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Original research article

Hemodialysis and biotransformation of erythrocyte epoxy fatty acids in peripheral tissue

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ABSTRACT

Cardiovascular disease is the leading cause of mortality in patients with renal failure. Red blood cells (RBCs) are potential reservoirs for epoxy fatty acids (oxylipins) that regulate cardiovascular function. Hemoglobin exhibits pseudo-lipoxygenase activity *in vitro*. We previously assessed the impact of single hemodialysis (HD) treatment on RBC epoxy fatty acids status in circulating arterial blood and found that eicosanoids in oxygenated RBCs could be particularly vulnerable in chronic kidney disease and hemodialysis. The purpose of the present study was to evaluate the differences of RBC epoxy fatty acids profiles in arterial and venous blood *in vivo* (AV differences) from patients treated by HD treatment. We collected arterial and venous blood samples in upper limbs from 12 end-stage renal disease (ESRD) patients (age 72±12 years) before and after HD treatment. We measured oxylipins derived from cytochrome P450 (CYP) monooxygenase and lipoxygenase (LOX)/CYP ω/(ω-1)-hydroxylase pathways in RBCs by LC–MS/MS tandem mass spectrometry. Our data demonstrate arteriovenous differences in LOX pathway metabolites in RBCs after dialysis, including numerous hydroxyeicosatetraenoic acids (HETEs), hydroxydocosahexaenoic acids (HDHAs) and hydroxyeicosapentaenoic acids (HEPEs). We detected more pronounced changes in free metabolites in RBCs after HD, as compared with the total RBC compartment. Hemodialysis treatment did not affect the majority of CYP and CYP ω/(ω-1)-hydroxylase products in RBCs. Our data indicate that erythro-metabolites of the LOX pathway are influenced by renal-replacement therapies, which could have deleterious effects in the circulation.

Abbreviation: AV, arteriovenous; CKD, chronic kidney disease; ESRD, end-stage renal disease; AA, arachidonic acid; EpOMEs, epoxyoctadecenoic acids; HETE, hydroxyeicosatetraenoic acid; LOX, lipoxygenase; PUFA, polyunsaturated fatty acid; CYP450, cytochrome P450; EDP, epoxydocosapentaenoic acid; EEQ, epoxyeicosatetraenoic acid; EET, epoxyeicosatrienoic acid; HDHA, hydroxydocosahexaenoic acid; HEPE, hydroxyeicosapentaenoic acid; DHETs, dihydroxyeicosatrienoic acids; DiHOMEs, dihydroxyctadecenoic acids; DiHDPAs, dihydroxydocosapentaenoic acids; DiHETEs, dihydroxyeicosatetraenoic acids; LOX, lipoxygenase; HD, hemodialysis; sEH, soluble epoxide hydrolase; EPA, eicosapentaenoic acid; LA, linolenic acid; DHA, docosahexaenoic acid; SD, standard deviation; ADPKD, autosomal dominant polycystic kidney disease; ATP, adenosine triphosphate; PPARs, peroxisome proliferator-activated receptors; ESAs, erythropoiesis-stimulating agents

1. Introduction

Chronic kidney disease (CKD) is an important risk factor for cardiovascular and all-cause mortality. Survival rates among ESRD hemodialysis (HD) patients are poor and most deaths are related to cardiovascular disease [1]. The reasons are unknown, but seem to

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involve non-traditional risk factors [1].

Anemia is a common consequence of CKD. Correcting low hemoglobin before HD initiation may improve survival by avoiding potential harms of chronic anemia [2]. Erythropoiesis-stimulating agents (ESAs) are widely used to avoid severe anemia in ESRD patients and to reduce the need for blood transfusions but not to normalize hemoglobin levels. However, multiple studies have shown that, among CKD patients (including those on hemodialysis), using ESAs to correct hemoglobin to normal increases the risk of adverse outcomes[3–6]. The reasons are unknown, but could go far beyond potential side effects of ESAs [7].

Measurements of lipid mediators and risk factors solely in plasma are suboptimal. Since such determinations invariably ignore RBCs that make up 3 kg of the circulating blood, that is 5-8% of human body weight. RBCs are potential reservoirs for vasodilatory epoxyeicosatrienoic acids (EETs) [8] (Fig. 1). The potential role of RBCs to the production of bioactive metabolites of the P450 (CYP) monooxygenase and lipoxygenase (LOX)/CYP ω/(ω-1)-hydroxylase pathways is three-fold. First, the conversion of arachidonic acid (AA) to EETs and hydroxyeicosatetraenoic acids (HETEs) by RBCs is a function of hemoglobin-activating oxygen in a monooxygenase-like fashion [9] (Fig. 1). Second, hemoglobin has been found to exhibit (quasi-) pseudo-lipoxygenase activity toward linoleic acid (LA) *in vitro,* which eventually leads to the production of lipid hydroperoxides generated from polyunsaturated fatty acids (Fig. 1) [10,11]. In these studies, hemoglobin at low concentrations (0.01-1 μM) catalyzes quasi-lipoxygenase reactions with a very high substrate specificity comparable to that of true lipoxygenases. Finally, RBCs possess a soluble epoxide hydrolase (sEH) that hydrates leukotriene A4 (LTA4) [8] and EETs to less bioactive diols [12,13] (Fig. 1). However, whether or not hemoglobin and sEH are capable to exhibit monooxygenase- and quasi-lipoxygenase reactions or hydrolysis of the metabolites in RBCs *in vivo* remains unknown.

We recently established the lipidomics approach for the analysis of oxylipins derived from CYP monooxygenase and LOX/CYP ω/(ω-1)-hydroxylase pathways in human RBCs [14] (Fig. 1). We detected pronounced changes in metabolite levels in circulating (oxygenated)

