Nutritional conditioning
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Summary and General Discussion
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“Give different drugs to different patients, for the sweet ones do not benefit everyone, nor do the astringent ones, nor are all the patients able to drink the same thing.”

- Hippocrates (circa 400 BC)

Already in the 4th century BC, the Greek physician Hippocrates recognized “natural diversities” between persons and instructed to give different drugs to different patients. This variability in drug response is often due to differences in drug metabolism and may result in treatment failure, side effects or even toxicity. Many factors contribute to variability in drug metabolism such as patient characteristics (e.g. age and body weight), pharmacologic and environmental factors and also nutritional status, such as fasting (Figure 1).1 Preclinical studies have shown that fasting can alter drug metabolism by modulating the activity of different metabolizing enzymes involved.2 In animals, short-term fasting, defined as the abstinence of food and drinks except water for a period of 24 to 72 hours, can increase drug metabolizing enzyme activity by activation of nuclear receptors such as the pregnane X receptor (PXR) and the constitutive androstane receptor (CAR).3,4 Interestingly, the role of short-term fasting on drug metabolism in general, and on individual enzymes involved in drug metabolism in particular, has hardly been studied in humans prior to the studies described in the current thesis.

This chapter summarizes the results of our studies investigating the effects of short-term fasting on drug metabolism. Furthermore, the main findings are discussed and placed in broader perspective.

Preclinical Study

Following a general introduction to this thesis (Chapter 1), Part I, Chapter 2, describes a preclinical study in mice in which the role of the nuclear receptor CAR in fasting-induced alterations in Cytochrome P450 (CYP)-mediated drug metabolism was investigated. CYP enzymes play an important role in drug metabolism because of their ability to catalyze the oxidative biotransformation of most drugs.5 The CYP enzymes which are important in human drug metabolism are predominantly expressed in the liver. The metabolic CYP enzyme activity in mice can be determined by measuring hepatic mRNA of the specific enzyme. In humans, CYP enzyme activity can be determined using a probe drug which is exclusively metabolized by this enzyme. Differences in concentrations of the probe drug then reflect the activity of the enzyme. In this preclinical study, both wild type and CAR -/- mice received an intraperitoneal administration of (1) five probe drugs for human CYP enzymes (caffeine (CYP1A2), metoprolol (CYP2D6), omeprazole (CYP2C19),
midazolam (CYP3A4) and S-warfarin (CYP2C9)) or (2) a sham injection. The sham injection contained saline and was administered to the control groups to determine the presence of a placebo effect caused by the injection itself. The mice were either fed ad libitum or fasted for 24 hours. Blood was sampled at predefined intervals to determine exposure of the probe drugs and hepatic mRNA expression was measured of the mouse orthologues of the five CYP enzymes. Fasting induced CAR mRNA expression in mice but had clear differential effects on the expression of CYP enzymes. Fasting decreased the expression of the mouse orthologues of human CYP1A2 and CYP2D6, whereas it increased the expression of the orthologues for CYP3A4 and CYP2C9. This indicates that the fasting-induced changes were largely independent of CAR. Furthermore, the fasting-induced alterations in CYP-mediated clearance of the probe drugs were largely independent of CAR. Besides the role of CAR in fasting induced regulation of drug metabolizing enzymes, additional preclinical research may also focus on other nuclear receptors such as PXR, AhR (aryl hydrocarbon receptor) and PPARα (peroxisome proliferator-activated receptor alpha).

**Figure 1:** Factors that contribute to variability in drug metabolism
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Effects of Fasting on Phase I Drug Metabolism

Part II focusses on the effects of fasting on phase I drug metabolism. In phase I, reactive and polar groups are introduced into their substrates by oxidation, reduction or hydrolysis. CYP enzymes are the most important enzymes in this phase of drug metabolism. In humans, five CYP isoforms predominantly present in the liver are frequently involved in drug metabolism: CYP1A2, CYP2D6, CYP2C9, CYP2C19 and CYP3A4. Together, these isoforms are responsible for more than 70% of all phase I dependent metabolism of drugs. In addition to the administration of a single CYP-specific probe drug at a time, it is possible to simultaneously administer multiple probe drugs, which is commonly known as the “cocktail approach”. In Chapter 3 the development and validation of an LC-MS/MS method for the simultaneous quantification of a five probe cocktail is described. Compared to other methods, this method only requires a single (1) and simplified (2) sample preparation step, less LC systems (3), and a small volume (10 μl) of plasma (4) for the quantification of the cocktail probe drugs. The method was validated for the quantification of the probe drugs administered at therapeutically low doses. Although no side effects have been reported of a single administration of the drug cocktail, the sensitivity of the method allows to further decrease the doses to subtherapeutic levels. The lower limits of quantification may even further be decreased by using additional sample preparation methods such as solid phase extraction (SPE) which can selectively remove interferences thereby improving the quantitation. This could allow microdosing of the five probe drugs (maximum dose of 100 μg). Microdosing has recently been introduced in clinical studies to minimize the risk of harmful events to human subjects. Another advantage of our new method is the small volume of blood required which allows testing the drug cocktail in a broader group of patients (e.g., elderly or children).

