
Pharmacokinetics of caffeine: A systematic analysis of reported data for application in metabolic phenotyping and liver function testing

Jan Grzegorzewski¹, Florian Bartsch¹, Adrian Köller¹, and Matthias König^{1,*}

¹ Institute for Theoretical Biology, Humboldt-University Berlin, Invalidenstraße 110, 10115 Berlin, Germany

Correspondence*:

Matthias König

koenigmx@hu-berlin.de

ABSTRACT

Caffeine is by far the most ubiquitous psychostimulant worldwide found in tea, coffee, cocoa, energy drinks, and many other beverages. Caffeine is almost completely metabolized in the liver by the cytochrome P-450 enzyme system mainly to 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 147 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 (i) the alteration of caffeine pharmacokinetics with smoking and oral contraceptive use; (ii) drug-drug interactions with caffeine as possible confounding factors of caffeine pharmacokinetics or source of adverse effects; (iii) alteration of caffeine pharmacokinetics in disease; and (iv) 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

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 U.S. 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 completely 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 between 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 daily consumption of caffeine-containing beverages between individuals. In addition, the cytochrome P450 1A2 (CYP1A2) activity in humans widely varies as well, between 5-6 fold (Schrenk et al., 1998). Both factors contribute to a wide range of caffeine plasma concentrations. CYP1A2 activity and protein amount, and consequently caffeine pharmacokinetics, are affected by environmental, genetic, and epigenetic factors (Klein et al., 2010). 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 a twin study (Rasmussen et al., 2002). However, satisfying genetic or epigenetic markers- on the CYP1A locus on chromosome 15 could not be found by follow-up studies (Jiang et al., 2006; Ghotbi et al., 2007; Gunes and Dahl, 2008; Klein et al., 2010; Yang et al., 2010). Other 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 oral contraceptive consumption 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 determined by other measures such as the Child-Turcotte-Pugh-Score (CTP) (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, 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 (Miners and Birkett, 1996; Fuhr et al., 1996; Faber et al., 2005; Hakooz, 2009). It gets 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 biotransformation is virtually confined to the liver (Amchin et al., 1999; Kalow and Tang, 1993; Drozdzik et al., 2018). Among those mentioned, CYP1A2 phenotyping has the most attention as CYP1A2 is among the most important hepatic enzymes

in general. It contributes 5–20% to the hepatic P450 pool and it is involved in the clearance of about 9% 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), but 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 correlate satisfactorily 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 performed in any of the above mentioned tissues but typically only at a single time point after drug administration. The MR of various metabolites at 4 hours 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 more and more 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 in concentration profiles. 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 may be established. Differences in clinical protocols (e.g. dosing amount, sampling tissue, and timing) have not been systematically analyzed in current literature. In the same manner, 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 MATERIAL AND METHODS

2.1 Data curation

Publications containing caffeine pharmacokinetics data were searched on PubMed and google scholar using combinations of keywords related to caffeine and pharmacokinetics. Most publications were retrieved by combining the keyword caffeine with one of the following keywords: pharmacokinetics, pk, plasma concentration, serum concentration, saliva, time profile, time-course, concentration-time profile, clearance, half-life, elimination rate, or area under the curve (AUC). Based on this initial corpus, additional publications were added by following references and citations. Pharmacokinetics data was curated manually for the pharmacokinetic database PK-DB (<https://pk-db.com/>) (Grzegorzewski et al.,

2020) using established workflows. The pharmacokinetics data was stored with relevant metadata on groups, individuals, interventions, and outputs. PK-DB supported hereby 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).

2.2 Data processing and filtering

Certain data processing and data filtering methods were applied in all analyses. Data included in the analyses was either measured in plasma, serum, or saliva (urinary measurements were excluded). The data was neither filtered based on application route (e.g. oral, intravenous, intramuscular) nor application form (e.g. tablet, capsule, solution) of caffeine, but all data independent of application route and form was used for analyses. Male subjects were assumed not to take any oral contraceptives. Data outliers from four studies (Stille et al., 1987; Harder et al., 1988, 1989; Balogh et al., 1992) were excluded from all analyses. All four studies probably originate from the same clinical investigation which was detected as outliers during the data curation process.

In Fig. 2A, B and Fig. 3A-D, the data was 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 Fig. 2, the depicted subjects were healthy. Substances with negligible caffeine-drug interactions were determined by an effect size analysis in Sec. 3.4. All other co-administrations and investigations containing multiple caffeine dosages were excluded.

In subplot Fig. 3A, included subjects were healthy. The area under the caffeine concentration curves measured at least up to 12 hours 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 Fig. 3C, 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 Fig. 4A-C, no data was excluded. In subplot Fig. 4D, 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

