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Physiologically based pharmacokinetic (PBPK) modeling for dynamical liver function tests and CYP phenotyping

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M. Sc. Jan GRZEGORZEWSKI

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Gutachter

1. Dr. Matthias König
2. Prof. Dr. Hans-Peter Herzel
3. Prof. Dr. Wilhelm Huisinga

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Prof. Dr. Julia von Blumenthal

Dekan der Lebenswissenschaftlichen Fakultät

Prof. Dr. Dr. Christian Ulrichs

Tag der mündlichen Prüfung

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Declaration of Authorship

I, M. Sc. Jan GRZEGORZEWSKI, declare that this thesis titled, "Physiologically based pharmacokinetic (PBPK) modeling for dynamical liver function tests and CYP phenotyping" and the work presented in it are my own. I confirm that:

- This work was done wholly or mainly while in candidature for a research degree at this University.
- Where any part of this thesis has previously been submitted for a degree or any other qualification at this University or any other institution, this has been clearly stated.
- Where I have consulted the published work of others, this is always clearly attributed.
- Where I have quoted from the work of others, the source is always given. With the exception of such quotations, this thesis is entirely my own work.
- I have acknowledged all main sources of help.
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"New directions in science are launched by new tools much more often than by new concepts. The effect of a concept-driven revolution is to explain old things in a new way. The effect of a tool-driven revolution is to discover new things that have to be explained."

Freeman Dyson

Abstract

Cytochrome P450 (CYP) phenotyping and dynamic liver function testing are essential methods in clinical practice. These methods utilize the pharmacokinetics (PK) of test substances and their metabolites to gain insight into the liver's metabolic capacity and the activity of enzymes and transporters. Despite an extensive body of literature, many aspects affecting liver function and CYP activity are not well understood. Liver function tests are not only influenced by numerous characteristics of a studied subject but also by the specifics of individual study procedures. A key challenge is to disentangle the various factors which influence the outcome of the measurements from each other to study their influence on the dynamic liver function and CYP phenotype. In this work, the challenge was addressed through meta-analysis and physiologically based pharmacokinetic (PBPK) modeling.

As a foundation, an open pharmacokinetics database (https://pk-db.com) was developed and pharmacokinetics data were curated for a wide range of test substances. To my knowledge, PK-DB currently contains the largest open pharmacokinetic dataset on substances used for phenotyping and dynamical liver function testing. The dataset allowed for identifying and quantifying demographic and racial bias (sex, ethnicity, age, health), reporting errors, and inconsistencies in pharmacokinetic literature.

Based on the data, a caffeine pharmacokinetics meta-analysis was conducted concerning various factors affecting liver function and CYP1A2 activity. In particular, meta-analysis and data integration solidified existing knowledge on the effects of smoking, oral contraceptives, multiple diseases, and co-medications on caffeine pharmacokinetics. Similarly, the measurement accuracy of caffeine concentration in saliva versus plasma was quantified, and the effect of dosing amount and sampling timing for phenotyping were analyzed.

In addition, the impact of CYP2D6 polymorphism was investigated. Therefore, a PBPK model of dextromethorphan (DXM) and its metabolites dextrorphan (DXO) and dextrorphan O-glucuronide (DXO-Glu) was developed, and calibrated and validated with pharmacokinetics data. The variability in CYP activity was modeled based on *in vitro* data. The model can predict individual plasma concentrations and urinary amounts of DXM, DXO, and DXO-Glu and the metabolic phenotype based on the individual's CYP2D6 genotype and physiological characteristics. The analyses suggest that most of the variability in the pharmacokinetics of dextromethorphan can be attributed to the variability in CYP2D6 and CYP3A4 enzyme kinetics. Among various other investigations, the influence of ethnicity on CYP2D6 activity was also investigated.

Contributions to PK data curation and PBPK model development were also made for other phenotyping and liver function test substances (chlorzoxazone, codeine, diazepam, galactose, indocyanine green (ICG), metoprolol, midazolam, omeprazole, pravastatin, simvastatin, talinolol, and torasemide). For ICG, in particular, the impact of hepatic blood flow, cardiac output, and body weight, as well as the survival probability after partial hepatectomy, were investigated

by PBPK modeling. Notably, the studying of the various test substances was only made possible by a systematic and standardized workflow that facilitated data integration, data sharing, the creation of reproducible PBPK models, and the standardized integration of data and models.

In conclusion, a pharmacokinetic database, methods, and workflows for the analysis of test compounds used in dynamical liver function testing and CYP phenotyping were established. Factors affecting CYP phenotyping and liver function testing were investigated by meta-analysis and PBPK modeling. The models developed in this effort have the potential to impact personalized medicine and to increase the precision of dynamic liver function tests in clinics.

Zusammenfassung

Die Phänotypisierung von Cytochrom P450 (CYP) und Leberfunktionstests sind wichtige Methoden in der klinischen Praxis. Diese Methoden nutzen die Pharmakokinetik (PK) von Testsubstanzen und ihren Metaboliten, um Einblicke in die Stoffwechselkapazität der Leber und in die Aktivität von Enzymen und Transportern zu gewinnen. Trotz umfangreicher Literatur sind viele Aspekte, die sich auf die Leberfunktion und die CYP-Aktivität auswirken, nicht gut verstanden. Diese Leberfunktionstests werden nicht nur von zahlreichen Proband:innenmerkmalen, sondern auch von den Besonderheiten der jeweiligen Untersuchung beeinflusst. Eine zentrale Herausforderung besteht darin, die verschiedenen Faktoren, die das Ergebnis der Messungen beeinflussen, voneinander zu trennen, um ihren jeweiligen Einfluss auf die dynamische Leberfunktion und den CYP-Phänotyp zu untersuchen. In dieser Arbeit wurde diese Herausforderung durch Metaanalysen und physiologisch basierte pharmakokinetische (PBPK) Modellierung angegangen.

Als Grundlage wurde eine offene Pharmakokinetik-Datenbank (https://pk-db.com) entwickelt und Pharmakokinetik-Daten für ein breites Spektrum von Testsubstanzen kuratiert. Meines Wissens enthält die PK-DB derzeit den größten offenen pharmakokinetischen Datensatz zu Substanzen, die für die Phänotypisierung und dynamische Leberfunktionstests verwendet werden. Der Datensatz ermöglichte die Identifizierung und Quantifizierung von demografischen und rassischen Bias (Geschlecht, ethnische Zugehörigkeit, Alter, Gesundheitszustand), Meldefehlern und Unstimmigkeiten in der Pharmakokinetik der berichteten Testsubstanzen.

Auf der Grundlage der Daten wurde eine Metaanalyse der Pharmakokinetik von Koffein im Hinblick auf verschiedene Faktoren durchgeführt, die die Leberfunktion und die CYP1A2-Aktivität beeinflussen. Insbesondere wurde das vorhandene Wissen über die Auswirkungen des Rauchens, der Einnahme oraler Verhütungsmittel, verschiedener Krankheiten und Begleitmedikationen auf die Pharmakokinetik von Koffein durch eine Metaanalyse und Datenintegration konsolidiert. Ebenso wurde die Messgenauigkeit der Koffeinkonzentration im Speichel im Vergleich zum Plasma quantifiziert und die Auswirkungen der Dosierungsmenge und des Zeitpunkts der Probenahme für die Phänotypisierung analysiert.

Darüber hinaus wurde der Einfluss des CYP2D6-Polymorphismus untersucht. Hierzu wurde ein PBPK-Modell für Dextromethorphan (DXM) und seine Metaboliten Dextrorphan (DXO) und Dextrorphan O-Glucuronid (DXO-Glu) entwickelt und mit den Pharmakokinetik-Daten kalibriert und validiert. Die Variabilität der CYP-Aktivität wurde auf der Grundlage von In-vitro-Daten modelliert. Das Modell ermöglichte die Vorhersage individueller Plasmakonzentrationen und Urinmengen von DXM, DXO und DXO-Glu und des metabolischen Phänotyps auf der Grundlage des CYP2D6-Genotyps und der physiologischen Merkmale der Person. Die Untersuchungen suggerieren, dass der größte Teil der Variabilität in der Pharmakokinetik von Dextromethorphan auf die Variabilität der CYP2D6- und CYP3A4-Enzymkinetik zurückgeführt werden kann. Neben verschiedenen

anderen Analysen wurde auch der Einfluss der ethnischen Zugehörigkeit auf die CYP2D6-Aktivität untersucht.

Beiträge zur Kuratierung von PK-Daten und zur Entwicklung von PBPK-Modellen wurden auch für andere Phänotypisierungs- und Leberfunktionstestsubstanzen geleistet (Chlorzoxazon, Kodein, Diazepam, Galaktose, Indocyaningrün (ICG), Metoprolol, Midazolam, Omeprazol, Pravastatin, Simvastatin, Talinolol und Torasemid). Insbesondere für ICG wurden die Auswirkungen auf den hepatischen Blutfluss, das Herzzeitvolumen und das Körpergewicht sowie die Überlebenswahrscheinlichkeit nach partieller Hepatektomie durch PBPK-Modellierung untersucht. Die Untersuchung der verschiedenen Testsubstanzen war nur durch einen systematischen und standardisierten Workflow möglich, welcher die Datenintegration, die gemeinsame Nutzung von Daten, die Erstellung reproduzierbarer PBPK-Modelle und die standardisierte Integration von Daten und Modellen umfasst.

Zusammengefasst wurden im Rahmen dieser Arbeit eine pharmakokinetische Datenbank, Methoden sowie Workflows für die Analyse von Prüfsubstanzen, die bei dynamischen Leberfunktionstests und der CYP-Phänotypisierung verwendet werden, erstellt. Dabei wurden Faktoren, die sich auf die CYP-Phänotypisierung und die Leberfunktionstests auswirken, mittels Metaanalyse und PBPK-Modellierung untersucht. Die dabei entwickelten Modelle können einen Beitrag zur personalisierten Medizin liefern und die Präzision dynamischer Leberfunktionstests in Kliniken erhöhen.

Contents

Chapter 1

Preface

The presented work is a cumulative dissertation consisting of three publications in peer-reviewed scientific journals with me as the primary author and several other scientific outcomes (publications, preprints, and bachelor theses) with non-primary contributions from my side. The methods in the work are computational, involving primarily mathematical modeling and software development. The objective was to contribute scientifically in two ways, first, by building open and accessible resources for mathematical modeling of pharmacokinetics and secondly, by computationally studying the cytochrome P450 (CYP) system and liver function in humans.

I believe in the high relevance of open data and code that is accessible in a FAIR manner, especially in the messy world of biology and the corporate world of pharmacology. Along that theme and regarding my first objective, I authored *"PK-DB: Pharmacokinetics Database for Individualized and Stratified Computational Modeling"* as the primary author and contributed to *"Ten Simple Rules for FAIR Sharing of Experimental and Clinical Data with the Modeling Community [preprint]"* as a co-author. The former is an ongoing project. All of the following computational investigations were only possible due to the existence of PK-DB and contributed to PK-DB as a byproduct. With respect to my second goal, I studied two closely related and highly relevant clinical and medical methods in this thesis, (i) dynamical liver function testing and (ii) *in vivo* CYP phenotyping. Both methods are performed by the administration of test substances in order to diagnose or monitor specific characteristics related primarily to the liver. Experimentally, they do not differ much, except for the application of different test substances and slightly modified protocols. The main differences are purpose and setting. Dynamical liver function tests are predominantly performed to quantify liver function, often in patients with liver-related diseases or in the context of liver surgery such as liver transplantation or hepatectomy. In contrast, *in vivo* CYP phenotyping primarily aims to characterize the cytochrome P450 detoxification system, for instance, to individualize drug dosing or study inter-individual differences in the pharmacokinetics of drugs. Due to the similarity of both methods, they were covered together in this thesis. The two publications, *"Pharmacokinetics of Caffeine: A Systematic Analysis of Reported Data for Application in Metabolic Phenotyping and Liver Function Testing"* and *"Physiologically based pharmacokinetic (PBPK) modeling of the role of CYP2D6 polymorphism for metabolic phenotyping with dextromethorphan"*, as well as the coauthored publications on indocyanine green-based liver function testing *"Physiologically Based Modeling of the Effect of Physiological and Anthropometric Variability on Indocyanine Green Based Liver Function Tests"* and *"Prediction of Survival After Partial Hepatectomy Using a Physiologically Based Pharmacokinetic Model of Indocyanine Green Liver Function Tests"*, are related to my aforementioned second objective. Other minor contributions are mentioned in suitable passages in the text.

This dissertation is structured into five chapters, Preface, Introduction, Results and Publications, Discussion and Outlook, and Appendix. The introduction is not

particularly technical and provides the necessary background in physiology, pharmacokinetics (PKs), and mathematical modeling. There, I introduce the most relevant concepts in pharmacokinetics, liver function testing, CYP phenotyping, and ordinary differential equation (ODE)-based modeling. In the third chapter, the results of my dissertation are presented, i.e., the three primary-authored publications in chronological order, followed by other related work. They build up on each other, starting from database engineering, over quantitative, and finally, to mechanistic modeling. The individual sections contain the original publication and a contextualization of the work with respect to liver function testing and CYP phenotyping, followed by additional analyses, which were not included in the publications. In the fourth chapter, the results are discussed and an outlook is provided on what I believe to be interesting follow-up investigations and how this work could potentially be translated into an application in healthcare. The final chapter contains supplementary information in the form of an appendix.

For all three publications, I carried out the mathematical analysis and wrote the software and the manuscript. For PK-DB, which is an ongoing project, I maintain the web service, curated data, and trained new collaborators. Up-to-date, 14 people contributed to PK-DB by curating data from over 700 publications, which makes PK-DB presumably the biggest openly available pharmacokinetic database. For the caffeine meta-analysis, the data curation was performed collaboratively by Florian Bartsch, Adrian Köller, Matthias König, and me. For the PBPK model of dextromethorphan, the data was collaboratively curated by Janosch Brandhorst and me. Matthias König supervised all of the projects and edited the manuscripts.

I am deeply grateful for Matthias' invaluable insights, guidance, and integrity in all aspects of my thesis and also the project prior to the thesis. Most of all, he sowed the seed for the, not so mundane after all, topic of standardization and automation. Without him, I would not have had the perseverance and vision to delay tackling scientific questions before having a solid reusable infrastructure in place. I am delighted to see that this infrastructure is being increasingly used and that I thereby contribute to and gain insights into related projects. Thank you, Matthias, all the members of König group, and the co-authors of my papers. Next, I also want to thank the members of the Institute of Theoretical Biology (ITB), it has been not only my scientific home for the last several years but also the place where I made new friends. In addition to that, I want to thank all the members of my dissertation committee for investing time and effort in the evaluation. Last but not least, I am deeply grateful for my supporting and caring friends, family, and Julia.

Chapter 2

Introduction

2.1 History and Relevance of Pharmacokinetics Modeling

The application of substances in order to cure diseases or increase well-being dates back at least 3000 years to ancient Egypt and Mesopotamia [RC07]. In some sense, it is probably as old as humanity or older as even some animals self-medicate, e.g., dogs eating grass in order to remove toxins [Shu14]. Nonetheless, it was not until the 19th century that pharmacology emerged as a scientific discipline that systematically studies the interactions between substances and cells, tissues, organs, and organisms. In the following decades, the science around what happens to substances due to processes in living organisms matured and was named "pharmacokinetics" (PK) [Nel61].

The first mathematical models in pharmacology emerged in the 20th century, with mathematical equations describing enzyme kinetics [MM13] and simple compartment models of drug elimination from the body [WT24]. In the sixties, these models progressed from one- to multi-compartment models leading to early physiologically-based pharmacokinetic (PBPK) models and allowed to more accurately describe the kinetics of compounds in the body [Wag81]. These models enabled for the first time to study the role of tissues and organs on the pharmacokinetics of substances, for instance, the impact of liver function on the pharmacokinetics of drugs. However, understanding the fate of drugs is not an end in itself. Medical scientists are primarily interested in treating diseases and hence investigating the effect of drugs on the body, named "pharmacodynamics" (PD). In order to study and predict drug treatment effects, both, PK and PD are relevant. Consequently, shortly after the emergence of the first PK models, the first PD models were developed and both coupled to so-called PK/PD models. Initially, they were utilized to investigate the impact of chemotherapy on cell survival rates [Jus73].

Nowadays, PK, PD, and PK/PD modeling have high relevance in various areas of science, clinical practice, and drug development, e.g., animal-to-human translation, dosage protocol selection in clinical trials, investigation of the effect of comedications, or the individualization of drug treatment. In any case, PK modeling is challenging, as it requires knowledge of mathematical modeling and underlying biology and physics.

2.2 Pharmacokinetics

Pharmacokinetics is the field in which the fate of substances applied to the human body is studied. The main interest hereby is how a substance administered to the body appears and disappears in tissues, plasma, and urine. Four main processes

contribute to the PK of a substance: absorption (how the drug reaches the body), distribution (how the drug distributes within the body), metabolism (how the drug is converted), and excretion (how the drug is eliminated), or short "ADME". In the following, each of the processes will be described in detail. A schematic overview of all the processes is shown in Fig. 2.1.

2.2.1 Absorbtion

Absorption describes the process of substances entering the body, which primarily refers to the steps until the compound reaches the systemic blood circulation. Drugs or test substances can be applied via various routes (e.g., intravenously, orally, intranasally, buccally, sublingually, rectally, or via inhalation). The choice of the application route depends on various considerations, such as the physicochemical properties of the drug, the onset of action, or the first-pass effect (i.e., only a part of the dose can reach the systemic circulation, which is described in detail below). Most drugs are applied orally and enter the body via the digestive tract, i.e., they are absorbed via the intestine after the passage through the stomach, see Fig. 2.1B. For some substances, the digestive process can already start in the oral cavity allowing them to be directly absorbed into the systemic circulation via the oral mucosa [YL93], and bypassing the first pass effect and accelerating the onset of action. These are the main advantages of buccal and sublingual applications by the way. In the oral cavity, the metabolism is initiated by enzymes contained in saliva. For solid application forms, the early absorption and metabolism in the oral mucosa are less important. Capsules and tablets do not significantly dissolve before entering the stomach. Extended-release tablets or drugs with low solubility do not even dissolve until reaching the intestine [Wei+05]. Low solubility is one of the biggest challenges in drug design as substances must be present in dissolved form at the site of absorption for it to occur [SGS12]. On the way through the gastrointestinal tract, the substances first enter the stomach. The high acidity of the gastric juice (i.e., $< 4pH$) can facilitate drug dissolution, metabolism, or degradation, more so for acid-labile substances (e.g., omeprazole). Subsequently, substances leave the stomach and enter the upper part of the intestine via gastric emptying. Most of the absorption occurs in the first two sections of the small intestine (i.e., duodenum and jejunum) via the enterocytes in the intestinal epithelium. To reach systemic circulation, substances must be transported from the intestinal lumen through the enterocytes to the intestinal blood.

Lipophilic drugs pass the intestinal epithelial barrier predominantly via transcellular transport, hydrophilic substances, on the other hand, typically bypass intracellular metabolism via paracellular transport. The physicochemical properties of the applied substances (i.e., electrical charge, lipophilicity, hydrophilicity) and various transporters (e.g., sodium-glucose cotransporter) regulate the absorption velocity across the apical plasma and basolateral membrane of the intestinal epithelial cells. Generally speaking, small and lipophilic chemicals can freely pass cell membranes while large or charged compounds only pass cell membranes with the aid of influx and efflux transporters such as the organic anion transporting polypeptides (OATPs) [KN09]. Transport proteins are also important at the later stages of the ADME processes, anytime large or charged molecules have to cross biological membranes, e.g., during bile secretion in the liver, tubular reabsorption and secretion in the kidneys, or the crossing of the blood-brain barrier.

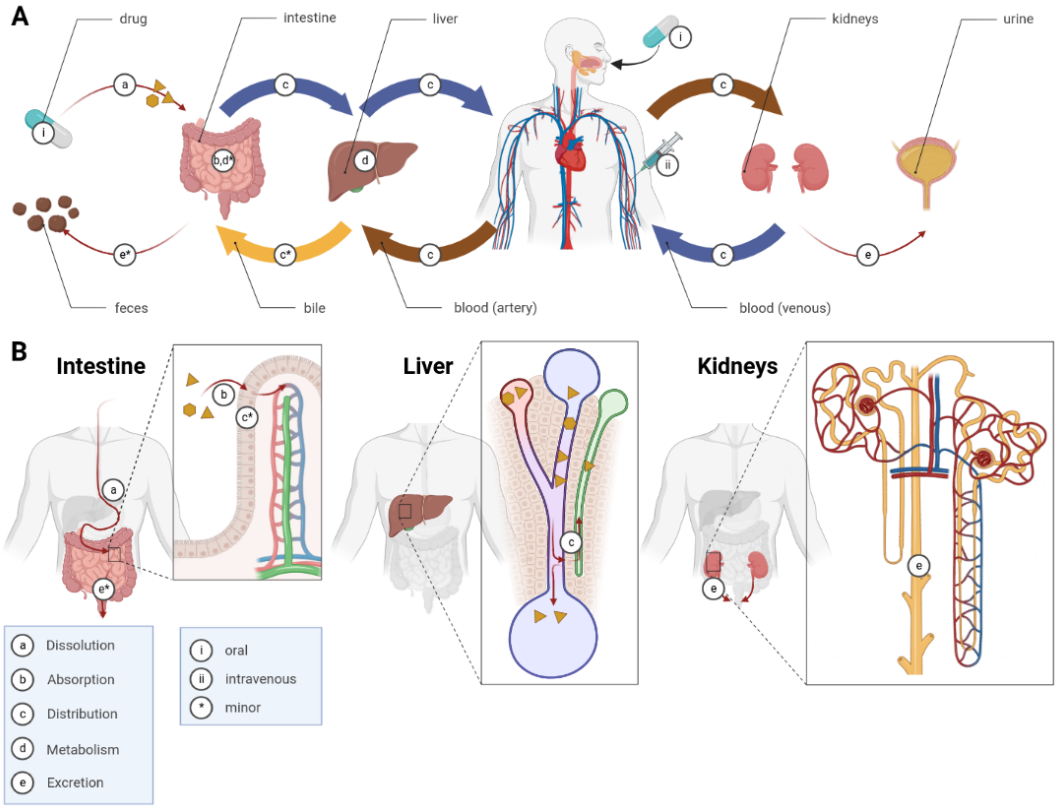

FIGURE 2.1: **Schematic overview of the ADME processes for intravenously and orally applied substances**: **A)** An illustration of the general roles of the central organs involved in absorption, distribution, metabolism, and excretion (ADME). The fate of a stereotypical drug is illustrated by arrows. The extent to which each of the tissues actually satisfies the depicted role depends on the properties of the specific substance. Narrow arrows depict the absorption and excretion from the body. Blue and brown arrows represent the venous and arterial blood flow, respectively. The yellow arrow depicts the bile flow. **B)** The role of the three central organs which are involved in the ADME process and a schematic representation of their "functional units". On the left, the dissolution and subsequent absorption over the intestinal epithelial cells. In the center, a liver sinusoid with blood flowing from top to bottom (blue), bile flowing in the opposite direction (green), and the metabolism taking place in the hepatocytes (light brown). On the right, the urinary excretion of metabolites through a nephron is shown. For more detail on all of the processes see the ADME section in the text. The illustration was created with https://biorender.com/.

The fraction of a given dose entering the systemic circulation is referred to as "bioavailability". Partial absorption of substances by the intestine as well as metabolization on the first pass through the intestinal wall and liver, the so-called "first-pass effect", contribute to reduced bioavailability. Incidentally, some highly lipophilic drugs are capable of bypassing the liver on the first pass by the mesenteric lymphatic circulation, which leads to higher bioavailability [Tru+07; Yáñ+11]. The bioavailability of a drug has high practical relevance for the route of application and dosing regime.

In summary, absorption regulates the amount and speed of the appearance of a substance in the systemic circulation that is subsequently available for distribution, metabolization, and excretion.

2.2.2 Distribution

Nutrients, oxygen, waste products, and other substances are transported by the blood through the body via the circulatory system, which comprises the heart, lungs, blood, and blood vessels, see Fig. 2.1A. Blood is essential for transport in the human body and accounts for approximately 10 percent of the body weight. At rest, the heart pumps about 5 [l/min] through the body, referred to as cardiac output. In a nutshell, the blood leaves the right ventricle of the heart, flows to the lungs, gets oxygenated, and continues through the arteries to the organs and into the tissues. The deoxygenated blood leaves the tissues and is carried through the veins back to the heart, and the cycle starts again. Orally applied drugs and substances (e.g., nutrients via food) reach the systemic circulation via the digestive system. Approximately 15 percent of the blood flow passes through the intestine and thereby absorbs the substances. The exiting nutrient-rich blood first passes through the portal vein and liver before circulating back to the heart. In contrast, intravenously applied substances directly enter the systemic circulation.

In addition to the systemic circulation, substances can circulate in the body via the "enterohepatic circulation". Certain substances are excreted from the liver in the bile which is secreted back into the gastrointestinal tract. Substances in the bile can subsequently be reabsorbed from the intestine and reach the liver again, closing the circle. Enterohepatic circulation is, however, less important in comparison to the systemic circulation and takes place on much longer time scales in the range of hours.

The physicochemical properties of the circulating compounds largely determine how freely they can cross various blood-tissue barriers but also how much binding to plasma proteins occurs. Almost all substances are at least partially bound to plasma proteins (e.g., albumin, gamma globulin). Bound and unbound states are typically in chemical equilibrium and described by the plasma binding coefficient. Proteins cannot passively cross cell membranes. So only the unbound fraction of the substance can enter cells and be metabolized or excreted. E.g., over 95 percent of the drug warfarin is bound to plasma proteins which results in a long half-life in the body (approximately 40 hours) for warfarin. Again, large and polarized molecules cannot pass cell membranes without efflux and uptake transporters. Approximately 10 percent of human genes are related to transporters, indicating their importance [YH15]. So due to the blood tissue barriers, accumulation of various drug compounds in specific areas of the body occurs. Lipophilic substances (e.g., polychlorinated biphenyls) tend to stay for prolonged times in areas of high lipid density, such as the fat tissue, leading to their dynamics behaving as if an additional slow compartment is present.

In essence, once a substance reaches the systemic circulation, it is non-trivially distributed to various places in the body, consequently, also to particular organs where metabolism and elimination occur.

2.2.3 Metabolism

Drug metabolism refers to the biochemical transformation of xenobiotics, and enzymes play a central role in that process. Most drug-metabolizing enzymes are located in the liver, more specifically in the main cell type of the liver, the hepatocytes.

Metabolic reactions are classified into three phases (I, II, and III). The phases do not necessarily occur in order, but ultimately the biotransformation or sequence of biotransformations evolved to have two major effects on the xenobiotic, (i) inactive the drug, and (ii) increase its water-solubility so that it can be subsequently excreted by the kidneys and bile [PL22]. Interestingly, for prodrugs like codeine, the phase I metabolism actually activates the drug. Phase I metabolism can be either oxidation, reduction, or hydrolysis. Among these, the most frequent reactions are oxidations catalyzed by cytochrome P450 enzymes (CYPs) [RG15]. CYPs are an ancient superfamily of enzymes present in all kingdoms of life, which evolved as a central detoxification system. In humans, they are involved in the metabolism of approximately 70-80 percent of all drugs in clinical use [ZS13]. The subset of six CYPs (CYP1A2, CYP3A4, CYP3A5, CYP2C9, CYP2C19, CYP2D6) metabolize 90 percent of the subset of drugs [LP07]. The second most frequent biotransformation of xenobiotics and the most important phase II metabolism is glucuronidation performed by the glucuronosyltransferases (UGT). Substrates undergoing glucuronidation result in products with highly increased charge and, therefore, high hydrophilicity, which facilitates the excretion via the bile and urine but requires efflux transporters for membrane transport [Yan+17].

The liver evolved as the main metabolizing organ in the body but some drug-metabolizing enzymes, such as certain isoforms of the cytochrome P450, are also present in the intestinal wall or the kidneys. Depending on the compound, these alternative routes of metabolization can play an important role, e.g., metabolization of midazolam by CYP3A4 in the intestine.

2.2.4 Excretion

Excretion refers to the process of elimination of drugs and their metabolites from the body. The four main routes of elimination are excretion via the kidneys in the urine, excretion via the colon as feces, removal of gaseous substances such as carbon dioxide via the lungs, and excretion via the skin as sweat.

Most drugs and also their metabolites are excreted via the kidneys in the urine. With approximately 0.5 percent of total body weight and 19 percent relative tissue blood flow, the kidneys are a relatively small but highly perfused organ. They account for several important tasks in the body (i.e., excretion of waste products, assisting in blood pH homeostasis, and blood pressure regulation). Each kidney contains approximately one million nephrons, the functional units of the kidney, which perform four roughly consecutive processes (i.e., filtration, reabsorption, secretion, and excretion), see Fig. 2.1B. Renal filtration takes place in the renal glomerulus, where parts of the blood (i.e., a fraction of protein-unbound blood compounds with less than 300 g/mol molecular weight) leave the blood vessel due to hydrostatic pressure into a downstream process which eventually results in urine. The adjustable difference in afferent and efferent glomerulus vessel diameter regulates the blood

pressure and the leaving blood fraction. Red blood cells and macromolecules such as most proteins can not pass the glomerular filtration barrier. During the filtration, important compounds (e.g., sodium, potassium, chloride, magnesium, amino acids, glucose, bicarbonate, and excess water) are initially filtered out but are subsequently reabsorbed back into the bloodstream (tubular reabsorption). In the opposing direction, various not yet filtered-out molecules (e.g., hydrogen ions, urea, creatine, penicillin) are secreted into the renal tubules. Ion absorption and secretion assist in blood pH homeostasis.

Substances that are not absorbed or metabolized during the passage through the intestine and colon are removed from the body within the feces, which also applies to substances contained in the bile. Biliary excretion is an important route, especially for lipophilic and large compounds (300-500 g/mol). The bile comprises 95 percent of water and 5 percent of organic and inorganic compounds (i.e., bile salts, phospholipids, amino acids, porphyrins, steroids, cholesterol, immune globulin A, enzymes, and due to biliary drug excretion also xenobiotics [Boy13]). Bile acids are synthesized from cholesterol by the hepatocytes and secreted into the bile canaliculus via highly regulated ABC transporters located at the apical membrane [DLR09]. The bile flows in opposite direction to the portal blood flow, see Fig.2.1B, into the bile bladder and subsequently via the common bile duct into the small intestine where it plays a vital role in the absorption of lipids, fat-soluble vitamins, and cholesterol [HH08]. Many components and even xenobiotics are recovered from the bile by reabsorption in the intestine resulting in enterohepatic circulation.

Mostly salt, heavy metals, and water can be excreted via the skin as sweat. Contrary to a widespread misconception, sweat is usually minorly involved in the removal of toxins and waste products [Bak19]. Its dominant function is thermoregulation. For practical matters, activities leading to sweating typically also lead to increased heart rate which in turn increases the cardiac output and clearance of xenobiotics [Cla+19].

2.3 Liver Function Testing and CYP Phenotyping

The health status and function of the liver can be examined using various methods, such as static and dynamic liver function tests, Fibroscan, and liver biopsy [WP14]. Fibroscan and liver biopsy allow to diagnose pathophysiological changes, while static and dynamic liver function tests quantify the function, which is the primary focus of the present work. Within the function tests, there are several variants that examine complementary aspects.

2.3.1 Liver Function Testing

First, it is important to understand that the liver is, in many ways, a special organ with various functions. The liver is the heaviest solid internal organ, accounting for about two percent of body weight [ICR02; JR13]. Even more impressive is its 18 percent contribution to the total resting energy consumption, which hints at its importance [Wan+10]. It is highly perfused, with over one-fourth of the total blood flow passing the liver [JR13], and the only organ that is supplied by a dual blood supply with about one-fourth oxygenated blood via the hepatic artery and three-fourths nutrient-rich deoxygenated blood via the portal vein. It has an exceptional regenerative capacity (e.g., after hepatectomy), as up to 80 percent of its volume can be recovered [Tru+07]. The high importance of the blood supply and regenerative

function of the liver is only logical considering its tasks. Besides its function of metabolizing xenobiotics and consequently its role in detoxification, the liver syntheses many plasma proteins and biochemicals necessary for digestion and growth, it has iron, vitamin, and energy storage capabilities, the latter in the form of glycogen, it regulates blood clotting, and it clears indigenous waste products like bilirubin. The responsible processes occur in each of the hundreds of thousands of liver lobules which are the functional units.

Each lobule is a hexagonal structure with its own blood supply. The blood flows through vascular channels, called sinusoids, from the outside, i.e., portal vein and hepatic artery, to the inside, i.e., central vein. The content in the blood flowing through the sinusoid gets bidirectionally exchanged with the adjacent metabolizing cells, the hepatocytes. Thereby passing the perisinusoidal space, the space between the sinusoidal endothelial cells and the hepatocytes. Of note, this space is majorly involved in fibrotic scaring leading to cirrhotic liver disease [San+21]. Not all compounds entering the hepatocytes are secreted back to the sinusoids. Some are secreted into the bile, which flows in an opposing direction to the blood via the bile canaliculi to the outside of the liver lobule until it finally reaches the common duct and the gall bladder. The whole process is depicted in the middle of Fig. 2.1B.

In the case of diseases and disorders in the liver, one or many aspects of the processes are malfunctioning often resulting in altered liver function. A common symptom, e.g., is bilirubin jaundice (i.e., the eyes' whites and the skin's getting yellowish) which is related to a problem with bilirubin excretion through the bile. Certain routinely performed liver function tests are grouped together under the name static liver function tests and are part of the comprehensive metabolic panel. These tests are characterized by the fact that they analyze the contents of the blood for specific biological molecules (biomarkers) which are related to different functions of the liver. Among these are alanine transaminase (ALT), aspartate aminotransferase (AST), gamma-glutamyl transferase (GGT), lactate dehydrogenase (LD), and alkaline phosphatase (ASP) which are enzymes released or leaked by the liver in case of a liver injury, blocked bile duct, or other liver diseases. Elevated levels of these biomarkers and bilirubin, a substance created during the breakdown of red blood cells and secreted into the bile, indicate liver-related problems. Decreased levels of other markers, i.e., total protein amounts, even more so albumin, indicate problems in hepatic protein synthesis. In essence, bilirubin informs about excretion, GGT and ASP inform about cholestasis, ALT and AST informs about liver damage, and albumin informs about synthesis capacity. The outcomes of the tests get compiled with other information to severity scores for evaluation of the liver, i.e., CHILD PUGH or MELD. These static tests, however, suffer high intra-individual variability and a lack of reproducibility [LSC08]. Another major problem of the static tests is the long half-lifes of the biomarkers resulting in delayed insights. Nonetheless, they are a very valuable tool for clinical evaluation due to their simplicity and general availability.

In contrast, dynamic liver function tests measure selective aspects of the capacity of the liver to eliminate and metabolize substances from the body, wich is often quantified by the elimination rate of the test substance in plasma, see e.g. Fig. 2.2A. Advantageously, these tests can be performed in short intervals and previous measurements can be used as a baseline to quantify the relative change. Therefore, they are particularly well suited for monitoring liver function in rapidly progressing diseases and changes in liver function in the context of liver surgery such as partial hepatectomy or liver transplantation. Historically, a multitude of test substances (e.g., bromosulfophthalein (BSP), caffeine, galactose, ICG, Lidocaine (MEGX), methacetin) have been utilized. Nowadays, ICG is by far the most widely applied substance.

Other substances are either not as practical or potentially hazardous, especially for critically ill patients [Sak07].

2.3.2 Phenotyping of Cytochrome P450 Enzymes

The capacity of the liver to eliminate substances from the body depends on substance-specific cellular mechanisms with metabolization via CYPs being one of the most important factors. 70-80 percent of prescribed drugs are metabolized by CYPs located in the hepatocytes. CYP activity is related to dynamical liver function and most of the procedure of *in vivo* CYP phenotyping resembles dynamical liver function testing. Caffeine, e.g., is used as well for dynamical liver function testing as for CYP1A2 phenotyping. For CYP phenotyping, the test substances are selected to be predominantly metabolized only by a single CYP isoform. A selection of typical CYP test substances can be found in Tab. 3.1.

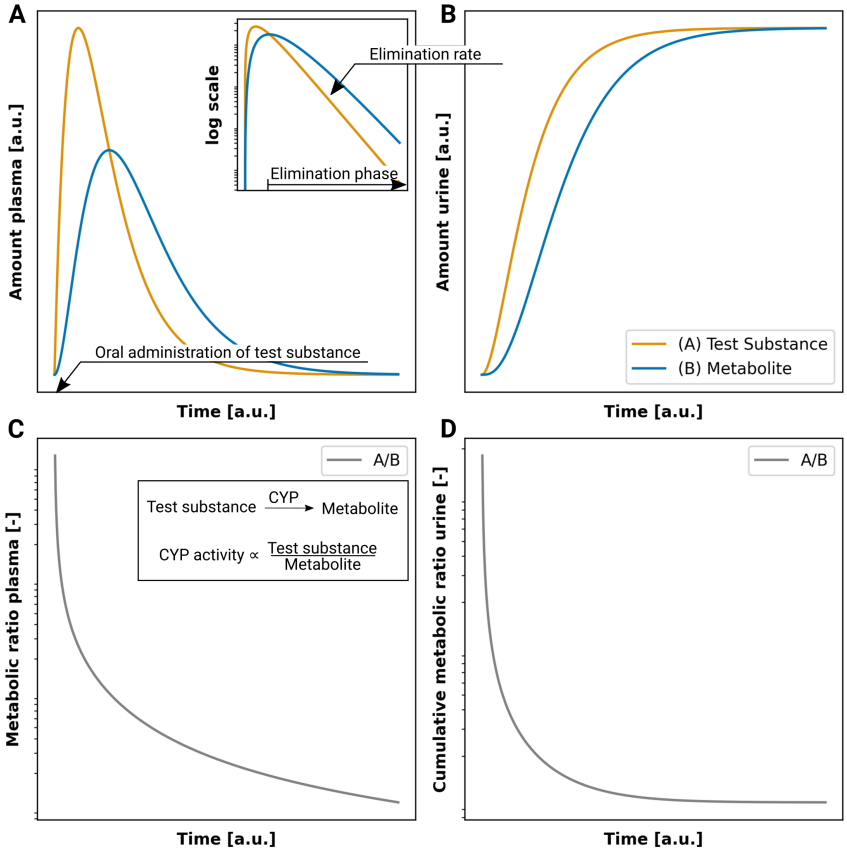

FIGURE 2.2: **Illustration of CYP phenotyping by (cumulative) metabolic ratios and liver function testing by elimination rates.** The example was generated by the simple dynamic model depicted in Fig. 2.3. **A)** Plasma amounts of the test substance A (orange) and metabolite B (blue).; **B)** Urine amounts of the test substance A (orange) and metabolite B (blue).; **C)** Metabolic ratio A/B in plasma; and **D)** The cumulative metabolic ratio of A/B in urine. The plasma and urinary amounts and metabolic ratios can be used to evaluate liver function and the enzymes involved in the conversion of A to B. Time and amounts are in arbitrary units [a.u.].

After the administration of the test substance, the amount of the drug and the main metabolite is measured either in the plasma and/or urine at single or multiple time points. Specifically, the concentration in blood or the amounts in the urine of the test substance and the metabolite produced by the CYP of interest are quantified

and the ratio between them is calculated. Often, due to its simplicity, the so-called (cumulative) metabolic ratio, i.e., the ratio of the cumulative drug and metabolite in the urine, is measured at a single time point. Small values of the metabolic ratio correspond to fast metabolizers and high values to poor metabolizers. This concept is visualized in Fig. 2.2. In the case of extensive phase 2 and 3 metabolisms resulting in multiple metabolites, the metabolic ratio is normally calculated by dividing the quantity of the test substance by all downstream metabolites. In blood, the sampling times are typically chosen to be in the range of the half-life of the test drug. Earlier sampling might be too much influenced by the absorption phase. Later sampling time on the other hand can decrease the accuracy of the measurement due to smaller substance amounts which might be difficult to quantify accurately. Additionally, metabolic ratios tend to shift slightly over time in plasma, which has to be accounted for when comparing results from different sampling times. In urine, the cumulative metabolic ratios tend to be more stable over time [Grz+22; GBK22].

An important concept in metabolic phenotyping is the so-called cocktail approach. Hereby, the activity of multiple enzymes (e.g., CYP isoforms) or transporters can be measured simultaneously by applying a cocktail of multiple probe drugs simultaneously. For this purpose, test substances are selected so that they have only minor interactions with other test substances and are specific for different enzymes.

2.3.3 Influencing Factors

Many factors related to the ADME process can potentially alter the results of dynamical liver function tests and the results of CYP phenotyping tests. As discussed earlier. In practice, test substances are selected to be primarily eliminated by the liver and for CYP phenotyping by particular CYPs in the liver. So, it should not be surprising that the results are first and foremost influenced, as intended, by the functional liver volume and CYP activity, respectively. As already noted, also dynamical liver function tests are influenced by substance-specific mechanisms on the cellular level. The effect of various factors will be exemplified below.

Important and well-study are drug-drug and drug-food interactions due to shared CYP450-mediated metabolic pathways. Various molecular mechanisms lead to inhibition or induction of the involved enzymes [Deo+20] (e.g., competitive inhibition, non-competitive inhibition). Examples are the inhibition of CYP3A4 by grapefruit juice or the induction of CYP1A2 by smoking [Grz+22].

In addition, genetic polymorphisms can have high relevance, as many enzymes and transporters are known to be polymorphic (e.g., CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, OATP1B1) [ZS13]. In this context, CYP2D6 is one of the most extensively studied enzymes. Until now, over 130 different CYP2D6 genetic variants have been identified with very different enzyme activities [GBK22]. The change in enzyme activity can be due to differences in binding affinities or protein abundance. Even for highly polymorphic enzymes like CYP2D6, there is a good correlation between CYP activity and protein amounts [Lee+09]. Much of the large intra-individual differences in liver function can be explained by the large variability in CYP protein amounts [Ach+14; Nin+18]. In addition, there is some evidence that CYPs are affected by the circadian clock [Fro09], resulting in changes in CYP protein amounts during the day.

Pathophysiological alternations are extremely important, especially in the organs involved in the ADME processes, which typically results in a reduction in clearance

of many drugs, like, e.g., pravastatin, with chronic renal insufficiency [LP22]. In dynamic liver function, the most severe changes obviously occur in liver diseases, particularly in late-stage liver disease [Grz+22]. This is of high relevance as liver-related chronic conditions (e.g., non-alcoholic and alcohol-related fatty liver diseases) and diseases (hepatitis, cirrhosis, hepatocellular carcinoma) are prevailing at a very high level worldwide, leading to approximately 2 million death per year, and on average 1.6 years of life lost [Asr+19]. In early-stage liver diseases, an increased amount of fat in the liver, called steatosis, typically does not change liver function, and most patients with steatotic livers never progress to the later stages. However, obesity is the number one risk factor for non-alcoholic fatty liver disease (NAFLD), 90 percent of obese and 65 percent of overweight patients are diagnosed with NAFLD [AC09], and extended amounts of adipose tissue also alter lipophilic substances' clearance rates. Testosterone used *in vitro* for the phenotyping of CYP3A4, e.g., sequesters into adipose tissue, which increases the apparent volume of distribution and thereby also the half-life.

Perfusion of the liver can be an important factor affecting the clearance of substances by the liver. See for instance the large effect of hepatic perfusion and cardiac output on the elimination of ICG [Köl21]. Steatosis can have as well a marked effect on the perfusion of the liver as do changes after liver surgery, e.g., portal hypertension after partial hepatectomy.

Anthropometric factors also play a notable role. Liver function is proportional to the functional liver volume; consequently, the natural variation in liver volume due to age, sex, heritage, and body size also affects liver function. Liver function deteriorates with age [Cie+16], as does kidney function with reduced glomerular filtration rate (GFR) by 4-12 mL/min per decade [Del+12]. Men tend to have slightly larger livers (approximately 8 percent) than women [Kra+03]. Increased alcohol consumption is also associated with larger livers but not necessarily with higher functional liver volume.

In summary, high inter-individual differences in liver function CYP activity are caused by many different factors. Besides very obvious interactions, the impact and interplay of the different factors quickly extend straightforward comprehensibility and require mathematical modeling even if only a small fraction of them are studied or controlled for *in vivo*.

2.4 Physiologically Based Pharmacokinetics (PBPK) modeling

Computational modeling is a unique tool to study complex systems. Generally speaking, there are two broad categories of computational models that can be used to model the pharmacokinetics of tests substances: (i) Phenomenological models (regression) that aim to describe the PK and (ii) models based on "first principles" that incorporate prior knowledge of the system. PBPK modeling belongs to the latter category and is very well suited to study liver function and CYP phenotypes based on the pharmacokinetics of test substances. In PBPK models, the ADME processes, such as metabolism in the liver or distribution via the blood flow are implemented via a system of ordinary differential equations (ODEs). The compartments (e.g. liver, kidney, and blood) in such models are assumed spatially homogeneous. In the model, they are connected via transport equations to account for the distribution, e.g., the systemic and biliary circulation. The state variables of the model correspond to the

amounts or concentrations of substances in the respective compartments and can be compared to *in vivo* measurements.

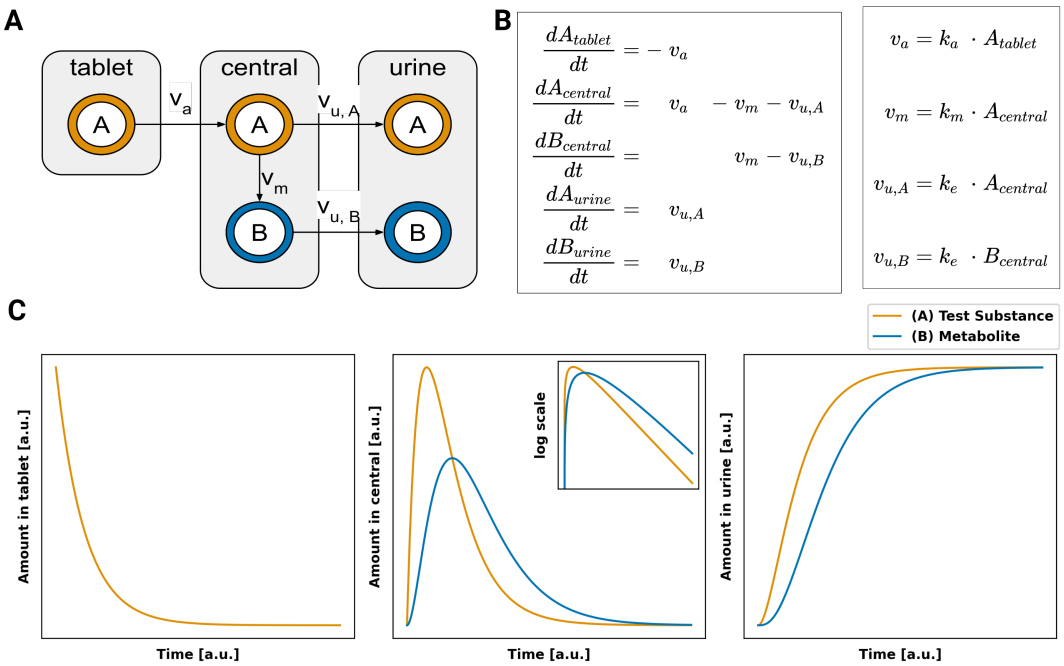

FIGURE 2.3: **Simple ODE-based pharmacokinetics model. A)** The system consists of three compartments (tablet, central, urine) that are connected via transport reactions. The model contains two substances the test substance A (orange); and the metabolite B (blue). The test substance A is metabolized to metabolite B in the central compartment. **B)** The resulting system of ordinary differential equations (ODEs). The rate of absorption, metabolism, and excretion (v_a, v_m, $v_{u,A}$, $v_{u,B}$) are modeled via irreversible mass-action kinetics. **C)** With an initial amount of $A_{tablet} = 10$ and rates $k_a = 1$, $k_m = 1$, and $k_e = 1$, all in [a.u.], the resulting amounts over time of the substances in the tablet, central, urine compartments are depicted.

In the following, the concept of modeling pharmacokinetics is described by the example of an administered test substance, see Fig. 2.3. In this example, the system consists of three compartments (i.e., tablet, central, and urine). The applied test substance (A) is absorbed from the tablet into the central compartment, where it can be transformed into a single metabolite (B). Both substances can be excreted from the central compartment into the urine compartment. All processes (absorption, metabolism, excretion) were implemented by first-order kinetics (irreversible mass-action kinetics), i.e., the speed of the process is proportional to the current concentration of the substance. This illustrative model is very much an oversimplification for didactic purposes. Nonetheless, it is complex enough to simulate and study concentration-time profiles of two substances in the central and the urinary compartment. This simple model is able to describe typical measurements taken for liver function testing (i.e., elimination rates calculated by plasma concentrations) or CYP phenotyping (i.e., metabolic ratios of parent drug and metabolite in plasma or urine). The model was used to simulate timecourses in plasma and urine to explain both concepts, see Fig. 2.2.

In this example, it is assumed that the concentration in the central compartment corresponds to the concentration in plasma. For the simulations, the compartments have a volume of 1 liter, transport rates and metabolic rates of 1 [1/hr], and the initial amount of the test substance in the tablet of 10 [mmol]. The model was implemented in the Systems Biology Markup Language (SBML), see Sec. 5, a free and

open model format capable of representing many different biological phenomena and best practices to facilitate model reusability and more [Huc+19; Kea+20].

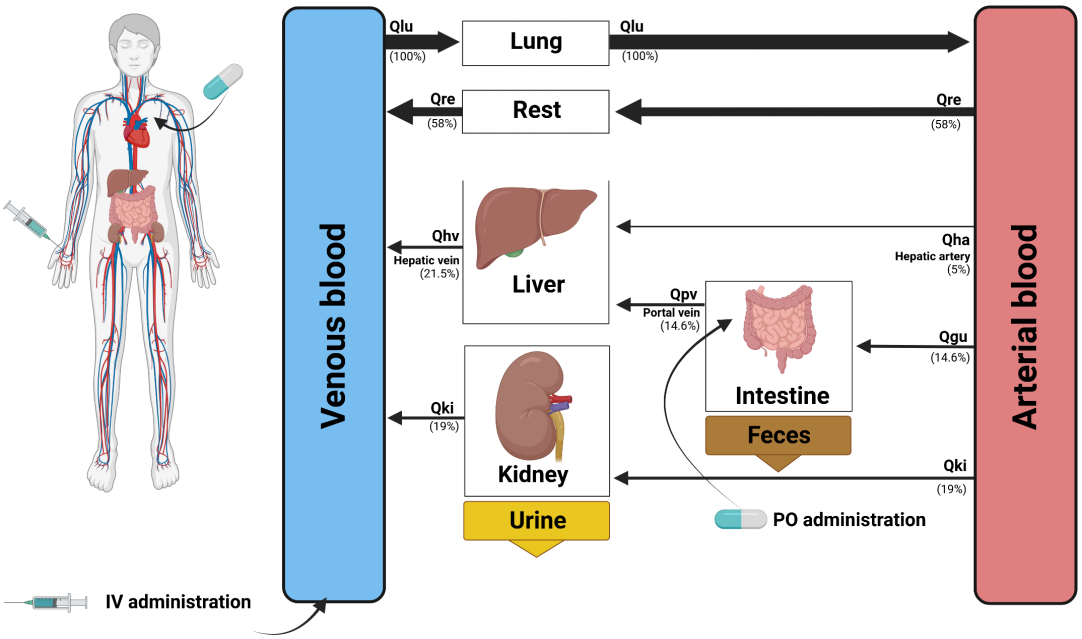

FIGURE 2.4: **Physiologically based pharmacokinetic (PBPK) model**. A generic PBPK model for a given probe substance. The arrows represent the blood flow between the different compartments, with the arrow width indicating the relative amounts. Intravenous (IV) administrations are typically conducted in the venous blood compartment, and orally applied drugs end up in the intestine compartment. The details of the tissue models for the liver, kidney, intestine, lung, and rest compartment are omitted. The figure was illustrated with https://biorender.com/.

For more sophisticated models, the body is subdivided into more spacial compartments, which are connected via transport equations. The compartments typically resemble the physiology, i.e., organs, smaller units within organs, or blood vessels. The blood flow to the organs is well described by constant rates based on the cardiac output, the circulatory connections, and the fraction of the blood flow to the individual organs, for an illustration of the generic model, see Fig. 2.4.

In the models implemented in this thesis, substance-specific tissue models are created and connected to the whole-body model. None-relevant organs are lumped into a rest compartment. The individual organs are modeled by at least two compartments, the plasma and tissue compartments. The transport between plasma and the tissues is typically modeled by a combination of passive and active transport, with the rate being dependent on the concentration difference between plasma and the tissue. As described in previous sections, the transport rate is thereby influenced by many factors, e.g., blood plasma binding, physicochemical properties of the substance, and activity of transporters. Many of the factors are, in principle, measurable, calculable, or at least estimable within reasonable ranges. This is similarly true for the metabolic capacity of enzymes responsible for the bio-transformation of the substances within the tissues such as the liver. The metabolism takes place inside the tissue. Depending on the substance, the kinetics of the transformation is modeled either by (irreversible or reversible) mass action, as in the simple example above,

$$v = k_{max} * [S] \tag{2.1}$$

or via Michaelis-Menten kinetics to account for the saturation of the enzymes.

$$v = \frac{V_{max}[S]}{K_m + [S]} \tag{2.2}$$

With the Michaelis-Menten coefficient ($K_m = \frac{k_2 + k_{-1}}{k_1}$) and maximal reaction velocity ($V_{max} = k_2[E] + [ES]$). The equation is derived from a reversible binding process (steady-state assumption) and irreversible catalytic process ($\mathrm{E + S} \underset{\mathrm{k_{-1}}}{\overset{\mathrm{k_1}}{\rightleftharpoons}} \mathrm{[ES]} \xrightarrow{\mathrm{k_2}} \mathrm{P + E}$). In the limit of $K_m \gg [S]$, the reaction exhibits first-order kinetics (e.g., caffeine metabolism), where a constant proportion of the drug concentration is eliminated, which leads to an exponential decline in the concentration over time. The reaction kinetics in the limit of $K_m \ll [S]$ result in zero-order kinetics (e.g., alcohol metabolism), where a constant amount is eliminated and resulting in a linear decline in the concentration over time. These reaction rates, depending on the kinetic parameters, is where much inter-individual biological variation can be observed.

