

CYP2E1 Activity Before and After Weight Loss in Morbidly Obese Subjects With Nonalcoholic Fatty Liver Disease

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Previous studies suggest that hepatic cytochrome P450 2E1 (CYP2E1) activity is increased in individuals with chronic alcoholism, nonalcoholic steatohepatitis (NASH), and morbid obesity, and may contribute to liver disease. We studied 16 morbidly obese subjects with varying degrees of hepatic steatosis and 16 normal-weight controls. Obese subjects were evaluated at baseline, 6 weeks, and 1 year after gastroplasty, a procedure that leads to weight loss. Hepatic CYP2E1 activity was assessed by determination of the clearance of chlorzoxazone (CLZ), an *in vivo* CYP2E1-selective probe. Liver biopsy tissue was obtained during surgery for histopathology. Both the total and unbound oral CLZ clearance (Cl_u/F) was elevated approximately threefold in morbidly obese subjects compared with controls ($P < .001$). The Cl_u/F was significantly higher among subjects with steatosis involving $>50\%$ of hepatocytes, compared with those with steatosis in $\leq 50\%$ of hepatocytes ($P = .02$). At postoperative week 6 and year 1, the median body mass index (BMI) of subjects who underwent gastroplasty decreased by 11% and 33%, total oral CLZ clearance declined by 16% ($P < .01$) and 46% ($P < .05$), and Cl_u/F decreased by 18% ($P < .05$) and 35% ($P = .16$), respectively. Moreover, those subjects with a year 1 BMI $< 30 \text{ kg/m}^2$ exhibited a median Cl_u/F that was 63% lower ($P = .02$) than the respective clearance for all other subjects. In conclusion, hepatic CYP2E1 activity is up-regulated in morbidly obese subjects. A positive association between the degree of steatosis and CYP2E1 activity preoperatively and between the extent of obesity and CYP2E1 activity postoperatively, suggests that CYP2E1 induction is related to or caused by hepatic pathology that results from morbid obesity. (HEPATOLOGY 2003;38:428-435.)

Cytochrome P450 2E1 (CYP2E1) is responsible for the metabolism and bioactivation of several therapeutic agents (e.g., halothane, enflurane, acetaminophen) and environmental toxicants (e.g.,

carbon tetrachloride, benzene, *N*-nitrosodimethylamine, ethanol).¹ In humans, CYP2E1 is found primarily in the liver, but extrahepatic expression has been described.¹ Liver function can be affected adversely by acute or chronic exposure to certain CYP2E1 substrates, some of which cause injury as the result of reactive oxygen formation. Thus, factors that influence steady-state *in vivo* CYP2E1 activity would have obvious implications in defining the risk of organ toxicity from exposure to compounds that undergo CYP2E1-mediated bioactivation.

Factors that induce CYP2E1 activity can be classified as having either an exogenous (e.g., isoniazid, ethanol, pyrazole) or endogenous (e.g., diabetes, fasting, hypophysectomy, obesity) origin.¹ For example, in rats, diet-induced obesity has been shown to increase hepatic CYP2E1 protein expression and enhance the metabolism of prototypical CYP2E1 substrates.² In addition, obesity represents a risk factor for acetaminophen-induced hepa-

Abbreviations: CYP2E1, cytochrome P450 2E1; NASH, nonalcoholic steatohepatitis; CLZ, chlorzoxazone; BMI, body mass index; 6-OH-CLZ, 6-hydroxy-chlorzoxazone; f_u , free fraction; Cl/F , oral clearance; Cl_u/F , unbound oral clearance.

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torenal toxicity,^{3,4} a phenomenon thought to be mediated in part by CYP2E1-catalyzed formation of a reactive metabolite, *N*-acetyl-*p*-benzoquinone imine.^{5,6}

There is some evidence for a causal relationship between obesity and associated CYP2E1-mediated liver disease risk in humans. Obesity represents an identified risk factor for organ injury associated with the administration of halothane⁷ and, presumably, this occurs through halothane bioactivation catalyzed by CYP2E1.⁸⁻¹⁰ Similarly, there are data suggesting a role for alcohol-inducible CYP2E1-catalyzed lipid peroxidation in the pathogenesis of alcoholic liver disease.¹

Morbid obesity is frequently associated with nonalcoholic fatty liver disease. In this setting, nonalcoholic fatty liver disease may range from steatosis alone to steatohepatitis with advanced fibrosis.¹¹ Fatty infiltration (most often severe) of the liver is present almost universally in morbidly obese subjects undergoing gastric bypass, and fibrosis or cirrhosis is present in about 10% of cases.¹² Recent studies have shown that there is an increase in hepatocellular CYP2E1 expression among patients with steatohepatitis,¹³ findings that are supported by data from an animal model of nonalcoholic steatohepatitis (NASH).¹⁴ Thus, it has been suggested that steatohepatitis associated with morbid obesity is mediated, in part, by CYP2E1 induction and CYP2E1-mediated oxidative injury.¹⁵