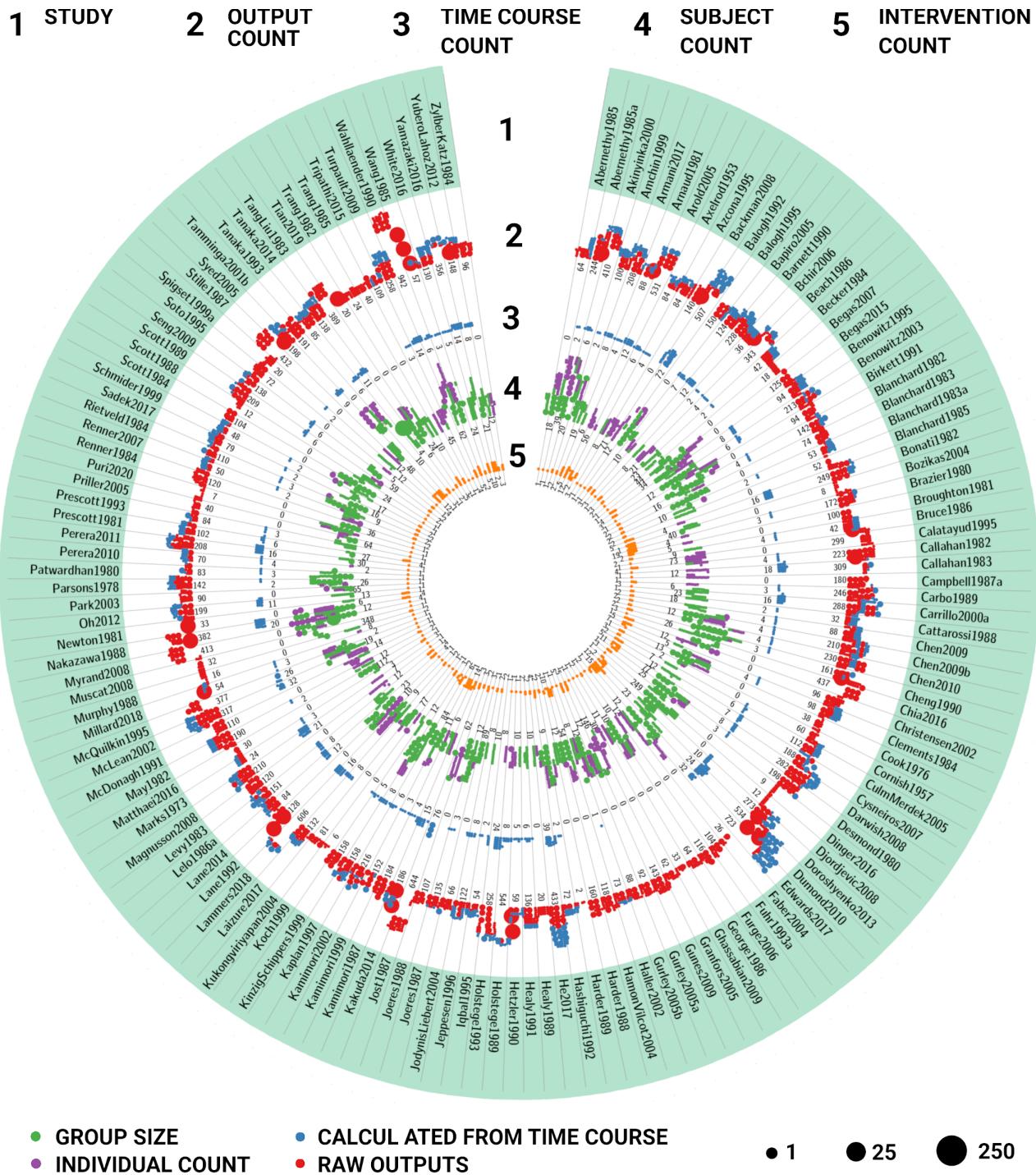


Figure 1. Overview of studies in the caffeine pharmacokinetics data set. The data set consists of 147 studies containing 1284 groups, 13093 individuals, 1632 interventions, 97563 outputs, and 4138 time-courses. The circular plot is structured in stripes and rings. Each stripe represents a different study, 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 (1) name of the study; (2) number of outputs (pharmacokinetics parameters and other measurements). Red dots represent reported data, blue dots data calculated from time-courses reported in the study; (3) number of time-courses; (4) number of participants. Purple dots represent participants with individual data, green dots represent collectively reported participants; (5) number of interventions applied to the participants in the study. For additional information see table Tab. 1.

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).

(continued)

Within this work, the first comprehensive open pharmacokinetics data set on caffeine was established. We demonstrate the value of the data set by application to multiple research questions relevant for metabolic phenotyping and liver function testing: (i) the effect of smoking and oral contraceptive use on caffeine elimination (Sec. 3.3); (ii) the interaction of caffeine with other drugs (Sec. 3.4), (iii) alteration of caffeine pharmacokinetics in disease (Sec. 3.5) and (iv) the applicability of caffeine as a salivary test substance by comparison of plasma and saliva data (Sec. 3.6). The data set integrates data originating from 147 publications (Fig. 1 and Tab. 1), with most of the publications corresponding to a distinct clinical trial. 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 (i) the characteristics of studied patient cohorts and subjects (e.g. age, body weight, smoking status, health status, fasting); (ii) the applied interventions (e.g. dosing, substance, route of application); (iii) measured pharmacokinetic time-courses; and (iv) pharmacokinetic parameters (e.g. clearance, half-life, area under the curve). In summary, data from 1284 groups and 13093 individuals is reported under 1632 interventions resulting in 97563 pharmacokinetic outputs and 4138 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 (Grzegorzewski et al., 2020). In the following we will first provide an overview over the detail of reporting of the studies in the data set, followed by example applications.

3.2 Reporting of pharmacokinetics data

The study design as well as quality and detail of reporting of results were very heterogeneous between studies. Major differences exist in the number of reported time-courses and number of study participants. Only a minority of the studies report individual participant data, whereas most studies report group data. In most studies, the application of a single dose of caffeine was studied. In the case of multiple interventions, mostly only one additional substance was co-administrated. The main categories of studies were either (i) comparing caffeine pharmacokinetics in two groups (e.g. healthy vs. disease), (ii) caffeine-drug interactions (comparing caffeine alone vs. caffeine and additional substance) (iii) phenotyping studies (including drug cocktails); or (iv) methodological studies (e.g. establishing mass spectroscopy protocol for quantification).

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 mostly reported in good detail. For the dosing with caffeine, the amount, route (e.g. oral, intravenous), form (tablet, capsule, solution), and the substance are mostly reported. Co-administrations of medication and other substances are often only mentioned qualitatively, skipping the amount, route, form, or exact timing of application.

The quantification protocol, i.e. quantification method (e.g. high-performance liquid chromatography, gas-liquid chromatography), the site of sampling (e.g. plasma, serum, saliva, urine), and the time points when samples were taken, was mostly reported in good detail. However, in some studies the quantification method and protocol are not explicitly mentioned but only via additional references, which complicates the curation.

The information on subject characteristics was less often reported in sufficient detail with large differences in the quality and quantity of reporting between studies. The information on sex, weight, and age was relatively often reported on group level. However, age and weight were rarely reported on an individual level. Other anthropometric factors such as height, BMI, and ethnicity are rarely reported. The genotype (gCYP1A2) or phenotype (pCYP1A2, pXO, pNAT2) of enzymes involved in the metabolism of caffeine, i.e. CYP1A2, xanthine oxidase (XO), or N-acetyltransferase type 2 (NAT2), are almost never reported, even though there is evidence that genetic variation can play an important role in caffeine

metabolism. It is worth noting that low-cost genotyping methods were not available for early studies included in the dataset. The information on other factors influencing the pharmacokinetics of caffeine is reported very heterogeneously. The strong influence of smoking and the use of oral contraceptives 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. Further markers related to cardiovascular health (e.g. blood pressure, cholesterol level, bilirubin level) are basically not reported. In case of cirrhosis, further information of severity is reported sparsely. Important information on the abstinence of caffeine or 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 only very rarely provided.

Most studies report pharmacokinetics outputs on caffeine. Data on the main product paraxanthine and secondary metabolites theobromine and theophylline 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. In summary, we established the largest freely available pharmacokinetics data set of caffeine so far consisting of data from 147 publications and clinical trials. By using a highly standardized data curation pipeline (established as part of PK-DB (Grzegorzewski et al., 2020)) high quality and consistency of the data could be achieved. This enabled us despite the heterogeneity in reporting to apply the data set to various research questions relevant for metabolic phenotyping and liver function tests. In the following, we demonstrate the value of the data set via multiple example applications consisting of (i) stratification of caffeine pharmacokinetics due to smoking and oral contraceptive use (Sec. 3.3), (ii) the interaction of drugs with caffeine (drug-drug interaction; Sec. 3.4); (iii) alteration of caffeine pharmacokinetics in disease (drug-disease interaction, Sec. 3.5; and (iv) metabolic phenotyping and liver function testing (Sec. 3.6).