Many of the parameters of PBPK models are based on physiological parameters or can be derived from physicochemical properties of the studied substances. These parameter values must be acquired from the literature. Unfortunately, not all parameters can be resolved, but remain uncertain (e.g., maximal rates in the *in vivo* situation are difficult to infer from *in vitro* data. Missing parameters are often resolved using parameter fitting based on pharmacokinetics data. In this method, parameter values are changed under the subjective to minimize a loss function which is a metric that describes the difference between simulation results and the data. The data is crucial not only for fitting model parameters but also in the model development process. Poor model performance can be due to poor parameter values and flawed model assumptions. Notably, the performance of the model should be evaluated on an independent data set. Otherwise, the model might end up highly overfitted.

In summary, many details should be considered when building predictive PBPK models, and explicitly crucial among them are pharmacokinetics data and standardization of data and models.

2.5 Objectives

The objective of the work was to address the following key open questions:

What does a standardized representation of the pharmacokinetics data as reported in literature look like, and is it possible to use a single data representation to investigate many different scientific questions?

Can a dataset of available pharmacokinetics data on liver function testing and CYP phenotyping be established?

Can the data be used to better understand what factors affect CYP phenotyping and liver function testing; (i) by systematically integrating data for caffeine; (ii) by PBPK modeling of dextromethorphan?

(to i) What are the shortcomings in reporting pharmacokinetics data in the area of CYP phenotyping and liver function testing?

(to ii) Despite the sparsity of available data, can the data be used to calibrate

and validate PKPB models for phenotyping and liver function testing?

(to ii) What would a systematic PBPK modeling workflow based on literature data look like?

Chapter 3

Results and Publications

3.1 Overview

The main results of this thesis consist of three publications with me as a primary author. They are presented in chronological order, followed by other relevant scientific outcomes. The three publications follow a natural progression from pharmacokinetic data to meta-analyses of data and finally to PBPK modeling informed by data. The chosen approach is empirically driven and shows a concept of how to perform pharmacokinetic modeling in the scenario of plenty of data from various data sources and with a large degree of heterogeneity (e.g., intervention protocol, sampling site and sampling timing, characteristics of the participants, substance quantification methods, selective reporting).

Along that theme, the first publication is titled *"PK-DB: pharmacokinetics database for individualized and stratified computational modeling"* and covers the first open pharmacokinetics database (https://pk-db.com). Within the scope of that publication and from prior investigations, an initial pharmacokinetics data corpus was established. PK-DB is an ongoing project, and additional pharmacokinetics data is continuously added to the database, mostly from the curation of existing pharmacokinetics literature.

It is difficult to draw comprehensive conclusions from a single investigation with small or medium sample size, even more so when large mathematical models are being used or high uncertainty is expected in the parameter values of the models used for the analysis. In the field of pharmacokinetics, clinical trials are quite elaborate and only a few large clinical trials are conducted. Seventy-five percent of the pharmacokinetic studies have fewer than thirty participants, and many of these studies have a case-control design, leading to half the effective sample size of subjects with similar characteristics, see Fig. 3.1B. Further, PBPK models are often quite large and difficult to parameterize, even if most of the parameters are physiologically constrained. In this kind of scenario, data integration from multiple studies comes as a very handy technique. Data integration and sophisticated techniques to account for heterogeneity in the data are traditionally applied in the domain of meta-analysis and systematic reviews. Consequently, the second publication is a meta-analysis which is titled, *"Pharmacokinetics of Caffeine: A Systematic Analysis of Reported Data for Application in Metabolic Phenotyping and Liver Function Testing"*. An important result from the analysis was the quantification of some influencing factors on liver function and CYP1A2 phenotype. Nevertheless, identifying and, more so, quantifying effects from heterogeneous data is very challenging with "simple" statistical methods. Still, the established workflows coming from systematic reviews and meta-analyses, e.g., PRISMA [Moh+09; Tri+18], proved to be very valuable and are currently only little noticed in the field of PBPK modeling.

PBPK is a perfect fit to study scientific questions if large heterogeneity is present in the data. This led to my third paper, titled *"Physiologically based pharmacokinetic (PBPK) modeling of the role of CYP2D6 polymorphism for metabolic phenotyping with dextromethorphan"*. In this approach, reported differences in the intervention protocol (e.g., route of application, amount of applied dextromethorphan, sampling timing), as well as characteristics of the subjects (e.g., CYP2D6 genotype, body weight), were directly accounted for in the model parameters which among other things led to insights on the role of CYP2D6 polymorphism for metabolic phenotyping.

As a result of the three investigations, a pharmacokinetic data and modeling workflow was established and applied to other studies on liver function or phenotyping using PBPK modeling with other primary investigators. In short, the workflow consists of: (1) a systematic PubMed and PKPDAI [GH+21] search via search queries; (2) sorting and reducing the retrieved literature corpus based on systematic eligibility and priority criteria; (3) curating data from eligible publications into PK-DB; (4) conducting meta-analysis or physiological-based pharmacokinetic modeling to address scientific questions. An overview of liver function and phenotyping-related data and projects addressed in this manner is provided in Tab. 3.1.

Test substance	Primary proteins	Reference publication	PK-DB studies	Primary PKPB modeler
caffeine	CYP1A2 (P05177)	[Grz+22]	147	M. König
chlorzoxazone	CYP2E1 (P05181)		23	J. Küttner
codeine / morphine	CYP2D6 (P10635)		42/12	J. Grzegorzewski
dextromethorphan	CYP2D6 (P10635) CYP3A4/5 (P08684, P20815)	[GBK22]	51	J. Grzegorzewski
diazepam	CYP3A4/5 (P08684, P20815)		28	D. Ke
galactose	galactokinase (P51570)		3	M. König
indocyanine green (ICG)	OATP1B3 (Q9NPD5)	[Köl21; KGK21; Köl+21]	51	A. Köller
metoprolol	CYP2D6 (P10635)		13	P. Ogata
midazolam	CYP3A4/5 (P08684, P20815)	[Dup20]	65	Y. Duport
omeprazole	CYP2C19 (P33261)	[Bal21]	16	S. Balci
pravastatin	OATP1B1 (Q9Y6L6)	[LP22]	33	H. Leal Pujol
simvastatin	CYP3A4/5 (P08684, P20815) OATP1B1 (Q9Y6L6)	[Bar20]	48	F. Bartsch
talinolol	P-glycoprotein (P08183)		13	B. S. Mallol
torasemide	CYP2C8 (P10632) CYP2C9 (P11712)		18	S. De Angelis

TABLE 3.1: **Overview of prioritized probe substances in PK-DB.** The primary protein (with UniProt identifier) refers to a transporter or CYP that modulates the elimination process for the test substance. References/publication refers to a publication or bachelor thesis conducted on the test substance. PK-DB studies and primary PBPK modeler describe the number of studies curated from the literature and present PK-DB, and the person modeling the test substance. The data was accessed on 2022-11-08.

3.2 Published work 1: Pharmacokinetics Database PK-DB

An open issue in the field of pharmacokinetics is the reusable storage of data from experimental and clinical studies, which is especially important for computational modeling. The need for a standardized representation of pharmacokinetics data led to the development of PK-DB, published in January 2021. The aim was to create an open online resource on pharmacokinetics that adheres to FAIR principles [Wil+16]. The database is in active use, and since its publication, it has grown to include pharmacokinetics content on many substances used for dynamical liver function testing and CYP phenotyping, see Tab. 3.1, as a result, it perfectly complements the theme of the thesis. Since the publication of PK-DB, the content of the database has increased by approximately 300 studies to a total of more than 700 studies, to which I have contributed in part, see Fig. 3.1A. It contains a representative cross-section of the literature on the clinical investigation in the field of pharmacokinetics focused on test compounds relevant for CYP phenotyping and liver function testing.

Some aspects of the content of the database are investigated in the following. The results probably generalize to the whole body of literature on PK clinical investigation and have not been published in the original publication. When looking at the content of PK-DB, it becomes apparent that demographic and racial bias exists in pharmacokinetics studies (e.g., sex, ethnicity, age, health). Specifically, studies in

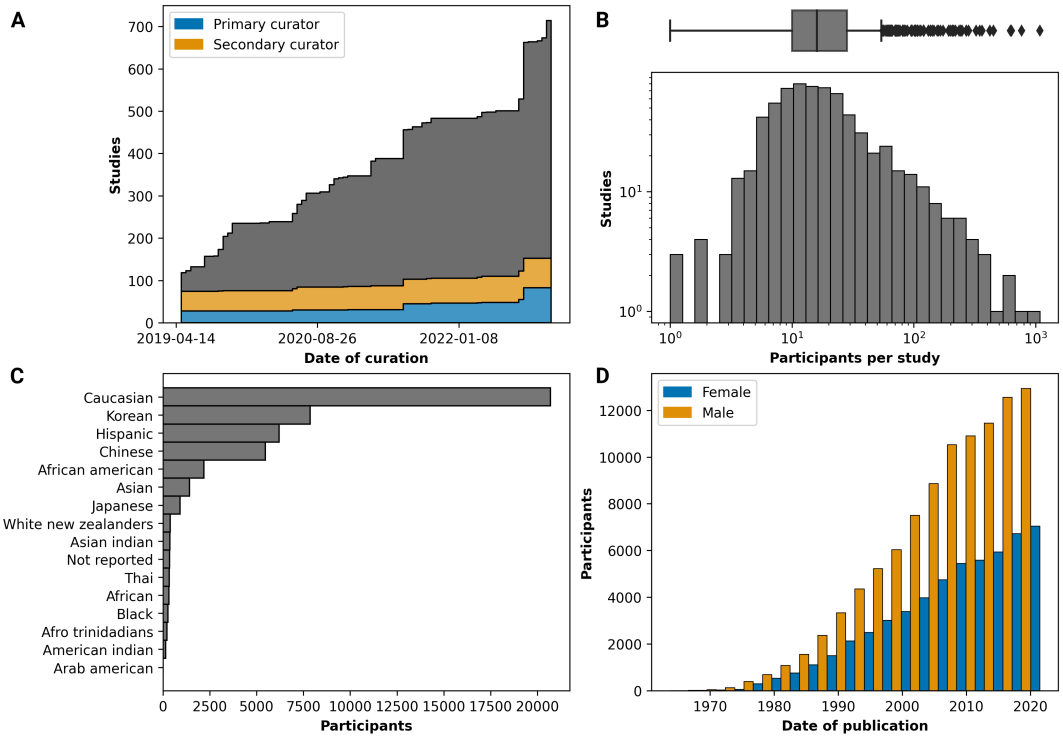

FIGURE 3.1: **PK-DB statistics**: **A)** Cumulative number of pharmacokinetic studies over time in PK-DB. Studies curated primarily by me (blue) and curated secondarily by me (orange); **B)** the distribution of participants in clinical trials in PK-DB, presented in log scale. 25 percent of studies have less than 10 participants ($Q_1 = 10$). 50 percent of studies have less than 16 participants ($Q_2 = 16$), and 25 percent of studies have more than 30 participants ($Q_3 = 30$); **C**) the total number of study participants in PK-DB, stratified by the reported ethnicity; **D)** the cumulative number of female (green) and male (orange) participants in pharmacokinetic studies versus the date of publication. The data was accessed on 2022-11-17.

PK-DB, published from 1970 until today, show a strong bias towards male participants, see Fig. 3.1A, with males being approximately twice as often studied in PK investigations as women. Regarding ethnicity, Caucasians are strongly overrepresented, see Fig. 3.1C. There is also a bias in the individual participant age between healthy and unhealthy subjects, see Fig. 3.2. Healthy subjects are, on average more than 20 years younger than unhealthy subjects.

In addition, most of the studies in the field of pharmacokinetics contain rather a moderate number of individuals, with 75 percent of them having less than 30 participants, see Fig. 3.1B. Small sample sizes are more prone to bias, often lack the diversity needed to represent the population accurately, and are also more likely to be impacted by random chance. This is especially problematic because large inter-individual variability exists in pharmacokinetics. In conclusion, the results based on the data should either take into account the confounding factors or be treated with some caution as they might not generalize very well.

In summary, a high-quality open online pharmacokinetics database was developed. Importantly, the data is enriched with the required meta-information for computational modeling and data integration.

Publication

PK-DB: pharmacokinetics database for individualized and stratified computational modeling
Grzegorzewski J, Brandhorst J, Green K, Eleftheriadou D, Duport Y, Bartsch F, Köller A, Ke DYJ, De Angelis S, König M. Nucleic Acids Res. 2021 Jan 8;49(D1):D1358-D1364. doi: 10.1093/nar/gkaa990. PMID: 33151297

The PK-DB frontend, API, and the source code of PK-DB were released alongside the publication. For the PAGE 2021 conference, a short video of me presenting a poster on PK-DB was made available as well:

- Source code: https://github.com/matthiaskoenig/pkdb
- Web application (API): https://pk-db.com/api/v1/swagger/
- Short tutorial on API: https://github.com/matthiaskoenig/pkdb/blob/develop/docs/pkdb_api.ipynb
- Web application (frontend): https://pk-db.com
- Poster presentation: https://www.youtube.com/watch?v=g6U47dc_nuY&t=4s

D1358–D1364 *Nucleic Acids Research, 2021, Vol. 49, Database issue* *Published online 5 November 2020*
doi: 10.1093/nar/gkaa990

PK-DB: pharmacokinetics database for individualized and stratified computational modeling

Jan Grzegorzewski[1], Janosch Brandhorst[1], Kathleen Green[2], Dimitra Eleftheriadou[1], Yannick Duport[3], Florian Barthorscht[1], Adrian Köller[1], Danny Yu Jia Ke[4], Sara De Angelis[5] and Matthias König[1,*]

[1]Institute for Theoretical Biology, Humboldt-University Berlin, Invalidenstraße 110, Berlin 10115, Germany, [2]Department of Biochemistry, University of Stellenbosch, Van der Byl Street, Stellenbosch 7600, South Africa, [3]Department of Mathematics and Computer Science, Freie Universität Berlin, Arnimallee 14, Berlin 14195, Germany, [4]Department of Biology, University of Ottawa, Ottawa, ON, Canada and [5]King's College London, Department of Biomedical Engineering & Imaging Sciences, London, UK

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ABSTRACT

A multitude of pharmacokinetics studies have been published. However, due to the lack of an open database, pharmacokinetics data, as well as the corresponding meta-information, have been difficult to access. We present PK-DB (https://pk-db.com), an open database for pharmacokinetics information from clinical trials. PK-DB provides curated information on (i) characteristics of studied patient cohorts and subjects (e.g. age, bodyweight, smoking status, genetic variants); (ii) applied interventions (e.g. dosing, substance, route of application); (iii) pharmacokinetic parameters (e.g. clearance, half-life, area under the curve) and (iv) measured pharmacokinetic time-courses. Key features are the representation of experimental errors, the normalization of measurement units, annotation of information to biological ontologies, calculation of pharmacokinetic parameters from concentration-time profiles, a workflow for collaborative data curation, strong validation rules on the data, computational access via a REST API as well as human access via a web interface. PK-DB enables meta-analysis based on data from multiple studies and data integration with computational models. A special focus lies on meta-data relevant for individualized and stratified computational modeling with methods like physiologically based pharmacokinetic (PBPK), pharmacokinetic/pharmacodynamic (PK/PD), or population pharmacokinetic (pop PK) modeling.

INTRODUCTION

The pharmacokinetics (PK) of drugs and medication, i.e. how the body after administration affects substances via absorption, distribution, metabolization, and elimination, are of great interest for medical research and drug development. The main measures in the field are concentration-time profiles and derived PK parameters from these time-courses like half-lifes or clearance rates. These measures strongly depend on the dosage and individual characteristics of the subject or group under investigation. Factors like age, weight, sex, smoking behavior, genetic variants or disease drive the large inter-individual variability in PK (1) making such meta-data indispensable for research in pharmacokinetics. The study of variability in drug exposure due to these covariates is an important field of research with a long history, generally referred to as population pharmacokinetics (2). Modern approaches go beyond classical population information by accounting for additional factors, for example, for genetic variants (3). This meta-information on subjects in combination with the main measures are the basis for individualized and stratified approaches in drug treatment which will potentially pave the road towards both precision dosing and precision medicine.

A multitude of PK studies have been published but despite the wealth of literature almost none of the data is accessible in a machine-readable format and certainly not with FAIR (findable, accessible, interoperable and reproducible) principles (4) in mind. The lack of transparency and reproducibility (5) in the field is ubiquitous. Currently the only way to retrieve this treasure is by digitizing and curating the pharmacokinetics information from publications. Despite the central role of PK in the medical and pharma field, no open freely accessible database of pharmacokinetics information exists so far. In addition, heterogeneity in the reporting of clinical study designs, pharmacokinetic measures, in-

*To whom correspondence should be addressed. Tel: +49 30 209398435; Fax: +49 30 209398435; Email: konigmatt@googlemail.com

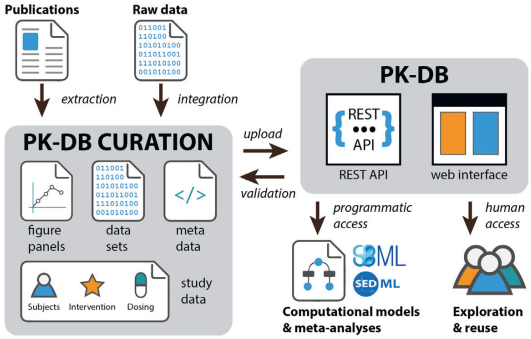

Figure 1. PK-DB overview. Schematic overview of the curation process and interaction with the PK-DB database. Data is either extracted from literature (digitization of figures and tables) or data sets are directly imported (from collaboration partners). Figure panels, data sets, meta-data and study information on subjects, interventions and dosing is curated. All data files and the study information are uploaded via REST endpoints. The curated data is checked against validation rules, data is normalized (e.g. units), and pharmacokinetic parameters are calculated. The uploaded study information can either be programmatically accessed via the REST API or via the web frontend.

dividual, and population-related meta-information further complicates data reuse and integration. Many studies only report a small fraction of the underlying data, e.g. individual data or prominent PK parameters are missing in most studies and even time-courses averaged over subjects within a group are only present in a subset of data.

For computational modeling, meta-analysis and most methods in machine learning a standardized and machine-readable representation of data is of major importance. PK data could be utilized in many different ways (6–8) if such a representation and corresponding database would exist. One of the various applications is pharmacokinetic modeling which provides a unique opportunity to integrate PK data and parameters from multiple clinical trials into a single predictive model.

These models can integrate PK-DB data on differences in the study protocol, the dosing, as well as individual, group and population characteristics and be parameterized and validated with the available time courses and pharmacokinetic parameters.

DESCRIPTION AND RESULTS

PK-DB (https://pk-db.com) is an open web-accessible database storing comprehensive information on pharmacokinetics studies consisting of PK data, PK parameters, and associated meta-information (see Figure 1 for a general overview).

Database statistics

PK-DB provides curated information on (i) characteristics of studied patient cohorts and subjects (e.g. age, bodyweight, smoking status, genetic variants); (ii) applied interventions (e.g. dosing, substance, route of application); (iii) concentration-time curves and (iv) parameters measured in PK studies (e.g. clearance, half-life and area under the curve). The focus so far of data curation has been on substances applied in dynamical liver function tests, benzodiazepines, statins, and studies of glucose metabolism.

PK-DB-v0.9.3 (9) consists of 512 studies containing 1457 groups, 6308 individuals, 1408 interventions, 73 017 outputs, 3148 time-courses and 37 scatters related to acetaminophen, caffeine, codeine, diazepam, glucose, midazolam, morphine, oxazepam, simvastatin or torasemide (see Figure 2, Supplementary Material 1 and Supplementary Material 2).

Design principles

Important features of PK-DB are the representation of experimental errors, the normalization of measurement units, annotation of information to biological ontologies, calculation of pharmacokinetic parameters from concentration-time profiles, a workflow for collaborative data curation, strong validation rules on the data, computational access via a REST API as well as human access via a web interface. Key principles in the design of PK-DB were:

Accessibility of data for computational modeling and data science. All data is available via REST endpoints as well as the web frontend allowing for simple integration of PK-DB data into existing workflows, e.g. for the building of computational models. The REST web service allows querying and retrieving all information from PK-DB in an automatic fashion. The major advantage of the REST API (https://pk-db.com/api/v1/swagger/) as a central access point is that it can be accessed programmatically independent of the programming language. In the following, we present various use cases to demonstrate the usefulness of this approach, e.g. creating an overview of the database content using R and circos (Figure 2), and meta-analyses of multiple studies using Python (Figure 4). The use of PK-DB data is facilitated by annotation of biological and medical concepts to respective ontologies. This enables the integration with additional data sets or computational models based on the semantic information, e.g., substances are annotated to ChEBI (10), and diseases to ncit, hp, doid and mondo (11–14). A special focus lies on meta-data for individualized and stratified computational modeling of pharmacokinetics with methods like physiologically based pharmacokinetic (PBPK), pharmacokinetic/pharmacodynamic (PK/PD) or population pharmacokinetic (pop PK) modeling.

Extensibility and generalizability. The PK-DB data model is not limited to a specific problem domain but allows simple extensions to other fields and experimental data sets, within the overall area of pharmacokinetics. Examples are extensible types for the group or individual characteristics currently represented in the database. Additional types can easily be added to cover the important information for a given problem domain.

Unit and data normalization. A key challenge in using data for computational modeling and data science are non-standardized units coming from different data sets. It requires time-consuming retrieval of this information from

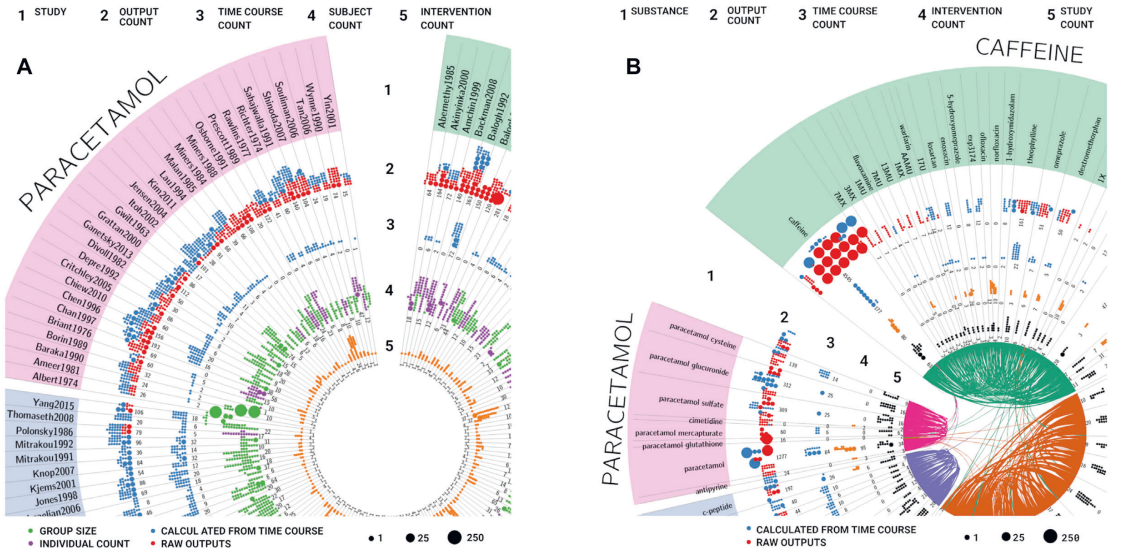

Figure 2. PK-DB content. (**A**) Studies. Overview of the study content in PK-DB. PK-DB-v0.9.3 (9) 512 studies containing 1457 groups, 6308 individuals, 1408 interventions, 73017 outputs, 3148 time-courses and 37 scatters related to acetaminophen, caffeine, codeine, diazepam, glucose, midazolam, morphine, oxazepam, simvastatin or torasemide. The circular plot is structured in stripes and rings, with each stripe representing a single study. In each ring, the counts of different data types are depicted. Dot size corresponds to the number of entries. The rings give an overview of the following information (1) name of the study; (2) number of outputs (PK parameters and other measurements). Red dots represent reported data, blue dots data calculated from time-courses; (3) number of time-courses; (4) number of participants. Purple dots represent participants with individual data, green dots represent participants which are reported as a group; (5) the number of interventions applied to the participants in the study. (**B**) Substances Overview of the substance content in PK-DB. Substances with very few entries (<2 studies) are excluded from the plot. The circular plot is structured in stripes and rings, with each stripe representing a different substance. Substances were clustered in five substance classes (caffeine, glucose, codeine, and paracetamol) by agglomerative clustering of the pair co-occurrence of substances within studies. Classes are labeled according to the most frequent substance within the class. Each co-occurrence of two substances is visualized by a connecting ribbon between the substances in the center. The rings describe the following information for the respective substance (1) name of the substance; (2) number of outputs (PK parameters and other measurements). Red dots represent reported data and blue dots represent data calculated from reported concentration-time profiles. (3) the number of time-courses; (4) number of applied interventions; (5) number of studies in which the substance occurred. For complete figures see Supplementary Material 1 and Supplementary Material 2.

the literature and error-prone conversion of units and corresponding data. PK-DB provides a solution to this issue. During upload the data is harmonized, e.g. data is converted between molar units and mass, using the molecular weight of the respective substances based on its ChEBI information (10). In addition, for all information stored the allowed units are defined (actual units must be convertible to these units).

Representation of time-course data. The main measures in pharmacokinetics studies are concentration-time curves of the administered substance and its metabolites. These time-courses are crucial for kinetic modeling, e.g. using PBPK or PK/PD models. Consequently, a central focus was on storing and analyzing such data efficiently.

Calculation of pharmacokinetic parameters. PK-DB calculates important PK parameters such as half-life, clearance or volume of distribution from the time-concentration profiles during data upload based on non-compartmental methods (15). Parameters are calculated based on linear regression of the logarithmic concentration values in the exponential decay phase (see example in Figure 3 and Table 1). Non-compartmental methods were chosen for comparison of calculated values with reported PK parameters in the literature, mainly calculated based on non-compartmental methods.

Data quality. Strong validation (e.g. of categoricals), minimum relevant information, instance cross-referencing and correct unit-dimensions ensure high quality of the curated data. Non-obvious curation mistakes (or respective errors in the reporting of the data) can be addressed by outlier identification in subsequent meta-analyses.

Access rights. PK-DB allows to keep studies privately or only share with certain collaborators. This allows sharing the study during the curation process only with trusted people with a simple option to make the study public. Some information is only accessible by a limited group of users due to copyright issues, e.g. for manually curated studies from the literature the underlying publication can only be made accessible if it is Open Access. A subset of studies is currently private because the underlying raw data from the clinical trial has not been published yet.

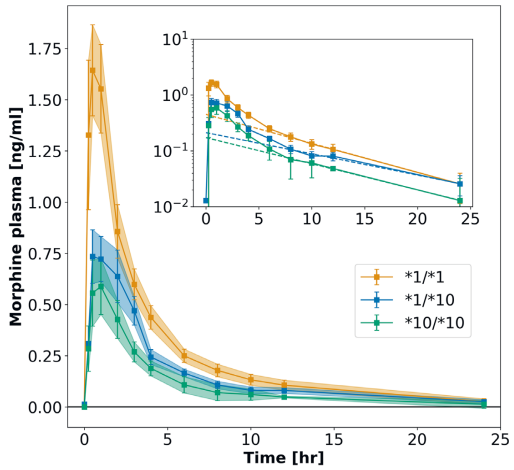

Figure 3. Calculation of pharmacokinetics data from time-courses. Concentration–time courses have been digitized from figures in the literature. This example shows morphine plasma time-courses after the application of codeine to three subgroups with different genotypes (16) (see https://pk-db.com/data/PKDB00111). PK parameters are calculated from reported concentration-time profiles using non-compartmental methods, e.g. the half-life (t_{half}) or elimination rate (k_{el}) of morphine. The exponential decay is used for the fitting of PK parameters (see inlet). Calculated and reported PK parameters for this example are listed in Table 1. Due to the unavailability of individual participant data in most pharmacokinetics studies, parameters have to be determined on the mean time–concentration curves (averaged over subjects in a given group). Data is mean ± standard error (SE).

Technology

The PK-DB backend is implemented in Python using the Django framework with Postgres as the underlying database system. For fast, full-text search most data is indexed with Elasticsearch. The provided REST API uses the Django-rest-framework with endpoints accessible from https://pk-db.com/api/. The web frontend (https://pk-db.com) is implemented in JavaScript based on the Vue.js framework interacting with the backend via the REST API. The complete PK-DB stack is distributed as docker-containers. PK-DB is licensed under GNU Lesser General Public License version 3 (LGPL-3.0) with source code available from https://github.com/matthiaskoenig/pkdb.

Curation workflow

PK-DB provides a collaborative curation interface which simplifies the upload and update of curated study information. A central component is to track all files and curation changes via Git version control. On changes, the files can automatically be uploaded and validated against a development server which provides direct feedback on missing information or curation errors (e.g. units of concentration measurements must be convertible to [g/l] or [mol/l]). A multitude of constraints have been defined as validation rules on the uploaded data instead of having the data model layer too restrictive. These validation rules are constantly updated based on curator feedback. Allowed choices in the data model are based on an internal ontology, which allows to update encodable information without the need to update the database backend.

The typical workflow for extracting data from the literature is depicted in Figure 1. At the beginning of the curation process, a body of literature is selected based on literature research for a given problem domain. Subsequently, the relevant (meta-)information is manually extracted from the literature and encoded in a standardized JSON format. Extracted data like concentration-time courses or PK parameters are stored as tabular data in spreadsheets. After finishing the initial curation process, a second curator is checking the data.

Curation is an iterative process involving multiple curators over time. Tracking changes to the curated data is therefore crucial. Instead of implementing such history and change tracking on database level with substantial overhead, we utilize the full set of Git features out of the box to track changes to our files. All curators work hereby on a shared Git repository. Private data can be tracked in separate private Git repositories.

Calculation of pharmacokinetic parameters

An important part of PK-DB is the automatic calculation of PK parameters from the reported concentration-time curves based on non-compartmental methods (15). Figure 3 and Table 1 illustrate the automatic calculation of PK parameters from concentration-time profiles for an example study. The authors were hereby interested in the influence of specific genetic alleles on the pharmacokinetics of codeine (16). In the study, information was limited to the averaged measures with variation (standard error within group), but no individual subject data was reported.

Calculated parameters are the area under the curve (AUC_{end}), the area under the curve extrapolated to infinity (AUC_{∞}), the concentration maximum (C_{max}), the time at concentration maximum (t_{max}), the half-life (t_{half}), the elimination rate (k_{el}), the clearance (Clearance) and the volume of distribution (V_d). The calculated values are in good agreement with the reported values (all lie within the reported standard deviations).

Mathematically correct, first the PK parameters should be calculated for each subject individually and subsequently be averaged. Unfortunately, this is not possible if only averaged data is reported. Consequently, as approximation PK parameters are calculated on the averaged time-courses. Due to the often very large inter-individual differences in pharmacokinetics the calculated values on average data can be notable different between reported and calculated parameters (Table 1, e.g. t_{half}). Even more fundamentally, the description of the data as averages with variations has inherent problems by assuming homogeneity of the data which often is not the case (17). Consequently, we strongly encourage the publication of individual subject data in PK studies.

A further limitation of PK studies is that often only a subset of pharmacokinetics information is reported. In the example displayed in Table 1 (16), the volume of distribution (V_d) and the elimination rate (k_{el}) are not reported, but can be calculated.

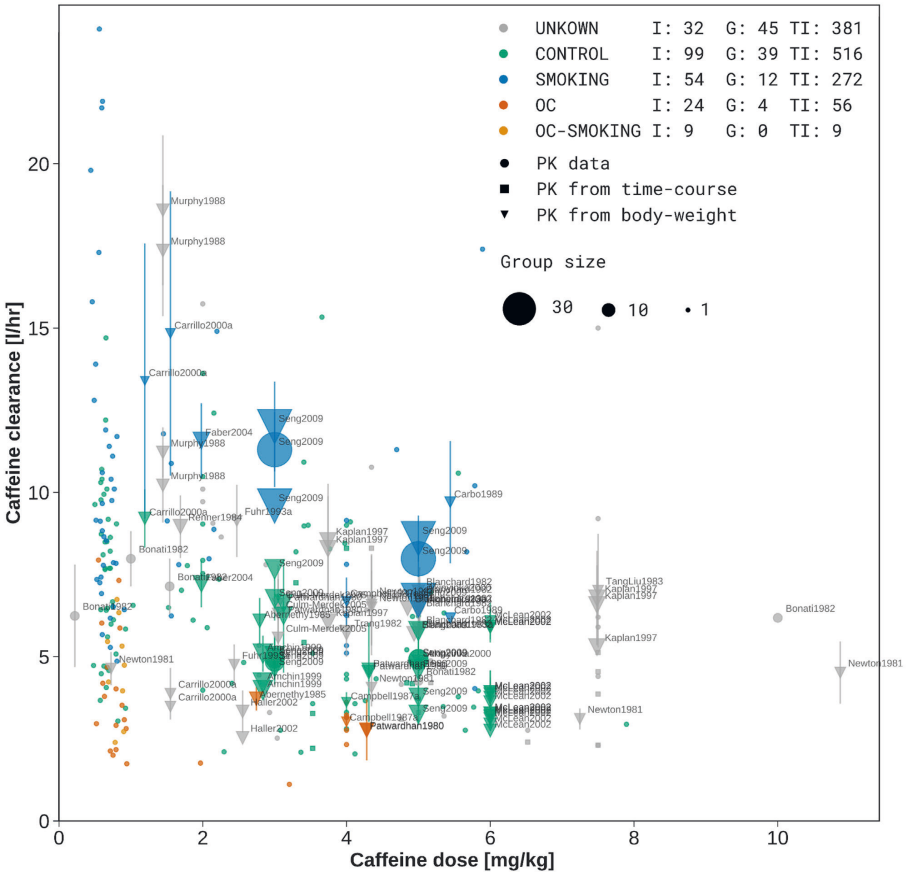

Figure 4. Meta-analysis of caffeine clearance depending on caffeine dose. Caffeine clearance is stratified based on reported smoking and oral contraceptive (OC) use. UNKNOWN (grey) data corresponds to unreported smoking and OC, CONTROL (green) are non-smokers not taking OC, SMOKING (blue) are smokers not taking OC, OC (dark orange) are non-smokers taking OC, and OC-SMOKING (light orange) are smokers taking oral contraceptives. For the stratification groups the number of individuals (I), number of groups (G) and number of total participants (TP) is provided in the legend. Individual and group data is depicted, with group size encoded as dot size. Data points from groups are labeled by the study identifier. Reported PK parameters are depicted as circles, PK parameters calculated from concentration-time profiles as squares, and PK parameters inferred from PK data and reported bodyweights of the participants as triangles (to convert to dose per bodyweight). Typically, dosing is reported in mass units and clearance in a volume per time. Sometimes both values are reported in bodyweight units. Here, all available data is harmonized. Suspicious data from four studies (18–21), very likely from a single clinical trial, was excluded.

Meta-analysis of caffeine

PK-DB allowed us for the first time to undertake an extensive and systematic analysis of the effect of lifestyle factors like smoking and oral contraceptive use on the clearance of caffeine combining data from multiple studies. For this use case, we integrated data from 44 studies, based on programmatic interaction with PK-DB via the REST API. By curating information about the respective patient characteristics (lifestyle factors), the actual interventions performed in the studies (dosing and route), and important information like the errors on the reported data we could gain a unique view on the strong and consistent effect of smoking and oral contraceptive use on the clearance of caffeine. The large variability between studies and individuals could be markedly reduced by accounting for lifestyle information.

Importantly, the meta-analysis allowed us to directly improve the curation status of many studies by easily detecting visible outliers in the data which could in most cases directly be backtracked to curation errors or incorrectly reported data (e.g. incorrect units) which were subsequently corrected in the database.

A positive aspect is that most of the reported studies are consistent. For instance with caffeine, most of the data was in line with each other with a single exception being Stille *et al.* (18). Here, a systematic bias in the data could be observed probably due to an analytic problem. Interestingly,

Table 1. Calculation of pharmacokinetic parameters. Comparison of PK parameters for morphine reported in a representative study of codeine application (16) (see https://pk-db.com/data/PKDB00111). PK parameters were calculated from mean concentration-time profiles (see Figure 3). Only data for the groups, no individual data was reported in the study

Parameter (unit)	Genotype	Reported $\overline{x_r} \pm SD$	PK-DB $\overline{x_c}$	Difference (%) $\frac{\overline{x_r}-\overline{x_c}}{\overline{x_r}}$
AUC_{end}	*1/*1	6.63 ± 2.07	6.24	5.88
(ng/ml · h)	*1/*10	3.77 ± 1.93	3.80	− 0.80
	*10/*10	2.65 ± 1.95	2.59	2.26
AUC_{∞}	*1/*1	8.52 ± 4.10	6.40	24.88
(ng/ml · h)	*1/*10	5.05 ± 3.30	3.97	21.39
	*10/*10	3.26 ± 2.43	2.68	17.79
C_{max}	*1/*1	2.06 ± 0.89	1.64	20.39
(ng/ml)	*1/*10	0.96 ± 0.42	0.73	23.96
	*10/*10	0.68 ± 0.50	0.59	13.24
t_{half}	*1/*1	9.40 ± 11.70	4.15	55.85
(h)	*1/*10	11.50 ± 11.10	4.76	58.61
	*10/*10	6.84 ± 5.46	4.66	31.87
t_{max}	*1/*1	0.64 ± 0.28	0.50	21.88
(h)	*1/*10	0.86 ± 0.52	0.50	41.86
	*10/*10	0.86 ± 0.52	1.00	− 16.28
k_{el}	*1/*1	–	0.17	–
(1/h)	*1/*10	–	0.15	–
	*10/*10	–	0.15	–

Reported PK parameters are presented as mean ± standard deviation (SD)
Unreported values displayed as (–)
AUC – area under the curve; C_{max} – maximum concentration; t_{half} – half-life; t_{max} – time of maximum concentration; k_{el} – elimination rate.

the same data set was published multiple times, overall in four publications all showing the same bias (18–21).

Data quality and validation

The integration of data from multiple studies and subsequent meta-analyses is a valuable procedure to identify curation errors which cannot be caught by validation rules alone. The combination of both, the validation rules and the meta-analyses helped to identify errors also in the reporting. In the following, we will give some examples of suspicious reported data detected by meta-analysis: Wang *et al.* (22) reported incorrect units; Seng et al. (23) calculated volumes per bodyweight incorrectly; In the publication of Carbo *et al.* (24) participant number 4 has a suspiciously high half-life and participant number 3 a suspiciously high clearance rate. It is unclear if this is a reporting error; In the publication of Beach *et al.* (25) nine smokers and two non-smokers have suspiciously very high clearance rates, again unclear if this is a reporting error. In the publication of Wu *et al.* (16) the concentration-time profiles and concentration maxima were reported with incorrect units.

Data validation and data integration via PK-DB allowed us to identify and correct these issues.

CONCLUSION AND DISCUSSION

PK-DB is the first open database for pharmacokinetics data and corresponding meta-information. We provide an important resource which allows storing pharmacokinetics information in a FAIR (findable, accessible, interoperable and reproducible) manner (4).

PK-DB is supported by the Federal Ministry of Education and Research (BMBF, Germany) within the research network Systems Medicine of the Liver (LiSyM) until 2021. PK-DB is a central part of the DFG funded project QuaLiPerf (Quantifying Liver Perfusion–Function Relationship in Complex Resection—A Systems Medicine Approach) with maintenance guaranteed until end of 2025.

We demonstrate the value of PK-DB via a stratified meta-analysis of pharmacokinetics studies for caffeine curated from literature which allows us to integrate and harmonize pharmacokinetics information from a wide range of studies and sources. By performing the curation for commonly applied drugs (codeine and paracetamol), for a substance used in liver function tests (caffeine), as well as for glucose we could demonstrate the applicability of PK-DB to a wide range of substances and gain insights into how well data is reported in the various fields.

PK-DB has many unique features compared to existing databases for pharmacokinetics information, such as the database for pharmacokinetics properties (26) or the recent pharmacokinetic database time-series data and parameters for 144 environmental chemicals (27). PK-DB allows to handles experimental errors, the normalization of measurement units, annotation of information to biological ontologies, calculation of pharmacokinetic parameters from concentration-time profiles, comes with a workflow for collaborative data curation, has strong validation rules on the data, and provides computational access via a REST API as well as human access via a web interface.

The reporting of data in the field of pharmacokinetics is very poor despite the main point of the publications being the reporting of the data. Without guidelines on minimal information for studies, it is very difficult to compare studies or integrate data from different sources. Incomplete and poor reporting of data in the field of pharmacokinetics has also been reported by others (28,29). As our analysis shows, even basic information, crucial for the interpretation and analyses of PK studies, are not reported in many publications. It is impossible to integrate and reuse such data. For instance, in the case of codeine, often not even the given dose can be retrieved from the publication because it is not clearly reported which substance was administered (codeine-sulfate, codeine-phosphate or codeine). Other examples are unreported bodyweights, so that conversions to doses per bodyweight are not possible.

Based on our work we have a set of important suggestions when publishing clinical studies in the field of pharmacokinetics: (i) Publish the actual data in a machine-readable format (e.g. a data table in the supplement); (ii) Publish the actual concentration-time curves, not only derived parameters; (iii) Provide data for individual subjects which is much more informative and allows to calculate all data for individuals and for groups; (iv) Provide at minimum information on (individual) patient characteristics which includes basic anthropometric information like age, bodyweight, sex, height, and the subset of important lifestyle factors known to alter pharmacokinetics (e.g. co-medication, oral contraceptive use, smoking status, alcohol consumption or for instance for CYP1A2 substrates like caffeine: methylxanthine consumption/abstinence); (v) Clearly describe the study protocol: Which substance was given in which dose, in which route (oral, intravenous), and in what form (tablet, capsule, solution), the more specific the information the better.

Prospective plans for PK-DB are to increase the scope by manual curation and semi-automatic approaches. Furthermore, workflows for the deposition of pharmacokinetics data upon publication will be established.

We envision that PK-DB will encourage better reporting of pharmacokinetics studies by providing means for data representation and integration and will improve reusability of pharmacokinetics information by providing PK data in a central database, and will facilitate data integration between studies and with computational models.

SUPPLEMENTARY DATA

Supplementary Data are available at NAR Online.

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Author contributions: J.G. and M.K. conceived the study, drafted the manuscript, implemented the software, and performed all analyses. J.G., M.K., J.B., K.G., D.E., Y.D., F.B., A.K., D.K., S.D.A. and D.E. curated data for PK-DB. All authors read, corrected and approved the manuscript.

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Conflict of interest statement. None declared.

REFERENCES

1. Polasek,T.M., Shakib,S. and Rostami-Hodjegan,A. (2018) Precision dosing in clinical medicine: present and future. *Expert. Rev. Clin. Immu.*, **11**, 743–746.
2. Aarons,L. (1991) Population pharmacokinetics: theory and practice. *Brit. J. Clin. Pharmaco.*, **32**, 669–670.
3. Lötsch,J., Skarke,C., Schmidt,H., Rohrbacher,M., Hofmann,U., Schwab,M. and Geisslinger,G. (2006) Evidence for morphine-independent central nervous opioid effects after administration of codeine: contribution of other codeine metabolites. *Clin. Pharm. Th.*, **79**, 35–48.
4. Wilkinson,M.D., Dumontier,M., Aalbersberg,I.J.J., Appleton,G., Axton,M., Baak,A., Blomberg,N., Boiten,J.-W., da Silva Santos,L.B., Bourne,P.E. *et al.* (2016) The FAIR Guiding Principles for scientific data management and stewardship. *Scientific Data*, **3**, 160018.
5. Ioannidis,J.P.A. (2019) Reproducible pharmacokinetics. *J. Pharmacokinet. Phar.*, **46**, 111–116.
6. Mould,D.R. and Upton,R.N. (2012) Basic concepts in population modeling, simulation, and model-based drug development. *CPT: Pharmacometrics Syst. Pharmacol.*, **1**, e6.
7. Mould,D.R. and Upton,R.N. (2013) Basic concepts in population modeling, simulation, and model-based drug development-part 2: introduction to pharmacokinetic modeling methods. *CPT: Pharmacometrics Syst. Pharmacol.*, **2**, e38.
8. Upton,R.N. and Mould,D.R. (2014) Basic concepts in population modeling, simulation, and model-based drug development: part 3-introduction to pharmacodynamic modeling methods. *CPT: Pharmacometrics Syst. Pharmacol.*, **3**, e88.
9. König,M. and Grzegorzewski,J. (2020) matthiaskoenig/pkdb: release notes for pkdb-v0.9.3.
10. Hastings,J., Owen,G., Dekker,A., Ennis,M., Kale,N., Muthukrishnan,V., Turner,S., Swainston,N., Mendes,P. and Steinbeck,C. (2016) ChEBI in 2016: improved services and an expanding collection of metabolites. *Nucleic Acids Res.*, **44**, D1214–D1219.
11. Sioutos,N., de Coronado,S., Haber,M.W., Hartel,F.W., Shaiu,W.-L. and Wright,L.W. (2007) NCI Thesaurus: a semantic model integrating cancer-related clinical and molecular information. *J. Biomed. Inform.*, **40**, 30–43.
12. Köhler,S., Carmody,L., Vasilevsky,N., Jacobsen,J.O.B., Danis,D., Gourdine,J.-P., Gargano,M., Harris,N.L., Matentzoglu,N., McMurry,J.A. *et al.* (2019) Expansion of the Human Phenotype Ontology (HPO) knowledge base and resources. *Nucleic Acids Res.*, **47**, D1018–D1027.
13. Kibbe,W.A., Arze,C., Felix,V., Mitraka,E., Bolton,E., Fu,G., Mungall,C.J., Binder,J.X., Malone,J., Vasant,D. *et al.* (2015) Disease Ontology 2015 update: an expanded and updated database of human diseases for linking biomedical knowledge through disease data. *Nucleic Acids Res.*, **43**, D1071–D1078.
14. Mungall,C.J., McMurry,J.A., Köhler,S., Balhoff,J.P., Borromeo,C., Brush,M., Carbon,S., Conlin,T., Dunn,N., Engelstad,M. *et al.* (2017) The Monarch Initiative: an integrative data and analytic platform connecting phenotypes to genotypes across species. *Nucleic Acids Res.*, **45**, D712–D722.
15. Gabrielsson,J. and Weiner,D. (2012) Non-compartmental analysis. *Methods Mol. Biol. (Clifton, N.J.)*, **929**, 377–389.
16. Wu,X., Yuan,L., Zuo,J., Lv,J. and Guo,T. (2014) The impact of CYP2D6 polymorphisms on the pharmacokinetics of codeine and its metabolites in Mongolian Chinese subjects. *Eur. J. Clin. Pharmacol.*, **70**, 57–63.
17. Hanin,L. (2017) Why statistical inference from clinical trials is likely to generate false and irreproducible results. *BMC Med. Res. Methodol.*, **17**, 127.
18. Stille,W., Harder,S., Mieke,S., Beer,C., Shah,P.M., Frech,K. and Staib,A.H. (1987) Decrease of caffeine elimination in man during co-administration of 4-quinolones. *J. Antimicrob. Chemoth.*, **20**, 729–734.
19. Harder,S., Staib,A.H., Beer,C., Papenburg,A., Stille,W. and Shah,P.M. (1988) 4-Quinolones inhibit biotransformation of caffeine. *Eur. J. Clin. Pharmacol.*, **35**, 651–656.
20. Harder,S., Fuhr,U., Staib,A.H. and Wolff,T. (1989) Ciprofloxacin-caffeine: a drug interaction established using in vivo and in vitro investigations. *Am. J. Med.*, **87**, 89S–91S.
21. Balogh,A., Harder,S., Vollandt,R. and Staib,A.H. (1992) Intra-individual variability of caffeine elimination in healthy subjects. *Int. J. Clin. Pharmacol. Ther. Toxicol.*, **30**, 383–387.
22. Wang,T., Kleber,G., Stellaard,F. and Paumgartner,G. (1985) Caffeine elimination: a test of liver function. *Klin. Wochenschr.*, **63**, 1124–1128.
23. Seng,K.-Y., Fun,C.-Y., Law,Y.-L., Lim,W.-M., Fan,W. and Lim,C.-L. (2009) Population pharmacokinetics of caffeine in healthy male adults using mixed-effects models. *J. Clin. Pharm. Ther.*, **34**, 103–114.
24. Carbó,M., Segura,J., De la Torre,R., Badenas,J.M. and Camí,J. (1989) Effect of quinolones on caffeine disposition. *Clin. Pharm. Th.*, **45**, 234–240.
25. Beach,C.A., Mays,D.C., Guiler,R.C., Jacober,C.H. and Gerber,N. (1986) Inhibition of elimination of caffeine by disulfiram in normal subjects and recovering alcoholics. *Clin. Pharm. Th.*, **39**, 265–270.
26. Moda,T.L., Torres,L.G., Carrara,A.E. and Andricopulo,A.D. (2008) PK/DB: database for pharmacokinetic properties and predictive in silico ADME models. *Bioinformatics (Oxford, England)*, **24**, 2270–2271.
27. Sayre,R.R., Wambaugh,J.F. and Grulke,C.M. (2020) Database of pharmacokinetic time-series data and parameters for 144 environmental chemicals. *Scientific Data*, **7**, 122.
28. Kanji,S., Hayes,M., Ling,A., Shamseer,L., Chant,C., Edwards,D.J., Edwards,S., Ensom,M.H.H., Foster,D.R., Hardy,B. *et al.* (2015) Reporting guidelines for clinical pharmacokinetic studies: the ClinPK statement. *Clin. Pharmacokinet.*, **54**, 783–795.
29. Dykstra,K., Mehrotra,N., Tornøe,C.W., Kastrissios,H., Patel,B., Al-Huniti,N., Jadhav,P., Wang,Y. and Byon,W. (2015) Reporting guidelines for population pharmacokinetic analyses. *J. Pharmacokinet. Phar.*, **42**, 301–314.