The drug chlorzoxazone (CLZ) has been used extensively as a selective *in vivo* probe of CYP2E1 activity. In addition to strong *in vitro* evidence,^{16,17} the enzyme selectivity of CLZ *in vivo* is shown by the pronounced reduction in oral CLZ clearance that is observed when a known mechanism-based CYP2E1 inhibitor, disulfiram, is administered as a single dose 10 hours before CLZ.¹⁸ The same disulfiram pretreatment regimen does not alter the *in vivo* activity of CYP2A6, CYP2D6, CYP2C9, CYP2C19, and CYP3A4.^{19,20} Consistent with data from an animal model of obesity,² moderately obese human subjects also exhibit increased *in vivo* CYP2E1 activity, as evidenced by a twofold increase in CLZ clearance above that of normal weight controls.²¹

In this study, we tested the hypotheses that *in vivo* CYP2E1 catalytic activity is elevated with morbid obesity and that this activity would be reduced by a surgical intervention that evokes significant weight loss and a trend toward normalization of other physiologic parameters. A secondary aim was to examine the relationship between CYP2E1 activity and a histopathologic assessment of hepatic steatosis that is associated with morbid obesity.

Patients and Methods

Human Subjects

This protocol was reviewed and approved by the University of Washington Human Subjects Internal Review Board. Sixteen morbidly obese men and women (defined as a body mass index [BMI, kg/m²] greater than 40) who were candidates for vertical banded gastroplasty were recruited and consented for the study before surgery. A matched number of men and women who were within 10% of ideal body weight were recruited and consented to serve as controls. Control and obese subjects were matched for ideal body weight. Prospective subjects with a history of alcohol abuse or current use (within the last 2 weeks) of prescription medications or any other product known or suspected to induce or inhibit CYP2E1 were excluded. Prospective control subjects with abnormal serum liver function tests also were excluded. Subjects were studied at the General Clinical Research Center and Operating Room Suite at the University of Washington Medical Center.

Experimental Protocols

Weight and height were obtained from all subjects, and the BMI was calculated on entry into the study. Ideal body weight was estimated based on a standard table accounting for height and gender (Documenta Geigy, 1973). For the obese group, several clinical parameters were collected as part of the presurgical evaluation, including measurements of glucose intolerance (fasting blood glucose and glycosylated hemoglobin A1c) and ketosis (quantitative urinary ketones).

For the preoperative study, the disposition of oral CLZ in morbidly obese subjects and control subjects was compared. For the longitudinal study, which examined the influence of weight loss from gastric bypass on CYP2E1 activity, postoperative and preoperative measurements for each subject were compared. Oral CLZ disposition was measured at baseline and approximately 6 weeks and 1 year after surgery.

For all CLZ disposition studies, a single oral 750-mg dose was administered at 8 a.m. with 8 oz of water after an overnight fast. Venous blood samples were obtained from an indwelling catheter just before and at 0.5, 1, 1.5, 2, 3, 4, 5, 6, 7, and 8 hours after the dose. Breakfast and lunch were given at approximately 1.5 hours and 4 hours after the dose, respectively. Urine was collected between 0 to 8 hours and 8 to 24 hours after drug administration. Volumes of urine for each collection period were measured and an aliquot taken for analysis. All plasma and urine samples were stored at -20°C until analysis for CLZ and 6-hydroxychlorzoxazone (6-OH-CLZ) concentrations. A

core needle biopsy specimen of the liver was obtained intraoperatively and used for histologic examination.

Histopathology of the Liver

Microscopic slides were prepared from liver biopsy specimens and stained with hematoxylin and eosin or trichrome blue. A hepatologist (K.V.K.) who was blinded to the results of the CLZ pharmacokinetic analysis was asked to rate the degree of fatty infiltration (0%-5%, 6%-25%, 26%-50%, 51%-75%, and >75%) using light microscopy at low magnification power. Additional descriptive reports from the attending pathologist were used to assess the presence of NASH, following the criteria of Brunt et al.²² A diagnosis of NASH was based on the presence of macrovesicular steatosis involving >5% of biopsy hepatocytes and two or more of the following features: pericellular or perivenular fibrosis, ballooning degeneration of hepatocytes, Mallory's hyaline, significant periportal or lobular inflammation, and lipogranulomas. Necroinflammatory grading of NASH was not performed.

