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# Correlation of Body Weight and Composition With Hepatic Activities of Cytochrome P450 Enzymes

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ABSTRACT

Obesity is associated with comorbidities of which pharmacological treatment is needed. Physiological changes associated with obesity may influence the pharmacokinetics of drugs, but the effect of body weight on drug metabolism capacity remains uncertain. The aim of this study was to investigate *ex vivo* activities of hepatic drug metabolizing CYP enzymes in patients covering a wide range of body weight. Liver biopsies from 36 individuals with a body mass index (BMI) ranging from 18 to 63 kg/m$^2$ were obtained. Individual hepatic microsomes were prepared and activities of CYP3A, CYP2B6, CYP2C8, CYP2D6, CYP2C9, CYP2C19 and CYP1A2 were determined. The unbound intrinsic clearance ($CL_{int,u}$) values for CYP3A correlated negatively with body weight ($r = -0.43$, $p < 0.01$), waist circumference ($r = -0.47$, $p < 0.01$), hip circumference ($r = -0.51$, $p < 0.01$), fat percent ($r = -0.41$, $p < 0.05$), fat mass ($r = -0.48$, $p < 0.01$) and BMI ($r = -0.46$, $p < 0.01$). Linear regression analysis showed that $CL_{int,u}$ values for CYP3A decreased with 5% with each 10% increase in body weight ($r^2 = 0.12$, $\beta = -0.558$, $p < 0.05$). There were no correlations between body weight measures and $CL_{int,u}$ values for the other CYP enzymes investigated. These results indicate reduced hepatic metabolizing capacity of CYP3A substrates in patients with increasing body weight.



## Introduction

Obesity, defined by a body mass index (BMI) of 30 kg/m$^2$ or above, is a growing global health issue.[1] Patients with obesity are at increased risk for developing comorbidities, such as cardiovascular disease, diabetes, musculoskeletal disorders and cancer,[2] and are therefore often in need of pharmacological treatment. Various obesity-related physiological changes may alter drug disposition, including increased amounts of adipose tissue and lean body mass, increased blood volume and cardiac output, altered gastrointestinal function and low-grade inflammation.[3,4] Also, changes in drug metabolizing capacity have been indicated based on altered pharmacokinetics of cytochrome P450 (CYP) substrates in patients with

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obesity compared with non-obese controls.[5–9] Such changes in pharmacokinetics may be secondary to physiological changes like increased blood flow to the eliminating organ, or directly due to changed CYP enzyme activity.

The CYP enzymes are involved in the metabolism of 70—80% of all drugs.[10] Of these, up to 50% are metabolized by the CYP3A subfamily, consisting mainly of the polymorphic enzymes CYP3A4 and the CYP3A5.[10,11] Brill et al. reported overall trends towards decreased clearance of CYP3A substrates and increased clearance for substrates of CYP1A2, CYP2C9, CYP2C19 and CYP2D6 in patients with obesity.[5] The liver is the main drug metabolizing organ and expresses a wide range of CYP isoforms,[12] but little data exist on the association between body weight/composition and hepatic activity of CYP enzymes. However, increasing BMI was associated with decreased protein expression of CYP3A4 in a study including liver samples from patients with obesity.[13] Knowledge about the effect of body weight and composition on hepatic CYP activities is relevant for increased insight into the variability in pharmacokinetics between individuals in need of pharmacological treatment.

The aim of this study was to investigate the correlation between body weight/composition and hepatic intrinsic clearance ($CL_{int}$) of CYP3A, CYP2B6, CYP2C8, CYP2D6, CYP2C9, CYP2C19 and CYP1A2 in patients covering a wide range of body weight.

## Materials and Methods

### *Chemicals*

Tris base, protease inhibitor cocktail tablets (Complete, Mini), HEPES buffer and reduced β-nicotinamide adenine dinucleotide phosphate (NADPH) were purchased from Sigma-Aldrich (St. Louis, MO). Sucrose and methanol were obtained from Merck (Kenilworth, NJ). Acetonitrile (ACN) was from Fisher Scientific (Waltham, MA). Ethylenediaminetetraacetic acid (EDTA) was purchased from Ferak (Berlin, Germany). Phosphate buffer was prepared from dipotassium hydrogen phosphate and potassium dihydrogen phosphate from Sigma-Aldrich. CYP probe substrates, metabolites and internal standard (5,5-diethyl-1,3-diphenyl-2-iminobarbituric acid) were prepared in-house or obtained from Sigma-Aldrich. High purity water was prepared with a Milli-Q® Water Purification System (Merck).