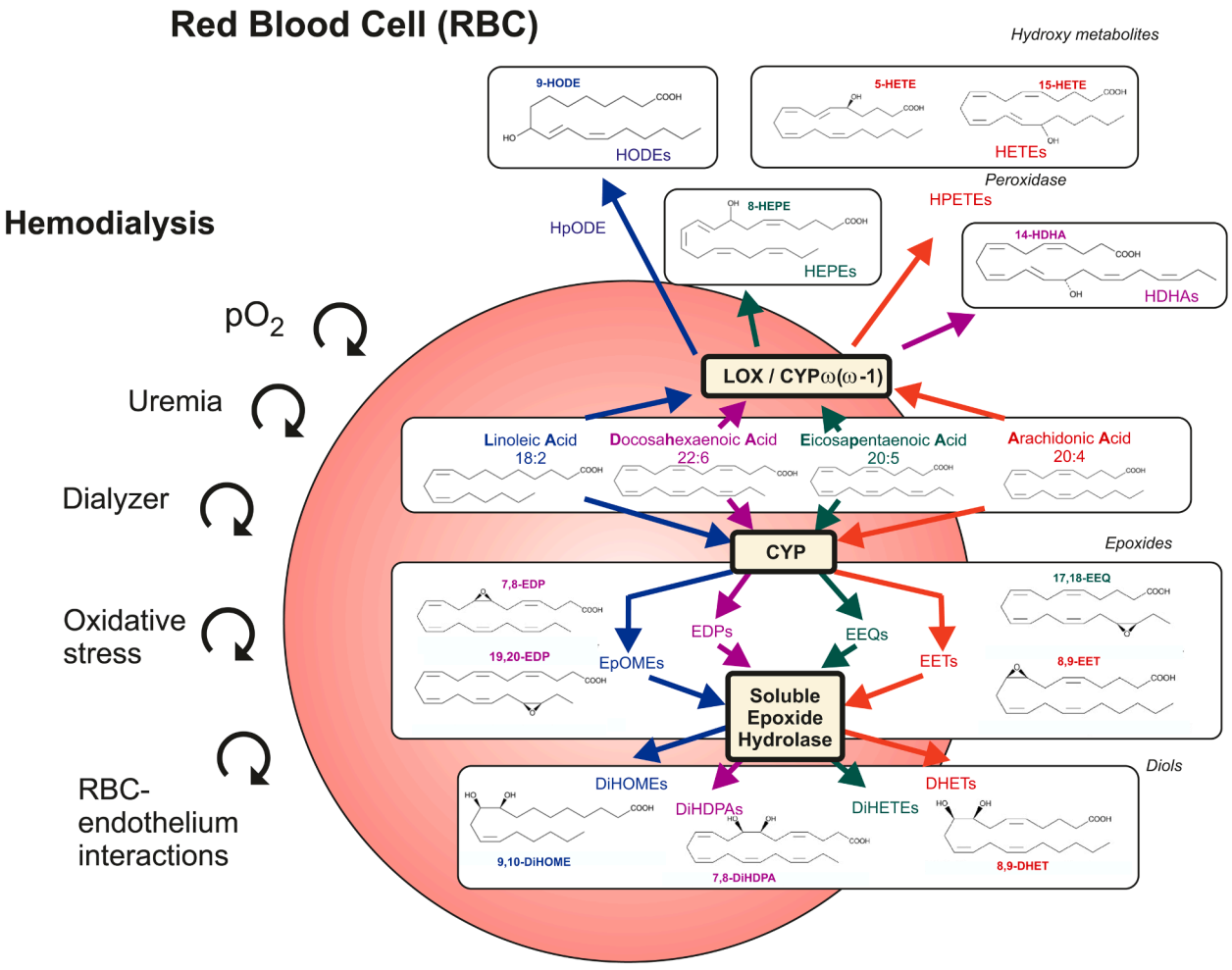

Fig. 1. Hypothetic influence of hemodialysis affecting the content of cytochrome P450 epoxygenase (CYP) and lipoxygenase (LOX) / CYP omega-hydroxylase metabolites in RBCs. CKD and hemodialysis is associated with red blood cell (RBC) deoxygenation in peripheral tissues, RBC-dialyzer interactions, shear stress, RBC-endothelial interactions and oxidative stress. The scheme illustrates the epoxide and hydroxy metabolites pathways studied. The polyunsaturated fatty acids (PUFA) linoleic (LA), arachidonic (AA), eicosapentaenoic (EPA) and docosahexaenoic acids (DHA) are converted to hydroperoxylinoleic acids (HpODEs), hydroxyoctadecadienoic acids (HODEs, e.g. 9-HODE), hydroxydocosahexaenoic acids (HDHAs, e.g. 14-HDHA), hydroperoxyeicosatetraenoic acids (HPETEs), hydroperoxyeicosatetraenoic acids (HEPEs, e.g. 8-HEPE) and hydroxyeicosatetraenoic acids (HETEs, e.g. 15-HETE) by LOX, CYP omega/(omega-1)-hydroxylase and peroxidase pathways. LA, AA, EPA and DHA are converted to epoxyoctadecenoic acids (EpOMEs, e.g. 9,10-EpOME), epoxyeicosatrienoic acid (EETs, e.g. 11,12-EET), epoxyeicosatetraenoic acids (EEQs, e.g. 11,11-EEQ) and epoxydocosapentaenoic acids (EDPs, e.g. 10,11-EDP and 16,17-EDP) by CYP, respectively. EpOMEs, EETs, EEQs and EDPs are converted to dihydroxyctadecenoic acids (DiHOMEs, e.g. 9,10-DiHOME), dihydroxyeicosatrienoic acids (DHETs, e.g. 11,12-DHET), dihydroxyeicosatetraenoic acids (DiHETEs) and dihydroxydocosapentaenoic acids (DiHDPAs, e.g. 10,11-DiHDPA), respectively, by the soluble epoxide hydrolase (sEH) enzyme. Modified from [14]. The metabolites measured within these pathways track the changes observed. Arrows demarcate metabolic pathways evaluated.

arterial RBCs after hemodialysis [14]. To gain information on lipid biotransformation in RBCs, we now collected arterial and venous blood samples in upper extremities from ESRD patients and tested the hypothesis that hemodialysis affects the arteriovenous difference of these metabolites *in vivo* (Fig. 2). We performed the experiments to better understand peripheral biotransformation of the erythro-metabolites, particularly whether or not the peripheral tissues, especially the muscles in the upper limbs, either produce, store or degrade part of the hydroxy and epoxy-metabolites in RBCs that pass through them. We are particularly interested to clarify which arteriovenous relationships are present in RBC oxylipin profiles during hemodialysis and in what way they (i.e. the AV differences) are modified by extracorporeal renal replacement therapy, which causes oxidative stress, chronic inflammation and blood-endothelial interactions (Fig. 2).

2. Material and methods

2.1. Subjects

The ethical committee of the Charité University Medicine approved the study and written informed consent was obtained. The study has been officially registered: (ClinicalTrials.gov, identifier: NCT03857984). Nine men and three non-pregnant women diagnosed with ESRD requiring regular hemodialysis treatment three times a week were enrolled, and all patients were older than 18 years of age. In order to be included in the study, patients needed to have a stable hemodialysis prescription. Dialysis was performed *via* native fistula or gore-tex grafts. Exclusion criteria were: poor patient compliance, hemoglobin level below 8.0 g/dL, or the presence of an active infection.

2.2. Research design

All patients were treated in a sitting position. The subjects used Polyflux 170H dialyzer (PAES membrane, Gambro) for dialysis treatment. The conditions of dialysis treatment were as follows: blood flow ≥250 mL/min, dialysate flow 500 mL/min, double needle puncture technique, dialysis time on average 4 h 15 min. Arterial blood samples were collected from the arteriovenous fistula or Gore-Tex grafts before the start of dialysis (Pre-HD) and 5-15 min before the end of dialysis (Post-HD), while peripheral venous blood was collected on the

