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Skin metabolism phase I and phase II enzymes in native and reconstructed human skin: a short review

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Understanding skin metabolism is important when considering drug discovery and safety assessment. This review compares xenobiotic skin metabolism in *ex vivo* skin to reconstructed human skin and reconstructed human epidermis models, concentrating on phase I and phase II enzymes. Reports on phase I enzymes are more abundant than for phase II enzymes with mRNA and protein expression far more reported than enzyme activity. Almost all of the xenobiotic metabolizing enzymes detected in human skin are also present in liver. However, in general the relative levels are lower in skin than in liver and fewer enzymes are reported.

Introduction

The skin is the largest organ in the human body and provides a protective barrier between the environment and our inner organs. In line with its barrier properties, the skin also has essential metabolic properties. This is of importance when considering drug discovery, identification of mode of action of drugs applied to skin and safety assessment of drugs and consumer products. Animal models have been extensively used in the preclinical phase of drug development and for safety testing. However, animal models are now recognized to poorly predict the human response as a result of differences in skin physiology and immunity [1,2]. This, together with ethical reasons, has resulted in an ongoing transition from animal testing to alternative testing strategies and the implementation of the 7th amendment to the EU Cosmetics Directive 76/768/EEC promoting reduction, refinement and, where possible, replacement of animal usage [3]. Already, human *ex vivo* skin explants and *in vitro* reconstructed human skin (RHS) models are widely being developed and implemented for risk assessment of drugs and corrosion, irritation, genotoxicity and sensitization testing of chemicals [4]. Importantly, with respect to metabolism, animal usage does not permit a focus on skin metabolism but rather metabolism within the entire animal, in particular the liver.

Because the liver is considered the most important organ for metabolizing systemic xenobiotic factors [5], in general metabolism data are derived from liver, and divided into three phases (phase I, phase II and transporters). Data have accumulated from animal, *in vitro* liver studies and *in silico* models: (i) phase I involves modification of xenobiotics by enzymes like the cytochrome P450 (CYP) family, aldehyde oxidases (AOs), aldehyde dehydrogenases (ALDHs), aldo-ketoreductases (AKRs), alcohol dehydrogenases (ADHs), esterases, flavin-containing monooxygenases (FMOs) and cyclooxygenases (COXs) [6]; (ii) phase II enzymes like gluthathione *S*-transferases (GSTs), UDP-glucuronosyltransferases (UGTs), sulfotransferases (SULTs), *N*-acetyltransferases (NATs) and methyltransferases (MTs) add polar groups to the phase I products to prepare them for excretion [7,8]; and (iii) transporters, ATP-binding cassette (ABC) and solute carrier (SLC) transporters export (altered) metabolites out of the cell [9]. Next to the enzymes involved in these phases, proteinases and peptidases also play a part in xenobiotic metabolism. Establishing the absorption, distribution and metabolism properties of xenobiotics in human skin is crucial for safety assessment of drugs and consumer products that are applied topically to the skin and also to understand the mode of action of drugs. Currently, studies on skin metabolism focus on enzymes and pathways identified from liver metabolism but how relevant these are for skin metabolism remains to be determined.

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This review focuses on the data available describing metabolic competency in native human skin (*ex vivo* human skin tissue explants), compared with *in vitro* 3D organotypic reconstructed human epidermis (RHE) and reconstructed human skin (RHS) models. In 2007, we performed an extensive review on the current knowledge of skin metabolism [10]. Here, we focus on data published after this period and with emphasis on the phase I and phase II enzymes involved in skin metabolism of xenobiotics.

Native human skin to study xenobiotic metabolism ex *vivo*

Skin consists of two main layers: the epidermis and the dermis. The upper air-exposed epidermis, with its rigid lipid-rich stratum corneum, has a major role in barrier function [10]. It consists predominantly of stratified, proliferating and differentiating keratinocytes (95%) with important innate immune cells such as Langerhans cells and mast cells being distributed throughout. The underlying dermis provides tensile strength and elasticity. Additionally, the dermis contains blood and lymph vessels, skin appendages (hair follicles, glands) and residential as well as infiltrating immune cells. Fibroblasts are dispersed throughout the dermis and are responsible for secreting the extracellular matrix. Freshly excised (*ex vivo*) skin has all of these attributes but, owing to the lack of blood supply, it has a limited viability of ~48 h. A short-term *ex vivo* skin explant culturing method was developed to study skin absorption, distribution, metabolism and excretion for topical and, to a lesser degree, systemic, chemical exposure [11,12]. This methodology is in agreement with the OECD guideline no. 28 describing skin absorption testing [13]. Here, we discuss studies describing phase I and phase II enzymes involved in xenobiotic skin metabolism in *ex vivo* skin explants.

Phase I enzymes in native human skin

CYPs, especially CYP1, CYP2, CYP3 and CYP4 subfamilies, are an important class of metabolizing enzymes that account for 75% of the total phase I metabolism in liver by producing reactive intermediates or activating drugs with their monooxygenase activity [5]. In human skin, members of the CYP1 family have been shown to be expressed. However, with regard to activity of CYP1 enzymes, controversy exists when using, among others, the well-known EROD (7-ethoxy resorufin *O*-deethylase) assay (Table 1). Some authors describe intermediate activity of CYP1 enzymes [14], whereas others were confronted with activity below the threshold limit of the assay [15]. Concerning the CYP2 and CYP3 subfamilies, mRNA has been detected [14,16–19] but the enzyme activity was generally around the limit of detection or undetectable [14,15]. Eilstein and colleagues showed some CYP2 activity in skin using the 7-methoxy-4-(trifluoromethyl)-coumarin *O*-demethylase (MFCOD) assay; however, this was again close to the detection limit of the assay [14]. CYP3 activity can be demonstrated by using the benzyloxyquinoline (BQ) assay [14,15]; but again the levels were around the detection limit. For most of the other CYP subfamily enzymes (e.g., CYP4, CYP7, CYP8, CYP11, CYP21, CYP26, CYP27, CYP39 and CYP51) no activity has been assessed whereas the mRNA levels have been shown to be present [16,18]. Another CYP enzyme mRNA: CYP2S1, was shown in human skin and could probably be considered to be involved in the reductive detoxification of several carcinogenic aromatic amines and heterocyclic *N*-hydroxylamines [16,20]. Despite the fact that mRNA for most CYPs is present in native skin and is even induced in some studies [21,22], the expression and activity is clearly lower than in the liver.

Other phase I enzymes have been studied in addition to CYPs. AOs facilitate the conversion of aldehydes into carboxylic acid and catalyze the hydrolyzation of heterocycles. AO is, next to CYPs, one of the most important enzymes in phase I metabolism in the liver [23]. In human skin, elevated AO mRNA was detected in buttock skin [16] and at protein levels in female breast skin [24], whereas enzyme activity has been shown in various skin sources [25]. To examine its activity, substrates like carbazeran and zoniporide were used. Conversion into 4-hydroxycarbazeran and into 2-oxo-zonporide was demonstrated, which could be reverted by the AO inhibitor hydralazine. These findings indicate that AOs can play a part in *ex vivo* skin metabolism [25].

ALDH catalyzes the conversion of aldehydes into carboxylic acid. Next to its catalytic function, ALDH plays a part as an antioxidant and facilitates UV–light absorption [26]. Detectable mRNA levels of several ALDH subtypes (i.e., 1, 2, 3 and 7), as well as protein of some of these enzymes in different explant sources, were shown [16,24]. Enzyme activity was reported in native skin only by Eilstein *et al.* [27]. The same applies to the AKR superfamily of proteins, which are structurally related and catalyze redox reactions on, for example, sugar aldehydes, quinones and keto-steroids [28]. In human skin, mRNA as well as protein of AKR1 (A and C) and AKR7 have been demonstrated in various explants [16,18,24] but, here, experiments on activity are lacking.