CLZ and 6-OH-CLZ Analyses

Plasma CLZ was analyzed by a previously described high-performance liquid chromatography (HPLC) method,¹⁸ with the following modifications: A single-step precipitation was employed by the addition of 100 μ L acetonitrile and 200 μ L 10% trichloroacetic acid/water to 200 μ L of plasma. Addition of an internal standard was omitted. After centrifugation for 10 minutes, 20 μ L of the supernatant was injected directly on an integrated high-performance liquid chromatography system (Hewlett-Packard 1050 series) fitted with a 4.6 \times 250 mm, 5 μ C18 reversed-phase column (Rainin, Deerfield, IL) and a C18 cartridge guard column (Alltech Associates, Woburn, MA). An isocratic mobile phase of 0.15% ammonium acetate (pH 4.7)/acetonitrile (63:37) at a flow rate of 1.0 mL/min was used and the effluent was monitored at 280 nm. Under these conditions, the CLZ retention time was 8.2 minutes. The total (conjugated and unconjugated) concentration of 6-OH-CLZ in plasma and urine was measured by a previously published method with minor modifications.²¹

CLZ Plasma Protein Binding

The binding of CLZ to plasma proteins was measured with pooled plasma obtained during each pharmacokinetic study. Each pooled plasma sample was spiked with additional CLZ to yield a final concentration of at least 40 μ g/mL. A 0.3-mL aliquot of spiked plasma was dialyzed against an equal volume of 67 mmol/L sodium phosphate

buffer, pH 7.4, in an equilibrium dialysis chamber separated by a minimum 12 kd membrane (Spectra/Por; Spectrum Medical Industries, Los Angeles, CA), at 37°C for 4 hours.²³ Samples were dialyzed in duplicate and CLZ concentrations in the plasma and buffer chambers were measured as described above. The free fraction (f_u) was calculated as a ratio of buffer to total plasma CLZ concentration.

CLZ Pharmacokinetic Analyses

Pharmacokinetic parameters were calculated from the plasma concentration-time curve and urine recovery data using standard methods.²⁴

Statistical Analyses

The relationship between the oral clearance of CLZ (Cl/F) and the independent variables BMI, gender, and diabetes, was examined by multivariate linear regression analysis (SPSS for Win95 v. 7.5, SPSS Inc., Chicago, IL). For evaluating the effects of obesity on *in vivo* CYP2E1 activity, differences in CLZ disposition variables between the obese and control groups were analyzed for significance with distribution-free tests (Mann-Whitney Test). The Friedman test was used for determining the significance of changes in CLZ disposition between the 3 treatment periods, preoperatively, at 6 weeks and 1 year after gastroplasty. The level of significance was set at $P < .05$ for pairwise comparisons of preoperative and postoperative periods with corrections for multiple comparisons. The relationship between oral CLZ clearance and the preoperative degree of fatty infiltration and NASH and postoperative 6-week ketonuria and 1-year BMI also were tested (Kruskal-Wallis and Mann-Whitney Tests).

Results

Effects of Morbid Obesity

Control and morbidly obese groups were matched for gender and ideal body weight. As expected, both the absolute body weight (median, 172 vs. 59 kg; $P < .001$) and BMI (59.5 vs. 21.0 kg/m², $P < .001$) of obese subjects was significantly higher than the corresponding values for control subjects (Table 1). Preoperative laboratory findings indicated diabetes (abnormal fasting blood glucose \geq 126 mg/dL and standardized hemoglobin A1c concentrations $>$ 7.1%²⁵) in 5 of 16 obese subjects (Table 2) with one subject requiring insulin treatment. One obese subject had abnormal serum liver enzyme levels and 2 were noted to have ketonuria, both \leq 40 mg/dL. Lab tests for glucose control and liver function were within normal limits for all control subjects (not shown).

Table 1. Baseline Chlorzoxazone Pharmacokinetic Parameters for Obese and Control Subjects

	Obese (Median [Range])	Control (Median [Range])
Weight (kg)	172* (104-273)	59 (48-93)
BMI (kg/m ²)	60* (45-100)	21 (18-26)
Cl/F (mL/min)	885* (328-2027)	248 (84-543)
f _{u(CLZ)} (%)	3.7* (2.9-6.7)	2.5 (1.1-4.0)
Cl _u /F (L/min)	27.5* (9.1-55.6)	9.9 (3.2-49.4)
f _{m(6-OH-CLZ)} (%)	68.1 (27.8-96.1)	59.6 (28.1-68.5)
AUC ratio (6-OH-CLZ/CLZ)	0.39* (0.06-0.87)	0.14 (0.03-0.40)

Abbreviation: f_{m(6-OH-CLZ)}, fraction of chlorzoxazone dose metabolized and excreted in urine as 6-OH-CLZ.

*Obese vs. control, Mann-Whitney U Test; $P < .001$.