This validated new method was used in the clinical trial described in Chapter 4 in which the effects of short-term fasting on CYP-mediated drug metabolism were studied. In a randomized cross-over study design, nine healthy subjects orally ingested a cocktail consisting of five CYP-specific probe-drugs (caffeine (CYP1A2), S-warfarin (CYP2C9), omeprazole (CYP2C19), metoprolol (CYP2D6) and midazolam (CYP3A4)) on two occasions: after an overnight 10h fast (control) and after 36h of fasting. In addition, the effects of 24h of fasting on hepatic mRNA expression of CYP-isoforms corresponding with the five studied CYP-enzymes in humans were studied in Wistar rats. In the healthy subjects, 36h of fasting increased oral caffeine clearance by 20% (p=0.03) which was in accordance with the fasting induced hepatic mRNA expression of Cyp1a2 in rats. Conversely, short-term fasting decreased oral S-warfarin clearance by 25% (p<0.001). This was in accordance with the fasting decreased hepatic mRNA expression of the CYP enzyme corresponding to human CYP2C9 in rats. Similar to CYP1A2, fasting increased hepatic mRNA expression of the orthologues of human CYP2C19, CYP2D6, and CYP3A4
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(p<0.05). However, short-term fasting did not affect the clearances of the corresponding probe drugs omeprazole, metoprolol and midazolam in humans. This discrepancy may be due to interspecies differences and illustrates, together with the study performed in mice (Chapter 2), that none of the animal species are completely similar to humans with respect to all CYP enzyme activities. Nonetheless, similarities are present for some specific CYP isoforms, for example Cyp1a2 and Cyp2c11 in male rats which may be used to study the effects of fasting on the corresponding enzymes CYP1A2 and CYP2C9 in humans. Furthermore, the results of this study demonstrate that short-term fasting in humans alters cytochrome P450-mediated drug metabolism in a non-uniform pattern.

Because the drug cocktail was administered orally in this study, the effect of fasting on the observed differences in exposure (AUC, area under the plasma concentration-time curve) might also be explained by other factors, rather than only by an effect of fasting on intrinsic clearance (CYP enzyme activity). These other potential factors include bioavailability (F) and/or protein binding. This is described by the well-stirred model of drug metabolism:

\[ \text{AUC}_{\text{oral}} = (F_{\text{abs}} \times F_{G} \times \text{Dose})/(f_{u} \times \text{Cl}_{\text{int}}) \]  
(Equation 1)

in which \(F_{\text{abs}}\) represents the fraction of administered drug absorbed into the gut wall; \(F_{G}\), the fraction that gets through the gut wall unchanged; \(f_{u}\) the fraction unbound drug in plasma and \(\text{Cl}_{\text{int}}\) the intrinsic CYP-mediated clearance. In order to distinguish between the effects of short-term fasting on these three factors, Chapters 5 and 6 studied the effects of fasting on bioavailability and protein binding of the cocktail drugs, respectively.

Chapter 5 presents the outcomes of a randomized, controlled, crossover study performed in healthy subjects in which the effects of short-term fasting on systemic CYP-mediated drug metabolism were studied. In order to distinguish between the effects of short-term fasting on bioavailability and systemic clearance, the previously used drug cocktail was administered on four occasions: oral (1) and intravenous (2) administration after an overnight 10-hours fast (control) and oral (3) and intravenous (4) administration after 36 hours of fasting. Short-term fasting increased systemic clearance of caffeine (17%, \(p=0.04\)) and metoprolol (13%, \(p<0.01\)), whereas it decreased \(S\)-warfarin clearance (19%, \(p<0.01\)). Although short-term fasting affected systemic clearance mediated by several CYP enzymes, fasting did not affect oral bioavailability of the five probe drugs. The findings are in line with the previous study (Chapter 4) in which the cocktail was administered orally. In this study, short-term fasting also altered metoprolol clearance, which is likely due to increased statistical power because of a larger sample size.