The ADH enzymes convert alcohols to ketones or aldehydes. There are five classes and the most important one in the liver is ADH1 [29]. Also, for this class of enzymes, in skin explants mRNA and protein expression have been shown [18,24]. Independent of these studies, in other studies it was suggested that ADH was active because conversion of cinnamaldehyde to cinnamic alcohol and cinnamic acid was realized [27,30]. Esterases are enzymes that catalyze hydrolysis reactions to split esters into an acid and alcohol. Esterases have a profound role in oxidation of drugs in the liver [31]. In human skin, esterase activity has been proven in several studies as discussed by Gibbs *et al.* [10]. More recently, carboxyl esterase mRNA has also been detected in various human skin explant models [16,24] but no activity assessment has been performed.

FMOs catalyze oxidation reactions. Substrates of FMOs vary and the main function is to oxidize xenobiotics to make them excretable [32]. FMO mRNA of subfamilies 1–5 has been detected in various skin sources [16–18]. Although the presence of FMO3 protein was shown [17], activity has not been detected. The COX enzymes are involved in inflammation but also play an important part in metabolism by catalyzing peroxidase reactions in which prostaglandins are present [33]. In two independent studies, COX mRNA was demonstrated in skin [34,35], whereas, by measuring PGE2 formation, one study was able to show COX activity [15].

Besides these main classes of phase I metabolizing enzymes, other dehydrogenases have been detected in human skin (Table 1). In short, Hu *et al.* detected mRNA of glucose-6-phosphate dehydrogenase 3, NAD(P) transhydrogenase, NAD(P)H quinone

TABLE 1

Detection of mRNA, protein and/or tested activity of the phase I metabolizing enzymes in native skin, 2D cell culture and skin models (RHE and RHS)

Phase I enzymes	*Ex vivo* native skin	2D KC cell cultures	Reconstructed human epidermis	Reconstructed human skin
Cytochrome P450	mRNA [14,16–19]	mRNA [17,53,61–63]$^{\&}$ [64]$^{\#}$	mRNA [18]$^{\alpha}$ [21]$^{\mu}$	mRNA [18]$^{\delta}$ [17,21]$^{\beta}$
CYP1A1	Activity [14]/no activity [15]	Protein [63]$^{\&}$ Activity [63]$^{\&}$ [64]$^{\#}$ [65]$^{@}$	Activity [14]$^{\alpha\ \pi}$ [48]$^{\lambda}$/no activity [49]$^{\mu}$	Activity [14] $^{\delta}$/no activity [49]$^{\beta}$
CYP1A2	mRNA [16,18] No activity [15]	mRNA [53,62]$^{\&}$ Activity [15]$^{\&\ \#\ @}$	mRNA [18]$^{\alpha}$ [16]$^{\mu}$ Activity [14]$^{\alpha\ \pi}$/no activity [49]$^{\mu}$	mRNA [18]$^{\delta}$ Activity [14]$^{\delta}$/no activity [49]$^{\beta}$
CYP1B1	mRNA [16–19] Activity [14]/no activity [15]	mRNA [17,53,61–63]$^{\&}$ [66]$^{\#}$ No activity [15]$^{\&\ \#\ @}$	mRNA [18]$^{\alpha}$ [16,21]$^{\mu}$ [48]$^{\lambda}$ Activity [14]$^{\alpha\ \pi}$	mRNA [18]$^{\delta}$ [17,21]$^{\beta}$ Activity [14]$^{\delta}$
CYP2A6	mRNA [16]	mRNA [53,67]$^{\&}$	–	mRNA [17]$^{\beta}$
CYP2B1	–	Activity [63]$^{\&}$ [65]$^{@}$	–	–
CYP2B6	mRNA [19] No activity [14,15]	mRNA [53,67]$^{\&}$ [67]$^{\#}$ Protein [63]$^{\&}$ No activity [15]$^{\&\ \#\ @}$	mRNA [18]$^{\alpha}$ Activity [14]$^{\alpha\ \pi}$/no activity [49]$^{\mu}$	mRNA [18]$^{\delta}$ Activity [14]$^{\delta}$/no activity [49]$^{\beta}$
CYP2C8	–	mRNA [53]$^{\&}$	mRNA [18]$^{\alpha}$	mRNA [18]$^{\delta}$
CYP2C9	mRNA [16,18]	mRNA [62]$^{\&}$	mRNA [18]$^{\alpha}$ [16]$^{\mu}$	mRNA [18]$^{\delta}$
CYP2C18	mRNA [16] No activity [14]	mRNA [62]$^{\&}$	mRNA [18]$^{\alpha}$ [16,21]$^{\mu\ \pi}$ [48]$^{\lambda}$ Activity [14]$^{\alpha\ \pi}$	mRNA [18]δ [21]$^{\beta}$ Activity [14]$^{\delta}$
CYP2C19	–	mRNA [62]$^{\&}$	mRNA [21]$^{\mu}$	–
CYP2D6	mRNA [16,18]	mRNA [17,62]$^{\&}$	mRNA [18]$^{\alpha}$ [16]$^{\mu}$	mRNA [18]$^{\delta}$ [17]$^{\beta}$
CYP2E1	mRNA [16–19] No activity [14]	mRNA [17,61–63,68]$^{\&}$ Protein [63,68]$^{\&}$ Activity [63]$^{\&}$	mRNA [18]$^{\alpha}$ [21]$^{\mu}$ Activity [14]$^{\alpha\ \pi}$	mRNA [18]$^{\delta}$ [17,21]$^{\beta}$ Activity [14]$^{\delta}$
CYP2F1	mRNA [18]	–	mRNA [18]$^{\alpha}$	mRNA [18]$^{\delta}$
CYP2J2	mRNA [16,18]	mRNA [62]$^{\&}$	mRNA [18]$^{\alpha}$ [16,21]$^{\mu}$	mRNA [18]$^{\delta}$ [21]$^{\beta}$
CYP2R1	mRNA [16]	mRNA [62]$^{\&}$	mRNA [16,48]$^{\mu\ \lambda}$	–
CYP2S1	mRNA [16,20]	mRNA [17,62]$^{\&}$ [69]$^{\#}$	mRNA [16,48]$^{\mu\ \lambda}$	mRNA [17]$^{\beta}$
CYP2U1	mRNA [16]	mRNA [62]$^{\&}$	mRNA [16]$^{\mu}$	–
CYP2W1	mRNA [16]	mRNA [62]$^{\&}$	mRNA [16]$^{\mu}$	–
CYP3A1	mRNA [17] No activity [15]	Protein [63]$^{\&}$ Activity [15,63]$^{\&}$/no activity [15]$^{\#\ @}$	Activity [15,49]$^{\mu}$	Activity [49]$^{\beta}$
CYP3A4	mRNA [17] Activity [15]	mRNA [53,62,67,68]$^{\&}$ Protein [63,68]$^{\&}$ Activity [15]$^{\#\ @}$/no activity [15]$^{\&}$	mRNA [21]$^{\mu}$ Activity [14,15]$^{\alpha\ \mu}$	mRNA [17,21]$^{\beta}$ Activity [14]$^{\delta}$
CYP3A5	mRNA [16,18,19] Activity [14]	mRNA [63,68]$^{\&}$ Protein [68]$^{\&}$	mRNA [18]$^{\alpha}$ [16,21]$^{\mu}$ [48]$^{\lambda}$ Activity [14]$^{\alpha\ \pi}$	mRNA [18]$^{\delta}$ [21]$^{\beta}$ Activity [14]$^{\delta}$
CYP3A7	mRNA [18] Activity [14]	–	mRNA [18]$^{\alpha}$ Activity [14]$^{\alpha\ \pi}$	mRNA [18]$^{\delta}$ Activity [14]$^{\delta}$
CYP3A43	mRNA [16]	–	–	–
CYP4A11	mRNA [16]	–	–	–
CYP4B1	mRNA [16,18]	mRNA [62]$^{\&}$	mRNA [18]$^{\alpha}$ [16,21]$^{\mu}$ [48]$^{\lambda}$	mRNA [18]$^{\delta}$ [21]$^{\beta}$
CYP4F2	mRNA [16]	–	mRNA [18]$^{\alpha}$ [48]$^{\lambda}$	mRNA [18]$^{\delta}$
CYP4F3	mRNA [16,18]	–	mRNA [18]$^{\alpha}$ [16]$^{\mu}$ [48]$^{\lambda}$	mRNA [18]$^{\delta}$
CYP4F8	mRNA [16,18]	–	mRNA [18]$^{\alpha}$ [16]$^{\mu}$	mRNA [18]$^{\delta}$
CYP4F11	–	–	mRNA [48]$^{\lambda}$	–
CYP4F12	mRNA [16,18]	–	mRNA [18]$^{\alpha}$ [16]$^{\mu}$	mRNA [18]$^{\delta}$
CYP4F22	mRNA [16]	–	mRNA [16]$^{\mu}$	–
CYP4V2	mRNA [16]	–	mRNA [16]$^{\mu}$	–
CYP4 × 2	mRNA [16]	–	–	–
CYP7B1	mRNA [16,18]	–	mRNA [18]$^{\alpha}$ [16]$^{\mu}$ [48]$^{\lambda}$	mRNA [18]$^{\delta}$
CYP8B1	mRNA [16]	–	–	–
CYP11A1	mRNA [18]	–	mRNA [18]$^{\alpha}$	mRNA [18]$^{\delta}$
CYP20A1	mRNA [16]	–	mRNA [16]$^{\mu}$	–
CYP21A2	mRNA [18]	–	mRNA [18]$^{\alpha}$	mRNA [18]$^{\delta}$
CYP24A1	–	–	mRNA [16]$^{\mu}$	–
CYP26A1	mRNA [18]	mRNA [53]$^{\&}$	mRNA [18]$^{\alpha}$	mRNA [18]$^{\delta}$
CYP26B1	mRNA [16]	–	mRNA [18]$^{\alpha}$ [16]$^{\mu}$ [48]$^{\lambda}$	mRNA [18]$^{\delta}$
CYP27A1	mRNA [18]	–	mRNA [18]$^{\alpha}$	mRNA [18]$^{\delta}$
CYP27B1	mRNA [16]	–	mRNA [18]$^{\alpha}$ [16]$^{\mu}$ [48]$^{\lambda}$	mRNA [18]$^{\delta}$
CYP27C1	mRNA [16]	–	mRNA [16]$^{\mu}$	–
CYP39A1	–	–	mRNA [18]$^{\alpha}$ [16]$^{\mu}$	mRNA [18]$^{\delta}$

