG**

ABSTRACT

Background: Several anatomic and physiologic changes occur after Roux-en-Y Gastric Bypass (RYGB) and its associated weight loss. At present, no single unified model can predict changes in drug metabolism associated with either RYGB surgery or weight loss.

5 **Objective:** The aim of this longitudinal human study was to measure the activity of the five most important Cytochrome P450 (CYP) involved in drug metabolism in patients with obesity before and after RYGB. Jejunal and liver biopsies obtained during bariatric surgery were used to measure CYPs amount and correlation between jejunal and hepatic content was estimated.

Settings: France University Hospital.

10 **Methods:** Eleven volunteers with a mean body mass index (BMI) of 44.1 (39.4-50.0) kg/m² participated in the study. CYP1A2, CYP2C9, CYP2C19, CYP2D6 and CYP3A4/A5 activities were measured with a cocktail approach before surgery (Visit 1), 5-8 weeks after surgery (Visit 2) and 25-30 weeks after surgery (Visit 3).

Results: CYP3A4/A5 and CYP2C9 metabolic ratio were transitorily and significantly increased 15 immediately after surgery (Visit 2 vs Visit 1). RYGB procedure does not lead to significant change in CYP activity 25-30 weeks after surgery (Visit 3 vs Visit 1). Samples obtained during surgery showed significant correlation between intestinal and liver contents of CYP2C9 and CYP3A4/A5. Except for liver CYP1A2 content, CYP metabolic activities were not correlated to their intestinal or liver contents.

20 **Conclusions:** This study showed that RYGB procedure does not lead to significant change in CYP activity 25-30 weeks after surgery. But CYP3A4/A5 and CYP2C9 activities were transitorily and significantly increased in the immediate postoperative context (< 1 month), representing a situation at risk of reduced drug exposure for several drugs having a narrow therapeutic window. In addition, considering high inter-individual variability in liver contents and activity of CYP3A4 and CYP2C9, 25 patients receiving drugs highly metabolized by these two CYP should be closely monitored in the immediate postoperative period.

Key-words: Roux-en-Y gastric bypass; drug metabolism; CYP450; pharmacokinetics; phenotypic exploration.

INTRODUCTION

The prevalence of obesity has increased in most parts of the world over the last decades ⁽¹⁾. The findings from the majority of long-term epidemiological studies indicate that being obese is dramatically associated with increased morbidity and mortality ⁽²⁾. Bariatric surgery is a well-known option for subjects with obesity to achieve and sustain weight loss. It has favourable effects on established cardiovascular risk factors and it reduces the rate of new cardiovascular disease events and cancers, as well as the overall mortality ^(3,4). As a consequence, the number of procedures has significantly increased worldwide during the last decade ⁽⁵⁾. Roux-en-Y gastric bypass (RYGB) remains one of the most common procedures performed worldwide and combines restrictive and malabsorptive procedures ⁽⁶⁾.

Therapeutic management during the perioperative period remains a concern, especially for patients treated with drugs having a narrow therapeutic range, drugs without well-established therapeutic drug monitoring or those without easily obtained pharmacodynamic marker of efficacy or tolerance for dosage adaptation ⁽⁷⁾.

Several anatomic and physiologic changes occur after RYGB and its associated weight loss. The new anatomy of the gastrointestinal tract as well as changes in body weight and metabolic status may influence pharmacokinetic (PK) parameters, e.g drug absorption, distribution, and clearance. But at present, no single unified model can predict changes in PK parameters associated with either RYGB surgery or weight loss ⁽⁸⁾. Each drug stands as an individual entity that needs to be investigated as such ⁽⁷⁾. Oral drug bioavailability and systemic clearance depend on their metabolism by drug-metabolizing enzymes (DME) dominated by cytochromes P450 (CYP) enzymes. They are mainly expressed within the gut and the liver and modifications in their activities can cause high interindividual variability in drug disposition with various pharmacological and toxicological consequences.

Although genotyping DME based on genomic DNA analysis has been extensively developed as an easy way to predict DME activity, phenotyping DME has been developed more recently due to the great improvement in the sensitivity and selectivity of analytical instrumentation. High performance liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) allowed assessing DME activities *in vivo* at a given time, information that would help in the choice of treatments and dose individualization ⁽⁹⁾. In contrast to genotyping methods, this method could also be used to assess simultaneously and quantitatively the effect of both genetic and environmental factors such as drug-drug interactions, disease-state, genetic variants and bariatric surgery on the activity of DME ^(10,11). The administration of a phenotyping cocktail containing several specific probe substrates of DME allows even the simultaneous evaluation of the activity of several DME ^(9,12).

The aim of the present study was to measure the activity of the five most important CYPs involved in drug metabolism in human, e.g. CYP1A2, CYP2C9, CYP2C19, CYP2D6 and CYP3A4/A5, assessed by a cocktail approach, in patients with obesity before and after RYGB. Moreover, jejunal and liver biopsies obtained during bariatric surgery were used to measure CYPs amount and correlation between jejunal and hepatic content was estimated.

MATERIAL AND METHODS

Study population

Volunteers with morbid obesity, aged from 18 to 60 years, involved in a medico-surgical obesity management program (Department of Nutrition and Department of Digestive and metabolic surgery, Ambroise Paré hospital, Boulogne-Billancourt, France) and candidates for a RYGB were included in the study. Exclusion criteria were as follow: Subjects with haemoglobin < 10 g/dl, renal or hepatic impairment (creatinine clearance < 60ml/min calculated with Cockcroft-Gault formula), liver transaminase levels > 3 times the upper limit of normal values), abnormal electrocardiogram, those usually treated with 2 drugs or more with inhibitory or inducing effect on DME used in the phenotyping cocktail, as recorded on the website (<http://www.drugbank.ca>), subjects with history of hypersensitivity to drugs used in the cocktail. Smokers were not included if they smoke more than 4 cigarettes a day.

All subjects gave their written informed consent. The protocol “SODA study” was approved by the Ethics Committee of Paris, France (CPP Ile de France I) and registered at the ClinicalTrials.gov website (EudraCT number 2009-010670-38, NCT00943969).