### *Patients*

Thirty-six patients enrolled in the COCKTAIL study (trial registration number NCT02386917) were included in the present analysis.[14] Liver biopsies were obtained during Roux-en-Y gastric bypass (RYGB) surgery (20 patients) or cholecystectomy (16 patients). Hepatic CYP activities from the RYGB patients have been described in previous work.[15] Anthropometric measurements and blood samples for clinical chemistry analyses were collected at the day before surgery and biopsy obtainment. Body weight and body composition were determined using the Inbody 720, Body Composition Analyzer (Biospace, Korea). Height was measured to the nearest 1 cm and BMI was calculated as weight in kilograms divided by the square of height in metres. Waist circumference and hip circumference were measured with a stretch-resistant tape parallel to the floor and midway between the 12th rib and the iliac crest, and around the widest portion of the buttocks, respectively. Patients undergoing RYGB were subjected to a three-week low energy diet (<1200 kcal/day) before surgery, while the cholecystectomy patients were not subjected to any defined diet. None of the patients received drugs known to alter the activities of the CYP enzymes investigated in the current study. This study complied with the Declaration of Helsinki. The study protocol was approved by the Regional Committee for Medical and Health Research Ethics (2013/2379/REK sørøst A), and all patients signed a written informed consent.

### *Blood Sample Analyses*

Clinical chemistry analyses were performed at Department of Laboratory Medicine, Vestfold Hospital Trust. Plasma concentrations of C-reactive protein (CRP) and high-sensitivity C-reactive Protein (hs-CRP) were measured with use of immunoturbidimetry (Advia Chemistry XPT systems, Siemens) at Fürst Medical Laboratory (Oslo, Norway). Plasma concentration of markers for inflammation representing various types of immune responses, i.e. C-C motif chemokine ligand (CCL)2 (monocyte chemo-attractant protein 1), CCL4 (macrophage inflammatory protein 1β), CCL11 (eotaxin), C-X-C motif chemokine 10 (CXCL)10 (interferon (IFN)-γ induced protein 10), granulocyte colony-stimulating factor (G-CSF), intracellular adhesion molecule 1 (ICAM-1), IFN-γ, interleukin (IL)-1β, IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, IL-12, IL-17, IL-18, IL-21, leptin, resistin, soluble CD40 ligand (sCD40L), soluble tumour vascular endothelial growth factor A (necrosis factor (TNF) receptor 1 (sTNFR1), TNF-α, VEGF-A) were analysed as a multiplex bead-based immunoassays (Bio-Techne, UK) based on xMAP technology (Luminex, Austin, TX) at the Department of Medical Biochemistry, Diakonhjemmet Hospital, Oslo. The manufacturer's analytical protocol was followed. Values below the lower limit of quantification were estimated to 10% of the lowest standard curve point.

### *Liver Biopsies*

Liver tissue was obtained from parenchyma, by cutting a sample close to the edge of the right liver lobe with cold scissors. The tissue sample was split in four and immediately transferred into individual cryo tubes, snap frozen in liquid nitrogen and stored at −80 °C until analysis.

### *Preparation of Microsomes and* Ex Vivo *CYP Activity Assay*

CYP activities were analysed with methods described in detail in previous work.[15] In short, liver homogenates were prepared with a Potter-Elvehjem homogenizator and microsomal fractions were isolated by differential centrifugation. Specific probe substrates in combined incubations were used for studying enzyme activities; midazolam (CYP3A), bupropion (CYP2B6), amodiaquine (CYP2C8), bufuralol (CYP2D6), diclofenac (CYP2C9), S-mephenytoin (CYP2C19) and phenacetin (CYP1A2). Incubations were carried out at 37 °C for 20 min, and each probe substrate was added in eight different concentrations. Reactions were terminated using ice-cold ACN containing internal standard. Metabolite concentrations (1-OH-midazolam, OH-bupropion, N-desethylamodiaquine, 1-OH-bufuralol, 4-OH-diclofenac, 4-OH-mephenytoin and paracetamol) were quantified against calibrators with liquid chromatography tandem mass spectrometry.