TABLE 1 (*Continued*)

Phase I enzymes	*Ex vivo* native skin	2D KC cell cultures	Reconstructed human epidermis	Reconstructed human skin
CYP46A1	–	–	mRNA [18]$^{\alpha}$	mRNA [18]$^{\delta}$
CYP51A1	mRNA [16]	–	mRNA [16]$^{\mu}$	–
Aldehyde oxidase	mRNA [16]	–	mRNA [16]$^{\mu}$	–
AO1	Protein [24]			
	Activity [25]			
Aldehyde dehydrogenase	mRNA [16]	Protein [24]$^{\#}$	mRNA [48]$^{\lambda}$	Activity [27]$^{\delta}$
ALDH	Protein [24]		Activity [27]$^{\alpha\ \pi}$	
ALDH1A1	mRNA [16]	–	mRNA [48]$^{\lambda}$	–
	Protein [24]			
ALDH1A3	mRNA [16]	–	mRNA [16]$^{\mu}$ [48]$^{\lambda}$	–
	Protein [24]			
ALDH1L1	mRNA [16]	–	–	–
	Protein [24]			
ALDH2	mRNA [16]	mRNA [65]$^{@}$	mRNA [16]$^{\mu}$ [48]$^{\lambda}$	–
	Protein [24]	Protein [24]$^{\#}$	Protein [24] $^{\alpha\ \mu\ \pi}$	
ALDH3A1	mRNA [16]	Protein [24]$^{\#}$	mRNA [16]$^{\mu}$	–
	Protein			
ALDH3A2	mRNA [16]	–	mRNA [16]$^{\mu}$	–
	Protein [24]		Protein [24]$^{\alpha}$	
ALDH3B1	mRNA [16]	–	mRNA [48]$^{\lambda}$	–
	Protein [24]			
ALDH3B2	mRNA [16]	–	mRNA [48]$^{\lambda}$	–
	Protein [24]			
ALDH4A1	–	–	mRNA [48]$^{\lambda}$	–
ALDH5A1	–	–	mRNA [48]$^{\lambda}$	–
ALDH6A1	–	–	mRNA [48]$^{\lambda}$	–
ALDH7A1	mRNA [16]	–	mRNA [16]$^{\mu}$ [48]$^{\lambda}$	–
			Protein [24]$^{\mu\ \pi}$	
ALDH9A1	Protein [24]	Protein [24]$^{\#}$	mRNA [48]$^{\lambda}$	–
			Protein [24]$^{\alpha\ \mu\ \pi}$	
Aldo-ketoreductase	mRNA [16,18]	Protein [24]$^{\#}$	mRNA [16]$^{\mu}$	–
AKR1A1	Protein [24]			
AKR1B1	–	–	Protein [24]$^{\alpha\ \mu\ \pi}$	–
AKR1C1	mRNA [16,18]	Protein [24]$^{\#}$	mRNA [18]$^{\alpha}$	mRNA [18]$^{\delta}$
	Protein [24]		Protein [24]$^{\alpha\ \mu\ \pi}$	–
AKR1C2	mRNA [16,18]	–	mRNA [18]$^{\alpha}$	mRNA [18]$^{\delta}$
	Protein [24]		Activity [18]$^{\alpha}$	
AKR1C3	mRNA [16]	–	mRNA [16]$^{\pi}$	–
AKR1C4	–	–	mRNA [18]$^{\alpha}$	–
AKR7A2	mRNA [16]	–	mRNA [16]$^{\pi}$	–
AKR7A3	mRNA [16]	–	mRNA [16]$^{\pi}$	–
Alcohol dehydrogenase	mRNA [18]	–	Activity [27]$^{\alpha\ \pi}$	Activity [27]$^{\delta}$
ADH1	Protein [24]			
ADH3	Protein [24]	–	Protein [24]$^{\alpha\ \mu\ \pi}$	–
ADH4	Protein [24]	–	–	–
ADH5	–	–	mRNA [48]$^{\lambda}$	–
ADH7	mRNA [18]	–	mRNA [18]$^{\alpha}$	mRNA [18]$^{\delta}$
	Activity [30]			
Esterase	Activity [70,71]	–	mRNA [48]$^{\lambda}$	Activity [14,50]$^{\beta\ \delta}$
ES			Activity [14,50]$^{\alpha\ \mu}$/no activity [50]$^{\pi}$	
Carboxyl esterase	mRNA [16]	–	mRNA [16,48]$^{\mu\ \lambda}$	–
CES1	Protein [24]			
CES2	mRNA [16]	mRNA [72]$^{\#}$	mRNA [16,48]$^{\mu\ \lambda}$	–
CES3	–	–	mRNA [16]$^{\mu}$	–
CES5	–	–	mRNA [48]$^{\lambda}$	–
Flavin-containing mono-oxygenase	mRNA [16–18]	–	mRNA [18]$^{\alpha}$	mRNA [18]$^{\delta}$ [17]$^{\beta}$
FMO1			Activity [49]$^{\mu}$	Activity [49]$^{\beta}$
FMO2	mRNA [17,18]	–	mRNA [18]$^{\alpha}$ [16]$^{\mu}$	mRNA [18]$^{\delta}$
FMO3	mRNA [16–18]	Protein [68]$^{\&}$	Activity [49]$^{\mu}$	Activity [49]$^{\beta}$
	Protein [17]			
FMO4	mRNA [17,18]	–	mRNA [18]$^{\alpha}$ [16]$^{\mu}$ [48]$^{\lambda}$	mRNA [18]$^{\delta}$
FMO5	mRNA [16–18]	mRNA [17]$^{\&}$	mRNA [18]$^{\alpha}$ [16]$^{\mu}$	mRNA [18]$^{\delta}$ [17]$^{\beta}$
Cyclooxygenase	mRNA [34,35]	Activity [15]$^{\&}$ [73]$^{\#}$	Activity [15]$^{\mu}$	–
COX1	Activity [15]			