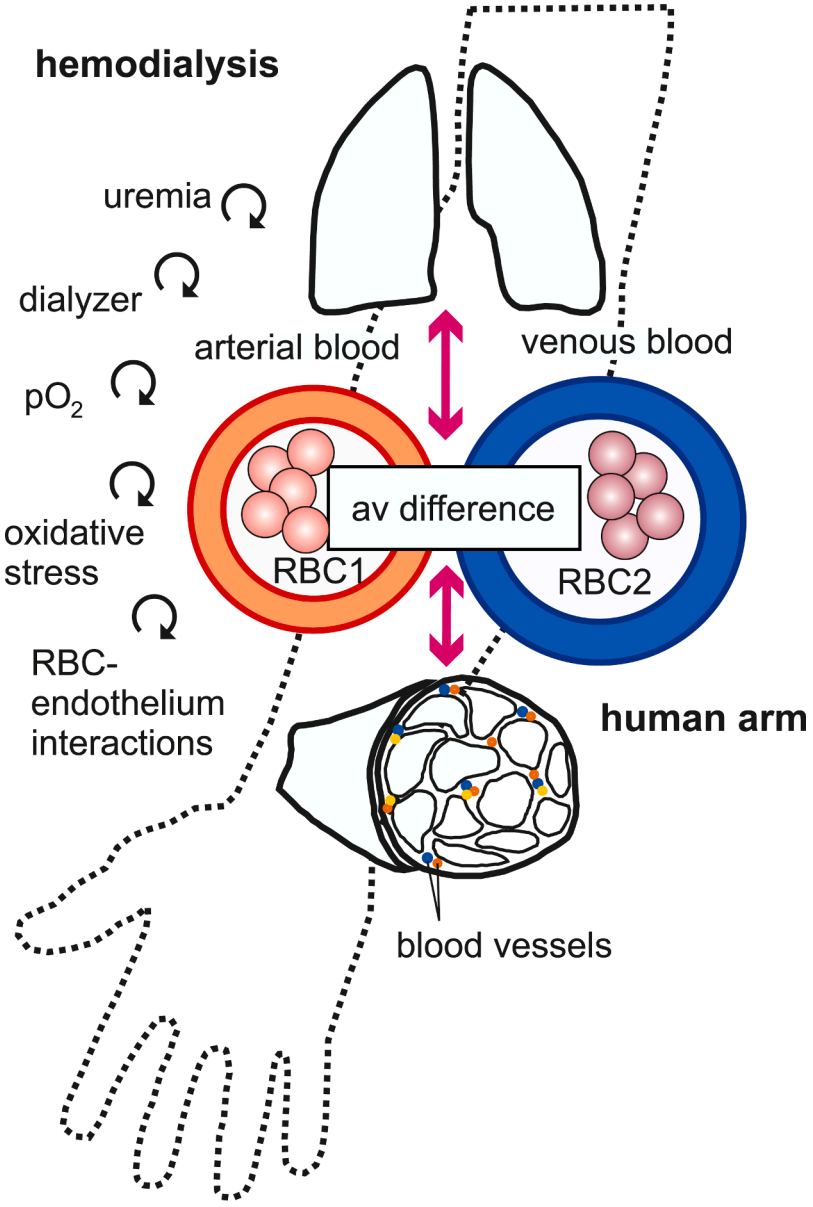

Fig. 2. A Simplified diagram of the relationship among different compartments. Central compartments (red blood cells, RBC1, RBC2) and peripheral compartments (organ tissues, especially upper limb muscle, with extracellular fluid, red blood cells (RBCs), etc.) are illustrated. The continuity between arterial and venous systems via pulmonary (top) and peripheral (bottom) upper limb muscle is indicated. Arterial and venous blood samples were taken before (pre-HD) and after HD (post-HD) treatment. Red blood cell fatty acid metabolites were measured in RBC1 and RBC2, i.e. arterial shunt and subcutaneous vein, respectively. It is obvious that the arteriovenous (av) difference is due to the fact that the peripheral tissues, especially the muscles, either produce, store or degrade part of the fatty acid metabolites in RBCs. RBCs may sense tissue O2 requirements via their degree of deoxygenation when they pass through the microcirculation and release vasodilatory compounds (e.g. adenosine triphosphate (ATP), CYP and LOX metabolites) to regulate blood flow.

ipsilateral extremity by subcutaneous arm vein puncture. The collection was performed at same time points to determine the arteriovenous difference of the RBC fatty acid metabolites (Fig. 2). It is obvious that the arteriovenous difference is due to the fact that the peripheral tissues, especially the muscles, either produce, store or degrade part of the RBC fatty acid metabolites that pass through the peripheral tissue. These metabolites may maintain adequate blood flow and oxygen delivery to the peripheral tissue. All blood samples were obtained through a 4°C pre-cooled EDTA vacuum extraction tube system. Glucose, lipoproteins, and triglycerides were measured by clinical routine methods in an accredited clinical laboratory.

2.3. Sample pre-processing and data analysis

Sample preparation and preprocessing, standardization, HPLC-MS detection and data processing were performed as described in [15,16]. All samples were analyzed for free and total RBC fatty acid metabolites [14,17].

2.4. Statistical analysis

Data are represented as mean ± standard deviation (SD). Descriptive statistics were obtained, and variables were checked for skewness and kurtosis to ensure that they met the normal distribution assumptions. In order to determine statistical significance, paired *t*-test or paired Wilcoxon test were used as appropriate to compare pre-HD *vs*. post-HD values. The arterio-venous difference is expressed as Δ, i.e. calculated by Δ = A - V. A *P* value of less than 0.05 was regarded as statistically significant. Statistical analysis was performed using SPSS Statistics software (IBM Corporation).

3. Results

3.1. Basic characteristics of the participants

Table 1 shows the clinical characteristics of the ESRD hemodialysis (HD) patients. The patients were diagnosed for the following conditions: focal segmental glomerulosclerosis (6 patients), IgA nephropathy (1 patients), renal amyloidosis (1 patient), hypertensive nephropathy (1 patient), ADPKD (autosomal dominant polycystic kidney disease) (1 patient), drug-induced kidney injury (1 patient), cystic kidneys (1 patient). All patients had major cardiovascular complications, i.e. cardiovascular and cerebrovascular events, and/or peripheral artery disease. The patients were not diabetic but had hyperlipidemia. Plasma glucose was 115.8 ± 36.7 mg/dL (normal range 60-110 mg/dl); total cholesterol, 221.8 ± 181.2 mg/dL (< 200 mg/dL); LDL-cholesterol, 101.2 ± 30.7 (< 130 mg/dL); HDL- cholesterol, 42.4 ± 9.9 (>35 mg/dL); triglycerides, 151.0 ± 77.4 (<200 mg/dL).

Table 1
Characteristics of hemodialysis (HD) patients (n=12).

	HD patients
Age (years)	72±12
Sex	
Male (n)	9
Female (n)	3
Body mass index (kg/m^2)	27±3.3
Race (n)	
	Caucasian = 12
Cause of end-stage renal disease	
Focal segmental glomerulosclerosis	6
IgA nephropathy (n)	1
Renal amyloidosis	1
Hypertension (n)	1
Drug induced (n)	1
ADPKD (n)	1
Cystic kidneys	1
Complications	
Cardiovascular (n)	12

Notes: Data are presented as mean ± SD or frequencies.

3.2. Effects of hemodialysis

The effects of hemodialysis treatment on total and free RBC hydroxy- and epoxy- fatty acids in ESRD HD patients are summarized (Tables 2, S1).