3.3 Published work 2: Pharmacokinetics of Caffeine

In this work, data integration and meta-analysis were performed to gain insights into the pharmacokinetics of caffeine in the context of metabolic phenotyping and liver function testing. Caffeine is the most common substance to phenotype CYP1A2 activity and also finds some application for general liver function testing. In terms of PK, caffeine is presumably one of the most studied substances, with a very large body of PK literature. The ubiquitous amounts of data motivated a systematic approach inspired by the guidelines for systematic reviews, named "Preferred Reporting Items for Systematic Reviews and Meta-Analyses" (PRISMA) [Moh+09; Tri+18]. By applying the principles and curating the available information in great detail, the data were harmonized and made available for the investigation of factors affecting the pharmacokinetics of caffeine and biases. In this work, a total of 141 clinical studies on caffeine pharmacokinetics were curated and made available via PK-DB. This makes it presumably the largest currently openly available dataset on caffeine pharmacokinetics. Only certain aspects of the data were used for the meta-analysis. The

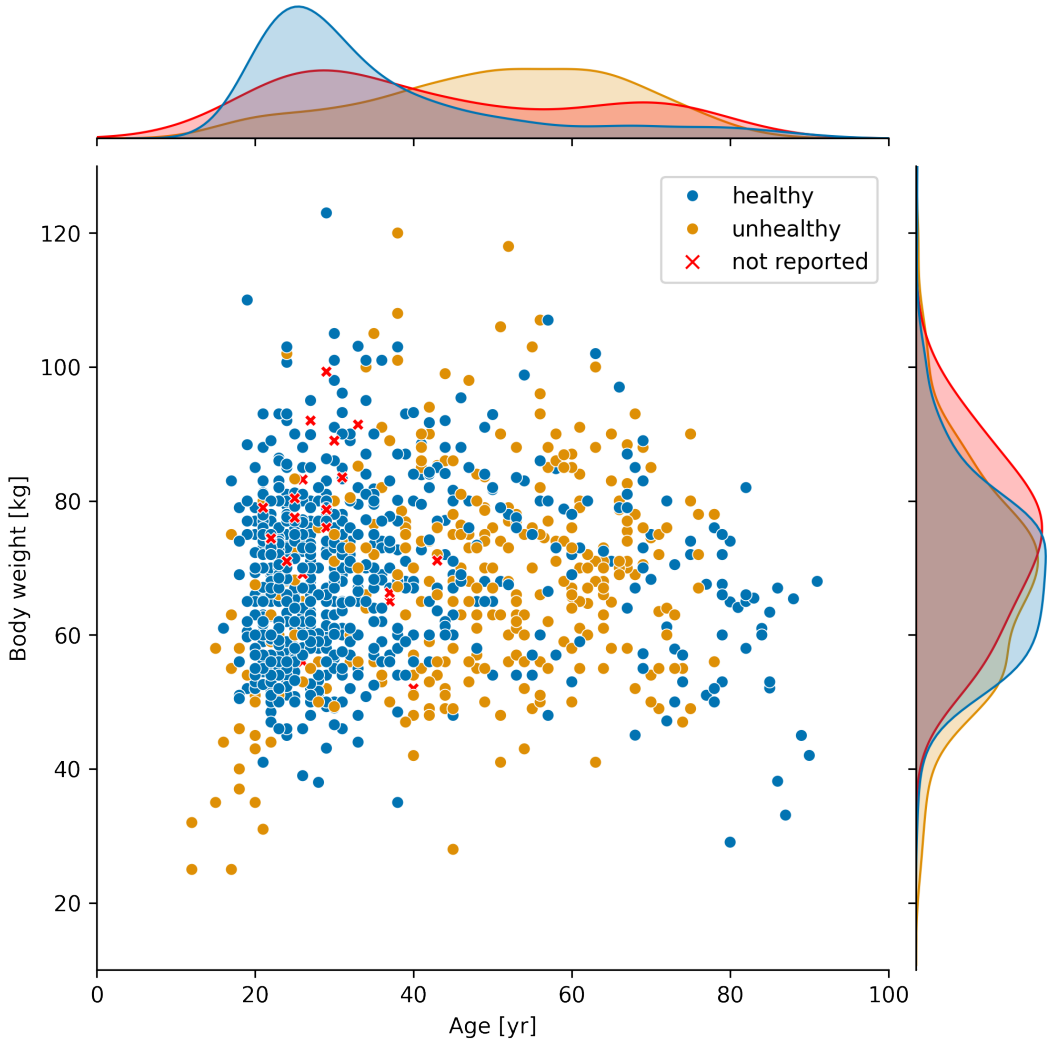

FIGURE 3.2: Correlation between age and body weight of individual subjects in PK-DB. Subjects are stratified based on their health status (i.e., healthy=blue, unhealthy=yellow, and not reported=red). Data were accessed on 2022-11-17.

extent of the dataset enabled the investigation of the state of caffeine reporting more generally. It was found that many factors which are known to influence the PK of caffeine were poorly reported. Further, the PKs themselves were rarely reported on an individual level but rather only stratified into heterogeneous groups, making it impossible to account for individual differences in the analysis. Time course data, the gold standard for PKPB modeling, was only infrequently reported. In terms of liver function testing and phenotyping, existing knowledge on the effects of smoking, the use of oral contraceptives, various diseases, and comedications on caffeine pharmacokinetics were solidified. Similarly, drug-disease, drug-drug interactions, and the accuracy of caffeine concentrations by salivary sampling in contrast to plasma sampling were quantified, and the effect of dosing amount and sampling timing for phenotyping (metabolic ratio, i.e., paraxanthine/caffeine in plasma) were analyzed.

Drug-disease interaction studies are usually designed as case-control studies. Subjects in the groups to be compared are selected so that the groups are as similar as possible apart from the disease of interest. Of course, this is only possible to a certain extent, and differences between groups can confound the results. For example, there are always some differences in age or weight between the groups. This is relevant as liver function, and even more so kidney function, deteriorates with age, and clearance is affected by body weight. These might be confounding factors in effect size analyses of drug-disease interaction studies. Obviously, there are many more relevant factors. From a meta-analysis of all individuals in PK-DB with reported body weight and age, it can be seen that there is a big difference between the average age of healthy and unhealthy subjects. Healthy subjects are, on average, over 20 years younger than ill subjects, see Fig. 3.2. Body weight, in contrast, does not differ very much. With PBPK modeling, it is possible to account for such differences between individuals and more accurately quantify the effect sizes.

The meta-analyses and data integration performed in this paper was only possible due to prior work on data standardization and very extensive amounts of curated data.

Publication

Pharmacokinetics of Caffeine: A Systematic Analysis of Reported Data for Application in Metabolic Phenotyping and Liver Function Testing
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The data used for the analysis can be accessed via PK-DB:

- PK-DB (frontend): `https://pk-db.com/data` [Open the search panel on the left and enter caffeine as the intervention substance.]
- PK-DB (data): `https://pk-db.com/api/v1/filter/?concise=false&download=true&interventions__substance=caffeine` [Warning: slow download, data processing can take up to a minute. Further, it is unclear if the URL will break at some time in the future due to major updates.]

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SYSTEMATIC REVIEW
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Pharmacokinetics of Caffeine: A Systematic Analysis of Reported Data for Application in Metabolic Phenotyping and Liver Function Testing

Jan Grzegorzewski, Florian Bartsch, Adrian Köller and Matthias König*

Institute for Theoretical Biology, Humboldt-University Berlin, Berlin, Germany

Caffeine is by far the most ubiquitous psychostimulant worldwide found in tea, coffee, cocoa, energy drinks, and many other beverages and food. Caffeine is almost exclusively metabolized in the liver by the cytochrome P-450 enzyme system to the main product paraxanthine and the additional products theobromine and theophylline. Besides its stimulating properties, two important applications of caffeine are metabolic phenotyping of cytochrome P450 1A2 (CYP1A2) and liver function testing. An open challenge in this context is to identify underlying causes of the large inter-individual variability in caffeine pharmacokinetics. Data is urgently needed to understand and quantify confounding factors such as lifestyle (e.g., smoking), the effects of drug-caffeine interactions (e.g., medication metabolized via CYP1A2), and the effect of disease. Here we report the first integrative and systematic analysis of data on caffeine pharmacokinetics from 141 publications and provide a comprehensive high-quality data set on the pharmacokinetics of caffeine, caffeine metabolites, and their metabolic ratios in human adults. The data set is enriched by meta-data on the characteristics of studied patient cohorts and subjects (e.g., age, body weight, smoking status, health status), the applied interventions (e.g., dosing, substance, route of application), measured pharmacokinetic time-courses, and pharmacokinetic parameters (e.g., clearance, half-life, area under the curve). We demonstrate via multiple applications how the data set can be used to solidify existing knowledge and gain new insights relevant for metabolic phenotyping and liver function testing based on caffeine. Specifically, we analyzed 1) the alteration of caffeine pharmacokinetics with smoking and use of oral contraceptives; 2) drug-drug interactions with caffeine as possible confounding factors of caffeine pharmacokinetics or source of adverse effects; 3) alteration of caffeine pharmacokinetics in disease; and 4) the applicability of caffeine as a salivary test substance by comparison of plasma and saliva data. In conclusion, our data set and analyses provide important resources which could enable more accurate caffeine-based metabolic phenotyping and liver function testing.

Keywords: caffeine, pharmacokinetics, smoking, oral contraceptives, drug-drug interactions, drug-disease interactions, CYP1A2, liver function test

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Edited by:
Feng Li,
Baylor College of Medicine, United States

Reviewed by:
Duygu Türközü Agagündüz, Gazi University, Turkey
Amelia Filippelli, University of Salerno, Italy

****Correspondence:***
Jan Grzegorzewski
grzegorj@hu-berlin.de

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1 INTRODUCTION

Caffeine is commonly found in tea, coffee, cocoa, energy drinks, and many other beverages. It is by far the most ubiquitous psychostimulant worldwide (Gilbert, 1984), with 85% of the United States population consuming caffeine daily (Mitchell et al., 2014). Among caffeine consumers, the average consumption is more than 200 mg of caffeine per day (Frary et al., 2005). Caffeine is mainly known for its stimulating properties but is also consumed for improved exercise performance and the treatment of various diseases (e.g., apnea in prematurity, hypersomnia). Two important applications of caffeine are liver function testing and metabolic phenotyping of cytochrome P450 1A2 (CYP1A2), N-acetyltransferase 2 (NA2), and xanthine oxidase (XO) (Wang et al., 1985; Jost et al., 1987; Wahlländer et al., 1990; Tripathi et al., 2015).

Caffeine is almost exclusively metabolized in the liver by the cytochrome P450 enzyme system with 3% or less being excreted unchanged in urine (Kot and Daniel, 2008). In humans, N-3 demethylation of caffeine (1,3,7-trimethylxanthine) to paraxanthine (1,7-dimethylxanthine) is the main reaction in the metabolism of caffeine, accounting for around 80–90% of caffeine demethylation. The reaction is exclusively mediated by the activity of the cytochrome P450 isoform 1A2 (CYP1A2) (Hakooz, 2009). The remainder of caffeine is metabolized to around 11 and 4% to the 1-demethylated product theobromine and 7-demethylated product theophylline, respectively (Lelo et al., 1986b; Kalow and Tang, 1993; Miners and Birkett, 1996; Amchin et al., 1999).

Large variation exists in the consumption of caffeine-containing beverages and food between individuals, which can induce CYP1A2 activity. In addition, CYP1A2 activity and protein amount are affected by environmental, genetic, and epigenetic factors (Klein et al., 2010) resulting in large variation between 5-6 fold in humans (Schrenk et al., 1998). These factors lead to a wide range of caffeine plasma concentrations and caffeine pharmacokinetics.

Sex does not significantly influence the CYP1A2 activity (Klein et al., 2010; Yang et al., 2010; Puri et al., 2020). A large heritability of CYP1A2 activity could be shown by two twin studies (Rasmussen et al., 2002; Matthaei et al., 2016). With excluded users of hormonal contraceptives and smokers, 89% of the variation in caffeine AUC was shown to be due to genetic effects and 8% specifically due to the CYP1A1/1A2 promoter polymorphism. In other studies, statistically significant genetic or epigenetic markers on the CYP1A locus on chromosome 15 could not be found (Moonen et al., 2004; Jiang et al., 2006; Ghotbi et al., 2007; Gunes and Dahl, 2008; Myrand et al., 2008; Klein et al., 2010; Yang et al., 2010). Genes regulating the expression and function of CYP1A2 and non-genetic factors could explain 42, 38, and 33% of CYP1A2 variation at activity, protein, and mRNA level, respectively (Klein et al., 2010). Lifestyle factors (e.g., smoking) and use of oral contraceptives have been shown to influence caffeine pharmacokinetics, as have pregnancy, obesity, alcohol consumption, and the coadministrations of drugs (e.g., fluvoxamine and pipmedic acid). Many diseases reduce the metabolic capabilities of patients. For caffeine which is predominantly metabolized by the liver, various liver diseases result in a strong reduction in caffeine clearance. The most profound reduction is observed in cirrhotic liver disease, correlating with the degree of hepatic impairment (Holstege et al., 1989; Park et al., 2003; Jodynis-Liebert et al., 2004; Tripathi et al., 2015).

Metabolic phenotyping of enzymes by probe drugs is a common method to evaluate the impact of lifestyle, drug-gene and drug-drug interactions, and other factors influencing enzyme activity. Caffeine is an established probe drug for CYP1A2, N-acetyltransferase 2 (NAT2), and xanthine oxidase (XO) metabolic activities (Fuhr et al., 1996; Miners and Birkett, 1996; Faber et al., 2005; Hakooz, 2009). It is rapidly and completely absorbed by the gastrointestinal tract, distributed throughout the total body water, has low plasma binding, as well as short half-life, negligible first-pass metabolism (Yesair et al., 1984), minimal renal elimination, excellent tolerability, and its biotransformation is virtually confined to the liver (Kalow and Tang, 1993; Amchin et al., 1999; Drozdzik et al., 2018). Caffeine is especially used for CYP1A2 phenotyping which contributes 5–20% to the hepatic P450 pool and is involved in the clearance of about 9% of clinically used drugs (Zanger and Schwab, 2013). The partial or systemic caffeine clearance measured in plasma is considered to be the gold standard for CYP1A2 phenotyping (Fuhr et al., 1996) since 95% of the systemic clearance of caffeine is estimated to be due to hepatic CYP1A2 (Amchin et al., 1999). Measurements in serum, saliva, and urine are extensively studied as well. In urine, sampling at multiple time points and precise timing are inherently difficult. Thus, clearance rates are typically calculated only from plasma, saliva, and serum samples. Measurements in saliva are not invasive and show good correlation with measurements in plasma (Callahan et al., 1982; Wahlländer et al., 1990). The metabolic ratio (MR) between various metabolites of caffeine is an established alternative measure for CYP1A2 enzyme activity (Hakooz, 2009). Analogously, the MR is measured in any of the above mentioned tissues though typically only at a single time point after drug administration. The MR of various metabolites at 4 h after dosing in plasma, saliva, and urine correlate well with the apparent caffeine clearance, 0.84, 0.82, 0.61, respectively (Carrillo et al., 2000). The MRs measured in plasma and urine have been historically most popular. However, measurements in saliva are routinely applied, especially in epidemiological studies (Tantcheva-Poór et al., 1999; Kukongviriyapan et al., 2004; Tripathi et al., 2015; Chia et al., 2016; Urry et al., 2016; Puri et al., 2020).

Despite the great potential of caffeine as a test substance for liver function tests and CYP1A2 based phenotyping, so far caffeine testing has not found widespread clinical adoption. For liver function tests, a major limiting factor is the large inter-individual variability. Data is urgently needed to understand and quantify confounding factors of caffeine pharmacokinetics such as lifestyle (e.g., smoking) and the effects of drug-drug interactions (e.g., drugs metabolized via CYP1A2) or how disease alters caffeine elimination. Based on such data, more accurate liver function tests and CYP1A2 phenotyping protocols could be established. Differences in

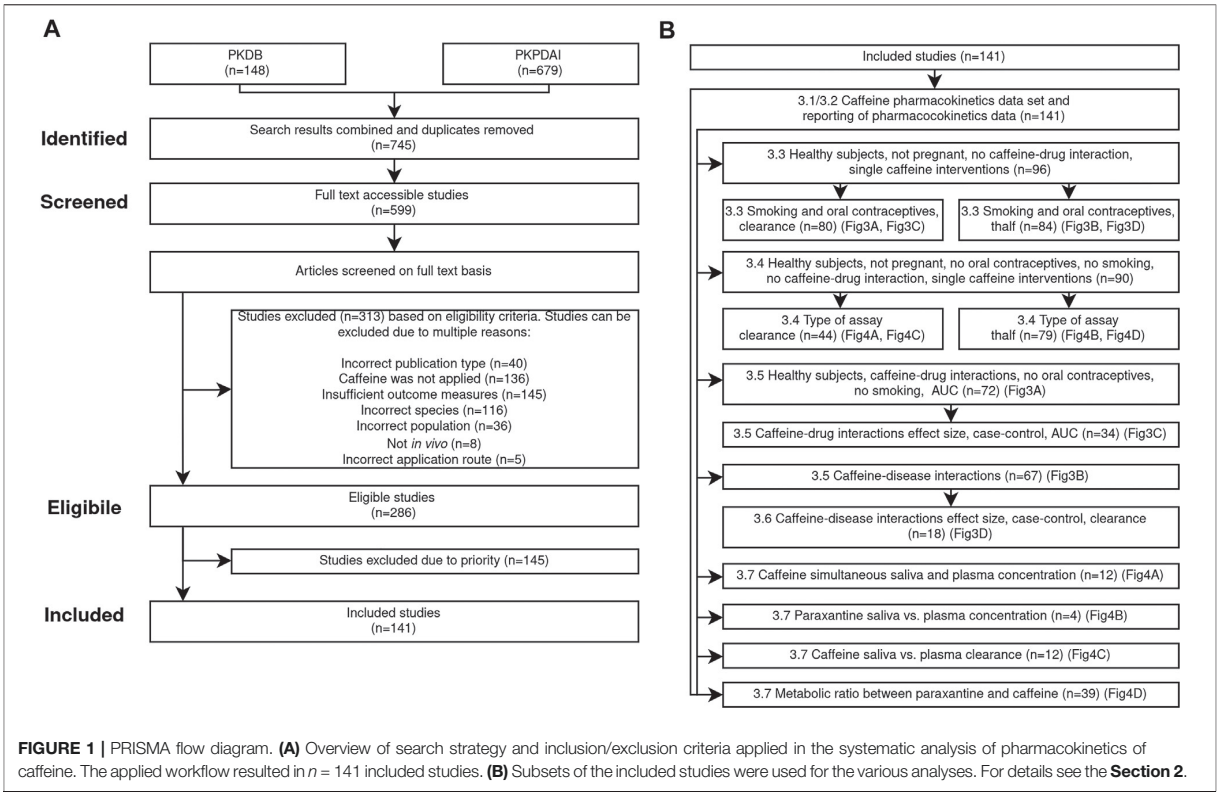

FIGURE 1 | PRISMA flow diagram. **(A)** Overview of search strategy and inclusion/exclusion criteria applied in the systematic analysis of pharmacokinetics of caffeine. The applied workflow resulted in $n = 141$ included studies. **(B)** Subsets of the included studies were used for the various analyses. For details see the **Section 2**.

clinical protocols (e.g., dosing amount, sampling tissue, and timing) have not been systematically analyzed in the literature. In addition, data on competing substances in the context of dynamical liver function tests (e.g., metacethin used in the LiMAx test (Rubin et al., 2017)) and CYP1A2 phenotyping is not accessible but absolutely imperative for a quantitative evaluation of these methods.

Caffeine pharmacokinetics have been investigated in a multitude of clinical trials, each with its own focus and research question. These studies have been reviewed in a broad scope, most recently in (Arnaud, 2011; Nehlig, 2018). Despite caffeine pharmacokinetics being highly studied in literature, no integrated pharmacokinetics data set exists so far and no systematic analysis of the reported data has been performed. The objective of this work was to fill this gap by providing the first comprehensive high-quality data set of reported data on caffeine pharmacokinetics and demonstrate its value via multiple example applications relevant for metabolic phenotyping and liver function testing based on caffeine.

2 MATERIALS AND METHODS

This study is guided by the Preferred Reporting Items for Systematic reviews and Meta-Analysis (PRISMA) statement and its extension for Scoping Reviews (PRISMA-ScR) (Liberati et al., 2009; Tricco et al., 2018). This work was conducted with the aim to answer the following research questions. 1) What is the current state of research on caffeine pharmacokinetics in adult humans in the context of metabolic phenotyping and liver function testing? 2) How homogeneous is the reporting? 3) How do caffeine dose and route impact caffeine pharmacokinetics? 4) What is the effect of smoking and oral contraceptive use on caffeine pharmacokinetics with respect to the caffeine dose? 5) What is the effect of coadministrations on caffeine pharmacokinetics with respect to the dosing amount and what are the effect sizes of caffeine-drug interactions? 6) What is the effect of diseases on caffeine pharmacokinetics with respect to the caffeine dose and what are the effect sizes of caffeine-disease interactions? 7) How do sampling time and tissue influence pharmacokinetics and phenotyping? The search, screening and filtering process are depicted in the PRISMA flow diagram in **Figure 1**.

2.1 Search Strategy

We searched the general purpose pharmacokinetics database PK-DB with the search query https://pk-db.com/api/v1/filter/?concise=false&download=true&interventions__substance=caffeine on 2021–10–06 and PKPDAI (Hernandez et al., 2021) with the search query https://app.pkpdai.com/?term=caffeine on 2021–10–06. PKPDAI is a dedicated search

engine for pharmacokinetics data based on PubMed. This search resulted in 745 studies from which 599 could be accessed as full text.

2.2 Eligibility Criteria

Studies were included or excluded based on the following eligibility criteria. Reviews or publications describing computational models were excluded. For a study to be eligible caffeine must be applied, it must report pharmacokinetics data for caffeine or its metabolites, data must be measured in humans, and individuals and groups must consisted of adults (age > =18 years), and data must be *in vivo* data. The application route of caffeine must be either oral, intravenous, or intramuscular. All application forms of caffeine (e.g., tablet, capsule, solution) are eligible. No restrictions were imposed on the dosing amount of caffeine or coadministrations. Relevant outcome measures are concentration-time profiles in plasma, serum, blood, and saliva of caffeine and metabolites and corresponding pharmacokinetic parameters. Studies containing pharmacokinetic parameters of caffeine and metabolites (i.e., clearance, maximum concentration, time of maximum concentration, half-life, AUC) and metabolic ratios of caffeine and its metabolites were included. In total 286 studies met our eligibility criteria, 313 studies were excluded for various reasons as described in **Figure 1**. Of the 286 studies, 145 were excluded due to low priority, e.g., if only urinary data was reported. Data was extracted and curated from the remaining 141 studies. During the curation process outliers from four studies (Stille et al., 1987; Harder et al., 1988, 1989; Balogh et al., 1992) were identified and excluded from all subsequent analyses. All four studies probably originate from the same clinical investigation.

2.3 Data Curation

Pharmacokinetics data was curated manually as part of the pharmacokinetics database PK-DB (https://pk-db.com/) (Grzegorzewski et al., 2020) using established workflows. The pharmacokinetics data was stored in combination with relevant metadata on groups, individuals, interventions, and outputs. PK-DB provided support in the curation process with strong validation rules, unit normalization, and automatic calculation of pharmacokinetic parameters from time-courses. As part of the curation process and the presented analyses, pharmacokinetic parameters and other commonly reported measurements were integrated from multiple studies. The meta-analyses and data integration allowed to identify and correct/remove outlier data which were mostly due to either curation errors or incorrect reporting. Pharmacokinetic parameters calculated from time-courses are included in the analyses. For more details see (Grzegorzewski et al., 2020).

2.4 Data Processing and Filtering

In **Figures 3A,B**, **Figures 5A–D**, and **Figure 4** the data is displayed in a similar manner. For collectively reported subjects, the group size and standard error is displayed as the marker size and error-bar, respectively. In the legend, (I), (G), and (TI) stand for individual participant data, the number of groups, and the total number of subjects, respectively. Data points are depicted as circles if reported equivalently in the source, as squares if calculated from concentration-time profiles and as triangles if inferred from corresponding pharmacokinetic data and body weights of the subjects. Typically, dosing is reported in mass units, AUC in mass per volume units, clearance in volume per time units, and half-life in time units. Occasionally, dosage, AUC, and clearance are reported in body weight units. In case of reported subject weight, the data is harmonized to similar units by multiplying with the reported weights.

In **Figure 3**, the depicted subjects were healthy. Male subjects were assumed to not take any oral contraceptives. Substances with negligible caffeine-drug interactions were determined by an effect size analysis in **Section 3.5**. All other co-administrations and investigations containing multiple caffeine dosages were excluded.

In subplot **Figure 5A**, included subjects were healthy. The area under the caffeine concentration curves measured at least up to 12 h after a single application of caffeine and AUC extrapolated to infinity were included. Multiple subsequent caffeine dosages were excluded, other administrations and co-administrations included. In subplot **Figure 5C**, healthy and non-healthy subjects were included. Single applications of caffeine or caffeine administrated as a cocktail with negligible drug-drug interactions were included. All other co-administrations and investigations containing multiple caffeine dosages were excluded.

In subplots **Figures 6A–C**, no data was excluded. In subplot **Figure 6D** and **Figure 4**, included subjects were healthy, non-smoking, non-pregnant, and non-oral contraceptive consumers. Interventions with caffeine administrated as a cocktail with negligible caffeine-drug interactions were included. All other co-administrations and investigations containing multiple caffeine dosages were excluded.

3 RESULTS

3.1 Caffeine Pharmacokinetics Data Set

Within this work, the first comprehensive open pharmacokinetics data set on caffeine was established. The data set integrates data from 141 publications (**Figure 2** and **Table 1**), with most of the publications corresponding to a distinct clinical trial. Studies were identified and included or excluded following the systematic approach described in the PRISMA flow diagram (**Figure 1A**). The focus of data curation was on pharmacokinetics data of caffeine, caffeine metabolites, and caffeine metabolic ratios in human adults. Importantly, the data set is enriched with meta-data on 1) the characteristics of studied patient cohorts and subjects (e.g., age, body weight, smoking status, health status, fasting); 2) the applied interventions (e.g., dosing, substance, route of application); 3) measured pharmacokinetic time-courses; and 4) pharmacokinetic parameters (e.g., clearance, half-life, area under the curve). In summary, data from 500 groups and 4,714 individuals is reported under 387 interventions resulting in 24,571 pharmacokinetic outputs and 846 time-courses. The data set is available via the pharmacokinetics database PK-DB (https://pk-db.com/) with a detailed description of the data structure provided in

TABLE 1 | Overview of curated studies. For each study the table shows which information was reported. Either, information was reported (✓), partially reported (⌀) or not reported at all (whitespace). The type of assay column contains reported information on quantification method for the concentration of caffeine or its metabolites (i.e., CC: Column chromatography, GC: Gas chromatography, CGC: Gas chromatography, GLC: Gas-liquid chromatography, GC MS: Gas chromatography mass spectrometry, HPLC: High performance liquid chromatography, HPLC MS/MS: High performance liquid chromatography-tandem mass spectrometry, RP-HPLC: Reversed-phase high-performance liquid chromatography, LC MS Liquid chromatography mass spectrometry, LC MS/MS Liquid chromatography-tandem mass spectrometry, HPLC-ESI-MS/MS: High-performance liquid chromatography-electrospray ionisation tandem mass spectrometry, ES MS/MS: Electrospray ionisation tandem mass spectrometry, UV/Vis: UV–visible spectrophotometry, EMIT: Enzyme multiplied immunoassay technique, ELISA: enzyme-linked immunosorbent assay, RIA: Radioimmunoassay).

BibKy	PKDB	Type of assay	Subjects	Groups	Sex	Age	Weight	Height	BMI	Ethnicity	Healthy	Medication	Smoking	Fast	Alcohol	Dose	Route	Form	pCYP1A2	pCYP3A2	pNAT2	pXO	Caffeine	Paraxanthine	Theophylline	Theobromine	13TU	17U	12U	37U	1X	1X	3X	3X	7X	7U	AFMU	AAMU	ADMU	AU	AXU
#	141		4,931	327	52	23	21	4	2	6	56	21	40	17	2	136	139	135	0	4	0	0	69	27	11	11	0	2	0	0	2	1	0	0	0	0	1	1	0	0	0
Abernethy and Todd (1985)	PKDB00001	HPLC	18	2	✓	✓	✓			✓	✓	⌀	✓			✓	✓	✓					⌀																			
Abernethy et al. (1985)	PKDB00427	HPLC	39	2	✓	⌀	⌀		⌀		✓	✓	✓	✓		✓	✓	✓					✓																		
Aklinyinka et al. (2000)	PKDB00002	HPLC	20	3	✓	✓	✓			✓	✓	⌀	✓			✓	✓	✓		⌀			✓	✓	⌀	⌀														
Amchin et al. (1999)	PKDB00003	HPLC	15	1	✓	✓	✓	✓			✓	✓	✓	✓		✓	✓	✓		⌀			✓	⌀		⌀		⌀			⌀	⌀						⌀		
Armant et al. (2017)	PKDB00428	HPLC MS/MS	19	1	⌀	⌀	⌀		⌀	⌀	⌀					✓	✓	✓					✓																	
Arnaud and Welsch (1982)	PKDB00429	RP-HPLC	6	1							⌀		⌀	⌀		⌀	✓	✓					✓	✓	✓	✓	⌀	⌀	⌀	⌀	⌀	⌀	⌀	⌀	⌀	⌀	⌀	⌀	⌀	⌀
Arold et al. (2005)	PKDB00430	HPLC	56	6	⌀	✓	✓	✓			✓		✓			✓	✓	✓					✓	⌀																
Axelrod and Reichenthal (1953)	PKDB00431	UV/Vis	3	0	⌀											✓	✓	✓					✓																	
Azcona et al. (1995)	PKDB00432	HPLC	8	1	⌀	⌀	⌀	⌀			⌀	⌀			⌀	✓	✓	✓					✓																	
Backman et al. (2008)	PKDB00004	HPLC	71	0	⌀	⌀	⌀		⌀		⌀	⌀	⌀		⌀	✓	✓	✓		⌀																			
Balogh et al. (1982)	PKDB00005	HPLC	12	1	✓		✓				✓	✓	✓			✓	✓	✓					✓																	
Balogh et al. (1995)	PKDB00006	HPLC	20	4	✓						✓		✓			✓	✓	✓					⌀																	
Bapiro et al. (2000)	PKDB00433	HPLC	10	3	✓						✓		✓			✓	✓	✓		⌀			✓	⌀																
Barnett et al. (1992)	PKDB00007	HPLC	6	0	⌀	⌀	⌀				⌀	⌀	⌀		⌀	✓	✓	✓					⌀																	
Bichl et al. (2006)	PKDB00434	HPLC	8	2	⌀	⌀	⌀		⌀		⌀			⌀		✓	✓	✓					✓																	
Beach et al. (1986)	PKDB00008	HPLC	21	4	✓						✓	✓	✓	✓		✓	✓	✓					⌀																	
Becker et al. (1984)	PKDB00435	HPLC	23	2	⌀						⌀	⌀				✓	✓	✓					✓		✓															
Begas et al. (2007)	PKDB00039	HPLC	44	5	⌀	⌀					⌀	⌀	⌀			✓	✓	✓		⌀	⌀	⌀																	
Begas et al. (2015)	PKDB00436	RP-HPLC	34	2	✓						✓	✓	⌀			✓	✓	✓		⌀			⌀	⌀				⌀			⌀	⌀					⌀			
Benowitz et al. (1995)	PKDB00010	HPLC	12	2	⌀	⌀					⌀	⌀	⌀			✓	✓	✓		⌀			✓	✓																
Benowitz et al. (2003)	PKDB00011	HPLC	12	3	✓						✓		✓	✓		✓	✓	✓			⌀	⌀	✓																	
Birkett and Miners (1991)	PKDB00012	HPLC	6	0	⌀	⌀	⌀				⌀		⌀			✓	✓	✓					⌀																	
Blanchard and Sawers (1983b)	PKDB00127	HPLC	16	2	⌀	⌀	⌀	⌀		⌀	⌀		⌀		⌀	✓	✓	✓					✓																	
Blanchard and Sawers (1983c)	PKDB00210	-	16	2	✓	✓					✓			✓	✓	✓	✓	✓					⌀																	
Blanchard and Sawers (1983a)	PKDB00013	HPLC	10	1	⌀	⌀	⌀		⌀	⌀	⌀	⌀	⌀	⌀	⌀	✓	✓	✓					✓																	
Blanchard et al. (1985)	PKDB00378	HPLC	12	2	⌀	⌀	⌀		⌀	⌀	⌀	⌀	⌀			✓	✓	✓					⌀	⌀	⌀	⌀	⌀	⌀	⌀	⌀		⌀	⌀	⌀		⌀	⌀				
Bonati et al. (1982)	PKDB00014	HPLC	4	1	✓		✓				✓		✓			✓	✓	✓					✓	✓	✓	✓		⌀			⌀		⌀						⌀		
Bouliou et al. (2004)	PKDB00437	HPLC	40	4	⌀	⌀					⌀		⌀			✓	✓	✓		⌀			⌀																	
Brazier et al. (1983)	PKDB00438	GC	4	0	⌀						⌀					✓	✓	✓					✓	⌀																
Broughton and Rogers (1981)	PKDB00439	GLC	5	0	⌀						⌀	⌀	⌀	⌀		✓	✓	✓					⌀																	
Bruce et al. (1986)	PKDB00440	HPLC	9	1	⌀						⌀				⌀	✓	✓	✓					✓	✓																
Calabaiyud et al. (1995)	PKDB00441	EMIT	73	2	✓	⌀					✓	✓	✓			✓	✓	✓					⌀																	
Callahan et al. (1982)	PKDB00442	HPLC	12	5			⌀				✓		✓	✓		✓	✓	✓					✓	⌀	⌀	⌀	⌀	⌀	⌀		⌀	⌀	⌀		⌀						
Callahan et al. (1983)	PKDB00443	HPLC	12	3	✓		✓				✓		✓	✓		✓	✓	✓					✓	✓		✓		✓	✓								✓			
Campbell et al. (1987)	PKDB00015	HPLC	15	3	✓	✓	✓			✓	✓		✓			✓	✓	✓					⌀				⌀			⌀	⌀						⌀			
Carbó et al. (1989)	PKDB00016	HPLC	6	1	✓	✓	✓				✓	✓	✓		✓	✓	✓	✓		⌀			⌀																	
Carrillo et al. (2000)	PKDB00017	HPLC	23	4	⌀	⌀	⌀				⌀	⌀	⌀			✓	✓	✓		⌀			⌀					✓											
Chen et al. (2003a)	PKDB00445	HPLC	16	1	⌀	⌀	⌀	⌀		⌀	⌀	⌀	⌀	⌀		✓	✓	✓		⌀			✓	✓																
Chen et al. (2003b)	PKDB00446	HPLC	12	1	✓					✓	✓	✓	✓	✓		✓	✓	✓			⌀	⌀	✓	✓																
Chen et al. (2010)	PKDB00447	HPLC	16	1	✓	✓	✓			✓	✓	✓	✓	✓		✓	✓	✓			⌀	⌀	✓	✓					⌀									⌀		
Cheng et al. (1992)	PKDB00018	HPLC	26	3	✓	⌀	⌀				✓		✓			✓	✓	✓					⌀																	
Chiu et al. (2016)	PKDB00448	HPLC	52	12	✓					✓	✓		✓			✓	✓	✓		⌀			⌀	⌀																
Christensen et al. (2002)	PKDB00449	HPLC	12	0	⌀	⌀	⌀				⌀	⌀	⌀			✓	✓	✓					⌀																	
Cook et al. (1970)	PKDB00450	RIA	13	0												✓	✓	✓					⌀																	
Cornish and Christman (1957)	PKDB00128	CC	2	0	⌀						⌀					✓	✓						⌀	⌀	⌀	⌀		⌀		⌀	⌀	⌀		⌀	⌀					
Culm-Merdek et al. (2005)	PKDB00019	HPLC	7	1	⌀	⌀	⌀				⌀		⌀			✓	✓	✓					✓	✓	⌀	⌀														
Cysewski et al. (2007)	PKDB00451	RP-HPLC	12	1	⌀						⌀	⌀	⌀			✓	✓	✓					✓	⌀	⌀	⌀														
Darwish et al. (2008)	PKDB00328	HPLC	77	3	⌀	⌀			⌀	⌀	⌀	⌀	⌀	⌀		✓	✓	✓					⌀																	
Desmond et al. (1982)	PKDB00384	HPLC	25	2	✓	⌀	⌀				✓	⌀	⌀	✓		✓	✓	✓					⌀																	
Dinger et al. (2016)	PKDB00452	LC MS/MS	6	1	⌀											✓	✓	✓					⌀																	
Djordjevic et al. (2008)	PKDB00453	RP-HPLC	249	12	⌀	⌀	⌀			⌀	⌀		⌀			✓	✓	✓		⌀																			
Dorshtyenko et al. (2013)	PKDB00138	LC MS	17	1	⌀	⌀			⌀		⌀		⌀		⌀	✓	✓	✓					⌀																	
Dumond et al. (2010)	PKDB00499	LC MS	23	1	⌀	⌀	⌀	⌀	⌀	⌀	⌀					✓	✓	✓			⌀	⌀	✓																	
Edwards et al. (2017)	PKDB00496	HPLC MS/MS	236	8	⌀	⌀	⌀	⌀	⌀	⌀	⌀	⌀		⌀		✓	✓	✓					✓																	
Faber and Fuhr (2004)	PKDB00032	HPLC	12	2	✓	✓	✓		✓		✓	✓	✓			✓	✓	✓					⌀																	
Fuhr et al. (1993)	PKDB00033	HPLC	12	2	✓	✓	✓	✓			✓		✓			✓	✓	✓					⌀																	
Furge and Rettke (2007)	PKDB00454	RP-HPLC	10	1									✓			✓	✓	✓		⌀			⌀	⌀																
George et al. (1985)	PKDB00034	HPLC	10	4	✓						✓	✓	✓		⌀	✓	✓	✓					⌀																	
Ghassabian et al. (2009)	PKDB00035	ES MS/MS	11	0	⌀	⌀					⌀	⌀				✓	✓	✓		⌀																			
Granfors et al. (2005)	PKDB00036	HPLC	30	2	✓	✓	✓				✓		✓			✓	✓	✓		⌀																			
Gunes et al. (2009)	PKDB00455	HPLC	146	0	⌀						⌀	⌀	⌀			✓	✓	✓	⌀	⌀																			
Gurley et al. (2005a)	PKDB00456	HPLC	12	1	✓	✓	✓				✓	✓	✓			✓	✓	✓		⌀																			
Gurley et al. (2005b)	PKDB00457	HPLC	12	1	✓	✓	✓				✓	✓	✓			✓	✓	✓		⌀																			
Haller et al. (2002)	PKDB00037	LC MS/MS	8	1	⌀		✓				✓		✓			✓	✓	✓					✓																	
Harmon-Vilcot et al. (2004)	PKDB00458	HPLC	54	2	✓	✓			✓		✓	✓				✓	✓	✓		⌀																			
Harder et al. (1988)	PKDB00038	HPLC	12	1	⌀		⌀				⌀	⌀	⌀			✓	✓	✓					⌀	⌀																
Harder et al. (1989)	PKDB00039	HPLC	12	1	⌀		⌀				⌀	⌀	⌀			✓	✓	✓					⌀																	
Hashiguchi et al. (1992)	PKDB00459	HPLC	9	1	✓	✓					✓		✓			✓	✓	✓					✓																	
He et al. (2017)	PKDB00460	LC MS/MS	8	1	✓						✓	✓	✓			✓	✓	✓					✓	⌀																

(Continued on following page)

TABLE 1 | (*Continued*) Overview of curated studies. For each study the table shows which information was reported. Either, information was reported (✓), partially reported (⊘) or not reported at all (whitespace). The type of assay column contains reported information on quantification method for the concentration of caffeine or its metabolites (i.e., CC: Column chromatography, GC: Gas chromatography, CGC: Capillary gas chromatography, GLC: Gas-liquid chromatography, GC MS: Gas chromatography mass spectrometry, HPLC: High performance liquid chromatography, HPLC MS/MS: High performance liquid chromatography-tandem mass spectrometry, RP-HPLC: Reversed-phase high-performance liquid chromatography, LC MS Liquid chromatography mass spectrometry, LC MS/MS Liquid chromatography-tandem mass spectrometry, HPLC-ESI-MS/MS: High-performance liquid chromatography-electrospray ionisation tandem mass spectrometry, ES MS/MS: Electrospray ionisation tandem mass spectrometry, UV/Vis: UV–visible spectrophotometry, EMIT: Enzyme multiplied immunoassay technique, ELISA: enzyme-linked immunosorbent assay, RIA: Radioimmunoassay).

Study	PKDB	Type of assay	Subjects	Groups	Sex	Age	Weight	Height	BMI	Ethnicity	Healthy	Medication	Smoking	Fast	Alcohol	Dose	Route	Form	pCYP1A2	pCYP1A2	pNAT2	pUG	Caffeine	Paraxanthine	Theophylline	Theobromine	137U	17U	13U	37U	1X	1X	3X	3X	7X	7X	7U	A1AU	A2AU	A3AU	A1U	A2U
Healy et al. (1989)	PKDB00040	RP-HPLC	10	1	⊘	⊘	⊘				⊘		⊘			✓	✓	✓					⊘	⊘																
Healy et al. (1991)	PKDB00041	RP-HPLC	16	1	⊘	⊘	⊘				⊘	⊘	⊘	⊘		✓	✓	✓		⊘			✓	✓															
Hetzler et al. (1992)	PKDB00042	RP-HPLC	10	1	⊘	⊘	⊘	⊘			⊘			⊘		✓	✓	✓		✓			✓	✓															
Holstege et al. (1989)	PKDB00461	HPLC	43	6	⊘	⊘					✓	✓				✓	✓	✓					✓	✓	✓	✓													
Holstege et al. (1993)	PKDB00462	HPLC	8	1	✓	⊘	⊘				✓		✓			✓	✓	✓					✓	✓	✓	✓													
Jeppesen et al. (1996)	PKDB00043	HPLC	8	1	⊘						⊘	⊘	⊘			✓	✓	✓					⊘	⊘	✓	⊘													
Jodynis-Liebert et al. (2004)	PKDB00382	HPLC	89	6	⊘	⊘	⊘				⊘	⊘	⊘			✓	✓	✓		⊘			⊘															
Joseris et al. (1987)	PKDB00385	GC	12	2	✓						✓	⊘		✓		✓	✓	✓					✓															
Joseris et al. (1988)	PKDB00044	GC	71	5	✓	⊘	⊘				✓	⊘	✓			✓	✓	✓					⊘															
Jost et al. (1987)	PKDB00463	EMIT	62	0	⊘	⊘	⊘				⊘		⊘			✓	✓	✓					✓															
Kakuda et al. (2014)	PKDB00137	LC MS/MS	14	1	⊘	⊘		⊘	⊘	⊘	⊘	⊘	⊘		⊘	✓	✓	✓					✓															
Kamimori et al. (1987)	PKDB00464	HPLC	6	2	✓	✓	✓				✓		✓	✓		✓	⊘	⊘					✓															
Kamimori et al. (1990)	PKDB00465	HPLC	11	3	✓	✓	✓				✓		✓	✓		✓	✓	✓					✓															
Kamimori et al. (2002)	PKDB00466	HPLC	84	1	⊘						⊘	⊘	⊘		⊘	✓	✓	✓					✓															
Kaplan et al. (1997)	PKDB00045	HPLC	12	1	✓	✓	✓				✓		✓			✓	✓	✓					✓	✓	⊘	✓													
Kiraly-Schippers et al. (1999)	PKDB00467	HPLC	12	1	⊘	⊘	⊘				⊘	⊘	⊘			✓	✓	✓					✓															
Koch et al. (1999)	PKDB00046	RP-HPLC	6	0		⊘	⊘				⊘					✓	✓	✓		⊘																		
Kokongkiatpapan et al. (2004)	PKDB00469	HPLC	77	9	✓	✓	⊘			⊘	⊘			✓	⊘	✓	✓	✓		⊘			⊘															
Laizure et al. (2017)	PKDB00470	HPLC-ESI-MS/MS	17	3	⊘	⊘	⊘				⊘	⊘	⊘			✓	✓	✓					⊘	✓	✓	✓													
Lammers et al. (2018)	PKDB00471	LC MS/MS	9	2	✓						✓				⊘	✓	✓	✓					✓															
Lane et al. (1992)	PKDB00472	RIA	10	2	✓	⊘					✓					✓	✓	✓					⊘															
Lane et al. (2014)	PKDB00341	HPLC	23	2	⊘	⊘				⊘	⊘	⊘	⊘			✓	✓	✓					✓	✓														
Lelo et al. (1986a)	PKDB00047	HPLC	6	1	✓						✓	✓	✓	✓		✓	✓	✓					✓	✓	✓	✓													
Levy and Zylber-Katz (1983)	PKDB00048	HPLC	12	0	⊘	⊘	⊘				⊘	⊘	⊘			✓	✓	✓					⊘															
Magnusson et al. (2008)	PKDB00049	HPLC MS/MS	7	0	⊘					⊘	⊘	⊘	⊘			✓	✓	✓		⊘			⊘	⊘														
Marks and Kelly (1973)	PKDB00338	UV/Vis	3	1	⊘									⊘		✓	✓	✓					✓															
Matthaei et al. (2016)	PKDB00473	HPLC	112	12	✓	⊘	⊘	⊘	⊘		✓			⊘		✓			⊘	⊘			⊘															
May et al. (1983)	PKDB00050	HPLC	12	2	✓						✓	✓	✓	✓		✓	✓	✓					⊘															
McDonagh et al. (1991)	PKDB00474	EMIT	103	8		⊘					⊘					✓	✓	✓					⊘															
McLean and Graham (2002)	PKDB00051	HPLC	14	2	⊘	⊘	⊘				⊘	⊘	⊘		⊘	✓	✓	✓					✓	✓	✓	✓													
McQuillin et al. (1995)	PKDB00475	RP-HPLC	19	0	⊘	⊘			⊘		⊘		⊘	⊘		✓	✓	✓		✓			✓	✓				✓			✓								
Millard et al. (2019)	PKDB00381	GC-MS,ELISA	2	0												✓	✓	✓	⊘				✓															
Murphy et al. (1988)	PKDB00052	HPLC	8	4	⊘	⊘	⊘				⊘	⊘	⊘			✓	✓	✓					⊘															
Muscat et al. (2008)	PKDB00476	HPLC	348	20	⊘					⊘	⊘		⊘				✓	✓		⊘																	
Myrand et al. (2008)	PKDB00497	HPLC	631	0	⊘	⊘	⊘	⊘		⊘	⊘		⊘			✓	✓	✓	⊘	⊘																	
Nakazawa and Tanaka (1988)	PKDB00477	HPLC	12	1	⊘	⊘	⊘				✓					✓	✓						✓	✓	✓	✓													
Newton et al. (1981)	PKDB00053	GLC	6	1	⊘	⊘	⊘				⊘		⊘	⊘		✓	✓	✓					⊘															
Oh et al. (2012)	PKDB00054	LC MS/MS	13	1							⊘					✓	✓	✓		⊘			✓	✓														
Park et al. (2003)	PKDB00055	GC	65	0	⊘	⊘	⊘				⊘	⊘	⊘			✓	✓	✓					⊘															
Parsons and Neims (1978)	PKDB00056	RIA	26	2	✓	✓					✓	✓	⊘			✓	✓	✓					✓															
Patwardhan et al. (1992)	PKDB00057	HPLC	31	3	⊘		⊘				⊘	⊘	⊘	⊘	⊘	✓	✓	✓					✓															
Pereira et al. (2010)	PKDB00478	HPLC	2	0	⊘	⊘	⊘				⊘		⊘			✓	✓	✓		⊘			✓	✓														
Pereira et al. (2011)	PKDB00058	HPLC	30	2	⊘						⊘	⊘	⊘			✓	✓	✓		⊘			✓	⊘														
Prillar (2005)	PKDB00479	HPLC	64	11	✓	⊘			⊘		✓		⊘			✓	✓	✓		⊘																	
Puri et al. (2020)	PKDB00480	Spectrophotometry	213	2	✓	✓					✓		✓			✓	✓	✓					⊘															
Renner et al. (1984)	PKDB00059	GLC	36	4	✓	✓	✓				✓		✓			✓	✓	✓					⊘															
Rietveld et al. (1984)	PKDB00481	GLC	9	0	⊘	⊘	⊘				⊘		⊘			✓	✓	✓					✓															
Sadiki et al. (2017)	PKDB00482	LC MS/MS	16	2							⊘	⊘	⊘			✓	✓	✓					⊘															
Schneider et al. (1999)	PKDB00483	HPLC	17	1	⊘						⊘	⊘	⊘			✓	✓	✓					✓															
Scott et al. (1984)	PKDB00484	RP-HPLC	42	0																			⊘															
Scott et al. (1988)	PKDB00485	HPLC	24	3	⊘	⊘	⊘				⊘	⊘	⊘	⊘		✓	✓	✓					⊘															
Scott et al. (1989)	PKDB00486	RP-HPLC	29	3	✓	⊘	⊘				✓	⊘				✓	✓	✓					✓	✓														
Seng et al. (2009)	PKDB00060	HPLC	59	3	⊘	⊘	⊘			⊘	⊘					✓	✓	✓					⊘															
Soto et al. (1995)	PKDB00487	HPLC	5	1	✓	✓	✓				✓	✓	✓			✓	✓	✓					⊘															
Spigset et al. (1999)	PKDB00061	HPLC	12	1	⊘	⊘	⊘				⊘	⊘	⊘	⊘		✓	✓	✓		✓			⊘															
Stille et al. (1987)	PKDB00062	HPLC	12	0	⊘		⊘				⊘	⊘	⊘			✓	✓	✓					⊘															
Syed et al. (2020)	PKDB00488	HPLC	48	3	⊘						⊘	⊘	⊘			✓	✓	✓					✓															
Tanaka et al. (1993)	PKDB00489	GC	10	1	⊘						⊘	⊘		⊘		✓	✓	✓					✓	✓	✓	✓													
Tanaka et al. (2014)	PKDB00136	HPLC-ESI-MS/MS	4	1	⊘						⊘					✓	✓	✓		✓			✓	✓														
Tang-Liu et al. (1983)	PKDB00126	RP-HPLC	6	1	✓		⊘				✓	✓		✓		✓	✓	✓					⊘	⊘	⊘	⊘	⊘	⊘	⊘	⊘		⊘	⊘	⊘		⊘	⊘				
Tian et al. (2018)	PKDB00490	HPLC	24	2	⊘				⊘		⊘		⊘			✓	✓	✓	⊘	⊘			⊘															
Trang et al. (1982)	PKDB00129	HPLC	10	1	⊘	⊘	⊘	⊘			⊘					✓	✓	✓					⊘															
Trang et al. (1985)	PKDB00491	HPLC	10	0	⊘						⊘	⊘	⊘			✓	✓	✓					⊘															
Tripathi et al. (2015)	PKDB00492	EMIT	45	5	✓	⊘					✓					✓	✓	✓					✓															
Turpault et al. (2009)	PKDB00063	LC MS/MS	30	1	⊘						⊘	⊘	⊘	⊘		✓	✓	✓		⊘			⊘	⊘														
Wahlländer et al. (1990)	PKDB00493	EMIT,RP-HPLC	62	10	✓	⊘					✓		⊘			✓	✓	✓					⊘															
Walther et al. (1983)	PKDB00500	GC	13	4	✓	⊘	⊘				✓					✓	✓	✓					✓															
Wang et al. (1980)	PKDB00383	CGC	27	3	⊘	⊘					⊘	⊘	⊘	⊘		✓	✓	✓					✓															
White et al. (2016)	PKDB00039	LC MS	24	1	⊘	⊘	⊘	⊘	⊘		⊘	⊘	⊘	⊘		✓	✓	✓					✓															
Yamazaki et al. (2017)	PKDB00494	LC MS	96	6	⊘	⊘	⊘		⊘	⊘	⊘	⊘				✓	✓	✓					✓															
Yuteri-Lahoz et al. (2012)	PKDB00495	HPLC	21	2	⊘	⊘	⊘	⊘		⊘	⊘		⊘	⊘		✓	✓	✓					✓	✓														
Zylber-Katz et al. (1984)	PKDB00065	HPLC	12	0	⊘	⊘	⊘				⊘	⊘	⊘			⊘	✓	✓					⊘															

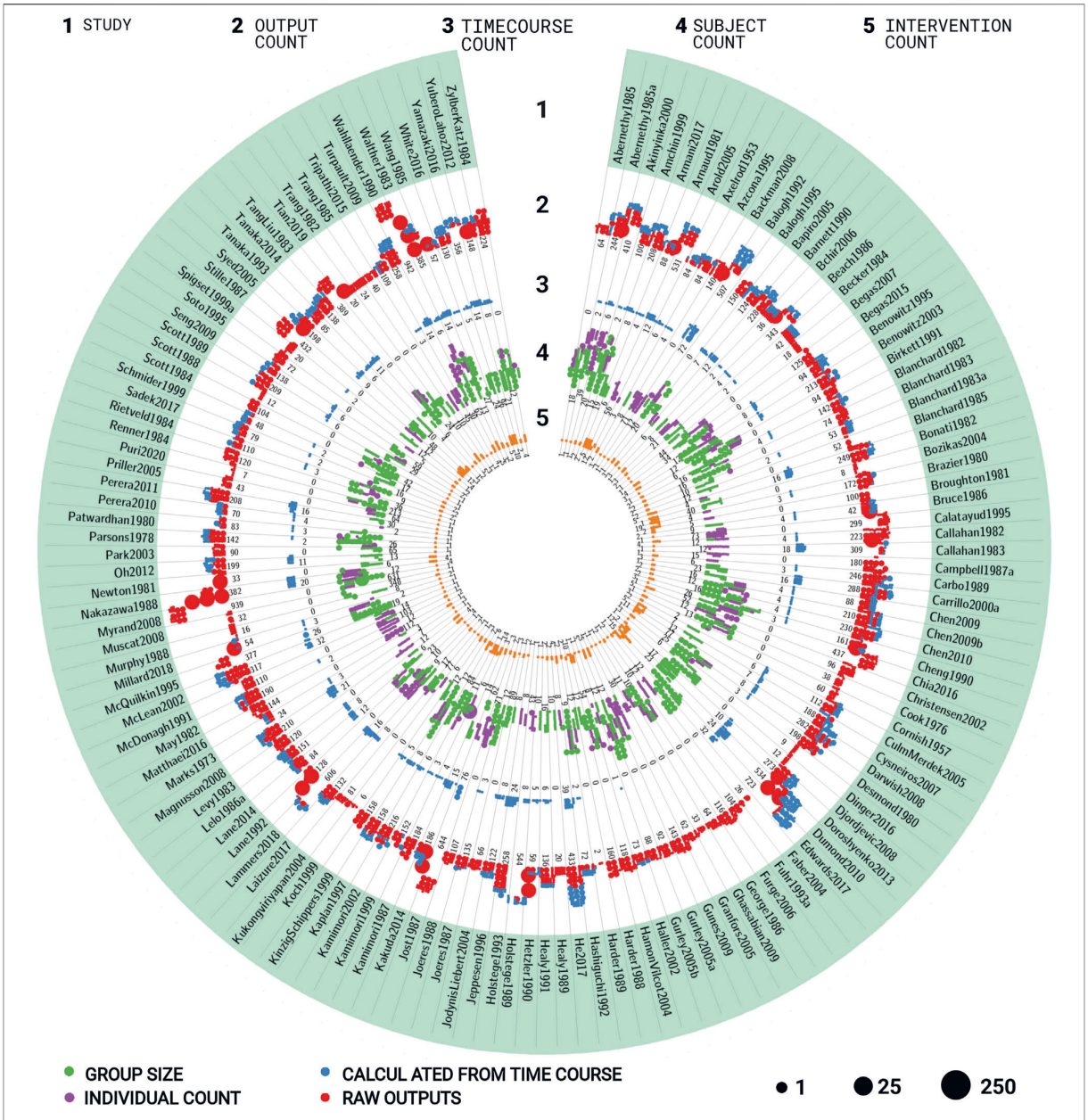

FIGURE 2 | Overview of studies in the caffeine pharmacokinetics data set. The data set consists of 141 studies containing 500 groups, 4,714 individuals, 387 interventions, 24 ,571 outputs, and 846 time-courses. The circular plot is structured in stripes and rings. Each stripe represents a different study, each ring the amount of different data types for the respective study. The dots represent the respective amount of data with the dot size corresponding to the number of entries per dot. The rings contain the following information for the respective study **(A)** name of the study; **(B)** number of outputs (pharmacokinetics parameters and other measurements). Red dots represent reported data, blue dots data calculated from time-courses reported in the study; **(C)** number of time-courses; **(D)** number of participants. Purple dots represent participants with individual data, green dots represent collectively reported participants; **(E)** number of interventions applied to the participants in the study. For additional information see **Table 1**.