Chapter 6 describes the results of a study performed to determine if fasting alters protein binding of the cocktail drugs. The study demonstrates that short-term fasting did not alter protein binding of caffeine, metoprolol and omeprazole compared to the
control intervention. This supports our previous findings that short-term fasting alters the pharmacokinetics of caffeine and metoprolol by affecting intrinsic CYP-mediated drug metabolism. However, additional research is warranted for the highly protein bound drugs midazolam and S-warfarin. Since in a significant number of samples free concentrations were below the detection limit, the results for both drugs are not fully certain and an effect of fasting on protein binding in addition to altered CYP3A4- and/or CYP2C9-mediated metabolism might be present. For future studies using midazolam or S-warfarin as a probe, the measurement of free instead of total plasma concentrations would therefore be preferred. This requires more sensitive analytical methods which are able to detect changes in the very low free plasma concentrations of this highly protein bound drug. For example by using additional sample preparation methods such as solid phase extraction (SPE). This would enable further optimization of the analytical method in terms of lowering the lower limit of quantification. At this moment, both the European Medicines Agency (EMA) and the US Food and Drug Administration (FDA) recommend S-warfarin as a suitable probe drug for CYP2C9 because of its selectivity for this specific enzyme and linear pharmacokinetics. However, since the availability of a highly sensitive method is of such great importance, one may question the suitability of midazolam and S-warfarin as probe drugs. The criteria for a suitable probe drug as mentioned in the FDA and EMA guidelines may well be expanded. An ideal probe drug should not only be enzyme selective (1) and have linear pharmacokinetics (2), but also have low protein binding (3), measurable by suitable analytical methods for quantification (4), (commercial) available (5), safe to administer (6) and, when used as a cocktail, the probe drugs should not interact with each other (7). Unfortunately, there are no ideal probes for CYP2C9 and CYP3A4. Remarkably, other probes mentioned in literature, such as tolbutamide or phenytoin (CYP2C9) or dextromethorphan (CYP3A4), are also highly protein bound. For research purposes, further optimization of the analytical method would therefore be the best option.

**Effects of Fasting on Phase II Drug Metabolism**

In *Part III*, the effects of short-term fasting of 36 hours on several drug metabolizing enzymes involved in phase II drug metabolism are presented. In *Chapter 7* the effects of two nutritional conditions, short-term fasting and a short-term high fat diet (HFD) on CYP3A4-mediated hydroxylation (phase I metabolism) and uridine diphosphate-glucuronosyltransferase (UGT)-mediated glucuronidation (phase II metabolism) are presented by studying the pharmacokinetics of midazolam as a probe (Figure 2). Interestingly, in contrast to our previous findings presented in chapters 4 and 5, short-term fasting increased systemic clearance of midazolam (CYP3A4) by 12% (p<0.01). This may be explained by the application of a different method to analyze the data in this study. After oral and intravenous administration of the drug cocktail containing midazolam
(chapters 4 and 5), data were analyzed using the nonlinear mixed-effects modeling software NONMEM. Instead of the more common noncompartmental analysis for this type of cross-over designed studies, nonlinear mixed effects compartmental modeling was preferred in order to accurately study time-dependent effects of fasting on drug metabolism.\(^7\) However, as illustrated in Figure 3 for the control versus short-term fasting intervention, significant differences were observed in the number of metabolite samples below the lower limit of quantification (BLOQ) between the interventions. Instead of excluding the BLOQ data from analysis which may bias the results, a different NONMEM method (M3 method) was used to account for these data.\(^6\) In the presence of a significant number of BLOQ samples (\(\geq 10\%\)), this method enables a more accurate fit and simulation of the data.\(^7\)