TABLE 1 (*Continued*)

Phase I enzymes	*Ex vivo* native skin	2D KC cell cultures	Reconstructed human epidermis	Reconstructed human skin
COX2	mRNA [34,35] Activity [15]	Activity [15]$^{\&}$ [73]$^{\#}$	Activity [15]$^{\mu}$	–
Sorbitol dehydrogenase	mRNA [16]	–	mRNA [18]$^{\alpha}$	mRNA [18]$^{\delta}$
Hydroxyacyl-coA dehydrogenase 2	–	–	mRNA [18]$^{\alpha}$	mRNA [18]$^{\delta}$
Glucose-6-phosphate dehydrogenase	mRNA [16]	–	mRNA [16]$^{\mu}$	–
NAD(P)H quinone dehydrogenase 1 & 2	mRNA [16]	–	mRNA [16]$^{\mu}$ [48]$^{\lambda}$	–
Nicotinamide nucleotide transhydrogenase	mRNA [16]	–	mRNA [16]$^{\mu}$	–
Carbonyl reductase 1	Protein [24]	Protein [24]$^{\#}$	Protein [24]$^{\alpha\ \mu\ \pi}$	–
Carbonyl reductase 3	Protein [24]	Protein [24]$^{\#}$	Protein [24]$^{\alpha\ \mu}$	–
NADH ubiquinone oxidoreductase	Protein [24]	–	Protein [24]$^{\alpha\ \mu\ \pi}$	–
Sulfide-quinone oxidoreductase	Protein [24]	–	Protein [24]$^{\alpha\ \mu\ \pi}$	–
NAD(P)H dehydrogenase 1	Protein [24]	–	Protein [24]$^{\mu}$	–
NAD(P)H dehydrogenase 2	Protein [24]	–	–	–
NADPH CYP reductase	–	–	Protein [24]$^{\alpha\ \mu}$	–
Hydroxysteroid 17-beta dehydrogenase 8	mRNA [18]	–	–	–
Epoxide hydrolase EPHX1	mRNA [16,18] Protein [24]	–	mRNA [18]$^{\alpha}$ [16]$^{\mu}$	mRNA [18]$^{\delta}$
EPHX2	mRNA [16,18]	–	mRNA [18]$^{\alpha}$ [16]$^{\mu}$	mRNA [18]$^{\delta}$
Glucosylceramide beta 3	mRNA [16]	–	mRNA [16]$^{\mu}$	–
Kynureninase	mRNA [16]	–	mRNA [16]$^{\mu}$	–
Steroid sulfatase	mRNA [18]	–	mRNA [18]$^{\alpha}$	mRNA [18]$^{\delta}$
Prostaglandin I2 synthase	mRNA [16]	–	–	–
Nitric oxide synthase NOS1	mRNA [18]	–	mRNA [18]$^{\alpha}$	mRNA [18]$^{\delta}$
NOS2A	mRNA [18]	–	mRNA [18]$^{\alpha}$	–
Ferredoxin 1	mRNA [18]	–	mRNA [16]$^{\mu}$	–
Gluthathione peroxidase 1, 2 & 3	mRNA [16]	–	mRNA [16]$^{\mu}$	–
Cytochrome B5 reductase 1, 2 & 3	mRNA [16]	–	mRNA [16]$^{\mu}$	–
Superoxide dismutase 2	mRNA [16]	–	mRNA [16]$^{\mu}$	–
Aryl hydrocarbon hydroxylase	–	Activity [74]$^{\&}$	–	–
Peroxidase	–	–	Activity [14]$^{\alpha\ \pi}$	Activity [14]$^{\delta}$
Hydroxysteroid dehydrogenase 17B10	–	–	mRNA [48]$^{\lambda}$	–
Dihydropyrimidine dehydrogenase	–	–	mRNA [48]$^{\lambda}$	–
Monoamine oxidase A	–	–	mRNA [48]$^{\lambda}$	–
Ubiquitin C-terminal hydrolase 1 & 3	–	–	mRNA [48]$^{\lambda}$	–
Prostaglandin-endoperoxide synthase 1 & 2	–	–	mRNA [48]$^{\lambda}$	–
Xylitol dehydrogenase	–	–	mRNA [48]$^{\lambda}$	–
Quinone oxidoreductase PIG3	–	–	Protein [24]$^{\mu\ \pi}$	–

Keratinocytes: & = primary; # = HaCat; @ = NCTC.
Reconstructed human epidermis models: α = EpiSkin^{TM}; μ = EpiDerm; π = SkinEthic^{TM} RHE; λ = ORS-RHE.
Reconstructed human skin models: β = Phenion FTM; δ = T-Skin^{TM} FTM.

dehydrogenases and sorbitol dehydrogenase in skin explants [16]. Also Luu-The *et al.* showed mRNA expression of hydroxysteroid 17-beta dehydrogenase, ferredoxin, steroid sulfatase and nitric oxide synthases [18]. Furthermore, van Eijl *et al.* showed, in their skin proteomics study, the expression of proteins like carbonyl reductases, NADH-ubiquinone oxidoreductase and sulfide quinone oxidoreductase [24]. For epoxide hydrolase, either the mRNA or protein was detected in various skin explants in different studies [16,18,24]. Because of fragmented data on the grouped and ungrouped phase I enzymes, where only mRNA or protein expression is described, a firm conclusion regarding their activity in native skin cannot be drawn.

Phase II enzymes in native human skin

Phase II metabolizing enzymes are typically involved in detoxification of a wide variety of chemicals, activation of drugs and cytoprotection against many xenobiotics. Below, the major phase II enzyme groups are reviewed (i.e., GST, UGT and *N*-acetyltransferase). Also, some light is shed on sulfotransferases and catechol *O*-methyltransferase in human explanted native skin (Table 2). The GST family of enzymes catalyzes the conjugation of xenobiotics with glutathione, which is an endogenous tripeptide [36]. By using 1-chloro-2,4-dinitrobenzene (CDNB), which is a broad-spectrum substrate of GSTs, the activity in human skin has been determined by different researchers in various human skin sources [14,17,37]. Besides the confirmed enzyme activity, mRNA expression of several subtypes (e.g., GST A, M, O, P, T and Z), as well as protein expression, has been described [16–18,24]. Some of these studies compare the skin data to that from liver; for example GST P expression is highly present in the skin but not in the liver [38], whereas GST A and M are low in skin but high in liver [39]. Expression of GST P appeared to be markedly upregulated in differentiated keratinocytes [40].

UGTs are enzymes that mediate the conjugation of glucuronide to endobiotics as well as xenobiotics [8]. They are important phase II enzymes because in humans 40–70% of all administered drugs are subjected to UGTs [41]. In human skin, UGT activity was shown by several research groups by measuring the formation of 4-MUG, by the conversion of 4-methylumbelliferone [14,17,37] and by measuring the glucuronidation of 7-hydroxycoumarin [42]. Next to activity, presence of mRNAs of other UGT subfamilies (e.g., UGT1A10 and UGT2Bs) has been demonstrated [16–18]. Despite the relatively low mRNA and protein expression, UGT activities measured in microsomes were similar between native skin and liver [37].