(Grzegorzewski et al., 2020). We demonstrate the value of the data set by its application to multiple research questions relevant for metabolic phenotyping and liver function testing (**Figure 1B**): 1) the effect of smoking and oral contraceptive use on caffeine elimination (**Section 3.3**); 2) the effect of the type of assay (**Section 3.4**); 3) the interaction of caffeine with other drugs

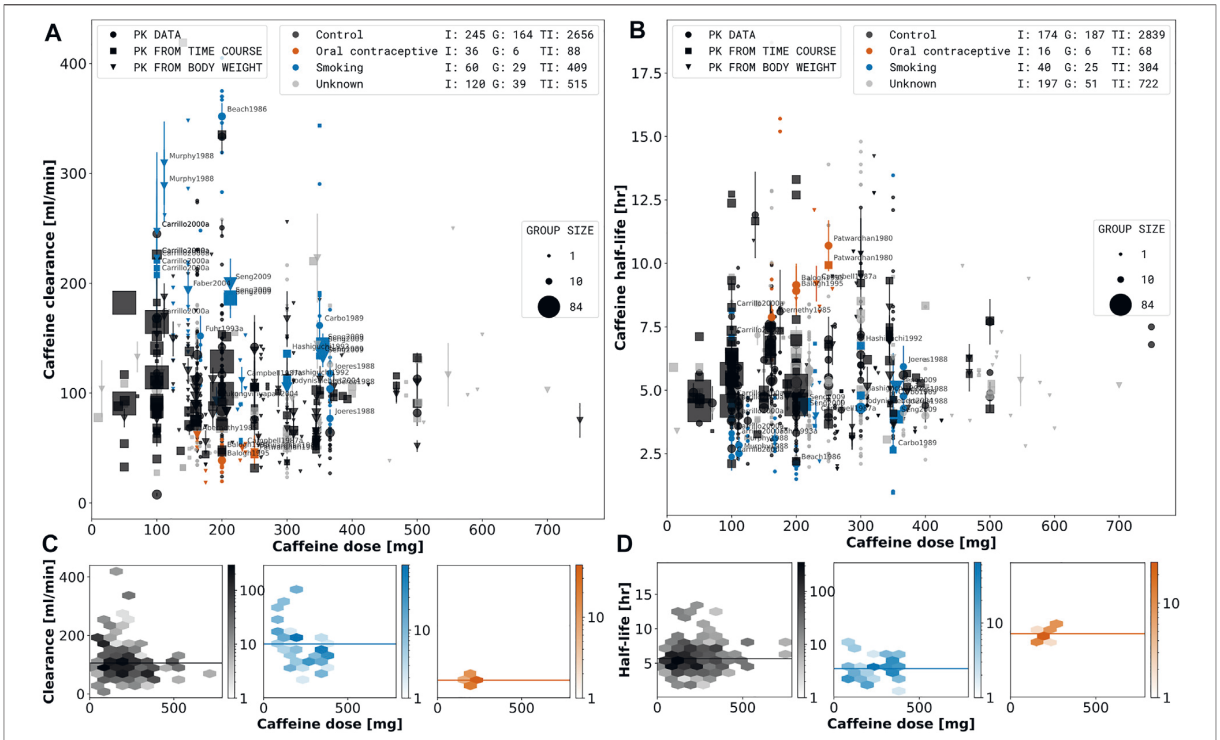

FIGURE 3 | Dose-dependent effect of smoking and oral contraceptive use on caffeine pharmacokinetics. A stratified meta-analysis of caffeine clearance **(A)** and half-life **(B)** depending on reported smoking and oral contraceptive use and dose was performed. Black: Control subjects are non-smokers and not taking oral contraceptives; Orange: Oral contraceptive users independent of smoking status (smokers and non-smokers). Blue: Smoking are smokers not consuming oral contraceptives. Grey: Unknown data correspond to subjects with unreported smoking and oral contraceptive status. Marker shape, and size describe the datatype and group size, respectively. Data representing smokers or oral contraceptive consumers is labeled by the respective study name. The hexagonal bin plots in the lower panel **(C, D)** correspond to the subset of data for the control, smoking, and oral contraceptive consuming subjects. The color intensity of each bin represents the number of subjects falling in a given hexagonal bin area. Data selection criteria and visualization are described in the **Section 2**.

(**Section 3.5**), 4) alteration of caffeine pharmacokinetics in disease (**Section 3.6**); and 5) the applicability of caffeine as a salivary test substance by comparison of plasma and saliva data (**Section 3.7**). In the following, we summarize the quality of reporting and provide example applications of the data set.

3.2 Reporting of Pharmacokinetics Data

The study design as well as quality and details of reporting of results were very heterogeneous between studies. Major differences exist in the study design, number of study participants, and number of reported time-courses. Many studies report some individual participants data (84/141) but only a minority of the studies report individual participant data for all study participants (6/84) (Axelrod and Reichenthal, 1953; Brazier et al., 1980; Rietveld et al., 1984; McQuilkin et al., 1995; Perera et al., 2010; Millard et al., 2018). Many studies report only aggregated data on group level (57/141). In most studies, the application of a single dose of caffeine was studied (129/141). In the case of multiple interventions (49/141), mostly one additional substance was co-administrated (33/141). The main categories of studies were either 1) case-control studies which compare caffeine pharmacokinetics in two groups (e.g., healthy vs Disease) (64/141), 2) crossover studies on caffeine-drug interactions (comparing caffeine alone vs caffeine and additional substance) (33/141) 3) studies on metabolic phenotypes (including drug cocktails) (42/141); or 4) methodological studies (e.g., establishing mass spectrometry protocol for quantification or new site of sampling).

Intervention protocols, i.e., the applied substances, form, dose, and timing of application was typically reported in good detail. In crossover studies, the difference between treatments was generally reported in good detail. For the dosing with caffeine, the amount (136/141), route (e.g., oral, intravenous) (140/141), form (tablet, capsule, solution) (136/141), and the substance (141/141) are typically reported. Co-administrations of medication and other substances are often mentioned qualitatively (28/49), skipping either the amount, route, form, or exact timing of application.

The quantification protocol, i.e., type of assay (e.g., high-performance liquid chromatography, gas-liquid

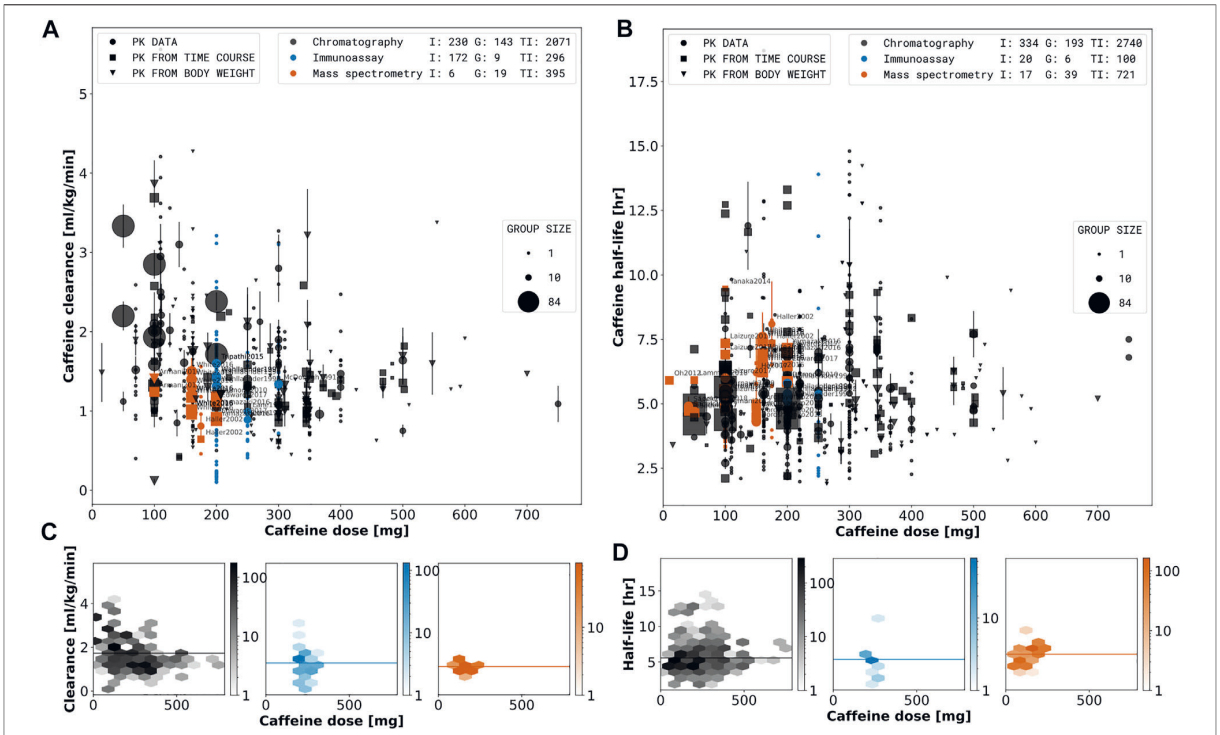

FIGURE 4 | Effect of type of assay on pharmacokinetic parameters. A stratified meta-analysis of caffeine clearance **(A)** and half-life **(B)** depending on reported assay type and dose. Orange: Measurements performed with mass spectrometry (LC MS, LC MS/MS, HPLC MS/MS, HPLC-ESI-MS/MS, ES MS/MS). Blue: Measurements performed with immunoassay (RIA, EMIT). Black: Separation performed with chromatography but quantification assay was not reported (HPLC, RP-HPLC, GLC, CGC, CC, GC). Marker shape, and size describe the datatype and group size, respectively. Data with reported quantification method are labeled by the respective study name. The hexagonal bin plots in the lower panel **(C, D)** correspond to the subset of data for the mass spectrometry quantification, immunoassay quantification and chromatography. The color intensity of each bin represents the number of subjects falling in a given hexagonal bin area. Data selection criteria and visualization are described in the **Section 2**.

chromatography) (140/141), the site of sampling (e.g., plasma, serum, saliva, urine) (141/141), and the time points when samples were taken (128/141), were mostly reported in good detail. However, in some studies the protocol is not mentioned explicitly but only via additional references, which complicates the curation. For a single study no information on the quantification method could be accessed (Blanchard and Sawers, 1983c). The reporting of type of assay is very heterogeneous with many studies not providing sufficient details (e.g., HPLC is often mentioned as the separation technique but the subsequent detection or quantification method is not stated).

The information on subject characteristics was less often reported in sufficient detail with large differences in the quality and quantity of reporting between studies. Any information on sex (131/141), weight (71/141), and age (89/141) was relatively often reported on group or individual level. However, age and weight were rarely reported on an individual level and often not even for all groups. Other anthropometric factors such as height (15/141), body mass index (BMI) (15/141), and ethnicity (25/141) are rarely reported. The genotype of CYP1A2 (gCYP1A2) is rarely measured or reported (5/141), even though there is evidence that genetic variation can play an important role in caffeine metabolism. Further, the nomenclature is not standardized. It is worth noting that low-cost genotyping methods were not available for early studies included in the data set. The phenotype (pCYP1A2, pXO, pNAT2) of enzymes involved in the metabolism of caffeine, i.e., CYP1A2, xanthine oxidase (XO), or N-acetyltransferase type 2 (NAT2) were investigated occasionally (38/141). The information on other factors influencing the pharmacokinetics of caffeine is reported very heterogeneously. The strong influence of smoking (105/141) and the use of oral contraceptives (42/141) on the enzyme activity of CYP1A2 and thereby on the apparent clearance of caffeine is covered relatively well in many publications. Health status and patient diseases are often covered (134/141). However, often categorized in broad and general disease classes, with more specific disease classification lacking. Markers related to cardiovascular health (e.g., blood pressure, cholesterol level, bilirubin level) are basically not reported in the context of caffeine pharmacokinetics. In case of cirrhosis, further information of severity is reported sparsely. Important information on the abstinence of caffeine or

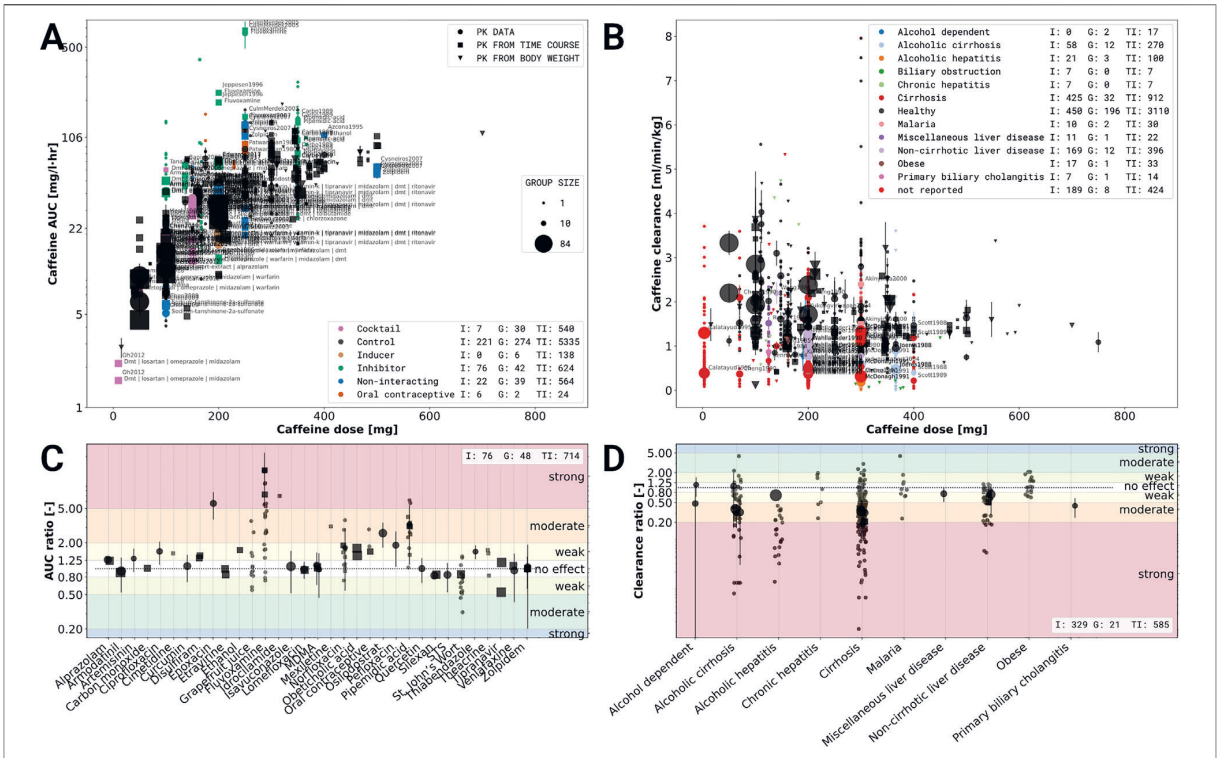

FIGURE 5 | Effects of caffeine-drug and caffeine-disease interactions. **(A)** Caffeine-drug interactions based on the caffeine area under the concentration curve (AUC). Data is stratified based on co-administration of drugs with caffeine and dose. Violet: caffeine administrated as part of a drug cocktail. Common co-administrations are dextrometorphan, metoprolol, midazolam, omeprazole, and warfarin.; Black: single caffeine administration (no co-administration); Brown: co-administration with an inducing effect on the elimination of caffeine; Green: co-administration with an inhibiting effect on the elimination of caffeine; Blue: co-administrations with no effect on the pharmacokinetics of caffeine; Orange co-administration of oral contraceptives. **(B)** Caffeine-disease interactions based on caffeine clearance and dose. Data was stratified based on the health status and reported diseases, with black data points corresponding to healthy subjects. **(C)** Effect sizes of caffeine-drug interaction for studies with a controlled study design, mostly randomized control trials (RCT). The effect size is based on the log AUC ratio between caffeine application alone and caffeine with co-administration of the respective drug. The drugs were characterized as having either a strong, moderate, weak or no effect. Strong, moderate and weak inhibitors increase the AUC ≥5-fold, ≥2 to <5-fold, ≥1.25 to <2-fold, respectively. **(D)** Effect size of caffeine-disease interactions for studies with a controlled study design, mostly case-controlled studies. The effect size is based on the log clearance ratio between subjects with and without a specific condition/disease. The diseases were characterized as having either a strong, moderate, weak or no effect. Strong, moderate and weak effect decreased the clearance by ≥80 percent, ≥50 to <80 percent and ≥20 to <50 percent, respectively. Data selection criteria and visualization are described in the **Section 2**.

methylxanthines and consumption of caffeine or other caffeine-containing beverages is often missing.

Individual-level reporting is essential for subsequent pharmacokinetic modeling, as biological mechanisms responsible for the pharmacokinetics strongly correlate with these factors and large inter-individual variability exists in caffeine pharmacokinetics. Despite the importance of individual subject data, information on individuals is rarely provided.

Most studies report pharmacokinetics outputs on caffeine (126/141). Data on the main product paraxanthine (46/141) and secondary metabolites theobromine (20/141) and theophylline (19/141) are reported sometimes. Additional metabolites such as 137U, 17U, 13U, 37U, 1X, 1U, 3X, 3U, 7X, 7U, AFMU, AAMU, ADMU, A1U, and A3U are seldom reported, mostly as urinary measurements and not directly but as part of a metabolic ratio.

3.3 Smoking and Oral Contraceptives

In a first analysis, we were interested in the effect of smoking and oral contraceptive use on the pharmacokinetics of caffeine in healthy subjects (see **Figure 3**). Both have repeatedly been reported as key exogenous factors affecting caffeine elimination. A main question was how reproducible the effect is and if by integrating data from multiple studies a more consistent picture of the effects can be gained. For the analysis the data set was stratified into smokers, oral contraceptive users and a control group (neither smoking nor using oral contraceptives). Smoking results in increased caffeine clearance (**Figures 3A,C**) and decreased half-life of caffeine elimination (**Figures 3B,D**) whereas oral contraceptive use has the opposite effect over a wide dose range of caffeine. An important result from our analysis is that a consistent and reproducible effect can be found over more than 50 years of pharmacokinetic research. With exception of a few outlier studies probably from a single

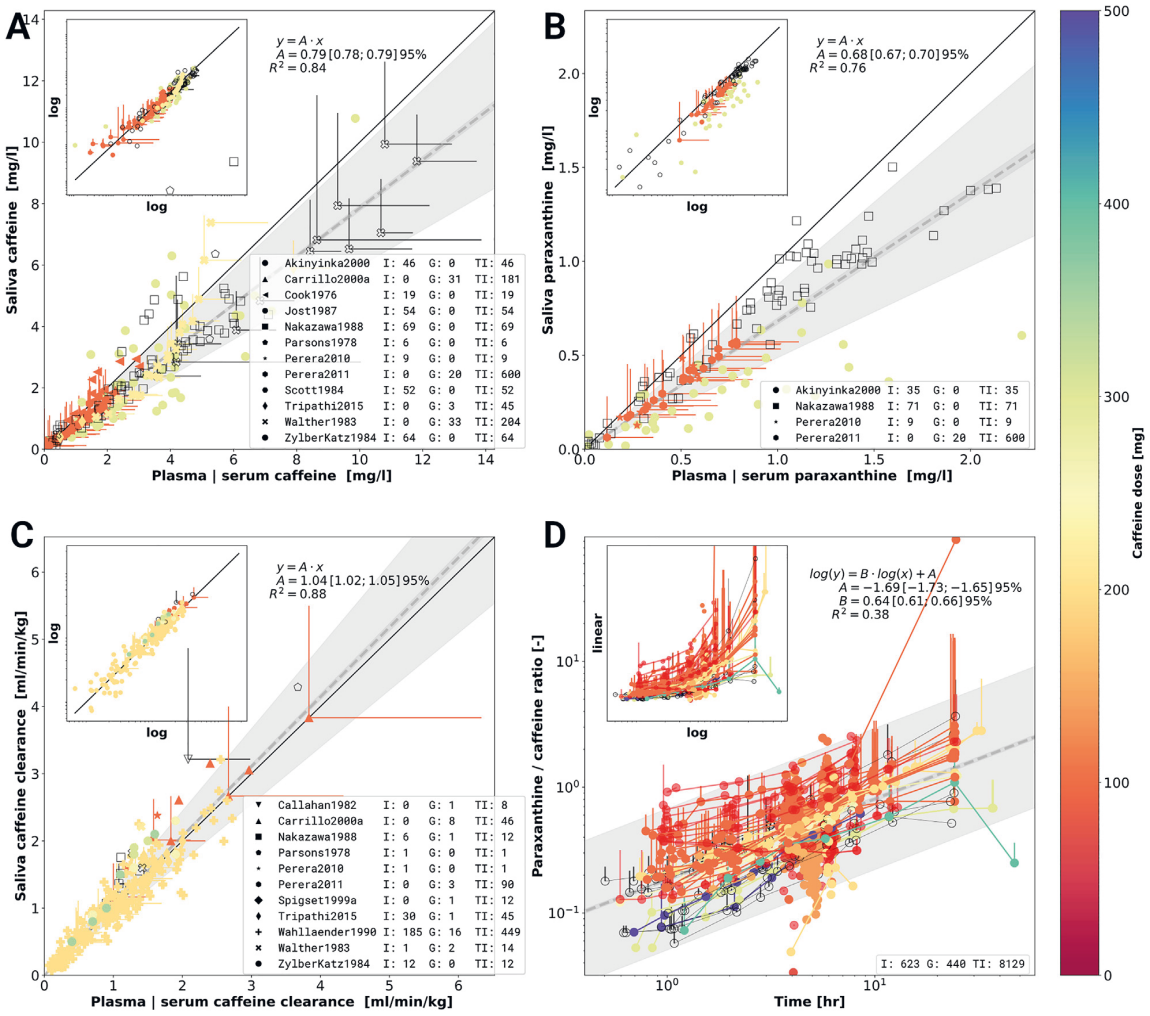

FIGURE 6 | Meta-analysis of caffeine and paraxanthine concentrations in plasma, serum and saliva. **(A)** Caffeine concentrations in saliva versus caffeine concentrations in plasma or serum. Individual data points come from a single investigation taken at identical times after caffeine dosing. Marker shape encodes the different study. Markers are color-coded by caffeine dose in mg. Empty markers correspond to data in which dosage was reported per body weight but no information on the subject weight was available. **(B)** Paraxanthine concentrations in saliva versus paraxanthine concentrations in plasma or serum analogue to **(A)**. **(C)** Caffeine clearance calculated from caffeine concentrations in saliva versus plasma or serum clearance. The panels A, B and C are in linear scale with a log-log inlet showing the same data. The dashed line in A, B and C represents a linear regression ($y = A \cdot x$) with wide shaded area being 95% confidence interval of the sample variability and narrow shaded area the 95% confidence interval of the fitted mean of the scaling factor A. **(D)** Time dependency of the metabolic ratio paraxanthine/caffeine. Metabolic ratios are measured in plasma, serum, or saliva. Data points belonging to a single time course from a study are connected via a line. The dashed line corresponds to the linear regression $\log(y) = B \cdot \log(t) + A$. Jitter was applied on the time axes for better visibility of overlapping points. Data selection criteria and visualization are described in the **Section 2**.

clinical trial (see methods) all data was highly consistent. This provides a strong argument for the applied methods and protocols.

Despite the large effect of smoking and oral contraceptive use on the pharmacokinetics of caffeine, the information is only reported for a subset of studies. Smoking as well as oral contraceptive use should be an exclusion criteria for subjects in studies of caffeine pharmacokinetics, due to the possible confounding effects. Importantly, in many groups smokers and non-smokers were mixed without reporting data for smokers and non-smokers separately. Without reporting of data on individuals or subgroups no stratification could be performed, which could strongly affect results if not balanced between groups. In summary, the integrative data analysis showed a consistent strong activating effect of smoking on caffeine elimination and an inhibiting effect of oral contraceptive use on caffeine elimination.

3.4 Effect of Type of Assay

The reported type of assays were curated systematically (see **Table 1** to study effects of the type of technique and assay on the reported pharmacokinetics data (see **Figure 4**, abbreviations explained in the legend of **Table 1**). Data affected by confounding factors (i.e., smoking, oral contraceptive use, caffeine-disease, and caffeine-disease interactions) was excluded. Based on our analysis no systematic difference between the reported type of assays could be detected. Immunoassays (RIA, EMIT, ELISA), mass spectrometry (LC MS, GC MS, LC MS/MS, HPLC MS/MS, HPLC-ESI-MS/MS, ES MS/MS), and chromatography with unspecified quantification method (HPLC, RP-HPLC, GLC, CGC, CC, GC) do not show any differences in half-life or clearance. Spectrophotometric methods (UV/Vis) are generally rare and were not applied in this data subset. Based on the analysis we conclude that no systematic correction was required to compare the data collected with the different types of assays.

3.5 Caffeine-Drug Interactions

An important question for metabolic phenotyping and liver function testing with caffeine is how the coadministration of other drugs and compounds affects caffeine clearance. Consequently, we studied in the second analysis the reported caffeine-drug interactions in the data set. The impact of drugs was quantified using the change in AUC between a coadministration and a respective control $log(\frac{AUC_{coadministration}}{AUC_{control}})$ (see **Figures 5A,C**).

Only case-controlled studies, mostly cross-over trials with a washout phase were included in the analysis. Corresponding controls were not matched across different studies. Overall coadministration data with AUC difference was available for 33 substances in our data set. In accordance with FDA, EMA and PMDA guidelines (Sudsakorn et al., 2020) we classified substances as inhibitors or inducers of caffeine clearance based on changes in AUC: FDA–Clinical DDI guidance: strong, moderate and weak inhibitors increase the AUC $\geq$5-fold, $\geq$2 to $<$5-fold, $\geq$1.25 to $<$2-fold, respectively; strong, moderate and weak inducers decrease the AUC by $\geq$80 percent, $\geq$50 to $<$80 percent and $\geq$20 to $<$50 percent, respectively.

Most substances do not affect the AUC of caffeine, with the exception of fluvoxamine, pipemidic acid and norfloxacin, which inhibit caffeine clearance. Tipranavir was the only substance showing a weak induction of caffeine clearance, but only in steady state dosing (not after a single dose) (Dumond et al., 2010)). Substances administered as a cocktail along side caffeine, aiming to phenotype several enzymes simultaneously did not affect the AUC of caffeine, substantiating the use of caffeine as part of a drug cocktail design (Turpault et al., 2009; Dumond et al., 2010; Oh et al., 2012; Doroshyenko et al., 2013; Kakuda et al., 2014; Tanaka et al., 2014; Armani et al., 2017; Edwards et al., 2017; Lammers et al., 2018). Our analysis shows that only a minority of studied drugs show an interaction with caffeine, confirming its value for phenotyping even under co-administration. As a side note, the protocols for studying caffeine-drug interactions were highly variable, e.g., the applied caffeine dose and the dose of the coadministrated substance varied between studies. Our results suggest that most medications can be safely consumed in combination with caffeine with exception of the antidepressant fluvoxamine, the antibacterial pipemidic acid and the antibiotic norfloxacin in which case caution is warranted.

3.6 Caffeine-Disease Interactions

An important question for using caffeine as a test substance for liver function testing and phenotyping, as well as for drugs metabolized via CYP1A2, is how disease affects the pharmacokinetics and elimination of caffeine. To study this question, we stratified caffeine clearance rates based on the reported disease of subjects and groups in the data set. To quantify the effect of disease the absolute clearance of caffeine (**Figure 5B**) and the logarithmic difference to a control group $log(\frac{AUC_{disease}}{AUC_{control}})$ (**Figure 5D**) were analyzed. The corresponding controls were not matched across different studies.

None of the reported diseases increased the clearance rate of caffeine. Most of the diseases contained in this data set are diseases of the liver (e.g., alcoholic cirrhosis, primary biliary cholangitis) or are known to affect the liver (e.g., alcohol dependent). Cirrhotic liver disease had moderate to strong effects on the caffeine clearance with large variability in the reported data. Malaria and obesity had no effect on clearance with caffeine. An issue in the study of caffeine-disease interaction is that control group and disease group are different subjects (no cross-over design). In addition diseases were reported very heterogeneously (e.g., either only cirrhosis or with underlying cause such as alcoholic cirrhosis).

3.7 Metabolic Phenotyping

An important question for metabolic phenotyping and liver function tests with caffeine is how saliva measurements correlate with plasma or serum caffeine measurements, in the following referred to as blood-based measurements. A good correlation would allow simple non-invasive phenotyping using saliva samples. To study this question we analyzed 1) the relationship of blood-based concentrations of caffeine and paraxanthine with their respective saliva concentrations (**Figures 6A,B**); and 2) how the caffeine clearance measured in saliva correlate to blood-based measurements (**Figure 6C**).

Systematic errors due to different dosing protocols and different clinical investigation seem to be minimal as the data from multiple studies shows very consistent results. Linear regressions were performed to quantify the relation between saliva and blood-based caffeine and paraxanthine measurements (see **Figures 6A–C**). The resulting scaling factors of saliva to blood-based concentration of caffeine and paraxanthine are 0.79 ± 0.01 and 0.68 ± 0.02 ($\bar{x} \pm$ SD), respectively. Pearson correlation coefficients between saliva and blood-based concentrations for caffeine and paraxanthine are 0.84 and 0.76, respectively.

When comparing saliva-based caffeine clearance against blood-based clearance (**Figure 6C**) an even stronger correlation of 0.88 with a scaling factor of 01.04 ± 0.02

($\bar{x} \pm$ SD) is observed. The integrated clearance data strongly indicates that clearance can either be calculated from saliva or blood-based measurements.

Paraxanthine/caffeine ratios are mainly used for metabolic phenotyping based on caffeine. Whereas most studies use 6 h to phenotype no clear consensus exists in the literature and metabolic ratios are reported for varying time points after caffeine application. Paraxanthine/caffeine ratios for caffeine administered either as a single dose or in a cocktail to healthy, non-smoking, non-pregnant, and non-oral contraceptive consuming subjects were investigated (**Figure 6D**). By applying this strict data filtering, the variability due to either smoking and oral contraceptive use (see **Section 3.3**), caffeine-drug interactions (see **Section 3.5**) or disease (see **Section 3.6**) could be removed from the metabolic ratios.

Early and late time-sampling are least suitable for phenotyping. At these time points concentrations are low, resulting in relatively high random errors and thus low single to noise ratio. In an early stage, the outcome of metabolic ratios are further influenced by the distribution phase of the substance and its absorption kinetics, both affected by form and route of administration. Main results are that the metabolic phenotyping with paraxanthine/caffeine ratios is strongly time dependent with increasing ratios with time; and that a clear caffeine-dose dependency exists in the phenotyping with smaller caffeine doses increasing the metabolic ratio. Our results show the importance of clear standardized protocols for metabolic phenotyping.

In summary, by integrating data from multiple studies we could show a very good correlation between saliva and plasma caffeine concentrations, paraxanthine concentrations and pharmacokinetic parameters calculated from saliva versus plasma concentrations. This pooled data provides a strong argument for caffeine phenotyping based on saliva samples. Furthermore, we analyzed the time dependency of paraxanthine/caffeine ratios often used for phenotyping of CYP1A2. Our time dependent correlation allows to correct caffeine/paraxanthine ratios depending on the time after application and caffeine dose.

4 DISCUSSION

Within this work, we performed a systematic data integration and multiple data analyses of reported data on caffeine pharmacokinetics in adults focusing on applications in metabolic phenotyping and liver function testing. To our knowledge, this is the largest open pharmacokinetics data set in humans with this kind of data being urgently needed to enable reproducible pharmacokinetics (Ioannidis, 2019). Data integration from multiple sources allows to solidify existing knowledge, increase statistical power, increase generalizability, and create new insights into the relationship between variables (Thacker, 1988). We show for instance, by systematically curating group and subject information on smoking status and oral contraceptive use a reproducible and consistent effect of smoking induction and inhibition via oral contraceptives over many studies and the complete dose regime of applied caffeine. Only a small subset of studies was specifically designed to study these questions (e.g., smoking by (Parsons and Neims, 1978; Benowitz et al., 2003; Backman et al., 2008; Gunes et al., 2009) and oral contraceptives by (Patwardhan et al., 1980; Rietveld et al., 1984; Abernethy and Todd, 1985)). Publicly providing comprehensive pharmacokinetics data in combination with detailed metadata allows to study new aspects of caffeine pharmacokinetics often not even anticipated by the original investigators. One example of such a new aspect is the assessment of dose and time-dependency of metabolic phenotyping via paraxanthine/caffeine ratios even if many of the data sets only report data for a single time point and dose.

Within this work we could confirm that oral contraceptives, smoking and drugs such as fluvoxamine or pipedemic acid alter the pharmacokinetics of caffeine, and that a saliva based metabolic phenotyping approach has very good correlation with blood based approaches, thereby solidifying existing knowledge. In addition novel aspects of the pharmacokinetics of caffeine could be elucidated. By data integration we could not only study the dose dependency of caffeine pharmacokinetics parameters, but also of the effects of smoking and oral contraceptives. For the first time we could show that both the induction of caffeine clearance by smoking and the reduction of caffeine clearance by oral contraceptives is an effect independent of the actual caffeine dose and that caffeine pharmacokinetics is affected by these confounding factors over a wide dose range. Important new results for the metabolic phenotyping with caffeine are our analysis of dose and time-dependency of paraxanthine/caffeine ratios. To our knowledge this time-dose-dependency has not been reported so far, and is especially relevant for phenotyping under very low doses such as cocktail approaches. Based on our results metabolic phenotyping data could be corrected for the dosage and time effects, thereby allowing to integrate data taken under various phenotyping protocols.

The dosage of a substance can have large effects on pharmacokinetic parameters. Using the data set we could evaluate the effect of caffeine dose on pharmacokinetic parameters systematically, e.g., on clearance (**Figure 3A**, **Figure 4A**, **Figure 5B**), half-life (**Figure 3B**, **Figure 4B**, **Figure 5B**), AUC (**Figure 5A**), or time-dependence of paraxanthine/caffeine ratios used in metabolic phenotyping (**Figure 6D**) by either directly using dose as a dimension of our analysis or by color coding based on dose. Caffeine dose has strong effects on the AUC of caffeine or the metabolic ratios of paraxanthine/caffeine whereas no clear effects on clearance or half-life could be observed. Our data set and analyses underline the importance of correcting for caffeine dose in the analysis of pharmacokinetic parameters and provide information for dosage corrections.

Various separation methods and detectors (mass spectrometry, UV/Vis, immunoassays or photodiode array) can be used for the quantification of caffeine metabolites and pharmacokinetics. The applied technique and assay can have large confounding effects. For instance cross-reactivity between caffeine and its metabolites have lead to false identification of concentrations by immunoassays (e.g., EMIT, ELISA) (Fligner and Opheim 1988). In earlier studies for example, EMIT methods showed a high cross-reactivity to paraxanthine (28%) Zysset et al.

(1984). Newer methods showed low cross reactivity towards paraxanthine (0.08%), however, high cross-reactivity towards theophyline (16%) (Carvalho et al., 2012). Based on our analysis no systematic difference between the reported type of assays could be detected (see **Figure 3**, **Figure 4**). This result is limited by the details of reporting with many studies not providing sufficient information on the quantification method.

An important outcome of our analysis are the very good correlations between saliva- and blood-based measurements for caffeine with 0.77 R^2 = 0.74 in very good agreement with data reported previously in individual studies 0.74 ± 0.1 R^2 = 0.98 (Akinyinka et al., 2000), 0.79 ± 0.05 R^2 = 0.96 (Zylber-Katz et al., 1984), 0.74 ± 0.08 (Newton et al., 1981), 0.74 ± 0.08 R^2 = 0.90 (Jost et al., 1987), 0.71 R^2 = 0.89 (Scott et al., 1984), 0.73 ± 0.06 (Walther et al., 1983), and for paraxanthine 0.68 R^2 = 0.76 compared to 0.77 R^2 = 0.91 (Nakazawa and Tanaka, 1988).

Several studies have shown that blood-derived pharmacokinetic parameters show excellent correlation with saliva-derived parameters (Newton et al., 1981; Akinyinka et al., 2000). We could confirm this observation when systematically analyzing the correlation between saliva- and plasma/serum-derived clearance. By integration of data from multiple studies we could increase the power of the conclusion and show the robustness of the reported correlation.

Overall we could show that the data from multiple studies are in very good agreement with each other after excluding data with confounding factors such as smoking or oral contraceptive use. These integrated results are a strong argument for saliva based metabolic phenotyping and liver function tests with caffeine, with sampling from saliva being convenient, painless, economical, without the requirement for special devices. Further, they allow simple repeated sampling as often required for pharmacokinetic research (Zylber-Katz et al., 1984).

Our systematic curation and analysis of reported caffeine data provided an overview of the current state and limitations of reporting of pharmacokinetic data. In summary, an accepted standard, minimum information guidelines, and standardized meta-data for the reporting of pharmacokinetics data of caffeine are missing. This finding is apparently not only true for the pharmacokinetics of caffeine but rather generally true for reporting of pharmacokinetics research. Major shortcomings in reporting are missing minimum information on factors that are known to influence the pharmacokinetics of caffeine (e.g., smoking and oral contraceptive status). Often not even basic subject information (e.g., weight, sex, or age) are reported. These factors are essential in the analysis of the pharmacokinetics of any substance *in vivo* (Stader et al., 2019). In general-purpose pharmacokinetic data sets, concentration-time profiles are the fundamental and most valuable data type. Common practice however is not to report the raw measured data but only derived pharmacokinetic parameters or metabolic ratios and not on individual participants level. Individual participants data have major advantages (Riley et al., 2010). We strongly advocate the reporting of all data on an individual level while including detailed anonymized meta information alongside the concentration-time profiles. Access to the individual raw data would enable data integration with different data sets and the stratification of the data under various aspects. There are recent efforts in creating a standard resource for that matter (Grzegorzewski et al., 2020).

Data integration and meta-analysis methods may be limited by selection bias, performance bias, detection bias, attrition bias, reporting bias and other biases (Higgins and Collaboration, 2020) but the extent of it in the field of pharmacokinetics is at large unknown (Ioannidis, 2019). Based on our data set we could evaluate the bias due to the type of assay and concluded that no bias could be found. Despite being the most comprehensive analysis so far, we could only present selected aspects mainly driven by the availability of reported data. Our focus in this study was on key factors affecting caffeine pharmacokinetics (smoking and oral contraceptive use), caffeine-drug and caffeine-disease interactions, as well as information relevant for the metabolic phenotyping and liver function testing with caffeine. Other important factors such as the pharmacogenetics of caffeine or urinary metabolic ratios have not been presented. Importantly, the corresponding data was curated and is readily available in PK-DB, but often very sparse (e.g., in case of genetic variants) or very heterogeneous (e.g., in case of urinary data). From our study (**Table 1** and other investigations Koonrungsesomboon et al. (2018), it is striking, how little caffeine pharmacogenetics data is captured in the literature despite high heritability of CYP1A2 activity (Rasmussen et al., 2002; Matthaei et al., 2016). Our systematic analysis identified this gap of knowledge and more research in this area is needed.

Despite many implemented measures to ensure high data quality (e.g., validation rules and checking of studies by multiple curators), we are aware that the created data set may contain mistakes. Please report such instances so that these can be resolved.

The scope of the presented data set is limited to caffeine pharmacokinetics in human adults. As identified by the systemic search, the data set is far from comprehensive. At very least further 145 studies are eligible for inclusion, but were classified as low priority for the analyses. Future work will include this data and extend our data curation effort towards children and infants. Beyond the 145 missing studies, additional studies with relevant data exist. Please contact us in such cases so that we can include these additional studies. Contribution of missing data is highly appreciated. Also if you want to contribute a caffeine data set of your own please get in contact.

Importantly, our results are not only applicable to caffeine, but many aspects can be translated to other substances metabolized via CYP1A2, e.g., to the LiMAx liver function tests based on the CYP1A2 substrate methacetin (Rubin et al., 2017). For instance based on our analysis we expect smoking and oral contraceptive use to be confounding factors of LiMAx tests, which should be recorded and be accounted for in the evaluation of liver function.

The large inter-individual variability in caffeine pharmacokinetics is a major limitation for metabolic phenotyping and liver function tests. Our work allowed for the first time to systematically evaluate the effect of key factors on caffeine pharmacokinetics such as smoking, oral contraceptive usage or caffeine-drug interactions. An important next step will be the development of methods to quantify and correct for these confounding factors. This could allow to reduce variability in caffeine based testing. A promising tool in this context are

physiological-based pharmacokinetics (PBPK) models (Jones and Rowland-Yeo, 2013) using information from the established data set as input for stratification and individualization. We could recently show that such an approach based on a similar data set for indocyanine green (ICG) allowed to account for important factors affecting ICG based liver function tests (Köller et al., 2021).

DATA AVAILABILITY STATEMENT

Publicly available datasets were analyzed in this study. This data can be found here: https://pk-db.com.

AUTHOR CONTRIBUTIONS

JG and MK designed the study, implemented and performed the analysis, curated the majority of caffeine studies and wrote the initial draft of the manuscript. FB and AK curated a subset of caffeine studies. All authors discussed the results. All authors contributed to and revised the manuscript critically.

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REFERENCES

Abernethy, D. R., and Todd, E. L. (1985). Impairment of Caffeine Clearance by Chronic Use of Low-Dose Oestrogen-Containing Oral Contraceptives. *Eur. J. Clin. Pharmacol.* 28, 425–428. doi:10.1007/BF00544361

Abernethy, D. R., Todd, E. L., and Schwartz, J. B. (1985). Caffeine Disposition in Obesity. *Br. J. Clin. Pharmacol.* 20, 61–66. doi:10.1111/j.1365-2125.1985.tb02799.x

Akinyinka, O. O., Sowunmi, A., Honeywell, R., and Renwick, A. G. (2000). The Effects of Acute Falciparum Malaria on the Disposition of Caffeine and the Comparison of Saliva and Plasma-Derived Pharmacokinetic Parameters in Adult Nigerians. *Eur. J. Clin. Pharmacol.* 56, 159–165. doi:10.1007/s002280050735

Amchin, J., Zarycranski, W., Taylor, K. P., Albano, D., and Klockowski, P. M. (1999). Effect of Venlafaxine on Cyp1a2-dependent Pharmacokinetics and Metabolism of Caffeine. *J. Clin. Pharmacol.* 39, 252–259.

Armani, S., Ting, L., Sauter, N., Darstein, C., Tripathi, A. P., Wang, L., et al. (2017). Drug Interaction Potential of Osilodrostat (Lci699) Based on its Effect on the Pharmacokinetics of Probe Drugs of Cytochrome P450 Enzymes in Healthy Adults. *Clin. Drug Investig.* 37, 465–472. doi:10.1007/s40261-017-0497-0

Arnaud, M. J. (2011). Pharmacokinetics and Metabolism of Natural Methylxanthines in Animal and Man. *Handb Exp. Pharmacol.* 200, 33–91. doi:10.1007/978-3-642-13443-2_3

Arnaud, M. J., and Welsch, C. (1982). "Theophylline and Caffeine Metabolism in Man," in *Theophylline and other Methylxanthines/Theophyllin und andere Methylxanthine. Methods in Clinical Pharmacology*. Editors N. Rietbrock, B. G. Woodcock, and A. H. Staib (Wiesbaden, Germany: Vieweg), 3, 135–148. doi:10.1007/978-3-663-05268-5_18

Arold, G., Donath, F., Maurer, A., Diefenbach, K., Bauer, S., Henneicke-von Zepelin, H. H., et al. (2005). No Relevant Interaction with Alprazolam, Caffeine, Tolbutamide, and Digoxin by Treatment with a Low-Hyperforin St John's Wort Extract. *Planta Med.* 71, 331–337. doi:10.1055/s-2005-864099

Axelrod, J., and Reichenthal, J. (1953). The Fate of Caffeine in Man and a Method for its Estimation in Biological Material. *J. Pharmacol. Exp. Ther.* 107, 519–523.

Azcona, O., Barbanoj, M. J., Torrent, J., and Jané, F. (1995). Evaluation of the central Effects of Alcohol and Caffeine Interaction. *Br. J. Clin. Pharmacol.* 40, 393–400. doi:10.1111/j.1365-2125.1995.tb04562.x

Backman, J. T., Schröder, M. T., and Neuvonen, P. J. (2008). Effects of Gender and Moderate Smoking on the Pharmacokinetics and Effects of the Cyp1a2 Substrate Tizanidine. *Eur. J. Clin. Pharmacol.* 64, 17–24. doi:10.1007/s00228-007-0389-y

Balogh, A., Harder, S., Vollandt, R., and Staib, A. H. (1992). Intra-individual Variability of Caffeine Elimination in Healthy Subjects. *Int. J. Clin. Pharmacol. Ther. Toxicol.* 30, 383–387.

Balogh, A., Klinger, G., Henschel, L., Börner, A., Vollanth, R., and Kuhnz, W. (1995). Influence of Ethinylestradiol-Containing Combination Oral Contraceptives with Gestodene or Levonorgestrel on Caffeine Elimination. *Eur. J. Clin. Pharmacol.* 48, 161–166. doi:10.1007/BF00192743

Bapiro, T. E., Sayi, J., Hasler, J. A., Jande, M., Rimoy, G., Masselle, A., et al. (2005). Artemisinin and Thiabendazole Are Potent Inhibitors of Cytochrome P450 1a2 (Cyp1a2) Activity in Humans. *Eur. J. Clin. Pharmacol.* 61, 755–761. doi:10.1007/s00228-005-0037-3

Barnett, G., Segura, J., de la Torre, R., and Carbó, M. (1990). Pharmacokinetic Determination of Relative Potency of Quinolone Inhibition of Caffeine Disposition. *Eur. J. Clin. Pharmacol.* 39, 63–69. doi:10.1007/BF02657060

Bchir, F., Dogui, M., Ben Fradj, R., Arnaud, M. J., and Saguem, S. (2006). Differences in Pharmacokinetic and Electroencephalographic Responses to Caffeine in Sleep-Sensitive and Non-sensitive Subjects. *C R. Biol.* 329, 512–519. doi:10.1016/j.crvi.2006.01.006

Beach, C. A., Mays, D. C., Guiler, R. C., Jacober, C. H., and Gerber, N. (1986). Inhibition of Elimination of Caffeine by Disulfiram in normal Subjects and Recovering Alcoholics. *Clin. Pharmacol. Ther.* 39, 265–270. doi:10.1038/clpt.1986.37

Becker, A. B., Simons, K. J., Gillespie, C. A., and Simons, F. E. (1984). The Bronchodilator Effects and Pharmacokinetics of Caffeine in Asthma. *N. Engl. J. Med.* 310, 743–746. doi:10.1056/NEJM198403223101202

Begas, E., Kouvaras, E., Tsakalof, A., Papakosta, S., and Asprodini, E. K. (2007). *In Vivo* evaluation of Cyp1a2, Cyp2a6, Nat-2 and Xanthine Oxidase Activities in a Greek Population Sample by the Rp-Hplc Monitoring of Caffeine Metabolic Ratios. *Biomed. Chromatogr.* 21, 190–200. doi:10.1002/bmc.736

Begas, E., Kouvaras, E., Tsakalof, A. K., Bounitsi, M., and Asprodini, E. K. (2015). Development and Validation of a Reversed-phase Hplc Method for Cyp1a2 Phenotyping by Use of a Caffeine Metabolite Ratio in Saliva. *Biomed. Chromatogr.* 29, 1657–1663. doi:10.1002/bmc.3475

Benowitz, N. L., Jacob, P., Mayan, H., and Denaro, C. (1995). Sympathomimetic Effects of Paraxanthine and Caffeine in Humans. *Clin. Pharmacol. Ther.* 58, 684–691. doi:10.1016/0009-9236(95)90025-X

Benowitz, N. L., Peng, M., and Jacob, P. (2003). Effects of Cigarette Smoking and Carbon Monoxide on Chlorzoxazone and Caffeine Metabolism. *Clin. Pharmacol. Ther.* 74, 468–474. doi:10.1016/j.clpt.2003.07.001

Birkett, D. J., and Miners, J. O. (1991). Caffeine Renal Clearance and Urine Caffeine Concentrations during Steady State Dosing. Implications for Monitoring

Caffeine Intake during Sports Events. *Br. J. Clin. Pharmacol.* 31, 405–408. doi:10.1111/j.1365-2125.1991.tb05553.x

Blanchard, J., and Sawers, S. J. (1983b). Comparative Pharmacokinetics of Caffeine in Young and Elderly Men. *J. Pharmacokinet. Biopharm.* 11, 109–126. doi:10.1007/BF01061844

Blanchard, J., Sawers, S. J., Jonkman, J. H., and Tang-Liu, D. D. (1985). Comparison of the Urinary Metabolite Profile of Caffeine in Young and Elderly Males. *Br. J. Clin. Pharmacol.* 19, 225–232. doi:10.1111/j.1365-2125.1985.tb02635.x

Blanchard, J., and Sawers, S. J. (1983c). Relationship between Urine Flow Rate and Renal Clearance of Caffeine in Man. *J. Clin. Pharmacol.* 23, 134–138. doi:10.1002/j.1552-4604.1983.tb02716.x

Blanchard, J., and Sawers, S. J. (1983a). The Absolute Bioavailability of Caffeine in Man. *Eur. J. Clin. Pharmacol.* 24, 93–98. doi:10.1007/BF00613933

Bonati, M., Latini, R., Galletti, F., Young, J. F., Tognoni, G., and Garattini, S. (1982). Caffeine Disposition after Oral Doses. *Clin. Pharmacol. Ther.* 32, 98–106. doi:10.1038/clpt.1982.132

Bozikas, V. P., Papakosta, M., Niopas, I., Karavatos, A., and Mirtsou-Fidani, V. (2004). Smoking Impact on Cyp1a2 Activity in a Group of Patients with Schizophrenia. *Eur. Neuropsychopharmacol.* 14, 39–44. doi:10.1016/s0924-977x(03)00061-0

Brazier, J. L., Descotes, J., Lery, N., Ollagnier, M., and Evreux, J. C. (1980). Inhibition by Idrocilamide of the Disposition of Caffeine. *Eur. J. Clin. Pharmacol.* 17, 37–43. doi:10.1007/BF00561675

Broughton, L. J., and Rogers, H. J. (1981). Decreased Systemic Clearance of Caffeine Due to Cimetidine. *Br. J. Clin. Pharmacol.* 12, 155–159. doi:10.1111/j.1365-2125.1981.tb01194.x

Bruce, M., Scott, N., Lader, M., and Marks, V. (1986). The Psychopharmacological and Electrophysiological Effects of Single Doses of Caffeine in Healthy Human Subjects. *Br. J. Clin. Pharmacol.* 22, 81–87. doi:10.1111/j.1365-2125.1986.tb02883.x

Calatayud, O., Rodríguez, M., Sánchez-Alcaraz, A., and Ibáñez, P. (1995). Caffeine Test Assessment for Measuring Liver Function in Critically Ill Patients. *J. Clin. Pharm. Ther.* 20, 23–29. doi:10.1111/j.1365-2710.1995.tb00621.x

Callahan, M. M., Robertson, R. S., Arnaud, M. J., Branfman, A. R., McComish, M. F., and Yesair, D. W. (1982). Human Metabolism of [1-methyl-14c]- and [2-14c]caffeine after Oral Administration. *Drug Metab. Dispos* 10, 417–423.

Callahan, M. M., Robertson, R. S., Branfman, A. R., McComish, M. F., and Yesair, D. W. (1983). Comparison of Caffeine Metabolism in Three Nonsmoking Populations after Oral Administration of Radiolabeled Caffeine. *Drug Metab. Dispos* 11, 211–217.