### *CYP Genotyping*

Analysis of *CYP2C9*, *CYP2C19*, *CYP2D6*, *CYP3A4*, *CYP3A5* and *CYP1A2* variant alleles were performed using Taqman-based real-time polymerase chain reaction assays implemented for routine pharmacogenetic analyses at the Center for Psychopharmacology, Diakonhjemmet Hospital. The following variant alleles were included in this study: *CYP2C9*: the reduced-function alleles **2* (rs1799853) and **3* (rs1057910); *CYP2C19*: the *null* alleles **2* (rs4244285), **3* (rs4986893) and **4* (rs28399504) and the gain-of-function allele **17* (rs12248560); *CYP2D6*: the *null* alleles **3* (rs35742686), **4* (rs3892097), **5* (whole gene deletion) and **6* (rs5030655), the reduced-function alleles **9* (rs5030656), **10*

(rs1065852) and **41* (rs28371725), as well as the increased-function allele (whole gene duplication); *CYP3A4*: the reduced-function allele **22* (rs35599367); *CYP3A5*: the *null* allele **3* (rs776746); *CYP1A2*: the increased induction allele **1F* (rs762551).

### Data Analysis

#### Enzyme Kinetics

Enzyme kinetic parameters were determined using untransformed data and GraphPad Prism 7® by fitting the reaction velocity versus substrate concentration data to the Michaelis-Menten model (equation (1)) or the substrate inhibition model (equation (2)):

$$v = \frac{V_{max} \times S}{K_m + S} \quad \text{Eq 1}$$

$$v = \frac{V_{max} \times S}{K_m + S\left(1 + \frac{S}{K_i}\right)} \quad \text{Eq 2}$$

where v is the velocity of the reaction, S is the substrate concentration, $V_{max}$ is the maximum velocity, $K_m$ is the Michaelis constant and $K_i$ is the inhibitor constant.

As the incubations were performed with varying concentrations of microsomal protein, $K_m$ values were adjusted for fraction of unbound drug ($fu_{mic}$). The $fu_{mic}$ values were predicted from physicochemical properties of the substrates and microsomal protein concentration using the Simcyp prediction tool.[16] Unbound intrinsic clearance ($CL_{int,u}$) was calculated from the ratio of $V_{max}$ to $K_{m,u}$.

#### Statistics

The relationship between variables was investigated using Spearman rank order correlation analysis. Simple linear regression analysis was performed to elucidate the relationship between body weight and CYP3A activity using log-transformed data. GraphPad Prism 7 and IBM SPSS Statistics, Version 25 (IBM Corp, USA) software were used.

**Table 1**
Patient Characteristics at the Day Before Surgery and Biopsy Obtainment.

| Characteristic | n = 36 |
|---|---|
| Sex (M/F) | 9/27 |
| Age (years) | 48 (20–62) |
| Body weight (kg) | 97 (47–166) |
| Body mass index (kg/m$^2$) | 36 (18–63) |
| Fat percent (%) | 43 (10–58) |
| Fat mass (kg) | 44 (8–96) |
| Waist circumference (cm) | 109 (34–142) |
| Hip circumference (cm) | 117 (41–179) |
| Waist-hip ratio | 0.90 (0.78–1.11) |
| Fat free mass (kg) | 58 (36–82) |
| Muscle mass (kg) | 32 (19–47) |
| Systolic blood pressure (mmHg) | 120 (103–172) |
| Diastolic blood pressure (mmHg) | 78 (65–106) |
| ASAT (U/L) | 26 (13–58) |
| ALAT (U/L) | 29 (0–89) |
| ALP (U/L) | 70 (27–110) |
| Albumin (g/L) | 40 (16–45) |
| Comorbidity | |
| Type 2 diabetes | 3 |
| Hypertension | 10 |
| Obstructive sleep apnea | 13 |
| Asthma | 6 |
| Cholelithiasis | 20 |
| Present smokers | 5 |

Data presented as median and range or absolute numbers.
ASAT, aspartate aminotransferase; ALAT, alanine aminotransferase; ALP, alkaline phosphatase.