3.2.1. Metabolites derived from LOX, CYP and ω/(ω-1)-hydroxylase pathways

Pre-HD

With exception of 16-HETE and 9-HODE, comparison between total LOX, CYP and CYP ω/(ω-1)-hydroxylase metabolites levels from arterial and venous blood showed no arteriovenous differences before HD treatment (pre-HD)., i.e. the levels of individual hydroxy- and epoxy-metabolites in RBCs were not significantly different in arterial vs. venous blood (Table 2A). These erythro-metabolites included 5-HETE, 8-HETE, 9-HETE, 11-HETE, 12-HETE, 15-HETE, 4-HDHA, 7-HDHA, 8-HDHA, 10-HDHA, 11-HDHA, 13-HDHA, 14-HDHA, 16-HDHA, 17-HDHA, 20-HDHA, 5-HEPE, 8-HEPE, 9-HEPE, 11-HEPE, 12-HEPE, 15-HEPE, 18-HEPE, 16-HETE, 17-HETE, 18-HETE, 19-HETE, 20-HETE, 9-HODE, 13-HODE, 22-HDHA, 20-HEPE, 5,6-EET, 8,9-EET, 11,12-EET, 14,15-EET, 5,6-DHET, 8,9-DHET, 11,12-DHET, 14,15-DHET, 9,10-EpOME, 12,13-EpOME, 9,10-DiHOME, 12,13-DiHOME, 7,8-EDP, 10,11-EDP, 13,14-EDP, 16,17-EDP, 19,20-EDP, 7,8-DiHDPA, 10,11-DiHDPA, 13,14-DiHDPA, 16,17-DiHDPA, 19,20-DiHDPA, 5,6-EEQ, 8,9-EEQ, 11,12-EEQ, 14,15-EEQ, 17,18-EEQ, 5,6-DiHETE, 8,9-DiHETE, 11,12-DiHETE, 14,15-DiHETE, 17,18-DiHETE (Table 2A). Similarly, free CYP expoxides and CYP ω/(ω-1)-hydroxylase metabolites (excluding 20-HETE) in RBCs showed no arteriovenous differences before HD treatment (pre-HD) (Table 2B). However, RBCs showed positive arteriovenous differences in content of numerous free LOX metabolites, including 8-HETE, 9-HETE, 11-HETE, 15-HETE, 17-HDHA, 20-HDHA, 8-HEPE, 9-HEPE, 11-HEPE, 15-HEPE and 18-HEPE (Table 2B).

Post-HD

Hemodialysis treatment caused positive arteriovenous differences in the majority of RBC LOX metabolites (Table 2A, B). The changes were particularly prominent in the free RBC compartment as compared with the total RBC compartment. Comparison of total erythro-LOX metabolites levels between arterial and venous blood showed arteriovenous differences after hemodialysis (post-HD) in 5-HETE, 8-HETE, 9-HETE, 7-HDHA, 8-HDHA, 10-HDHA, 14-HDHA, 8-HEPE, 9-HEPE and 12-HEPE (Table 2A). With exception of 5-HETE, RBCs showed arteriovenous differences post-HD in all hydroxy-metabolites measured in the free RBC compartment (Table 2B). These metabolites included 8-HETE, 9-HETE, 11-HETE, 12-HETE, 15-HETE, 4-HDHA, 7-HDHA, 8-HDHA, 10-HDHA, 11-HDHA, 13-HDHA, 14-HDHA, 16-HDHA, 17-HDHA, 20-HDHA, 5-HEPE, 8-HEPE, 9-HEPE, 11-HEPE, 12-HEPE, 15-HEPE and 18-HEPE. The effects were predominantly caused by changes in the venous blood (Table S1). Together, the findings indicate that hemodialysis causes profound arteriovenous differences in hydroxy-metabolites derived from LOX pathways in RBCs during peripheral perfusion of upper limb.

3.2.2. Epoxides derived from CYP monooxygenase pathways

Pre-HD

With exception of 5,6-DiHETE, comparison between total CYP epoxides levels in RBCs from arterial and venous blood showed no arteriovenous differences before HD treatment (pre-HD) (Table 2A). Similar results were found in the analyses of free erythro-epoxides (Table 2B).

As shown in Fig. 1, the main pathway of EET, EpOME, EEQ and EDP metabolism in many cells is conversion to DHETs, DiHOMEs, DiHETEs, and DiHDPAs by the soluble epoxide hydrolase enzyme (sEH). Since ESRD might have caused EET, EpOME, EEQ, and EDP production rapidly

Table 2
Effects of hemodialysis on hydroxy- and epoxy-metabolites in the CKD patients before (pre-HD) and at cessation (post-HD) of hemodialysis (n=12 each).

