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Modeling the effects of commonly used drugs on human metabolism

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Metabolism contributes significantly to the pharmacokinetics and pharmacodynamics of a drug. In addition, diet and genetics have a profound effect on cellular metabolism with respect to both health and disease. In the present study, we assembled a comprehensive, literature-based drug metabolic reconstruction of the 18 most highly prescribed drug groups, including statins, anti-hypertensives, immunosuppressants and analgesics. This reconstruction captures in detail our current understanding of their absorption, intracellular distribution, metabolism and elimination. We combined this drug module with the most comprehensive reconstruction of human metabolism, Recon 2, yielding Recon2_DM1796, which accounts for 2803 metabolites and 8161 reactions. By defining 50 specific drug objectives that captured the overall drug metabolism of these compounds, we investigated the effects of dietary composition and inherited metabolic disorders on drug metabolism and drug–drug interactions. Our main findings include: (a) a shift in dietary patterns significantly affects statins and acetaminophen metabolism; (b) disturbed statin metabolism contributes to the clinical phenotype of mitochondrial energy disorders; and (c) the interaction between statins and cyclosporine can be explained by several common metabolic and transport pathways other than the previously established CYP3A4 connection. This work holds the potential for studying adverse drug reactions and designing patient-specific therapies.

Introduction

Drugs are known to undergo two distinct phases in the process of metabolism and elimination. The phase I reactions are functionalization reactions that serve to expose the functional group of the parent drug molecule for subsequent transformations often resulting in its activation or inactivation (e.g. oxidation, reduction and hydrolysis reactions). The phase II reactions are conjugation reactions (Fig. 1) in which the metabolized derivative combines with glucuronic acid (via UDP-glucuronyl transferases, UGTs), sulfate (via sulfotransferases) or acetyl groups (via acetyltransferases), resulting in the formation of highly polar compounds that can then be readily excreted in the feces or urine [1]. Drug molecules require specific membrane transport proteins. For example, ATP-binding cassettes (ABCs) are usually involved in drug export, organic anion-transporting polypeptides involved in import [2,3]. Studying a drug should involve both its pharmacokinetics (including the absorption of oral drugs from the gastrointestinal tract, their distribution to various organs, metabolism for activation and degradation, and elimination; the acronym for this process is ADME) and its pharmacodynamics (the interaction of the drug with the entire physiological system, including

Abbreviations
ABC, ATP-binding cassette; FBA, flux balance analysis; HMG-CoA, 3-hydroxy-3-methylglutaryl-coenzyme A; IEM, inborn errors of metabolism; OAT, organic anion-transporter; OATP, organic anion-transporter polypeptide; TCA, tricarboxylic acid; UGT, UDP-glucuronyl transferases.

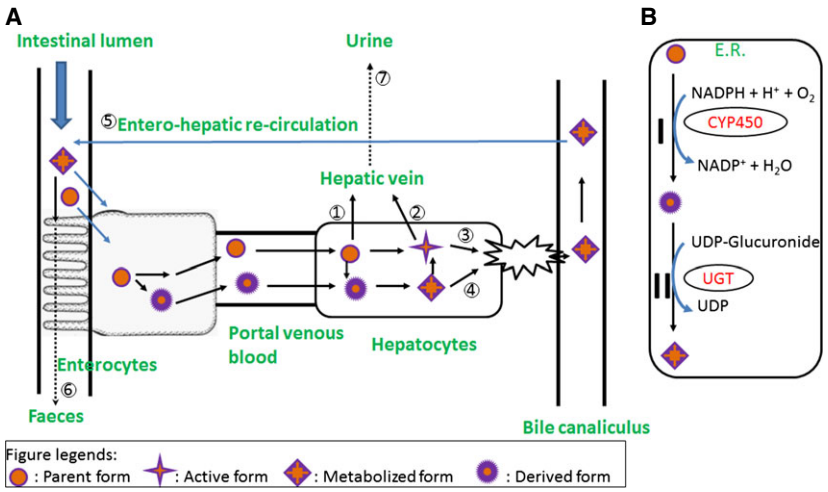


Fig. 1. A simplified overview of drug metabolism and elimination routes. (A) Orally administered drugs are primarily absorbed via the enterocytes of the small intestine, via specific transporters (e.g. *SLCO2B1*; GeneID: 11309). Once inside the enterocyte, phase I and phase II drug metabolizing enzymes may act on the parent form of the drug. Alternatively, the parent form may enter the portal venous blood. Specific hepatic uptake transporters (e.g. *SLCO1B1*; GeneID: 10599) take up the drug and its derivatives for further metabolism. The majority of the drugs undergo activation from the pro-drug to the active form in the liver and can then either exit into the hepatic vein to be exposed to systemic circulation for targeted action or enter the biliary route. Drugs can (1) enter the hepatic vein in either their parent form or (2) in their activated form, and they can also enter (3) the bile. On the other hand, drugs can undergo extensive metabolism in the liver to be released into both the hepatic vein and (4) the bile. Once inside the bile canaliculus, the drugs can either enter into (5) the enterohepatic re-circulation or be excreted in 6) the feces. Drugs exposed to the systemic circulation usually exit through (7) the renal route, via the urine. (B) Drugs undergo biotransformation from a lipophilic state to a polar state, which further aids in their easy excretion. A phase I reaction is shown (i.e. the oxidation of a drug molecule that is catalyzed by enzymes of the cytochrome P450 family) (CYP450), followed by a phase II reaction (i.e. glucuronidation catalyzed by UGT). Both of these reactions occur in the endoplasmic reticulum (ER) of either the enterocytes or the hepatocytes.

its intended target), which, together, initiate its biochemical and physiological responses in the body [1]. Various factors can affect drug action, such as age, gender, genetics, diet, disease and hormonal status, which can either potentiate or inhibit the required drug effect [1]. For example, the interaction of drugs with certain dietary components (e.g. grape fruit juice, watercress, vitamin C and cruciferous vegetables) or feeding states (e.g. a fully-fed state versus a starvation state) is well documented [4,5]. Additionally, disease states, particularly those involving the liver, have been known to interfere with the actions of a drug [1]. Furthermore, reports have shown the association of inborn errors of metabolism (IEMs) with drugs, after the administration of either a single drug or multiple drugs [6–8]. IEMs are metabolic disorders that are characterized by a specific gene mutation leading to a metabolic block in downstream metabolic pathways.

A genome-scale metabolic reconstruction captures the genomic, physiological and biochemical knowledge of the target organism [9]. More than 100 genome-scale metabolic reconstruction have been published, including for humans [10]. The most recent human metabolic reconstruction, Recon 2 [10], represents a significant expansion of content and knowledge compared to the previous version, Recon 1 [11]. Recon 2 comprises 7440 reactions, distributed over eight subcellular compartments/organelles (the cytoplasm, mitochondria, nucleus, endoplasmic reticulum, peroxisomes, lysosomes, Golgi apparatus and extracellular space) and 1789 unique genes. The reactions represent biochemical transformations occurring in any human cell, making Recon 2 highly compliant for studying drug metabolism, which occurs in both hepatic and extrahepatic tissues (e.g. the small intestine, kidney, lung and heart) [12]. Both the current human metabolic reconstruction and its predecessor have been used to investigate the role of IEMs [10,13,14], off-target drug effects [15] and to predict novel drug targets [16–23]. Furthermore, the liver-specific metabolic network, HepatoNet1 [24], has been combined with a whole-body physiology-based pharmacokinetic model to study the toxic effects of drugs [25]. To date, no study has investigated the metabolic load of drug metabolism itself using a genome-scale human metabolic reconstruction.

In the present study, we investigate *in silico* whether the genetic background and the dietary intake of an individual will alter the efficiency with which a drug is

converted into its active form or cleared from the body. Therefore, we assembled a comprehensive, literature-based drug metabolic reconstruction that allowed for the analysis of the effects of dietary composition and inherited metabolic disorders on drug metabolism and drug–drug interactions.

Results

The present study describes a manually assembled metabolic reconstruction for the five most highly prescribed drug groups. This reconstruction captures in detail our current understanding of the absorption, intracellular distribution, metabolism and elimination of drugs belonging to these five groups. We combined this drug module with the recently published community-driven human metabolic reconstruction, Recon 2, and used the combined reconstruction and *in silico* model, Recon2_DM1796, to study: (a) the role of diet in the metabolization of drugs; (b) the effect of pre-existing genetic deficiencies on drug metabolic patterns; and (c) the effect of pre-existing genetic deficiencies on drug–drug metabolic interactions.