**Figure 2:** Simplified metabolic pathway of midazolam

Furthermore, this study demonstrates that short-term fasting decreased UGT-mediated midazolam metabolism to 1-OH-midazolam-glucuronide by 13\% (p<0.01). In contrast, a short-term high fat diet did not affect midazolam metabolism.
In order to study the effects of fasting on other phase II drug metabolizing enzymes then UGT, such as sulfotransferases (SULT) and glutathione-S-transferases (GST), acetaminophen (paracetamol) was used as a probe. First, Chapter 8 describes the development and validation of an LC-MS/MS method to simultaneously quantify acetaminophen and six of its main metabolites. In clinical practice, acetaminophen plasma concentrations are quantified in case of an overdose which can cause hepatotoxicity. Quantification is usually performed by immunoassay or enzymatic colorimetric techniques because these methods are relatively simple and require short run times per sample. Despite a number of advantages, these methods have some limitations. For example, these methods only quantify the parent compound acetaminophen, but acetaminophen-induced hepatotoxicity is known to be caused by its CYP-mediated metabolites of which NAPQI is the most
important (Figure 4). The validated LC-MS/MS method can serve as a good reference method, especially in the case of immunoassay-interfering substances in the analyte sample that generate false-positive results and for the quantification of acetaminophen metabolites that are important in acetaminophen-induced hepatotoxicity. However, additional research is warranted to study the role of the metabolites including corresponding therapeutic and toxic plasma concentration ranges, instead of only acetaminophen, as marker(s) for acetaminophen-induced hepatotoxicity.

Figure 4: Simplified metabolic pathway of acetaminophen

Finally, Chapter 9 presents the results of a randomized, controlled, crossover study in healthy subjects in which the effects of short-term fasting (36 hours) on exposure of the parent compound acetaminophen and six of its main metabolites have been studied after oral administration of 1000 mg (2 tablets of 500 mg) of acetaminophen. Short-term fasting decreased the apparent clearance of acetaminophen by 10% (p<0.001) and of its sulfation and oxidation mediated metabolites acetaminophen-sulfate (17%, p<0.001), 3-cysteiny1-acetaminophen (12%, p<0.001) and 3-methoxy-acetaminophen (12%, p<0.001), respectively (Figure 4). The decreased apparent clearances of 3-cysteinyl-acetaminophen and 3-methoxy-acetaminophen reflect increased exposure of both metabolites which is probably due to an increased fraction of acetaminophen metabo-
lized to these metabolites by induction of the Cytochrome P450 enzymes involved. Similarly, the increased exposure of acetaminophen-sulfate can either be due to (1) increased activity of sulfotransferases (SULT) which catalyze the metabolism of acetaminophen to acetaminophen-sulfate and/or (2) decreased activity of transporter proteins involved in active secretion of acetaminophen-sulfate, such as Mrp2 and Bcrp. Additional research should be performed to study the effects of fasting on these, and on other, drug transporter proteins which are also regulated by nuclear receptors.

Short-term fasting did not affect the exposure of the UGT-mediated metabolite acetaminophen-glucuronide. This would appear to be in contrast with the finding that short-term fasting decreased UGT-mediated midazolam metabolism (Chapter 7). However, different UGT isoforms are involved in the metabolism of midazolam and acetaminophen. Midazolam is predominantly metabolized by UGT1A4 and UGT2B4/2B7, whereas acetaminophen is metabolized by UGT1A1 and UGT1A6. Therefore, our findings imply that short-term fasting differentially affects UGT isoforms.

Unfortunately, the effects of short-term fasting on GST-mediated metabolism could not be evaluated since all plasma concentrations of the GST-mediated metabolite of acetaminophen (acetaminophen-glutathione) were below the lower limit of quantification (10 μg/L). Similar findings of immeasurable acetaminophen-glutathione concentrations have been reported after acetaminophen administration in neonates and morbidly obese patients. This may be due to the relatively short half-life of this metabolite in humans compared with other species due to a relatively high expression of gamma-glutamyl transpeptidase, which is involved in the conversion of acetaminophen-glutathione to 3-cysteinyl-acetaminophen. Another option to study the effects of fasting on GST-mediated drug metabolism would be to measure total GST activity using a dedicated GST assay kit (e.g. Sigma Aldrich, Poole, United Kingdom). However, this assay does not discriminate between different GST-isoforms which may also be differentially affected by fasting as shown for CYP- and UGT-enzymes.

This study demonstrates that short-term fasting increases acetaminophen exposure and the exposure of its CYP-mediated metabolites in humans. The CYP-mediated metabolite 3-cysteinyl-acetaminophen is derived from the hepatotoxic metabolite NAPQI, but also the CYP-mediated metabolite 3-methoxy-acetaminophen has been associated with acetaminophen related hepatotoxicity. Therefore, the increase in exposure of both metabolites implies that fasting increases the risk of acetaminophen induced hepatotoxicity in humans. Additional research on the clinical relevance of this finding is recommended.
Fasting Differentially Affects Drug Metabolizing Enzymes