CLZ Pharmacokinetics and In Vivo CYP2E1 Activity

A summary of CLZ pharmacokinetic parameters in obese and control subjects is presented in Table 1. The median CLZ Cl/F was 3.6-fold greater in the obese group compared with the control group (885 vs. 248 mL/min, $P < .001$). There was considerable interindividual variability with this parameter for both nonobese and obese subjects. Stepwise linear regression analysis using the entire study population indicated that BMI was strongly and significantly correlated with CLZ Cl/F ($r^2 = 0.75$, $P < .001$). Adjusting for gender slightly improved the correlation, with men having a slightly higher clearance than women (r^2 change = 0.04, $P = .027$). A plot of the relationship between the most significant variable (BMI) and CLZ Cl/F is shown in Fig. 1.

CLZ was found to be highly protein-bound (~98%), and obesity was associated with a 50% increase in the plasma f_u (median, 3.7 vs. 2.5%, $P < .001$). After correction for this effect, the unbound Cl/F of CLZ, Cl_u/F, (which accounts for differences in plasma protein binding) still was significantly greater (median, 27.5 vs. 9.95 L/min, $P < .001$) in the obese group compared with controls (Table 1).

Other pharmacokinetic parameters also suggested altered CYP2E1-dependent metabolic clearance with mor-

Table 2. Baseline Glucose Control in Morbidly Obese Subjects

	Preoperative Laboratory Test Values		
	Observed Range	Normal Range	Abnormal
Fasting blood glucose (mg/dL)	90-282	62-122	8/16 (50%)
Glycosylated hemoglobin (standardized %)	4.2-11.6	4.0-6.0	7/16 (44%)
Urine ketones (mg/dL)	0-40	0	2/16 (13%)
Diabetes*	—	—	5/16 (31%)

*Diabetes defined as the presence of abnormal fasting blood glucose ≥ 126 mg/dL and standardized glycosylated hemoglobin levels $>7.1\%$.²⁵

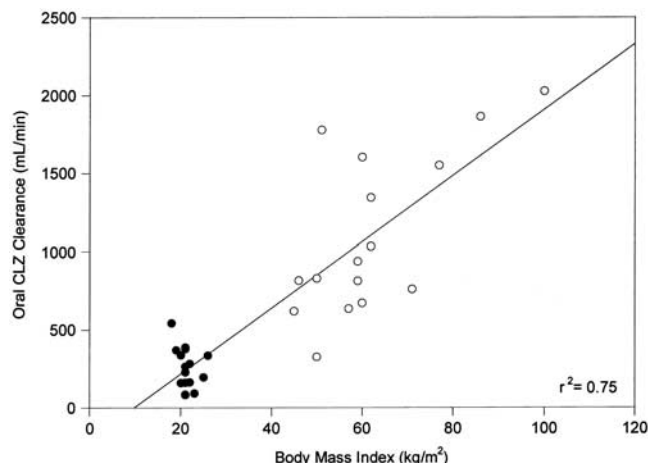


Fig. 1. Correlation of oral CLZ clearance with BMI. **Open boxes** represent obese subjects studied preoperatively. **Closed boxes** represent nonobese subjects.

bid obesity. The 6-OH-CLZ to CLZ area under the curve ratio after administration of a single oral dose of the parent drug represents the ratio of the metabolite formation clearance to the metabolite clearance (mediated by uridine 5'-diphosphate-glucuronosyltransferase). This parameter, shown in Table 1, also was significantly greater in the obese subjects compared with controls (median, 0.39 vs. 0.14, $P = .001$).

Hepatic Histopathologic Correlates of CYP2E1 Activity in Obesity

All morbidly obese subjects studied had characteristic hepatic pathology associated with morbid obesity. Each had macrovesicular fatty infiltration involving more than 5% of hepatocytes in the biopsy specimen (assessed by light microscopy) and 31% had findings consistent with NASH. One subject had cirrhosis (confirmed by trichrome staining) associated with steatohepatitis. When subjects were grouped by the degree of fatty infiltration, there was a trend toward higher unbound oral CLZ clearance (Cl_u/F) with increasing severity of steatosis ($P = .06$, Kruskal-Wallis Test) (Fig. 2). In addition, the unbound Cl/F was significantly higher among subjects with steatosis involving $>50\%$ of hepatocytes compared with those with steatosis in $\leq 50\%$ of hepatocytes ($P = .02$) (Table 3). Although not statistically significant, the median Cl_u/F for the 5 subjects with NASH was approximately 54% higher than the median parameter for subjects without NASH (Table 3).