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 Fig. 2). Both, have repeatedly been reported as key exogenous factors affecting caffeine elimination. A main question was hereby 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 (Fig. 2A,C) and decreased half-life of caffeine elimination (Fig. 2B,D) whereas oral contraceptive use has the opposite effect over a wide dose range of caffeine. A key result from this 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 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 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 independently. Without reporting of data on individuals or the subgroups no stratification could be performed and these factors could strongly affect results if not balanced between groups. In

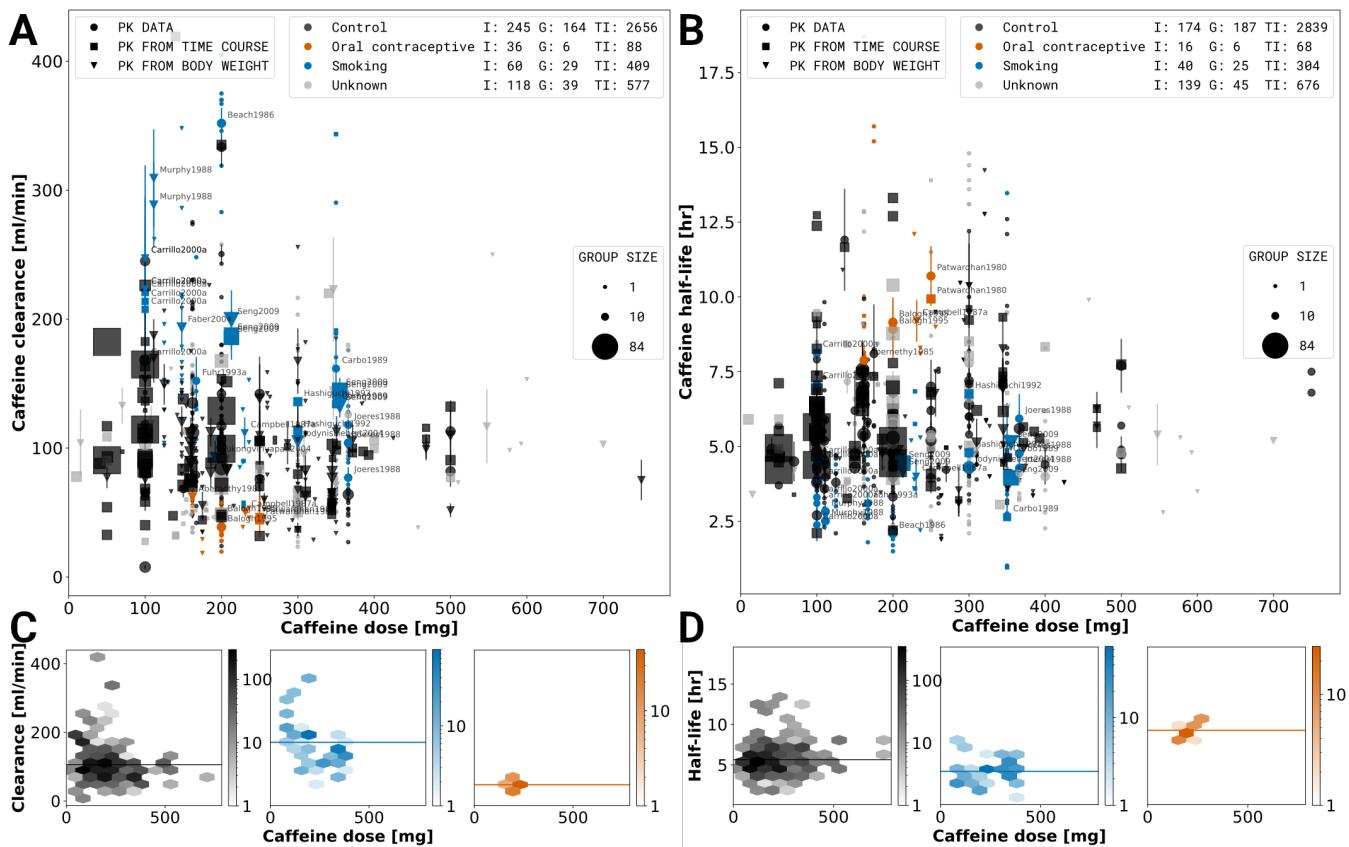


Figure 2. Dose-dependent effect of smoking and oral contraceptive use on caffeine pharmacokinetics. A stratified meta-analysis of caffeine clearance (A) and half-life (B) depending on reported smoking and oral contraceptive use was performed. Black: Control subjects are non-smokers and not taking oral 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 methods section.

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 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 looked in the second analysis at reported caffeine-drug interactions in the data set. The impact of drugs on the caffeine AUC based on reported co-administrations was quantified, using the AUC and AUC difference between a coadministration and a respective control $\log(\frac{AUC_{coadministration}}{AUC_{control}})$ for evaluation (see Fig. 3A,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