Study design

Three visits took place at the Center of Clinical Investigation of Saint Louis Hospital, before surgery (Visit 1), 5-8 weeks after surgery (Visit 2) and 25-30 weeks after surgery (Visit 3). The design of the three visits for phenotyping was similar. Subjects were required to abstain from caffeine, St. John’s Wort products and/or grapefruit juice for at least 72 h before the study session. At each visit, different clinical parameters were verified and, if necessary patients were excluded for the following reasons: Systolic blood pressure <90mmHg or diastolic blood pressure <45mmHg, oxygen saturation <92%, respiratory frequency <10/min, cardiac frequency <50 or >110/min, abnormal electrocardiogram. Then, each subject ingested the 5 probe drugs orally with 125 mL of water after an overnight fast and baseline blood sample was obtained. Composition of the cocktail was as follows: 100 mg of caffeine (oral liquid formulation), 20 mg of omeprazole

(tablet), 50 mg of losartan (tablet), 30 mg of dextromethorphan hydrobromide (oral liquid formulation), and 2 mg of midazolam (liquid formulation).

Blood samples were collected at several times following the oral administration of the cocktail: 15, 30, 45, 60, 90 minutes, 2, 4, and 8 hours. Patients were given their first meal 2 h after cocktail administration and a second meal 4 hours later. Urine was collected for 8 hours from the time of cocktail administration. Plasma and urine were harvested and frozen at -20°C until assayed.

Analytical Method

Determination of the five probe drugs and their metabolites in plasma and urine

The cocktail substrates and their CYP-specific metabolites (caffeine and paraxanthine for CYP1A2, omeprazole and 5-hydroxyomeprazole (OH-omeprazole) for CYP2C19, dextromethorphan and dextrorphan for CYP2D6 and midazolam and 1-hydroxymidazolam (OH-midazolam) for CYP3A4/5) were quantified in plasma using a validated LC-MS/MS method operating in dual electrospray ionization mode, as previously described⁽¹³⁾.

For measuring CYP2C9 activity, losartan and its metabolite (E-3174) were quantified in urine samples using a fully validated LC-MS/MS bioanalytical method. The chromatographic system was composed of two LC-20ADXR high pressure elution pumps with an autosampler (SIL-20ACXR) and column oven (CTO-20CA) set at 40°C . Detection was carried out using a QTRAP mass spectrometer (Applied Biosystem, Les Ulis, France). To a sample (100 μL of urine), 10 μL of internal standard (Cibenzoline at 1 $\mu\text{g}/\text{L}$ in methanol) and 1.3 ml of chloroform/isopropanol (95/5, v/v) were added. Following agitation for 10 minutes and centrifugation, the organic phase is evaporated under a gentle stream of nitrogen at 50°C . The residue was reconstituted with water/methanol (50/30, v/v) and vortex mixed before injection into chromatographic system. A volume of 10 μL was injected on an upisphere Strategy C18 column (150 *2.1 mm, 3 μ). The mobile phase was a mixture of ammonium formate (2 mM + 0.2% formic acid) buffer and acetonitrile (containing also 2 mM ammonium formate and 0.2 % formic acid). Total run time was 14 min without equilibration time between runs. The MS conditions were as followed: positive electrospray ionization mode (ESI+) with a source temperature at 500°C and ion-spray voltage of

5.5 kV. Multiple Reaction Monitoring (MRM) was performed with two transitions per compound and one for the IS. Reference standards of all compounds were obtained as pure powder from AlsaChim (Illkirch Graffenstaden, France). This method was validated according to EMA recommendations. The assay was linear over the validated concentration ranges of 10-200 µg/L for losartan and E3174. The best calibration curves fitting were obtained using linear regression with a weighting factor of 1/concentration ($r^2 > 0.995$) for the two compounds. The bias was < 15%. Intra-day and inter-day precision (% RSD) were < 10 and 12%, respectively. According to the different compounds, the matrix effect was between 87-108 %. The lower limit of quantitation for both losartan and its metabolite (E-3174) was 5 ng/ml.

10 ***Pharmacokinetic analysis***

Plasma concentrations versus time of each parent drug and its metabolite obtained from each subject were submitted to a non-compartmental pharmacokinetic analysis using WinNonlin® (version 4.1, Pharsight Corporation, Cary, NC, USA). The area under the plasma concentration–time curve ($AUC_{0-C_{last}}$) from time zero to the last time point (C_{last}), where plasma levels for the compounds of interest were adequately determined, was calculated by the linear up and log down trapezoidal rule. Metabolic ratio of each CYP pathway, except for CYP2C9 measured with urine, was calculated by dividing the $AUC_{0-C_{last}}$ of the metabolite to that of the parent drug. Values for the maximum plasma concentration (C_{max}) and time to C_{max} (T_{max}) were obtained directly from the observed individual plasma concentration–time curves. Metabolic ratio for CYP2C9 was the ratio of the amount of the metabolite (E-3174) to losartan in urine collected for 8 h after losartan administration.

Jejunal and hepatic CYP determination

Preparation of liver and jejunum microsomal fractions

25 Hepatic or jejunum tissues obtained during surgery were suspended and lysed by crushing in buffer containing 10 mM Tris-HCl (pH 7.4), 250 mM sucrose and a complete™ protease inhibitor cocktail tablet (Roche, Basel, Switzerland) with Ultra-Turrax T25 instrument (IKA, Germany). The lysate

was ultracentrifuged at 100 000g during 1 h at 5°C in order to discard the cytosol. The pellet was resuspended in 10 mM Tris-HCl (pH 7.4) buffer containing 250 mM sucrose. After another step of Ultra-Turrax crushing, the lysate was centrifuged at 10 800g during 25 min at 5°C. The supernatants were collected and ultracentrifuged again at 100 000g, 1h at 5°C to obtain the “crude
5 membrane” fraction. The resulting pellet was suspended in the Tris-HCl buffer and total proteins were quantified with BCA kit.