**Table 2**
Distribution of *CYP3A4*, *CYP3A5*, *CYPD26*, *CYP2C9*, *CYP2C19* and *CYP1A2* Diplotypes and Likely Phenotypes.

| Enzyme | Diplotype | Likely Phenotype | n | Reference |
|---|---|---|---|---|
| CYP3A4 | **1/*1* | NM | 34 | [35] |
| | **1/*22* | IM | 2 | |
| CYP3A5 | **1/*3* | IM | 2 | [36] |
| | **3/*3* | PM | 34 | |
| CYP2D6 | **1/*1* | NM | 10 | [37] |
| | **1/*9* | NM | 6 | |
| | **1/*10* | NM | 1 | |
| | **1/*41* | NM | 5 | |
| | **1/*4* | IM | 9 | |
| | **4/*9* | IM | 1 | |
| | **4/*10* | IM | 1 | |
| | **4/*5* | PM | 1 | |
| | **4/*6* | PM | 1 | |
| | **5/*5* | PM | 1 | |
| CYP2C9 | **1/*1* | NM | 25 | [38] |
| | **1/*2* | IM | 10 | |
| | **3/*3* | PM | 1 | |
| CYP2C19 | **17/*17* | UM | 2 | [39] |
| | **1/*17* | RM | 11 | |
| | **1/*1* | NM | 12 | |
| | **1/*2* | IM | 8 | |
| | **2/*17* | IM | 2 | |
| | **2/*2* | PM | 1 | |
| CYP1A2 | **1/*1* | NM | 2 | [40,41] |
| | **1/*1F* | Hyperinducer | 18 | |
| | **1F/*1F* | Hyperinducer | 16 | |

NM, normal metabolizer; IM, intermediate metabolizer; PM, poor metabolizer; RM, rapid metabolizer; UM, ultrarapid metabolizer.

## Results

Patient characteristics and diplotype/likely phenotype distribution for *CYP3A4*, *CYP3A5*, *CYP2D6*, *CYP2C9*, *CYP2C19* and *CYP1A2* are given in Tables 1 and 2, respectively.

Enzyme kinetic parameters for the hepatic CYP-mediated reactions are presented in Table 3. For CYP2B6, CYP2C8, CYP2D6, CYP2C19 and CYP1A2, enzyme activities were not quantifiable in all samples due to low tissue amounts and/or low activities (Table 3).

**Table 3**
Enzyme Kinetic Parameters (Given as Median and Range) for Metabolite Formation Representative of CYP3A, CYP2B6, CYP2C8, CYP2D6, CYP2C9, CYP2C19 and CYP1A2 Activities in Liver Microsomes.

| Enzyme | n | Parameter | Median (range) |
|---|---|---|---|
| CYP3A | 36 | $V_{max}$ | 0.175 (0.073–0.534) |
| | | $K_{m,u}$ | 4 (3–10) |
| | | $CL_{int,u}$ | 43 (11–126) |
| CYP2B6 | 35 | $V_{max}$ | 0.068 (0.016–0.445) |
| | | $K_{m,u}$ | 51 (20–189) |
| | | $CL_{int,u}$ | 1.2 (0.2–11.9) |
| CYP2C8 | 35 | $V_{max}$ | 0.462 (0.123–0.963) |
| | | $K_{m,u}$ | 3 (1–13) |
| | | $CL_{int,u}$ | 124 (31–604) |
| CYP2D6 | 35 | $V_{max}$ | 0.013 (0.005–0.038) |
| | | $K_{m,u}$ | 28 (10–56) |
| | | $CL_{int,u}$ | 0.5 (0.1–1.6) |
| CYP2C9 | 36 | $V_{max}$ | 1.008 (0.058–1.865) |
| | | $K_{m,u}$ | 23 (10–68) |
| | | $CL_{int,u}$ | 40 (6–140) |
| CYP2C19 | 26 | $V_{max}$ | 0.041 (0.010–0.117) |
| | | $K_{m,u}$ | 32 (8–107) |
| | | $CL_{int,u}$ | 1.3 (0.2–2.9) |
| CYP1A2 | 35 | $V_{max}$ | 0.177 (0.048–0.335) |
| | | $K_{m,u}$ | 50 (17–575) |
| | | $CL_{int,u}$ | 2.6 (0.6–9.3) |

$V_{max}$, maximum velocity (nmol/min/mg protein); $K_{m,u}$, unbound Michaelis constant (μM); $CL_{int,u}$, unbound intrinsic clearance (μL/min/mg protein).