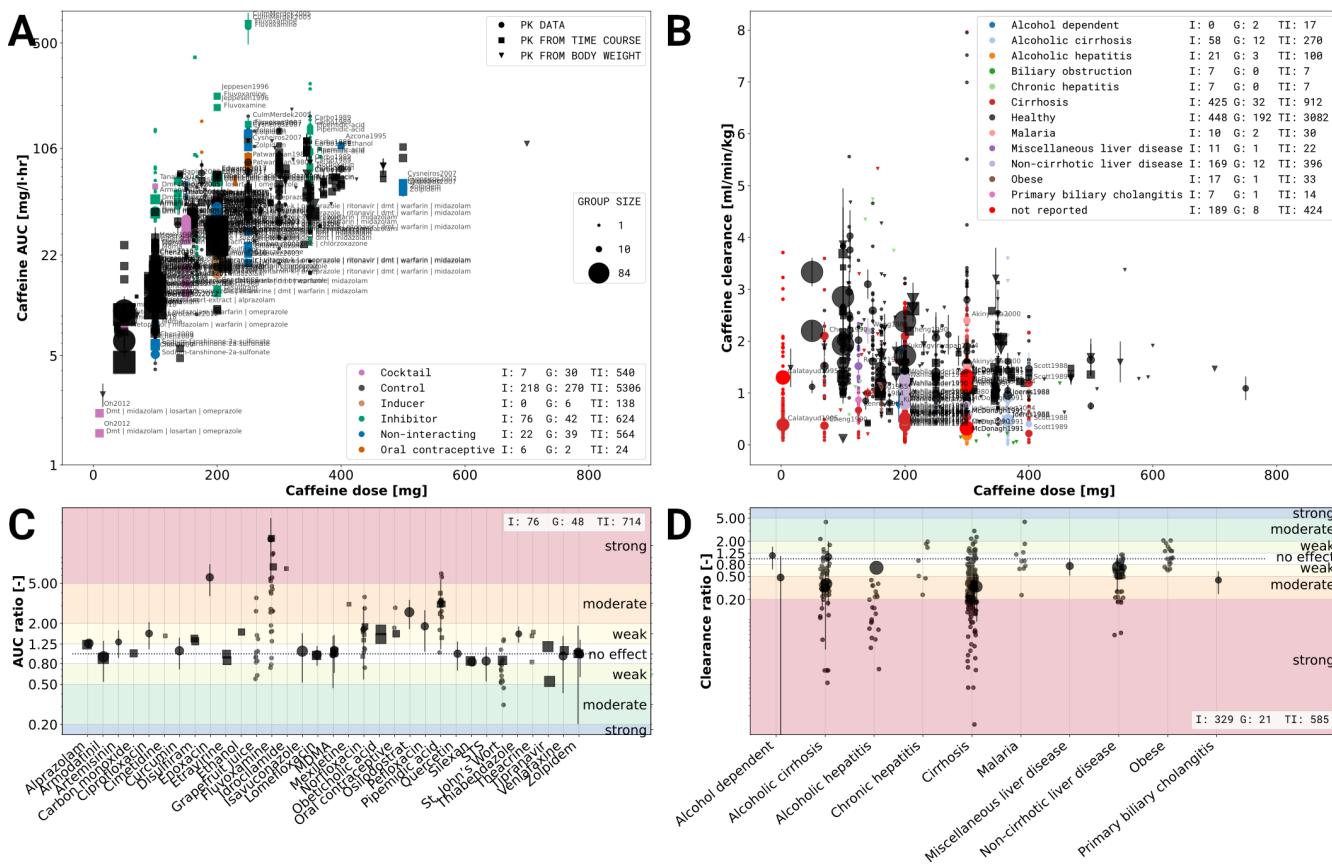


Figure 3. Effects of caffeine-drug and caffeine-disease interactions. A) Caffeine-drug interactions based on the caffeine area under the concentration curve (AUC). Data is stratified based on co-administration of drugs with caffeine. Violet: caffeine administrated as part of a drug cocktail. Common co-administrations are dextromethorphan, 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. 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 \geq 5-fold, \geq 2 to $<$ 5-fold, \geq 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 \geq 80 percent, \geq 50 to $<$ 80 percent and \geq 20 to $<$ 50 percent, respectively. Data selection criteria and visualization are described in the methods section.

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 all 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. 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 coadministered 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.

3.5 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 the question, we stratified caffeine clearance rates based on the reported disease of subjects and groups in the data set. For evaluation of the disease effect the absolute clearance of caffeine (Fig. 3B) and the logarithmic difference to a control group $\log(\frac{AUC_{disease}}{AUC_{control}})$ (Fig. 3D) was 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 at least 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 disease were reported very heterogeneously (e.g. either only cirrhosis or with underlying cause such as alcoholic cirrhosis).

3.6 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 (i) the dependency of blood-based concentrations of caffeine and paraxanthine on their respective saliva concentrations (Fig. 4A,B); and (ii) how the caffeine clearance measured in saliva correlate to blood-based measurements (Fig. 4C).

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 Fig. 4A-C). The resulting scaling factors of saliva to blood-based concentration of caffeine and paraxanthine are 0.77 ± 0.02 and 0.68 ± 0.01 ($\bar{x} \pm SD$), respectively. Pearson correlation coefficients between saliva and blood-based concentrations for caffeine and paraxanthine are 0.74 and 0.76, respectively.

When comparing saliva-based caffeine clearance against blood-based clearance (Fig. 4C) an even even stronger correlation of 0.88 with a scaling factor of 01.03 ± 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 hours to phenotype no clear consensus exists in the literature and metabolic ratios are reported for very different time points after caffeine application. Paraxanthine/caffeine ratios for caffeine