Absolute quantification of CYP by LC-MS/MS

Samples of microsomal fractions (50 µg of total protein) were solubilized in 7M guanidine hydrochloride, reduced with 320 mM dithiothreitol and alkylated with 270mM iodoacetamide. The
10 alkylated protein was precipitated with methanol-chloroform and resolubilized in 8 M urea, 0.1 M Tris-HCl containing 0.05% of Protease MAX™ (Promega, Madison, WI). The solubilized protein was diluted with 0.1 M Tris-HCl and treated with Lys-C endoprotease (Wako Pure Chemical Industries) for 2h at 37°C and digested with trypsin (Promega, Madison, WI) at 37°C overnight. The stable isotope-labelled peptide mixture was spiked in tryptic digests as internal standard to
15 reach a 12.5 fmol/µL final concentration. At last, the tryptic digests were acidified prior to LC-MS/MS quantitation ⁽¹⁴⁾.

Preparation of calibration standards and quality control (QC) samples

The calibration standards and QC samples were prepared with the *in silico* ⁽¹⁵⁾ selected signature peptides of each protein (Proteomedix Frontiers Co. Ltd). Eight non-zero standards were prepared
20 by serial dilutions and analysed to plot the calibration curves (0.125, 0.250, 1.25, 2.50, 12.5, 25.0, 62.5 and 125 fmol/µL). QC samples were prepared at three concentration levels (0.250, 5.00 and 100 fmol/µL).

LC-MS/MS assay

Samples were analysed using a Shimadzu UHPLC system Nexera 2 (Shimadzu, Kyoto, Japan)
25 coupled to an API 5500 triple quadrupole mass spectrometer (AB Sciex, Framingham, MA). The chromatography was performed on a XBridge BEH130 C18 (3.5 µm, 1x100 mm) column (Waters, Milford, MA) at 25°C in a gradient mode (2-60% B for 55 min, 60-100% B for 1 min, 100% B for

2 min and 100-2 % B in 2 min) under a flow rate of 50 μ L/min. The mobile phases were water with 0.1% of formic acid (A) and acetonitrile with 0.1% of formic acid (B) ⁽¹⁴⁾. Samples were ionized with TurboIonSpray (TIS) source and ions were detected in MRM scan mode.

Data processing for calculation of protein abundance

5 The data (chromatogram peak area) were obtained using the AB Sciex software Analyst 1.6. (AB Sciex, Framingham, MA). The peptides (unlabelled and labelled) were quantified following four specific MRM transitions obtained from the same precursor ion by calculating the ratios unlabelled peptide peak area/labelled peptide peak area. Each MRM channel corresponded to a pair of MRM transitions obtained with the same fragmentation. The concentration of the peptide was directly
10 related to the concentration of the protein of interest (fmol/ μ g) and was determined by calculating the mean concentrations obtained from the 4 MRM channels. CYP protein abundance was presented as mean \pm SD, minimal and maximal values, and interindividual variability was illustrated by dividing the maximal value by the minimal one.

15 **Data analysis**

Characteristics of the population over time were presented as mean and standard deviation (SD), minimum and maximum.

Pharmacokinetic parameters of the population over time were presented as mean and standard deviation (SD) or median, minimum and maximum and compared before and after surgery using a
20 Mixed model of ANOVA for normally distributed repeated measures. Data were log-transformed when necessary. If the procedure failed a non-parametric rank test was used. Multivariate normality was assessed using Mardia's test. We performed pairwise comparisons with a Dunnett correction to keep the Type I error at 5% overall.

All significance levels were two-sided and fixed at 5% and the statistical analyses were performed
25 using SAS software version 9.4 (SAS Institute Inc, Cary, NC).

RESULTS

Patients follow-up

As indicated in the patient flowchart (Figure 1), eleven volunteers participated in the study, a man and ten women, with a mean age of 36.7 (25-50) years and a mean body mass index (BMI) of 44.1 (39.4-50.0) kg/m² (Table 1). None of them used medication interacting with drug metabolism pathways evaluated by the cocktail during all test periods. Eight of them successfully completed the three study sessions. One woman was excluded before the Visit 2 due to the diagnosis of a chronic myeloid leukemia. Two additional women did not participate to the Visit 3 for the following reasons: one woman was lost of follow up and the other woman presented with bradycardia the day of the third visit.

At 25-30 weeks after surgery (Visit 3), the average of total weight loss was 25% of the initial body weight. All biological parameters improved after surgery, especially liver transaminases. The main clinical and biological characteristics of the patients who underwent the three visits are showed in Table 1.

Pharmacokinetic data for CYP substrates before and after surgery

The table 2 shows the main PK parameters of CYP probe substrates and their metabolites for each period and for the overall population. Average plasma concentrations of caffeine, dextromethorphan, midazolam, omeprazole and their respective metabolites are represented in Figure 2.

C_{max} significantly increased while T_{max} significantly decreased between pre and postoperative periods for caffeine (CYP1A2), omeprazole (CYP2C19), and midazolam (CYP3A4/5), indicating a more rapid oral absorption of these drugs after surgery.

Metabolic ratio were also determined between pre and postoperative periods to study the effect of surgery on CYP activity during the intestinal and hepatic first pass metabolism. CYP3A4/5 activity significantly increased between preoperative and postoperative period and this was statistically

significant only at 5-8 weeks after surgery. CYP2C9 activity also increased significantly between preoperative period and 5-8 weeks after surgery. In contrast, CYP activities were similar between pre and the two postoperative periods for CYP1A2 (caffeine), CYP2C19 (omeprazole), and CYP2D6 (dextromethorphan) (Table 2).

5

Intestinal and liver CYP content

Table 3 shows the protein abundance of the respective CYP investigated in our study. Except for CYP2C9, the inter-individual variability in liver CYP content was higher than that in the intestine. CYP1A2 intestinal content was below the limit of quantification in all our samples, but quantified in liver for all patients. CYP1A2 liver content had the largest variability in protein expression (16-
10 fold), followed by CYP3A4 liver content (13.2-fold), in comparison with the other CYP. CYP2C9 and CYP3A4 liver contents were higher than those of the other CYP measured in this study. Although detected in the vast majority of samples, CYP2C9 and CYP2C19 intestinal contents were much lower than those of CYP3A4. For CYP2C9 and CYP3A4, intestine and liver
15 contents were significantly correlated (Figure 3).