Effects of Weight Loss After Gastric Surgery

Postoperative Morphometric and Laboratory Measurements. Two obese subjects dropped out of the research study for personal reasons, 1 before the 6-week

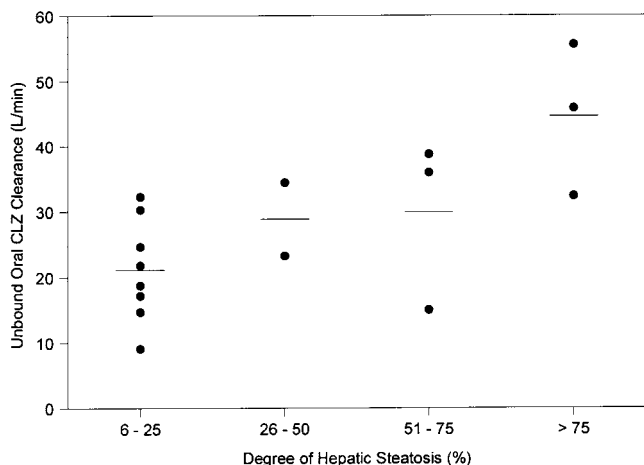


Fig. 2. Segregation of Cl_u/F by degree of hepatic fatty infiltration in morbidly obese subjects. Each point represents a preoperative measurement from a single subject. The **solid lines** represent the median value for the indicated group. Hepatic steatosis defined according to percent of parenchymal cell involvement: mild, 6% to 25%; mild to moderate, 26% to 50%; moderate to severe, 51% to 75%; severe, >75%.

evaluation and 1 before the year 1 evaluation. Therefore, 14 subjects completed the study and are included in the longitudinal analysis. Changes in BMI and glycosylated hemoglobin (Hgb A1c) occurring during the study are shown in Fig. 3. As expected, a relatively profound loss of weight occurred within the first 6 weeks after surgery. This weight loss was one-fourth of the total average weight lost over 12 months, yet it occurred in one eighth of the time studied. One year postoperatively, the median BMI was 43 kg/m², with a median reduction from baseline of 33% (Table 4 and Fig. 3). Six of 14 subjects had a BMI <40 kg/m², and 4 of these achieved a BMI <30 kg/m². Body weight was relatively constant during the 3- to 4-month period preceding the year 1 pharmacokinetic evaluation.

Table 3. Association Between Preoperative Hepatic Steatosis, NASH, and CYP2E1 Activity

	Cl/F^* (mL/min)	Cl_u/F^* (L/min)
Steatosis		
(>50%)‡	1,401 (673-1,778) [P = .08]	37.3 (15.0-55.6) [P = .02]
(≤50%)	922 (328-1345)	22.6 (9.1-34.5)
NASH†		
(+)‡	1,550 (673-1,550) [P = .23]	36.0 (15.0-45.8) [P = .13]
(-)	817 (622-2027)	23.3 (9.1-55.6)

*Median (range) of values reported.

†Defined as the presence of macrovesicular steatosis and two additional features of steatohepatitis, as defined under Methods (based on criteria of Brunt et al.²²).

‡A distribution-free test (Mann-Whitney, 2-tailed test) was used to compare groups with >50% and ≤50% steatosis, and groups with or without NASH.

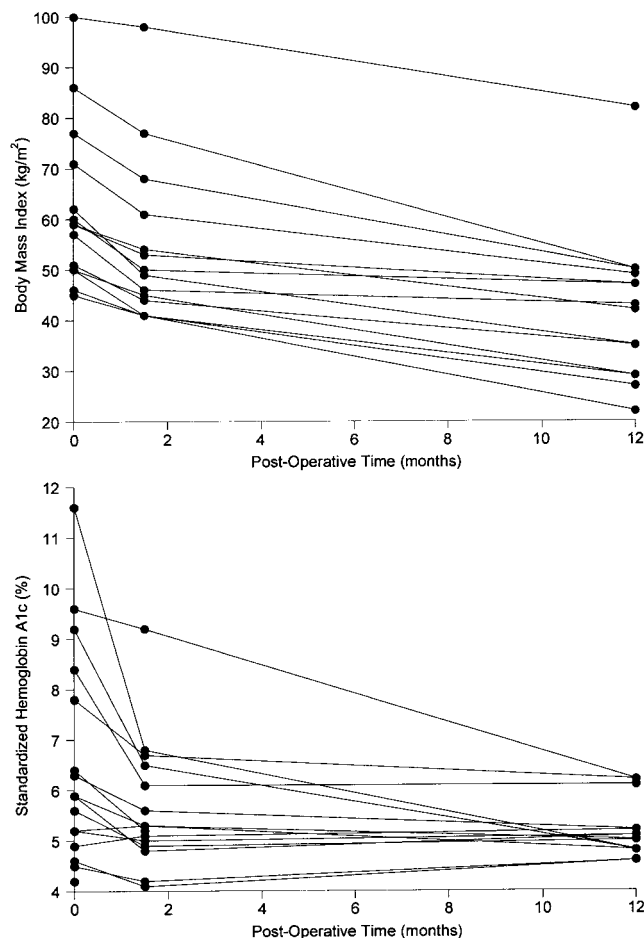


Fig. 3. Six-week and 1-year postoperative changes in BMI (**upper panel**) and percentage hemoglobin A1c-standardized (**lower panel**) in morbidly obese subjects after gastric surgery. Normal range for BMI is 18 to 26 kg/m². The normal range for standardized hemoglobin A1c is 4.0% to 6.0%.