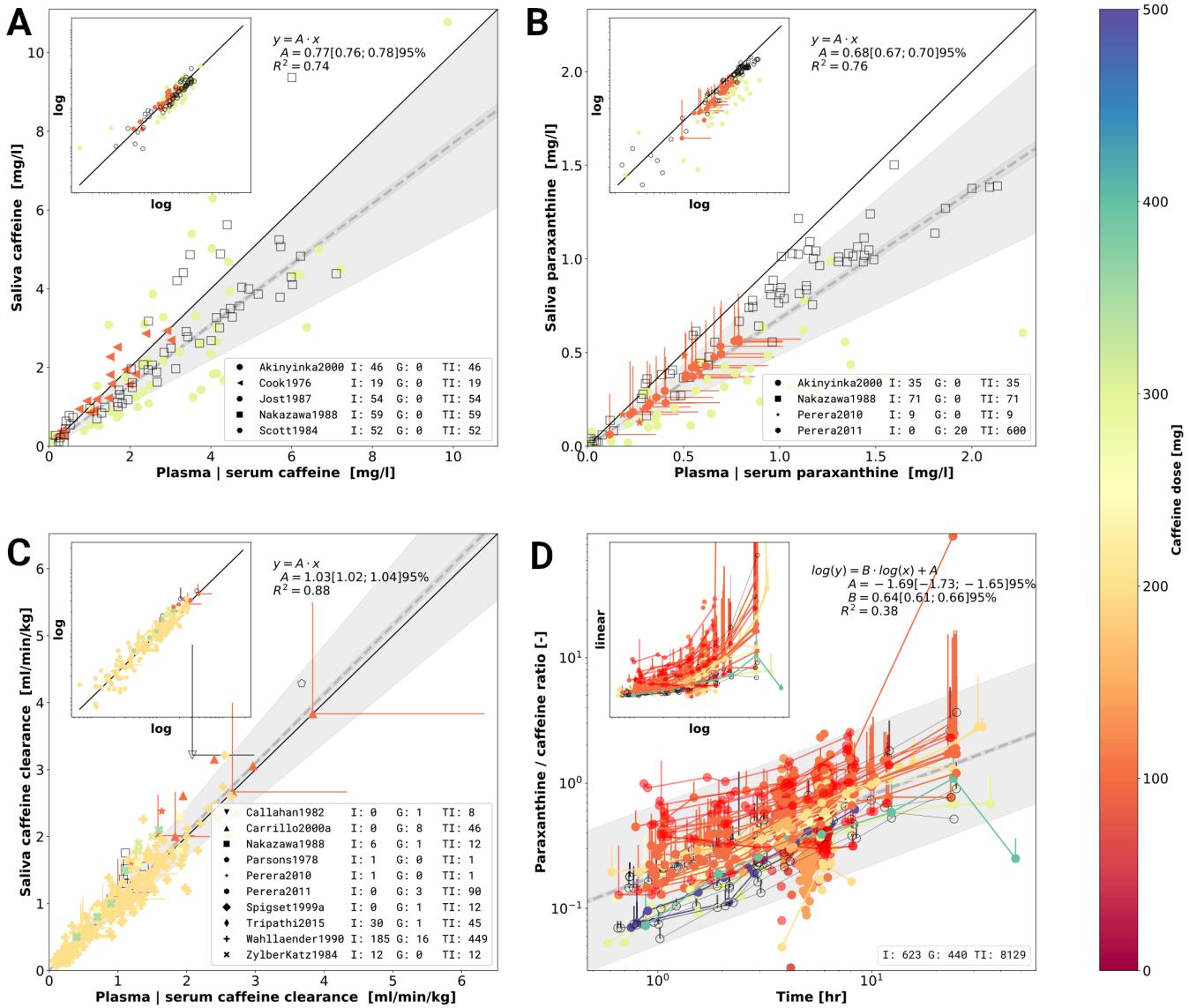


Figure 4. Meta-analysis of caffeine and paraxanthine concentrations in plasma, serum and saliva.

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 inset 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. Data is 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 methods section.

administered either as a single dose or in a cocktail to healthy, non-smoking, non-pregnant, and non-oral contraceptive consuming subjects were investigated (Fig. 4D). By applying this strict data filtering, the

variability due either smoking and oral contraceptive use (see Sec. 3.3), caffeine-drug interactions (see Sec. 3.4) or disease (see Sec. 3.5) could be removed from the metabolic ratios.

Early and late time-sampling are worst suited 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 the a systematic data integration and data analysis of reported data on caffeine pharmacokinetics in adults with focus on applications in metabolic phenotyping and liver function testing. To our knowledge, this is the largest such data set and analysis so far. Data integration from multiple sources allows to solidify existing knowledge, can increase statistical power, increase generalizability and create new insights into the relationship between variables (Thacker, 1988). For instance, by systematically curating group and subject information on smoking status and oral contraceptive use, we could show a reproducible and consistent effect of smoking induction and inhibition via oral contraceptives over many studies and the complete dose regime of applied caffeine, besides only a small subset of studies being specifically designed to study these questions (e.g. smoking by (Benowitz et al., 2003; Gunes et al., 2009; Parsons and Neims, 1978; Backman et al., 2008) and oral contraceptives by (Patwardhan et al., 1980; Abernethy and Todd, 1985; Rietveld et al., 1984). Providing data as open data with sufficient metadata allows to apply data in new contexts often not even anticipated by the original investigators. One example of such a new context is the determination of dose and time-dependency of metabolic phenotyping via paraxanthine/caffeine ratios besides many of the data sets only reporting data for a single time point and dose.

Like all data integration and meta-analysis methods limitations due to sampling bias, biased outcome interpretation and inadequate data may exist (Thacker, 1988). 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 has been 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). 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. Future work will extend our data curation effort towards children and infants and include studies with animal experiment. In addition, despite trying to be as comprehensive as possible 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.

The large inter-individual variability in caffeine pharmacokinetics is a major limitation for metabolic phenotyping and liver function tests. Our work allowed to 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 which would allow 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).

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 $0.74 \pm 0.1 R^2=0.98$ (Akinyinka et al., 2000), $0.79 \pm 0.05 R^2=0.96$ (Zylber-Katz et al., 1984), 0.74 ± 0.08 (Newton et al., 1981), $0.74 \pm 0.08 R^2=0.90$ (Jost et al., 1987), $0.71 R^2=0.89$ (Scott et al., 1984) 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 (Akinyinka et al., 2000; Newton et al., 1981). We could confirm this observation when systematically analyzing the correlation between saliva- and plasma/serum-derived clearance. 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, and allowing simple repeated sampling as required for pharmacokinetic research (Zylber-Katz et al., 1984).

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). E.g. based on our analysis we would 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.

Our systematic curation and analysis of reported caffeine data provided an overview over 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. 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).

CONFLICT OF INTEREST STATEMENT

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.

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.

FUNDING

JG, FB, AK and JG were supported by the Federal Ministry of Education and Research (BMBF, Germany) within the research network Systems Medicine of the Liver (LiSyM, grant number 031L0054). MK, FB and AK were supported by the German Research Foundation (DFG) within the Research Unit Programme FOR 5151 "QualiPerF (Quantifying Liver Perfusion-Function Relationship in Complex Resection - A Systems Medicine Approach)" by grant number 436883643.

DATA AVAILABILITY STATEMENT

The data sets analyzed for this study can be found in <https://pk-db.com/>.

Permission to Reuse and Copyright

Figures, tables, and images will be published under a Creative Commons CC-BY licence and permission must be obtained for use of copyrighted material from other sources (including re-published/adapted/modified/partial figures and images from the internet). It is the responsibility of the authors to acquire the licenses, to follow any citation instructions requested by third-party rights holders, and cover any supplementary charges.

ACKNOWLEDGMENTS

We acknowledge support by the BMBF-funded de.NBI Cloud within the German Network for Bioinformatics Infrastructure (de.NBI) (031A537B, 031A533A, 031A538A, 031A533B, 031A535A, 031A537C, 031A534A, 031A532B) for the PK-DB infrastructure.

REFERENCES

- Abernethy, D. R. and Todd, E. L. (1985). Impairment of caffeine clearance by chronic use of low-dose oestrogen-containing oral contraceptives. *European journal of clinical pharmacology* 28, 425–8
- Abernethy, D. R., Todd, E. L., and Schwartz, J. B. (1985). Caffeine disposition in obesity. *British journal of clinical pharmacology* 20, 61–6