Although preclinical studies suggest that short-term fasting increases the activity of nuclear receptors thereby increasing the activity of drug metabolizing enzymes, the clinical studies presented in this thesis demonstrate that short-term fasting differentially affects enzymes which are important in human drug metabolism. Short-term fasting increases the activity of phase I drug metabolizing enzymes CYP1A2, CYP2D6 and CYP3A4 (reflected by increased systemic clearances of caffeine, metoprolol and midazolam, respectively) whereas it, presumably, decreases the activity of CYP2C9 (reflected by decreased S-warfarin clearance). Furthermore, short-term fasting increases the activity of CYP enzymes involved in the conversion of acetaminophen towards its hepatotoxic metabolites. Regarding phase II drug metabolizing enzymes, short-term fasting decreases SULT-mediated (acetaminophen) and UGT1A4/2B4/2B7 mediated (midazolam) metabolism but not UGT1A1/UGT1A6 (acetaminophen) mediated metabolism. Fasting induced regulation of drug metabolizing enzymes seems to be more complex than expected from preclinical research and possibly consists of (multiple) positive and negative feedback loops. Additional research is recommended to further reveal the molecular and pharmacological mechanisms underlying our observations and the clinical implications of our findings.

Clinical Relevance

The results presented in this thesis demonstrate that short-term fasting contributes to variability in drug metabolism. However, it is questionable to what extent this is clinically relevant, since the effects of fasting found appear to be small (10-20 %). For most drugs these effects of fasting found within subjects are much smaller than the between-subject (interindividual) variability in drug metabolism. Nevertheless, this might be relevant for patients with fasting related consequences such as weight loss and cachexia using drugs with a small therapeutic range and/or in combination with other factors that contribute to variability in drug metabolism (Figure 1). Examples of drugs with a small therapeutic range which are metabolized by enzymes affected by short-term fasting are certain antidepressants (e.g., amitriptyline (CYP2D6/CYP2C19), clomipramine (CYP1A2/CYP2D6/CYP2C19), some atypical antipsychotics (e.g. clozapine (CYP1A2), olanzapine (UGT/CYP1A2/CYP2D6) and phenytoin (anti-epileptic agent, CYP2C9/CYP2C19).

Our findings may also be applicable to oral cytotoxic agents which are commonly used in oncology and extensively metabolized by drug metabolizing enzymes. Many of these cytotoxic agents are approved to be administered at fixed doses. In oncological patients with fasting related problems this flat-fixed dose regimen may theoretically result in treatment failure or, conversely, in toxicity.


**Personalized Nutrition**

Besides considering short-term fasting as a factor that contributes to variability in drug response, there is growing evidence for short-term fasting applied as therapy. As for medicine, Hippocrates already advocated the practice of fasting in the 4th century BC, believing that to eat when sick would be to feed the illness. However, it took centuries to get a better understanding of nutrition, including fasting, applied as therapy and/or to improve health. Today, research is performed in different fields to personalize nutrition. For example by organizations such as the Dutch organisation for applied scientific research (TNO) which collaborates with different parties such as food producers and universities to implement personalized nutrition at large scale because this improves people’s health behavior to prevent diseases such as obesity and diabetes. Nutrition also draws significant public attention because diet, together with exercise, remains the mainstay of lifestyle change. Although a lot of diets are not evidence based and often replaced by others shortly after introduction, a promising fasting based diet developed by Longo et al. was recently discussed in one of the leading medical journals JAMA. Subjects had to restrict their calories by 60 % for five consecutive days a month over three months to get the benefits of the so-called “fasting-mimicking diet”. This diet is based on the interesting hypothesis that post-fasting activates stem cells which drive the health and longevity benefits of this diet.

Furthermore, recent studies have shown that short-term fasting can have a positive effect in the treatment of cancer. By short-term fasting, the susceptibility to chemotherapy can differ between healthy somatic and cancer cells, a phenomenon called differential stress resistance. It is likely that the effects of short-term fasting will be enhanced if the period of fasting is prolonged, but although easily prescribed, adherence to a fasting protocol will be challenging if not impossible in cases of serious and/or terminal diseases. Therefore, clinical trials are now being performed in the field of oncology with low protein fasting mimicking diets to ease the burden of prolonged fasting.