Reconstruction of drug metabolism

We present the first extensive metabolic reconstruction of the five most commonly used drug groups (Tables 1 and S1). The reconstruction was built through an extensive manual curation of the available scientific literature. These drug groups comprise 18 drug compounds (Table 2): (a) the statin group, which is composed of eight anti-hypercholesterolemic compounds; (b) the anti-hypertensive group, composed of losartan, torasemide and nifedipine; (c) the immunosuppressant group, composed of cyclosporine and tacrolimus; (d) the analgesic group, which is composed of ibuprofen and acetaminophen; and (e) a miscellaneous group, consisting of anti-diabetic (gliclazide), sedative (midazolam) and anti-hyperuricemic (allopurinol) drugs. Information from more than 250 peer-reviewed research articles and books has been used to capture the known metabolic fate of these drugs in great detail in the small intestine and the liver (Tables 1 and S1). Below, we provide a short summary of the various drug metabolism and transport pathways included in the reconstruction. A description of the possible elimination routes is provided in Table S1 and Fig. 1.

Table 1. Statistics of the drug reconstruction module and Recon2_DM1796 (drug module + Recon 2).

Components	Drug module	Recon2_DM1796
Total number of metabolic reactions	187	4489
Total number of transport reactions	386	2782
Number of exchange/ demand reactions	148	890
Total number of unique metabolites	210 (177 drug metabolites)	2803
Total number of unique genes	57	1796 (seven new genes)
Number of compartments	5	8
References	259[a]	> 1500[a]

[a] Peer-reviewed journal articles, primary literature and books.

The statin group

Statins are a group of drugs that target the key enzyme of the *de novo* cholesterol biosynthetic pathway: 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase (EC 1.1.1.88, EC 1.1.1.34). They principally comprise eight drug compounds (Table 2) and are mainly used to treat hypercholesterolemia and coronary atherosclerosis, with the liver as the target organ [26]. Most of the currently used statins have some adverse drug actions. For example, rhabdomyolysis is a common side effect of cerivastatin, which led to its withdrawal in 2001 [27]. Although all statins are administered in their active form, lovastatin and simvastatin are given as pro-drugs [26]. All statins exhibit two chemically different forms, an acid and a lactone form, with the acid form being biologically more potent [28,29]. The chief metabolic reactions/pathways in which these forms participate are: (a) oxidation; (b) β-oxidation (pravastatin, fluvastatin); (c) glucuronidation; (d) interconversion between acid and lactone forms; (e) isomerization; (f) aromatization (pravastatin, fluvastatin); and (g) demethylation (cerivastatin and rosuvastatin; Fig. 2A). These reactions are most often catalyzed by enzymes encoded by genes of the *CYP* and *UGT* families, as well as genes involved in fatty acid oxidation (Tables 2 and S1). The drug module captures all of the known metabolic pathways of each of these statin drugs in the form of 94 metabolic reactions, leading to the formation of eight metabolites by lovastatin, 12 by simvastatin, 10 by atorvastatin, 13 by pravastatin, 15 by fluvastatin, seven by cerivastatin, three by rosuvastatin and five by pitavastatin (Table S1). The metabolism of these statins is important for their conversion into active forms to act on the target enzyme, as well as for their effective elimination. Most of the conversion of statins into their

Table 2. Drugs included in the drug reconstruction with their corresponding metabolic and transport genes.

Drug group	Drug common name	ATC code	Genes encoding metabolic and transport proteins	References
Statins	Lovastatin	C10AA02	*CYP3A4* (GeneID: 1576), *CYP3A5* (GeneID: 1577), *CYP2C8* (GeneID: 1558), *ABCB1* (GeneID:5243)	[2,111–113]
	Simvastatin	C10AA01	*CYP3A4* (GeneID: 1576), *CYP3A5* (GeneID: 1577), *UGT1A1* (GeneID:54658), *UGT1A3* (GeneID:54659), *ABCB1* (GeneID:5243)	[114,115]
	Atorvastatin	C10AA05	*CYP3A4* (GeneID: 1576), *CYP3A5* (GeneID: 1577), *CYP2C8* (GeneID: 1558), *UGT1A3* (GeneID: 54659), *UGT1A4* (GeneID: 54657), *UGT1A1* (GeneID: 54658), *UGT1A8* (GeneID: 54576), *UGT2B7* (GeneID: 7364), *UGT1A9* (GeneID: 54600), *SLCO1B1* (GeneID: 10599), *SLCO2B1* (GeneID: 11309), *ABCB1* (GeneID: 5243)	[112,115–117]
	Pravastatin	C10AA03	*CYP3A4* (GeneID: 1576), *CYP3A5* (GeneID: 1577), *SLCO1B1* (GeneID: 10599), *SLCO2B1* (GeneID: 11309), *ABCB11* (GeneID: 8647), *ABCB1* (GeneID: 5243), *ABCC2* (GeneID: 1244), *ABCG2* (GeneID: 9429)	[2,111,118]
	Fluvastatin	C10AA04	*CYP3A4* (GeneID: 1576), *CYP3A5* (GeneID: 1577), *CYP2C8* (GeneID: 1558), *CYP2C9* (GeneID: 1559), *CYP2D6* (GeneID: 1565), *CYP2C19* (GeneID: 1557), *SLCO1B3* (GeneID: 28234), *SLCO2B1* (GeneID: 11309), *ABCB11* (GeneID: 8647)	[112,119,120]
	Cerivastatin	C10AA06	*CYP3A4* (GeneID: 1576), *CYP3A5* (GeneID: 1577), *CYP2C8* (GeneID: 1558), *UGT1A1* (GeneID: 54658), *UGT1A3* (GeneID: 54659), *SLCO1B1* (GeneID: 10599), *ABCB11* (GeneID: 8647), *ABCB1* (GeneID: 5243), *ABCC2* (GeneID: 1244), *ABCG2* (GeneID: 9429)	[2,112,121]
	Pitavastatin	C10AA08	*CYP3A4* (GeneID: 1576), *CYP3A5* (GeneID: 1577), *CYP2C8* (GeneID: 1558), *CYP2D6* (GeneID: 1565), *UGT1A1* (GeneID: 54658), *UGT1A3* (GeneID: 54659), *UGT2B7* (GeneID: 7364), *UGT1A4* (GeneID: 54657), *UGT1A6* (GeneID: 54578), *SLCO1B3* (GeneID: 28234), *SLCO2B1* (GeneID: 11309), *ABCB11* (GeneID: 8647), *ABCG2* (GeneID: 9429)	[2,122,123]
	Rosuvastatin	C10AA07	*CYP2C9* (GeneID: 1559), *CYP2C19* (GeneID: 1557), *SLCO1B*1 (GeneID: 10599), *SLCO1B3* (GeneID: 28234), *SLCO2B1* (GeneID: 11309), *ABCG2* (GeneID: 9429)	[2,124,125]
Anti-hypertensive	Losartan	C09CA01	*CYP3A4* (GeneID: 1576), *CYP2C9* (GeneID: 1559), *UGT1A3* (GeneID:54659), *UGT2B7* (GeneID:7364), *UGT1A10* (GeneID: 54575), *UGT1A1* (GeneID: 54658), *UGT1A4* (GeneID: 54657), *UGT1A7* (GeneID: 54577), *UGT1A8* (GeneID: 54576), *UGT1A9* (GeneID: 54600), *UGT2B17* (GeneID: 7367), *ABCB1* (GeneID: 5243)	[2,126,127]
	Torasemide	C03CA04, C03CA01	*CYP2C9* (GeneID: 1559), *SLCO1B1* (GeneID: 10599), *SLC22A6* (GeneID: 9356)	[2,32,96]
	Nifedipine	C08CA05	*CYP3A4* (GeneID: 1576), *CYP3A5* (GeneID: 1577)	[128,129]
Immunosuppressants	Cyclosporine A	L04AD01, S01XA18	*CYP3A4* (GeneID: 1576), *CYP3A5* (GeneID: 1577), *ABCB1* (GeneID: 5243)	[2,37]
	Tacrolimus	D11AX14, L04AA05	*CYP3A4* (GeneID: 1576), *CYP3A5* (GeneID: 1577), *ABCB1* (GeneID: 5243)	[2,38]
Analgesics	Ibuprofen	C01EB16	*ACOT2* (GeneID: 10965), *ACOT4* (GeneID: 122970), *ACOT11* (GeneID: 26027), *ACOT8* (GeneID: 10005), *AMACR* (GeneID: 23600), *UGT1A3* (GeneID: 54659), *UGT1A9* (GeneID: 54600), *UGT2B7* (GeneID: 7364), *CYP2C9* (GeneID: 1559), *CYP2C8* (GeneID: 1558),	[41,43,45,51,130]

Table 2. (Continued).