The four SULT subfamily members (i.e., 1, 2, 4 and 6) mediate most sulfonation of molecules with nucleophilic centers by conjugating them to the sulfuric acid group of PAPS (3′-phosphoadenosine 5′-phosphosulfate) coenzymes, and are therefore essential for metabolism of several endogenous compounds [5]. Two of the above-mentioned studies also reported presence of SULT1 and SULT2 mRNA [16,18] and one study reported SULT activity [42]. Presence of mRNA coding for the PAPS proteins (PAPSS1 and PAPSS2) in human skin was also reported [16]. Presence and activity of SULT and PAPS in human native skin indicate their possible importance in endogenous skin metabolism.

Detoxification of chemicals has also been described by NATs via *N*-acetylation as well as metabolic activation via *O*-acetylation. NATs catalyze the conjugation of an acetyl group to a substrate, which can be, for example, an aromatic amine, a hydrazine or a sulfonamide, to create immunogenic inactive metabolites. Humans have several NAT genes [7]. In human skin, NAT activity was shown in several studies by use of different substrates by different groups: procainamide [42], *para*-aminobenzoïc acid and *N*-acetyl-*p*-phenylendiamine [14,43,44], *p*-toluïdine [37] and 2,4-toluenediamine [45]. Furthermore, in other studies only the NAT1 and NAT5 mRNA was detected in native skin [16–18]. Although NAT protein expression is relatively low in the skin (0.01%) [24], this is in agreement with the content found in the liver [46]. Catechol *O*-methyltransferase (COMT) enzymes catalyze the transfer of a methyl group to catecholamines [8]. Presence of COMT mRNA was reported in buttock skin and female breast skin [16,18]. In a large study of 89 skin donors the methylation activity of COMT enzymes was assessed [42]. It was concluded that not all skins show methylation activity of dopamine, which was neither age nor gender related [42].

In addition to this large group of enzymes, other phase II enzymes have been detected in native skin. However, these enzymes were only detected either at mRNA or protein levels, no activity was described [16,24]. They include, among others, cysteine conjugate b-lyase, thiopurine methyltransferase and glutamate-cysteine ligase for only mRNA, or γ-glutamyltransferase 7, glutathione synthetase, catalase, glutathione peroxidase 3 and peroxiredoxins at the protein level [24].

In summary, for native *ex vivo* skin expression of a total of 85 phase I enzymes at mRNA level and 26 at protein level has been shown. Activity was investigated for 15 enzymes and only in eight of them was activity confirmed. For five tested enzymes no activity could be detected, whereas mRNA and/or protein was detected (Table 3). Furthermore, for two enzymes a discrepancy in detected activity is reported, whereas mRNA expression was confirmed (Table 1). Similarly, for phase II enzymes mRNA and protein expression were detected in 32 and 13 enzymes, respectively. Activity was tested for five enzymes and all were confirmed (Table 2). This indicates that phase I enzymes are best studied in *ex vivo* skin samples compared with the phase II enzymes; but the tested activity is, in general, more robust and reproducible in phase II enzymes.

Reconstructed human skin to study xenobiotic metabolism *in vitro*

Because the use of freshly excised skin for metabolic studies has clear limitations with regard to its viability and availability for large-scale studies in academia and industry, attention is now focusing on the use of *in vitro* skin reconstructs and, in particular, commercially available skin reconstructs. In this review, we describe two types of skin reconstructs, both of which are commercially available: reconstructed human skin (RHS) and reconstructed human epidermis (RHE) (Table 4) [47]. RHS, also known as full-thickness bilayered models or skin equivalents, most-resembles human native skin (Fig. 1). RHS consists of stratified keratinocytes grown on a fibroblast-populated dermal matrix. Culture at the air–liquid interphase results in a proliferating stratum basale differentiating into a stratum spinosum, stratum granulosum and, finally, a stratum corneum as the keratinocytes migrate upward (Fig. 1b). RHE models are similar to RHS models but do not have the dermal component and therefore lack fibroblasts (Fig. 1c).

Phase I enzymes in reconstructed human epidermis and skin models

As with the *ex vivo* excised skin, several studies have looked into CYP enzymes in RHS and RHE models (Table 1). For CYP1A and CYP1B, activity as well as the presence of mRNA was examined in multiple studies [14,16–18,21,48,49]. For RHS (T-Skin™ FTM, which was previously called EpiSkin™ FTM) and RHE (EpiSkin™, SkinEthic™ RHE, ORS-RHE) models, mRNA was detected and activity demonstrated by using the EROD assay [14,18,48]. Also

TABLE 2

Detection of mRNA, protein and/or tested activity of the phase II metabolizing enzymes in native skin, 2D cell culture and skin models (RHE and RHS)