- 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. *European journal of clinical pharmacology* 56, 159–65
- 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. *Journal of clinical pharmacology* 39, 252–9
- Armani, S., Ting, L., Sauter, N., Darstein, C., Tripathi, A. P., Wang, L., et al. (2017). Drug interaction potential of osilodrostat (Ici699) based on its effect on the pharmacokinetics of probe drugs of cytochrome p450 enzymes in healthy adults. *Clinical drug investigation* 37, 465–472. doi:10.1007/s40261-017-0497-0
- Arnaud, M. J. (2011). Pharmacokinetics and metabolism of natural methylxanthines in animal and man. *Handbook of experimental pharmacology*, 33–91doi:10.1007/978-3-642-13443-2_3
- [Dataset] Arnaud, M. J. and Welsch, C. (1982). Theophylline and caffeine metabolism in man
- 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 medica* 71, 331–7
- Axelrod, J. and Reichenthal, J. (1953). The fate of caffeine in man and a method for its estimation in biological material. *The Journal of pharmacology and experimental therapeutics* 107, 519–23
- Azcona, O., Barbanjo, M. J., Torrent, J., and Jané, F. (1995). Evaluation of the central effects of alcohol and caffeine interaction. *British journal of clinical pharmacology* 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. *European journal of clinical pharmacology* 64, 17–24
- Balogh, A., Harder, S., Vollandt, R., and Staib, A. H. (1992). Intra-individual variability of caffeine elimination in healthy subjects. *International journal of clinical pharmacology, therapy, and toxicology* 30, 383–7
- 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. *European journal of clinical pharmacology* 48, 161–6
- 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. *European journal of clinical pharmacology* 61, 755–61
- Barnett, G., Segura, J., de la Torre, R., and Carbó, M. (1990). Pharmacokinetic determination of relative potency of quinolone inhibition of caffeine disposition. *European journal of clinical pharmacology* 39, 63–9
- 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. *Comptes rendus biologies* 329, 512–9
- 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. *Clinical pharmacology and therapeutics* 39, 265–70
- Becker, A. B., Simons, K. J., Gillespie, C. A., and Simons, F. E. (1984). The bronchodilator effects and pharmacokinetics of caffeine in asthma. *The New England journal of medicine* 310, 743–6

- 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. *Biomedical chromatography : BMC* 21, 190–200
- 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. *Biomedical chromatography : BMC* 29, 1657–63
- Benowitz, N. L., Jacob, P. r., Mayan, H., and Denaro, C. (1995). Sympathomimetic effects of paraxanthine and caffeine in humans. *Clinical pharmacology and therapeutics* 58, 684–91
- Benowitz, N. L., Peng, M., and Jacob, P. (2003). Effects of cigarette smoking and carbon monoxide on chlorzoxazone and caffeine metabolism. *Clinical pharmacology and therapeutics* 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. *British journal of clinical pharmacology* 31, 405–8
- Blanchard, J. and Sawers, S. J. (1983a). The absolute bioavailability of caffeine in man. *European journal of clinical pharmacology* 24, 93–8
- Blanchard, J. and Sawers, S. J. (1983b). Comparative pharmacokinetics of caffeine in young and elderly men. *Journal of pharmacokinetics and biopharmaceutics* 11, 109–26
- Blanchard, J. and Sawers, S. J. (1983c). Relationship between urine flow rate and renal clearance of caffeine in man. *Journal of clinical pharmacology* 23, 134–8
- 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. *British journal of clinical pharmacology* 19, 225–232. doi:10.1111/j.1365-2125.1985.tb02635.x
- Bonati, M., Latini, R., Galletti, F., Young, J. F., Tognoni, G., and Garattini, S. (1982). Caffeine disposition after oral doses. *Clinical pharmacology and therapeutics* 32, 98–106
- 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. *European neuropsychopharmacology : the journal of the European College of Neuropsychopharmacology* 14, 39–44
- Brazier, J. L., Descotes, J., Lery, N., Ollagnier, M., and Evreux, J. C. (1980). Inhibition by idrocilamide of the disposition of caffeine. *European journal of clinical pharmacology* 17, 37–43. doi:10.1007/BF00561675
- Broughton, L. J. and Rogers, H. J. (1981). Decreased systemic clearance of caffeine due to cimetidine. *British journal of clinical pharmacology* 12, 155–9
- Bruce, M., Scott, N., Lader, M., and Marks, V. (1986). The psychopharmacological and electrophysiological effects of single doses of caffeine in healthy human subjects. *British journal of clinical pharmacology* 22, 81–7
- Calatayud, O., Rodríguez, M., Sánchez-Alcaraz, A., and Ibáñez, P. (1995). Caffeine test assessment for measuring liver function in critically ill patients. *Journal of clinical pharmacy and therapeutics* 20, 23–9
- 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 metabolism and disposition: the biological fate of chemicals* 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 metabolism and disposition: the biological fate of chemicals* 11, 211–7

- Campbell, M. E., Spielberg, S. P., and Kalow, W. (1987). A urinary metabolite ratio that reflects systemic caffeine clearance. *Clinical pharmacology and therapeutics* 42, 157–65
- Carbó, M., Segura, J., De la Torre, R., Badenas, J. M., and Camí, J. (1989). Effect of quinolones on caffeine disposition. *Clinical pharmacology and therapeutics* 45, 234–40
- 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. *Therapeutic drug monitoring* 22, 409–17
- Cattarossi, L., Colacino, V., Janes, A., LoGreco, P., Rubini, S., Zilli, M., et al. (1988). Oral versus intramuscular loading of caffeine in idiopathic apnoea of prematurity. *European journal of pediatrics* 148, 262–3
- 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. *The Annals of pharmacotherapy* 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; the fate of foreign compounds in biological systems* 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. *Clinical and experimental pharmacology & physiology* 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. *Clinical pharmacology and therapeutics* 47, 516–24
- 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. *Biopharmaceutics & drug disposition* 37, 168–81
- 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). *Clinical pharmacology and therapeutics* 71, 141–52
- Clements, J. A., Critchley, J. A., and Prescott, L. F. (1984). The role of sulphate conjugation in the metabolism and disposition of oral and intravenous paracetamol in man. *British journal of clinical pharmacology* 18, 481–5
- 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. *The Journal of pharmacology and experimental therapeutics* 199, 679–686
- Cornish, H. H. and Christman, A. A. (1957). A study of the metabolism of theobromine, theophylline, and caffeine in man. *The Journal of biological chemistry* 228, 315–23
- 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. *British journal of clinical pharmacology* 60, 486–93
- 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. *Clinical pharmacology and therapeutics* 82, 54–62

- Darwish, M., Kirby, M., Robertson, P. J., and Hellriegel, E. T. (2008). Interaction profile of armodafinil with medications metabolized by cytochrome p450 enzymes 1a2, 3a4 and 2c19 in healthy subjects. *Clinical pharmacokinetics* 47, 61–74
- Desmond, P. V., Patwardhan, R. V., Johnson, R. F., and Schenker, S. (1980). Impaired elimination of caffeine in cirrhosis. *Digestive diseases and sciences* 25, 193–7
- 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. *Toxicology letters* 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. *European journal of clinical pharmacology* 64, 381–5
- 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 metabolism and disposition: the biological fate of chemicals* 41, 987–93
- 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. *Clinical pharmacology and therapeutics* 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. *Clinical pharmacology and therapeutics* 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. *Advances in therapy* 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. *Clinical pharmacology and therapeutics* 76, 178–84
- Faber, M. S., Jetter, A., and Fuhr, U. (2005). Assessment of cyp1a2 activity in clinical practice: why, how, and when? *Basic & clinical pharmacology & toxicology* 97, 125–134. doi:10.1111/j.1742-7843.2005.pto_973160.x
- 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. *Journal of the American Dietetic Association* 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. *British journal of clinical pharmacology* 35, 431–6
- 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. *Biochemistry and molecular biology education : a bimonthly publication of the International Union of Biochemistry and Molecular Biology* 35, 138–44
- 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. *Clinical and experimental pharmacology*