Campbell, M. E., Spielberg, S. P., and Kalow, W. (1987). A Urinary Metabolite Ratio that Reflects Systemic Caffeine Clearance. *Clin. Pharmacol. Ther.* 42, 157–165. doi:10.1038/clpt.1987.126

Carbó, M., Segura, J., De la Torre, R., Badenas, J. M., and Camí, J. (1989). Effect of Quinolones on Caffeine Disposition. *Clin. Pharmacol. Ther.* 45, 234–240. doi:10.1038/clpt.1989.23

Carrillo, J. A., Christensen, M., Ramos, S. I., Alm, C., Dahl, M. L., Benitez, J., et al. (2000). Evaluation of Caffeine as an *In Vivo* Probe for Cyp1a2 Using Measurements in Plasma, Saliva, and Urine. *Ther. Drug Monit.* 22, 409–417. doi:10.1097/00007691-200008000-00008

Carvalho, J. J., Weller, M. G., Panne, U., and Schneider, R. J. (2012). Monitoring Caffeine in Human Saliva Using a Newly Developed ELISA. *Anal. Lett.* 45, 2549–2561. doi:10.1080/00032719.2012.696226

Chen, Y., Liu, W. H., Chen, B. L., Fan, L., Han, Y., Wang, G., et al. (2010). Plant Polyphenol Curcumin Significantly Affects Cyp1a2 and Cyp2a6 Activity in Healthy, Male Chinese Volunteers. *Ann. Pharmacother.* 44, 1038–1045. doi:10.1345/aph.1M533

Chen, Y., Tu, J. H., He, Y. J., Zhang, W., Wang, G., Tan, Z. R., et al. (2009a). Effect of Sodium Tanshinone Ii a Sulfonate on the Activity of Cyp1a2 in Healthy Volunteers. *Xenobiotica* 39, 508–513. doi:10.1080/00498250902951763

Chen, Y., Xiao, P., Ou-Yang, D. S., Fan, L., Guo, D., Wang, Y. N., et al. (2009b). Simultaneous Action of the Flavonoid Quercetin on Cytochrome P450 (Cyp) 1a2, Cyp2a6, N-Acetyltransferase and Xanthine Oxidase Activity in Healthy Volunteers. *Clin. Exp. Pharmacol. Physiol.* 36, 828–833. doi:10.1111/j.1440-1681.2009.05158.x

Cheng, W. S., Murphy, T. L., Smith, M. T., Cooksley, W. G., Halliday, J. W., and Powell, L. W. (1990). Dose-dependent Pharmacokinetics of Caffeine in Humans: Relevance as a Test of Quantitative Liver Function. *Clin. Pharmacol. Ther.* 47, 516–524. doi:10.1038/clpt.1990.66

Chia, H. Y., Yau, W. P., and Ho, H. K. (2016). Establishing Population Distribution of Drug-Metabolizing Enzyme Activities for the Use of Salivary Caffeine as a Dynamic Liver Function Marker in a Singaporean Chinese Population. *Biopharm. Drug Dispos* 37, 168–181. doi:10.1002/bdd.2006

Christensen, M., Tybring, G., Mihara, K., Yasui-Furokori, N., Carrillo, J. A., Ramos, S. I., et al. (2002). Low Daily 10-mg and 20-mg Doses of Fluvoxamine Inhibit the Metabolism of Both Caffeine (Cytochrome P4501a2) and Omeprazole (Cytochrome P4502c19). *Clin. Pharmacol. Ther.* 71, 141–152. doi:10.1067/mcp.2002.121788

Cook, C. E., Tallent, C. R., Amerson, E. W., Myers, M. W., Kepler, J. A., Taylor, G. F., et al. (1976). Caffeine in Plasma and Saliva by a Radioimmunoassay Procedure. *J. Pharmacol. Exp. Ther.* 199, 679–686.

Cornish, H. H., and Christman, A. A. (1957). A Study of the Metabolism of Theobromine, Theophylline, and Caffeine in Man. *J. Biol. Chem.* 228, 315–323. doi:10.1016/s0021-9258(18)70714-x

Culm-Merdek, K. E., von Moltke, L. L., Harmatz, J. S., and Greenblatt, D. J. (2005). Fluvoxamine Impairs Single-Dose Caffeine Clearance without Altering Caffeine Pharmacodynamics. *Br. J. Clin. Pharmacol.* 60, 486–493. doi:10.1111/j.1365-2125.2005.02467.x

Cysneiros, R. M., Farkas, D., Harmatz, J. S., von Moltke, L. L., and Greenblatt, D. J. (2007). Pharmacokinetic and Pharmacodynamic Interactions between Zolpidem and Caffeine. *Clin. Pharmacol. Ther.* 82, 54–62. doi:10.1038/sj.clpt.6100211

Darwish, M., Kirby, M., Robertson, P., and Hellriegel, E. T. (2008). Interaction Profile of Armodafinil with Medications Metabolized by Cytochrome P450 Enzymes 1a2, 3a4 and 2c19 in Healthy Subjects. *Clin. Pharmacokinet.* 47, 61–74. doi:10.2165/00003088-200847010-00006

Desmond, P. V., Patwardhan, R. V., Johnson, R. F., and Schenker, S. (1980). Impaired Elimination of Caffeine in Cirrhosis. *Dig. Dis. Sci.* 25, 193–197. doi:10.1007/BF01308138

Dinger, J., Woods, C., Brandt, S. D., Meyer, M. R., and Maurer, H. H. (2016). Cytochrome P450 Inhibition Potential of New Psychoactive Substances of the Tryptamine Class. *Toxicol. Lett.* 241, 82–94. doi:10.1016/j.toxlet.2015.11.013

Djordjevic, N., Ghotbi, R., Bertilsson, L., Jankovic, S., and Aklillu, E. (2008). Induction of Cyp1a2 by Heavy Coffee Consumption in Serbs and Swedes. *Eur. J. Clin. Pharmacol.* 64, 381–385. doi:10.1007/s00228-007-0438-6

Doroshyenko, O., Rokitta, D., Zadoyan, G., Klement, S., Schläfke, S., Dienel, A., et al. (2013). Drug Cocktail Interaction Study on the Effect of the Orally Administered Lavender Oil Preparation Silexan on Cytochrome P450 Enzymes in Healthy Volunteers. *Drug Metab. Dispos* 41, 987–993. doi:10.1124/dmd.112.050203

Drozdzik, M., Busch, D., Lapczuk, J., Müller, J., Ostrowski, M., Kurzawski, M., et al. (2018). Protein Abundance of Clinically Relevant Drug-Metabolizing Enzymes in the Human Liver and Intestine: A Comparative Analysis in Paired Tissue Specimens. *Clin. Pharmacol. Ther.* 104, 515–524. doi:10.1002/cpt.967

Dumond, J. B., Vourvahis, M., Rezk, N. L., Patterson, K. B., Tien, H. C., White, N., et al. (2010). A Phenotype-Genotype Approach to Predicting Cyp450 and P-Glycoprotein Drug Interactions with the Mixed Inhibitor/inducer Tipranavir/ritonavir. *Clin. Pharmacol. Ther.* 87, 735–742. doi:10.1038/clpt.2009.253

Edwards, J. E., Eliot, L., Parkinson, A., Karan, S., and MacConell, L. (2017). Assessment of Pharmacokinetic Interactions between Obeticholic Acid and Caffeine, Midazolam, Warfarin, Dextromethorphan, Omeprazole, Rosuvastatin, and Digoxin in Phase 1 Studies in Healthy Subjects. *Adv. Ther.* 34, 2120–2138. doi:10.1007/s12325-017-0601-0

Faber, M. S., and Fuhr, U. (2004). Time Response of Cytochrome P450 1a2 Activity on Cessation of Heavy Smoking. *Clin. Pharmacol. Ther.* 76, 178–184. doi:10.1016/j.clpt.2004.04.003

Faber, M. S., Jetter, A., and Fuhr, U. (2005). Assessment of Cyp1a2 Activity in Clinical Practice: Why, How, and when? *Basic Clin. Pharmacol. Toxicol.* 97, 125–134. doi:10.1111/j.1742-7843.2005.pto_973160.x

Fligner, C. L., and Opheim, K. E. (1988). Caffeine and its Dimethylxanthine Metabolites in Two Cases of Caffeine Overdose: a Cause of Falsely Elevated Theophylline Concentrations in Serum. *J. Anal. Toxicol.* 12, 339–343. doi:10.1093/jat/12.6.339

Frary, C. D., Johnson, R. K., and Wang, M. Q. (2005). Food Sources and Intakes of Caffeine in the Diets of Persons in the united states. *J. Am. Diet. Assoc.* 105, 110–113. doi:10.1016/j.jada.2004.10.027

Fuhr, U., Klittich, K., and Staib, A. H. (1993). Inhibitory Effect of Grapefruit Juice and its Bitter Principal, Naringenin, on Cyp1a2 Dependent Metabolism of Caffeine in Man. *Br. J. Clin. Pharmacol.* 35, 431–436. doi:10.1111/j.1365-2125.1993.tb04162.x

Fuhr, U., Rost, K. L., Engelhardt, R., Sachs, M., Liermann, D., Belloc, C., et al. (1996). Evaluation of caffeine as a test drug for cyp1a2, nat2 and cyp2e1 phenotyping in man by *In Vivo* versus *In Vitro* correlations. *Pharmacogenetics* 6, 159–176. doi:10.1097/00008571-199604000-00003

Furge, L. L., and Fletke, K. J. (2007). Hplc Determination of Caffeine and Paraxanthine in Urine: An Assay for Cytochrome P450 1a2 Activity. *Biochem. Mol. Biol. Educ.* 35, 138–144. doi:10.1002/bmb.28

George, J., Murphy, T., Roberts, R., Cooksley, W. G., Halliday, J. W., and Powell, L. W. (1986). Influence of Alcohol and Caffeine Consumption on Caffeine Elimination. *Clin. Exp. Pharmacol. Physiol.* 13, 731–736. doi:10.1111/j.1440-1681.1986.tb02414.x

Ghassabian, S., Chetty, M., Tattam, B. N., Chem, M. C., Glen, J., Rahme, J., et al. (2009). A High-Throughput Assay Using Liquid Chromatography-Tandem Mass Spectrometry for Simultaneous *In Vivo* Phenotyping of 5 Major Cytochrome P450 Enzymes in Patients. *Ther. Drug Monit.* 31, 239–246. doi:10.1097/FTD.0b013e318197e1bf

Ghotbi, R., Christensen, M., Roh, H. K., Ingelman-Sundberg, M., Aklillu, E., and Bertilsson, L. (2007). Comparisons of Cyp1a2 Genetic Polymorphisms, Enzyme Activity and the Genotype-Phenotype Relationship in Swedes and Koreans. *Eur. J. Clin. Pharmacol.* 63, 537–546. doi:10.1007/s00228-007-0288-2

Gilbert, R. (1984). The Methylxanthine Beverages and Foods: Chemistry, Consumption, and Health Effects. *Prog. Clin. Biol. Res.* 158, 1–413.

Gonzalez Hernandez, F., Carter, S. J., Iso-Sipilä, J., Goldsmith, P., Almousa, A. A., Gastine, S., et al. (2021). An Automated Approach to Identify Scientific Publications Reporting Pharmacokinetic Parameters. *Wellcome Open Res.* 6, 88. doi:10.12688/wellcomeopenres.16718.1

Granfors, M. T., Backman, J. T., Laitila, J., and Neuvonen, P. J. (2005). Oral Contraceptives Containing Ethinyl Estradiol and Gestodene Markedly Increase Plasma Concentrations and Effects of Tizanidine by Inhibiting Cytochrome P450 1a2. *Clin. Pharmacol. Ther.* 78, 400–411. doi:10.1016/j.clpt.2005.06.009

Grzegorzewski, J., Brandhorst, J., Green, K., Eleftheriadou, D., Duport, Y., Barthorscht, F., et al. (2020). Pk-db: Pharmacokinetics Database for Individualized and Stratified Computational Modeling. *Nucleic Acids Res.* 49, D1358–D1364. doi:10.1093/nar/gkaa990

Gunes, A., and Dahl, M. L. (2008). Variation in Cyp1a2 Activity and its Clinical Implications: Influence of Environmental Factors and Genetic Polymorphisms. *Pharmacogenomics* 9, 625–637. doi:10.2217/14622416.9.5.625

Gunes, A., Ozbey, G., Vural, E. H., Uluoglu, C., Scordo, M. G., Zengil, H., et al. (2009). Influence of Genetic Polymorphisms, Smoking, Gender and Age on Cyp1a2 Activity in a Turkish Population. *Pharmacogenomics* 10, 769–778. doi:10.2217/pgs.09.22

Gurley, B. J., Gardner, S. F., Hubbard, M. A., Williams, D. K., Gentry, W. B., Cui, Y., et al. (2005a). Clinical Assessment of Effects of Botanical Supplementation on Cytochrome P450 Phenotypes in the Elderly: St John's Wort, Garlic Oil, Panax Ginseng and Ginkgo Biloba. *Drugs Aging* 22, 525–539. doi:10.2165/00002512-200522060-00006

Gurley, B. J., Gardner, S. F., Hubbard, M. A., Williams, D. K., Gentry, W. B., Khan, I. A., et al. (2005b). *In Vivo* effects of goldenseal, kava kava, black cohosh, and valerian on human cytochrome p450 1a2, 2d6, 2e1, and 3a4/5 phenotypes. *Clin. Pharmacol. Ther.* 77, 415–426. doi:10.1016/j.clpt.2005.01.009

Hakooz, N. M. (2009). Caffeine Metabolic Ratios for the *In Vivo* Evaluation of Cyp1a2, N-Acetyltransferase 2, Xanthine Oxidase and Cyp2a6 Enzymatic Activities. *Curr. Drug Metab.* 10, 329–338. doi:10.2174/138920009788499003

Haller, C. A., Jacob, P., and Benowitz, N. L. (2002). Pharmacology of Ephedra Alkaloids and Caffeine after Single-Dose Dietary Supplement Use. *Clin. Pharmacol. Ther.* 71, 421–432. doi:10.1067/mcp.2002.124523

Hamon-Vilcot, B., Simon, T., Becquemont, L., Poirier, J. M., Piette, F., and Jaillon, P. (2004). Effects of Malnutrition on Cytochrome P450 1a2 Activity in Elderly Patients. *Therapie* 59, 247–251. doi:10.2515/therapie:2004048

Harder, S., Fuhr, U., Staib, A. H., and Wolff, T. (1989). Ciprofloxacin-caffeine: a Drug Interaction Established Using *In Vivo* and *In Vitro* Investigations. *Am. J. Med.* 87, 89S–91S. doi:10.1016/0002-9343(89)90031-4

Harder, S., Staib, A. H., Beer, C., Papenburg, A., Stille, W., and Shah, P. M. (1988). 4-quinolones Inhibit Biotransformation of Caffeine. *Eur. J. Clin. Pharmacol.* 35, 651–656. doi:10.1007/BF00637602

Hashiguchi, M., Fujimura, A., Ohashi, K., and Ebihara, A. (1992). Diurnal Effect on Caffeine Clearance. *J. Clin. Pharmacol.* 32, 184–187. doi:10.1002/j.1552-4604.1992.tb03824.x

He, H., Ma, D., Crone, L. B., Butawan, M., Meibohm, B., Bloomer, R. J., et al. (2017). Assessment of the Drug-Drug Interaction Potential between Theacrine and Caffeine in Humans. *J. Caffeine Res.* 7, 95–102. doi:10.1089/jcr.2017.0006

Healy, D. P., Polk, R. E., Kanawati, L., Rock, D. T., and Mooney, M. L. (1989). Interaction between Oral Ciprofloxacin and Caffeine in normal Volunteers. *Antimicrob. Agents Chemother.* 33, 474–478. doi:10.1128/aac.33.4.474

Healy, D. P., Schoenle, J. R., Stotka, J., and Polk, R. E. (1991). Lack of Interaction between Lomefloxacin and Caffeine in normal Volunteers. *Antimicrob. Agents Chemother.* 35, 660–664. doi:10.1128/aac.35.4.660

Hetzler, R. K., Knowlton, R. G., Somani, S. M., Brown, D. D., and Perkins, R. M. (19901985). Effect of Paraxanthine on Ffa Mobilization after Intravenous Caffeine Administration in Humans. *J. Appl. Physiol. (1985)* 68, 44–47. doi:10.1152/jappl.1990.68.1.44

Holstege, A., Kurz, M., Weinbeck, M., and Gerok, W. (1993). Excretion of Caffeine and its Primary Degradation Products into Bile. *J. Hepatol.* 17, 67–73. doi:10.1016/s0168-8278(05)80523-9

Holstege, A., Staiger, M., Haag, K., and Gerok, W. (1989). Correlation of Caffeine Elimination and Child's Classification in Liver Cirrhosis. *Klin Wochenschr* 67, 6–15. doi:10.1007/BF01736528

Ioannidis, J. P. A. (2019). Reproducible Pharmacokinetics. *J. Pharmacokinet. Pharmacodyn* 46, 111–116. doi:10.1007/s10928-019-09621-y

Jeppesen, U., Loft, S., Poulsen, H. E., and Brśen, K. (1996). A Fluvoxamine-Caffeine Interaction Study. *Pharmacogenetics* 6, 213–222. doi:10.1097/00008571-199606000-00003

Jiang, Z., Dragin, N., Jorge-Nebert, L. F., Martin, M. V., Guengerich, F. P., Aklillu, E., et al. (2006). Search for an Association between the Human Cyp1a2 Genotype and Cyp1a2 Metabolic Phenotype. *Pharmacogenet Genomics* 16, 359–367. doi:10.1097/01.fpc.0000204994.99429.46

Jodynis-Liebert, J., Flieger, J., Matuszewska, A., and Juszczyk, J. (2004). Serum Metabolite/caffeine Ratios as a Test for Liver Function. *J. Clin. Pharmacol.* 44, 338–347. doi:10.1177/0091270004263468

Joeres, R., Klinker, H., Heusler, H., Epping, J., and Richter, E. (1987). Influence of Mexiletine on Caffeine Elimination. *Pharmacol. Ther.* 33, 163–169. doi:10.1016/0163-7258(87)90046-5

Joeres, R., Klinker, H., Heusler, H., Epping, J., Zilly, W., and Richter, E. (1988). Influence of Smoking on Caffeine Elimination in Healthy Volunteers and in Patients with Alcoholic Liver Cirrhosis. *Hepatology* 8, 575–579. doi:10.1002/hep.1840080323

Jones, H., and Rowland-Yeo, K. (2013). Basic Concepts in Physiologically Based Pharmacokinetic Modeling in Drug Discovery and Development. *CPT Pharmacometrics Syst. Pharmacol.* 2, e63. doi:10.1038/psp.2013.41

Jost, G., Wahlländer, A., von Mandach, U., and Preisig, R. (1987). Overnight Salivary Caffeine Clearance: a Liver Function Test Suitable for Routine Use. *Hepatology* 7, 338–344. doi:10.1002/hep.1840070221

J. P. T. Higgins and C. Collaboration (Editors) (2020). *Cochrane Handbook for Systematic Reviews of Interventions*. second edition edn (Hoboken, NJ: Wiley-Blackwell).

Kakuda, T. N., Van Solingen-Ristea, R. M., Onkelinx, J., Stevens, T., Aharchi, F., De Smedt, G., et al. (2014). The Effect of Single- and Multiple-Dose Etravirine on a Drug Cocktail of Representative Cytochrome P450 Probes and Digoxin in Healthy Subjects. *J. Clin. Pharmacol.* 54, 422–431. doi:10.1002/jcph.214

Kalow, W., and Tang, B. K. (1993). The Use of Caffeine for Enzyme Assays: a Critical Appraisal. *Clin. Pharmacol. Ther.* 53, 503–514. doi:10.1038/clpt.1993.63

Kamimori, G. H., Joubert, A., Otterstetter, R., Santaromana, M., and Eddington, N. D. (1999). The Effect of the Menstrual Cycle on the Pharmacokinetics of Caffeine in normal, Healthy Eumenorrheic Females. *Eur. J. Clin. Pharmacol.* 55, 445–449. doi:10.1007/s002280050654

Kamimori, G. H., Karyekar, C. S., Otterstetter, R., Cox, D. S., Balkin, T. J., Belenky, G. L., et al. (2002). The Rate of Absorption and Relative Bioavailability of Caffeine Administered in Chewing Gum versus Capsules to normal Healthy Volunteers. *Int. J. Pharm.* 234, 159–167. doi:10.1016/s0378-5173(01)00958-9

Kamimori, G. H., Somani, S. M., Knowlton, R. G., and Perkins, R. M. (1987). The Effects of Obesity and Exercise on the Pharmacokinetics of Caffeine in Lean and Obese Volunteers. *Eur. J. Clin. Pharmacol.* 31, 595–600. doi:10.1007/BF00606637

Kaplan, G. B., Greenblatt, D. J., Ehrenberg, B. L., Goddard, J. E., Cotreau, M. M., Harmatz, J. S., et al. (1997). Dose-dependent Pharmacokinetics and Psychomotor Effects of Caffeine in Humans. *J. Clin. Pharmacol.* 37, 693–703. doi:10.1002/j.1552-4604.1997.tb04356.x

Kinzig-Schippers, M., Fuhr, U., Zaigler, M., Dammeyer, J., Rüsing, G., Labedzki, A., et al. (1999). Interaction of Pefloxacin and Enoxacin with the Human Cytochrome P450 Enzyme Cyp1a2. *Clin. Pharmacol. Ther.* 65, 262–274. doi:10.1016/S0009-9236(99)70105-0

Klein, K., Winter, S., Turpeinen, M., Schwab, M., and Zanger, U. M. (2010). Pathway-targeted Pharmacogenomics of Cyp1a2 in Human Liver. *Front. Pharmacol.* 1, 129. doi:10.3389/fphar.2010.00129

Koch, J. P., ten Tusscher, G. W., Koppe, J. G., and Guchelaar, H. J. (1999). Validation of a High-Performance Liquid Chromatography Assay for Quantification of Caffeine and Paraxanthine in Human Serum in the Context of Cyp1a2 Phenotyping. *Biomed. Chromatogr.* 13, 309–314. doi:10.1002/(SICI)1099-0801(199906)13:4<309:AID-BMC881>3.0.CO;2-J

Köller, A., Grzegorzewski, J., Tautenhahn, H.-M., and König, M. (2021). Prediction of Survival after Partial Hepatectomy Using a Physiologically Based Pharmacokinetic Model of Indocyanine green Liver Function Tests. *bioRxiv*. doi:10.1101/2021.06.15.448411

Koonrungsesomboon, N., Khatsri, R., Wongchompoo, P., and Teekachunhatean, S. (2018). The Impact of Genetic Polymorphisms on CYP1A2 Activity in Humans: A Systematic Review and Meta-Analysis. *Pharmacogenomics J.* 18, 760–768. doi:10.1038/s41397-017-0011-3

Kot, M., and Daniel, W. A. (2008). Caffeine as a Marker Substrate for Testing Cytochrome P450 Activity in Human and Rat. *Pharmacol. Rep.* 60, 789–797.

Kukongviriyapan, V., Senggunprai, L., Prawan, A., Gaysornsiri, D., Kukongviriyapan, U., and Aiemsa-Ard, J. (2004). Salivary Caffeine Metabolic Ratio in Alcohol-dependent Subjects. *Eur. J. Clin. Pharmacol.* 60, 103–107. doi:10.1007/s00228-004-0734-3

Laizure, S. C., Meibohm, B., Nelson, K., Chen, F., Hu, Z. Y., and Parker, R. B. (2017). Comparison of Caffeine Disposition Following Administration by Oral Solution (Energy Drink) and Inspired Powder (Aeroshot) in Human Subjects. *Br. J. Clin. Pharmacol.* 83, 2687–2694. doi:10.1111/bcp.13389

Lammers, L. A., Achterbergh, R., Romijn, J. A., and Mathôt, R. A. A. (2018). Short-term Fasting Alters Pharmacokinetics of Cytochrome P450 Probe Drugs: Does Protein Binding Play a Role? *Eur. J. Drug Metab. Pharmacokinet.* 43, 251–257. doi:10.1007/s13318-017-0437-7

Lane, J. D., Steege, J. F., Rupp, S. L., and Kuhn, C. M. (1992). Menstrual Cycle Effects on Caffeine Elimination in the Human Female. *Eur. J. Clin. Pharmacol.* 43, 543–546. doi:10.1007/BF02285099

Lane, S. D., Green, C. E., Schmitz, J. M., Rathnayaka, N., Fang, W. B., Ferré, S., et al. (2014). Comparison of Caffeine and D-Amphetamine in Cocaine-dependent Subjects: Differential Outcomes on Subjective and Cardiovascular Effects, Reward Learning, and Salivary Paraxanthine. *J. Addict. Res. Ther.* 5, 176. doi:10.4172/2155-6105.1000176

Lelo, A., Birkett, D. J., Robson, R. A., and Miners, J. O. (1986a). Comparative Pharmacokinetics of Caffeine and its Primary Demethylated Metabolites Paraxanthine, Theobromine and Theophylline in Man. *Br. J. Clin. Pharmacol.* 22, 177–182. doi:10.1111/j.1365-2125.1986.tb05246.x

Lelo, A., Miners, J. O., Robson, R. A., and Birkett, D. J. (1986b). Quantitative Assessment of Caffeine Partial Clearances in Man. *Br. J. Clin. Pharmacol.* 22, 183–186. doi:10.1111/j.1365-2125.1986.tb05247.x

Levy, M., and Zylber-Katz, E. (1983). Caffeine Metabolism and Coffee-Attributed Sleep Disturbances. *Clin. Pharmacol. Ther.* 33, 770–775. doi:10.1038/clpt.1983.105

Liberati, A., Altman, D. G., Tetzlaff, J., Mulrow, C., Gøtzsche, P. C., Ioannidis, J. P., et al. (2009). The PRISMA Statement for Reporting Systematic Reviews and Meta-Analyses of Studies that Evaluate Healthcare Interventions: Explanation and Elaboration. *BMJ* 339, b2700. doi:10.1136/bmj.b2700

Magnusson, M. O., Dahl, M. L., Cederberg, J., Karlsson, M. O., and Sandström, R. (2008). Pharmacodynamics of Carbamazepine-Mediated Induction of Cyp3a4, Cyp1a2, and Pgp as Assessed by Probe Substrates Midazolam, Caffeine, and Digoxin. *Clin. Pharmacol. Ther.* 84, 52–62. doi:10.1038/sj.clpt.6100431

Marks, V., and Kelly, J. F. (1973). Absorption of Caffeine from tea, Coffee, and Coca Cola. *Lancet* 1, 827. doi:10.1016/s0140-6736(73)90625-9

Matthaei, J., Tzvetkov, M. V., Strube, J., Sehrt, D., Sachse-Seeboth, C., Hjelmborg, J. B., et al. (2016). Heritability of Caffeine Metabolism: Environmental Effects Masking Genetic Effects on Cyp1a2 Activity but Not on Nat2. *Clin. Pharmacol. Ther.* 100, 606–616. doi:10.1002/cpt.444

May, D. C., Jarboe, C. H., VanBakel, A. B., and Williams, W. M. (1982). Effects of Cimetidine on Caffeine Disposition in Smokers and Nonsmokers. *Clin. Pharmacol. Ther.* 31, 656–661. doi:10.1038/clpt.1982.91

McDonagh, J. E., Nathan, V. V., Bonavia, I. C., Moyle, G. R., and Tanner, A. R. (1991). Caffeine Clearance by Enzyme Multiplied Immunoassay Technique: a Simple, Inexpensive, and Useful Indicator of Liver Function. *Gut* 32, 681–684. doi:10.1136/gut.32.6.681

McLean, C., and Graham, T. E. (2002). Effects of Exercise and thermal Stress on Caffeine Pharmacokinetics in Men and Eumenorrheic Women. *J. Appl. Physiol.*93, 1471–1478. doi:10.1152/japplphysiol.00762.2000

McQuilkin, S. H., Nierenberg, D. W., and Bresnick, E. (1995). Analysis of Within-Subject Variation of Caffeine Metabolism when Used to Determine Cytochrome P4501a2 and N-Acetyltransferase-2 Activities. *Cancer Epidemiol. Biomarkers Prev.* 4, 139–146.

Millard, J. T., Passang, T., Ye, J., Kline, G. M., Beachy, T. M., Hepburn, V. L., et al. (2018). Genotype and Phenotype of Caffeine Metabolism: A Biochemistry Laboratory experiment. *J. Chem. Educ.* 95, 1856–1860. doi:10.1021/acs.jchemed.8b00318

Miners, J. O., and Birkett, D. J. (1996). The Use of Caffeine as a Metabolic Probe for Human Drug Metabolizing Enzymes. *Gen. Pharmacol.* 27, 245–249. doi:10.1016/0306-3623(95)02014-4

Mitchell, D. C., Knight, C. A., Hockenberry, J., Teplansky, R., and Hartman, T. J. (2014). Beverage Caffeine Intakes in the u.S. *Food Chem. Toxicol.* 63, 136–142. doi:10.1016/j.fct.2013.10.042

Moonen, H. J., Moonen, E. J., Maas, L., Dallinga, J. W., Kleinjans, J. C., and de Kok, T. M. (2004). CYP1A2 and NAT2 Genotype/phenotype Relations and Urinary Excretion of 2-Amino-1-Methyl-6-Phenylimidazo[4,5-B]pyridine (PhIP) in a Human Dietary Intervention Study. *Food Chem. Toxicol.* 42, 869–878. doi:10.1016/j.fct.2004.01.010

Murphy, T. L., McIvor, C., Yap, A., Cooksley, W. G., Halliday, J. W., and Powell, L. W. (1988). The Effect of Smoking on Caffeine Elimination: Implications for its Use as a Semiquantitative Test of Liver Function. *Clin. Exp. Pharmacol. Physiol.* 15, 9–13. doi:10.1111/j.1440-1681.1988.tb01003.x

Muscat, J. E., Pittman, B., Kleinman, W., Lazarus, P., Stellman, S. D., and Richie, J. P. (2008). Comparison of Cyp1a2 and Nat2 Phenotypes between Black and white Smokers. *Biochem. Pharmacol.* 76, 929–937. doi:10.1016/j.bcp.2008.07.024

Myrand, S. P., Sekiguchi, K., Man, M. Z., Lin, X., Tzeng, R. Y., Teng, C. H., et al. (2008). Pharmacokinetics/genotype Associations for Major Cytochrome P450 Enzymes in Native and First- and Third-Generation Japanese Populations: Comparison with Korean, Chinese, and Caucasian Populations. *Clin. Pharmacol. Ther.* 84, 347–361. doi:10.1038/sj.clpt.6100482

Nakazawa, K., and Tanaka, H. (1988). pharmacokinetics of Caffeine and Dimethylxanthines in Plasma and Saliva. *Yakugaku Zasshi* 108, 653–658. doi:10.1248/yakushi1947.108.7_653

Nehlig, A. (2018). Interindividual Differences in Caffeine Metabolism and Factors Driving Caffeine Consumption. *Pharmacol. Rev.* 70, 384–411. doi:10.1124/pr.117.014407

Newton, R., Broughton, L. J., Lind, M. J., Morrison, P. J., Rogers, H. J., and Bradbrook, I. D. (1981). Plasma and Salivary Pharmacokinetics of Caffeine in Man. *Eur. J. Clin. Pharmacol.* 21, 45–52. doi:10.1007/BF00609587

Oh, K. S., Park, S. J., Shinde, D. D., Shin, J. G., and Kim, D. H. (2012). High-sensitivity Liquid Chromatography-Tandem Mass Spectrometry for the Simultaneous Determination of Five Drugs and Their Cytochrome P450-specific Probe Metabolites in Human Plasma. *J. Chromatogr. B Analyt Technol. Biomed. Life Sci.* 895-896, 56–64. doi:10.1016/j.jchromb.2012.03.014

Park, G. J., Katelaris, P. H., Jones, D. B., Seow, F., Le Couteur, D. G., and Ngu, M. C. (2003). Validity of the 13c-Caffeine Breath Test as a Noninvasive, Quantitative Test of Liver Function. *Hepatology* 38, 1227–1236. doi:10.1053/jhep.2003.50475

Parsons, W. D., and Neims, A. H. (1978). Effect of Smoking on Caffeine Clearance. *Clin. Pharmacol. Ther.* 24, 40–45. doi:10.1002/cpt197824140

Patwardhan, R. V., Desmond, P. V., Johnson, R. F., and Schenker, S. (1980). Impaired Elimination of Caffeine by Oral Contraceptive Steroids. *J. Lab. Clin. Med.* 95, 603–608.

Perera, V., Gross, A. S., and McLachlan, A. J. (2010). Caffeine and Paraxanthine Hplc Assay for Cyp1a2 Phenotype Assessment Using Saliva and Plasma. *Biomed. Chromatogr.* 24, 1136–1144. doi:10.1002/bmc.1419

Perera, V., Gross, A. S., Xu, H., and McLachlan, A. J. (2011). Pharmacokinetics of Caffeine in Plasma and Saliva, and the Influence of Caffeine Abstinence on Cyp1a2 Metrics. *J. Pharm. Pharmacol.* 63, 1161–1168. doi:10.1111/j.2042-7158.2011.01326.x

Priller, S. (2005). Cytochrome P450 1a2 Phenotyping for Student Laboratories. *Pharm. Education J.* doi:10.1080/15602210500193219

Puri, B. K., Heard, C. R., and Monro, J. A. (2020). Is There a Sex Difference in Adult Salivary Clearance of Caffeine (1,3,7-Trimethylpurine-2,6-Dione)? *J. Oral Biol. Craniofac. Res.* 10, 20–22. doi:10.1016/j.jobcr.2020.01.010

Rasmussen, B. B., Brix, T. H., Kyvik, K. O., and Brøsen, K. (2002). The Interindividual Differences in the 3-demthylation of Caffeine Alias Cyp1a2 Is Determined by Both Genetic and Environmental Factors. *Pharmacogenetics* 12, 473–478. doi:10.1097/00008571-200208000-00008

Renner, E., Wietholtz, H., Huguenin, P., Arnaud, M. J., and Preisig, R. (1984). Caffeine: a Model Compound for Measuring Liver Function. *Hepatology* 4, 38–46. doi:10.1002/hep.1840040107

Rietveld, E. C., Broekman, M. M., Houben, J. J., Eskes, T. K., and van Rossum, J. M. (1984). Rapid Onset of an Increase in Caffeine Residence Time in Young Women Due to Oral Contraceptive Steroids. *Eur. J. Clin. Pharmacol.* 26, 371–373. doi:10.1007/BF00548769

Riley, R. D., Lambert, P. C., and Abo-Zaid, G. (2010). Meta-analysis of Individual Participant Data: Rationale, Conduct, and Reporting. *BMJ* 340, c221. doi:10.1136/bmj.c221

Rubin, T. M., Heyne, K., Luchterhand, A., Jan Bednarsch, J., W R Vondran, F., Polychronidis, G., et al. (2017). Kinetic Validation of the LiMAx Test during 10 000 Intravenous 13C-Methacetin Breath Tests. *J. Breath Res.* 12, 016005. doi:10.1088/1752-7163/aa820b

Sadek, P., Pan, X., Shepherd, P., Malandain, E., Carney, J., and Coleman, H. (2017). A Randomized, Two-Way Crossover Study to Evaluate the Pharmacokinetics of Caffeine Delivered Using Caffeinated Chewing Gum versus a Marketed Caffeinated Beverage in Healthy Adult Volunteers. *J. Caffeine Res.* 7, 125–132. doi:10.1089/jcr.2017.0025

Schmider, J., Brockmöller, J., Arold, G., Bauer, S., and Roots, I. (1999). Simultaneous Assessment of Cyp3a4 and Cyp1a2 Activity *In Vivo* with Alprazolam and Caffeine. *Pharmacogenetics* 9, 725–734. doi:10.1097/01213011-199912000-00007

Schrenk, D., Brockmeier, D., Mörike, K., Bock, K. W., and Eichelbaum, M. (1998). A Distribution Study of Cyp1a2 Phenotypes Among Smokers and Non-smokers in a Cohort of Healthy Caucasian Volunteers. *Eur. J. Clin. Pharmacol.* 53, 361–367. doi:10.1007/s002280050394

Scott, N. R., Chakraborty, J., and Marks, V. (1984). Determination of Caffeine, Theophylline and Theobromine in Serum and Saliva Using High-Performance Liquid Chromatography. *Ann. Clin. Biochem.* 21 (Pt 2), 120–124. doi:10.1177/000456328402100208

Scott, N. R., Stambuk, D., Chakraborty, J., Marks, V., and Morgan, M. Y. (1989). The Pharmacokinetics of Caffeine and its Dimethylxanthine Metabolites in Patients with Chronic Liver Disease. *Br. J. Clin. Pharmacol.* 27, 205–213. doi:10.1111/j.1365-2125.1989.tb05352.x

Scott, N. R., Stambuk, D., Chakraborty, J., Marks, V., and Morgan, M. Y. (1988). Caffeine Clearance and Biotransformation in Patients with Chronic Liver Disease. *Clin. Sci. (Lond)* 74, 377–384. doi:10.1042/cs0740377

Seng, K. Y., Fun, C. Y., Law, Y. L., Lim, W. M., Fan, W., and Lim, C. L. (2009). Population Pharmacokinetics of Caffeine in Healthy Male Adults Using Mixed-Effects Models. *J. Clin. Pharm. Ther.* 34, 103–114. doi:10.1111/j.1365-2710.2008.00976.x

Soto, J., Alsar, M. J., and Sacristan, J. A. (1995). Assessment of the Time Course of Drugs with Inhibitory Effects on Hepatic Metabolic Activity Using Successive Salivary Caffeine Tests. *Pharmacotherapy* 15, 781–784.

Spigset, O., Hägg, S., Söderström, E., and Dahlqvist, R. (1999). The Paraxanthine: caffeine Ratio in Serum or in Saliva as a Measure of Cyp1a2 Activity: when Should the Sample Be Obtained? *Pharmacogenetics* 9, 409–412. doi:10.1097/00008571-199906000-00019

Stader, F., Siccardi, M., Battegay, M., Kinvig, H., Penny, M. A., and Marzolini, C. (2019). Repository Describing an Aging Population to Inform Physiologically Based Pharmacokinetic Models Considering Anatomical, Physiological, and Biological Age-dependent Changes. *Clin. Pharmacokinet.* 58, 483–501. doi:10.1007/s40262-018-0709-7

Stille, W., Harder, S., Mieke, S., Beer, C., Shah, P. M., Frech, K., et al. (1987). Decrease of Caffeine Elimination in Man during Co-administration of 4-quinolones. *J. Antimicrob. Chemother.* 20, 729–734. doi:10.1093/jac/20.5.729

Sudsakorn, S., Bahadduri, P., Fretland, J., and Lu, C. (2020). 2020 Fda Drug-Drug Interaction Guidance: A Comparison Analysis and Action Plan by Pharmaceutical Industrial Scientists. *Curr. Drug Metab.* 21, 403–426. doi:10.2174/1389200221666200620210522

Syed, S. A., Kamimori, G. H., Kelly, W., and Eddington, N. D. (2005). Multiple Dose Pharmacokinetics of Caffeine Administered in Chewing Gum to normal Healthy Volunteers. *Biopharm. Drug Dispos* 26, 403–409. doi:10.1002/bdd.469

Tanaka, E., Ishikawa, A., Yamamoto, Y., Osada, A., Tsuji, K., Fukao, K., et al. (1993). Comparison of Hepatic Drug-Oxidizing Activity after Simultaneous Administration of Two Probe Drugs, Caffeine and Trimethadione, to Human Subjects. *Pharmacol. Toxicol.* 72, 31–33. doi:10.1111/j.1600-0773.1993.tb01335.x

Tanaka, S., Uchida, S., Inui, N., Takeuchi, K., Watanabe, H., and Namiki, N. (2014). Simultaneous Lc-Ms/ms Analysis of the Plasma Concentrations of a Cocktail of 5 Cytochrome P450 Substrate Drugs and Their Metabolites. *Biol. Pharm. Bull.* 37, 18–25. doi:10.1248/bpb.b13-00401

Tang-Liu, D. D., Williams, R. L., and Riegelman, S. (1983). Disposition of Caffeine and its Metabolites in Man. *J. Pharmacol. Exp. Ther.* 224, 180–185.

Tantcheva-Poór, I., Zaigler, M., Rietbrock, S., and Fuhr, U. (1999). Estimation of Cytochrome P-450 Cyp1a2 Activity in 863 Healthy Caucasians Using a Saliva-Based Caffeine Test. *Pharmacogenetics* 9, 131–144.

Thacker, S. B. (1988). Meta-analysis. A Quantitative Approach to Research Integration. *JAMA* 259, 1685–1689. doi:10.1001/jama.259.11.1685

Tian, D. D., Natesan, S., White, J. R., Paine, M. F., and Paine, M. F. (2019). Effects of Common Cyp1a2 Genotypes and Other Key Factors on Intraindividual Variation in the Caffeine Metabolic Ratio: An Exploratory Analysis. *Clin. Transl Sci.* 12, 39–46. doi:10.1111/cts.12598

Trang, J. M., Blanchard, J., Conrad, K. A., and Harrison, G. G. (1985). Relationship between Total Body Clearance of Caffeine and Urine Flow Rate in Elderly Men. *Biopharm. Drug Dispos* 6, 51–56. doi:10.1002/bdd.2510060107

Trang, J. M., Blanchard, J., Conrad, K. A., and Harrison, G. G. (1982). The Effect of Vitamin C on the Pharmacokinetics of Caffeine in Elderly Men. *Am. J. Clin. Nutr.* 35, 487–494. doi:10.1093/ajcn/35.3.487

Tricco, A. C., Lillie, E., Zarin, W., O'Brien, K. K., Colquhoun, H., Levac, D., et al. (2018). PRISMA Extension for Scoping Reviews (PRISMA-ScR): Checklist and Explanation. *Ann. Intern. Med.* 169, 467–473. doi:10.7326/M18-0850

Tripathi, A., Tiwari, B., Patil, R., Khanna, V., and Singh, V. (2015). The Role of Salivary Caffeine Clearance in the Diagnosis of Chronic Liver Disease. *J. Oral Biol. Craniofac. Res.* 5, 28–33. doi:10.1016/j.jobcr.2014.12.003

Turpault, S., Brian, W., Van Horn, R., Santoni, A., Poitiers, F., Donazzolo, Y., et al. (2009). Pharmacokinetic Assessment of a Five-Probe Cocktail for Cyps 1a2, 2c9, 2c19, 2d6 and 3a. *Br. J. Clin. Pharmacol.* 68, 928–935. doi:10.1111/j.1365-2125.2009.03548.x

Urry, E., Jetter, A., and Landolt, H. P. (2016). Assessment of Cyp1a2 Enzyme Activity in Relation to Type-2 Diabetes and Habitual Caffeine Intake. *Nutr. Metab. (Lond)* 13, 66. doi:10.1186/s12986-016-0126-6

Wahlländer, A., Mohr, S., and Paumgartner, G. (1990). Assessment of Hepatic Function. Comparison of Caffeine Clearance in Serum and Saliva during the Day and at Night. *J. Hepatol.* 10, 129–137. doi:10.1016/0168-8278(90)90041-o

Walther, H., Banditt, P., and Köhler, E. (1983). Significance of Caffeine Values in Serum, Saliva and Urine-Ddetermination of Pharmacokinetic Data by Non-invasive Methods in Psychopharmacologic Studies. *Pharmacopsychiatria* 16, 166–170. doi:10.1055/s-2007-1019492

Wang, T., Kleber, G., Stellaard, F., and Paumgartner, G. (1985). Caffeine Elimination: a Test of Liver Function. *Klin Wochenschr* 63, 1124–1128. doi:10.1007/BF02291094

White, J. R., Padowski, J. M., Zhong, Y., Chen, G., Luo, S., Lazarus, P., et al. (2016). Pharmacokinetic Analysis and Comparison of Caffeine Administered Rapidly or Slowly in Coffee Chilled or Hot versus Chilled Energy Drink in Healthy Young Adults. *Clin. Toxicol.* 54, 308–312. doi:10.3109/15563650.2016.1146740

Yamazaki, T., Desai, A., Goldwater, R., Han, D., Howieson, C., Akhtar, S., et al. (2017). Pharmacokinetic Effects of Isavuconazole Coadministration with the Cytochrome P450 Enzyme Substrates Bupropion, Repaglinide, Caffeine, Dextromethorphan, and Methadone in Healthy Subjects. *Clin. Pharmacol. Drug Dev.* 6, 54–65. doi:10.1002/cpdd.281

Yang, X., Zhang, B., Molony, C., Chudin, E., Hao, K., Zhu, J., et al. (2010). Systematic Genetic and Genomic Analysis of Cytochrome P450 Enzyme Activities in Human Liver. *Genome Res.* 20, 1020–1036. doi:10.1101/gr.103341.109

Yesair, D. W., Branfman, A. R., and Callahan, M. M. (1984). Human Disposition and Some Biochemical Aspects of Methylxanthines. *Prog. Clin. Biol. Res.* 158, 215–233.

Yubero-Lahoz, S., Pardo, R., Farre, M., Mathuna, B. Ó., Torrens, M., Mustata, C., et al. (2012). Changes in Cyp1a2 Activity in Humans after 3,4-methylenedioxymethamphetamine (Mdma, Ecstasy) Administration Using Caffeine as a Probe Drug. *Drug Metab. Pharmacokinet.* 27, 605–613. doi:10.2133/dmpk.dmpk-12-rg-032

Zanger, U. M., and Schwab, M. (2013). Cytochrome P450 Enzymes in Drug Metabolism: Regulation of Gene Expression, Enzyme Activities, and Impact of Genetic Variation. *Pharmacol. Ther.* 138, 103–141. doi:10.1016/j.pharmthera.2012.12.007

Zylber-Katz, E., Granit, L., and Levy, M. (1984). Relationship between Caffeine Concentrations in Plasma and Saliva. *Clin. Pharmacol. Ther.* 36, 133–137. doi:10.1038/clpt.1984.151

Zysset, T., Wahlländer, A., and Preisig, R. (1984). Evaluation of Caffeine Plasma Levels by an Automated Enzyme Immunoassay (EMIT) in Comparison with a High-Performance Liquid Chromatographic Method. *Ther. Drug Monit.* 6, 348–354. doi:10.1097/00007691-198409000-00016

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

3.4 Published work 3: PBPK Model of Dextromethorphan

In this work, a PBPK model of dextromethorphan and its metabolites was developed. Dextromethorphan is the most common test substance used to metabolically phenotype CYP2D6. With the aid of the model, various aspects relevant to CYP phenotyping were investigated. Most importantly, the effect of the CYP2D6 genotype on the phenotype was investigated. In contrast to the caffeine meta-analysis, reported differences in the intervention protocol and individual characteristics could be accounted for by PKPK modeling. The created model was validated by an extensive amount of pharmacokinetics data from 36 clinical studies. Again, the data was made accessible via PKDB, which makes it presumably the largest openly available dataset on dextromethorphan pharmacokinetics.

The validated model suggests that almost all the inter-individual variability in the urinary cumulative metabolic ratio (UCMR) is due to inter-individual variability in CYP2D6 and CYP3A4 enzyme kinetics. Further, the dataset and the model indicate very good robustness of the UCMR against the intervention protocol (i.e., application form, dosing amount, dissolution rate, and sampling time) and good robustness against physiological variation of the subjects, also for the impact of impaired renal function. This was a concern beforehand [SBN00]. Further, the distribution of UCMR and the risk of genotype-phenotype mismatch were estimated for different biogeographical populations.

Additionally, to the analyses in the publication, the impact of CYP3A4 and CYP2D6 induction or inhibition on the UCMR distribution were investigated, see Fig. 3.3. It is advised to read the publication first before this inhibition study as it builds up on its content. As described in the publication, Km and Vmax values for CYP3A4 and CYP2D6 were sampled based on the distribution of *in vitro* data. The CYP2D6 Km and Vmax were assumed to be affected by the CYP2D6 genotype, which was implemented via the activity score. The inhibition/induction was introduced in the model by a Km scan. A higher Km (i.e., binding affinity) results in a slower metabolism and vice versa. The model suggests that the inhibition of CYP3A4, e.g., by grapefruit juice, might be particularly dangerous for already poor CYP2D6 metabolizers as they then lack the ability to compensate for the low CYP2D6 activity. If validated experimentally, this would be of clinical relevance.

In summary, the first open dataset on dextromethorphan pharmacokinetics with a focus on genotype-phenotype association was created and used to build an openly available and validated PBPK model of dextromethorphan, provided in SBML, which can be used for individual CYP2D6 phenotype prediction and potentially have an impact in personalized dosing of drugs metabolized by CYP2D6. The chosen workflow from data curation, data representation, and data-driven modeling builds crucially on the previous two publications.

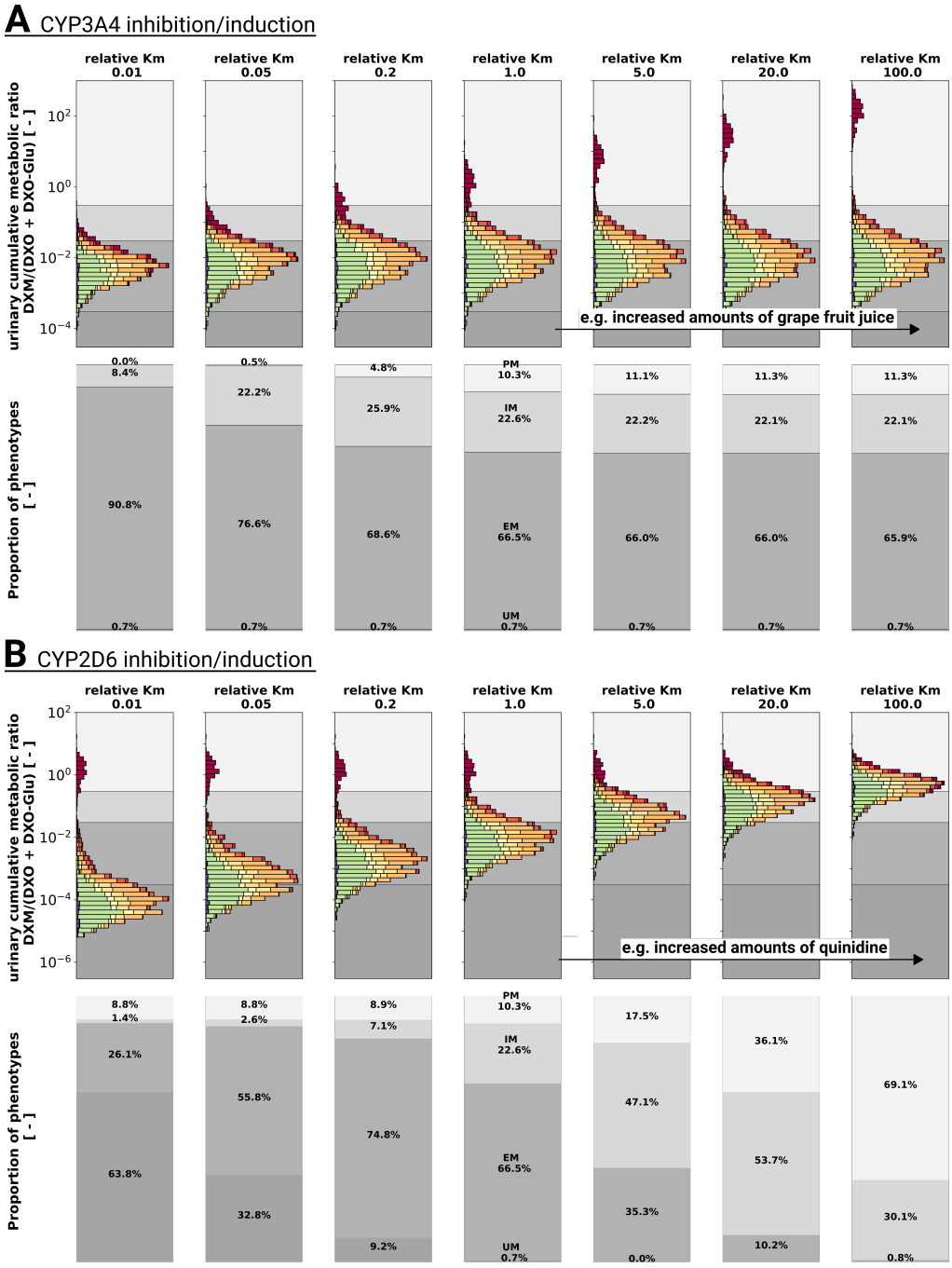

FIGURE 3.3: The effect of inhibition and induction of CYP3A4 **(A)** and CYP2D6 **(B)** via a relative change in the Michaelis constant, e.g., due to drug-drug interactions, on the distributions of UCMR and the proportion of phenotypes.