Drug group	Drug common name	ATC code	Genes encoding metabolic and transport proteins	References
			CYP3A4 (GeneID: 1576), *CYP2C19* (GeneID: 1557), *SLC22A6* (GeneID: 9356)	
	Acetaminophen	N02BE01	*SULT1* (GeneID: 6817), *UGT1A1* (GeneID: 54658), *UGT1A6* (GeneID: 54578), *UGT1A9* (GeneID: 54600), *UGT1A10* (GeneID: 54575), *CYP2E1* (GeneID: 1571), *CYP1A2* (GeneID: 1544), *CYP2A6* (GeneID: 1548), *CYP2D6* (GeneID: 1565), *CYP3A4* (GeneID: 1576), *ABCC3* (GeneID: 8714), *MRP2* (GeneID: 1244), *BCRP* (GeneID: 9429), *ABCB1* (GeneID: 5243)	[2,49,50,131,132]
Miscellaneous	Gliclazide	A10BB09	*CYP2C9* (GeneID: 1559), *CYP2C18* (GeneID: 1562), *CYP2C19* (GeneID: 1557), *CYP2C8* (GeneID: 1558), *CYP2D6* (GeneID: 1565)	[56,57]
	Midazolam	N05CD08	*CYP3A4* (GeneID: 1576), *CYP3A5* (GeneID: 1577), *UGT1A4* (GeneID: 54657), *UGT2B4* (GeneID: 7363), *UGT2B7* (GeneID: 7364), *ABCB1* (GeneID: 5243)	[58]
	Allopurinol	M04AA01	*XDH* (GeneID: 7498), *AOX1* (GeneID: 316)	[55]

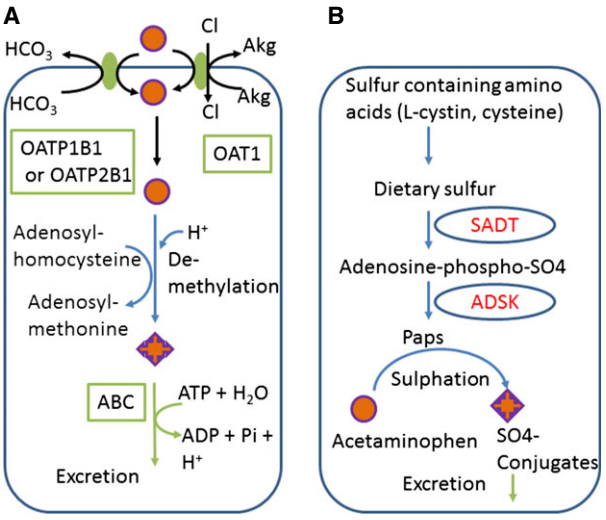


Fig. 2. Involvement of endogenous metabolites in the xenobiotic metabolism. (A) *S*-adenosyl-homocysteine is an important cofactor for the demethylation reactions of a majority of drugs, including, cerivastatin, rosuvastatin, cyclosporine, nifedipine and tacrolimus. Additionally, the drug transporters highlighted in green involve co-substrates that are important endogenous metabolites (e.g. α-ketoglutarate in the TCA cycle). (B) Sulfur-containing amino acids are a source of endogenous 3′-phosphonato-5′-adenylyl sulfate (paps), which is involved in the sulfation of acetaminophen metabolites, ultimately leading to their excretion. The sulfur flows from the dietary sources to paps and then to acetaminophen metabolites. SADT, sulfate adenylyl transferase (EC 2.7.7.4); ADSK, adenylyl-sulfate kinase (EC-2.7.1.25).

derivative metabolites occurs in the liver. Small intestinal enterocytes also contribute in part to the oxidation, interconversion between acid and lactone forms, as well as isomerization of lovastatin, atorvastatin and pravastatin. Distinct membrane transporters exist for all of the discussed statins, including for lovastatin and simvastatin, which are lipophilic statins that can cross cell membranes with ease.

The transporters are from the organic anion-transporter polypeptide (OATPs) family, the organic anion-transporter (OATs) family or the ABC family. Different representatives are known to transport different statins (Table 2). The various drug-derived compounds of all the statins follow both biliary and renal routes (Figs 1 and 2A). Generally, the transporters of the renal route are OATPs and the transporters of the biliary route are ABCs [3]. Alternatively, a few statins and their metabolites can also undergo enterohepatic re-circulation (e.g. pravastatin) [30]. The drug reconstruction captures 103 extracellular transport reactions for the transport of statins and their metabolites between the cytosol and the extracellular space.

The anti-hypertensive group

The anti-hypertensives are represented by three drugs (Table 2) and are mainly used in the treatment of hypertension, congestive heart failure and angina. Losartan inhibits the binding of angiotensin II, a peptide hormone, to the angiotensin II type 1 receptor. Torasemide inhibits the $Na^+/K^+/2Cl^-$ transport system in the kidney and nifedipine blocks L-type calcium channels. The chief metabolic pathways contained in the drug reconstruction are: (a) oxidation; (b) glucuronidation (losartan); (c) tolyl methyl hydroxylation

(torasemide); (d) lactonization (nifedipine); and (e) demethylation (nifedipine) (Fig. 2A). These are pathways represented by 14 metabolic reactions, catalyzed by enzymes encoded by the *CYP2C* and *CYP3A* gene families (Tables 2 and S1). These reactions lead to the formation of seven metabolites for losartan, three metabolites for torasemide and four metabolites for nifedipine. The oxidation of the parent forms of the drugs, such as losartan and torasemide, produces active metabolites (losartan-E3174/losartan-M6 [31]; torasemide-M3 [32]), which are equally active against their respective targets. The three anti-hypertensives and their derived metabolites are mostly excreted via the renal route. However, losartan in the parent form has also been recovered in bile, where it is transported with the ABCB1 transporter (GeneID: 5243) [2]. Although the majority of the efflux transporters remain unknown, the hepatic uptake transporter has been identified as the organic anion transporter, OAT1 (*SLC22A6*; GeneID: 9356) [33]. A total of 24 extracellular transport reactions were captured for this group in the drug reconstruction.

The immunosuppressant group

Cyclosporine A and tacrolimus are the representatives of the immunosuppressant group used in our drug reconstruction. Both of these drugs are administered to inhibit interleukin 2 transcription and T-lymphocyte activation. Cyclosporine A binds to cyclophilin, a peptidyl prolyl isomerase [34,35], whereas tacrolimus binds to the tacrolimus-binding protein. These drug–receptor complexes inhibit calcineurin, a protein phosphatase, and thus restrain a number of transcriptional events [36]. Immunosuppressants are used in rheumatoid arthritis, severe psoriasis and to prevent graft rejection. Metabolic pathways for these drugs include: (a) oxidation (cyclosporine, tacrolimus); (b) demethylation (cyclosporine, tacrolimus) (Fig. 2A); and (c) sulfation and glucuronidation (cyclosporine). These reactions are catalyzed by enzymes encoded by *CYP3A4* (GeneID: 1576) and *CYP3A5* (GeneID: 1577) for both drugs [37,38], leading to the formation of 14 metabolites by cyclosporine and eight metabolites by tacrolimus. These pathways are described with 27 metabolic reactions in the drug reconstruction. Precise transporters for cyclosporine and tacrolimus have not been identified; however, both of these drugs are relatively lipophilic with high permeability, and their metabolites follow both renal and biliary routes for excretion [39,40]. Consequently, 44 extracellular transport reactions were defined (Table S1). Details of their elimination routes are provided in Fig. 1.

The analgesic group

Analgesics are used for pain relief. Ibuprofen and acetaminophen are the most commonly used drugs of this group. Although ibuprofen inhibits both cyclooxygenase 1 and 2 (EC 1.14.99.1) [41], acetaminophen selectively inhibits cyclooxygenase 2 [42] and is therefore widely used for fever and pain. Ibuprofen mainly undergoes: (a) chiral inversion; (b) glucuronidation; and (c) oxidation [43,44]. These transformations are catalyzed by racemase (EC 5.1.99.4) [45], UDP-glucuronosyltransferases (EC 2.4.1.17) [46] and mono-oxygenase (EC 1.14.14.1) [47], which are encoded by multiple genes of the *ACOT*, *UGT* and *CYP* families, respectively (Tables 2 and S1). The metabolism of acetaminophen can be broadly classified as both: (a) non-oxidative and (b) oxidative. The majority of the drug (approximately 80%) undergoes non-oxidative metabolism [48], which consists of sulfation, catalyzed by sulfotransferase 1 (*SULT1*; GeneID: 6817, EC 2.8.2.1) (Fig. 2B) [49], and glucuronidation, catalyzed by UDP-glucuronosyltransferases that are encoded by various *UGTs* (Table 2) [50]. By contrast, the oxidative metabolism is catalyzed via cytochrome P450 family proteins (i.e. the CYP1, CYP2 and CYP3 families) (Tables 2 and S1). Accordingly, the drug reconstruction captures 32 metabolic reactions for the analgesic group, comprising 16 different metabolites each from ibuprofen and acetaminophen. With respect to the elimination route, ibuprofen and its metabolites primarily follow the renal route, whereas < 1% of the parent drug follows the biliary route [44]. Although ibuprofen can diffuse into the intestinal cells, the organic anion transporter, OAT1 (*SLC22A6*; GeneID: 9356) mediates its transport in the kidney [51] and liver [52]. In the case of acetaminophen, both a passive transport process [48] and active transport via various ABC transporters (Table 2) have been reported [2,48]. The majority of acetaminophen metabolites follow the renal route, whereas a few follow the biliary route (e.g. sulfate conjugates). Additionally, acetaminophen-glutathione conjugates undergo enterohepatic re-circulation [48]. In total, 38 extracellular transport reactions are included for this group in the drug reconstruction (Table S1). Details of their elimination routes are provided in Fig. 1.