Amount ng/g	pre-HD Arterial	pre-HD Venous	p value, t test (# paired Wilcoxon test)	pre-HD $\Delta_{A\text{-}V}$	post-HD Arterial	post-HD Venous	p value, t test (# paired Wilcoxon test)	post-HD $\Delta_{A\text{-}V}$
Panel A: Total metabolite levels.								
LOX metabolites								
5-HETE	192.57 ±25.35	198.56 ±32.71	0.511	-5.99 ±30.53	207.64 ±48.27	173.93 ±31.81	0.003	33.71 ±30.31
8-HETE	56.64 ±11.68	56.70 ±9.36	0.991	-0.05 ±15.81	63.03 ±12.45	56.56 ±9.36	0.038	6.47 ±9.50
9-HETE	154.21 ±19.56	159.42 ±18.96	0.388 #	-5.21 ±25.47	171.28 ±40.78	136.62 ±24.32	0.008	34.65 ±37.45
11-HETE	178.41 ±41.70	179.03 ±46.16	0.948	-0.61 ±31.52	184.55 ±66.18	171.73 ±57.79	0.34	12.83 ±44.57
12-HETE	413.36 ±143.96	469.21 ±325.75	0.638 #	-55.85 ±297.34	610.90 ±502.51	352.44 ±141.85	0.071 #	258.47 ±497.92
15-HETE	328.57 ±59.64	331.87 ±65.68	0.844	-3.30 ±56.56	337.74 ±92.64	306.33 ±85.33	0.097	31.41 ±60.08
4-HDHA	115.75 ±40.87	113.58 ±38.50	0.754	2.17 ±23.38	113.09 ±53.8986	104.4464 ±43.3149	0.263	8.6433 ±25.3880
7-HDHA	24.96 ±8.22	25.15 ±4.15	0.388 #	-0.19 ±7.02	28.55 ±8.34	22.08 ±5.51	0.013	6.47 ±7.53
8-HDHA	29.24 ±9.97	27.28 ±9.25	0.373	1.95 ±7.29	33.09 ±12.20	26.17 ±10.39	0.003	6.92 ±6.43
10-HDHA	20.09 ±6.25	20.06 ±5.02	0.988	0.02 ±4.88	21.58 ±6.45	18.34 ±6.65	0.029	3.24 ±4.48
11-HDHA	37.79 ±24.86	38.53 ±21.33	0.806	-0.73 ±10.17	34.92 ±28.61	35.36 ±22.06	0.935	-0.44 ±18.05
13-HDHA	45.76 ±17.61	47.91 ±19.64	0.523	-2.14 ±11.24	46.84 ±22.55	43.02 ±21.02	0.301	3.82 ±12.21
14-HDHA	45.92 ±18.73	48.43 ±15.56	0.53 #	-2.51 ±16.62	55.82 ±33.11	37.95 ±10.82	0.034 #	17.87 ±33.36
16-HDHA	58.53 ±27.04	59.80 ±25.79	0.804	-1.28 ±17.38	56.87 ±28.82	54.85 ±28.15	0.663	2.02 ±15.62
17-HDHA	71.77 ±30.09	70.33 ±27.14	0.725	1.44 ±13.80	70.20 ±31.59	64.84 ±30.06	0.28	5.37 ±16.39
20-HDHA	143.35 ±63.29	141.64 ±55.32	0.868	1.71 ±34.73	142.83 ±66.65	130.52 ±60.98	0.257	12.31 ±35.68
5-HEPE	14.85 ±6.92	13.80 ±5.68	0.519	1.05 ±5.44	15.04 ±5.91	12.94 ±5.66	0.167	2.11 ±4.94
8-HEPE	2.09±0.85	2.20 ±0.60	0.631	-0.11 ±0.77	2.56 ±0.89	1.85 ±0.72	0.042	0.71 ±1.07
9-HEPE	5.35 ±2.36	5.14 ±1.65	0.74	0.22 ±2.19	6.44 ±2.45	4.45 ±1.91	0.02	1.99 ±2.54
11-HEPE	21.94 ±16.25	23.29 ±13.88	0.655	-1.35 ±10.19	21.15 ±16.16	20.49 ±13.96	0.854	0.66 ±12.11
12-HEPE	38.22 ±21.81	42.08 ±19.71	0.754 #	-3.86 ±19.33	49.19 ±44.12	24.26 ±9.95	0.023 #	24.93 ±44.72
15-HEPE	6.88 ±3.40	7.37 ±2.93	0.485	-0.49 ±2.35	7.62 ±2.84	6.16 ±3.07	0.056	1.46 ±2.37
18-HEPE	20.23 ±7.43	19.27 ±6.61	0.587	0.96 ±5.95	20.58 ±7.48	17.87 ±8.10	0.108	2.71 ±5.38
CYP ω/(ω−1) metabolites								
16-HETE	11.35 ±4.51	14.09 ±5.15	0.015 #	-2.74 ±3.03	11.13 ±5.28	10.90 ±4.83	0.877	0.23 ±5.03
17-HETE	0.23 ±0.08	0.26 ±0.07	0.16	-0.03 ±0.06	0.23 ±0.07	0.21 ±0.06	0.361	0.02 ±0.07
18-HETE	0.64 ±0.26	0.67 ±0.20	0.587	-0.03 ±0.17	0.68 ±0.26	0.63 ±0.23	0.546	0.04 ±0.24
19-HETE	0.32 ±0.11	0.35 ±0.14	0.136 #	-0.03 ±0.07	0.35 ±0.17	0.28 ±0.10	0.182 #	0.07 ±0.17
20-HETE	0.589 ±0.138	0.63 ±0.24	0.695 #	-0.05 ±0.15	0.64 ±0.15	0.53 ±0.18	0.03	0.10 ±0.14
9-HODE	95.46 ±20.97	105.22 ±15.95	0.027	-9.76 ±13.27	112.39 ±38.96	94.99 ±25.11	0.239 #	17.40 ±43.98
13-HODE	108.45 ±26.10	117.85 ±16.26	0.142	-9.40 ±20.58	131.67 ±51.38	103.24 ±31.34	0.06 #	28.43 ±58.70
22-HDHA	0.26 ±0.14	0.33 ±0.23	0.182 #	-0.06 ±0.19	0.25 ±0.13	0.23 ±0.14	0.616	0.02 ±0.15
20-HEPE	0.26 ±0.14	0.10 ±0.17	1 #	0.01 ±0.04	0.05 ±0.07	0.05 ±0.10	0.893 #	0.00 ±0.08
CYP epoxy-metabolites								
5,6-EET	62.22 ±68.30	69.46 ±65.90	0.308 #	-7.24 ±21.79	91.36 ±99.99	56.52 ±63.89	0.347 #	34.84 ±83.99
8,9-EET	62.07 ±16.10	65.90 ±13.95	0.524	-3.83 ±20.17	76.50 ±22.04	63.37 ±14.10	0.067	13.13 ±22.35
11,12-EET	69.88 ±14.41	75.19 ±19.91	0.513	-5.30 ±27.17	84.18 ±25.51	69.94 ±15.93	0.136 #	14.24 ±28.98
14,15-EET	92.07 ±25.43	103.57 ±31.77	0.306	-11.49 ±37.08	109.32 ±40.86	94.90 ±24.38	0.182 #	14.41 ±42.81
5,6-DHET	2.57 ±0.60	2.84 ±0.76	0.084 #	-0.27 ±0.47	2.68 ±0.86	2.24 ±0.50	0.085	0.43 ±0.80
8,9-DHET	3.44 ±1.64	3.66 ±1.67	0.209 #	-0.21 ±0.79	3.48 ±1.62	3.03 ±1.50	0.099 #	0.45 ±1.15
11,12-DHET	1.66 ±0.64	1.66 ±0.63	0.875 #	0.00 ±0.44	1.76 ±0.48	1.49 ±0.47	0.004	0.27 ±0.26

(*continued on next page*)

Table 2 (*continued*)