**Table 4**
Correlation Coefficients (Spearman's rho) Between Body Weight/Composition and $CL_{int,u}$ (Unbound Intrinsic Clearance) for Reactions Mediated by CYP3A, CYP2B6, CYP2C8, CYP2D6, CYP2C9, CYP2C19 and CYP1A2.

| Body Weight/Composition | $CL_{int,u}$ in HLM | | | | | | |
|---|---|---|---|---|---|---|---|
| | CYP3A n = 36 | CYP2B6 n = 35 | CYP2C8 n = 35 | CYP2D6 n = 35 | CYP2C9 n = 36 | CYP2C19 n = 26 | CYP1A2 n = 35 |
| Body weight | **−0.43**[b] | −0.01 | −0.15 | −0.05 | −0.03 | 0.05 | −0.27 |
| Waist circumference | **−0.47**[b] | −0.05 | −0.23 | −0.07 | −0.03 | 0.00 | −0.32 |
| Hip circumference | **−0.51**[b] | −0.07 | −0.22 | −0.14 | −0.00 | 0.04 | −0.31 |
| Waist/hip ratio | −0.23 | 0.14 | −0.21 | 0.02 | −0.06 | −0.10 | −0.16 |
| Fat percent | **−0.41**[a] | −0.06 | −0.24 | −0.16 | −0.01 | 0.15 | −0.31 |
| Fat mass | **−0.48**[b] | −0.05 | −0.26 | −0.13 | −0.03 | 0.12 | −0.33 |
| Fat free mass | −0.22 | −0.01 | −0.02 | −0.03 | −0.02 | −0.07 | −0.19 |
| Muscle mass | −0.19 | 0.02 | −0.03 | 0.00 | −0.02 | −0.05 | −0.19 |
| Body mass index | **−0.46**[b] | −0.07 | −0.20 | −0.08 | 0.03 | 0.13 | −0.31 |

[a] $p < 0.05$.
[b] $p < 0.01$.

Activity of CYP3A in the liver samples showed significant correlations with parameters defining body weight and composition. The $CL_{int,u}$ values for the CYP3A-mediated reaction were negatively correlated with body weight, waist- and hip-circumference, fat percent, fat mass and BMI (Table 4). In line with this, a negative correlation between body weight and $V_{max}$ values ($r = -0.36$, $p < 0.05$) and a positive correlation between BMI and $K_m$ values ($r = 0.41$, $p < 0.05$) were observed. A linear regression analysis using log-transformed data showed that with a 10% increase in body weight, hepatic $CL_{int,u}$ values for CYP3A decreased with 5% ($r^2 = 0.12$, $\beta = -0.558$, $p < 0.05$, Fig. 1). To further study whether this decrease in hepatic CYP3A activity could be related to inflammation status, correlations between $CL_{int,u}$ and systemic levels of immunological markers were assessed. The $CL_{int,u}$ values for the CYP3A-mediated reaction were negatively correlated with plasma concentrations of IL-6 ($r = -0.34$, $p < 0.05$) (Fig. 2). It should be noted that while plasma levels of IL-6 were below 7 pg/mL in the majority of study subjects, considerably higher levels (>100 pg/mL) were detected in three individuals. The same individuals also deviated from the other patients with respect to elevated levels of CCL4, CCL11, CD40L, IFN-γ, IL-1β, IL-2, IL-4, IL-5, IL-8, IL-10, IL-12, IL-17, IL-18, IL-21, TNF-α and VEGF-A. A stronger correlation between CYP3A activities and IL-6 levels was found when excluding these three patients from the analysis ($r = -0.54$, $p < 0.01$) (Fig. 2). Also, CRP levels in three other patients were >10 mg/L and too high for quantification with the hs-CRP assay. When excluding these from the analysis, hepatic CYP3A activities correlated negatively with plasma levels of hs-CRP ($r = -48$, $p < 0.01$) (Fig. 3). There were also positive correlations between body weight and systemic levels of IL-6 ($r = 0.45$, $p < 0.01$) and hs-CRP ($r = 0.57$, $p < 0.001$). The hepatic activity of CYP3A did not correlate with other circulating inflammatory markers (Supplementary Table 1).