Phase II enzymes	*Ex vivo* native skin	2D KC cell cultures	Reconstructed human epidermis	Reconstructed human skin
Gluthatione S-transferases GST	Activity [14,17,37]	mRNA [65]$^{@}$ Activity [17,37,75]$^{\&}$ [37]$^{\#}$ [37]$^{@}$	Activity [37]$^{\mu}$ [14]$^{\alpha\ \pi}$	Activity [14]$^{\delta}$ [17]$^{\beta}$
GST A1	mRNA [17] Protein [24]	–	mRNA [16]$^{\mu}$ [48]$^{\lambda}$ Protein [24]$^{\alpha\ \mu\ \pi}$	–
GST A3	mRNA [17]	–	–	–
GST A4	–	–	mRNA [16]$^{\mu}$ [48]$^{\lambda}$	–
GST K1	–	–	mRNA [48]$^{\lambda}$	–
GST M1	mRNA [16] Protein [24]	–	mRNA [48]$^{\lambda}$ Protein [24]$^{\alpha\ \mu\ \pi}$	–
GST M2	mRNA [16]	–	mRNA [16,48]$^{\mu\ \lambda}$	–
GST M3	mRNA [16]	–	mRNA [16,48]$^{\mu\ \lambda}$	–
GST M4	mRNA [16]	–	mRNA [16,48]$^{\mu\ \lambda}$	–
GST M5	mRNA [16,18]	–	mRNA [16,48]$^{\mu\ \lambda}$	–
GST O	Protein [24]	Protein [24]$^{\#}$	mRNA [48]$^{\lambda}$ Protein [24]$^{\alpha\ \mu}$	–
GST P1	mRNA [16–18] Protein [24]	mRNA [17,75]$^{\&}$ Protein [24]$^{\#}$	mRNA [18]$^{\alpha}$ [16]$^{\mu}$ [48]$^{\lambda}$ Protein [24]$^{\alpha\ \mu\ \pi}$	mRNA [18]$^{\delta}$ [17]$^{\beta}$
GST T1	mRNA [18] Protein [24]	–	mRNA [18]$^{\alpha}$ [48]$^{\lambda}$	mRNA [18]$^{\delta}$
GST T2	–	–	mRNA [48]$^{\lambda}$	–
GST Z1	mRNA [16]	–	mRNA [16]$^{\mu}$	–
UDP-glucuronosyltransferases	mRNA [16]	mRNA [17]$^{\&}$	Activity [37,49]$^{\mu}$ [14]$^{\alpha\ \pi}$ [48]$^{\lambda}$	Activity [14]$^{\delta}$ [17,49]$^{\beta}$
UGT	Activity [14,17,37,42]	Activity [17,37]$^{\&}$ [37]$^{\#}$ [37]$^{@}$		
UGT1A1	–	–	mRNA [48]$^{\lambda}$	–
UGT1A3	–	–	mRNA [18]$^{\alpha}$ [48]$^{\lambda}$	–
UGT1A4	–	–	mRNA [18]$^{\alpha}$	–
UGT1A5	–	–	mRNA [18]$^{\alpha}$ [48]$^{\lambda}$	–
UGT1A6	–	–	mRNA [18]$^{\alpha}$ [16]$^{\mu}$ [48]$^{\lambda}$	–
UGT1A7	–	–	mRNA [18]$^{\alpha}$ [48]$^{\lambda}$	–
UGT1A8	–	–	mRNA [16]$^{\alpha\ \mu}$ [48]$^{\lambda}$	–
UGT1A9	–	–	mRNA [18]$^{\alpha}$	–
UGT1A10	mRNA [17,18]	–	mRNA [18]$^{\alpha}$ [16]$^{\mu}$ [48]$^{\lambda}$	mRNA [17]$^{\beta}$
UGT2	mRNA [16]	–	–	–
UGT2B4	mRNA [18]	–	mRNA [18]$^{\alpha}$	mRNA [18]$^{\delta}$
UGT2B7	–	–	mRNA [18]$^{\alpha}$	–
UGT2B10	–	–	mRNA [18]$^{\alpha}$	–
UGT2B11	–	–	mRNA [18]$^{\alpha}$	–
UGT2B15	mRNA [18]	–	mRNA [18]$^{\alpha}$	–
UGT2B17	mRNA [18]	–	mRNA [18]$^{\alpha}$	mRNA [18]$^{\delta}$
UGT2B28	mRNA [18]	–	mRNA [18]$^{\alpha}$	mRNA [18]$^{\delta}$
Sulfotransferases	mRNA [16]	Activity [75]$^{\&}$	Protein [24]$^{\alpha\ \pi}$	–
SULT1	Activity [42]			
SULT1A1	mRNA [18]	–	mRNA [16]$^{\alpha\ \mu}$ Activity [48]$^{\lambda}$/no activity [14]$^{\alpha\ \pi}$	mRNA [18]$^{\delta}$ No activity [14]$^{\delta}$
SULT1A3	–	–	mRNA [48]$^{\lambda}$	–
SULT1A4	–	–	mRNA [16]$^{\mu}$	–
SULT1A5	–	–	mRNA [48]$^{\lambda}$	–
SULT1A8	–	–	mRNA [48]$^{\lambda}$	–
SULT1B1	mRNA [18]	–	mRNA [18]$^{\alpha}$	mRNA [18]$^{\delta}$
SULT1C2	–	–	mRNA [18]$^{\alpha}$	–
SULT1E1	mRNA [18]	–	mRNA [18]$^{\alpha}$ [16]$^{\mu}$ [48]$^{\lambda}$	mRNA [18]$^{\delta}$
SULT2B1	mRNA [18]	–	mRNA [18]$^{\alpha}$ [16]$^{\mu}$ [48]$^{\lambda}$ Protein [24]$^{\alpha\ \mu\ \pi}$	–
N-acetylytransferases	mRNA [16–18]	mRNA [17,76]$^{\&}$ [51,77]$^{\#}$	mRNA [18]$^{\alpha}$ [16]$^{\mu}$ [48]$^{\lambda}$	mRNA [18]$^{\delta}$ [17]$^{\beta}$
NAT1	Activity [14,37,42,45]	Activity [37,45,51]$^{\&}$ [37,51]$^{\#}$ [37,52]$^{@}$	Activity [37,45,49]$^{\mu}$ [14]$^{\alpha\ \pi}$	Activity [14]$^{\delta}$ [45,49]$^{\beta}$
NAT5	mRNA [18]	–	mRNA [18]$^{\alpha}$	mRNA [18]$^{\delta}$
NAT10	–	–	Protein [24]$^{\alpha}$	–
Catechol O-methyltransferase	mRNA [16,18]	–	mRNA [18]$^{\alpha}$ [16]$^{\mu}$ [48]$^{\lambda}$	mRNA [18]$^{\delta}$

TABLE 2 (*Continued*)

Phase II enzymes	*Ex vivo* native skin	2D KC cell cultures	Reconstructed human epidermis	Reconstructed human skin
	Activity [42]		Protein [24]$^{\alpha\ \mu\ \pi}$	
Thiopurine S-methyltransferase	mRNA [16]	–	mRNA [16]$^{\mu}$ [48]$^{\lambda}$	–
Steroid sulfatase	–	–	mRNA [18]$^{\alpha}$	–
Glutamate-cysteine ligase catalytic subunit	mRNA [16]	–	mRNA [16]$^{\mu}$	–
N-acetyltransferase 20	–	–	mRNA [48]$^{\lambda}$	–
Short chain dehydrogenase/reductase 1 & 2	–	–	mRNA [18]$^{\alpha}$	–
Hydroxy-steroid dehydrogenase 11B1 & 2	–	–	mRNA [18]$^{\alpha}$	–
Catalase	Protein [24]	Protein [24]#	Protein [24]$^{\alpha\ \mu\ \pi}$	–
Peroxiredoxin 1	Protein [24]	Protein [24]#	Protein [24]$^{\alpha\ \mu\ \pi}$	–
Peroxiredoxin 2	Protein [24]	Protein [24]#	Protein [24]$^{\alpha\ \mu\ \pi}$	–
Peroxiredoxin 5	Protein [24]	Protein [24]#	Protein [24]$^{\alpha\ \mu\ \pi}$	–
Peroxiredoxin 6	Protein [24]	Protein [24]#	Protein [24]$^{\alpha\ \mu\ \pi}$	–
Glutathione synthetase	mRNA [16] Protein [24]	Protein [24]#	Protein [24]$^{\mu\ \pi}$	–
Glutathione peroxidase 3	Protein [24]	–	–	–
Arsenite methyltransferase	–	–	mRNA [48]$^{\lambda}$	–
Guanidinoacetate methyltransferase	–	–	mRNA [48]$^{\lambda}$	–
Nicotinamide *N*-methyltransferase	–	–	mRNA [48]$^{\lambda}$	–
Dolichyl-diphosphooligosaccharide	–	–	mRNA [48]$^{\lambda}$	–
Cysteine conjugate b-lyase 1	mRNA [16]	–	mRNA [48]$^{\lambda}$	–
3'-phosphoadenosine 5'-phosphosulfate synthases PAPSS1	mRNA [16]	–	mRNA [16]$^{\mu}$	–
PAPSS2	mRNA [16]	–	–	–
γ-Glutamyl transferase 7	mRNA [16] Protein [24]	–	–	–
Spermine N1-acetyltransferase 1	–	–	mRNA [48]$^{\lambda}$	–

Keratinocytes: & = primary; # = HaCat; @ = NCTC.
Reconstructed human epidermis models: α = EpiSkin™; μ = EpiDerm; π = SkinEthic RHE; λ = ORS-RHE.
Reconstructed human skin models: β = Phenion FTM; δ = T-Skin™ FTM.

TABLE 3

Phase I metabolizing enzymes: number of enzymes detected at mRNA, protein, tested activity or no activity (data obtained from Tables 1,2)

Phase I enzymes	*Ex vivo* native skin	2D KC cell cultures	Reconstructed human epidermis	Reconstructed human skin
mRNA	85	23	94	43
Protein	26	15	14	0
Activity	8	7	15	12
No activity	5	2	0	0
Conflicting activity/no activity result	2	2	4	3

TABLE 4

Human skin reconstructs currently commercially available on which testing of compounds has been performed for the purpose of metabolism assessment

Static models	Name	Cell types used	Dermal matrix (cells)	Refs
RHS	Phenion FTM	PK	Collagen (fibroblast)	[78]
	T-Skin™ FTM	PK	Collagen (fibroblast)	[18]
RHE	EpiSkin™	PK	Collagen (no cells)	[18]
	SkinEthic™ RHE	PK	–	[18]
	EpiDerm	PK	–	[78]
	ORS-RHE (epiCS)	ORS cells	–	[79]

Abbreviations: RHSreconstructed human skin; RHEreconstructed human epidermis; FTMfull thickness model; PKprimary keratinocytes; ORSouter root sheet keratinocytes.