Publication

Physiologically based pharmacokinetic (PBPK) modeling of the role of CYP2D6 polymorphism for metabolic phenotyping with dextromethorphan
Grzegorzewski J, Brandhorst J, König M. Front. Pharmacol., 24 October 2022, Sec. Pharmacogenetics and Pharmacogenomics doi: 10.3389/fphar.2022.10290. PMID: 36353484

Alongside the publication, the PK-DB frontend, API, and source code of PK-DB were published. For the PAGE 2021 conference, a short video of me presenting a poster on PK-DB was published:

- PK-DB (frontend): https://pk-db.com/data [Open the search panel on the left and enter dextromethorphan and dextromethorphan hydrobromide as the intervention substances.]
- PK-DB (data): https://pk-db.com/api/v1/filter/?concise=false&download=true&interventions__substance_sid__in=dmthbr__dmt [Warning: slow download, data processing can take up to a minute. Further, it is unclear if the URL will break at some time in the future due to major updates.]
- PBPK model of DXM in SBML under CC-BY 4.0 license: https://github.com/matthiaskoenig/dextromethorphan-model

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EDITED BY
Luis Abel Quiñones,
University of Chile, Chile

REVIEWED BY
Ismael Lares-Asseff,
Instituto Politécnico Nacional (IPN), Mexico
Zifei Qin,
First Affiliated Hospital of Zhengzhou University, China

*CORRESPONDENCE
Jan Grzegorzewski,
grzegorj@hu-berlin.de

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Physiologically based pharmacokinetic (PBPK) modeling of the role of CYP2D6 polymorphism for metabolic phenotyping with dextromethorphan

Jan Grzegorzewski*, Janosch Brandhorst and Matthias König

Institute for Theoretical Biology, Institute of Biology, Humboldt University, Berlin, Germany

The cytochrome P450 2D6 (CYP2D6) is a key xenobiotic-metabolizing enzyme involved in the clearance of many drugs. Genetic polymorphisms in CYP2D6 contribute to the large inter-individual variability in drug metabolism and could affect metabolic phenotyping of CYP2D6 probe substances such as dextromethorphan (DXM). To study this question, we (i) established an extensive pharmacokinetics dataset for DXM; and (ii) developed and validated a physiologically based pharmacokinetic (PBPK) model of DXM and its metabolites dextrorphan (DXO) and dextrorphan O-glucuronide (DXO-Glu) based on the data. Drug-gene interactions (DGI) were introduced by accounting for changes in CYP2D6 enzyme kinetics depending on activity score (AS), which in combination with AS for individual polymorphisms allowed us to model CYP2D6 gene variants. Variability in CYP3A4 and CYP2D6 activity was modeled based on *in vitro* data from human liver microsomes. Model predictions are in very good agreement with pharmacokinetics data for CYP2D6 polymorphisms, CYP2D6 activity as described by the AS system, and CYP2D6 metabolic phenotypes (UM, EM, IM, PM). The model was applied to investigate the genotype-phenotype association and the role of CYP2D6 polymorphisms for metabolic phenotyping using the urinary cumulative metabolic ratio (UCMR), DXM/(DXO + DXO-Glu). The effect of parameters on UCMR was studied *via* sensitivity analysis. Model predictions indicate very good robustness against the intervention protocol (i.e. application form, dosing amount, dissolution rate, and sampling time) and good robustness against physiological variation. The model is capable of estimating the UCMR dispersion within and across populations depending on activity scores. Moreover, the distribution of UCMR and the risk of genotype-phenotype mismatch could be estimated for populations with known CYP2D6 genotype frequencies. The model can be applied for individual prediction of UCMR and metabolic phenotype based on CYP2D6 genotype. Both, model and database are freely available for reuse.

KEYWORDS

dextromethorphan (DXM), CYP2D6, physiologically based pharmacokinetic model (PBPK), pharmacokinetics, pharmacogenomics (PGx), metabolic phenotype

1 Introduction

The cytochrome P450 (CYPs) superfamily of enzymes has a central role in the clearance of many substances and drugs, with the isoform 2D6 (CYP2D6) being one of the most important xenobiotic-metabolizing enzymes. CYP2D6 is involved in the clearance of around 20% of the most prescribed drugs (Saravanakumar et al., 2019) including antiarrhythmics having a small therapeutic range (e.g., flecainide, procainamide, mexiletine), anticancer agents (e.g., tamoxifen), antidepressants (e.g., citalopram, fluoxetine, duloxetine: venlafaxine), antipsychotics (e.g., aripiprazole, haloperidol, thioridazine), β-blockers (metoprolol), analgesics (tramadol, oxycodone, codeine), and antitussives (dextromethorphan) (Hurtado et al., 2020; Kibaly et al., 2021). CYP2D6-mediated drug response exhibits a particularly large inter-individual variability which poses a challenge for personalized dosage of medication by underdosing on the one hand and toxic side effects on the other. The activity of CYP2D6 is known to be majorly dependent on genetic variants (Berm et al., 2013; Preskorn et al., 2013; Shah and Smith, 2015) with polymorphism of CYP2D6 being related to the risk of adverse effects, non-response during treatment, and death by drug intoxication (Gasche et al., 2004; Kawanishi et al., 2004; Rau et al., 2004; Zackrisson et al., 2010).

In the late 70 s, a polymorphism in debrisoquine hydroxylation (Mahgoub et al., 1977) and sparteine oxidation (Eichelbaum et al., 1979) was discovered and subsequently attributed to allelic variants of the CYP2D6 gene. In the following years, CYP2D6 became one of the most studied drug-metabolizing enzymes. Genetic variants were classified into distinct phenotypes and subjects carrying combinations of these variants were categorized as poor metabolizer (gPM), intermediate metabolizer (gIM), extensive metabolizer (gEM), and ultra rapid metabolizer (gUM) (Zanger et al., 2004; Gaedigk et al., 2017). This classification is based on the relationship between genetic variants and CYP2D6-mediated drug response. For these genetically predicted phenotypes, we use the "g" nomenclature as they can be easily confused with the actual *in vivo* metabolic phenotype, determined based on pharmacokinetic measurements after the administration of CYP2D6 test drugs. Nowadays, the CYP2D6 activity score (AS) system, a more refined metric, is often applied to characterize genotype-phenotype associations (Gaedigk et al., 2018a). In the system, discrete values between 0 and 1 are assigned to gene variants. The final activity score is calculated by the sum of the activity scores of both alleles. For instance, a person with diplotype *1/*3 (the variant *1 has an AS of 1 and the variant *3 has no activity with an AS of 0) has an overall AS of 1. Higher activity scores than 2 and additional complexity arise from copy number variation (CNV), chimeras, and hybrids with the pseudo gene CYP2D7. This can result in ambiguities and difficulties in the assignment of the correct diplotype and activity score (Gaedigk et al., 2007; Nofziger and Paulmichl, 2018; Gaedigk et al., 2019). Of note, AS specifics are still under heavy debate and regularly updated (Caudle et al., 2020). A multitude of population studies have been conducted to identify and associate allele variants with metabolic phenotypes within and across populations (Gaedigk et al., 2017). Over 130 CYP2D6 star (*) allele haplotypes have been identified and subsequently cataloged by the Pharmacogene Variation (PharmVar) Consortium into PharmGKB with their respective activity score contribution (Gaedigk et al., 2018b; Whirl-Carrillo et al., 2021).

Various methods exist for the metabolic phenotyping based on test substances. The gold standard is plasma concentration sampling of probe substances and their metabolites at various time points after the administration. (Partial) clearance rates and the relative enzyme activities can be calculated from these plasma time profiles. Simplified methods have been established for many probe substances which do not require repeated sampling of blood, e.g., the (cumulative) metabolic ratios between the probe substance and one or several of its metabolites at a single time point in blood, plasma, or urine are utilized as such proxy measures. Large-scale population studies often tend to employ urinary ratios of metabolites. Alternatively, sampling of saliva and breath are worth considering (De Kesel et al., 2016). Probe substances for metabolic phenotyping of CYP2D6 are debrisoquine, dextromethorphan, metoprolol, or sparteine (Frank et al., 2007; Fuhr et al., 2007). Bufuralol is less popular but well suited for *in vitro* investigations due to its fluorescent properties (Zanger et al., 2004). Although debrisoquine and sparteine have excellent properties for CYP2D6 phenotyping, they have been withdrawn from clinical use in most countries and are therefore no longer readily available. Frequently in use for the phenotyping of CYP2D6 activity are metoprolol and dextromethorphan.

Dextromethorphan (DXM) is an over-the-counter, antitussive, non-narcotic, synthetic analog of codeine affecting the activity of numerous channels and receptors in the brain that trigger the cough reflex (Silva and Dinis-Oliveira, 2020). It is generally well-tolerated, considered safe in therapeutic dosage, and easily available (Fuhr et al., 2007). Besides therapeutic purposes, DXM is most commonly applied as a probe substance for CY2D6 phenotyping, alone or with other probe substances in a cocktail. DXM can be administered orally and intravenously, has low bioavailability (≈50%) and a rapid first-pass effect in the intestine and liver. Typically only about half of

the dose is recovered in urine over at least 12 h after administration, primarily as glucuronides (Schadel et al., 1995; Capon et al., 1996; Tennezé et al., 1999; Strauch et al., 2009). In the systemic circulation, ≈ 55–65% of DXM is non-specifically bound to plasma proteins (Lutz and Isoherranen, 2012; Taylor et al., 2016).

The biotransformation of DXM is mostly confined to the liver, where DXM is O-demethylated by CYP2D6 to the active metabolite dextrorphan (DXO). Subsequently to O-demethylation, most of the DXO is rapidly transformed *via* UDP-glucuronosyltransferase (UGT) to dextrorphan O-glucuronide (DXO-Glu) and excreted *via* the urine. In individuals without any functional variant of CYP2D6, the metabolization of DXM to DXO is extremely slow but still present. Apparently, the O-demethylation is not exclusively mediated by CYP2D6, and it has been demonstrated *in vitro* that O-demethylation of DXM can be marginally mediated by CYP3A4, CYP3A5 and CYP2C9 (von Moltke et al., 1998; McGinnity et al., 2000; Takashima et al., 2005; Yu and Haining, 2001). In line with this observation, inhibition of CYP2D6, e.g., barely affects poor metabolizer (Pope et al., 2004). The second pathway of DXM metabolization goes *via* N-demethylation to 3-methoxymorphinan which is mainly catalyzed *via* CYP3A4. Subsequently, 3-methoxymorphinan and DXO are biotransformed to 3-hydroxymorphinan which is then rapidly transformed *via* glucuronidation to hydroxymorphian O-glucuronide and excreted in the urine. The urinary cumulative metabolic ratio (UCMR) of DXM to its metabolites DXM/(DXO + DXO-Glu) is a widely applied measure for the *in vivo* CYP2D6 phenotyping.

An crucial question for metabolic phenotyping and liver function testing is how CYP2D6 polymorphisms affect the pharmacokinetics of DXM and metabolic phenotyping based on DXM, such as the UCMR. The objective of this work was to answer this question by the means of physiologically based pharmacokinetic (PBPK) modeling of DXM.

2 Material and methods

2.1 Pharmacokinetics database of DXM

Pharmacokinetics data of DXM was systematically curated from literature for model development, parameterization, and validation. Curation efforts were mainly focused on concentration-time profiles of DXM, DXO, and DXO-Glu in plasma or serum and their amounts or ratios in urine. The data is accompanied by metadata on the investigated subjects and groups (e.g., CYP2D6 genotype or activity score) and the applied intervention (e.g., dose and application form of DXM). All data was curated using an established curation pipeline (Grzegorzewski et al., 2022) and is available *via* the pharmacokinetics database PK-DB (https://pk-db.com) (Grzegorzewski et al., 2021). As a first step, a PubMed search for the pharmacokinetics of dextromethorphan in combination with genotyping and/or phenotyping was performed with the search query https://pubmed.ncbi.nlm.nih.gov/?term=dextromethorphan+AND+%28phenotype+OR+phenotyping%29+AND+genotype. The literature corpus was extended with drug cocktail studies from PK-DB (Grzegorzewski et al., 2022), secondary literature from references, and results from PKPDAI with the search query https://app.pkpdai.com/?term=dextromethorphan (Gonzalez Hernandez et al., 2021). Data was selected and curated based on eligibility criteria, see below. During the curation process, the initial corpus was updated by additional publications from the references and citations. A subset of the studies only reported pharmacokinetic parameters without timecourses. These studies were curated but not further used in the following analyses.

To be eligible, studies had to report *in vivo* pharmacokinetics data for adult (age ≥18) humans after administration of DXM or DXM hydrobromide. The application route of DXM was restricted to oral (PO) or intravenous (IV). All application forms (e.g., tablet, capsule, solution) were accepted. No restrictions were imposed on the dosing amount of DXM or coadministrations of other substances. Studies containing coadminstrations that inhibit or induce the pharmacokinetics of DXM were identified during the modeling process and excluded. The relevant outcome measures are concentration-time profiles in plasma, serum, and urine amounts of DXM, DXM metabolites, or metabolic ratios of metabolites such as UCMR. Studies containing pharmacokinetic parameters of DXM and its metabolites (e.g., clearance, half-life, AUC) and (urinary cumulative) metabolic ratios of DXM and its metabolites were included. Data containing timecourses and CYP2D6 genotype information were prioritized. Non-healthy subjects were excluded if the disease is known to affect the pharmacokinetics of DXM or DXM metabolites. Study B from the PhD thesis of Frank (2009) highly deviates from the remaining data and was therefore excluded. Further, Wyen et al. (2008) was identified as a duplicate of Study E from the PhD thesis of Frank (2009) and excluded. The final set of curated studies used in the presented analyses is provided in Table 1.

For the selection and evaluation of studies from the literature, the PRISMA-ScR guidelines were adopted where applicable (Tricco et al., 2018). The initial corpus contained 404 studies. After screening, application of eligibility criteria, and prioritization, a total of 47 studies were curated (see Supplementary Figure S1). Of these studies, 36 contained data used in the present work (Table 1).

2.2 PBPK model of DXM

The PBPK model of DXM, DXO, and DXO-Glu (Figure 1) was encoded in the Systems Biology Markup Language (SBML) (Hucka et al., 2019; Keating et al., 2020). For development and visualization, sbmlutils (König, 2021b) and cy3sbml (König et al.,

TABLE 1 Clinical studies with pharmacokinetics used for model evaluation.

Reference	PK-DB	PMID	DXM application	Dosing protocol	Description
Abdelrahman et al. (1999)	PKDB00573	10340911	DXM	Oral (syrup): 0.3 mg/kg	Investigation of terbinafine as a CYP2D6 inhibitor *in vivo*
Abduljalil et al. (2010)	PKDB00574	20881950	DXM hydrobromide	Oral (capsule): 30 mg	Assessment of activity levels for CYP2D6*1, CYP2D6*2, and CYP2D6*41 genes by population pharmacokinetics of dextromethorphan
Armani et al. (2017)	PKDB00428	10340911	DXM (in cocktail)	Oral (NR): 30 mg	The antitussive effect of dextromethorphan in relation to CYP2D6 activity
Barnhart. (1980)	PKDB00575	7423506	DXM hydrobromide	Oral (capsule): 30 mg	The urinary excretion of dextromethorphan and three metabolites in dogs and humans
Capon et al. (1996)	PKDB00576	8841152	DXM hydrobromide	Oral (NR): 30 mg	The antitussive effect of dextromethorphan in relation to CYP2D6 activity
Chen et al. (2017)	PKDB00577	28512430	DXM	Oral (tablet): 15 mg + water 300 ml	CYP2D6 phenotyping using urine, plasma, and saliva metabolic ratios to assess the impact of CYP2D6*10 on inter-individual variation in a Chinese population
Chládek et al. (2000)	PKDB00578	11214771	DXM hydrobromide	Oral (syrup): 30 mg	*In vivo* indices of CYP2D6 activity: comparison of dextromethorphan metabolic ratios in 4-h urine and 3-h plasma
Demirbas et al. (1998)	PKDB00579	9840216	DXM hydrobromide	Oral (sustained release tablet): 60 mg	Bioavailability of dextromethorphan (as dextrorphan) from sustained release formulations in the presence of guaifenesin in human volunteers
Dorado et al. (2017)	PKDB00580	28271978	DXM	Oral (NR): 15 mg	Lessons from Cuba for global precision medicine: CYP2D6 genotype is not a robust predictor of CYP2D6 ultrarapid metabolism
Doroshyenko et al. (2013)	PKDB00138	23401474	DXM (in cocktail)	Oral (capsule): 30 mg	Drug metabolism and disposition: the biological fate of chemicals
Duedahl et al. (2005)	PKDB00597	15661445	DXM	Intravenous: 0.5 mg/kg	Intravenous dextromethorphan to human volunteers: relationship between pharmacokinetics and anti-hyperalgesic effect
Dumond et al. (2010)	PKDB00499	20147896	DXM (in cocktail)	Oral (solution): 30 mg	A phenotype-genotype approach to predicting CYP450 and P-glycoprotein drug interactions with the mixed inhibitor/inducer tipranavir/ritonavir
Edwards et al. (2017)	PKDB00496	28808886	DXM (in cocktail)	Oral (capsule): 30 mg	Assessment of pharmacokinetic interactions between obeticholic acid and caffeine, midazolam, warfarin, dextromethorphan, omeprazole, rosuvastatin, and digoxin in phase 1 studies in healthy subjects
Eichhold et al. (1997)	PKDB00596	-	DXM hydrobromide	Oral (syrup): 30 mg	Determination of dextromethorphan and dextrorphan in human plasma by liquid chromatography/tandem mass spectrometry
Eichhold et al. (2007)	PKDB00581	16930908	DXM hydrobromide	Oral (solution): 20 mg	Simultaneous determination of dextromethorphan, dextrorphan, and guaifenesin in human plasma using semi-automated liquid/liquid extraction and gradient liquid chromatography tandem mass spectrometry
Frank (2009)	PKDB00582	-	DXM hydrobromide (in cocktail)	Oral (capsule): 30 mg	Evaluation of pharmacokinetic metrics for phenotyping of the human CYP2D6 enzyme with dextromethorphan
Gaedigk (2013)	PKDB00583	24151800	DXM	Oral (syrup): 0.3 mg/kg	Complexities of CYP2D6 gene analysis and interpretation
Hou et al. (1991)	PKDB00584	2015730	DXM hydrobromide	Oral (capsule): 50 mg	Salivary analysis for determination of dextromethorphan metabolic phenotype
Hu et al. (2011)	PKDB00585	21050887	DXM hydrobromide	Oral (sustained release tablet): 30 mg	Floating matrix dosage form for dextromethorphan hydrobromide based on gas forming technique: *in vitro* and *in vivo* evaluation in healthy volunteers
Jones et al. (1996)	PKDB00586	8873685	DXM hydrobromide	Oral (syrup): 30 mg	Determination of cytochrome P450 3A4/5 activity *in vivo* with dextromethorphan N-demethylation
Köhler et al. (1997)	PKDB00587	9429230	DXM	Oral (syrup): 20 mg	CYP2D6 genotype and phenotyping by determination of dextromethorphan and metabolites in serum of healthy controls and of patients under psychotropic medication
López et al. (2005)	PKDB00588	16249913	DXM hydrobromide	Oral (syrup): 30 mg	CYP2D6 genotype and phenotype determination in a Mexican Mestizo population

(Continued on following page)

TABLE 1 (*Continued*) Clinical studies with pharmacokinetics used for model evaluation.

Reference	PK-DB	PMID	DXM application	Dosing protocol	Description
Lenuzza et al. (2016)	PKDB00598	25465228	DXM (in cocktail)	Oral (tablet): 18 mg	Safety and pharmacokinetics of the (CIME) Combination of Drugs and Their Metabolites after a single oral dosing in healthy volunteers
Montané Jaime et al. (2013)	PKDB00589	23394389	DXM hydrobromide	Oral (NR): 30 mg + water	Characterization of the CYP2D6 gene locus and metabolic activity in Indo- and Afro-Trinidadians: discovery of novel allelic variants
Myrand et al. (2008)	PKDB00497	18231117	DXM (in cocktail)	Oral (NR): 30 mg	Pharmacokinetics/genotype associations for major cytochrome P450 enzymes in native and first- and third-generation Japanese populations: comparison with Korean, Chinese, and Caucasian populations
Nagai et al. (1996)	PKDB00590	8830977	DXM hydrobromide	Oral (tablet): 30 mg	Pharmacokinetics and polymorphic oxidation of dextromethorphan in a Japanese population
Nakashima et al. (2007)	PKDB00599	17652181	DXM hydrobromide	Oral (tablet): 30 mg	Effect of cinacalcet hydrochloride, a new calcimimetic agent, on the pharmacokinetics of dextromethorphan: *in vitro* and clinical studies
Nyunt et al. (2008)	PKDB00591	18362694	DXM	Oral (tablet): 30 mg	Pharmacokinetic effect of AMD070, an Oral CXCR4 antagonist, on CYP3A4 and CYP2D6 substrates midazolam and dextromethorphan in healthy volunteers
Oh et al. (2012)	PKDB00054	22483397	DXM (in cocktail)	Oral (NR): 2 mg	High-sensitivity liquid chromatography-tandem mass spectrometry for the simultaneous determination of five drugs and their cytochrome P450-specific probe metabolites in human plasma
Pope et al. (2004)	PKDB00592	15342614	DXM	Oral (capsule): 30 mg; 45 mg; 60 mg	Pharmacokinetics of dextromethorphan after single or multiple dosing in combination with quinidine in extensive and poor metabolizers
Qiu et al. (2016)	PKDB00600	27023460	DXM hydrobromide	Oral (tablet): 15 mg	Effects of the Chinese herbal formula "Zuojin Pill" on the pharmacokinetics of dextromethorphan in healthy Chinese volunteers with CYP2D6*10 genotype
Schadel et al. (1995)	PKDB00593	7593709	DXM	Oral (capsule): 30 mg	Pharmacokinetics of dextromethorphan and metabolites in humans: influence of the CYP2D6 phenotype and quinidine inhibition
Schoedel et al. (2012)	PKDB00594	22283559	DXM	Oral (capsule): twice daily for 8 days; 30 mg	Randomized open-label drug-drug interaction trial of dextromethorphan/quinidine and paroxetine in healthy volunteers
Tamminga et al. (2001)	PKDB00498	11829201	DXM hydrobromide	Oral (tablet): 22 mg	The prevalence of CYP2D6 and CYP2C19 genotypes in a population of healthy Dutch volunteers
Yamazaki et al. (2017)	PKDB00494	27273149	DXM (in cocktail)	Oral (NR): 30 mg	Pharmacokinetic Effects of isavuconazole coadministration with the cytochrome P450 enzyme substrates bupropion, repaglinide, caffeine, dextromethorphan, and methadone in healthy subjects
Zawertailo et al. (2010)	PKDB00595	20041473	DXM	Oral (capsule): 3 mg/kg	Effect of metabolic blockade on the psychoactive effects of dextromethorphan

NR: not reported, DXM: dextromethorphan.

2012; König and Rodriguez, 2019) were used. The model utilizes ordinary differential equations (ODE) which were numerically solved by sbmlsim (König, 2021a) based on the high-performance SBML simulator libroadrunner (Somogyi et al., 2015; Welsh et al., 2022). It is available in SBML under CC-BY 4.0 license from https://github.com/matthiaskoenig/dextromethorphan-model. Within this work, version 0.9.5 of the model was used (Grzegorzewski and König, 2022).

The model is hierarchically organized with submodels coupled using hierarchical model composition (Smith et al., 2015). The top layer represents the whole body with organs and tissues connected *via* the blood flow. The lower layer describes substance-related processes within the tissues. Tissues with minor influence on the pharmacokinetics of DXM, DXO, or DXO-Glu are lumped into the 'rest' compartment. Intravenous and oral application of DXM appears in the venous and intestinal compartments, respectively. A fraction of DXM is absorbed *via* the intestinal wall into the systemic circulation. The remainder is excreted *via* the feces. The plasma concentration is evaluated at the median cubital vein.

The distribution of DXM, DXO, and DXO-Glu between plasma and tissue compartments is based on tissue-to-plasma partition coefficients (K_p) and the corresponding rates of tissue distribution (f_{tissue}).

The metabolism of DXM only includes processes relevant for the simulation of the reported pharmacokinetics data (see Figures 1B,C). Routes of minor contribution such as

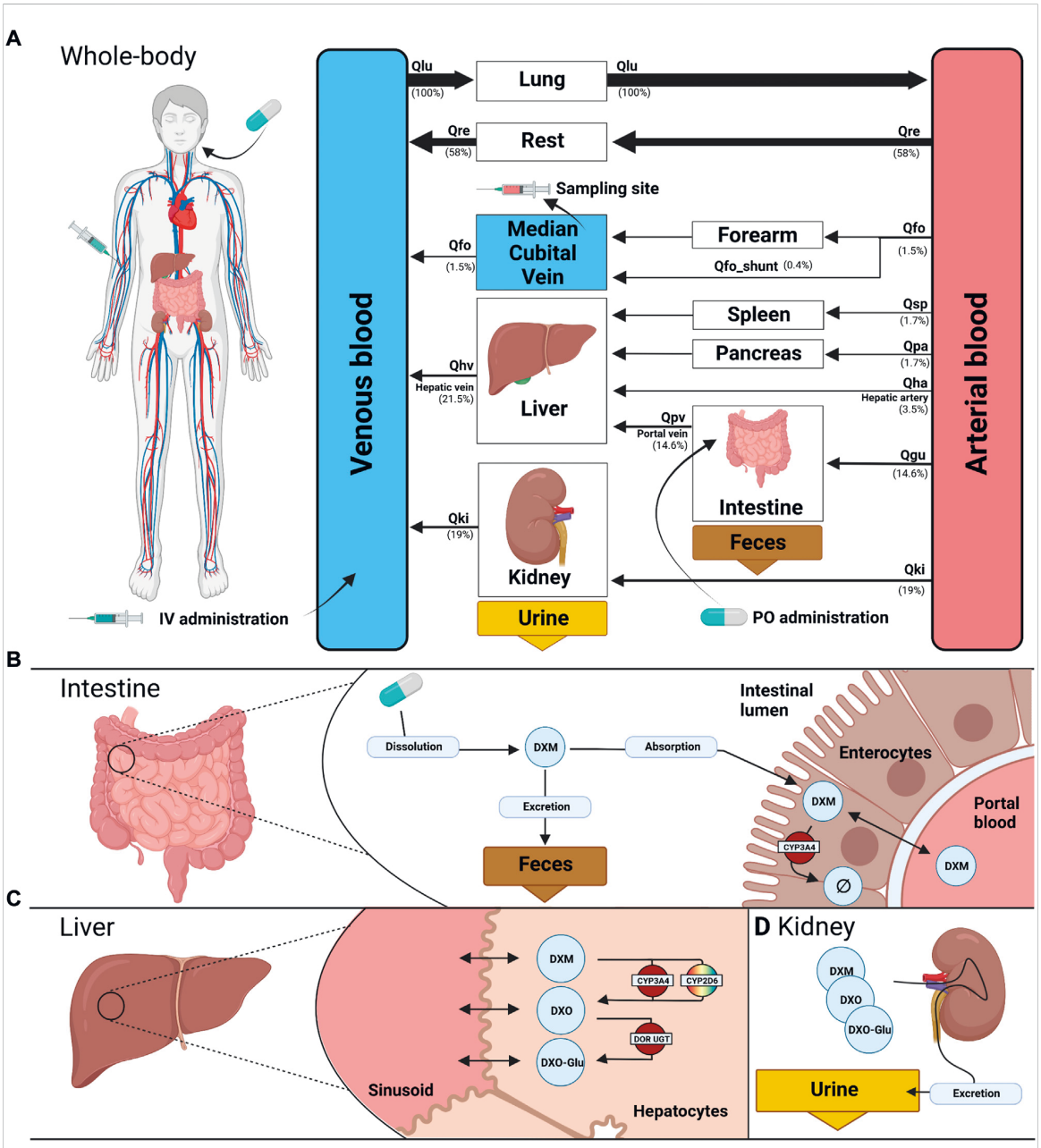

FIGURE 1
PBPK model of dextromethorphan (DXM). **(A)** whole body model consisting of liver, kidney, intestine, forearm, lung and the rest compartment. Organs with minor relevance are not modeled explicitly and lumped into the rest compartment. Organs are coupled *via* the systemic circulation with arrow width proportional to relative blood flow. DXM can be administered intravenously (IV) or orally (PO) with DXM appearing in the venous blood or intestine, respectively. **(B)** intestine model consisting of dissolution, absorption and excretion of DXM. Only a fraction of DXM is absorbed with the remainder excreted in the feces. First pass metabolism of DXM *via* CYP3A4 in the intestine reduces the amount of DXM appearing in the circulation. **(C)** liver model consisting of DXM → DXO conversion *via* CYP2D6 and CYP3A4 and subsequent glucuronidation to DXO-Glu. **(D)** kidney model for the urinary excretion of DXM, DXO, and DXO-Glu. Created with BioRender.com.

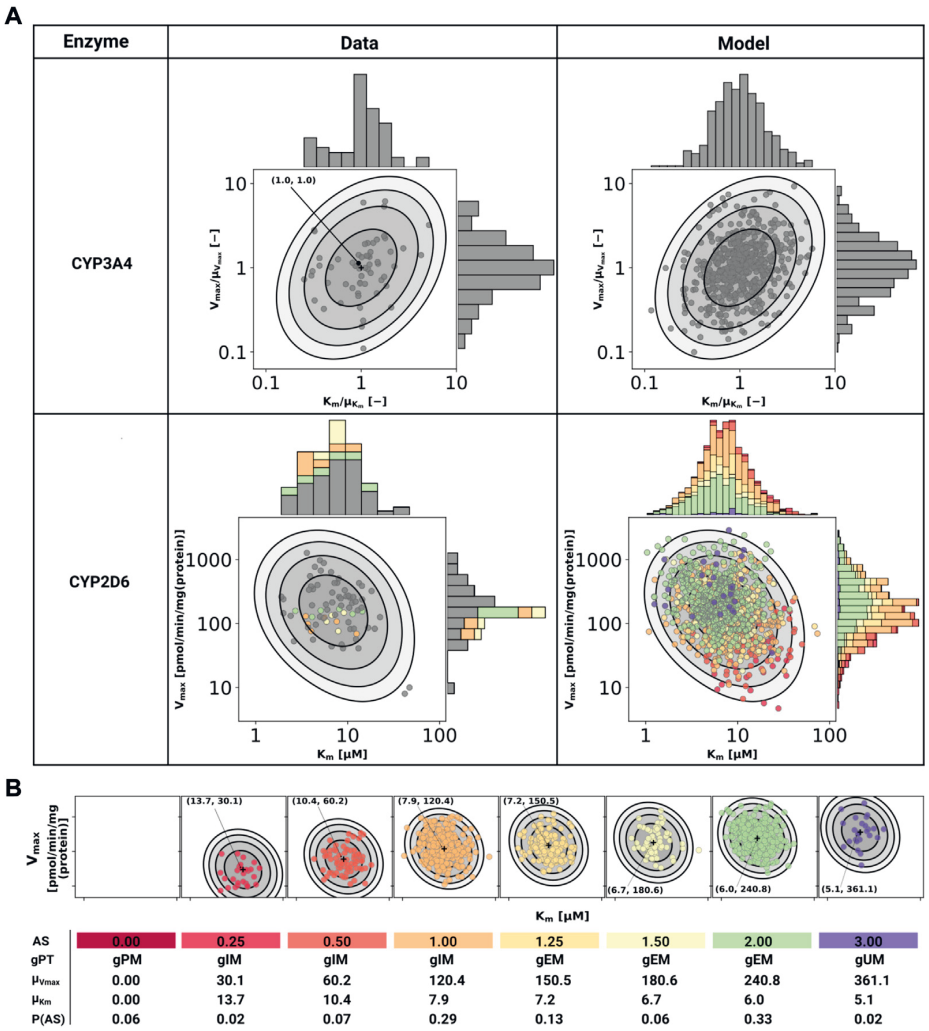

FIGURE 2
Model of CYP3A4 and CYP2D6. **(A)** CYP3A4 and CYP2D6 distributions. Conversion of DXM → DXO *via* CYP3A4 and CYP2D6 are modeled *via* Michaelis-Menten kinetics. Variability was included *via* two-dimensional lognormal distributions of Michaelis-Menten coefficient (K_m) and maximum rate of reaction (V_{max}). The distribution parameters were determine by fitting to *in vitro* data in human liver microsomes. Variability of CYP3A4 parameters was measured by midazolam (Yang et al., 2012), variability of CYP2D6 parameters *via* DXM (Yang et al., 2012; Storelli et al., 2019a). To transfer the CYP3A4 data from midazolam to DXM normalized values were used. The distribution of CYP2D6 was modeled as a mixture model of the underlying activity scores as depicted in **(B)**. The model CYP3A4 and CYP2D6 distributions were sampled with each point corresponding to a combination of V_{max} and K_m. CYP2D6 data was color-coded by the respective activity score. **(B)** CYP2D6 activity score model. CYP2D6 activity was modeled *via* a mixture model of individual activity scores. With increasing activity score the V_{max} for the DXM → DXO conversion increases and the μ_{Km} for DXM decreases, i.e., reaction velocity and affinity for the substrate increase. The table provides AS, genetic phenotype (gPT), mean V_{max}, mean K_m, and AS frequency in curated UCMR data (P (AS)). In case of AS = 0.0 CYP2D6 has no activity for the DXM → DXO conversion.

N-demethylation of DXM in the liver were neglected. Metabolic reactions take place in the intestine and liver and are modeled using irreversible Michaelis-Menten reaction kinetics of the form $v = V_{\max} \cdot \frac{S}{S+Km}$, with $V_{\max}$ and K_m for CYP3A4 and CYP2D6 sampled from distributions as described below. The conversion of DXM to DXO can be either catalyzed *via* CYP2D6 (main route) or CYP3A4 (minor route) in the liver. Reactions with other products than DXM, DXO, and DXO-Glu were modeled as annihilation, i.e. the products of the reaction are not modeled

explicitly. DXM, DXO, and DXO-Glu are eliminated into the urine *via* renal excretion.

A subset of model parameters was fitted by minimizing the distance between model predictions and subsets of the data in Figure 4, Figure 5, Figure 6, Figure 7, Figure 8, and Figure 9.

2.3 CYP3A4 and CYP2D6

CYP3A4 and CYP2D6 variability was modeled *via* correlated bivariate lognormal distributions fitted to *in vitro* data for CYP2D6 (Yang et al., 2012; Storelli et al., 2019a) and CYP3A4 (Yang et al., 2012), respectively. The data was log10 transformed and a Gaussian, parameterized by the mean (μ) and standard deviation, was fitted by maximum likelihood estimation. The multivariate distribution was realized by a Gaussian copula which in turn was parameterized by Kendall's tau correlation coefficient from the data (see Figure 2 for data and model).

In order to model the effect of the CYP2D6 AS on the activity, V_{max} was assumed to be proportional to the AS, $V_{max} \propto AS$ and K_m was scaled by the activity score along the first principle component of log10(K_m) and log10(V_{max}) (principal component regression). To model the effect of genetic polymorphisms of CYP2D6, pharmacogenetic variants in the CYP2D6 gene were mapped to their AS and the total activity calculated as the sum of the activity of the two alleles. The genotype-phenotype definitions (i.e. allele variant to AS mapping) were used from PharmGKB (https://www.pharmgkb.org/page/cyp2d6RefMaterials, accessed on 2022-01-10) (Whirl-Carrillo et al., 2021) (Supplementary Table S1).

The stochastic model of CYP2D6 kinetics for a given population consists of a mixture model comprised from the models for each AS weighted by their respective frequency P(AS), i.e., $P(V_{max}, K_m) = \sum_{AS} P(AS) P(V_{max}, K_m|AS)$. To simulate a given AS, the respective K_m and V_{max} values were used (see Figure 2). The variability in pharmacokinetics was simulated by sampling K_m and V_{max} from the CYP3A4 and CYP2D6 distributions. Distributions of CYP3A4 and CYP2D6 parameters were assumed to be statistically independent. To simulate different populations, the AS frequencies for the respective biogeographical population were used from PharmGKB (https://www.pharmgkb.org/page/cyp2d6RefMaterials, accessed on 2022-01-10) (Whirl-Carrillo et al., 2021) (Supplementary Table S2).

2.4 CYP2D6 metabolic phenotype

The metabolic phenotypes ultrarapid metabolizer (UM), extensive metabolizer (EM), intermediate metabolizer (IM), and poor metabolizer (PM) were assigned based on the urinary cumulative metabolic ratio of DXM to total dextrorphan UCMR = $\frac{DXM}{DXO+DXO-Glu}$ with the following cutoffs: PM: UCMR ≥0.3, IM: 0.03 ≤ UCMR<0.3, EM: 0.0003 ≤ UCMR<0.03, UM: UCMR<0.0003. Some studies reported the extensive metabolizer as normal metabolizer (NM) with identical cutoffs to the EM. Such data was labeled as EM.

2.5 Sensitivity analysis

A local sensitivity analysis of the effect of model parameters on the UCMR was performed. Individual model parameters (p_i) were varied in both directions by 10% from the base model value ($p_{i,-\Delta} \xleftarrow{10\%} p_{i,0} \xrightarrow{10\%} p_{i,\Delta}$) and the change in the state variable describing the UCMR at 8 h (q) was recorded. The local sensitivity (S (q, p_i, AS)) was calculated for a range of ASs (0, 0.25, 0.5, 1, 1.25, 1.5, 2.0, 3.0) by the following formula:

$$S(q, p_i, AS) = \frac{1}{2} \cdot \frac{q(p_{i,\Delta}, AS) - q(p_{i,-\Delta}, AS)}{p_{i,0}} \quad (1)$$

3 Results

Within this work, a physiologically based pharmacokinetic (PBPK) model of DXM was developed and applied to study the role of the CYP2D6 polymorphism on the pharmacokinetics of DXM and metabolic phenotyping using DXM.

3.1 Pharmacokinetics database of DXM

For the development and evaluation of the model, a large pharmacokinetics dataset of DXM and its metabolites, consisting of 36 clinical studies, was established (Table 1). Most of the studies investigated either drug-gene interactions (DGI), drug-drug interactions (DDI), or the interplay of both (i.e. drug-drug-gene interactions). The large majority of studies applied DXM orally (n = 35), whereas only a single publication studied DXM pharmacokinetics after intravenous application (n = 1) (Duedahl et al., 2005). The application form (i.e., solution, syrup, capsule, table), the used DXM dose (2 mg–3 mg/kg), and coadministrations (i.e., phenotyping cocktail, quinidine, cinacalcet hydrochloride, zuojin) vary between studies, as do sampling times and sampled tissues (i.e., urine, plasma, serum). Importantly, plenty of individual UCMR measurements with corresponding CYP2D6 genotype information are contained within this dataset (n = 11 studies). To our knowledge, this is the first large freely available dataset of pharmacokinetics data for DXM with all data accessible from the pharmacokinetics database (PK-DB) (Grzegorzewski et al., 2021).

TABLE 2 Model parameters in PBPK model of DXM. The complete information is available from the model repository. The prefixes GU__, LI__,KI__, correspond to the intestine/gut, liver, and kidneys, respectively. Values are either adopted from the references or fitted (F). During the robustness analysis of UCMR, various parameters were scanned (S) and a local sensitivity (SA) was performed, see Section 3.5.

Parameter	Description	References	Value	Unit	F	S	SA
BW	Body weight	ICRP (2002) (male)	75	kg			✓
HEIGHT	Height	ICRP (2002) (male)	170	cm			✓
HR	Heart rate		70	1/min			✓
HRrest	Heart rate (resting)		70	1/min			✓
COBW	Cardiac output per bodyweight	ICRP (2002); de Simone et al. (1997)	1.548	ml/s/kg		✓	✓
HCT	Hematocrit	Vander (2001); Herman (2016) (upper range male)	0.51	-			
Kp_fo_dxm	Tissue/plasma partition coefficient DXM forearm		10	-	✓		✓
f_shunting_forearm	Shunting in forearm		0.2795	-	✓		
FVgu	Gut fractional tissue volume	Jones and Rowland-Yeo (2013); ICRP (2002)	0.0171	l/kg			✓
FVki	Kidney fractional tissue volume	Jones and Rowland-Yeo (2013); ICRP (2002)	0.0044	l/kg			✓
FVli	Liver fractional tissue volume	Jones and Rowland-Yeo (2013); ICRP (2002)	0.021	l/kg		✓	✓
FVlu	Lung fractional tissue volume	Jones and Rowland-Yeo (2013); ICRP (2002)	0.0076	l/kg			✓
FVsp	Spleen fractional tissue volume	Jones and Rowland-Yeo (2013); ICRP (2002)	0.0026	l/kg			✓
FVpa	Pancreas fractional tissue volume	Jones and Rowland-Yeo (2013); ICRP (2002)	0.01	l/kg			✓
FVfo	Fore arm fractional tissue volume		0.0048	l/kg	✓		✓
FVve	Venous fractional tissue volume	Jones and Rowland-Yeo (2013); ICRP (2002)	0.0514	l/kg			✓
FVar	Arterial fractional tissue volume	Jones and Rowland-Yeo (2013); ICRP (2002)	0.0257	l/kg			✓
FVpo	Portal fractional tissue volume	Jones and Rowland-Yeo (2013); ICRP (2002)	0.001	l/kg			✓
FQgu	Gut fractional tissue blood flow	Jones and Rowland-Yeo (2013)	0.146	-			✓
FQki	Kidney fractional tissue blood flow	Jones and Rowland-Yeo (2013)	0.19	-			✓
FQh	Hepatic (venous side) fractional tissue blood flow	Jones and Rowland-Yeo (2013)	0.215	-			
FQlu	Lung fractional tissue blood flow	Jones and Rowland-Yeo (2013)	1	-			✓
FQsp	Spleen fractional tissue blood flow	Jones and Rowland-Yeo (2013)	0.017	-			✓
FQfo	Fore arm fractional tissue blood flow	RNAO (2022)	0.0146	-			✓
FQpa	Pancreas fractional tissue blood flow	ICRP (2002)	0.017	-			✓
ftissue_dxm	Vmax tissue distribution DXM		1000	l/min	✓		✓
Kp_dxm	Tissue/plasma partition coefficient DXM		8.7346	-	✓	✓	✓
Ka_dis_dxm	DXM rate of dissolution and stomach passage		0.0217	1/hr	✓		✓
Mr_dxo	Molecular weight DXO	CHEBI:29133	257.3707	g/mole			
ftissue_dxo	Vmax tissue distribution DXO		100	l/min	✓		✓
Kp_dxo	Tissue/plasma partition coefficient DXO		4	-	✓		✓
Mr_dxo_glu	Molecular weight DXO_glu	CHEBI:32645	433.4948	g/mole			
ftissue_dxo_glu	Vmax tissue distribution DXO_glu		3	l/min	✓		✓
Kp_dxo_glu	Tissue/plasma partition coefficient DXO_glu		0.08	-	✓		✓
KI__DXMEX_k	DXM urinary excretion rate		0.017	1/min	✓		✓
KI__DXOEX_k	DXO urinary excretion rate		0.3	1/min	✓		✓
KI__DXOGLUEX_k	DXO glucuronide urinary excretion rate		10	1/min	✓		✓
LI__DXMCYP2D6_Vmax	DXM CYP2D6 Vmax		0.003	mmol/min/l	✓		✓

(Continued on following page)

TABLE 2 (*Continued*) Model parameters in PBPK model of DXM. The complete information is available from the model repository. The prefixes GU__, LI__,KI__, correspond to the intestine/gut, liver, and kidneys, respectively. Values are either adopted from the references or fitted (F). During the robustness analysis of UCMR, various parameters were scanned (S) and a local sensitivity (SA) was performed, see Section 3.5.

Parameter	Description	References	Value	Unit	F	S	SA
LI__DXMCYP2D6_Km	DXM CYP2D6 Km	Storelli et al. (2019a); Yang et al. (2012)	0.0079	mM			✓
LI__cyp2d6_ac	CYP2D6 activity score		0.0–3.0	-			✓
LI__lambda_1	Slope of Km by principal component regression of (Km, Vmax) in log space	Storelli et al. (2019a); Yang et al. (2012)	-0.4	-	✓		
LI__DXMCYP3A4_Vmax	Vmax of DXO formation by CYP3A4		0.0004	mmol/min/l	✓		✓
LI__DXMCYP3A4_Km	Km of DXO formation by CYP3A4	Yu and Haining (2001)	0.157	mM			✓
LI__DXOUGT_Vmax	DXO UGT Vmax (glucuronidation)		0.8953	mmol/min/l	✓		✓
LI__DXOUGT_Km	DXO UGT Km (glucuronidation)	Lutz and Isoherranen (2012)	0.69	mM			✓
GU__F_dxm	Fraction absorbed DXM	Schadel et al. (1995)	0.55	-			✓
GU__Ka_abs_dxm	Ka_abs absorption DXM		3.4285	1/hr	✓		✓
GU__DXMCYP3A4_Vmax	DXM CYP3A4 Vmax		0.0002	mmol/min/l	✓		✓
GU__DXMCYP3A4_Km	DXM CYP3A4 Km	Kerry et al. (1994); Yu and Haining (2001)	0.7	mM			✓
PODOSE	DXM oral dose			mg		✓	✓

3.2 PBPK model of DXM

Within this work, a PBPK model was developed (Figure 1) to study the role of CYP2D6 polymorphism on DXM pharmacokinetics and metabolic phenotyping with DXM. Important model parameters are provided in Table 2. The model is organized hierarchically, with the top layer representing the whole body (Figure 1A) consisting of the liver, kidney, intestine, forearm, lung, and the rest compartment. Organs with minor relevance are not modeled explicitly and lumped into the rest compartment. Organs are coupled *via* the systemic circulation. DXM can be administered intravenously (IV) or orally (PO) with DXM appearing in the venous blood or intestine, respectively. The intestinal model (Figure 1B) describes dissolution, absorption and excretion of DXM. Only a fraction of DXM is absorbed, with the remainder excreted in the feces. DXM enters the circulatory system by crossing the enterocytes of the intestinal wall. First pass metabolism of DXM *via* CYP3A4 N-demethylation in the intestine reduces the amount of DXM appearing in the systemic circulation. In the liver model (Figure 1C), DXM gets transformed *via* O-demethylation to DXO and subsequently transformed to DXO-Glu. The reactions are modeled by Michaelis Menten kinetics and characterized with K_m and V_{max} values. O-demethylation takes place *via* CYP3A4 and CYP2D6. The K_m and V_{max} of CYP2D6 is modulated *via* the AS, details can be found in Section 3.3. The kidney model (Figure 1D) describes the urinary excretion of DXM, DXO, and DXO-Glu.

The model allows to predict concentrations and amounts of DXM, DXO, and DXO-Glu depending on CYP2D6 polymorphism, CYP2D6 diplotype, and CYP2D6 AS with amounts and concentrations of DXM, DXO, and DXO-Glu being evaluated in urine or the median cubital vein (plasma).

To our knowledge, this is the first freely accessible, reproducible, and reusable PBPK model of DXM with the model available in SBML from https://github.com/matthiaskoenig/dextromethorphan-model.

3.3 CYP3A4 and CYP2D6 variability

Cytochrome P450 enzymes exhibit enormous inter-individual variability in enzyme activity. To account for this variability a stochastic model of CYP2D6 and CYP3A4 activity based on bivariate lognormal distributions of K_m and V_{max} was developed and fitted to experimental data from human liver microsomes (Yang et al., 2012; Storelli et al., 2019a) (see Figure 2).

For the CYP2D6 model, the V_{max} is assumed to be linearly related to the AS with $AS = 0$ having no CYP2D6 activity. The dispersion of K_m and V_{max} are assumed to be constant for all activity scores. For the mixture model, the frequencies of the individual activity scores P (AS) are adopted from our curated dataset (i.e., relative amount of subjects with reported activity scores and UCMRs). With increasing AS the maximal reaction velocity (V_{max}) of DXM conversion *via* CYP2D6 increases as does the affinity for the substrate DXM (K_m decreases). The

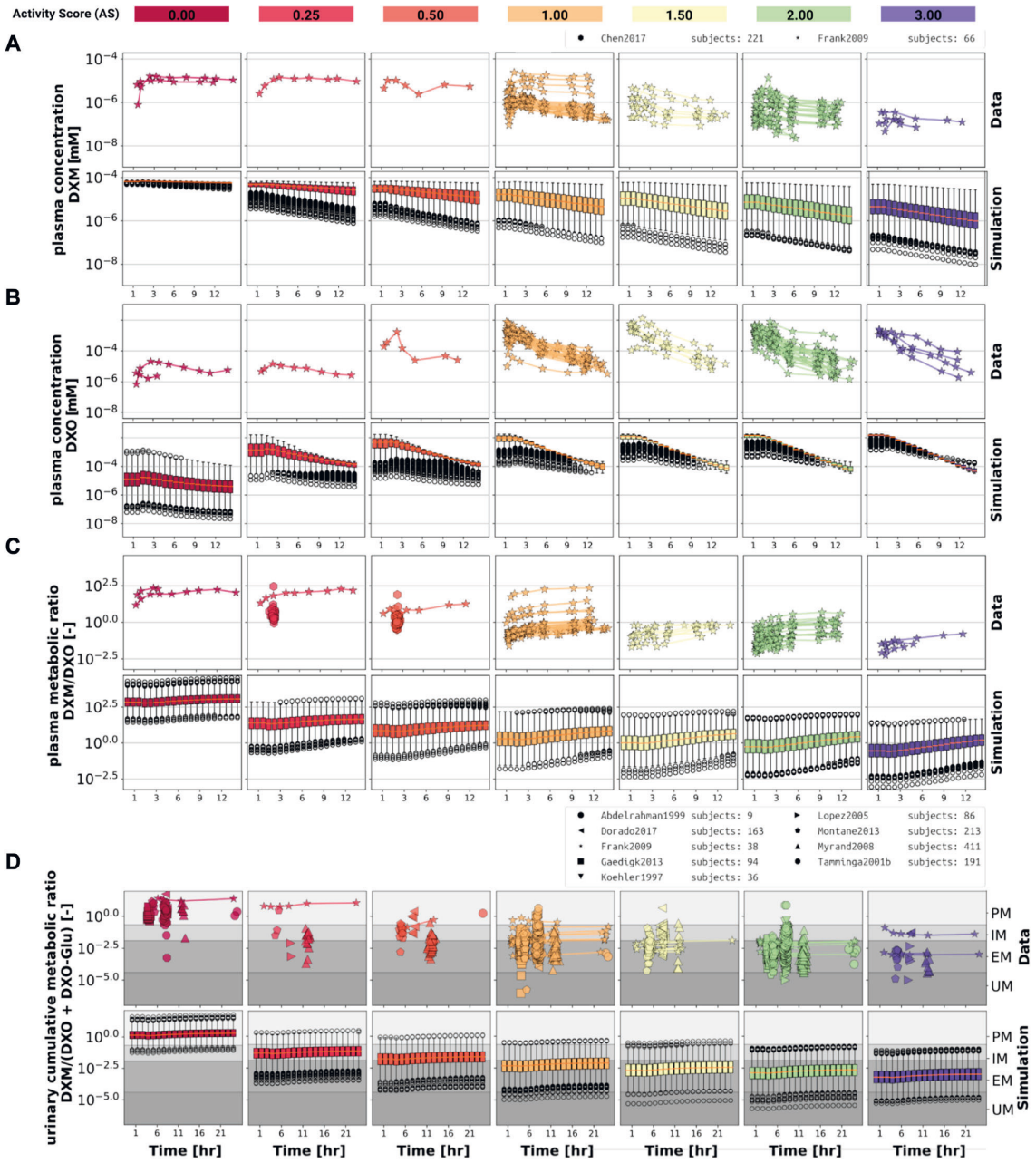

FIGURE 3
Time-dependency of DXM pharmacokinetics by activity score. **(A)** DXM plasma concentration, **(B)** DXO plasma concentration, **(C)** DXM/DXO plasma ratio, **(D)** UCMR (DXM/(DXO + DXO-Glu) in urine). Depicted is a subset of data in which 30 mg of DXM was applied orally. The upper rows in the panels depict the data in healthy adults from (Köhler et al., 1997; Abdelrahman et al., 1999; Tamminga et al., 2001; López et al., 2005; Myrand et al., 2008; Frank, 2009; Gaedigk, 2013; Montané Jaime et al., 2013; Chen et al., 2017; Dorado et al., 2017). Cocktail studies are included. Studies containing coadminstrations with established drug-drug interactions are excluded. The lower rows depict the respective simulation results. To visualize the large variability in the simulation box plots showing the quartiles along side the median and outliers for selected time points are used. Variables changed in the simulation are the CYP3A4 and CYP2D6 reaction parameters K_m and V_{max} according to the distributions in Figure 2. For the different activity scores the respective CYP2D6 activity score model was used.