The hepatic $CL_{int,u}$ values for reactions mediated by CYP2B6, CYP2C8, CYP2D6, CYP2C9, CYP2C19 and CYP1A2 were not significantly correlated with body weight or composition variables (Table 4).

No further significant correlations between $CL_{int,u}$ values and body weight and composition variables were found when repeating the correlation analyses after dividing the individuals into subgroups based on genotypes or likely phenotypes, or when excluding smokers (CYP1A2) (data not shown).

## Discussion

The main findings of this study were that hepatic $CL_{int,u}$ values for CYP3A decreased with increasing body weight, and that the $CL_{int,u}$ values were most strongly correlated with waist/hip circumference and fat mass, indicative of excessive fat accumulation, rather than with fat free mass or muscle mass. The former is in line with the negative correlation between BMI and hepatic CYP3A protein expression in patients with obesity (BMI from 34 to 64 kg/m$^2$) found in a previous study.[13] An obvious strength of the present study, however, is that liver tissue was obtained from patients with a wide range of BMI (18−63 kg/m$^2$), i.e. patients with normal weight, overweight, or mild to severe obesity.

In agreement with our finding, *in vivo* studies have shown reduced oral clearance of CYP3A substrates in patients with obesity compared with non-obese individuals, suggesting reduced CYP3A

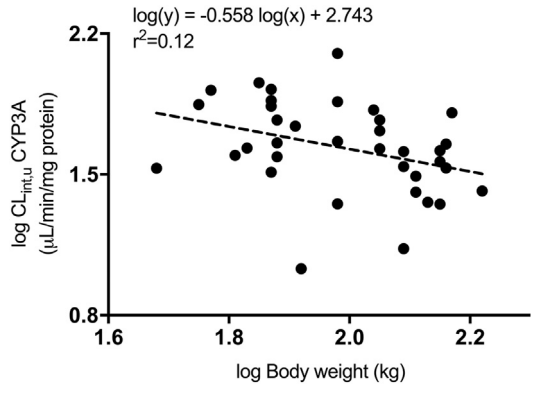


**Fig. 1.** Linear regression model showing the relationship between $CL_{int,u}$ (unbound intrinsic clearance) for CYP3A (Y) and body weight (X) using log transformed data ($r^2 = 0.12$, $\beta = -0.558$, $p < 0.05$).

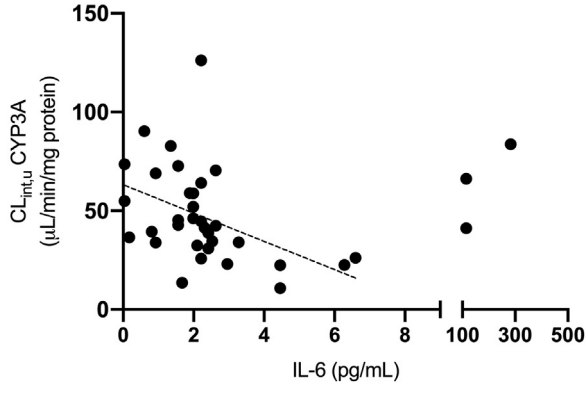


**Fig. 2.** Correlation between systemic concentrations of IL-6 (interleukin-6) and $CL_{int,u}$ (unbound intrinsic clearance) for CYP3A ($r = -0.34$, $p < 0.05$). A stronger correlation was found when removing the three outliers with IL-6 concentrations >100 pg/mL ($r = -0.54$, $p < 0.01$).

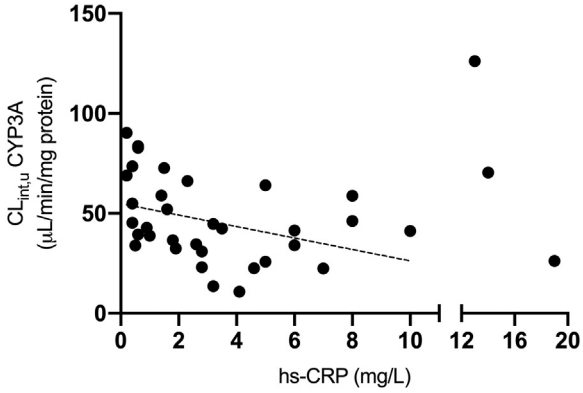


**Fig. 3.** Correlation between systemic concentrations of hs-CRP (high-sensitivity C-reactive protein) and $CL_{int,u}$ (unbound intrinsic clearance) for CYP3A ($r = -0.32$, $p > 0.05$). A stronger correlation was found when removing three individuals with CRP concentrations >10 mg/L measured with a separate assay ($r = -0.48$, $p < 0.01$).