Correlation between CYP intestinal and liver contents with their metabolic activity before surgery

CYP1A2 liver contents were significantly correlated with CYP1A2 activity ($r=0.65$, $p=0.0487$).
20 There was also a trend for the correlation between CYP2C19 liver contents and activity that did not reach significance ($r=0.69$, $p=0.09$). None of the other CYP liver and intestine contents were correlated to their metabolic activity evaluated before surgery.

DISCUSSION

In exploring the activity of several CYPs in subjects with obesity undergoing RYGB before and after surgery, we observed that CYP3A4/A5 and CYP2C9 metabolic ratio were transiently and significantly increased immediately after surgery (Visit 2 vs Visit 1). Thanks to samples obtained during surgery we showed significant correlations between intestinal and liver contents of CYP2C9 and CYP3A4. Except for liver CYP1A2 content, CYP metabolic activities were not correlated to their intestinal or liver contents.

Due to changes in the gastrointestinal anatomy, lean and fat mass weight loss, and improvement in metabolic status and inflammation, RYGB may alter the pharmacokinetics of several drugs and consequently influence their disposition and pharmacodynamics. In this study, we mainly focused on the changes in CYPs activity following RYGB surgery. Altered CYPs expression and activity may indeed alter both the extent of oral absorption and the systemic elimination of CYP substrates. CYP2C9 and CYP3A4/A5 metabolic activities were increased between the pre- and the postoperative periods but they varied over time. In both cases, CYP2C9 and CYP3A4/5 activities were significantly higher in the 5-8 weeks postoperative period in comparison with the preoperative period but they returned to baseline values at 25-30 weeks after surgery. In accordance, several studies showed similar CYP1A2, 2C9, 2C19, CYP2D6 or 3A4/5 activity between patients who undergone RYGB and their non-surgical controls, or in the same patients between pre and postoperative periods^{(10)(16,17)}. But Brill *et al.* showed that midazolam systemic clearance was 1.7 times higher one year after surgery when compared to preoperative period, suggesting that losing weight after RYGB may have increased hepatic CYP3A4/A5 activity⁽¹⁸⁾. Mitrov-Winkelmoen *et al.* showed a lower omeprazole exposure in patients with obesity at least 6 weeks after RYGB surgery suggesting either reduced omeprazole bioavailability and/or higher systemic clearance due to a higher CYP2C19 activity⁽¹⁹⁾.

Overall, our study together with all other cited studies are not in favour of a significant change in the CYP activity 6 months after RYGB and thereafter in comparison with preoperative period. The lack of consequence of the shunt of one part of the jejunum is in accordance with microsomal

studies suggesting that intestinal metabolism contributes minimally to the overall first-pass effect of orally administered substrates ⁽²⁰⁾. But it cannot be excluded that reduced intestinal CYP metabolism may increase liver CYP expression and activity, as observed in animals after gastrectomy ⁽²¹⁾. The transitory increased CYP3A4/A5 and CYP2C9 activity at one month after surgery may reflect such adaptation. However, the intraindividual variability in CYP activity was high considering the three periods, illustrating how many individuals' factors may be involved. Body composition, inflammatory status, steatosis, and dietary intake vary greatly between individuals and influence CYP activity ^(22,23). With surgery induced weight loss, these factors may induce significant change in CYP activity from a period to another at individual level.

10 The rate (velocity) of drug absorption, illustrated by the values of C_{max} and T_{max} after oral administration, increased in postoperative period in comparison with preoperative period. As already discussed in many studies and systematic reviews, the rapid emptying of the small gastric pouch may account for faster transfer of drug molecules into the jejunum, inducing earlier absorption of orally administered medications except for sustained-release formulations ^(7,10). It may thus influence the effect of some drugs whose pharmacodynamics effects are directly related to their peak plasma concentrations, even if the overall drug exposure (AUC) is not necessarily increased ^(7,24). Whether drug oral bioavailability is altered in patients with obesity who undergone RYGB surgery remains a matter of debate as rare studies explored pharmacokinetics of drugs after their administration by both intravenous and oral routes in patients with RYGB ⁽²⁵⁾. In addition, as bioavailability may be different between obese and non-obese subjects ⁽²⁵⁾, such studies should be systematically performed before and after surgery and in comparison with normal weight subjects in order to demonstrate changes in bioavailability induced by obesity and surgery, respectively.

Expression profiles of CYP in human tissues have mainly been examined at mRNA level and rare studies compared intestinal and liver CYP amounts in paired samples of patients ^(26,27,28,29). Here, we found similar CYP contents than those previously reported in another study ⁽³⁰⁾. A large interindividual variability in CYP content in the intestine and in the liver was observed in our study in accordance with data in the literature ^(22,26,27,28,29,31). The relative CYP abundance in liver is in

accordance with that reported in the literature, CYP2C9 and CYP3A4 being the most common CYP represented⁽³²⁾. However, CYP3A4 liver contents were lower than those of CYP2C9 in our study, suggesting that obesity might reduce CYP3A4 expression, in accordance with some animal and human studies showing reduced CYP3A4 contents related to hepatic steatosis^(33,34). But Brill *et al.* recently demonstrated that CYP3A4 activity assessed using midazolam substrate was not different between obese and non-obese subjects⁽²⁵⁾. The relative CYP abundance in intestine was similar to those reported by Galetin *et al.*⁽³²⁾. In our study, CYP3A4 protein content was about 1.3-fold higher in hepatic than in intestinal samples, while those of CYP2C9, CYP2C19, CYP2D6 were 21.5-, 3- and 21-fold higher, respectively. Thanks to paired intestinal and liver specimens of patients with digestive cancer, Von Richter *et al.* reported that CYP3A4 content in enterocytes was 3 times higher than that of liver specimens, suggesting the role of inflammation markers and underlying disease condition in intestinal and liver CYP expression⁽²⁶⁾. Conversely, CYP3A4 liver content seems to be reduced with obesity^(33,34). Apart from this study and to the best of our knowledge, comparison in paired samples has not been reported in humans.