- & physiology 13, 731–6
- 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. *Therapeutic drug monitoring* 31, 239–46
- 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. *European journal of clinical pharmacology* 63, 537–546. doi:10.1007/s00228-007-0288-2
- Gilbert, R. (1984). The methylxanthine beverages and foods: chemistry, consumption, and health effects. *Progress in clinical and biological research* 158, 1–413
- 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. *Clinical pharmacology and therapeutics* 78, 400–11
- 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 research* 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. *Clinical pharmacology and therapeutics* 77, 415–426. doi:10.1016/j.clpt.2005.01.009
- Hakooz, N. M. K. (2009). Caffeine metabolic ratios for the in vivo evaluation of cyp1a2, n-acetyltransferase 2, xanthine oxidase and cyp2a6 enzymatic activities. *Current drug metabolism* 10, 329–338. doi:10.2174/138920009788499003
- Haller, C. A., Jacob, P. r., and Benowitz, N. L. (2002). Pharmacology of ephedra alkaloids and caffeine after single-dose dietary supplement use. *Clinical pharmacology and therapeutics* 71, 421–32
- 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. *The American journal of medicine* 87, 89S–91S
- Harder, S., Staib, A. H., Beer, C., Papenburg, A., Stille, W., and Shah, P. M. (1988). 4-quinolones inhibit biotransformation of caffeine. *European journal of clinical pharmacology* 35, 651–6
- Hashiguchi, M., Fujimura, A., Ohashi, K., and Ebihara, A. (1992). Diurnal effect on caffeine clearance. *Journal of clinical pharmacology* 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. *Journal of caffeine research* 7, 95–102
- 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. *Antimicrobial agents and chemotherapy* 33, 474–8
- Healy, D. P., Schoenle, J. R., Stotka, J., and Polk, R. E. (1991). Lack of interaction between lomefloxacin and caffeine in normal volunteers. *Antimicrobial agents and chemotherapy* 35, 660–4
- Hetzler, R. K., Knowlton, R. G., Somani, S. M., Brown, D. D., and Perkins, R. M. r. (1990). Effect of paraxanthine on ffa mobilization after intravenous caffeine administration in humans. *Journal of applied physiology (Bethesda, Md. : 1985)* 68, 44–7
- Holstege, A., Kurz, M., Weinbeck, M., and Gerok, W. (1993). Excretion of caffeine and its primary degradation products into bile. *Journal of hepatology* 17, 67–73
- Holstege, A., Staiger, M., Haag, K., and Gerok, W. (1989). Correlation of caffeine elimination and child's classification in liver cirrhosis. *Klinische Wochenschrift* 67, 6–15
- Iqbal, N., Ahmad, B., Janbaz, K. H., Gilani, A. U., and Niazi, S. K. (1995). The effect of caffeine on the pharmacokinetics of acetaminophen in man. *Biopharmaceutics & drug disposition* 16, 481–7
- Jeppesen, U., Loft, S., Poulsen, H. E., and Bršen, K. (1996). A fluvoxamine-caffeine interaction study. *Pharmacogenetics* 6, 213–22
- 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. *Pharmacogenetics and genomics* 16, 359–367. doi:10.1097/01.fpc.0000204994.99429.46
- Jodynisi-Liebert, J., Flieger, J., Matuszewska, A., and Juszczak, J. (2004). Serum metabolite/caffeine ratios as a test for liver function. *Journal of clinical pharmacology* 44, 338–47
- Joeres, R., Klinker, H., Heusler, H., Epping, J., and Richter, E. (1987). Influence of mexiletine on caffeine elimination. *Pharmacology & therapeutics* 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 (Baltimore, Md.)* 8, 575–9
- Jones, H. and Rowland-Yeo, K. (2013). Basic concepts in physiologically based pharmacokinetic modeling in drug discovery and development. *CPT: pharmacometrics & systems pharmacology* 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 (Baltimore, Md.)* 7, 338–344. doi:10.1002/hep.1840070221
- 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. *Journal of clinical pharmacology* 54, 422–31
- Kalow, W. and Tang, B. K. (1993). The use of caffeine for enzyme assays: a critical appraisal. *Clinical pharmacology and therapeutics* 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. *European journal of clinical pharmacology* 55, 445–9
- Kamimori, G. H., Karyekar, C. S., Otterstetter, R., Cox, D. S., Balkin, T. J., Belenk, G. L., et al. (2002). The rate of absorption and relative bioavailability of caffeine administered in chewing gum versus capsules to normal healthy volunteers. *International journal of pharmaceutics* 234, 159–67

- 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. *European journal of clinical pharmacology* 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. *Journal of clinical pharmacology* 37, 693–703
- 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. *Clinical pharmacology and therapeutics* 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. *Frontiers in pharmacology* 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. *Biomedical chromatography : BMC* 13, 309–14
- 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
- Kot, M. and Daniel, W. A. (2008). Caffeine as a marker substrate for testing cytochrome p450 activity in human and rat. *Pharmacological reports : PR* 60, 789–797
- Kukongviriyapan, V., Senggunprai, L., Prawan, A., Gaysornsiri, D., Kukongviriyapan, U., and Aiems-Ard, J. (2004). Salivary caffeine metabolic ratio in alcohol-dependent subjects. *European journal of clinical pharmacology* 60, 103–7
- 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. *British journal of clinical pharmacology* 83, 2687–2694
- 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? *European journal of drug metabolism and pharmacokinetics* 43, 251–257
- Lane, J. D., Steege, J. F., Rupp, S. L., and Kuhn, C. M. (1992). Menstrual cycle effects on caffeine elimination in the human female. *European journal of clinical pharmacology* 43, 543–6
- 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. *Journal of addiction research & therapy* 5, 176
- 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. *British journal of clinical pharmacology* 22, 177–82
- Lelo, A., Miners, J. O., Robson, R. A., and Birkett, D. J. (1986b). Quantitative assessment of caffeine partial clearances in man. *British journal of clinical pharmacology* 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. *Clinical pharmacology and therapeutics* 33, 770–5
- 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. *Clinical pharmacology and therapeutics* 84, 52–62
- Marks, V. and Kelly, J. F. (1973). Absorption of caffeine from tea, coffee, and coca cola. *Lancet (London, England)* 1, 827
- 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. *Clinical pharmacology and therapeutics* 100, 606–616
- May, D. C., Jarboe, C. H., VanBakel, A. B., and Williams, W. M. (1982). Effects of cimetidine on caffeine disposition in smokers and nonsmokers. *Clinical pharmacology and therapeutics* 31, 656–61
- 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. *Journal of applied physiology (Bethesda, Md. : 1985)* 93, 1471–8
- 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 epidemiology, biomarkers & prevention : a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology* 4, 139–46
- 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. *Journal of Chemical Education* 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. *General pharmacology* 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 and chemical toxicology : an international journal published for the British Industrial Biological Research Association* 63, 136–42
- 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. *Clinical and experimental pharmacology & physiology* 15, 9–13
- 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. *Biochemical pharmacology* 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. *Clinical pharmacology and therapeutics* 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 : Journal of the Pharmaceutical Society of Japan* 108, 653–8
- Nehlig, A. (2018). Interindividual differences in caffeine metabolism and factors driving caffeine consumption. *Pharmacological reviews* 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. *European journal of clinical pharmacology* 21, 45–52
- 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. *Journal of chromatography. B,*