**Future Perspectives**

A better understanding of the effects of fasting can improve the efficacy and safety of pharmacotherapy for the individual patient. In order to optimize drug treatment in oncology, it would be of interest to further study the effects of low protein diets on differential stress resistance, but also to study the effects of these fasting mimicking diets on the metabolism of the drugs administered during these diets. In particular, research should focus on anti-cancer drugs that are metabolized by phase I or phase II drug metabolizing enzymes which are affected by fasting such as the anti-mitotic cytostatics paclitaxel and docetaxel (CYP3A4) or the topoisomerase inhibitors etoposide (CYP2C9, CYP3A4) and irinotecan (CYP3A4, UGT1A1). Irinotecan is used in the treatment of metastatic colon cancer. It is a prodrug which needs to be metabolized by carboxyl
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Esterase to the active metabolite SN-38 to exert its anti-tumour activity. SN-38 is further metabolized by UGT1A1 to the inactive metabolite SN-38-glucuronide. Irinotecan is not entirely metabolized to SN-38. Another fraction of the drug is directly metabolized by CYP3A4 to inactive metabolites. Previously, Mathijssen et al have shown that St. John’s wort, which increases CYP3A4 enzyme activity, increases the formation of the CYP3A4-mediated inactive metabolites thereby decreasing the exposure of the active metabolite SN-38. Their findings indicate that patients on irinotecan treatment should refrain from taking this herb. Although the studies presented in this thesis did not show an effect of short-term fasting on UGT1A1-mediated clearance (acetaminophen), short-term fasting increased CYP3A4-mediated clearance (midazolam). Despite the proposed beneficial effects of fasting-mimicking diets in terms of differential stress resistance, we therefore hypothesize that short-term fasting could decrease the exposure of the active metabolite SN-38 which may result in treatment failure.

Furthermore, the results presented in this theses demonstrate that short-term fasting is not the only factor contributing to variability in drug metabolism. This is also true for other factors mentioned such as patient characteristics, physiological and genetic factors (Figure 1). Following completion of the Human Genome Project a lot of research has been performed in the era of pharmacogenetics. However, pharmacogenetics alone, and also when studied together with patient characteristics (e.g. age and sex), does not adequately describe all variability in drug response. Therefore, there is growing interest in so called systems pharmacology which is based on interdisciplinary translational science. Systems pharmacology is an holistic approach to pharmacology that aims to develop a global understanding of the interaction between pathophysiology and drug action in humans to explain, simulate and predict the net clinical drug response. It approaches different factors that contribute to variability in drug metabolism as networks of interaction and uses bioinformatics and statistical techniques (e.g. physiologically based pharmacokinetic models (PBPK)) to integrate and interpret these interactions. Since fasting related consequences such as weight loss or cachexia are common in patients, especially in patients with advanced cancer, additional research should be performed to study the effects of short-term fasting together with other factors known to contribute to variability in drug metabolism by using a systems pharmacological approach.

Increasing knowledge on factors contributing to variability in drug metabolism such as short-term fasting will improve personalized medicine, in which drug therapy is tailored to the individual patient, by further characterization of different patient groups. This will also result in smaller patient groups which impedes performance of the usually large phase III randomized controlled clinical trials (RCTs) that are designed to assess the effectiveness of new treatments. RCTs are still considered important in the evidence based development and registration of drugs. However, changes in the field of clinical trials but also at regulatory level are required to let personalized medicine become a clinical success.
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From a clinical perspective, characterization of a patient’s variability in drug metabolism can be useful to optimize individual treatment outcome. The drug cocktail described in this thesis can be used to phenotype drug metabolism by CYP enzymes in humans. However, the determination of complete area under the plasma concentration-time curves (AUCs) will be too invasive and time consuming for the clinical setting. Therefore, a limited sampling strategy should be developed to be able to characterize the pharmacokinetics of a typical patient using only one or two blood samples after administration of the cocktail.34

Another valuable tool for tailoring the dose of prescribed drugs to the individual characteristics of a patient is therapeutic drug monitoring (TDM). TDM is based on the measurement and interpretation of drug concentrations in e.g. plasma or serum and aims to improve patient care by adjusting the dose of a drug to achieve concentration levels within an established therapeutic range.35 Although this requires the availability of appropriate analytical methods, it can also be used to control compliance which is another, non-physiological, factor contributing to variability in drug response.

Conclusion
The studies presented in this thesis provide evidence that short-term fasting contributes to variability in human drug metabolism. Although variability in drug metabolism can not be attributed to the effects of fasting alone, short-term fasting may be relevant for drugs with a small therapeutic range and/or in combination with other factors that contribute to variability in drug metabolism.

Short-term fasting is part of a complex interplay of factors affecting drug response. Knowledge of the effects of short-term fasting contributes to personalized medicine in which drug therapy is tailored to the individual patient and drug response can be predicted accurately leading to optimized health care.
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