The significant correlation between intestinal and liver CYP3A4 and CYP2C9 content suggests similar regulation pathways in subjects with obesity. Comparing 10 paired intestine-liver samples, Lin *et al.* reported no significant correlation between hepatic and jejunal CYP3A4 mRNA and protein expression⁽³⁵⁾. Conversely, the lack of correlation for CYP2D6 and CYP2C19 liver and intestinal content may be due to the fact that for some specimens, CYP content is below the limit of quantification.

To the best of our knowledge, no study has correlated CYP *in vivo* activity with their intestinal and liver content. It has been mainly performed at microsomal level in order to predict first pass drug metabolism and clearance with contradictory results due to the source of tissue, the methods of measurement in CYP expression and the isolation methods^(36,37).

Several studies have demonstrated a relationship between genetics and/or intestinal CYP mRNA and drug concentrations^(23,31,38,39,40,41) and it has been admitted that CYP intestinal and liver protein content is involved in the variability in CYP activity.

In our study, except for liver CYP1A2, CYP activities are not correlated to their intestinal or liver content. First, liver and intestine are not the only organs containing CYPs in the organism ^(42,43). Secondly, the tissues containing CYPs have physiological particularities that are not taken into account while measuring only CYP content, such as organ blood flow.

5 To explore CYP activity before and after RYGB, we used a prospective longitudinal design that minimizes the variance related to interindividual variations in drug disposition by allowing individuals to serve as their own control. In addition, this is the first study exploring simultaneously the role of CYP liver and intestine content in CYP activity. However, our study has several limitations. This is an exploratory pilot study with a small sample size, and all subjects did not
10 complete the entire study protocol. Given the high variability in pharmacokinetics parameters, our results should be replicated in another clinical study. Since this study was performed in patients with morbid obesity, these results cannot be generalized to the overall population.

In conclusion, this study showed that RYGB procedure does not lead to significant change in CYP
15 activity 25-30 weeks after surgery. But CYP3A4/A5 and CYP2C9 activities were transitorily and significantly increased in the immediate postoperative context (< 1 month). This represents a situation at risk of reduced drug exposure for several drugs that are substrates of CYP2C9 and/or CYP3A4 and having a narrow therapeutic window such as immunosuppressant drugs (tacrolimus, cyclosporine,...), antivitamin K drugs (acenocoumarol, warfarin, fluindione), antiarrhythmic drugs
20 (disopyramide, amiodarone), statins, tyrosine kinase inhibitors and others. In addition, considering high inter-individual variability in liver contents and activity of CYP3A4 and CYP2C9, patients receiving drugs highly metabolized by these CYP should be also closely monitored in the immediate postoperative period.

25 **The authors have no conflict of interest to declare related with the present study.**

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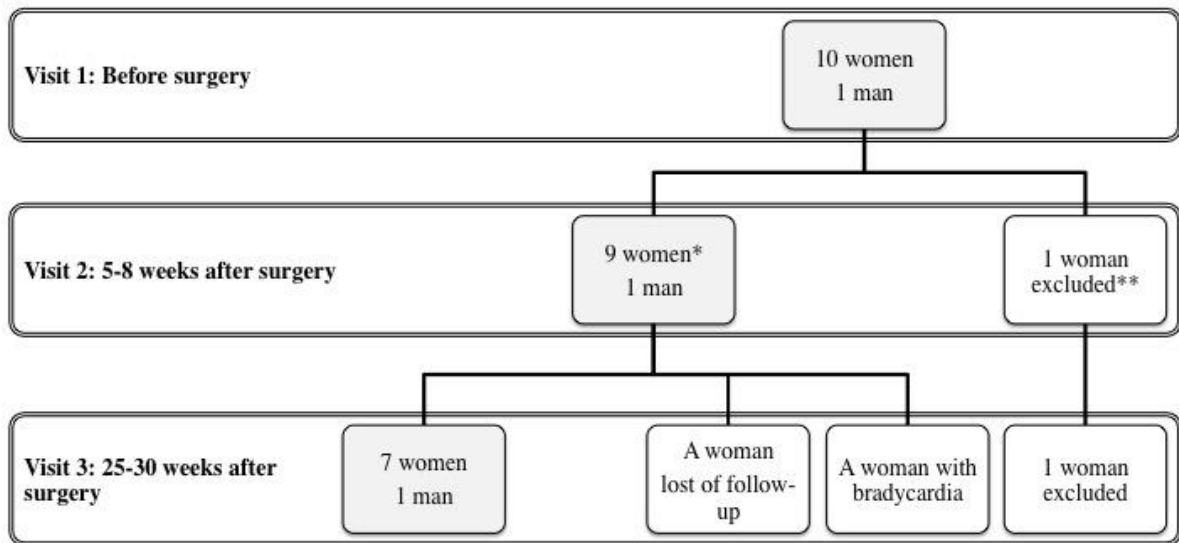
Figure legends

Figure 1. Study Flowchart

5 **Figure 2.** Pharmacokinetics of parent drugs and their metabolite in patients at visits 1, 2 or 3.

Values are mean \pm SEM of individual plasma concentrations of caffeine (A), paraxanthine (B), dextromethorphan (C), dextrorphan (D), midazolam (E), OH-midazolam (F), omeprazole (G) and OH-omeprazole (H)

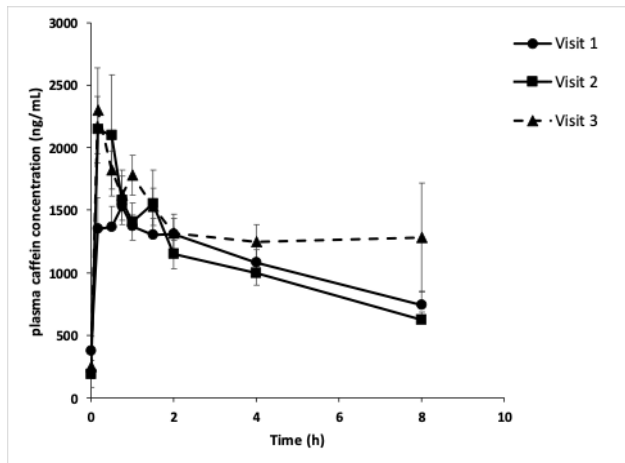
10 **Figure 3.** Correlation between intestinal and liver CYP2C9 and CYP3A4 contents.