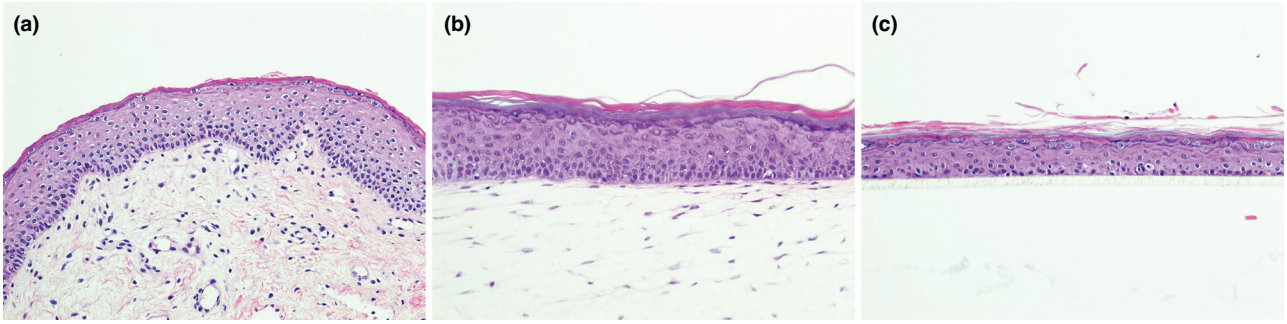

FIGURE 1

Comparison of human skin and skin reconstructs. (a) Native human skin biopsy illustrating the epidermis and dermis. (b) Reconstructed human skin: epidermis on fibroblast-populated collagen hydrogel. (c) Reconstructed human epidermis grown on a porous membrane. Magnification 200 ×.

for the other RHS (Phenion FTM) and RHE (EpiDerm) models, CYP1A and CYP1B mRNA was detected but activity in these cultures was below the limit of detection [17,21,49]. Considering the CYP2 subfamily, mRNA of several subtypes was identified in all models tested [16–18,21,48]. Activity, by contrast, for some of these CYP2 enzymes, was only found in T-Skin™ FTM, EpiSkin™ and SkinEthic™ RHE with the MFCOD assay [14]. Jackh and colleagues examined CYP2B activity in EpiDerm and Phenion FTM but did not detect any activity [49]. With regard to CYP3A subfamily activity, specific activity for CYP3A1, 4, 5 and 7 was confirmed in T-Skin™ FTM, EpiSkin™, EpiDerm and SkinEthic™ RHE models, using the BFCOD assay (benzyloxy-4-trifluoromethylcoumarin-*O*-debenzyloxylase) [14,15,18,49]. Furthermore, presence of mRNA of other CYPs such as CYP4, 7, 21 or 51 was demonstrated in a majority of the models but no activity has been tested so far (Table 1) [16,18,21,48]. The only study that reported some data on AO enzymes in a skin reconstruct was done by Hu *et al.* [16]. They showed the presence of mRNA in an EpiDerm model. However, the proteomic study conducted by van Eijl *et al.* did not detect any AO protein in EpiSkin™, EpiDerm or SkinEthic™ RHE models [24].

For ALDH and its subfamily, mRNA and/or protein was shown in RHS (T-Skin™ FTM) and all RHE models [16,24,48]. In a short report by Eilstein *et al.* [27], ALDH activity was compared between S9 fraction of cells from skin models and liver cells and they showed that, by using cinnamic alcohol on T-Skin™ FTM, EpiSkin™ and SkinEthic™ RHE, low activity could be detected in these skin models. For phase I enzymes belonging to the AKR family mRNA and/or protein was detected in one RHS (T-Skin™ FTM) and some RHE (EpiSkin™, EpiDerm, SkinEthic™ RHE) models [16,18,24]. With regard to enzyme activity, only the EpiSkin™ model confirmed AKR1C2 activity [18]. Presence of protein expression in RHE and RHS, and activity in RHS, indicate that keratinocytes do show AKR activity.

Looking at ADH enzyme subtypes, mRNA expression was shown in some (EpiSkin™, ORS-RHE) and protein in other (EpiSkin™, EpiDerm, SkinEthic™ RHE) RHE models [24,48]. For the RHS model, mRNA was observed for T-Skin™ FTM. Also, ADH activity was compared between S9 fraction of cells from several models and liver cells and was shown to be either low or not present at all [18,27]. Esterase activity has been reported for EpiDerm by Bätz *et al.* by measuring the conversion of prednicarbate and fluorescein diacetate to prednisolone and fluorescein, respectively. In another RHE model (SkinEthic™ RHE) this activity was absent [50]. A study by Eilstein *et al.*, as well as Bätz *et al.*, confirmed esterase activity in RHS and some RHE (EpiSkin™, SkinEthic™ RHE) models by measuring deacetylation of 4-methylumbelliferyl acetate (4-MUAc) [14,50]. Similar to native skin, for carboxylesterase enzymes only mRNA has been found in RHE models (EpiDerm, ORS-RHE); but no protein or activity has been tested so far [16,48].

Enzyme activity of the FMO family, FMO 1 and FMO 3, was shown in EpiDerm and in Phenion FTM by assessing oxidation of benzydamine [49]. Furthermore, mRNA for those and other FMO types was found in RHS models and in RHE models (EpiSkin™, EpiDerm, ORS-RHE) but protein was not found in any model and activity was not tested for these enzymes [16–18,48]. COX activity was demonstrated by Götz *et al.* in the EpiDerm model by determining the formation of PGE2. Basal COX rates were ten-times lower than observed in native skin, but addition of 3-methylcholanthrene (3-MC) could double the activity in the EpiDerm model [15], whereas keratinocytes in monolayer did not show this increase. 3-MC is known as a prototypical arylhydrocarbon receptor agonist and CYP inducer.

Besides the main classes of dehydrogenases, mRNA of other dehydrogenase enzymes was detected in some of the skin reconstructs (Table 1). For example hydroxyacyl-CoA dehydrogenase and sorbitol dehydrogenase mRNA were detected in a RHS model (T-Skin™ FTM) [18], whereas glucose-6-phosphate dehydrogenase, NAD(P)H quinone dehydrogenase 1 and nicotinamide nucleotide transhydrogenase were found in some RHE models (EpiSkin™, EpiDerm, ORS-RHE) [16,48]. van Eijl *et al.* demonstrated in their proteomics study the protein presence of carbonyl reductase 1 and 3, NADH ubiquinone oxidoreductase, sulfide-quinone oxidoreductase, NAD(P)H dehydrogenase 1 and NADPH CYP reductase in EpiSkin™, EpiDerm and SkinEthic™ RHE models [24]. Whether or not these and many other ungrouped phase I enzymes are active enzymes in the skin needs to be determined in future studies.

When considering these results for phase I enzymes, together with the results described above for freshly excised human skin, it appears that, for a majority of the metabolizing phase I enzymes, the mRNA and protein can be detected in many skin models, but the activity remains difficult to test and quantify. It is most possible that phase I enzymes, in particular the CYP family, could play a smaller part in metabolism in human skin than in liver because levels and activity appear lower than in liver studies [15,24,27,49].