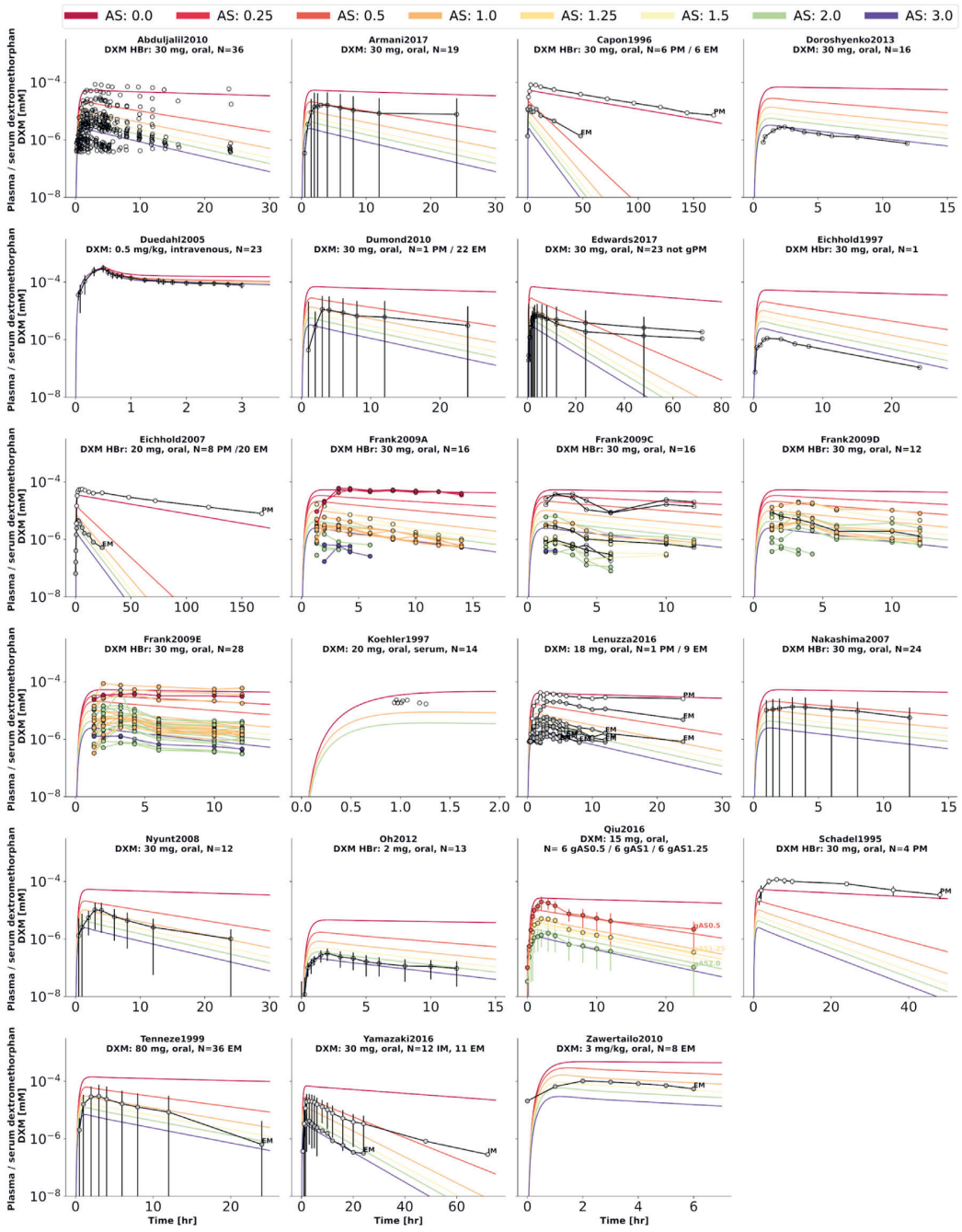

FIGURE 4
Dextromethorphan (DXM) concentration in plasma or serum. Studies were simulated according to the reported dosing protocol. In case of available activity score information the clinical data is color coded accordingly. Information on metabolizer phenotype (UM, EM, IM, PM) is provided where reported. Data from (Schadel et al., 1995; Capon et al., 1996; Eichhold et al., 1997, 2007; Köhler et al., 1997; Tennezé et al., 1999; Duedahl et al., 2005; Nakashima et al., 2007; Nyunt et al., 2008; Frank, 2009; Abduljalil et al., 2010; Dumond et al., 2010; Zawertailo et al., 2010; Oh et al., 2012; Doroshyenko et al., 2013; Lenuzza et al., 2016; Qiu et al., 2016; Armani et al., 2017; Edwards et al., 2017; Yamazaki et al., 2017).

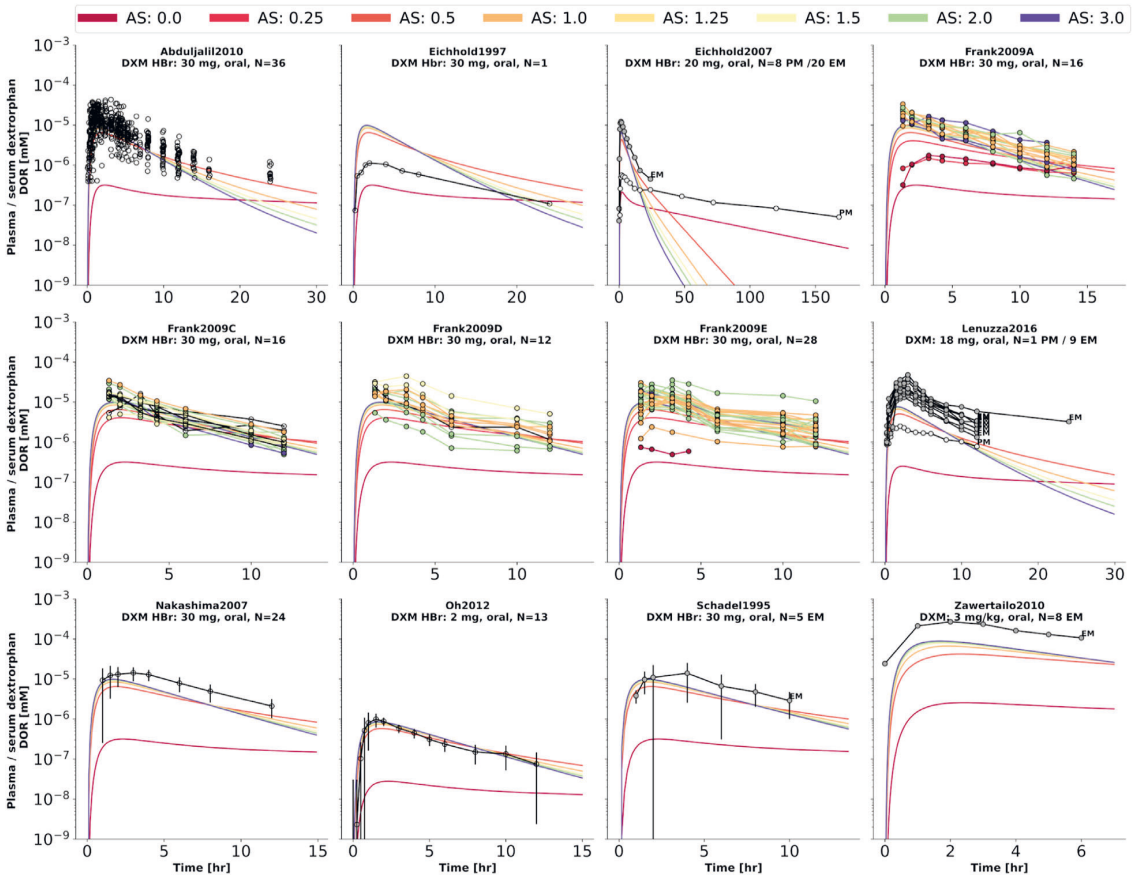

FIGURE 5
Dextrorphan (DXO) concentration in plasma or serum. Studies were simulated according to the reported dosing protocol. In case of available activity score information the clinical data is color coded accordingly. Information on metabolizer phenotype (UM, EM, IM, PM) is provided where reported. Data from (Schadel et al., 1995; Eichhold et al., 1997, 2007; Nakashima et al., 2007; Frank, 2009; Abduljalil et al., 2010; Zawertailo et al., 2010; Oh et al., 2012; Lenuzza et al., 2016).

models of CYP3A4 and CYP2D6 are capable of reproducing the data from the literature, but limited information on CYP2D6 genetics within the data hinders the validation of the AS-specific model.

As motivated in the introduction, even subjects carrying no functional variant of the CYP2D6 gene do metabolize DXM to DXO, however extremely slow. This was implemented in the model *via* a secondary O-demethylation *via* CYP3A4 with mean K_m for DXM adopted from Yu and Haining (2001). The dispersion of K_m and V_{max} is assumed to be identical to the one measured by midazolam in Storelli et al. (2019a) and Yang et al. (2012).

The resulting CYP3A4 and CYP2D6 enzyme model was coupled to the PBPK model and allowed to account (i) for the variability in DXM pharmacokinetics due to the variability in CYPs parameters and (ii) the effect of the AS on CYP2D6 activity and consequently DXM pharmacokinetics.

3.4 Effect of CYP2D6 activity score on DXM pharmacokinetics

Model performance was visually assessed for common pharmacokinetic measurements (i.e., DXM, DXO, DXM/DXO in plasma, and DXM/(DXO + DXO-Glu) in urine) and for subjects with reported AS or diplotype (Figure 3). For each AS, a virtual population based on 2,000 K_m and V_{max} samples was created from the stochastic models of CYP3A4 and CYP2D6 model. For every AS, an oral application of 30 mg DXM was simulated and compared to the corresponding data.

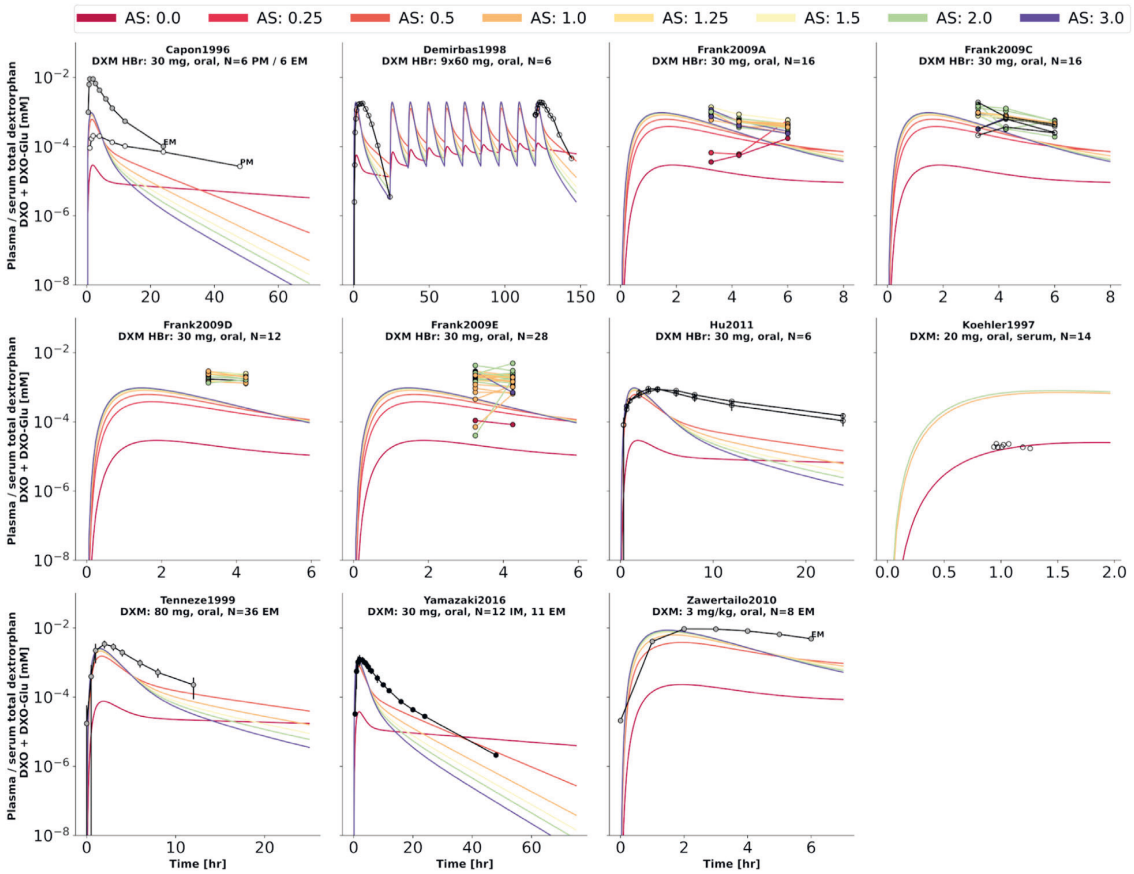

FIGURE 6
Total dextrorphan (DXO + DXO-Glu) concentration in plasma or serum. Studies were simulated according to the reported dosing protocol. In case of available activity score information the clinical data is color coded accordingly. Information on metabolizer phenotype (UM, EM, IM, PM) is provided where reported. Data from (Capon et al., 1996; Köhler et al., 1997; Demirbas et al., 1998; Tennezé et al., 1999; Zawertailo et al., 2010; Hu et al., 2011; Yamazaki et al., 2017).

The model predicts large relative variance within a AS group as well as across different AS groups. With increasing AS, and consequently CYP2D6 activity, plasma DXM decreases (Figure 3A), plasma DXO increases (Figure 3B) and the plasma DXM/DXO decreases (Figure 3C) in very good agreement with the data (Frank, 2009; Chen et al., 2017). The large variability within a AS group is a consequence of the large variability of K_m and V_{max} in CYP2D6 activity of a single AS (see Figure 2). The large overlap between distributions of adjacent AS results in a large overlap in the pharmacokinetics between neighboring AS.

The UCMR (Figure 3D) is very stable over time with a good agreement with the data. With increasing AS, the UCMR decreases and thereby shifts from PM *via* IM to the EM metabolic phenotype. The UCMR data was pooled independently of the amount of applied DXM (in contrast to A-C only using data from 30 mg oral application) and compared to the simulation as the UCMR endpoint is very robust against the given dose (see Section 3.5).

Overall the model predictions of DXM pharmacokinetics depending on AS are in very good agreement with the available data despite the limited availability of pharmacokinetics timecourses for the low AS 0, 0.25, and 0.5.

To further evaluate the model performance, simulations were compared to pharmacokinetics data for DXM in plasma or serum (Figure 4), DXO in plasma or serum (Figure 5), and DXO + DXO-Glu in plasma or serum (Figure 6), DXM in urine (Figure 7), DXO + DXO-Glu in urine (Figure 8), and the UCMR (Figure 9). With expected variability in mind, the model is capable to reproduce all data from the pharmacokinetics dataset. Minor shortcomings of the model are faster kinetics of DXO + DXO-Glu in plasma (Figure 6).

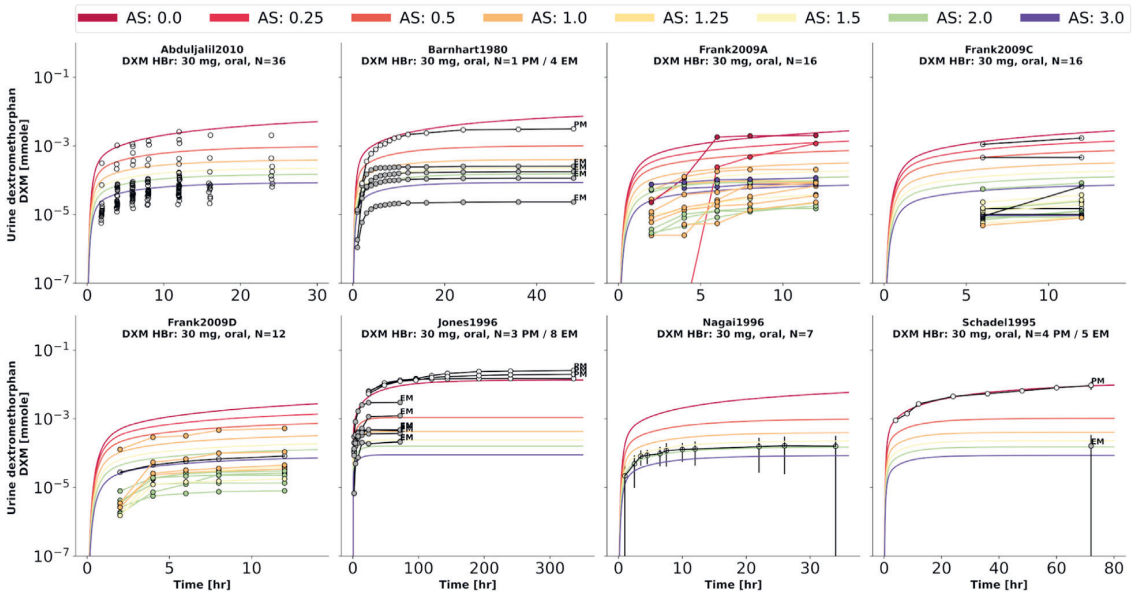

FIGURE 7
Dextromethorphan (DXM) amount in urine. Studies were simulated according to the reported dosing protocol. In case of available activity score information the clinical data is color coded accordingly. Information on metabolizer phenotype (UM, EM, IM, PM) is provided where reported. Data from (Barnhart, 1980; Schadel et al., 1995; Jones et al., 1996; Nagai et al., 1996; Frank, 2009; Abduljalil et al., 2010).

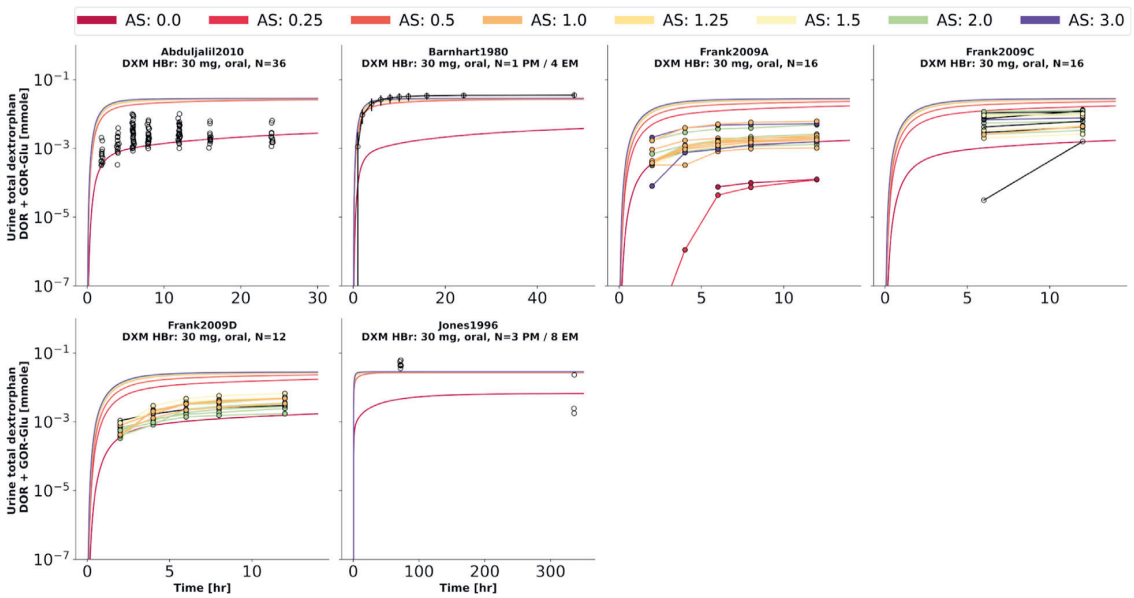

FIGURE 8
Total dextrorphan (DXO + DXO-Glu) amount in urine. Studies were simulated according to the reported dosing protocol. In case of available activity score information the clinical data is color coded accordingly. Information on metabolizer phenotype (UM, EM, IM, PM) is provided where reported. Data from (Barnhart, 1980; Jones et al., 1996; Frank, 2009; Abduljalil et al., 2010).

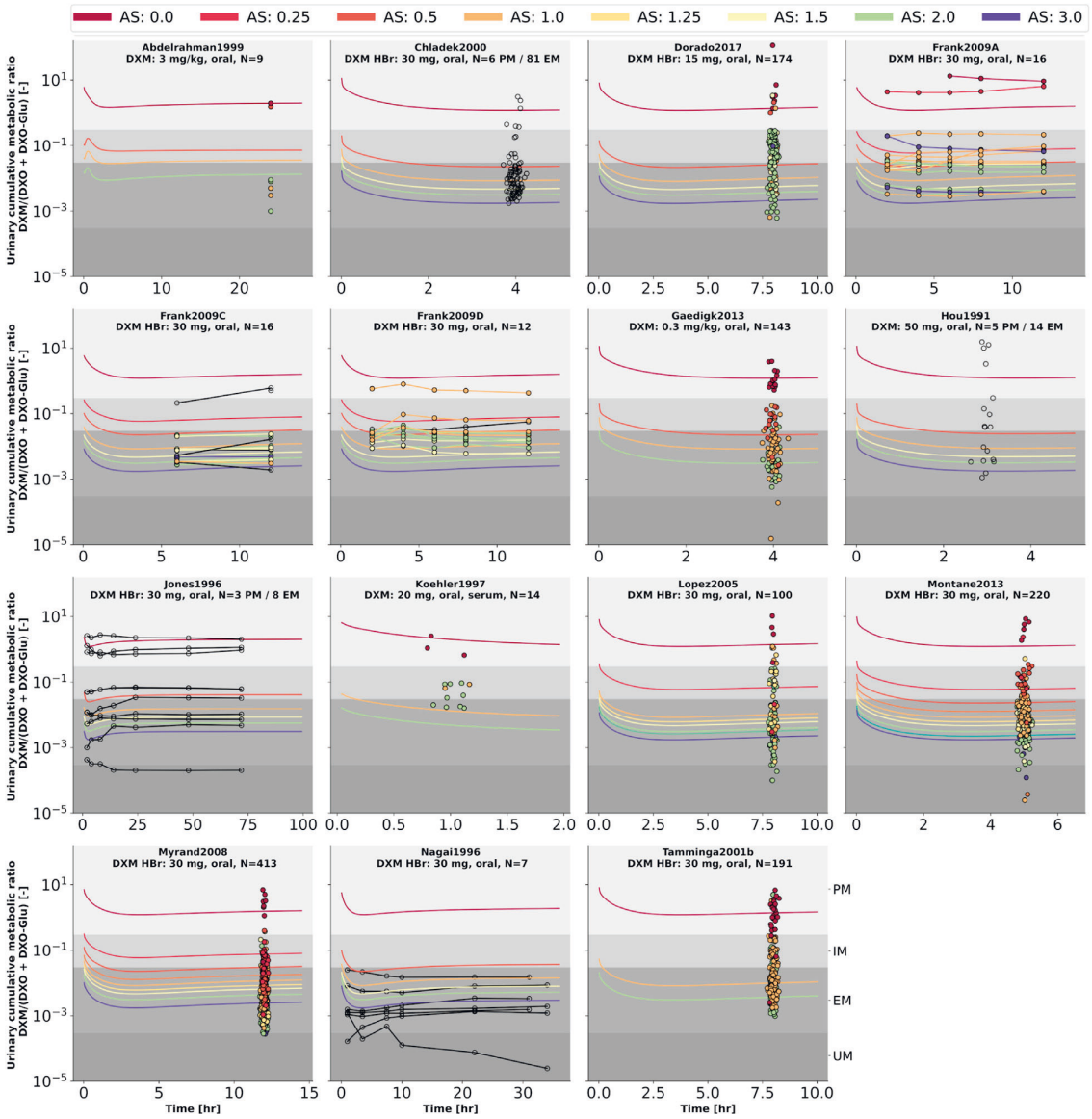

FIGURE 9
Cumulative metabolic ratio between dextormethorphan and total dextrorphan (DXM/(DXO + DXO-GLU)) in urine (UCMR). Studies were simulated according to the reported dosing protocol. In case of available activity score information the clinical data is color coded accordingly. Information on metabolizer phenotype (UM, EM, IM, PM) is provided where reported. Data from (Hou et al., 1991; Jones et al., 1996; Nagai et al., 1996; Köhler et al., 1997; Abdelrahman et al., 1999; Chládek et al., 2000; Tamminga et al., 2001; López et al., 2005; Myrand et al., 2008; Frank, 2009; Gaedigk, 2013; Montané Jaime et al., 2013; Dorado et al., 2017). The metabolic phenotype definitions for UM, EM, IM, PM are depicted as gray areas.

3.5 Effect of parameters on metabolic phenotyping *via* UCMR

Analysis of the effect of parameter changes on UCMR is highly relevant as it can help to identify potential confounding factors and bias in UCMR based phenotyping. Of special importance is the question if there is a dependency on the genetic polymorphism (activity score) of these effects.

To answer this question, model parameters (i.e., liver volume, cardiac output, tissue-to-plasma partition coefficient of DXM, and

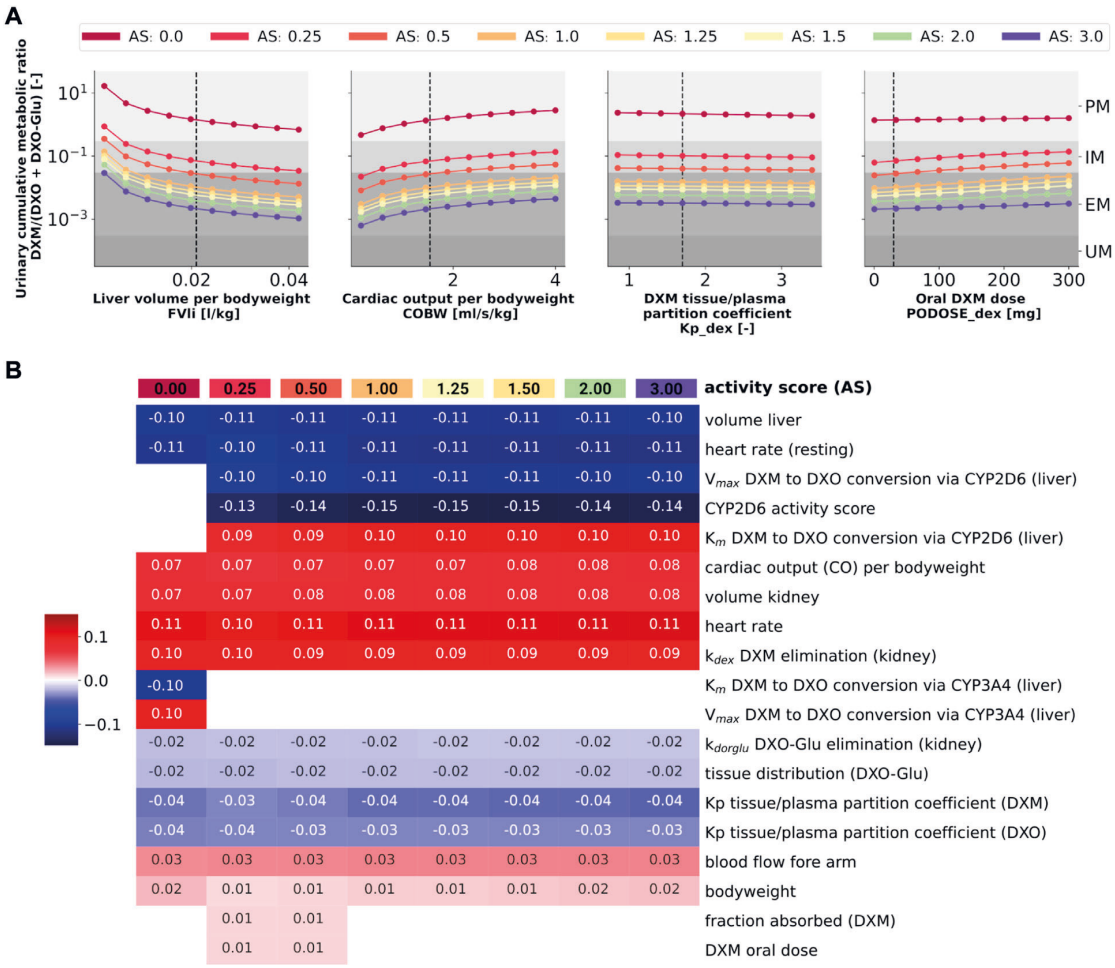

FIGURE 10
Sensitivity analysis of UCMR by activity score. **(A)** Dependency of UCMR (urinary cumulative ratio of DXM/(DXO-Glu) after 8 h and 30 mg oral DXM) on selected physiological parameters and the DXM dose. Parameter scans were performed for all activity scores. Reference model parameters are depicted as dashed lines. **(B)** Sensitivity analysis of model parameters. To systematically study the effect of parameter changes the local sensitivity of UCMR were calculated for all activity scores. Parameters were varied 10% in both direction around the reference parameter value and the relative change of UCMR was calculated (insensitive parameters with relative change of UCMR smaller than 1% were omitted). Positive sensitivities are depicted in red, negative sensitivities in blue. Parameters were sorted *via* agglomerative clustering. Representative parameters of the clusters (i.e., liver volume per bodyweight, cardiac output per bodyweight, DXM tissue/plasma partition coefficient, and oral DXM dose) are depicted in A. The local sensitivity for the activity scores in B corresponds to the normalized slope at the dashed lines in A.

oral dose) were changed in reasonable ranges and the effect on UCMR at 8 h after the application of 30 mg of DXM was investigated (Figure 10A). Independent of the AS, UCMR increased with increasing liver volume and decreased with increasing cardiac output. A change in the tissue-to-plasma partition coefficient of DXM or the amount of oral DXM barely affected the UCMR.

CYP2D6 phenotyping by UCMR is very stable over time as demonstrated in the time course predictions (see 3D and Figure 9) and robust against changes in factors related to the intervention protocol (i.e. dosing amount of DXM, dissolution rate) and to some extent against changes in physiological parameters (see local sensitivity analysis of UCMR in Figure 10B).

Liver volume, heart rate, cardiac output, kidney volume, and kidney elimination rate of DXM altered the UCMR with a similar magnitude as the CYP2D6 reaction parameters. However, the biological variation in these physiological

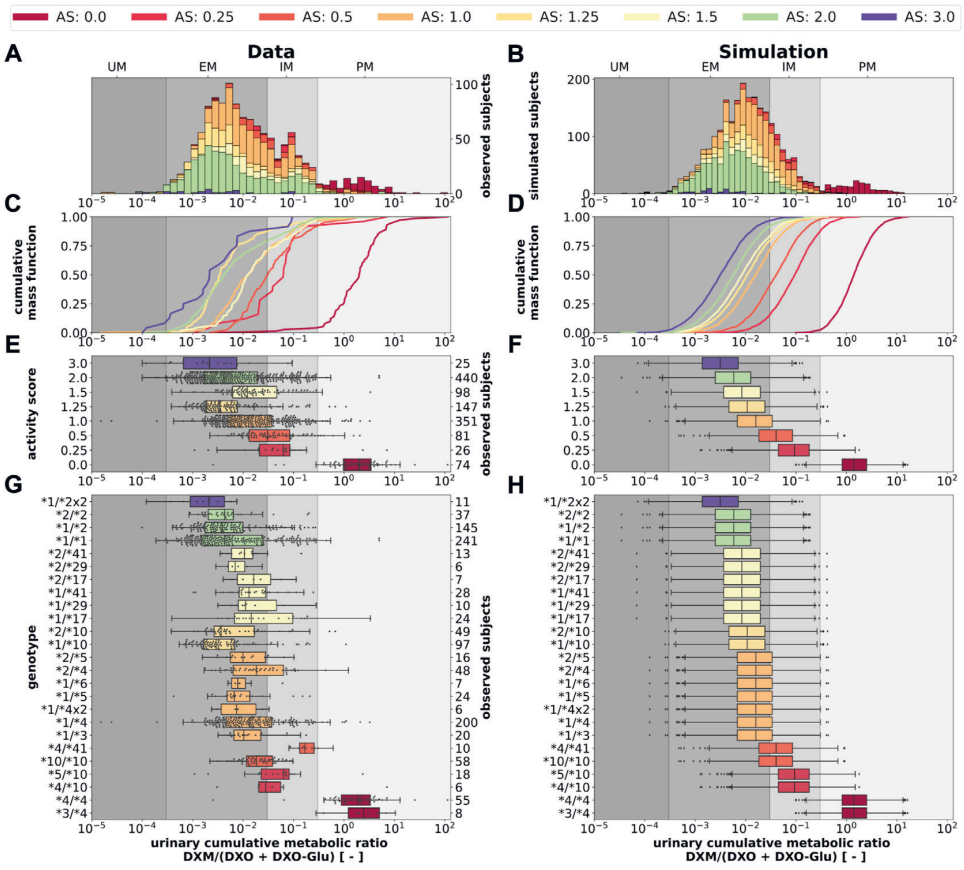

FIGURE 11
CYP2D6 genotype-, activity score association of the UCMR. Simulation of urinary cumulative ratio of DXM/(DXO-Glu) (UCMR) based on activity score frequencies. UCMR data was measured at least 4 h after the application of DXM (hydrobromide) in healthy adults. Cocktail studies were included in the analysis. Studies containing coadminstrations with established drug-drug interactions were excluded. The ranges for metabolic phenotypes (UM, EM, IM, PM) are depicted as gray shaded areas. For timecourse UCMRs, only the latest measurement after administration was included. Data from (Köhler et al., 1997; Abdelrahman et al., 1999; Tamminga et al., 2001; López et al., 2005; Myrand et al., 2008; Frank, 2009; Gaedigk, 2013; Montané Jaime et al., 2013; Dorado et al., 2017). **(A)** Histogram of UCMR data stratified by CYP2D6 activity score. **(B)** Corresponding simulation results (UCMR at 8 h) from the Monte Carlo simulation with random variables being the enzyme reaction parameter (i.e., K_m, V_{max}). See details in Figure 2. **(C)** Empirical CMFs stratified by the activity scores. **(D)** Corresponding simulated CMFs stratified by CYP2D6 activity scores. **(E)** Box plots of observed UCMRs stratified by CYP2D6 activity scores. **(F)** Box plots of simulated UCMRs stratified by the activity scores. **(G)** Box plots of observed UCMRs stratified by CYP2D6 diplotypes. **(H)** Box plots of simulated UCMRs stratified by CYP2D6 diplotypes. For D, F, and H, 2,000 samples were simulated for each activity score whereas in B and D a two-fold oversampling with the CYP2D6 activity score frequencies from the UCMR data was performed.

parameters is orders of magnitude lower. The sensitivity analysis showed no effect of UGT $V_{\max}$ and K_m on UCMR which is the reason why inter-individual variability of UGT activity was not further investigated in this work. Local sensitivity of UCMR was almost identical at different AS values for almost all parameters, i.e., the effect of physiological parameters is of similar relative magnitude independent of AS. For AS = 0, our model assumptions of minor DXM metabolism by CYP3A4 lead to UCMR not being modulated by CYP2D6 but rather by CYP3A4 activity. Nonetheless, even across studies with non-standardized intervention protocols the UCMR measurements seam to be a good but not perfect endpoint to quantify and compare CYP2D6 enzyme activity. Importantly, our analysis indicates that UCMR measurements can be pooled even across investigations with different intervention protocols (as for instance performed in Figure 3D). This still may lead to biases and errors, e.g., due to differences in the quantification protocol.

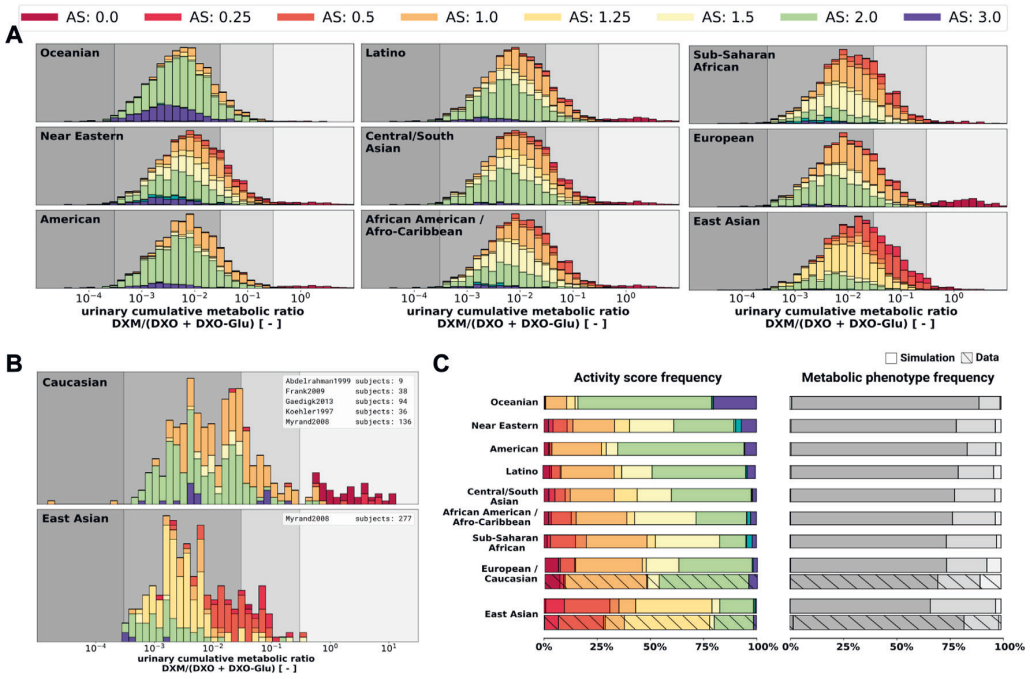

FIGURE 12
UCMR distributions for biogeographical populations **(A)** Simulated UCMR distributions at 8 h for various biogeographical populations based on reported CYP2D6 activity score frequencies as reported in PharmGKB (Whirl-Carrillo et al., 2021). Frequencies are provided in the Supplementary Table S2. **(B)** Reported UCMRs depending on activity score for Caucasians and East Asians from (Köhler et al., 1997; Abdelrahman et al., 1999; Myrand et al., 2008; Frank, 2009; Gaedigk, 2013). Cocktail studies were included in the analysis. Studies containing coadminstrations with established drug-drug interactions were excluded. **(C)** Simulated activity score frequency and metabolic phenotype frequency for the populations and comparison with data for Caucasian and East Asian populations (hatched bars).

3.6 Effect of CYP2D6 polymorphisms and activity score on UCMR

Next, we tested if the model is able to predict UCMR distributions for given genotypes and AS (Figure 11). Model predictions based on underlying genotype frequencies were compared with the experimental data. UCMR distributions for individual AS groups are well described by the model. The AS impacts the UCMR, with increasing AS resulting in an decrease in UCMR. However, individual AS distributions heavily overlap, as expected, due to the large overlap in CYP2D6 parameter distributions between different AS. The predicted distributions tend to be slightly narrower than the actual data. Possible reasons are many fold (e.g., omitted physiological variation, omitted variation in UGT activity, difficulties in correct genotype assignment, unknown effect modifiers, and biases).

The AS system could be refined to better describe the data. The categorization of CYP2D6 genotypes into discrete activity values (i.e., 0, 0.25, 0.5, 1) is an oversimplification, a continuous activity score would probably perform better. The model and data indicate that gUM ($AS \geq 3$) is a very unreliable predictor for ultra rapid metabolism and only gPMs ($AS = 0$) are almost perfectly distinguishable from other metabolizers, see Figures 11C–F.

Another strength of the presented model is that it enables the prediction of the *in vivo* phenotype of subjects based on *in vitro* data.

3.7 Population variability in UCMR

Finally, the model was also capable to predict UCMR distributions for different biogeographical populations (Figure 12) based on the underlying AS frequencies (Supplementary Table S2). Based on the reported frequencies, the UCMR distributions were simulated at 8 h after the application of 30 mg DXM for Oceanian, Near Eastern, American, Latino, Central/South Asian, African American/Afro-Caribbean, Sub-Saharan African, European, and East Asian populations (Figure 12A). Data for Caucasian and East Asian populations (Figure 12B) was used for validation of the predictions (Figure 12C). The data is in good agreement with measurements of Caucasians and East Asians as reported by

Abdelrahman et al. (1999); Frank (2009); Gaedigk (2013); Köhler et al. (1997); Myrand et al. (2008).

4 Discussion

During the last 20 years various modeling approaches and software solutions were utilized to investigate various aspects of DXM pharmacokinetics, e.g., using GastroPlus (Bolger et al., 2019), P-Pharm (Moghadamnia et al., 2003), SAS (Ito et al., 2010; Chiba et al., 2012), SimCYP (Dickinson et al., 2007; Ke et al., 2013; Sager et al., 2014; Chen et al., 2016; Rougée et al., 2016; Adiwidjaja et al., 2018; Storelli et al., 2019b; Machavaram et al., 2019), MATLAB (Kim et al., 2017), or PK-Sim (Rüdesheim et al., 2022). However, most of the work is difficult/impossible to validate or to build up on due to a lack of accessibility of models and software, and platform-dependency of the models. Here, we provide an openly accessible, reproducible and platform-independent whole-body model of DXM metabolism, which facilitates reusability, extensibility, and comparability.

Apart from that, modeling work which aims for high empirical evidence relies on trustworthy supporting real world data. More and independent sources of data are highly beneficial for the scientific outcomes. For that matter, guidelines like PRISMA for reporting transparency, completeness, and accuracy find very broad endorsement in the field of systematic reviews and meta analysis. The present work faces somewhat similar challenges for the evaluation and selection of data from literature. Therefore, PRISMA-ScR guidelines were adopted where applicable. With this approach, bias within the used dataset could be mitigated or at least identified. Importantly, we supplement our open and accessible model with a large, open, and accessible database of pharmacokinetics data.

The presented PBPK model is able to predict the DXM metabolism of populations and individuals based on their CYP2D6 genotype. It is probably the first model capable to predict individual UCMRs and the expected distributions of UCMR. Moreover, it can reproduce a broad range of reported clinical data on DXM and enables better intuition on how to interpret DXM related pharmacokinetics. E.g., an important message is that CYP2D6 activity is not the only modulator of UCMR, as can be seen by the large variability in activity score and overlap between activity scores. UCMR as a proxy of CYP2D6 metabolic phenotype should therefore be interpreted carefully. The model shows that for extremely low CYP2D6 activity the UCMR is not primarily governed by the CYP2D6 activity. This is consistent with the finding that CYP2D6 inhibition merely affects PMs (Pope et al., 2004).

The current version of the model is already very valuable, still there is plenty of room for improvement. By providing the data and model in open and standardized formats we enable and encourage these improvements by model extensions and updates.

Many of the physiological parameters in the model were fitted or estimated although they could be measured in principle. E.g., relatively low DXM concentrations in plasma suggest substantial extra-vascular binding of DXM. However, tissue-plasma partition coefficients (Kp) are difficult to assess and only limited data is available. Steinberg et al. (1996) reported brain levels to be 68-fold higher and cerebrospinal fluid levels 4-fold lower than serum levels, respectively. Others estimated Kp ~ 1.65 from n-octanol-water partition coefficients and again others suggested additional trapping mechanisms (i.e. lysosomal trapping) (Bolger et al., 2019). In the model, the DXO-Glu kinetics is a bit to rapid (see Figure 6), probably due to the decision to model tissue distribution uniformly for all organs (i.e., identical K_p and f_{tissue}). We decided for a more parsimonious model. Glucuronides, however, are generally much more polar than their respective non-glucuronides which result in less plasma binding, higher urinary excretion, lower lipid-solubility, and higher water-solubility. Transport into different tissues is affected differently by polarity.

Most important for model improvements would be additional *in vitro* measurements on the association between CYP2D6 genotype and phenotype which are very limited in literature (Storelli et al., 2019a; Ning et al., 2019; Dalton et al., 2020). Furthermore, simultaneous *in vitro* and UCMR measurements do not exist the literature. Both would be very important for the validation of the AS system and the development of new models which e.g. take into account structural variation (Dalton et al., 2020). For instance, with the AS system alone it is not possible to explain why CYP2D6 is inhibited differently for different genotypes Qiu et al. (2016).

In conclusion, we developed and validated a PBPK model of DXM and applied it to study the effect of the CYP2D6 polymorphism on metabolic phenotyping.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding author. All clinical data of dextromethorphan pharmacokinetics that was used in this work can be found in PK-DB available from https://pk-db.com.

Author contributions

JG and MK designed the study, developed the computational model, implemented and performed the analysis, and wrote the initial draft of the manuscript. JB

provided support with data curation. All authors discussed the results. All authors contributed to and revised the manuscript critically.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphar.2022.1029073/full#supplementary-material

References

Abdelrahman, S., Gotschall, R., Kauffman, R., Leeder, J., and Kearns, G. (1999). Investigation of terbinafine as a CYP2D6 inhibitor *in vivo*. *Clin. Pharmacol. Ther.* 65, 465–472. doi:10.1016/S0009-9236(99)70065-2

Abduljalil, K., Frank, D., Gaedigk, A., Klaassen, T., Tomalik-Scharte, D., Jetter, A., et al. (2010). Assessment of activity levels for CYP2D6*1, CYP2D6*2, and CYP2D6*41 genes by population pharmacokinetics of dextromethorphan. *Clin. Pharmacol. Ther.* 88, 643–651. doi:10.1038/clpt.2010.137

Adiwidjaja, J., Boddy, A. V., and McLachlan, A. J. (2018). A strategy to refine the phenotyping approach and its implementation to predict drug clearance: A physiologically based pharmacokinetic simulation study. *CPT. Pharmacometrics Syst. Pharmacol.* 7, 798–808. doi:10.1002/psp4.12355

Armani, S., Ting, L., Sauter, N., Darstein, C., Tripathi, A. P., Wang, L., et al. (2017). Drug interaction potential of osilodrostat (LCI699) based on its effect on the pharmacokinetics of probe drugs of cytochrome P450 enzymes in healthy adults. *Clin. Drug Investig.* 37, 465–472. doi:10.1007/s40261-017-0497-0

Barnhart, J. W. (1980). The urinary excretion of dextromethorphan and three metabolites in dogs and humans. *Toxicol. Appl. Pharmacol.* 55, 43–48. doi:10.1016/0041-008X(80)90218-5

Berm, E. J. J., Risselada, A. J., Mulder, H., Hak, E., and Wilffert, B. (2013). Phenoconversion of cytochrome P450 2D6: The need for identifying the intermediate metabolizer genotype. *J. Clin. Psychiatry* 74, 1025. doi:10.4088/JCP.13lr08555

Bolger, M. B., Macwan, J. S., Sarfraz, M., Almukainzi, M., and Löbenberg, R. (2019). The irrelevance of *in vitro* dissolution in setting product specifications for drugs like dextromethorphan that are subject to lysosomal trapping. *J. Pharm. Sci.* 108, 268–278. doi:10.1016/j.xphs.2018.09.036

Capon, D. A., Bochner, F., Kerry, N., Mikus, G., Danz, C., and Somogyi, A. A. (1996). The influence of CYP2D6 polymorphism and quinidine on the disposition and antitussive effect of dextromethorphan in humans. *Clin. Pharmacol. Ther.* 60, 295–307. doi:10.1016/S0009-9236(96)90056-9

Caudle, K. E., Sangkuhl, K., Whirl-Carrillo, M., Swen, J. J., Haidar, C. E., Klein, T. E., et al. (2020). Standardizing *CYP2D6* genotype to phenotype translation: Consensus recommendations from the clinical pharmacogenetics implementation consortium and Dutch pharmacogenetics working group. *Clin. Transl. Sci.* 13, 116–124. doi:10.1111/cts.12692

Chen, R., Rostami-Hodjegan, A., Wang, H., Berk, D., Shi, J., and Hu, P. (2016). Application of a physiologically based pharmacokinetic model for the evaluation of single-point plasma phenotyping method of CYP2D6. *Eur. J. Pharm. Sci.* 92, 131–136. doi:10.1016/j.ejps.2016.07.001

Chen, R., Zheng, X., and Hu, P. (2017). CYP2D6 phenotyping using urine, plasma, and saliva metabolic ratios to assess the impact of CYP2D6·10 on interindividual variation in a Chinese population. *Front. Pharmacol.* 8, 239. doi:10.3389/fphar.2017.00239

Chiba, K., Kato, M., Ito, T., Suwa, T., and Sugiyama, Y. (2012). Inter-individual variability of *in vivo* CYP2D6 activity in different genotypes. *Drug Metab. Pharmacokinet.* 27, 405–413. doi:10.2133/dmpk.dmpk-11-rg-078

Chládek, J., Zimová, G., Beránek, M., and Martínková, J. (2000). *In-vivo* indices of CYP2D6 activity: Comparison of dextromethorphan metabolic ratios in 4-h urine and 3-h plasma. *Eur. J. Clin. Pharmacol.* 56, 651–657. doi:10.1007/s002280000218

Dalton, R., Lee, S.-B., Claw, K. G., Prasad, B., Phillips, B. R., Shen, D. D., et al. (2020). Interrogation of CYP2D6 structural variant alleles improves the correlation between CYP2D6 genotype and CYP2D6-mediated metabolic activity. *Clin. Transl. Sci.* 13, 147–156. doi:10.1111/cts.12695

De Kesel, P. M. M., Lambert, W. E., and Stove, C. P. (2016). Alternative sampling strategies for cytochrome P450 phenotyping. *Clin. Pharmacokinet.* 55, 169–184. doi:10.1007/s40262-015-0306-y

de Simone, G., Devereux, R. B., Daniels, S. R., Mureddu, G., Roman, M. J., Kimball, T. R., et al. (1997). Stroke volume and cardiac output in normotensive children and adults. Assessment of relations with body size and impact of overweight. *Circulation* 95, 1837–1843. doi:10.1161/01.cir.95.7.1837

Demirbas, S., Reyderman, L., and Stavchansky, S. (1998). Bioavailability of dextromethorphan (as dextrorphan) from sustained release formulations in the presence of guaifenesin in human volunteers. *Biopharm. Drug Dispos.* 19, 541–545.

Dickinson, G. L., Rezaee, S., Proctor, N. J., Lennard, M. S., Tucker, G. T., and Rostami-Hodjegan, A. (2007). Incorporating *in vitro* information on drug metabolism into clinical trial simulations to assess the effect of CYP2D6 polymorphism on pharmacokinetics and pharmacodynamics: Dextromethorphan as a model application. *J. Clin. Pharmacol.* 47, 175–186. doi:10.1177/0091270006294279

Dorado, P., González, I., Naranjo, M. E. G., de Andrés, F., Peñas-Lledó, E. M., Calzadilla, L. R., et al. (2017). Lessons from Cuba for global precision medicine: *CYP2D6* genotype is not a robust predictor of CYP2D6 ultrarapid metabolism. *OMICS A J. Integr. Biol.* 21, 17–26. doi:10.1089/omi.2016.0166

Doroshyenko, O., Rokitta, D., Zadoyan, G., Klement, S., Schläfke, S., Dienel, A., et al. (2013). Drug cocktail interaction study on the effect of the orally administered lavender oil preparation silexan on cytochrome P450 enzymes in healthy volunteers. *Drug Metab. Dispos.* 41, 987–993. doi:10.1124/dmd.112.050203

Duedahl, T. H., Dirks, J., Petersen, K. B., Romsing, J., Larsen, N.-E., and Dahl, J. B. (2005). Intravenous dextromethorphan to human volunteers: Relationship between pharmacokinetics and anti-hyperalgesic effect. *Pain* 113, 360–368. doi:10.1016/j.pain.2004.11.015

Dumond, J. B., Vourvahis, M., Rezk, N. L., Patterson, K. B., Tien, H.-C., White, N., et al. (2010). A phenotype–genotype Approach to predicting CYP450 and P-glycoprotein drug interactions with the mixed inhibitor/inducer tipranavir/ritonavir. *Clin. Pharmacol. Ther.* 87, 735–742. doi:10.1038/clpt.2009.253

Edwards, J. E., Eliot, L., Parkinson, A., Karan, S., and MacConell, L. (2017). Assessment of pharmacokinetic interactions between obeticholic acid and caffeine, midazolam, warfarin, dextromethorphan, omeprazole, rosuvastatin, and digoxin in phase 1 studies in healthy subjects. *Adv. Ther.* 34, 2120–2138. doi:10.1007/s12325-017-0601-0

Eichelbaum, M., Spannbrucker, N., Steincke, B., and Dengler, H. J. (1979). Defective N-oxidation of sparteine in man: A new pharmacogenetic defect. *Eur. J. Clin. Pharmacol.* 16, 183–187. doi:10.1007/BF00562059

Eichhold, T. H., Greenfield, L. J., Hoke, S. H., and Wehmeyer, K. R. (1997). Determination of dextromethorphan and dextrorphan in human plasma by liquid chromatography/tandem mass spectrometry. *J. Mass Spectrom.* 32, 1205–1211. doi:10.1002/(SICI)1096-9888(199711)32:11¡1205::AID-JMS579¿3.0.CO;2-C

Eichhold, T. H., McCauley-Myers, D. L., Khambe, D. A., Thompson, G. A., and Hoke, S. H. (2007). Simultaneous determination of dextromethorphan, dextrorphan, and guaifenesin in human plasma using semi-automated liquid/liquid extraction and gradient liquid chromatography tandem mass spectrometry. *J. Pharm. Biomed. Anal.* 43, 586–600. doi:10.1016/j.jpba.2006.07.018

Frank, D. (2009). "Evaluation of pharmacokinetic metrics for phenotyping of the human CYP2D6 enzyme with dextromethorphan," (Germany: Rheinische Friedrich-Wilhelms-Universität Bonn). Ph.D. thesis.

Frank, D., Jaehde, U., and Fuhr, U. (2007). Evaluation of probe drugs and pharmacokinetic metrics for CYP2D6 phenotyping. *Eur. J. Clin. Pharmacol.* 63, 321–333. doi:10.1007/s00228-006-0250-8

Fuhr, U., Jetter, A., and Kirchheiner, J. (2007). Appropriate phenotyping procedures for drug metabolizing enzymes and transporters in humans and their simultaneous use in the "cocktail" approach. *Clin. Pharmacol. Ther.* 81, 270–283. doi:10.1038/sj.clpt.6100050

Gaedigk, A. (2013). Complexities of *CYP2D6* gene analysis and interpretation. *Int. Rev. Psychiatry* 25, 534–553. doi:10.3109/09540261.2013.825581

Gaedigk, A., Dinh, J., Jeong, H., Prasad, B., and Leeder, J. (2018a). Ten years' experience with the CYP2D6 activity score: A perspective on future investigations to improve clinical predictions for precision therapeutics. *J. Pers. Med.* 8, 15. doi:10.3390/jpm8020015

Gaedigk, A., Eklund, J. D., Pearce, R. E., Leeder, J. S., Alander, S. W., Phillips, M. S., et al. (2007). Identification and characterization of CYP2D6*56B, an allele associated with the poor metabolizer phenotype. *Clin. Pharmacol. Ther.* 81, 817–820. doi:10.1038/sj.clpt.6100125

Gaedigk, A., Ingelman-Sundberg, M., Miller, N. A., Leeder, J. S., Whirl-Carrillo, M., Klein, T. E., et al. (2018b). The pharmacogene variation (PharmVar) consortium: Incorporation of the human cytochrome P450 (CYP) allele nomenclature database. *Clin. Pharmacol. Ther.* 103, 399–401. doi:10.1002/cpt.910

Gaedigk, A., Sangkuhl, K., Whirl-Carrillo, M., Klein, T., and Leeder, J. S. (2017). Prediction of CYP2D6 phenotype from genotype across world populations. *Genet. Med.* 19, 69–76. doi:10.1038/gim.2016.80

Gaedigk, A., Turner, A., Everts, R. E., Scott, S. A., Aggarwal, P., Broeckel, U., et al. (2019). Characterization of reference materials for genetic testing of CYP2D6 alleles: A GeT-RM collaborative project. *J. Mol. Diagn.* 21, 1034–1052. doi:10.1016/j.jmoldx.2019.06.007

Gasche, Y., Daali, Y., Fathi, M., Chiappe, A., Cottini, S., Dayer, P., et al. (2004). Codeine intoxication associated with ultrarapid CYP2D6 metabolism. *N. Engl. J. Med.* 351, 2827–2831. doi:10.1056/NEJMoa041888

Gonzalez Hernandez, F., Carter, S. J., Iso-Sipilä, J., Goldsmith, P., Almousa, A. A., Gastine, S., et al. (2021). An automated approach to identify scientific publications reporting pharmacokinetic parameters. *Wellcome Open Res.* 6, 88. doi:10.12688/wellcomeopenres.16718.1

Grzegorzewski, J., Bartsch, F., Köller, A., and König, M. (2022). Pharmacokinetics of caffeine: A systematic analysis of reported data for application in metabolic phenotyping and liver function testing. *Front. Pharmacol.* 12, 752826. doi:10.3389/fphar.2021.752826

Grzegorzewski, J., and König, M. (2022). Physiologically based pharmacokinetic (PBPK) model of dextromethorphan. bibkey Grzegorzewski2022_dex_model. doi:10.5281/zenodo.7025683

Grzegorzewski, J., Brandhorst, J., Green, K., Eleftheriadou, D., Duport, Y., Barthorscht, F., et al. (2021). PK-DB: Pharmacokinetics database for individualized and stratified computational modeling. *Nucleic Acids Res.* 49, D1358–D1364. doi:10.1093/nar/gkaa990

Herman, I. P. (2016). *Physics of the human body*. Charm: Springer.