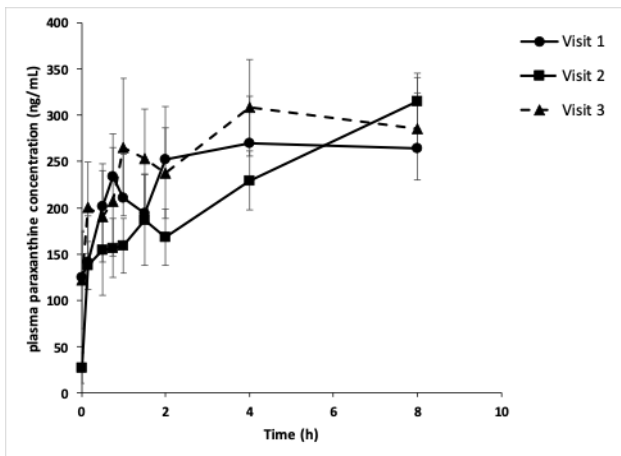
*One woman was not able of taking all drugs orally

**Diagnosis of chronic myeloid leukemia

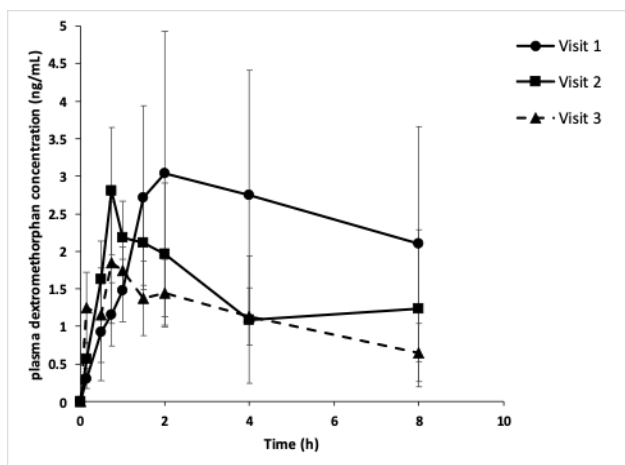
SODA-Figure 1. Flow Chart



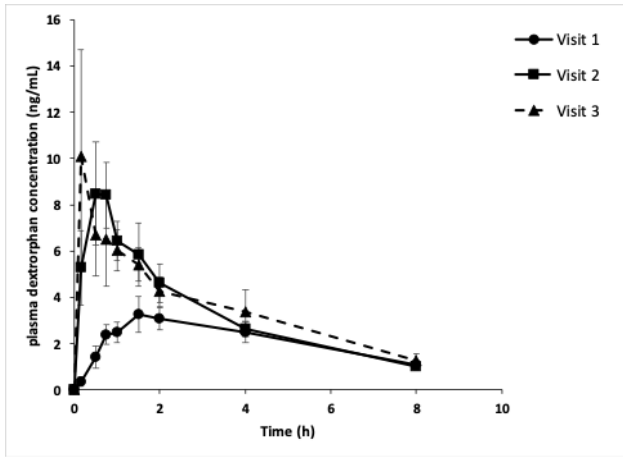
SODA_Figure 2A



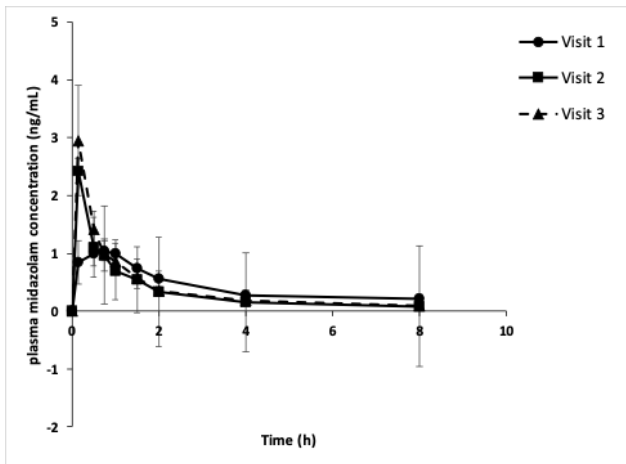
SODA_Figure 2B



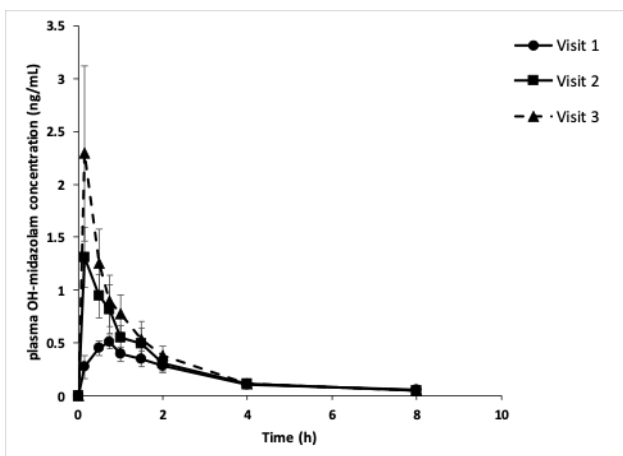
SODA_Figure 2C



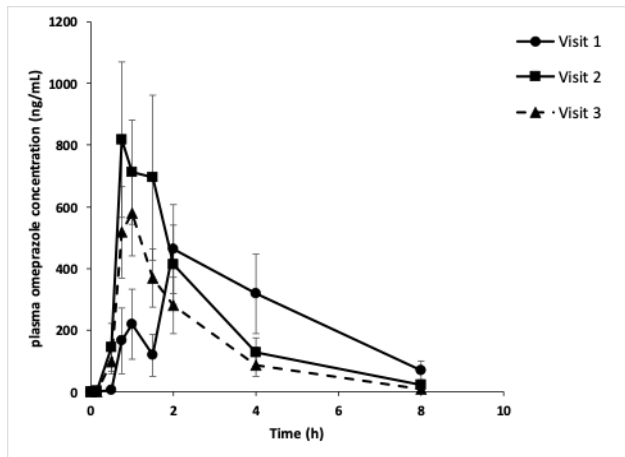
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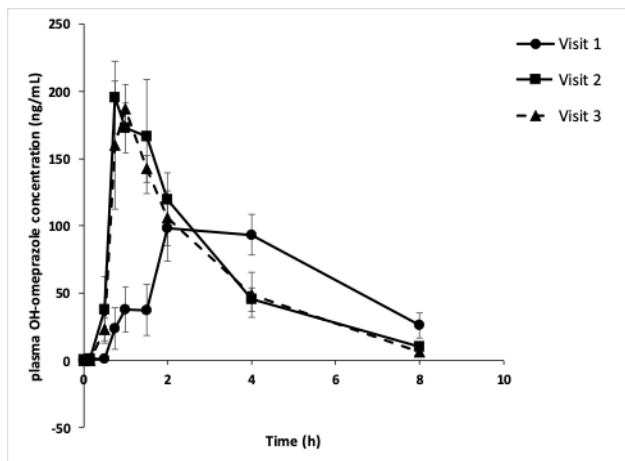
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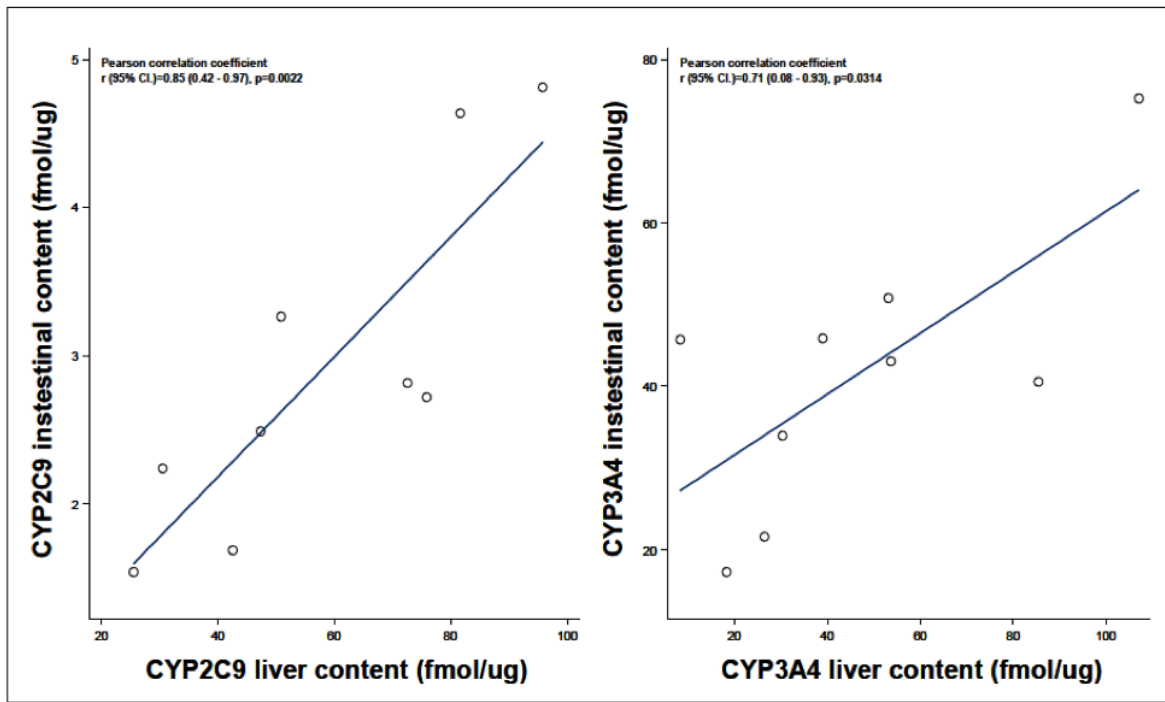
SODA_Figure 2F



SODA_Figure 2G



SODA_Figure 2H



SODA_Figure 3

Table 1 Clinical and Biological Characteristics of the population over time

	Before Surgery	5-8 weeks after surgery	25-30 weeks after surgery
Weight (kg)			
Mean \pm SD	124.5 \pm 17.4	108.5 \pm 16.3	91.9 \pm 15.1