The miscellaneous group

This group consists of three drug compounds: (a) the oral hypoglycemic agent gliclazide, which targets K^+/ATP channel and helps in controlling basal glucose level by acting on glycogen synthase (EC 2.4.1.11) and

fructose-2,6-bisphosphatase (EC 2.7.1.105) [53]; (b) the sedative midazolam, which produces antegrade amnesia by causing γ-aminobutyrate accumulation [54]; and (c) the anti-hyperuricemic agent allopurinol, which targets the urate producing enzyme xanthine oxidase (EC 1.17.3.2) [55]. These drugs primarily undergo: (a) oxidation and (b) conjugation with either UDP-ribose (allopurinol) or UDP-glucuronate (gliclazide, midazolam), and these reactions are catalyzed by: (a) oxidoreductases for allopurinol [55] and (b) monooxygenase (EC 1.14.14.1) for gliclazide and midazolam. The enzymes that catalyze these reactions are encoded by genes of the *CYP2C/2D* family for gliclazide [56,57] and by the *CYP3A* family for midazolam [58] (Table 2). Various *UGTs* catalyze the conjugation reactions (Tables 2 and S1). The metabolism of this group in the drug reconstruction has been covered in the form of 20 reactions, involving 10 metabolites from gliclazide, six from midazolam and three from allopurinol. The elimination routes for gliclazide and its metabolites are both renal (60–70%) and biliary (10–20%) [59], although elimination is exclusively renal for midazolam, allopurinol and their metabolites [55,60]. Although gliclazide and midazolam are highly lipophilic [53,61], allopurinol transporters are considered to be concentrative nucleoside transporters (*SLC* family) [62]. All of these transport mechanisms have been represented with 37 reactions in the drug module.

Promiscuity in drug transporters

Drugs require transport proteins both for uptake and exit from the cell. During the reconstruction process, we encountered various promiscuous drug transporters (Table S1). Promiscuity in drug transporters refers to sharing of transport protein by multiple drugs [63]. We discuss two transport proteins: (a) P-glycoprotein (*ABCB1*; GeneID: 5243) and (b) the solute carrier organic anion transporter family member 1B1 (*SLCO1B1*; GeneID: 10599) that recognize statins as well as drugs from other groups. The P-glycoprotein has a broad specificity and, in the drug reconstruction, recognizes lovastatin, simvastatin, atorvastatin, pravastatin, fluvastatin, cerivastatin and pitavastatin from the statin group; immunosuppresants such as cyclosporine and tacrolimus; and also losartan, acetaminophen, midazolam [2]. The *SLC* gene family comprises major drug transporters, whereby the SLCO1B1 not only transport statins [2], such as atorvastatin, pravastatin, cerivstatin and rosuvastatin, but also the anti-hypertensive torasemide [64]. Another transport protein worth mentioning is PEPT1 (*SLC15A1*; GeneID: 6564), which recognizes at least 400 different di- and tri-petides, including drugs such as antivirals, antibiotics and angiotensin-converting enzyme inhibitors [2]. Of the reconstructed drugs, PEPT1 has been shown to interact with ibuprofen in rat jejunum [65]. Interestingly, P-glycoprotein substrates, specifically statins and cyclosporine, mediate clinically relevant drug–drug interactions in transplant patients, much of which has been attributed to their sharing of transport protein (see below for details).

Modeling drug metabolism under different dietary regimes

The drug reconstruction module was combined with Recon 2 [10] yielding Recon2_DM1796, where DM stands for drug module, followed by the total number of unique genes contained in the combined metabolic network (Table 1). This combined reconstruction was used to assess the role of diet and genetics in drug metabolism.

The effect of diet on the synthesis and clearance of different drug molecules

We were interested in evaluating the role of the diet in the metabolism of different drugs. Therefore, we simulated three different dietary regimes: an average American diet [66], a balanced diet [67,68] and a vegetarian diet [69], Table S2. We defined 50 drug objectives representing the synthesis or breakdown of the various drug metabolites and their derivatives (Table 3). To avoid any metabolic interaction between the drugs, only one drug was given at a time. Flux balance analysis (FBA) [70] was then performed on Recon2_DM1796. Although the majority of the drug objectives (44 of 50; 88%) had the same maximal flux values under all three dietary conditions (Table S3), a considerable difference was found for six of the objectives. Compared with the average American diet, a 93% flux reduction in the vegetarian diet and a 92% flux reduction in the balanced diet were observed for the glucuronidation reactions of cerivastatin-M1, rosuvastatin, and for the spontaneous conversion of glucuronide to the lactone form of pitavastatin. Moreover, a 95% flux reduction in the conversion of the pro-drug to its active drug was predicted for lovastatin and simvastatin, under both the vegetarian and balanced diets. Additionally, the sulfation of acetaminophen had a low flux value only under the vegetarian diet (i.e. a 63% reduction compared to the average American and balanced diet) (Table 3). To analyze this increased excretion effect of sulfated-acetaminophen metabolites,

Table 3. Drug metabolic objectives. D1, average American diet; D2, balanced diet; D3, vegetarian diet. ✓, the corresponding task carried a nonzero flux value; ↓, reduced flux value for the corresponding task. Additionally, the effect of all drugs on the biomass reaction was tested (Table S3).

Drug group	Drug metabolic objectives	D1	D2 versus D1	D3 versus D1
Statins	Conversion of pro-durg lovastatin to active hydroxyacid form	✓	↓	↓
	Oxidation of lovastatin to 6-β-hydroxy-lovastatin	✓	✓	✓
	Oxidation of lovastatin to 6-exomethylene-lovastatin	✓	✓	✓
	Conversion of simvastatin pro-drug to active form	✓	↓	↓
	Conversion of 6-β-hydroxy simvastatin lactone to acid form	✓	✓	✓
	Conversion of 6-β-hydroxy-methyl simvastatin lactone to acid form	✓	✓	✓
	Conversion of 6-β-carboxy simvastatin lactone to acid form	✓	✓	✓
	Oxidation of atorvastatin acid to 4-hydroxy-atorvastatin-acid	✓	✓	✓
	Oxidation of atorvastatin acid to 2-hydroxy-atorvastatin-acid	✓	✓	✓
	Isomerization of pravastatin to 3-α-iso-pravastatin	✓	✓	✓
	Isomerization of pravastatin to 6-epi-pravastatin	✓	✓	✓
	β-oxidation of 3-*S*-hydroxy-pravastatin-CoA to tetranor-CoA	✓	✓	✓
	Oxidation of fluvastatin to 5-hydroxy-fluvastatin	✓	✓	✓
	Oxidation of fluvastatin to 6-hydroxy-fluvastatin	✓	✓	✓
	Conversion of fluvastatin-CoA to des-isoproylpropionic-acid-fluvastatin-CoA	✓	✓	✓
	Hydroxylation of cerivastatin to M23	✓	✓	✓
	Hydroxylation of cerivastatin-M1 to M24	✓	✓	✓
	Glucuronidation of cerivastatin-M1	✓	↓	↓
	Glucuronidation of rosuvastatin	✓	↓	↓
	Secretion of *N*-desmethyl-rosuvastatin	✓	✓	✓
	Conversion of glucuronide to pitavastatin-lactone	✓	↓	↓
Anti-hypertensives	Oxidation of losartan to active metabolite EXP3174 (M6)	✓	✓	✓
	Glucuronidation of losartan to losartan-M7	✓	✓	✓
	Formation of losartan-N1-glucuronide	✓	✓	✓
	Tolyl methyl hydroxylation of torasemide	✓	✓	✓
	Oxidation of torasemide-M1 to M5	✓	✓	✓
	Oxidation of nifepine to nitropyridine metabolite	✓	✓	✓
	Oxidation of acid metabolite of nifedipine	✓	✓	✓
Immunosuppressants	Oxidation of cyclosporine to AM1	✓	✓	✓
	Oxidation of cyclosporine to AM9	✓	✓	✓
	Glucuronidation of AM1c-cyclosporine	✓	✓	✓
	Sulfation of cyclosporine for excretion	✓	✓	✓
	Demthylation of tacrolimus to 13-*O*-desmethyl tacrolimus	✓	✓	✓
	Demethylation of tacrolimus to 15-*O*-desmethyl tacrolimus	✓	✓	✓
	Oxidation of tacrolimus	✓	✓	✓
Analgesics	Chiral conversion of R to S form of iburpofen	✓	✓	✓
	Oxidation of *S*-3-hydroxy ibuprofen to *S*-carboxy ibuprofen	✓	✓	✓
	Secretion of 1-hydroxy-ibuprofen	✓	✓	✓
	Secretion of 1-hydroxy *S*-ibuprofen-glucuronide	✓	✓	✓
	Glucuronidation of acetaminophen	✓	✓	✓
	Sulfation of acetaminophen	✓	✓	↓
Miscellaneous	Oxidation of gliclazide to methyl-hydroxy-gliclazide	✓	✓	✓
	Oxidation of methyl-hydroxy-gliclazide to carboxy-gliclazide	✓	✓	✓
	Secretion of 6-β-OH-gliclazide-glucuronide	✓	✓	✓
	Oxidation of midazolam to 1-OH-midazolam	✓	✓	✓
	Oxidation of midazolam to 4-OH-midazolam	✓	✓	✓
	Glucuronidation of 1-OH-midazolam	✓	✓	✓
	Oxidation of allopurinol to oxypurinol	✓	✓	✓
	Conjugation of oxypurinol to oxypurinol-1-riboside	✓	✓	✓
	Conjugation of oxypurinol to oxypurinol-7-riboside	✓	✓	✓