14,15-DHET	2.29 ±0.35	2.29 ±0.38	0.956	-0.01 ±0.48	2.35 ±0.35	2.14 ±0.27	0.177	0.21 ±0.49
9,10-EpOME	37.19 ±15.89	40.17 ±11.39	0.386	-2.99 ±11.46	54.47 ±28.71	40.01 ±17.56	0.128	14.46 ±30.45
12,13-EpOME	39.69 ±12.91	43.09 ±8.73	0.385	-3.40 ±13.02	55.17 ±21.02	43.09 ±13.08	0.071 #	12.08 ±24.98
9,10-DiHOME	1.98 ±1.28	2.13 ±1.43	0.638 #	-0.15 ±0.74	2.72 ±2.06	2.36 ±1.93	0.638 #	0.35 ±2.03
12,13-DiHOME	3.49 ±1.35	3.36 ±1.56	0.583 #	0.13 ±1.04	4.50 ±2.83	4.57 ±2.95	0.347 #	-0.07 ±2.67
7,8-EDP	39.28 ±19.86	40.07 ±18.50	0.695 #	-0.79 ±21.07	44.29 ±16.39	39.12 ±13.12	0.241	5.16 ±14.42
10,11-EDP	18.95 ±8.56	20.22 ±7.14	0.613	-1.27 ±8.45	25.07 ±9.45	18.32 ±5.64	0.03	6.75 ±9.39
13,14-EDP	17.88 ±9.99	19.76 ±10.12	0.569	-1.88 ±11.10	18.31 ±7.98	14.44 ±4.46	0.144	3.88 ±8.54
16,17-EDP	32.72 ±16.513	36.67 ±15.08	0.474	-3.96 ±18.48	27.36 ±14.54	21.28 ±9.22	0.182 #	6.08 ±14.78
19,20-EDP	23.01 ±10.21	25.17 ±10.13	0.48 #	-2.16 ±12.00	32.01 ±11.74	22.38 ±6.48	0.019 #	9.63 ±12.86
7,8-DiHDPA	1.15 ±0.50	1.29 ±0.47	0.262	-0.14 ±0.40	1.32 ±0.71	1.22 ±0.48	0.596	0.10 ±0.62
10,11-DiHDPA	0.76 ±0.17	0.74 ±0.14	0.713	0.02 ±0.17	0.75 ±0.19	0.70 ±0.17	0.449	0.05 ±0.21
13,14-DiHDPA	0.85 ±0.33	0.77 ±0.21	0.464	0.08 ±0.38	0.87 ±0.39	0.89 ±0.38	0.859	-0.02 ±0.31
16,17-DiHDPA	0.48 ±0.16	0.49 ±0.12	0.831	-0.01 ±0.15	0.48 ±0.19	0.479 ±0.11	0.898	0.01 ±0.16
19,20-DiHDPA	1.19 ±0.43	1.09 ±0.28	0.402	0.10 ±0.39	1.28 ±0.35	1.05 ±0.25	0.108	0.23 ±0.45
5,6-EEQ	ns	ns	ns	ns	ns	ns	ns	ns
8,9-EEQ	3.10 ±2.48	2.69 ±1.17	0.58 #	0.40 ±2.45	4.22 ±2.60	2.76 ±1.40	0.028 #	1.46 ±2.12
11,12-EEQ	3.64 ±2.59	3.68 ±2.02	0.875 #	-0.04 ±3.04	4.89 ±1.72	3.58 ±1.29	0.029	1.30 ±1.80
14,15-EEQ	2.83 ±1.68	2.75 ±1.41	0.814 #	0.08 ±2.03	3.78 ±1.71	2.54 ±0.95	0.032	1.25 ±1.76
17,18-EEQ	4.74 ±4.03	4.09 ±2.08	0.937 #	0.65 ±3.02	7.42 ±6.13	4.67 ±3.85	0.071 #	2.75 ±5.54
5,6-DiHETE	2.61 ±0.98	1.71 ±0.46	0.011	0.90 ±1.03	2.21 ±0.73	2.09 ±0.49	0.875 #	0.12 ±0.83
8,9-DiHETE	0.22 ±0.17	0.17 ±0.06	0.272 #	0.05 ±0.14	0.21 ±0.09	0.19 ±0.05	0.695 #	0.02 ±0.10
11,12-DiHETE	3.80 ±2.55	3.56 ±1.68	0.777	0.24 ±2.90	5.30 ±2.66	4.70±3.49	0.388 #	0.60 ±4.32
14,15-DiHETE	0.13 ±0.10	0.11 ±0.04	0.937 #	0.020 ±0.10	0.05 ±0.07	0.05 ±0.10	0.041 #	0.04 ±0.06
17,18-DiHETE	0.44 ±0.17	0.39 ±0.11	0.219	0.05 ±0.14	0.43 ±0.16	0.45 ±0.22	1 #	-0.02 ±0.22
Panel B: Free metabolite levels.								
LOX metabolites								
5-HETE	2.50 ±1.67	2.51 ±2.93	0.638 #	-0.01 ±3.70	2.42 ±1.42	1.47 ±1.60	0.071 #	0.95 ±1.84
8-HETE	2.38 ±0.61	1.77 ±0.46	0.006	0.61 ±0.62	2.38 ±0.85	1.39 ±0.56	0.003	0.99\ ±0.88
9-HETE	2.49 ±1.30	1.75 ±0.81	0.011	0.74 ±0.84	1.99 ±0.87	1.28 ±0.60	0.023	0.71 ±0.88
11-HETE	3.10 ±1.21	2.29 ±1.06	0.012 #	0.81 ±0.91	2.76 ±1.19	1.76 ±0.65	0.016	1.00 ±1.17
12-HETE	544.04 ±231.17	425.17 ±210.46	0.077	118.86 ±211.26	555.84 ±336.12	336.43 ±213.05	0.015	219.41 ±252.60
15-HETE	6.88 ±1.86	5.21 ±2.07	0.003	1.66 ±1.55	7.45 ±2.70	4.50 ±1.79	0.001	2.95 ±2.21
4-HDHA	0.23 ±0.11	0.20 ±0.09	0.169	0.03 ±0.07	0.21 ±0.09	0.13 ±0.07	0.001	0.08 ±0.06
7-HDHA	0.38 ±0.15	0.34 ±0.28	0.289 #	0.04 ±0.25	0.28 ±0.18	0.16 ±0.05	0.01 #	0.12 ±0.16
8-HDHA	4.17 ±3.73	3.59 ±4.05	0.433 #	0.59 ±1.87	3.74 ±3.54	2.02 ±1.93	0.035	1.72 ±2.37
10-HDHA	1.41 ±0.86	1.13 ±0.78	0.06 #	0.28 ±0.44	1.11 ±0.65	0.69 ±0.40	0.017	0.42 ±0.50
11-HDHA	5.39 ±3.80	4.41 ±3.20	0.099 #	0.98±1.80	3.57 ±2.41	2.18 ±1.47	0.031	1.38 ±1.87
13-HDHA	0.82 ±0.67	0.67 ±0.73	0.05 #	0.16 ±0.25	0.52 ±0.33	0.32 ±0.16	0.003 #	0.20 ±0.24
14-HDHA	30.72 ±21.13	25.10 ±18.55	0.117 #	5.62 ±11.16	23.18 ±17.75	14.14 ±9.38	0.043	9.04 ±13.07
16-HDHA	2.21 ±2.64	1.77 ±3.69	0.06 #	0.44 ±4.67	3.68 ±5.34	1.47 ±2.16	0.002 #	2.21 ±3.28
17-HDHA	1.84 ±1.02	1.40 ±0.82	0.028 #	0.44 ±0.59	1.46 ±0.70	0.86 ±0.40	0.001	0.60 ±0.47
20-HDHA	2.27 ±1.06	1.84 ±1.06	0.039	0.44 ±0.64	2.39 ±1.38	1.35 ±0.60	0.003 #	1.03 ±0.99
5-HEPE	0.52 ±0.29	0.50 ±0.61	0.347 #	0.02 ±0.69	0.51 ±0.48	0.23 ±0.17	0.041 #	0.28 ±0.48
8-HEPE	0.11 ±0.07	0.07 ±0.04	0.002 #	0.03 ±0.04	0.09 ±0.07	0.05 ±0.02	0.003 #	0.04 ±0.06
9-HEPE	0.32 ±0.27	0.21 ±0.13	0.006 #	0.11 ±0.17	0.25 ±0.22	0.13 ±0.04	0.006 #	0.12 ±0.21
11-HEPE	0.70 ±0.55	0.49 ±0.43	0.008 #	0.21 ±0.20	0.56 ±0.36	0.34 ±0.21	0.003 #	0.21 ±0.19
12-HEPE	43.51 ±25.87	35.57 ±23.18	0.102	7.94 ±15.42	31.16 ±21.78	18.21 ±11.77	0.034	12.95 ±17.70
15-HEPE	0.42 ±0.24	0.32 ±0.17	0.023 #	0.09 ±0.14	0.35 ±0.21	0.23 ±0.08	0.019 #	0.12 ±0.18
18-HEPE	0.60 ±0.28	0.41 ±0.14	0.013	0.19 ±0.22	0.57 ±0.33	0.33 ±0.12	0.003 #	0.24 ±0.28
CYP ω/(ω−1) metabolites								
16-HETE	0.09 ±0.03	0.08 ±0.02	0.084	0.01 ±0.02	0.10 ±0.03	0.08 ±0.01	0.272 #	0.01 ±0.03
17-HETE	ns	ns	ns #	ns	ns	ns #	ns	ns
18-HETE	0.04 ±0.01	0.04 ±0.01	0.798	0.00 ±0.01	0.045 ±0.02	0.04 ±0.02	0.35	0.01 ±0.03
19-HETE	0.01 ±0.01	0.00±0.01	1 #	0	0.01 ±0.02	0.01 ±0.02	1 #	0.00 ±0.01
20-HETE	0.07 ±0.07	0.13 ±0.08	0.013 #	-0.06 ±0.06	0.06 ±0.06	0.10 ±0.09	0.131 #	-0.04 ±0.07
9-HODE	24.96 ±16.21	45.28 ±99.25	0.209 #	-20.31 ±85.34	14.67±8.45	12.19±8.95	0.084 #	2.48 ±7.13
13-HODE	19.85 ±10.47	37.06 ±77.53	0.272 #	-17.21 ±71.57	12.38 ±7.03	9.17 ±6.28	0.041 #	3.20 ±5.05
22-HDHA	0.08 ±0.07	0.07 ±0.04	0.583 #	0.01 ±0.08	0.05 ±0.02	0.04 ±0.02	0.534 #	0.00 ±0.02
20-HEPE	0.03 ±0.04	0.03 ±0.05	0.866 #	0.00 ±0.06	0.01 ±0.03	0.03 ±0.04	0.138 #	-0.02 ±0.03
CYP epoxy-metabolites								