Min, Max	95.0, 163.0	86.0, 147.0	73.0, 124.0
Total Body Weight loss (%)			
Mean \pm SD		12.4 \pm 4.5	25.2 \pm 5.4
Min, Max		8.6, 24.3	14.0, 32.5
BMI (kg/m²)			
Mean \pm SD	44.1 \pm 3.6	38.9 \pm 3.8	32.8 \pm 3.0
Min, Max	39.4, 50.0	34.4, 45.7	26.6, 36.2
AST (UI/L)			
Mean \pm SD	24.0 \pm 7.3	24.4 \pm 24.4	14.5 \pm 4.5
Min, Max	17.0, 42.0	13.0, 89.0	8.0, 22.0
ALT (UI/L)			
Mean \pm SD	35.7 \pm 9.8	51.3 \pm 83.6	15.0 \pm 4.6
Min, Max	19.0, 52.0	11.0, 285.0	10.0, 22.0
PAL (UI/L)			
Mean \pm SD	79.1 \pm 17.9	66.6 \pm 17.7	65.7 \pm 15.4
Min, Max	53.0, 100.0	35.0, 91.0	40.0, 87.0
Creatinine (mmol/L)			
Mean \pm SD	68.0 \pm 18.7	63.3 \pm 16.8	63.4 \pm 14.8
Min, Max	48.0, 117.0	46.0, 94.0	52.0, 98.0
Cholesterol (mmol/L)			
Mean \pm SD	4.9 \pm 0.8	3.9 \pm 0.8	4.1 \pm 0.9
Min, Max	3.6, 6.5	3.0, 5.6	3.1, 5.5
Triglycerides (mmol/L)			
Mean \pm SD	1.4 \pm 0.6	1.1 \pm 0.5	0.9 \pm 0.5
Min, Max	0.6, 2.6	0.6, 2.0	0.6, 1.9