we performed a robustness analysis under the vegetarian diet (Fig. 3). We observed a linear increase in secretion of all the above six acetaminophen metabolites with increased uptake of L-cystine, further confirming the simulation results. These modeling results are consistent with expectations that diet does influ-

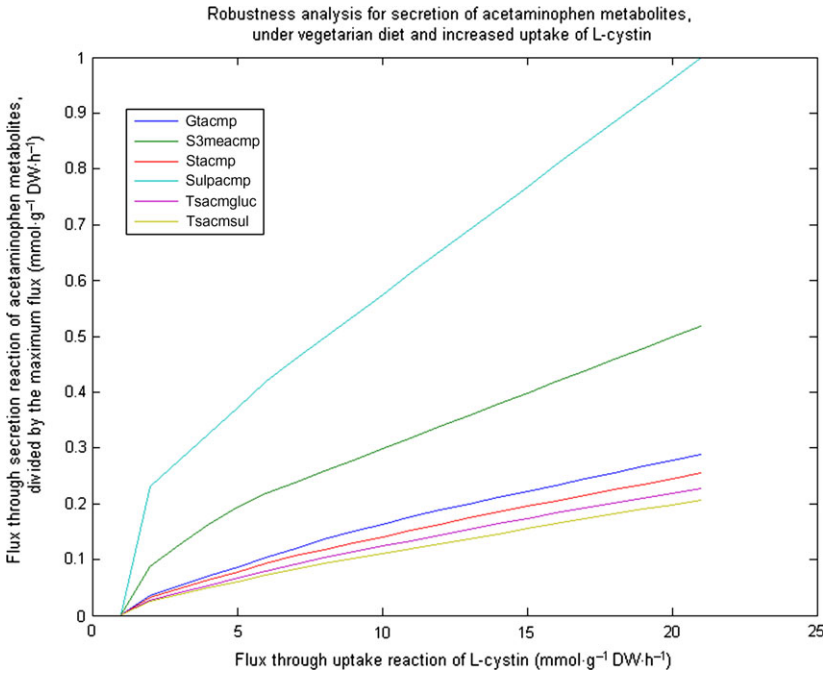


Fig. 3. Robustness analysis of acetaminophen metabolites under vegetarian diet and increased uptake rate of L-cystin. All six sulfated-acetaminophen metabolites followed increased secretion pattern as the L-cystin uptake rate increased. gtacmp, glucuronide-thiomethyl-acetaminophen conjugate; s3meacmp: sulfate-conjugate-3-methoxy-acetaminophen; stacmp, sulfate-conjugate of thiomethyl-acetaminophen; sulpacmp, sulfate-conjugate-acetaminophen; tsacmgluc, thiomethyl-sulfoxide-acetaminophen-glucuronide; tsacmsul, thiomethyl-sulfoxide-acetaminophen-sulfate. For more details on metabolites, see Table S1.

ence the capability of the human body to activate and eliminate these drugs [4,71–73].

Effect of the dietary composition on drug excretion and key cellular pathways

Although the diet can influence drug metabolism, we can now use the model to investigate how drug metabolism could affect cellular metabolism. We therefore performed flux variability analysis (FASTFVA) [74], which computes the possible flux range for each reaction of interest (e.g. the minimally and maximally feasible flux values), given the particular simulation constraints. Changes in the flux range upon altered simulation conditions (e.g. diet composition or drug uptake) are one way of identifying how such models compensate for these alterations. We computed the secretion pattern of Recon2_DM1796 for various drug metabolites (124 of 177; the remaining 53 are either internal metabolites or not secreted) under the three different dietary conditions. Each drug was given individually in a particular diet, allowing for the comparison of diet and drug-specific models. As expected, all drug metabolites known to be excreted were found to be secreted under all three dietary conditions. In most cases, the diet composition did not alter the excretion flux. However, when comparing the vegetarian diet with the average American diet, six out of 124 (5%) drug metabolites were found to have a reduced flux (with a reduction of between 62% and 81%) through the secretion reactions. All six metabolites were acetaminophen metabolites. These observations are consistent with our previous results (i.e. there was 63% flux reduction in the flux through sulfation of the acetaminophen reaction under the vegetarian diet only).

We also analyzed the optimal flux through 260 cellular tasks (Table S4) by introducing inhibition of the drug target reactions as constraints under an average American diet and optimizing each task at a time. A task was termed 'affected' if either null or reduced (relative to no-drug condition) flux was obtained through the respective objective. Only 13 of the 18 drugs had metabolic targets (Table S4). Hence, we only analyzed the effect of these drugs. Although the majority of the tasks were unaffected, all of the eight statins affected 10 of 260 cellular tasks, followed by allopurinol (three of 260) and the analgesics (acetaminophen and ibuprofen), which affected only one cellular task. Gliclazide and torasemide did not affect any of the tasks because they target transport reactions. Transport reactions are highly redundant in Recon2_DM1796, and a global metabolic reconstruction may not be the most suitable approach for capturing the effect of drugs targeting transport reactions.

The statins affected the cellular task of cholesterol synthesis and related reactions (i.e. synthesis of farnesyl-diphosphate, dolichol, coenzyme Q10 and bile acids). All of these compounds require mevalonate as their precursor, which is produced by HMG-CoA reductase (EC 1.1.1.34, EC 1.1.1.88), the statin target

enzyme (Fig. 4A). Farnesyl-diphosphate is derived from mevalonate in a seven-step reaction and is used to synthesize dolichol. The coenzyme Q10 [75–77] is synthesized from tyrosine and farnesyl-diphosphate in multiple reaction steps. Mevalonate further forms cholesterol, which is a precursor for bile acids. Therefore, statins also affect these related pathways by inhibiting HMG-CoA reductase. In the case of allopurinol, uric acid synthesis was affected (Fig. 4B) and the analgesics affected thromboxin synthesis (Fig. 4C). These effects are in accordance with their respective drug targeted action (Table S4) and it is important that a computational model can capture such relationships.

The effect of genotype on drug metabolism under the average American diet

To study the relationship between drug metabolism in healthy and disease conditions, we performed a single gene deletion [78] for each of the 1796 metabolic genes