The abrupt weight loss during the first 6 weeks after surgery was associated frequently with ketonuria (6 of 14) and resulted presumably from a marked negative energy balance. Only two subjects exhibited elevated Hgb A1c at year 1, and there was a reduction from the preoperative values in all subjects (Fig. 3). There was no evidence of ketosis at year 1, consistent with the stabilization of weight loss and improvement in glucose control.

Postoperative CLZ Pharmacokinetics. The average percent of the CLZ dose that was recovered as 6-hydroxy metabolite did not differ significantly when the 2 postoperative periods were compared with the preoperative baseline (Table 4), although recovery values for all periods were highly variable (Fig. 4).

There were significant reductions in oral CLZ clearance at week 6 (median decrease of 16%, $P < .01$) and year 1 (median decrease of 46%, $P < .05$) postoperatively (Table 4 and Fig. 5A). There was no significant change in the plasma CLZ f_u at week 6, but by year 1 there was a

Table 4. Postoperative Chlorzoxazone Pharmacokinetic Parameters

	Baseline (Median [Range])	Week 6 (Median [Range])	Year 1 (Median [Range])
Weight (kg)	172 (104-273)	145‡ (95-247)	118‡ (61-208)
BMI (kg/m ²)	59 (45-100)	50‡ (41-98)	43‡ (22-82)
Cl/F (mL/min)	885 (328-2027)	712† (285-2055)	524* (228-1617)
f _u (CLZ) (%)	3.8 (2.9-6.7)	4.3 (2.4-7.1)	3.3* (1.7-4.8)
Cl _u /F (L/min)	26.8 (9.11-55.6)	16.6* (7.70-45.0)	19.5 (7.65-50.4)
f _m (6-OH-CLZ) (%)	68.1 (27.8-96.1)	52.8 (26.7-98.4)	62.6 (28.5-88.7)

NOTE. Preoperative baseline vs. postoperative week 6 or year 1, Friedman Test (n = 14 completed all three phases).

Abbreviation: f_m(6-OH-CLZ), fraction of chlorzoxazone dose metabolized and excreted in urine as 6-OH-CLZ.

*P < .05

†P < .01

‡P < .001

significant decrease compared with preoperative baseline (median decrease of 25%, P < .05). In addition, there was no significant postoperative change in the recovery of 6-OH-CLZ in urine (Table 4) or the plasma CLZ T_{max} (time of peak concentration) for oral CLZ absorption (~2 hours).

With respect to the Cl_u/F, there was a median decrease of 18% (P < .05) at week 6, and by year 1, the median change was -35%. However, the year 1 median change from baseline was not statistically significant (P = .16) as a result of large increases in CLZ Cl/F from the preoperative period in two subjects (Fig. 5B). An explanation for these outliers is unclear, but urinary recovery data from these individuals did not indicate an unusually low fraction of dose absorbed at year 1. For the remainder of obese study subjects, the unbound CLZ plasma clearance paralleled the return of BMI toward normal values. There was a trend for a greater reduction in Cl_u/F with greater

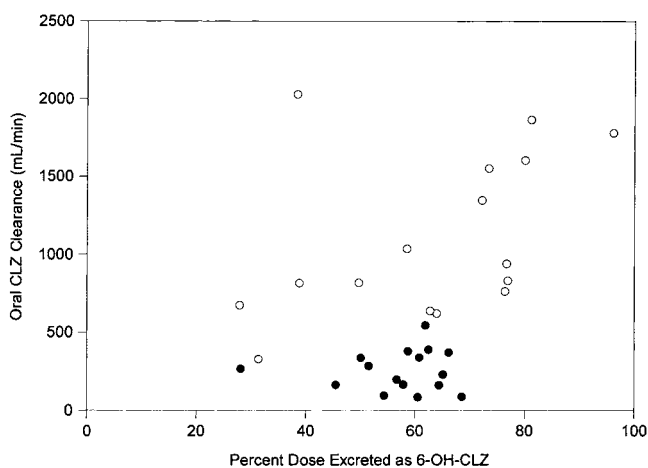


Fig. 4. Relationship between fraction of the administered CLZ dose excreted as 6-OH-CLZ and CLZ clearance. **Open circles** represent obese subjects. **Closed circles** represent non-obese subjects.

absolute change in BMI ($r = 0.44$; $P < .01$). In addition, the 4 subjects with a year 1 BMI <30 kg/m² all exhibited total and unbound CLZ clearances (228-494 mL/min and 7.7-14.1 L/min, respectively) that were comparable to the range observed for nonobese controls (84-543 mL/min and 3.2-49.4 L/min). Moreover, the median total and unbound CLZ clearances for subjects with a 1-year BMI <30 kg/m² were approximately 54% and 63% lower (P = .02 and .02, respectively) than the respective median values for all other subjects with a BMI ≥30 kg/m² (Table 5).