- Analytical technologies in the biomedical and life sciences 895-896, 56–64
- Park, G. J.-H., 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 (Baltimore, Md.)* 38, 1227–36
- Parsons, W. D. and Neims, A. H. (1978). Effect of smoking on caffeine clearance. *Clinical pharmacology and therapeutics* 24, 40–5
- Patwardhan, R. V., Desmond, P. V., Johnson, R. F., and Schenker, S. (1980). Impaired elimination of caffeine by oral contraceptive steroids. *The Journal of laboratory and clinical medicine* 95, 603–8. PIP: TJ: JOURNAL OF LABORATORY AND CLINICAL MEDICINE.
- Perera, V., Gross, A. S., and McLachlan, A. J. (2010). Caffeine and paraxanthine hplc assay for cyp1a2 phenotype assessment using saliva and plasma. *Biomedical chromatography : BMC* 24, 1136–44
- 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. *The Journal of pharmacy and pharmacology* 63, 1161–8
- Prescott, L. F., Critchley, J. A., Balali-Mood, M., and Pentland, B. (1981). Effects of microsomal enzyme induction on paracetamol metabolism in man. *British journal of clinical pharmacology* 12, 149–53
- Prescott, L. F., Yoovathaworn, K., Makarananda, K., Saivises, R., and Sriwatanakul, K. (1993). Impaired absorption of paracetamol in vegetarians. *British journal of clinical pharmacology* 36, 237–40
- Priller, S. (2005). Cytochrome p450 1a2 phenotyping for student laboratories. *Pharmacy Education journal*
- 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)? *Journal of oral biology and craniofacial research* 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-demethylation of caffeine alias cyp1a2 is determined by both genetic and environmental factors. *Pharmacogenetics* 12, 473–478. doi:10.1097/00008571-200208000-00008
- Renner, B., Clarke, G., Grattan, T., Beisel, A., Mueller, C., Werner, U., et al. (2007). Caffeine accelerates absorption and enhances the analgesic effect of acetaminophen. *Journal of clinical pharmacology* 47, 715–26
- Renner, E., Wietholtz, H., Huguenin, P., Arnaud, M. J., and Preisig, R. (1984). Caffeine: a model compound for measuring liver function. *Hepatology (Baltimore, Md.)* 4, 38–46
- 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. *European journal of clinical pharmacology* 26, 371–3. PIP: TJ: EUROPEAN JOURNAL OF CLINICAL PHARMACOLOGY.
- Rubin, T. M., Heyne, K., Luchterhand, A., Bednarsch, J., W R Vondran, F., Polychronidis, G., et al. (2017). Kinetic validation of the limax test during 10 000 intravenous , javax.xml.bind.jaxbelement@10fb8f50, c-methacetin breath tests. *Journal of breath research* 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. *Journal of caffeine research* 7, 125–132
- 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, 725–34

- 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. *European journal of clinical pharmacology* 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. *Annals of clinical biochemistry* 21 (Pt 2), 120–124. doi:10.1177/000456328402100208
- Scott, N. R., Stambuk, D., Chakraborty, J., Marks, V., and Morgan, M. Y. (1988). Caffeine clearance and biotransformation in patients with chronic liver disease. *Clinical science (London, England : 1979)* 74, 377–84
- 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. *British journal of clinical pharmacology* 27, 205–13
- 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. *Journal of clinical pharmacy and therapeutics* 34, 103–14
- 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, 409–12
- 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. *Clinical pharmacokinetics* 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. *The Journal of antimicrobial chemotherapy* 20, 729–34
- 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. *Current drug metabolism* 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. *Biopharmaceutics & drug disposition* 26, 403–9
- Tamminga, W. J., Wemer, J., Oosterhuis, B., de Zeeuw, R. A., de Leij, L. F., and Jonkman, J. H. (2001). The prevalence of cyp2d6 and cyp2c19 genotypes in a population of healthy dutch volunteers. *European journal of clinical pharmacology* 57, 717–722. doi:10.1007/s002280100359
- 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. *Pharmacology & toxicology* 72, 31–3
- 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. *Biological & pharmaceutical bulletin* 37, 18–25
- Tang-Liu, D. D., Williams, R. L., and Riegelman, S. (1983). Disposition of caffeine and its metabolites in man. *The Journal of pharmacology and experimental therapeutics* 224, 180–5

- 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., John R, 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. *Clinical and translational science* 12, 39–46
- 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. *The American journal of clinical nutrition* 35, 487–94
- 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. *Biopharmaceutics & drug disposition* 6, 51–6
- 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. *Journal of oral biology and craniofacial research* 5, 28–33
- 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. *British journal of clinical pharmacology* 68, 928–35
- Urry, E., Jetter, A., and Landolt, H.-P. (2016). Assessment of cyp1a2 enzyme activity in relation to type-2 diabetes and habitual caffeine intake. *Nutrition & metabolism* 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. *Journal of hepatology* 10, 129–137. doi:10.1016/0168-8278(90)90041-o
- Wang, T., Kleber, G., Stellaard, F., and Paumgartner, G. (1985). Caffeine elimination: a test of liver function. *Klinische Wochenschrift* 63, 1124–8
- White, J. R. J., 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. *Clinical toxicology (Philadelphia, Pa.)* 54, 308–12
- 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. *Clinical pharmacology in drug development* 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 research* 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. *Progress in clinical and biological research* 158, 215–233
- Yubero-Lahoz, S., Pardo, R., Farre, M., Mathuna, B. O., 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 metabolism and pharmacokinetics* 27, 605–13
- Zanger, U. M. and Schwab, M. (2013). Cytochrome p450 enzymes in drug metabolism: regulation of gene expression, enzyme activities, and impact of genetic variation. *Pharmacology & therapeutics* 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. *Clinical pharmacology and therapeutics* 36, 133–7