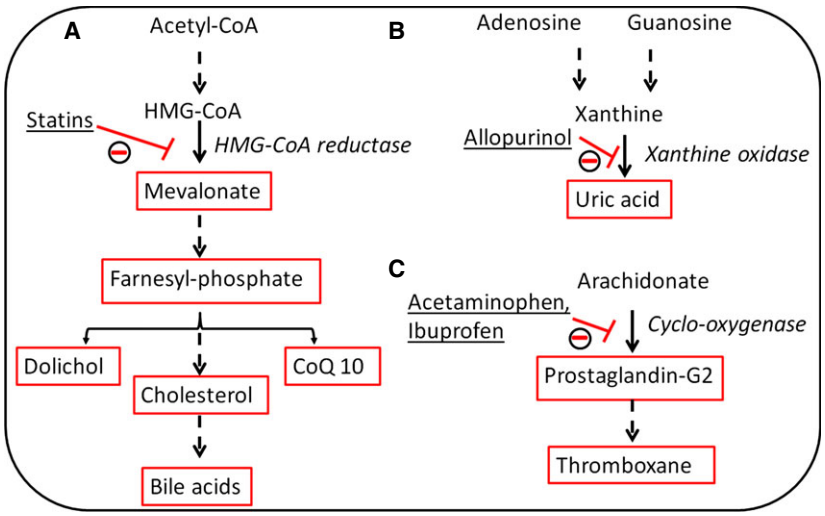
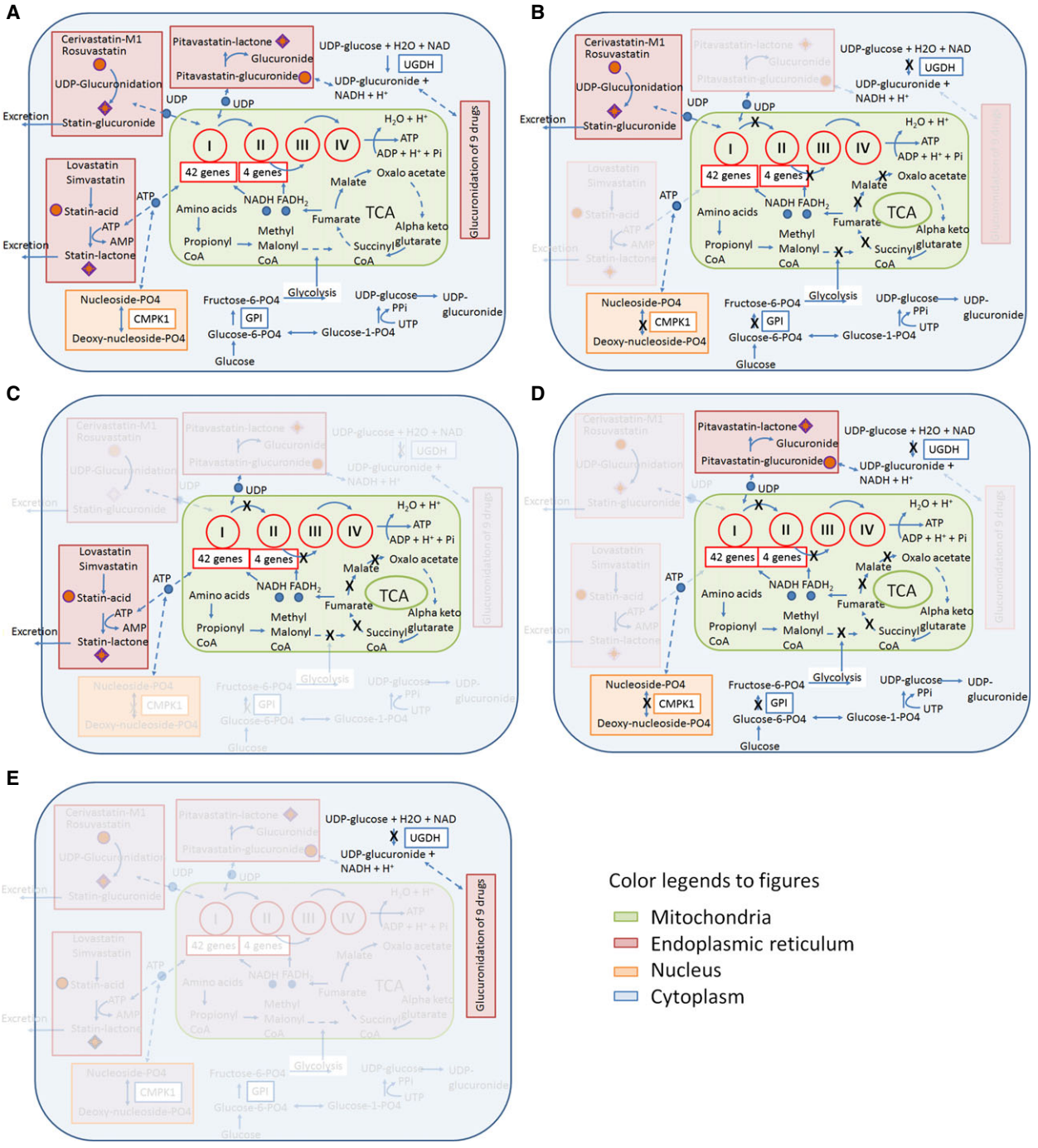


Fig. 4. The effect of drugs administration on key cellular pathways. (A) Effect of statins on cholesterol metabolism and related reactions. The synthesis of cholesterol involves a multistep reaction from the precursor acetyl-CoA molecule. The key enzyme of this pathway is HMG-CoA reductase enzyme (EC 1.1.1.34, EC 1.1.1.88), which produces mevalonate, and which is also the statin target. Mevalonate is the precursor for the synthesis of farnesyl-phosphate, dolichol, cholesterol and CoQ10. Because bile acids are also formed from cholesterol, the inhibition by statins also affects their synthesis. (B) Effect of allopurinol on purine metabolism. Allopurinol targets the xanthine oxidase (EC 1.17.3.2), which results in lack of synthesis of uric acid. (C) Effect of acetaminophen and ibuprofen on eicosanoid metabolism. These analgesics target the cyclo-oxygenase (EC 1.14.99.1). Eicosanoids, such as arachidonate, produce prostaglandins and thromboxane. Hence, analgesics affect the synthesis of these metabolites. The inhibition effects of these drugs on the enzymes and on successive steps (red) are shown.

Fig. 5. The effect of single gene deletion on the drug objectives under an average American diet. (A) The inter-relationships between gene deletions and drug objectives are shown. Areas with a colored background represent different intracellular compartments: green, mitochondria; red, endoplasmic reticulum; orange, nucleus; blue, cytoplasm. The majority of the gene deletions that affected the highest number of statin metabolic reactions belonged to the mitochondrial electron transport chain. Only energy and glucuronidation metabolic pathways are shown because their blockage (via a single gene deletion) affected the highest number of drug objectives. In the drug–drug interaction study, the TCA cycle and electron transport chain genes affected the transport reactions of atorvastatin and cyclosporine, although they only affected the metabolic reaction of atorvastatin. Similarly, the *CMPK1* and *UGDH* gene deletions affected the metabolic reactions of atorvastatin and cyclosporine but only the transport reactions of atorvastatin. These genes are thus shared between atorvastatin and cyclosporine for their complete metabolism and elimination. (B–E) Unique drug objectives that are affected by specific gene deletions, and their metabolic connections. (B) Glucuronidation of cerivastatin M1 and rosuvastatin affected by deletion of genes of the mitochondrial electron transport chain, TCA cycle genes, UDP-glucose-6-dehydrogenase, glucose-6-phosphate isomerase gene and the *CMPK1* gene. (C) Activation of lovastatin and atorvastatin pro-drug forms is affected by the deletion of genes involved in energy metabolism. (D) Conversion of glucuronide to lactone form of pitavastatin is affected by the deletion of genes involved in UDP-glucuronide formation (i.e. UDP-glucose-6-dehydrogenase), as well as genes involved in energy and amino acid metabolism. (E) Glucuronidation of nine drugs was affected by deletion of the gene directly involved in UDP-glucuronide formation (i.e. *UGDH*).



captured in Recon2_DM1796 and computed the maximally feasible flux through each of the drug objectives under the average American diet condition. An objective was deemed 'affected' by a single gene deletion when its flux was reduced by at least 20% compared to the healthy model. Overall, we found that the majority of single gene deletions did not affect any drug objective. However, 72 of the 1796 (4%) gene deletions altered the flux through at least one of the 34 drug objectives (Fig. 5 and Table S5). Sixteen objectives were not affected by any gene deletions. The deletion of the gene encoding the cytidine monophosphate kinase (*CMPK1*; GeneID: 51727, EC 2.7.4.14) affected the highest number of drug objectives (i.e. 26), highlighting the importance of this reaction for the metabolism of numerous drug molecules. Indeed, CMPK1

phosphorylates uridine-monophosphate to UDP, which is subsequently converted into UTP by the nucleoside-diphosphate kinase. UTP is required for the synthesis of UDP-glucuronate, an important co-factor in glucuronidation reactions for the majority of the modeled drugs (Fig. 5). The highest number of single gene deletions altered the flux through the drug objectives for the spontaneous conversion of the glucuronide to the lactone form of pitavastatin (53 knockout genes), glucuronidation of cerivastatin-M1 and rosuvastatin (50 gene knockouts each).

We analyzed the relevance of these single gene deletions to known diseases by mapping the recently published compendium of inborn errors of metabolism [79] to Recon2_DM1796. We found that 25 of the 73 (34%) gene deletions corresponded to 12 unique IEMs (Table S6). Interestingly, 80% of the affected genes (20 of 25) were found to encode proteins involved in energy metabolism [electron transport chain, inosine-monophosphate synthesis, pyrimidine metabolism, tricarboxylic acid (TCA) cycle and glycolysis] and their deletions are therefore predicted to have a more significant effect on the metabolism of the statin drugs. An additional 56% of the IEM associated gene deletions (14 of 25) were found to encode complex I and II proteins of the mitochondrial electron transport chain (Fig. 5).