Accumulation of ketone bodies in liver and blood is associated with CYP2E1 induction in animals. Although 6 of 14 obese subjects exhibited ketonuria during the week 6 period, the median total and unbound CLZ clearance for these individuals was not appreciably different from corresponding median values for all other subjects (Cl/F, 920 vs. 658 mL/min; Cl_u/F, 22.4 vs. 16.0 L/min) (Table 5).

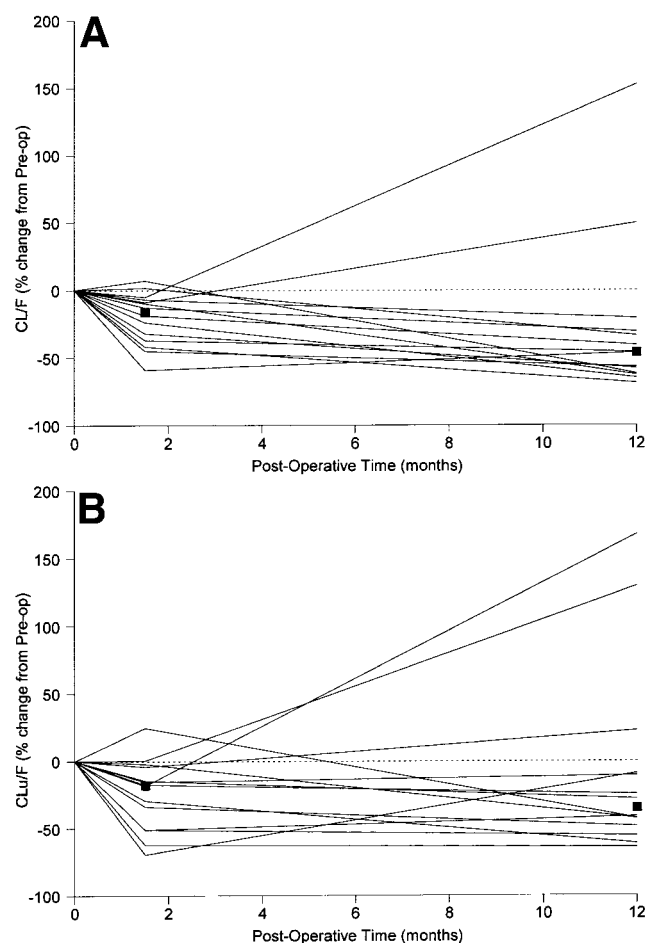


Fig. 5. Postoperative changes in total (A) and unbound (B) oral CLZ clearance. The week 6 and year 1 parameter change (compared with baseline) for individual subjects is represented by a **connecting line**. **Square symbols** represent the median value for the entire group at each postoperative timepoint.

Table 5. Association Between Postoperative Ketosis, BMI, and CYP2E1 Activity

	Cl/F* (mL/min)	Cl _u /F* (L/min)
Ketonuria (6 wk)†		
(+)	920 (425-2027) [P = .40]	22.4 (9.9-28.9) [P = .48]
(-)	658 (285-1394)	16.0 (7.7-45.0)
BMI (1 y)†		
(≥30 kg/m ²)	686 (367-1617) [P = .02]	23.0 (7.6-50.4) [P = .02]
(<30 kg/m ²)	317 (228-494)	8.5 (7.7-14.1)

*Median (range) of values reported.

†A distribution-free test (Mann-Whitney, 2-tailed test) was used to compare groups with or without ketonuria at postoperative week 6, and groups with a BMI ≥30 kg/m² and <30 kg/m² at postoperative year 1.

Discussion

The metabolic clearance of oral CLZ was found to be markedly elevated in individuals suffering from morbid obesity in comparison to nonobese control subjects. This finding is in agreement with results reported by O'Shea et al. in a study of subjects with moderate obesity.²¹ We also observed a positive relationship between the severity of hepatic steatosis and Cl_u/F (Fig. 2) as well as a marked reduction in Cl_u/F after gastroplasty (Table 4 and Fig. 5). These results, coupled with the observation that serial biopsy specimens in morbidly obese subjects show reversal of hepatic steatosis after gastroplasty,²⁶ suggest a causal relationship between CYP2E1 induction and hepatic fatty infiltration.