TABLE 5

Phase II metabolizing enzymes: number of enzymes detected at mRNA, protein, tested activity or no activity (data obtained from Tables 1,2)

Phase II enzymes	*Ex vivo* native skin	2D KC cell cultures	Reconstructed human epidermis	Reconstructed human skin
mRNA	32	4	54	12
Protein	13	8	14	0
Activity	5	4	3	3
No activity	0	0	0	1
Conflicting activity/no activity result	0	0	1	0

Phase II enzymes in reconstructed human epidermis and skin models

As described above, phase II metabolizing enzymes typically play a part in detoxification and cytoprotection against many xenobiotics (Table 2). GSTs are one of the largest and best-studied phase II metabolizing enzymes of the liver [36,39]. In addition to native skin, the activity of GST was also confirmed in RHS models with the broad-spectrum GST CDNB assay (Table 2) [14,17,37]. Furthermore, mRNA and/or protein of several GST subtypes was detected in these RHS models [17,18,24]. By comparing enzyme activity found in RHS models to some of the RHE models (EpiSkin™, EpiDerm, SkinEthic™ RHE) [14,37] and mRNA and/or protein presence of other GST subtypes, presence of fibroblasts in the dermis of the RHS models seems not to play a large part in the GST activity as suggested before [17].

Phase II also includes UGT enzymes that facilitate glucoronation. Both RHS and all RHE models showed that UGT activity is present when using the substrate 4-methylumbelliferone (4-MU) [14,17,37,48,49]. In addition, Götz *et al.* showed activity on the substrate UDP-glucuronic acid (UDPGA) in the EpiDerm model [37]. mRNA of several other UGT subtypes (UGT1Ax and UGT2Bx) was detected in both RHS and some RHE (EpiSkin™, EpiDerm, ORS-RHE) models [16–18,48] (Table 2).

In comparison to the native skin where SULT enzyme activity was present, enzyme activity was only confirmed in the ORS-RHE model [48]. For T-Skin™ FTM, EpiSkin™ and SkinEthic™ RHE models there was no SULT enzyme activity detected [14]. mRNA of several subtypes of SULT1 and SULT2, however, has been detected in T-Skin™ FTM and EpiSkin™ [16,18] and protein for EpiSkin™, EpiDerm and SkinEthic™ RHE [24]. As mentioned above, PAPS coenzyme aids in conjugation activity of SULTs. PAPS mRNA and protein was detected only in the EpiDerm model [16], but protein expression was not tested for any of the models [24]. Why the activity of SULT enzymes is lacking in the RHS and RHE models (except in ORS-RHE), whereas the mRNA and proteins are present, is not understood [14].

NAT activity was shown in all tested models [14,37,45,49,51,52]. To illustrate this, different substrates have been used: for example (*N*-acetylation of) PABA [14,49], *p*-toluidine [37] and *N*-(3-amino-4-methyl-phenyl)acetamide (M1) [45]. The latter was also used as a NAT1 inhibitor (cyanamide) to confirm that the conversion was due to the activity of NAT1. Presence of mRNA of different NAT subtypes was also examined and confirmed in all models, except the SkinEthic™ RHE which was not tested [16–18]. NAT10 protein was detected only in the EpiSkin™ model [24]. Also, expression of mRNA and protein of other phase II metabolizing enzymes was detected in the reconstructed skin models. In short, among others, mRNA of catechol *O*-methyltransferase, thiopurine *S*-methyltransferase, steroid sulfatase, short-chain dehydrogenase/reductase and hydroxy-steroid dehydrogenase were detected in EpiSkin™ and EpiDerm [16,18,48]. At the protein level, in EpiSkin™, EpiDerm and SkinEthic™ RHE, expression of catalase and peroxiredoxin 1–6 was shown. Only for EpiDerm and SkinEthic™ RHE, glutathione synthetase protein expression was confirmed [24].

In summary, in *in vitro* skin models, mRNA expression was shown for 94 phase I enzymes (Table 3). In RHE models, which have been investigated more extensively, the detected number of enzymes was more ($n = 94$) compared with the RHS models ($n = 43$). More phase I proteins have been shown with RHE ($n = 14$) compared with RHS ($n = 0$), whereas enzyme activity was similar in numbers in RHE ($n = 15$) and RHS ($n = 12$). In RHE and RHS models, disagreement exists on activity of some enzymes. With regard to phase II enzymes, the detected number of enzymes (at the mRNA and protein level) in RHE is much more compared with the RHS models, which could be explained by the extensive data available for RHE compared to RHS (Table 5). Disagreement on activity was seen for one enzyme in a RHE model.

Metabolism in conventional submerged keratinocyte cultures

Finally, the literature was reviewed for studies describing phase I and phase II enzymes in conventional submerged keratinocytes grown in tissue culture dishes (Tables 1,2). Whereas mRNA and protein expression were reported, the numbers of enzymes detected were few compared with native *ex vivo* skin and reconstructed skin models (Table 3). Notably, for the 15 phase I enzymes in which activity was investigated, seven enzymes showed activity and two did not. For another two there was discrepancy between studies. Some of these studies describe an absence of activity whereas mRNA and/or protein might have been detected. Similarly, for phase II enzymes, mRNA and protein expression were reported; but again the numbers were very few compared with native *ex vivo* skin and reconstructed skin models (Table 5). These results indicate that, although gene transcription and protein translation of some enzymes can already occur in undifferentiated keratinocytes, the majority of genes can become active in differentiated epidermis.

Concluding remarks and future perspectives

Overall, whole skin contains many metabolizing enzymes that facilitate a range of biotransformation reactions. Almost all of the

xenobiotic metabolizing enzymes tested and detected in human skin are based on liver metabolism. All chemicals used to characterize skin metabolic capabilities come from liver studies. Furthermore, certain chemical activities are not well characterized upfront and might have appeared low in skin because the probes or substrate chosen were not appropriate. In general, available data on skin metabolism indicate that basal CYP-dependent biotransformation occurs at a very low level in unexposed conditions unless it is enhanced by an external trigger [49]. Furthermore, considering that native skin is a compartmented organ, the presence of any activity was shown in the epidermal layer (keratinocytes) rather than in dermal fibroblasts when comparing RHE and RHS models (also noted in other reviews [53]). Moreover, because the current skin models do not contain biological complexity, for example hair follicles where high CYP activities were observed [54,55], data with current models might not illustrate precisely the location of enzyme and extent of activity. Notably, the phase II metabolizing enzymes, with the exception of UGTs, are generally expressed at much higher levels than the phase I metabolizing enzymes. This is in good agreement with the role of the skin as an important organ involved in detoxification and elimination of xenobiotic compounds (e.g., by sweat glands) [56,57]. Taking all the data from skin together, in comparison with liver enzyme expression and activity, skin demonstrates ~300-fold lower phase I enzyme expression and activity [24]. Although there are some differences in shown activity between CYP enzymes and other phase I enzymes (e.g., esterases and COX) [15,27], the metabolic activity of skin is still considered relevant from a toxicological standpoint when considering the size of these organs.

It is arguably a significant challenge to extrapolate the *in vitro* model data to *ex vivo* explant data, which is then subsequently related to the *in vivo* situation where data for human skin metabolism is virtually nonexistent. As is done for liver metabolism, skin *in vitro* models for skin metabolism need to ensure enzyme activity is present so that *in vitro* to *in vivo* extrapolation is meaningful. The development of new drugs requires studies on pharmacokinetics and pharmacodynamics in appropriate preclinical models recapitulating the human native skin situation in terms of mRNA and enzyme expression, and most importantly enzyme activity. In terms of pharmacokinetic and pharmacodynamic evaluation of drugs, organ-on-a-chip technological advances in the field of tissue engineering and microfluidics are expected to provide a relevant physiological human system for testing substances within a single organ and between organs in the future [58]. In recent years, skin-on-a-chip technology has become more advanced [47,59,60]. Although more research and validation of the technology is required, some groups have already established well-performing in-house systems [59,60] and it has now to be shown whether these models are metabolically active.

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