Atorvastatin and cyclosporine drug–drug interaction: single gene deletion and secretion profile

One of the most fascinating aspects of drug metabolism is the ability of different drugs to interact with each other when administered simultaneously. To study the ability of Recon2_DM1796 to capture the metabolic interactions of known drugs, we chose to study the interaction between statins and immunosuppressants. These drugs are known to exhibit considerable interactions, often leading to rhabdomyolysis in patients [80–83]. The joint administration of atorvastatin and cyclosporine was simulated under conditions of the average American diet. We predicted that 21 drug metabolites can be secreted, seven of which were derived from atorvastatin and 11 from cyclosporine. No change in the maximally feasible flux rate was predicted for these secretion exchange reactions when the two drugs were given together or separately. We also investigated the effect of the genotype on this drug interaction by comparing simulations with and without the gene deletion. We computed the maximally feasible flux through all 80 associated drug reactions (i.e. reactions involving metabolism of these drugs) and found that 48% of the reactions (38 of 80) were affected by 58 of the 1796 gene deletions (Fig. 5 and Table S7). Interestingly, 46 of the 58 (79%) affected genes encoded proteins of the mitochondrial electron transport chain and these alterations reduced the flux through the reactions of atorvastatin metabolism and the transport of the two drugs. Additionally, the gene deletions for cytidine monophosphate kinase (GeneID: 51727) and UDP-glucose 6-dehydrogenase (GeneID: 7358) affected the metabolic reactions of both drugs but only altered the elimination reactions of atorvastatin (Fig. 5).

Discussion

The presented drug module represents a comprehensive, manually assembled collection of metabolic reactions for 18 of the most commonly used drugs. In conjunction with the most recent human metabolic reconstruction, we investigated the role of dietary regime and genetic background on drug metabolism. Our findings are that: (a) diet plays a crucial role in the metabolism and elimination of acetaminophen and statins; (b) disturbed statin metabolism may contribute to the phenotypic effects of mitochondrial energy disorders; and (c) metabolic interactions between atorvastatin and cyclosporine in individuals with inherited metabolic diseases may occur as a result of common metabolic and transport reactions, using common metabolites and transport proteins.

Diet has been known to play both a direct and indirect role in drug metabolism, either by inducing/inhibiting phase I enzymes (e.g. garlic inhibits CYP3A4 activity [72], whereas caffeine induces CYP1A1/1A2 activity [73]) or by affecting drug transport systems (e.g. fruit juices and herbal supplements induce P-glycoprotein activity [4,71]). Additionally, the gut microbiota modulate drug metabolism either alone or in combination with ingested dietary ingredients [84]. In the present study, we analyzed the effect of three different dietary regimes on various drug metabolic pathways. The metabolism of acetaminophen and statins was predicted to be greatly altered depending on the diet. For example, the simulated vegetarian diet was low in L-cystin (i.e. a disulfide formed from two cysteine molecules), which resulted in a reduced excretion capability for the six acetaminophen metabolites, as well as a reduced flux through the acetaminophen sulfation reaction, compared to the two other diets (Table 3). Because the simulated vegetarian diet contained only L-cystin and not L-cysteine, the L-cystin is either acted upon by glutathione oxido-reductase (EC 1.8.4.4) or thioredoxin-disulfide reductase (EC 1.8.1.9) to release the cysteine molecules. The sulfur contained in cysteine then enters various metabolic

pathways, including the biosynthesis of phosphoadenylyl sulfate, an important cofactor in the sulfation reaction of acetaminophen (Fig. 2B), in which the sulfate conjugate of acetaminophen is formed by the action of sulfo-transferase 1 (EC 2.8.2.1). Only 30–50% of an administered acetaminophen dosage is recovered in the urine in the form of sulfate conjugates [48]. The lack of sulfur-containing amino acids and sulfur deficient diets have been shown to cause reduced acetaminophen elimination [85], hepatic necrosis and liver injury in rats [86]. Furthermore, consistent with our predictions, apiaceous vegetable diets, which have high levels of carrots, parsnips, celery, dill and parsley, and which are generally low in sulfur-containing compounds, have been reported to result in reduced activity in the acetaminophen metabolic enzymes [87].

The cellular energy state has been shown to influence drug metabolism. In particular, energy deficiencies have been reported to result in the decreased clearance and elimination of antipyrine and aminopyrine drugs [88], as well as phenazone and theophylline drugs [5]. We observed an average flux reduction of 80% for the five drug objectives of the numerous statins under balanced and vegetarian diets (Table 3). When analyzed for the major energy source, under these diets, the average American diet used more carbohydrates (e.g. starch, maltose, lactose, sucrose) and lipids (e.g. triglycerides) as a result of their higher input (Table S2). Carbohydrates are directly fed into energy production by their conversion into glucose. Lipids yield ATP via the lipolysis of triglycerides, followed by the β-oxidation of the resulting fatty acids. The balanced diet utilized uniquely more essential fatty acids, such as α-linolenic acid and eicosatetraenoic acid. Additionally, the higher utilization of retinol was common between balanced and vegetarian diets. The retinol-utilizing reactions generated NADH (electron carrier for ATP synthesis) via retinol dehydrogenase (EC 1.1.1.105) and retinal dehydrogenase (EC 1.2.1.36). This shift in favorable energy precursors is determined by the particular dietary condition and therefore there was a marked difference in the efficacy of statin metabolism between the three diets. The dependence of statins on energy is mainly for their elimination via ABC transporters and for UDP synthesis, which is in turn needed for the subsequent glucuronidation reactions. When the general cellular reactions were analyzed, the average American diet resulted in much higher flux through ATP synthase, the complex I–IV reactions of the electron transport chain and the biomass reactions, implying that a higher cellular energy level is available for growth and nongrowth associated functions under this diet. Hence, we suggest that a diet that supplies a rich source of energy to the cell favors efficient drug metabolism and ultimately drug excretion, as observed for the statins.

Myopathy is associated with 0.1% of the patient population on statin monotherapy, which may lead to rhabdomyolysis (i.e. severe muscle damage, myoglobinuria, acute renal failure and death) when combined with other drugs, such as cyclosporine, digoxin and erythromycin. This severe adverse drug reaction may be a result of the complex interactions between these drugs and disease [89]. Atorvastatin and cyclosporine are commonly prescribed after organ transplantation and, subsequent to their concomitant usage, rhabdomyolysis has been reported in both renal [77] and cardiac [90] transplant patients. Various mechanisms of statin–cyclosporine interactions have been proposed, and the interactions are considered to stem from shared metabolic pathways because both drugs are CYP3A4 substrates, and have common elimination routes via P-glycoprotein [91]. Based on our simulations, we propose that additional metabolic routes, such as UDP-glucose 6-dehydrogenase (EC 1.1.1.22) and UMP-CMP kinase (EC 2.7.4.14), play a significant role in this drug–drug interaction. The UMP-CMP kinase provides UDP for the synthesis of UDP-glucuronate, which is required for the glucuronidation of these drugs. Statin-induced myopathy may also result from certain genetic predispositions, as observed in individuals with IEMs causing muscle disease/exercise intolerance, such as McArdle disease (OMIM: 232600), CPT-II deficiency (OMIM: 255110), myoadenylate deaminase deficiency (OMIM: 102770) [92] and a number of neuromuscular disorders [93]. Simultaneously, genetic polymorphisms in a number of *CYP*, *OATP* and *APOE* family genes have been linked to myopathic outcomes after statin administration [94]. We predict that a major fraction of the gene knockouts (79%) encoding proteins of the mitochondrial electron transport chain affected both the metabolic and transport reactions of atorvastatin and cyclosporine, highlighting their dependence on cellular energy for elimination. We thus propose that specific mutations in such genes (Fig. 5) could alter the interaction profile of these drugs, leading to energy deficiency, which could further contribute to their adverse drug reactions.

Disease conditions known to influence drug metabolism include liver disease, endocrine disorders and various types of infections [1,95]. Only a limited number of studies have analyzed the effect of drugs on individuals with particular inherited metabolic diseases. The examples include: (a) statins and lactic acidosis (OMIM: 245400) [7]; (b) anti-epileptic drugs and dihydrolipoamide dehydrogenase deficiency (OMIM: 238331) [8]; (c) methotrexate, trimethoprim and niacin,

as well as hyperhomocysteinemia (OMIM: 603174); (d) drugs known to induce hemolytic conditions (e.g. antimalarials, sulfonamides, antipyretics) and G6PD deficiency (OMIM: 305900) [96]; (e) sulfanol, aminopyrine and pyrazinamide, as well as hepatic porphyria [6]; and (f) thiopurine drugs and thiopurine *S*-methyltransferase deficiency (OMIM: 610460). We showed that the metabolism of statins was most affected by the deletion of various genes, most of which encode for proteins involved in energy metabolism (Fig. 5). Indeed, 46 of these genes encode for the NADH and succinate dehydrogenases and complexes I and II of the electron transport chain. Furthermore, the predicted reduction of statin metabolism in lower energy diets is because all statins and most of their derivatives require ATP for their elimination, which occurs via the ABC transport proteins. Additionally, energy is required for the activation of fluvastatin and pravastatin and for interconversion between the acid and lactone forms of atorvastatin, simvastatin and lovastatin. At the same time, even though our simulations of enzyme deficiencies were carried out under the energy-rich average American diet, deletions of genes involved in energy metabolism reduced the energy metabolism of the corresponding models. We therefore propose that the observed adverse effect of statins on individuals with mitochondrial energy disorders may be a result of compromised levels of cellular ATP.