(*continued on next page*)

Table 2 (*continued*)

5,6-EET	0.67 ±0.33	0.56 ±0.33	0.388 #	0.11 ±0.44	1.01 ±0.49	0.80 ±0.33	0.239 #	0.21 ±0.58
8,9-EET	1.37 ±0.77	1.43 ±1.02	0.846	-0.05 ±0.89	1.48 ±0.88	1.41 ±0.68	0.816	0.07 ±0.95
11,12-EET	0.80 ±0.31	0.59 ±0.26	0.099 #	0.22 ±0.41	1.19 ±0.60	0.94 ±0.38	0.288	0.25 ±0.78
14,15-EET	0.86 ±0.33	0.67 ±0.33	0.158 #	0.19 ±0.43	1.41 ±0.62	1.02 ±0.32	0.09	0.39 ±0.73
5,6-DHET	0.03 ±0.02	0.03 ±0.01	0.607	0.00 ±0.02	0.03 ±0.01	0.03 ±0.02	0.06 #	0.01 ±0.02
8,9-DHET	0.09 ±0.02	0.09 ±0.03	0.653	0.00 ±0.03	0.08 ±0.02	0.08 ±0.02	0.347 #	0.00 ±0.02
11,12-DHET	0.07±0.04	0.07 ±0.04	0.388 #	0.00 ±0.01	0.07 ±0.05	0.06 ±0.03	0.158 #	0.01 ±0.03
14,15-DHET	0.09 ±0.03	0.09 ±0.04	0.209 #	-0.01 ±0.02	0.09 ±0.04	0.08 ±0.03	0.272 #	0.01 ±0.03
9,10-EpOME	4.02 ±1.61	5.90 ±8.91	0.814 #	-1.88 ±8.52	4.25 ±2.09	4.28 ±2.38	0.638 #	-0.02 ±2.16
12,13-EpOME	4.46 ±1.80	6.87 ±10.61	0.814 #	-2.41 ±10.19	4.42 ±1.84	4.68 ±2.43	0.68	-0.26 ±2.13
9,10-DiHOME	0.912 ±0.49	1.16 ±0.88	0.388 #	-0.24 ±0.69	0.83 ±0.69	1.03 ±0.91	0.023 #	-0.21 ±0.27
12,13-DiHOME	1.46 ±0.77	1.90 ±1.33	0.308 #	-0.44 ±1.25	1.55 ±1.30	1.92 ±1.68	0.136 #	-0.37 ±0.51
7,8-EDP	0.32 ±0.14	0.24 ±0.14	0.158 #	0.08 ±0.17	0.39 ±0.24	0.29 ±0.15	0.116	0.09 ±0.19
10,11-EDP	0.14 ±0.06	0.14 ±0.09	0.638 #	0.00 ±0.09	0.18 ±0.11	0.15 ±0.07	0.388 #	0.03 ±0.09
13,14-EDP	0.13 ±0.07	0.13 ±0.12	0.937 #	0.00 ±0.10	0.13 ±0.09	0.12 ±0.08	0.53 #	0.01 ±0.08
16,17-EDP	0.24 ±0.09	0.21 ±0.11	0.367 #	0.03 ±0.10	0.27 ±0.16	0.22 ±0.12	0.182 #	0.05 ±0.13
19,20-EDP	0.20 ±0.08	0.18 ±0.10	0.48 #	0.02 ±0.11	0.19 ±0.11	0.21 ±0.08	0.568	-0.02 ±0.10
7,8-DiHDPA	0.03 ±0.01	0.03 ±0.01	0.225	0.00 ±0.01	0.05 ±0.01	0.03 ±0.01	0.565	0.00 ±0.01
10,11-DiHDPA	0.03 ±0.02	0.03 ±0.02	0.05 #	0	0.03 ±0.02	0.02 ±0.02	0.308 #	0.00 ±0.01
13,14-DiHDPA	0.04 ±0.01	0.04 ±0.01	0.347 #	0.00 ±0.01	0.04 ±0.01	0.04 ±0.01	0.477 #	0.00 ±0.01
16,17-DiHDPA	0.05 ±0.01	0.05 ±0.01	0.199	0.00 ±0.01	0.05 ±0.01	0.05 ±0.01	0.787	0.00 ±0.01
19,20-DiHDPA	0.31 ±0.16	0.29 ±0.17	0.638 #	0.02 ±0.17	0.47 ±0.37	0.31 ±0.18	0.136 #	0.16 ±0.36
5,6-EEQ	ns	ns	ns	ns	ns	ns	ns	ns
8,9-EEQ	0.13 ±0.07	0.09 ±0.05	0.121	0.03 ±0.07	0.120 ±0.14	0.17 ±0.08	0.583 #	0.03 ±0.14
11,12-EEQ	0.10 ±0.09	0.11 ±0.08	0.814 #	0.00 ±0.11	0.17 ±0.13	0.11 ±0.07	0.388 #	0.05 ±0.13
14,15-EEQ	0.10 ±0.07	0.09 ±0.06	0.814 #	0.01 ±0.08	0.16 ±0.12	0.13 ±0.08	0.814 #	0.03 ±0.14
17,18-EEQ	0.13 ±0.10	0.13 ±0.10	0.875 #	0.00 ±0.11	0.21 ±0.17	0.12 ±0.05	0.099 #	0.09 ±0.16
5,6-DiHETE	0.07 ±0.03	0.06 ±0.03	0.641	0.01 ±0.05	0.08 ±0.04	0.05 ±0.03	0.062	0.03 ±0.05
8,9-DiHETE	0.01 ±0.01	0.01 ±0.01	0.109 #	0	0.01 ±0.01	0.01 ±0.01	1 #	0.00 ±0.01
11,12-DiHETE	0.02 ±0.01	0.02 ±0.01	0.327 #	0.00 ±0.01	0.02 ±0.01	0.01 ±0.00	0.023	0.01 ±0.01
14,15-DiHETE	0.01 ±0.01	0.01 ±0.01	0.875 #	0	0.02 ±0.00	0.01 ±0.00	0.198	0.00 ±0.01
17,18-DiHETE	0.07 ±0.02	0.07 ±0.03	0.986	0.00 ±0.03	0.08 ±0.03	0.07 ±0.03	0.695 #	0.00 ±0.03

Notes: A, arterial blood; V, venous blood. A-V difference; arteriovenous difference. Mean±SD.

degraded to their diols, we next analyzed the sums of the individual CYP epoxy-metabolites and their diols (Table 3). With exception of 5,6-EEQ and 5,6-DiHETE, we found that ESRD was not associated with differences in the sums in arterial *vs*. venous blood, i.e. there was no arteriovenous differences in the total levels of the erythro-metabolites (Table 3A). Moreover, we calculated diol/epoxide ratios of the erythro-epoxides (Table 3A) and found that the four classes of epoxy-metabolites are unequally hydrolyzed to appear in RBCs in the arterial circulation. We found that EEQs are better metabolized into their diols (ratio of DiHETEs/EEQs; 0.5667±0.1934) than EETs, EDPs and EPOMEs (ratios of those diols/epoxy-metabolites, 0.0390±0.0209, 0.0395 ±0.0210 and 0.0752±0.0361, respectively; Dunn's multiple comparison test, P>0.05) (Table 3). In fact, the following order of ratios was identified: DiHETEs/EEQs > DiHOMEs/EpOMEs = DiHDPA/EDPs = DHETs/EETs (Dunn's multiple comparison test, $P<0.05$). This pattern was also found for the erythro-metabolites in the venous blood, as shown (Table 3A), and there was no arteriovenous difference in these parameters (Table 3A). Moreover, similar results were found for free erythrocyte epoxy-metabolites (Table 3B). Together, the findings indicate that CYP epoxy-metabolites are unequally hydrolyzed by sEH in arterial and venous blood *in vivo* [14,18]. However, there is no arteriovenous difference in the erythro-epoxides before hemodialysis.