Hou, Z.-Y., Pickle, L. W., Meyer, P. S., and Woosley, R. L. (1991). Salivary analysis for determination of dextromethorphan metabolic phenotype. *Clin. Pharmacol. Ther.* 49, 410–419. doi:10.1038/clpt.1991.48

Hu, L., Li, L., Yang, X., Liu, W., Yang, J., Jia, Y., et al. (2011). Floating matrix dosage form for dextromethorphan hydrobromide based on gas forming technique: *In vitro* and *in vivo* evaluation in healthy volunteers. *Eur. J. Pharm. Sci.* 42, 99–105. doi:10.1016/j.ejps.2010.10.010

Hucka, M., Bergmann, F. T., Chaouiya, C., Dräger, A., Hoops, S., Keating, S. M., et al. (2019). The systems biology markup language (SBML): Language specification for level 3 version 2 core release 2. *J. Integr. Bioinform.* 16, 0021. doi:10.1515/jib-2019-0021

Hurtado, I., García-Sempere, A., Peiró, S., and Sanfélix-Gimeno, G. (2020). Increasing trends in opioid use from 2010 to 2018 in the region of valencia, Spain: A real-world, population-based study. *Front. Pharmacol.* 11, 612556. doi:10.3389/fphar.2020.612556

ICRP (2002). Basic anatomical and physiological data for use in radiological protection: Reference values. A report of age- and gender-related differences in the anatomical and physiological characteristics of reference individuals. ICRP Publication 89. *Ann. ICRP* 32, 5–265.

Ito, T., Kato, M., Chiba, K., Okazaki, O., and Sugiyama, Y. (2010). Estimation of the interindividual variability of cytochrome 2D6 activity from urinary metabolic ratios in the literature. *Drug Metab. Pharmacokinet.* 25, 243–253. doi:10.2133/dmpk.25.243

Jones, D. R., Gorski, J. C., Haehner, B. D., O'Mara, E. M., and Hall, S. D. (1996). Determination of cytochrome P450 3A4/5 activity *in vivo* with dextromethorphan N-demethylation. *Clin. Pharmacol. Ther.* 60, 374–384. doi:10.1016/S0009-9236(96)90194-0

Jones, H., and Rowland-Yeo, K. (2013). Basic concepts in physiologically based pharmacokinetic modeling in drug discovery and development. *CPT. Pharmacometrics Syst. Pharmacol.* 2, e63. doi:10.1038/psp.2013.41

Kawanishi, C., Lundgren, S., Agren, H., and Bertilsson, L. (2004). Increased incidence of CYP2D6 gene duplication in patients with persistent mood disorders: Ultrarapid metabolism of antidepressants as a cause of nonresponse. A pilot study. *Eur. J. Clin. Pharmacol.* 59, 803–807. doi:10.1007/s00228-003-0701-4

Ke, A. B., Nallani, S. C., Zhao, P., Rostami-Hodjegan, A., Isoherranen, N., and Unadkat, J. D. (2013). A physiologically based pharmacokinetic model to predict disposition of CYP2D6 and CYP1A2 metabolized drugs in pregnant women. *Drug Metab. Dispos.* 41, 801–813. doi:10.1124/dmd.112.050161

Keating, S. M., Waltemath, D., König, M., Zhang, F., Dräger, A., Chaouiya, C., et al. (2020). SBML Level 3: An extensible format for the exchange and reuse of biological models. *Mol. Syst. Biol.* 16, e9110. doi:10.15252/msb.20199110

Kerry, N., Somogyi, A., Bochner, F., and Mikus, G. (1994). The role of CYP2D6 in primary and secondary oxidative metabolism of dextromethorphan: *In vitro* studies using human liver microsomes. *Br. J. Clin. Pharmacol.* 38, 243–248. doi:10.1111/j.1365-2125.1994.tb04348.x

Kibaly, C., Alderete, J. A., Liu, S. H., Nasef, H. S., Law, P.-Y., Evans, C. J., et al. (2021). Oxycodone in the opioid epidemic: High 'liking', 'wanting', and abuse liability. *Cell. Mol. Neurobiol.* 41, 899–926. doi:10.1007/s10571-020-01013-y

Kim, E.-Y., Shin, S.-G., and Shin, J.-G. (2017). Prediction and visualization of CYP2D6 genotype-based phenotype using clustering algorithms. *Transl. Clin. Pharmacol.* 25, 147–152. doi:10.12793/tcp.2017.25.3.147

Köhler, D., Härtter, S., Fuchs, K., Sieghart, W., and Hiemke, C. (1997). CYP2D6 genotype and phenotyping by determination of dextromethorphan and metabolites in serum of healthy controls and of patients under psychotropic medication. *Pharmacogenetics* 7, 453–461. doi:10.1097/00008571-199712000-00003

König, M., Dräger, A., and Holzhütter, H.-G. (2012). CySBML: A cytoscape plugin for sbml. *Bioinforma. Oxf. Engl.* 28, 2402–2403. doi:10.1093/bioinformatics/bts432

König, M. (2021a). Matthiaskoenig/sbmlsim: 0.1.14 - SBML simulation made easy. Available at: https://zenodo.org/record/5531088. [Accessed: 2022-08-23].

König, M., and Rodriguez, N. (2019). Matthiaskoenig/cy3sbml: Cy3sbml-v0.3.0 - SBML for cytoscape. Available at: https://zenodo.org/record/3451319. [Accessed: 2022-08-23].

König, M. (2021b). Sbmlutils: Python utilities for SBML. Available at: https://zenodo.org/record/6599299. [Accessed: 2022-08-23].

Lenuzza, N., Duval, X., Nicolas, G., Thévenot, E., Job, S., Videau, O., et al. (2016). Safety and pharmacokinetics of the CIME combination of drugs and their metabolites after a single oral dosing in healthy volunteers. *Eur. J. Drug Metab. Pharmacokinet.* 41, 125–138. doi:10.1007/s13318-014-0239-0

López, M., Guerrero, J., Jung–Cook, H., and Alonso, M. E. (2005). CYP2D6 genotype and phenotype determination in a Mexican Mestizo population. *Eur. J. Clin. Pharmacol.* 61, 749–754. doi:10.1007/s00228-005-0038-2

Lutz, J. D., and Isoherranen, N. (2012). Prediction of relative *in vivo* metabolite exposure from *in vitro* data using two model drugs: Dextromethorphan and omeprazole. *Drug Metab. Dispos.* 40, 159–168. doi:10.1124/dmd.111.042200

Machavaram, K. K., Endo-Tsukude, C., Terao, K., Gill, K. L., Hatley, O. J., Gardner, I., et al. (2019). Simulating the impact of elevated levels of interleukin-6 on the pharmacokinetics of various CYP450 substrates in patients with neuromyelitis optica or neuromyelitis optica spectrum disorders in different ethnic populations. *AAPS J.* 21, 42. doi:10.1208/s12248-019-0309-y

Mahgoub, A., Idle, J. R., Dring, L. G., Lancaster, R., and Smith, R. L. (1977). Polymorphic hydroxylation of Debrisoquine in man. *Lancet (London, Engl.* 2, 584–586. doi:10.1016/s0140-6736(77)91430-1

McGinnity, D. F., Parker, A. J., Soars, M., and Riley, R. J. (2000). Automated definition of the enzymology of drug oxidation by the major human drug metabolizing cytochrome P450s. *Drug Metab. Dispos.* 28, 1327–1334.

Moghadamnia, A. A., Rostami-Hodjegan, A., Abdul-Manap, R., Wright, C. E., Morice, A. H., and Tucker, G. T. (2003). Physiologically based modelling of inhibition of metabolism and assessment of the relative potency of drug and metabolite: Dextromethorphan vs . dextrorphan using quinidine inhibition: Dextromethorphan vs. dextrorphan using quinidine inhibition. *Br. J. Clin. Pharmacol.* 56, 57–67. doi:10.1046/j.1365-2125.2003.01853.x

Montané Jaime, L. K., Lalla, A., Steimer, W., and Gaedigk, A. (2013). Characterization of the *CYP2D6* gene locus and metabolic activity in Indo- and Afro-Trinidadians: Discovery of novel allelic variants. *Pharmacogenomics* 14, 261–276. doi:10.2217/pgs.12.207

Myrand, S., Sekiguchi, K., Man, M., Lin, X., Tzeng, R.-Y., Teng, C.-H., et al. (2008). Pharmacokinetics/genotype Associations for major cytochrome P450 enzymes in native and first- and third-generation Japanese populations: Comparison with Korean, Chinese, and caucasian populations. *Clin. Pharmacol. Ther.* 84, 347–361. doi:10.1038/sj.clpt.6100482

Nagai, N., Kawakubo, T., Kaneko, F., Ishii, M., Shinohara, R., Saito, Y., et al. (1996). Pharmacokinetics and polymorphic oxidation of dextromethorphan in a Japanese population. *Biopharm. Drug Dispos.* 17, 421–433.

Nakashima, D., Takama, H., Ogasawara, Y., Kawakami, T., Nishitoba, T., Hoshi, S., et al. (2007). Effect of cinacalcet hydrochloride, a new calcimimetic agent, on the pharmacokinetics of dextromethorphan: *In vitro* and clinical studies. *J. Clin. Pharmacol.* 47, 1311–1319. doi:10.1177/0091270007304103

Ning, M., Duarte, J. D., Stevison, F., Isoherranen, N., Rubin, L. H., and Jeong, H. (2019). Determinants of cytochrome P450 2D6 mRNA levels in healthy human liver tissue. *Clin. Transl. Sci.* 12, 416–423. doi:10.1111/cts.12632

Nofziger, C., and Paulmichl, M. (2018). Accurately genotyping *CYP2D6* : Not for the faint of heart. *Pharmacogenomics* 19, 999–1002. doi:10.2217/pgs-2018-0105

Nyunt, M. M., Becker, S., MacFarland, R. T., Chee, P., Scarborough, R., Everts, S., et al. (2008). Pharmacokinetic effect of AMD070, an oral CXCR4 antagonist, on CYP3A4 and CYP2D6 substrates midazolam and dextromethorphan in healthy volunteers. *J. Acquir. Immune Defic. Syndr.* 47, 559–565. doi:10.1097/QAI.0b013e3181627566

Oh, K.-S., Park, S.-J., Shinde, D. D., Shin, J.-G., and Kim, D.-H. (2012). High-sensitivity liquid chromatography-tandem mass spectrometry for the simultaneous determination of five drugs and their cytochrome P450-specific probe metabolites in human plasma. *J. Chromatogr. B Anal. Technol. Biomed. Life Sci.* 895, 56–64. doi:10.1016/j.jchromb.2012.03.014

Pope, L. E., Khalil, M. H., Berg, J. E., Stiles, M., Yakatan, G. J., and Sellers, E. M. (2004). Pharmacokinetics of dextromethorphan after single or multiple dosing in combination with quinidine in extensive and poor metabolizers. *J. Clin. Pharmacol.* 44, 1132–1142. doi:10.1177/0091270004269521

Preskorn, S. H., Kane, C. P., Lobello, K., Nichols, A. I., Fayyad, R., Buckley, G., et al. (2013). Cytochrome P450 2D6 phenoconversion is common in patients being treated for depression: Implications for personalized medicine. *J. Clin. Psychiatry* 74, 614–621. doi:10.4088/JCP.12m07807

Qiu, F., Liu, S., Miao, P., Zeng, J., Zhu, L., Zhao, T., et al. (2016). Effects of the Chinese herbal formula "Zuojin Pill" on the pharmacokinetics of dextromethorphan in healthy Chinese volunteers with CYP2D6*10 genotype. *Eur. J. Clin. Pharmacol.* 72, 689–695. doi:10.1007/s00228-016-2048-7

Rau, T., Wohlleben, G., Wuttke, H., Thuerauf, N., Lunkenheimer, J., Lanczik, M., et al. (2004). CYP2D6 genotype: Impact on adverse effects and nonresponse during treatment with antidepressants-a pilot study. *Clin. Pharmacol. Ther.* 75, 386–393. doi:10.1016/j.clpt.2003.12.015

RNAO (2022). Nursing best practice guideline by nurses' association of ontario: Appendix g: Tools for assessing anxiety, depression, and stress. Available at: https://bpgmobile.rnao.ca/sites/default/files/Appendix%20C%20Vein%20Anatomy.pdf. [Accessed: 2022-08-23].

Rougée, L. R. A., Mohutsky, M. A., Bedwell, D. W., Ruterbories, K. J., and Hall, S. D. (2016). The impact of the hepatocyte-to-plasma pH gradient on the prediction of hepatic clearance and drug-drug interactions for CYP2D6 substrates. *Drug Metab. Dispos.* 44, 1819–1827. –1827. doi:10.1124/dmd.116.071761

Rüdesheim, S., Selzer, D., Fuhr, U., Schwab, M., and Lehr, T. (2022). Physiologically-based pharmacokinetic modeling of dextromethorphan to investigate interindividual variability within CYP2D6 activity score groups. *CPT. Pharmacometrics Syst. Pharmacol.* 11, 494–511. doi:10.1002/psp4.12776

Sager, J. E., Lutz, J. D., Foti, R. S., Davis, C., Kunze, K. L., and Isoherranen, N. (2014). Fluoxetine- and norfluoxetine-mediated complex drug-drug interactions: *In vitro* to *in vivo* correlation of effects on CYP2D6, CYP2C19, and CYP3A4. *Clin. Pharmacol. Ther.* 95, 653–662. doi:10.1038/clpt.2014.50

Saravanakumar, A., Sadighi, A., Ryu, R., and Akhlaghi, F. (2019). Physicochemical properties, biotransformation, and transport pathways of established and newly approved medications: A systematic review of the top 200 most prescribed drugs vs. the FDA-approved drugs between 2005 and 2016. *Clin. Pharmacokinet.* 58, 1281–1294. –1294. doi:10.1007/s40262-019-00750-8

Schadel, M., Wu, D., Otton, S. V., Kalow, W., and Sellers, E. M. (1995). Pharmacokinetics of dextromethorphan and metabolites in humans: Influence of the CYP2D6 phenotype and quinidine inhibition. *J. Clin. Psychopharmacol.* 15, 263–269. doi:10.1097/00004714-199508000-00005

Schoedel, K. A., Pope, L. E., and Sellers, E. M. (2012). Randomized open-label drug-drug interaction trial of dextromethorphan/quinidine and paroxetine in healthy volunteers. *Clin. Drug Investig.* 32, 157–169. doi:10.2165/11599870-000000000-00000

Shah, R. R., and Smith, R. L. (2015). Addressing phenoconversion: The achilles' heel of personalized medicine. *Br. J. Clin. Pharmacol.* 79, 222–240. doi:10.1111/bcp.12441

Silva, A. R., and Dinis-Oliveira, R. J. (2020). Pharmacokinetics and pharmacodynamics of dextromethorphan: Clinical and forensic aspects. *Drug Metab. Rev.* 52, 258–282. doi:10.1080/03602532.2020.1758712

Smith, L. P., Hucka, M., Hoops, S., Finney, A., Ginkel, M., Myers, C. J., et al. (2015). SBML level 3 package: Hierarchical model composition, version 1 release 3. *J. Integr. Bioinform.* 12, 268. doi:10.2390/biecoll-jib-2015-268

Somogyi, E. T., Bouteiller, J.-M., Glazier, J. A., König, M., Medley, J. K., Swat, M. H., et al. (2015). libRoadRunner: A high performance SBML simulation and analysis library. *Bioinforma. Oxf. Engl.* 31, 3315–3321. doi:10.1093/bioinformatics/btv363

Steinberg, G. K., Bell, T. E., and Yenari, M. A. (1996). Dose escalation safety and tolerance study of the N-methyl-d-aspartate antagonist dextromethorphan in neurosurgery patients. *J. Neurosurg.* 84, 860–866. doi:10.3171/jns.1996.84.5.0860

Storelli, F., Desmeules, J., and Daali, Y. (2019a). Genotype-sensitive reversible and time-dependent CYP2D6 inhibition in human liver microsomes. *Basic Clin. Pharmacol. Toxicol.* 124, 170–180. doi:10.1111/bcpt.13124

Storelli, F., Desmeules, J., and Daali, Y. (2019b). Physiologically-based pharmacokinetic modeling for the prediction of CYP2D6-mediated gene–drug–drug interactions. *CPT. Pharmacometrics Syst. Pharmacol.* 8, 567–576. doi:10.1002/psp4.12411

Strauch, K., Lutz, U., Bittner, N., and Lutz, W. K. (2009). Dose–response relationship for the pharmacokinetic interaction of grapefruit juice with dextromethorphan investigated by human urinary metabolite profiles. *Food Chem. Toxicol.* 47, 1928–1935. doi:10.1016/j.fct.2009.05.004

Takashima, T., Murase, S., Iwasaki, K., and Shimada, K. (2005). Evaluation of dextromethorphan metabolism using hepatocytes from CYP2D6 poor and extensive metabolizers. *Drug Metab. Pharmacokinet.* 20, 177–182. doi:10.2133/dmpk.20.177

Tamminga, W., Wemer, J., Oosterhuis, B., de Zeeuw, R., de Leij, L., and Jonkman, J. (2001). The prevalence of CYP2D6 and CYP2C19 genotypes in a population of healthy Dutch volunteers. *Eur. J. Clin. Pharmacol.* 57, 717–722. doi:10.1007/s002280100359

Taylor, C. P., Traynelis, S. F., Siffert, J., Pope, L. E., and Matsumoto, R. R. (2016). Pharmacology of dextromethorphan: Relevance to dextromethorphan/quinidine (Nuedexta®) clinical use. *Pharmacol. Ther.* 164, 170–182. doi:10.1016/j.pharmthera.2016.04.010

Tennezé, L., Verstuyft, C., Becquemont, L., Poirier, J. M., Wilkinson, G. R., and Funck-Brentano, C. (1999). Assessment of CYP2D6 and CYP2C19 activity *in vivo*

in humans: A cocktail study with dextromethorphan and chloroguanide alone and in combination. *Clin. Pharmacol. Ther.* 66, 582–588. doi:10.1053/cp.1999.v66.103401001

Tricco, A. C., Lillie, E., Zarin, W., O'Brien, K. K., Colquhoun, H., Levac, D., et al. (2018). PRISMA extension for scoping reviews (PRISMA-ScR): Checklist and explanation. *Ann. Intern. Med.* 169, 467–473. doi:10.7326/M18-0850

Vander, J. S., A. (2001). *Human physiology: The mechanisms of body function*. New York City: McGraw-Hill higher education.

von Moltke, L. L., Greenblatt, D. J., Grassi, J. M., Granda, B. W., Venkatakrishnan, K., Schmider, J., et al. (1998). Multiple human cytochromes contribute to biotransformation of dextromethorphan *in-vitro*: Role of CYP2C9, CYP2C19, CYP2D6, and CYP3A. *J. Pharm. Pharmacol.* 50, 997–1004. doi:10.1111/j.2042-7158.1998.tb06914.x

Welsh, C., Xu, J., Smith, L., König, M., Choi, K., and Sauro, H. M. (2022). libRoadRunner 2.0: A high-performance sbml simulation and analysis library. arxiv. doi:10.48550/arXiv.2203.01175

Whirl-Carrillo, M., Huddart, R., Gong, L., Sangkuhl, K., Thorn, C. F., Whaley, R., et al. (2021). An evidence-based framework for evaluating pharmacogenomics knowledge for personalized medicine. *Clin. Pharmacol. Ther.* 110, 563–572. doi:10.1002/cpt.2350

Wyen, C., Fuhr, U., Frank, D., Aarnoutse, R. E., Klaassen, T., Lazar, A., et al. (2008). Effect of an antiretroviral regimen containing ritonavir boosted lopinavir on intestinal and hepatic CYP3A, CYP2D6 and P-glycoprotein in HIV-infected patients. *Clin. Pharmacol. Ther.* 84, 75–82. doi:10.1038/sj.clpt.6100452

Yamazaki, T., Desai, A., Goldwater, R., Han, D., Howieson, C., Akhtar, S., et al. (2017). Pharmacokinetic effects of isavuconazole coadministration with the cytochrome P450 enzyme substrates bupropion, repaglinide, caffeine, dextromethorphan, and methadone in healthy subjects. *Clin. Pharmacol. Drug Dev.* 6, 54–65. doi:10.1002/cpdd.281

Yang, J., He, M. M., Niu, W., Wrighton, S. A., Li, L., Liu, Y., et al. (2012). Metabolic capabilities of cytochrome P450 enzymes in Chinese liver microsomes compared with those in Caucasian liver microsomes: Hepatic cytochromes P450 of Chinese and Caucasians. *Br. J. Clin. Pharmacol.* 73, 268–284. doi:10.1111/j.1365-2125.2011.04076.x

Yu, A., and Haining, R. L. (2001). Comparative contribution to dextromethorphan metabolism by cytochrome P450 isoforms *in vitro*: Can dextromethorphan be used as a dual probe for both CTP2D6 and CYP3A activities? *Drug Metab. Dispos.* 29, 1514–1520.

Zackrisson, A. L., Lindblom, B., and Ahlner, J. (2010). High frequency of occurrence of CYP2D6 gene duplication/multiduplication indicating ultrarapid metabolism among suicide cases. *Clin. Pharmacol. Ther.* 88, 354–359. doi:10.1038/clpt.2009.216

Zanger, U. M., Raimundo, S., and Eichelbaum, M. (2004). Cytochrome P450 2D6: Overview and update on pharmacology, genetics, biochemistry. *Naunyn. Schmiedeb. Arch. Pharmacol.* 369, 23–37. doi:10.1007/s00210-003-0832-2

Zawertailo, L. A., Tyndale, R. F., Busto, U., and Sellers, E. M. (2010). Effect of metabolic blockade on the psychoactive effects of dextromethorphan. *Hum. Psychopharmacol.* 25, 71–79. doi:10.1002/hup.1086

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Supplementary Material

1 SUPPLEMENTARY TABLES AND FIGURES

Allele	Activity score (AS)
*1	1.0
*1x2	2.0
*2	1.0
*2x2	2.0
*3	0
*4	0
*4x2	0
*5	0
*6	0
*10	0.25
*17	0.5
*29	0.5
*41	0.5

Table S1. CYP2D6 allele-phenotype association. The phenotype of CYP2D6 allele variants are characterized by the activity value adopted from PharmGKB.

Activity Score	Sub-Saharan African	African American & Afro-Caribbean	European	Near Eastern	East Asian	Central & South Asian	American	Latino	Oceanian
0	1.53	2.33	6.47	2.20	0.86	2.34	2.18	3.12	0.38
0.25	1.38	1.17	0.80	2.01	8.10	2.65	0.42	0.93	0.31
0.5	10.90	9.29	6.72	6.34	19.82	4.71	1.03	3.84	0.17
0.75	4.77	2.29	0.41	2.69	3.94	2.24	0.10	0.56	0.04
1	26.45	23.46	31.01	18.82	7.28	19.94	22.04	23.76	9.57
1.25	3.64	3.62	1.81	6.80	33.15	10.35	2.14	3.37	3.90
1.5	28.04	28.43	15.16	19.93	3.45	15.44	5.10	13.67	1.32
2	11.40	23.38	34.03	27.14	14.62	36.09	56.27	42.02	61.14
2.25	0.32	0.22	0.05	0.88	0.69	0.26	0.10	0.15	0.60
2.5	2.44	1.69	0.46	2.56	0.07	0.39	0.24	0.59	0.20
3	1.86	2.68	1.99	6.49	0.60	1.81	5.15	3.57	18.37
4	0.08	0.08	0.03	0.42	0.01	0.02	0.12	0.08	1.41
Phenotype									
UM	0.4	0.4	0.5	0.7	0.3	0.5	0.7	0.7	1.1
EM	73.7	76.6	73.8	78.2	66.3	77.5	83.3	79.1	88.5
IM	23.7	20.2	19.1	18.5	30.9	19.0	13.5	16.8	9.9
PM	2.2	2.7	6.7	2.7	2.6	2.9	2.4	3.5	0.5

Table S2. Proportion [%] of CYP2D6 activity scores and simulated metabolic phenotypes by biogeographical group.

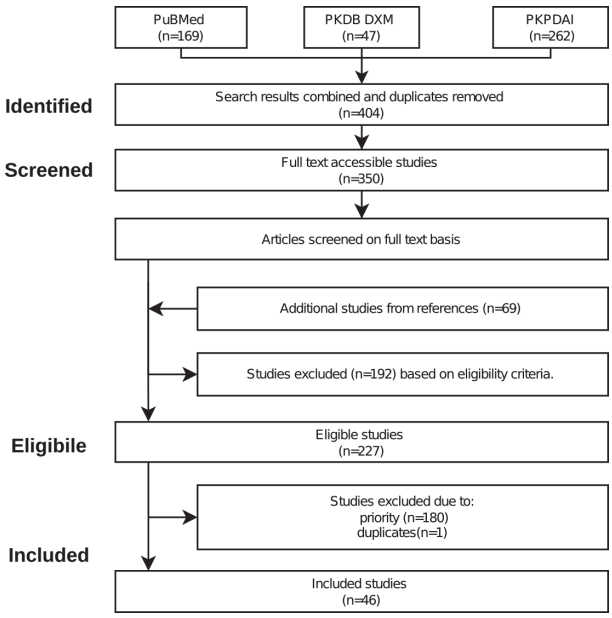

Figure S1. PRISMA flow diagram. Overview of the data selection for the pharmacokinetics dataset used in this work. PuBMed, PKDB, and PKPDAI were utilized for the literature search on DXM pharmacokinetics. Applied eligibility criteria resulted in 227 studies of which 46 were curated for this work. The process is described in detail in the Materials and Methods section.

3.5 Additional Publications and Preprints

Apart from the presented work, significant contributions were made to two publications and a preprint [Kön+21; Köl+21; Köl21], of which I am not the primary author.

Ten Simple Rules for FAIR Sharing of Experimental and Clinical Data with the Modeling Community [Preprint]
König M., **Grzegorzewski J.**, Golebiewski M., Hermjakob H., Hucka M., Olivier B.; Keating S., Nickerson D., Schreiber F., Sheriff R., Waltemath D. Preprints 2021, 2021080303 , doi: 10.20944/preprints202108.0303.v1.

In König et al. [Kön+21], we aim to clarify the best practice for sharing experimental and clinical data with the simulation modeling community. Prior to the preprint, I have seen many publications on pharmacokinetics, have built a database that aims to reflect all important aspects of pharmacokinetics data, and have systematically analyzed shortcomings in the reporting of caffeine pharmacokinetics. Insights from these investigations found their way into the manuscript. In summary, we advocate to:

1. share your data in a human and machine-accessible manner;
2. disseminate license and attribution information with data;
3. store your data in an open and standardized format and check that the format is used correctly;
4. use domain-specific formats and databases;
5. share raw and processed data. Share as much as possible, but not more;
6. add metadata (data about your data) to make data findable and comprehensible;
7. share code and workflows for data processing with the data;
8. archive data and code in a persistent manner accessible via a DOI;
9. disseminate information on experimental and computational methods and protocols with the data;
10. lean back and enjoy the additional credit and citations.

Physiologically Based Modeling of the Effect of Physiological and Anthropometric Variability on Indocyanine Green Based Liver Function Tests
Köller A, **Grzegorzewski J**, König M., Front Physiol. 2021 Nov 22,12:757293, doi: 10.3389/fphys.2021.757293, PMID: 34880776.

In this paper, we used PBPK modeling to analyze the effects of various factors on the elimination of indocyanine green (ICG) by the liver. ICG is a commonly used test compound in dynamic liver function tests. In the study, we could show that factors such as hepatic blood flow, cardiac output, and body weight can affect the elimination of ICG by the liver and contribute to inter-individual variability. The results of the study may help improve the power of ICG-based liver function tests and move toward an individualized evaluation of liver function.

Prediction of Survival After Partial Hepatectomy Using a Physiologically Based Pharmacokinetic Model of Indocyanine Green Liver Function Tests
Köller A, **Grzegorzewski J**, Tautenhahn H-M, König M; . Front Physiol. 2021 Nov 22;12:730418, doi: 10.3389/fphys.2021.730418, PMID: 34880771.

In this paper, we used PBPK modeling to analyze/predict the effect of partial hepatectomy on the elimination of indocyanine green (ICG) by the liver. Without refitting the ICG PKDB model used in [Köl+21], we could accurately predict postoperative ICG pharmacokinetics and individual likelihoods of survival after liver resection.

Chapter 4

Discussion and Outlook

In this work, we established the first open pharmacokinetics database, curated extensive amounts of pharmacokinetics data, and built a pipeline for meta-analysis and PBPK modeling. This pipeline was used to investigate various questions in the context of dynamic liver function testing and CYP phenotyping. The results of this work might translate into the clinics and personalized medicine.

The importance and effectiveness of data and workflow standardization became apparent from work in the thesis. This is true on many layers. On the experimental side, measurements with comparable subject characteristics and intervention protocol are easier to integrate, resulting in bias being less of a concern. Nonetheless, it could also be shown that with PBPK modeling, many of the differences which can lead to changes in pharmacokinetics can be accounted for. Therefore, standardized intervention protocols are desirable but not strictly necessary for PBPK modeling.

Next, there is enormous room for improvement in the reporting of pharmacokinetic data and results. The deficits are quite comprehensively shown in our caffeine meta-analysis. In my experience, the deficits are also broadly present in publications on clinical trials for other test substances and are a general problem in the field of pharmacokinetics. Much of the available data for modeling comes from publications on clinical trials. The data is hidden in tables and figures, and text. The original researchers typically chose a presentation of the data to convey a specific scientific finding. Individual time course data, the gold standard for PBPK modeling, are mostly unnecessary for that purpose. Unsurprisingly, they are barely reported. There is presumably a lack of awareness that such data can find secondary use by the modeling community and help to answer questions the study was not designed for. Hopefully, PK-DB and the corresponding publication can increase such awareness. I imagine that shallow data availability statements can be replaced by links to repositories containing anonymized raw data in future publications on pharmacokinetics trials.

On the modeling side, standardized and programming language agnostic model formats like SBML prove to be very valuable. They enable streamlining the modeling process and make it feasible to reuse, compare, improve, and share mathematical models. Among many advantages, updates on reusable components translate to all models which use the component, and components from PBPK models are particularly well suited to be reused as the physiology remains the same for different subjects. Model annotation with additional information in the form of metadata and the use of persistent identifiers to databases is yet another important piece in the puzzle of standardization. Models without metadata are very difficult to comprehend, e.g., often suffer from cryptic or ambiguous variable names, which results in a lack of reusability.

Our meta-analysis on caffeine solidified the knowledge of influencing factors of caffeine pharmacokinetics. Prior to the analysis, the effect size of numerous factors

remained less clear due to the limited scope and insufficient power of individual clinical trials. A better understanding of how various factors alter caffeine PK is potentially very valuable in the clinics for liver function testing and phenotyping but also for individual caffeine consumption for indulgence. For instance, many people are aware of the potential side effects (e.g., disruption of the sleep/wake cycle) due to excessive caffeine consumption, especially late in the day. Enhancing personal well-being may be possible by adapting caffeine consumption according to individual lifestyle factors, such as smoking habits or the use of oral contraceptives. Many of the lessons learned from the study of caffeine can be directly transferred to the meta-analysis of other substances used in the context of liver function testing.

As part of the work, we established a dextromethorphan model for CYP2D6 phenotyping, which provides many testable predictions with real-world clinical implications. As a next step, the results coming from the PKPB model should be validated experimentally. For that, it would be beneficial to conduct *in vitro* reaction kinetics, CYP2D6 genotyping, and pharmacokinetics measurements in plasma and urine on the same subjects. These types of measurements would be important to improve our understanding of the relationship between the CYP2D6 genotype and CYP2D6-mediated metabolic activity. This could potentially lead to the development of more sophisticated genotype-phenotype association models containing information on the structural variations, see [Dal+20]. Further, the computational model could be validated and improved to be actually used in the clinic or for personalized medicine. A direct application of the model is to better understand the metabolization of other CYP2D6 substances, for instance, of the β-blocker metoprolol in an ongoing project.

The developed modeling pipeline established in this thesis resulted in the development of PBPK models of additional test substances, e.g., codeine, ICG, galactose, omeprazole, chlorzoxazone, metoprolol, pravastatin, simvastatin. Various factors influencing liver function and CYP phenotyping are investigated with the respective models. Similar to the dextromethorphan model, they provide many testable predictions with real-world clinical implications. In combination, they cover many of the enzymes and transporters that strongly influence the pharmacokinetics of commonly used drugs. Exciting is the potential of integrating these models into a holistic model that accounts for more and more factors that affect the pharmacokinetics of a wide range of drugs. Such a model has the potential to be used as a digital pharmacokinetic twin, which would allow the prediction of individual pharmacokinetics of a subject for a large set of drugs. To truly harness the potential of such a model-informed individualized medicine approach, I believe a paradigm shift towards greatly increased availability of large amounts of data is necessary, similar to that which is occurring with the advent of AI. Digital pharmacokinetic twins rely on experimental measurements to parameterize the individual models, e.g., using CYP phenotyping via test substances. Judging from the curated data and the models implemented in this thesis, a large part of the variation in the pharmacokinetics of substances seems to be explainable by the difference in enzyme and/or transporter activities. It seems that the influencing factors (e.g., lifestyle, genetics, ...) alter the pharmacokinetics of many drugs mostly via changes in the CYP and transporter activities. Also, the high variability of CYP2D6 activity between subjects with the same CYP2D6 polymorphism indicates that genotyping cannot replace phenotyping for now and that the genotype is only one factor of many affecting CYP activity.

Thus, CYP (and other enzymes and transporters) phenotyping has great potential to become a standard test and presumably one of the most important measurements to inform personalized medicine in the future. For that matter, the cocktail

approach is a perfect fit. The application of multiple substances to measure multiple enzyme and transporter activities simultaneously can be used to predict the pharmacokinetics of drugs. The cocktail approach is well-established in the clinic. Nevertheless, it does not find an application on a broad scale. There are several challenges for a wider application which makes the measurement more complicated than, e.g., basic metabolic panel testing. One of the challenges is the time required between cocktail administration and the measurement, typically in the range of a few hours. In addition, the quantification is currently quite expensive and time-consuming as they are commonly performed by high-performance liquid chromatography. Finally, individualizing the pharmacokinetics of drugs is no silver bullet in personalized medicine. Pharmacodynamics also plays an important role, especially in the context of drug tolerance.

In summary, in this work, we established the methods and workflows to model cocktail studies and liver function tests which have the potential to have a great impact on personalized medicine and to increase the precision of dynamic liver function tests in the clinics.

Chapter 5

Appendix

5.1 Simple SBML model for liver function testing

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Bibliography

[AC09] Curtis K. Argo and Stephen H. Caldwell. "Epidemiology and Natural History of Non-Alcoholic Steatohepatitis". In: *Clinics in Liver Disease* 13.4 (Nov. 2009), pp. 511–531. ISSN: 1557-8224. DOI: 10.1016/j.cld.2009.07.005.

[Ach+14] Brahim Achour et al. "Simultaneous Quantification of the Abundance of Several Cytochrome P450 and Uridine 5′-Diphospho-Glucuronosyltransferase Enzymes in Human Liver Microsomes Using Multiplexed Targeted Proteomics". In: *Drug Metabolism and Disposition* 42.4 (Apr. 2014), pp. 500–510. ISSN: 0090-9556, 1521-009X. DOI: 10.1124/dmd.113.055632.

[Asr+19] Sumeet K. Asrani et al. "Burden of Liver Diseases in the World". In: *Journal of Hepatology* 70.1 (Jan. 2019), pp. 151–171. ISSN: 1600-0641. DOI: 10.1016/j.jhep.2018.09.014.

[Bak19] Lindsay B. Baker. "Physiology of Sweat Gland Function: The Roles of Sweating and Sweat Composition in Human Health". In: *Temperature: Multidisciplinary Biomedical Journal* 6.3 (July 2019), pp. 211–259. ISSN: 2332-8940. DOI: 10.1080/23328940.2019.1632145.

[Bal21] Sükrü Balci. "Computational Modelling of Omeprazole - Pharmacokinetics and Pharmacodynamics". Bachelor's Thesis. Oct. 2021.

[Bar20] Florian Bartsch. "Computational Modelling of Simvastatin - Effects on HMG-CoA Reductase Activity and Cholesterol Bachelor Thesis". Bachelor's Thesis. Nov. 2020.

[Boy13] James L. Boyer. "Bile Formation and Secretion". In: *Comprehensive Physiology* 3.3 (July 2013), pp. 1035–1078. ISSN: 2040-4603. DOI: 10.1002/cphy.c120027.

[Cie+16] Kasia P. Cieslak et al. "Liver Function Declines with Increased Age". In: *HPB: the official journal of the International Hepato Pancreato Biliary Association* 18.8 (Aug. 2016), pp. 691–696. ISSN: 1477-2574. DOI: 10.1016/j.hpb.2016.05.011.

[Cla+19] Guido Claessen et al. "Heart Rate Reserve in Fontan Patients: Chronotropic Incompetence or Hemodynamic Limitation?" In: *Journal of the American Heart Association* 8 (May 2019). DOI: 10.1161/JAHA.119.012008.

[Dal+20] Rachel Dalton et al. "Interrogation of CYP2D6 Structural Variant Alleles Improves the Correlation Between CYP2D6 Genotype and CYP2D6-Mediated Metabolic Activity." In: *Clinical and translational science* 13.1 (Jan. 2020), pp. 147–156. ISSN: 1752-8062 1752-8054. DOI: 10.1111/cts.12695.

[Del+12] Pierre Delanaye et al. "Normal Reference Values for Glomerular Filtration Rate: What Do We Really Know?" In: *Nephrology, Dialysis, Transplantation: Official Publication of the European Dialysis and Transplant Association - European Renal Association* 27.7 (July 2012), pp. 2664–2672. ISSN: 1460-2385. DOI: 10.1093/ndt/gfs265. pmid: 22802582.

[Deo+20] Malavika Deodhar et al. "Mechanisms of CYP450 Inhibition: Understanding Drug-Drug Interactions Due to Mechanism-Based Inhibition in Clinical Practice". In: *Pharmaceutics* 12.9 (Sept. 2020), p. 846. ISSN: 1999-4923. DOI: 10.3390/pharmaceutics12090846.

[DLR09] Paul A. Dawson, Tian Lan, and Anuradha Rao. "Bile Acid Transporters". In: *Journal of Lipid Research* 50.12 (Dec. 2009), pp. 2340–2357. ISSN: 0022-2275. DOI: 10.1194/jlr.R900012-JLR200.

[Dup20] Yannick Duport. "Computational Modelling of Midazolam Clearance: Effect of Inhibitors and Inducers". Bachelor's Thesis. Aug. 2020.

[Fro09] Oren Froy. "Cytochrome P450 and the Biological Clock in Mammals". In: *Current Drug Metabolism* 10.2 (Feb. 2009), pp. 104–115. ISSN: 1389-2002. DOI: 10.2174/138920009787522179.

[GBK22] Jan Grzegorzewski, Janosch Brandhorst, and Matthias König. "Physiologically Based Pharmacokinetic (PBPK) Modeling of the Role of CYP2D6 Polymorphism for Metabolic Phenotyping with Dextromethorphan". In: *Frontiers in Pharmacology* 13 (2022). ISSN: 1663-9812. DOI: 10.3389/fphar.2022.1029073.

[GH+21] Ferran Gonzalez Hernandez et al. "An Automated Approach to Identify Scientific Publications Reporting Pharmacokinetic Parameters". In: *Wellcome Open Research* 6 (2021), p. 88. ISSN: 2398-502X. DOI: 10.12688/wellcomeopenres.16718.1.

[Grz+22] Jan Grzegorzewski et al. "Pharmacokinetics of Caffeine: A Systematic Analysis of Reported Data for Application in Metabolic Phenotyping and Liver Function Testing". In: *Frontiers in Pharmacology* 12 (2022). ISSN: 1663-9812. DOI: 10.3389/fphar.2021.752826.

[HH08] A. F. Hofmann and L. R. Hagey. "Bile Acids: Chemistry, Pathochemistry, Biology, Pathobiology, and Therapeutics". In: *Cellular and molecular life sciences: CMLS* 65.16 (Aug. 2008), pp. 2461–2483. ISSN: 1420-682X. DOI: 10.1007/s00018-008-7568-6.

[Huc+19] Michael Hucka et al. "The Systems Biology Markup Language (SBML): Language Specification for Level 3 Version 2 Core Release 2". In: *Journal of Integrative Bioinformatics* (2019). DOI: 10/gncdt3.

[ICR02] ICRP. "Basic Anatomical and Physiological Data for Use in Radiological Protection: Reference Values. A Report of Age- and Gender-Related Differences in the Anatomical and Physiological Characteristics of Reference Individuals. ICRP Publication 89." In: *Annals of the ICRP* 32.3-4 (2002), pp. 5–265. ISSN: 0146-6453. ppublish.

[JR13] Hm Jones and K. Rowland-Yeo. "Basic Concepts in Physiologically Based Pharmacokinetic Modeling in Drug Discovery and Development." In: *CPT: pharmacometrics & systems pharmacology* 2 (Aug. 2013), e63. ISSN: 2163-8306. DOI: 10/gftvdn. epublish.

[Jus73] William J. Jusko. "A Pharmacodynamic Model for Cell-Cycle-Specific Chemotherapeutic Agents". In: *Journal of Pharmacokinetics and Biopharmaceutics* 1.3 (June 1973), pp. 175–200. ISSN: 0090-466X. DOI: 10.1007/BF01062346.

[Kea+20] Sarah M. Keating et al. "SBML Level 3: An Extensible Format for the Exchange and Reuse of Biological Models." In: *Molecular systems biology* 16.8 (Aug. 2020), e9110. ISSN: 1744-4292. DOI: 10/gncdt5. ppublish.

[KGK21] Adrian Köller, Jan Grzegorzewski, and Matthias König. "Physiologically Based Modeling of the Effect of Physiological and Anthropometric Variability on Indocyanine Green Based Liver Function Tests". In: *Frontiers in Physiology* 12 (2021). ISSN: 1664-042X.

[KN09] A. Kalliokoski and M. Niemi. "Impact of OATP Transporters on Pharmacokinetics." In: *British journal of pharmacology* 158.3 (Oct. 2009), pp. 693–705. ISSN: 1476-5381. DOI: 10/c4qt7j. ppublish.

[Köl21] Adrian Köller. "A Physiologically Based Model of Indocyanine Green Liver Function Tests - Effects of Physiological Factors, Hepatic Disease and Hepatic Surgery Bachelor Thesis". Bachelor's Thesis. Mar. 2021.

[Köl+21] Adrian Köller et al. "Prediction of Survival After Partial Hepatectomy Using a Physiologically Based Pharmacokinetic Model of Indocyanine Green Liver Function Tests". In: *Frontiers in Physiology* 12 (2021). ISSN: 1664-042X.

[Kön+21] Matthias König et al. *Ten Simple Rules for Sharing Experimental and Clinical Data with the Modeling Community*. Aug. 2021. DOI: 10.20944/preprints202108.0303.v1.

[Kra+03] Wolfgang Kratzer et al. "Factors Affecting Liver Size". In: *Journal of Ultrasound in Medicine* 22.11 (2003), pp. 1155–1161. ISSN: 1550-9613. DOI: 10.7863/jum.2003.22.11.1155.

[Lee+09] Su-Jun Lee et al. "Discovery of Novel Functional Variants and Extensive Evaluation of *CYP2D6* Genetic Polymorphisms in Koreans". In: *Drug Metabolism and Disposition* 37.7 (July 2009), pp. 1464–1470. ISSN: 0090-9556, 1521-009X. DOI: 10.1124/dmd.108.022368.

[LP07] Tom Lynch and Amy Price. "The Effect of Cytochrome P450 Metabolism on Drug Response, Interactions, and Adverse Effects". In: *American Family Physician* 76.3 (Aug. 2007), pp. 391–396.

[LP22] Helena Leal Pujol. "A Physiologically Based Model of Pravastatin - The Role of Genotypes and Hepatic or Renal Impairment on the Pharmacokinetics of Pravastatin". Bachelor's Thesis. May 2022.

[LSC08] Mariana Lazo, Elizabeth Selvin, and Jeanne M. Clark. "Brief Communication: Clinical Implications of Short-Term Variability in Liver Function Test Results". In: *Annals of Internal Medicine* 148.5 (Mar. 2008), pp. 348–352. ISSN: 1539-3704. DOI: 10.7326/0003-4819-148-5-200803040-00005.

[MM13] Leonor Michaelis and Maud Menten. "Die Kinetik der Invertinwirkung". In: *Biochemische Zeitschrift* (1913).

[Moh+09] David Moher et al. "Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement". In: *PLoS Medicine* 6.7 (July 2009), e1000097. ISSN: 1549-1676. DOI: 10/bq3jpc.

[Nel61] E Nelson. "Kinetics of Drug Absorption, Distribution, Metabolism, and Excretion". In: *Journal of pharmaceutical sciences* 50 (Mar. 1961). ISSN: 0022-3549. DOI: 10.1002/jps.2600500302.

[Nin+18] Miaoran Ning et al. "CYP2D6 Protein Level Is the Major Contributor to Interindividual Variability in CYP2D6-Mediated Drug Metabolism in Healthy Human Liver Tissue". In: *Clinical Pharmacology & Therapeutics* 104.5 (Nov. 2018), pp. 974–982. ISSN: 00099236. DOI: 10.1002/cpt.1032.

[PL22] Simone Phang-Lyn and Valerie A. Llerena. "Biochemistry, Biotransformation". In: *StatPearls*. Treasure Island (FL): StatPearls Publishing, 2022.

[RC07] F. P. Retief and L. Cilliers. "Mesopotamian Medicine". In: *South African Medical Journal = Suid-Afrikaanse Tydskrif Vir Geneeskunde* 97.1 (Jan. 2007), pp. 27–30. ISSN: 0256-9574.

[RG15] Slobodan Rendic and F. Peter Guengerich. "Survey of Human Oxidoreductases and Cytochrome P450 Enzymes Involved in the Metabolism of Xenobiotic and Natural Chemicals". In: *Chemical Research in Toxicology* 28.1 (Jan. 2015), pp. 38–42. ISSN: 1520-5010. DOI: 10.1021/tx500444e.

[Sak07] Samir G. Sakka. "Assessing Liver Function". In: *Current Opinion in Critical Care* 13.2 (Apr. 2007), pp. 207–214. ISSN: 1070-5295. DOI: 10.1097/MCC.0b013e328012b268.

[San+21] Carlos Sanz-García et al. "The Space of Disse: The Liver Hub in Health and Disease". In: *Livers* 1.1 (Mar. 2021), pp. 3–26. ISSN: 2673-4389. DOI: 10.3390/livers1010002.

[SBN00] Daniel S. Streetman, Joseph S. Jr Bertino, and Anne N. Nafziger. "Phenotyping of Drug-Metabolizing Enzymes in Adults: A Review of in-Vivo Cytochrome P450 Phenotyping Probes". In: *Pharmacogenetics and Genomics* 10.3 (Apr. 2000), pp. 187–216. ISSN: 1744-6872. DOI: 10.1097/00008571-200004000-00001.

[SGS12] Ketan T. Savjani, Anuradha K. Gajjar, and Jignasa K. Savjani. "Drug Solubility: Importance and Enhancement Techniques". In: *ISRN Pharmaceutics* 2012 (July 2012), pp. 1–10. ISSN: 2090-6153. DOI: 10.5402/2012/195727.

[Shu14] Joel Shurkin. "News Feature: Animals That Self-Medicate". In: *Proceedings of the National Academy of Sciences of the United States of America* 111.49 (Dec. 2014), pp. 17339–41. DOI: 10.1073/PNAS.1419966111.

[Tri+18] Andrea C. Tricco et al. "PRISMA Extension for Scoping Reviews (PRISMA-ScR): Checklist and Explanation". In: *Annals of Internal Medicine* 169.7 (Oct. 2018), pp. 467–473. ISSN: 1539-3704. DOI: 10/gfd8vk.

[Tru+07] Stéphanie Truant et al. "Remnant Liver Volume to Body Weight Ratio > or =0.5%: A New Cut-off to Estimate Postoperative Risks after Extended Resection in Noncirrhotic Liver". In: *Journal of the American College of Surgeons* 204.1 (Jan. 2007), pp. 22–33. ISSN: 1072-7515. DOI: 10.1016/j.jamcollsurg.2006.09.007.

[Wag81] J. G. Wagner. "History of Pharmacokinetics". In: *Pharmacology & Therapeutics* 12.3 (1981), pp. 537–562. ISSN: 0163-7258. DOI: 10.1016/0163-7258(81)90097-8.

[Wan+10] ZiMian Wang et al. "Specific Metabolic Rates of Major Organs and Tissues across Adulthood: Evaluation by Mechanistic Model of Resting Energy Expenditure1234". In: *The American Journal of Clinical Nutrition* 92.6 (Dec. 2010), pp. 1369–1377. ISSN: 0002-9165. DOI: 10.3945/ajcn.2010.29885.

[Wei+05] Werner Weitschies et al. "Impact of the Intragastric Location of Extended Release Tablets on Food Interactions". In: *Journal of Controlled Release* 108.2 (Nov. 2005), pp. 375–385. ISSN: 0168-3659. DOI: 10.1016/j.jconrel.2005.08.018.

[Wil+16] Mark D. Wilkinson et al. "The FAIR Guiding Principles for Scientific Data Management and Stewardship". In: *Scientific Data* 3.1 (Mar. 2016), p. 160018. ISSN: 2052-4463. DOI: 10.1038/sdata.2016.18.

[WP14] Julius Wilder and Keyur Patel. "The Clinical Utility of FibroScan® as a Noninvasive Diagnostic Test for Liver Disease". In: *Medical Devices (Auckland, N.Z.)* 7 (May 2014), pp. 107–114. ISSN: 1179-1470. DOI: 10.2147/MDER.S46943.

[WT24] EMP Widmark and J Tandberg. "Über Die Bedingungen Für Die Akkumulation Indifferenter Narkotika. Theoretische Berechnungen". In: (1924).

[Yáñ+11] Jaime A. Yáñez et al. "Intestinal Lymphatic Transport for Drug Delivery". In: *Advanced Drug Delivery Reviews* 63.10 (Sept. 2011), pp. 923–942. ISSN: 0169-409X. DOI: 10.1016/j.addr.2011.05.019.

[Yan+17] Guangyi Yang et al. "Glucuronidation: Driving Factors and Their Impact on Glucuronide Disposition". In: *Drug metabolism reviews* 49.2 (May 2017), pp. 105–138. ISSN: 0360-2532. DOI: 10.1080/03602532.2017.1293682.

[YH15] Nicole J. Yang and Marlon J. Hinner. "Getting Across the Cell Membrane: An Overview for Small Molecules, Peptides, and Proteins". In: *Methods in molecular biology (Clifton, N.J.)* 1266 (2015), pp. 29–53. ISSN: 1064-3745. DOI: 10.1007/978-1-4939-2272-7_3.

[YL93] Hiroshi Yamahara and Vincent H.L. Lee. "Drug Metabolism in the Oral Cavity". In: *Advanced Drug Delivery Reviews* 12.1-2 (Oct. 1993), pp. 25–39. ISSN: 0169409X. DOI: 10.1016/0169-409X(93)90039-7.

[ZS13] Ulrich M. Zanger and Matthias Schwab. "Cytochrome P450 Enzymes in Drug Metabolism: Regulation of Gene Expression, Enzyme Activities, and Impact of Genetic Variation". In: *Pharmacology & Therapeutics* 138.1 (Apr. 2013), pp. 103–141. ISSN: 01637258. DOI: 10.1016/j.pharmthera.2012.12.007.