activity.[5,7,17,18] Further substantiating the correlation between body weight and CYP3A activity, increased clearance of midazolam was reported in patients one year after bariatric surgery, indicative of recovery of hepatic CYP3A activity after weight loss.[19,20] However, many other factors associated with obesity, like increased liver volume and increased hepatic blood flow, may also affect drug clearance in these patients. This was suggested to explain the lack of a significant difference in midazolam clearance in a study comparing patients with severe obesity and normal weight volunteers.[6] The effect of obesity on elimination of CYP3A substrates is thus likely to depend on the specific drug in use. Furthermore, as observed in the present study, it should be highlighted that the interindividual variability in CYP3A capacity is considerable, and many other factors like sex, diet, genetics and diseases may also influence the overall CYP3A activity. Nevertheless, the finding in liver tissue from individual patients in the present study, support the hypothesis of obesity-induced suppression of hepatic CYP3A activity.

The $CL_{int,u}$ values obtained for the other CYP enzymes investigated in the present study did not show significant correlations with body weight or body composition variables, suggesting that hepatic activities of these isoforms are not altered to any relevant degree by changes in body weight and altered body composition. In line with this, a limited number of *in vivo* studies have so far not provided conclusive evidence for altered pharmacokinetics of CYP1A2, CYP2D6, CYP2C9 and CYP2C19 substrates in patients with obesity.[5] For some of the CYP isoforms this may in part be due to activities being largely determined by genetic variability, possibly making them less susceptible to environmental and disease-related factors.

The mechanisms behind the correlations between increased body weight/composition variables and enzyme kinetic parameters found in the present study are not clear and likely to be multifactorial. Obesity is associated with chronic low grade inflammation, and the levels of circulating inflammatory cytokines, such as TNF-α, IL-6 and IL-1β, and acute phase reactants, like CRP, are increased compared with non-obese individuals.[21,22] In the present study, hepatic activity of CYP3A was negatively correlated with systemic IL-6 levels. In agreement with this, treatment with IL-6 has been shown to suppress the expression and activity of CYP3A in primary human hepatocytes and a liver cell model *in vitro*.[23–27] Furthermore, IL-6 was recently found to be negatively correlated with 4-β-hydroxycholesterol, an endogenous marker of CYP3A activity *in vivo*, in patients with rheumatoid arthritis.[28] Although possibly confounded by the existing positive correlation between IL-6 and body weight, the present study supports the speculation that inflammatory status, e.g. increased IL-6 levels, may partly mediate the obesity-induced hepatic CYP3A suppression. Another possible contributor to the decreased CYP3A activity is intrahepatic fat accumulation. Altered expression and activity of CYP enzymes have been reported in patients with non-alcoholic fatty liver disease (NAFLD),[29–32] a condition strongly associated with obesity.[33] Recently, 2.7- and 4.1-fold lower $CL_{int}$ values for the 1-hydroxylation of midazolam (CYP3A) were reported in human liver microsomes from deceased organ donors with non-alcoholic fatty liver and non-alcoholic steatohepatitis.[32] As we did not investigate liver tissue samples histologically in the present study, we could not determine the prevalence of NAFLD in this patient population. However, the patients with severe obesity were subject to a three-week low energy diet prior to surgery and biopsy obtainment, which has been shown to significantly reduce liver volume and hepatic fat content.[34] It is therefore possible that the magnitude of reduction in hepatic CYP3A activity with increasing body weight was underestimated in the present study due to a potential recovery in CYP3A activity during the period on diet.

In conclusion, this study showed that hepatic CYP3A activity decreased with increasing body weight, while the activities of CYP2B6, CYP2C8, CYP2D6, CYP2C9, CYP2C19 and CYP1A2 in the liver were not associated with varying BMI.

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## Appendix A. Supplementary Data

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