Histologic data from this study reflected the distribution of hepatic pathology typically observed in the morbidly obese.^{12,27} Subjects with the greatest severity of hepatic steatosis (≥50%) exhibited the highest CYP2E1 activity and may be at greater risk for CYP2E1-dependent oxidative liver injury than those with milder (<50%) fatty infiltration. Moreover, the 4 subjects with BMIs below the threshold of obesity (<30 kg/m²) 1 year after gastroplasty all exhibited an unbound CLZ clearance that was within the range of values for the nonobese control group (Tables 1 and 5). In contrast, among subjects with a BMI ≥30 kg/m² at 1 year, only 1 of 10 exhibited an unbound Cl/F within the range of controls. Thus, hepatic CYP2E1 activity was correlated highly with obesity before and after gastroplasty. Given the positive relationship between obesity and hepatic steatosis before gastroplasty, we speculate that those subjects with elevated CYP2E1 activity postoperatively (1 year) continued to have hepatic steatosis. A postoperative liver biopsy would help address this hypothesis and might be clinically justified in future studies, given the findings of the present study.

There is some evidence to suggest that CYP2E1 induction may occur in humans with poorly controlled diabetes.²⁸ In addition, results from experiments conducted

with human hepatocytes indicate that insulin may suppress the expression of hepatic CYP2E1,²⁹ and that insulin resistance, rather than steatosis, may lead to an induction of steady-state hepatic CYP2E1 content. However, in our morbidly obese population, the presence of preoperative diabetes (elevated fasting blood glucose and glycosylated hemoglobin concentration) was not an independent predictor of CYP2E1 activity. Nevertheless, it is possible that our study size was too small to detect a significant association.

The observation of increased CYP2E1 activity *in vivo* in morbidly obese subjects is supported by the finding of increased CYP2E1 immunohistochemical staining associated with areas of steatosis in biopsies from subjects with NASH.¹³ However, the difference in the Cl/F of CLZ between obese and nonobese subjects also could be attributed to a reduction in the fraction of dose absorbed by obese subjects. The assumption of complete absorption cannot be addressed definitively because of the lack of a suitable intravenous form of CLZ. However, mass balance calculations indicate that ~60% of the oral dose given to normal-weight and morbidly obese subjects was recovered in the urine as 6-OH-CLZ and its glucuronide, and there was no difference between groups. An increase in the apparent Cl/F of the magnitude observed for morbidly obese subjects derived solely from a decrease in drug absorption would imply a large and uniform decrease in the fraction of the dose recovered as 6-OH-CLZ, which was not observed. In fact, there was a trend for greater average recovery in obese subjects when compared with controls (Fig. 4). Thus, the present findings are more consistent with an increase in CYP2E1-mediated formation of 6-OH-CLZ with morbid obesity than altered oral CLZ absorption.

Another variable that should be considered in the interpretation of the CLZ elimination data is the unbound plasma fraction. Extensive plasma protein binding can affect the Cl/F of a drug such as chlorzoxazone by altering the concentration of unbound drug at the P450 enzyme active site. Indeed, a major fraction of the increase in oral CLZ clearance observed in morbidly obese subjects was explained by the increase in plasma f_u. However, the median values for the unbound clearance in the morbidly obese group were still more than 2.5-fold greater than the respective value for control subjects (Table 1), indicating an increase in intrahepatocellular CYP2E1-mediated CLZ metabolism. Our finding that the Cl_u/F for the morbidly obese group at postoperative year 1 was still significantly higher than controls (Tables 1 and 4) can be explained by the fact that most morbidly obese subjects remained obese at year 1. Indeed, the postoperative year 1 mean BMI (43 kg/m²) and mean oral CLZ clearance (679

mL/min) observed in this study was very similar to the mean parameters (42 kg/m² and 741 mL/min) reported by O'Shea et al.²¹

We noted that obese subjects with NASH exhibited higher median CYP2E1 activity than did those without NASH (Table 3). Indeed, there are data supporting a possible role for CYP2E1 in the pathogenesis of liver disease with morbid obesity. Free fatty acids represent endogenous substrates for CYP2E1.³⁰ One possibility is that the binding of these substrates to enzyme might result in an increase in uncoupled oxidation and generation of oxygen-formed radicals and hydrogen peroxide, and subsequent intracellular membrane damage. Whether an increase in CYP2E1 activity is actually involved in the pathophysiologic processes in morbid obesity or simply represents an epiphenomenon merits further investigation. It is possible that up-regulation of CYP2E1 activity establishes a vicious cycle of accelerated liver injury when increased oxidative stress and significant steatosis is present. If so, our data provide supportive evidence that weight loss in morbidly obese individuals is associated with down-regulation of CYP2E1 activity and, thus, may have a beneficial effect in patients with nonalcoholic fatty liver disease.

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