In the present study, we describe a drug module that can be readily combined with the human metabolic reconstruction to investigate the effect of drug metabolism on the overall cellular metabolism. The *in silico* model not only captured known drug actions, but also suggested biochemical insights that could aid effective drug metabolism. Moreover, the tissue-specific metabolism of drugs can also be derived from the current module, provided that the precise uptake and secretion transporters and metabolic enzymes are sufficiently known. This in turn could aid in the development of more individualized and personalized medications. Furthermore, this information will also serve as a starting point for investigating adverse drug reactions and their biochemical basis.

Materials and methods

Reconstruction of drug metabolism

Drugs were chosen in accordance with qualifying criteria. The drugs selected were: (a) primarily administered via the oral route; (b) among the most commonly used/highly prescribed agents; (c) able to undergo extensive metabolic reactions, including first-pass extrahepatic metabolism prior to excretion; and (d) able to exhibit significant interactions with other drugs. A thorough manual curation of the scientific literature was performed to obtain information on the absorption, distribution, biotransformation, elimination, therapeutic usage, clinical dosage, adverse drug reactions and associated transporters of the selected drugs. Additional information was obtained from PharmGKB [97], DrugBank [98], Human Metabolome Database [99], Chemical Entities of Biological Interest (ChEBI) [100] and PubChem [101]. All drug compounds were drawn using MARVINSKETCH, version 5.7.0, 2011 (http://www.chemaxon.com). The elemental formula, charge and chemical structure were thoroughly checked against the databases and scientific literature. Genome annotations were obtained from EntrezGene [102] and protein information was obtained from the Uniprot [103] and BRENDA [104] databases and used along with the scientific literature for assigning gene–protein-reaction associations to metabolic and transport reactions. The reconstruction methodology was kept in accordance with the reconstruction protocol [105].

Collection of dietary constituents

Diet information was collected from the US Department of Agriculture through the Agricultural Research Service [66]. All nutrients were converted to common units ($g \cdot day^{-1}$). The average American diet (≥ 2 years) was collected for both males and females [66]. A balanced diet representing the recommended dietary reference intakes of micronutrients and macronutrients [67,68] was also assembled. The dietary reference intake reflects the minimum requirements advised to be taken daily to maintain a healthy lifestyle. The values were taken for males aged between 30 and 50 years. The total fat and cholesterol intake for the balanced diet was also represented [106,107]. Vegetarian diet information was also collected [69] and the serving size was calculated to be three cups, as suggested by the Centers for Disease Control and Prevention (http://www.cdc.gov/nutrition/index.html). The vegetarian diet consisted of a mixture of corn, lima beans, snap beans, green peas and carrots (with salt). We did not represent the energy value of the food (i.e. the caloric load) as a result of a disparity between the various sources and a lack of specific information. The total carbohydrate content in this diet was converted into the starch and dextrin uptake because polysaccharides are the most abundant carbohydrate component present in our diet [68]. Disaccharides represented 60% and monosaccharides represented 40% of the total sugars because free monosaccharides are not a significant part of the diet [68] (Table S2).

Combining the drug module with the human metabolic reconstruction

The drug reconstruction was assembled and converted into a mathematical model using RBIONET [108] as a reconstruc-

tion environment in conjunction with the reconstruction protocol established previously [105]. All reactions were checked for mass and charge balance and corrected manually. Thereafter, the reconstruction was combined with the recently published community-driven global reconstruction of human metabolism, Recon 2 [10]. Both matrices were merged and analyzed for dead-end metabolites and network gaps using COBRA toolbox [75]. Gaps were filled by reviewing the scientific literature. Furthermore, the biomass reaction was tested for a nonzero, positive flux value, and non-mass balanced reactions were checked.

Simulations

The drug and dietary ingredient dosage values were converted to common units ($g \cdot day^{-1}$). Because the small intestine acts as a gateway for the passage of all ingested components into the body, the model inputs were formulated as the dry weight (g) of small intestinal enterocytes (i.e. in $mmol \cdot gDW,enterocytes^{-1} \cdot h^{-1}$), as described previously [109]. The intestinal absorption rates of each diet and drug compound were collected from the literature. The lower and upper bounds of the corresponding exchange reactions were fixed accordingly (Table S2). Additionally, the lower and upper bounds of all the model reactions were raised by 10^3 to avoid the error range (i.e. values below 10^{-5}) of MATLAB (MathWorks, Inc., Natick, MA, USA). We obtained diet- and drug-specific models and tested them for the 50 drug metabolic objectives using FBA [70]. Consequently, the models were also tested for a functional biomass under each of these conditions.

Drug objectives and secretion reactions

Drugs objectives were chosen to represent the overall metabolism of each drug, including their transport reactions. However, in cases where a major part of the drug was known to undergo a specific metabolic pathway (e.g. 80% of acetaminophen was known to undergo non-oxidative metabolism), the relevant pathway was chosen. All drug metabolites derived from the parent form of the drug were constrained to be 'only secreted' into the extracellular space (i.e. the corresponding exchange reactions were set to have a lower bound) (lb = 0).

FBA

Assuming steady-state conditions where the sum of the input fluxes equaled the sum of the output fluxes, FBA follows $S.v = 0$, where S represents the stoichiometric matrix of size $m \times n$ (where m is the number of metabolites and n is the number of reactions in the network). In this relationship, v is a flux vector of size $n \times 1$, containing a flux value of v_i, for each reaction i, in the network. Under the given simulation conditions, we used FBA to maximize a given objective function, as defined by the constraints applied to the model.

To identify the basic metabolic differences between the three diets under different drug dosage conditions, we computed the optimal flux vector at the same time as minimizing the Euclidean norm of the internal fluxes.

Robustness analysis

This method uses FBA to analyze the sensitivity of the specific objective reaction to another chosen reaction [70]. In this method, the flux through chosen reaction is varied and the optimal flux value through the objective reaction is calculated. Under vegetarian diet constraints, the flux through the uptake reaction for L-cystin was varied between 1 $mmol \cdot gDW^{-1} \cdot h^{-1}$ and 20 $mmol \cdot gDW^{-1} \cdot h^{-1}$, and the optimal flux value through the secretion of acetaminophen metabolites was calculated (Fig. 3).

Fast flux variability analysis

Flux variability analysis [110] uses FBA to maximize and minimize each reaction in the model and was performed using FASTFVA [74].

Single gene deletion analysis

Generally, reactions in metabolic networks are represented with gene–protein-reaction associations, which are Boolean relationships between genes that encode the enzymes/proteins catalyzing the reactions. These relationships make use of 'or' when isozymes are involved and 'and' when multimeric enzyme complexes are involved. In a single gene deletion [75], those reactions associated with an 'and' relationship or encoded by a single gene were inactivated by setting the corresponding reaction bounds to zero ($lb = ub = 0$ $mmol \cdot gDW^{-1} \cdot h^{-1}$) using FBA to maximize the given objective function. This procedure was performed for all network genes.

All fluxes were computed ($mmol \cdot gDW^{-1} \cdot h^{-1}$). All simulations were carried out using the MATLAB programming environment and the TOMLAB (TomOpt Inc., Seattle, WA, USA) linear programming solver with COBRA [75].

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Author contributions

SS designed and performed the experiments, analyzed data and wrote the paper. IT designed the experiments, analyzed data and wrote the paper. HH and RF developed analytical tools.

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Supporting information

Additional supporting information may be found in the online version of this article at the publisher's web site:

Table S1. Drug Reconstruction: Rxns, Mets: Drug reconstruction content (reaction content), drug reconstruction content (metabolite content).

Table S2. Diet formulations: Nutrient composition of balanced diet, average American diet, and vegetarian diet. All the model inputs reported are the constraints fixed on lower bounds of the exchange reactions.

Table S3. FBA of drug objectives: List of metabolic tasks, references, and computed values.

Table S4. DrugEffect_cellularTasks: Drug targets in the Recon2_DM1796, and effect of drugs on the key cellular objectives

Table S5. Genotype_Drug effect: Effect of genotype on drug metabolism under average American diet

Table S6. Mapping of IEMs: List of inborn errors of metabolism correspoding to the single gene deletions of Recon2_DM1796

Table S7. Genotype_Drug interaction: Effect of genotype on atorvastatin-cyclosporine metabolism under average American diet, administered together.