HDL (mmol/L)			
Mean \pm SD	1.2 \pm 0.2	1.1 \pm 0.2	1.2 \pm 0.1
Min, Max	0.9, 1.5	0.9, 1.4	1.1, 1.4
LDL (mmol/L)			
Mean \pm SD	3.1 \pm 0.6	2.3 \pm 0.6	2.5 \pm 0.8
Min, Max	2.3, 4.4	1.6, 3.5	1.7, 3.6

Legends : kg : kilogram ; mmol : millimoles ; L : liter ; SD : standard deviation ; Min, Max : minimum, maximum ; BMI : body mass index ; AST : aspartate aminotransferase ; ALT : alanine aminotransferase ; PAL : alkaline phosphatase ; HDL : high density lipoprotein ; LDL : low density lipoprotein

Table 2. Mean pharmacokinetic parameters before and after surgery

	Before Surgery	5-8 weeks after surgery	25-30 weeks after surgery
<u>CYP1A2</u>			
Cmax caffeine			
Mean ± SD	1438 ± 375	2461 ± 1157*	2214 ± 750
Min, Max	775, 1966	747, 4764	973, 3441
Tmax caffeine			
Median	0.8	0.3	0.2
Min, Max	0.2, 1.5	0.2, 4.0	0.2, 4.0
AUC caffeine			
Mean ± SD	5422 ± 2133	6730 ± 2088	7635 ± 2755
Min, Max	220, 2726	681, 2288	341, 2732
CYP1A2 activity			
Mean ± SD	0.2 ± 0.2	0.2 ± 0.1	0.2 ± 0.1
Min, Max	0.1, 0.7	0.1, 0.4	0.1, 0.3
<u>CYP2C19</u>			
Cmax OMZ			
Mean ± SD	519 ± 444	1017 ± 882*	655 ± 449
Min, Max	75, 1600	206, 2780	196, 1350
Tmax OMZ			
Median	2.0	0.9*	1**
Min, Max	1.0, 8.0	0.5, 1.5	0.8, 1.0
AUC OMZ			
Mean ± SD	424 ± 163	478 ± 250	440 ± 214
Min, Max	196, 686	242, 1097	244, 902
CYP2C19 activity			
Mean ± SD	0.5 ± 0.4	0.6 ± 0.6	0.8 ± 0.6
Min, Max	0.1, 1.5	0.1, 1.7	0.2, 1.9
<u>CYP3A4/5</u>			
Cmax MID			

Mean ± SD	1.4 ± 1.0	2.5 ± 1.4	3.0 ± 2.6
Min, Max	0.4, 4.3	0.7, 4.9	0.7, 9.0
Tmax MID			
Mean ± SD	0.5	0.2*	0.2**
Min, Max	0.2, 1.0	0.2, 0.8	0.2, 0.5
AUC MID			
Mean ± SD	3.3 ± 2.9	2.7 ± 1.2	3.1 ± 2.0
Min, Max	0.7, 11.6	0.4, 4.4	1.2, 7.7
CYP3A4/5 activity			
Mean ± SD	0.5 ± 0.2	1.2 ± 1.1*	0.9 ± 0.3
Min, Max	0.2, 0.8	0.3, 4.1	0.6, 1.4
CYP2D6			
Cmax DXM			
Mean ± SD	8.7 ± 16.1	13.5 ± 30.5	3.4 ± 1.2
Min, Max	0.5, 51.6	1.4, 99.9	2.1, 5.4
Tmax DXM			
Median	1.5	0.9	1.5
Min, Max	0.5, 8.0	0.5, 8.0	0.5, 8.0
AUC DXM			
Mean ± SD	17.2 ± 33.5	13.4 ± 20.6	8.3 ± 5.0
Min, Max	0.5, 116.7	1.8, 65.2	2.4, 16.8
CYP2D6 activity			
Mean ± SD	4.6 ± 7.1	4.6 ± 3.4	4.1 ± 2.7
Min, Max	0.0, 25.1	0.0, 11.1	1.1, 7.3
CYP2C9[§]			
CYP2C9 activity			
Mean ± SD	0.6 ± 0.3	1.0 ± 0.5*	0.7 ± 0.5
Min, Max	0.2, 1.0	0.2, 1.8	0.1, 1.5

Legends

CYP : cytochrome P450, SD : standard deviation ; Min, Max : minimum, maximum ; Cmax : maximal concentration (ng/mL), Tmax : time to reach Cmax (h); AUC: area under the curve (ng.h/mL); OMZ : omeprazole ; MID : midazolam ; DXM : dextromethorphan ;

CYP activity: metabolite AUC/parent AUC in blood samples or in urine for CYP2C9 activity
 * p<0.05 between before and 5-8 weeks after surgery, ** p<0.05 between before and 25-30 weeks after surgery.

§ CYP2C9 activity: losartan and its metabolites were measured in urines.

Table 3. Intestinal and liver CYP contents

	Liver content (fmol/ug)	Intestinal content (fmol/ug)
CYP1A2		
n (BLQ)	11 (0)	11 (11)
Mean \pm SD	14.1 \pm 10.4	-
Min, Max	2.2, 35.4	-
Variability	16.0	-
CYP2C9		
n (BLQ)	9 (2)	11 (0)
Mean \pm SD	58.0 \pm 24.3	2.7 \pm 1.1
Min, Max	25.5, 95.8	1.3, 4.8
Variability	3.8	3.7
CYP2C19		
n (BLQ)	8 (3)	9 (2)
Mean \pm SD	2.5 \pm 1.8	0.5 \pm 0.2
Min, Max	0.5, 5.3	0.2, 0.9
Variability	11.7	3.8
CYP2D6		
n (BLQ)	8 (3)	5 (6)
Mean \pm SD	11.8 \pm 5.2	0.6 \pm 0.2
Min, Max	5.4, 22.0	0.3, 0.8
Variability	4.1	2.4
CYP3A4		
n (BLQ)	9 (2)	11 (0)
Mean \pm SD	46.8 \pm 32.2	36.7 \pm 18.7
Min, Max	8.1, 107.0	13.1, 75.2
Variability	13.2	5.7

n : number of samples

Min, Max : minimum, maximum

Variability =Max/Min

BLQ : below the limit of quantification