Post-HD

Hemodialysis treatment did not cause arteriovenous differences in the majority of RBC epoxides (Table 2A, B). The total levels of following epoxy-metabolites were not different in arterial *vs*. venous blood (Table 2A): 5,6-EET, 8,9-EET, 11,12-EET, 14,15-EET, 5,6-DHET, 8,9-DHET, 14,15-DHET, 9,10-EpOME, 12,13-EpOME, 9,10-DiHOME, 12,13-DiHOME, 7,8-EDP, 13,14-EDP, 16,17-EDP, 7,8-DiHDPA, 10,11-DiHDPA, 13,14-DiHDPA, 16,17-DiHDPA, 19,20-DiHDPA, 5,6-EEQ,17,18-EEQ, 5,6-DiHETE, 8,9-DiHETE, 11,12-DiHETE and 17,18-DiHETE. However, hemodialysis caused positive arteriovenous differences in total levels of few epoxy-metabolites, i.e. 11,12-DHET, 10,11-EDP, 19,20-EDP, 8,9-EEQ, 11,12-EEQ, 14,14-EEQ and 14,15-DiHETE in RBCs (Table 2A). These differences were observed in the total but not free RBC compartment (Table 2B). We found that ratios of diols/epoxides of all four CYP eicosanoid subclasses were not affected by dialysis (Tables 3A, B, S2). Together, the findings indicate that hemodialysis does not affect the majority of CYP epoxy-metabolites (i.e. EETs, EpOMEs, numerous EDPs and EEQs and their diols) to cause arteriovenous differences in RBCs during peripheral perfusion (post-HD). Nevertheless, the findings indicate that the muscles in the upper limbs can either produce, store or degrade part of some individual epoxy-metabolites in RBCs (few EEQs and EDPs) that pass through them.

4. Discussion

Peripheral perfusion is passage of blood to the extremities of the body. Our study is the first study to assess biotransformation of eicosanoids in RBCs in peripheral perfusion *in vivo*. To gain insight into RBC oxylipin metabolism, we evaluated the differences of RBC oxylipins levels in arterial and venous blood of upper limbs from uremic patients treated by HD treatment. Our data demonstrate that RBCs of ESRD patients show significant arteriovenous differences (AV differences) in numerous eicosanoids specifically derived from the LOX pathway. The observed arteriovenous differences were caused by the dialysis treatment itself (post-HD), since we did not detect arteriovenous differences between the metabolite levels before HD treatment (pre-HD).The changes were particularly prominent in the free RBC compartment as compared with the total RBC compartment. The extent to which the RBC LOX metabolites exhibit beneficial or detrimental cardiovascular effects remains to be explored. To our surprise, we have seen no evidence that peripheral tissues, specifically the muscles in the upper limbs, either produce, store or degrade a wide range of CYP epoxides (e.g. EETs) or CYP ω/(ω-1)-hydroxylase products in RBCs that pass through them. We therefore suggest that RBC eicosanoids particularly derived from the LOX pathway (but not CYP epoxide/ CYP ω/(ω-1)-hydroxylase pathways) in peripheral perfusion constitute a fraction of lipid mediators,

Table 3
Effects of hemodialysis on epoxides and their respective diol ratios in the CKD patients before (pre-HD) and at cessation (post-HD) of hemodialysis (n=12 each).

Amount ng/g	pre-HD Arterial	pre-HD Venous	p value, t test (# paired Wilcoxon test)	pre-HD Δ_{A-V}	post-HD Arterial	post-HD Venous	p value, t test (# paired Wilcoxon test)	pre-HD Δ_{A-V}
Panel A: Total metabolite levels.								
5,6-EET + 5,6-DHET	64.79 ±67.79	72.30 ±68.41	0.308 #	-7.51 ±21.72	94.04 ±99.69	58.76 ±63.49	0.308 #	35.28 ±83.82
8,9-EET + 8,9-DHET	65.52 ±15.88	69.56 ±14.81	0.509	-4.04 ±20.53	79.98 ±22.55	66.40 ±13.53	0.055	13.58 ±21.94
11,12-EET + 11,12-DHET	71.55 ±14.37	76.85 ±20.73	0.513	-5.30 ±27.18	85.94 ±25.69	71.43 ±15.93	0.099 #	14.51 ±29.01
14,15-EET + 14,15-DHET	94.36 ±25.30	105.86 ±33.03	0.306	-11.50 ±37.09	111.66 ±40.97	97.05 ±24.40	0.182 #	14.62 ±43.00
12,13-EpOME + 12,13-DiHOME	43.18 ±13.43	46.45 ±9.57	0.399	-3.27 ±12.92	59.67 ±23.23	47.66 ±15.27	0.084 #	12.01 ±26.84
9,10-EpOME + 9,10-DiHOME	39.16 ±16.90	42.30 ±13.10	0.374	-3.14 ±11.74	57.19 ±30.62	42.38 ±19.35	0.099 #	14.81 ±32.19
5,6-EEQ + 5,6-DiHETE	2.62 ±0.99	1.71 ±0.48	0.011 #	0.90 ±1.03	2.22 ±0.73	2.10 ±0.48	0.875 #	0.12 ±0.83
8,9-EEQ + 8,9-DiHETE	3.31 ±2.64	2.87 ±1.25	0.814 #	0.45 ±2.56	4.43 ±2.67	2.95 ±1.43	0.028 #	1.47 ±2.19
11,12-EEQ + 11,12-DiHETE	7.43 ±4.77	7.24 ±3.22	0.903	0.20 ±5.52	10.19 ±3.67	8.29 ±3.82	0.256	1.90 ±5.49
14,15-EEQ + 14,15-DiHETE	2.96 ±1.75	2.87 ±1.49	0.754 #	0.10 ±2.07	3.94 ±1.76	2.66 ±0.99	0.03	1.29 ±1.79
17,18-EEQ + 17,18-DiHETE	5.19 ±4.14	4.48 ±2.19	0.937 #	0.70 ±3.15	7.86 ±6.18	5.12 ±3.80	0.136 #	2.74 ±5.63
7,8-EDP + 7,8-DiHDPA	40.43 ±20.07	41.36 ±19.51	0.884	-0.92 ±21.37	45.61 ±16.82	40.35 ±13.32	0.243	5.26 ±14.78
10,11-EDP + 10,11-DiHDPA	19.71 ±8.67	20.96 ±7.45	0.622	-1.25 ±8.53	25.82 ±9.47	19.03 ±5.58	0.029	6.79 ±9.37
13,14-EDP + 13,14-DiHDPA	18.73 ±10.27	20.53 ±10.58	0.594	-1.80 ±11.36	19.18 ±8.02	15.32 ±4.45	0.151	3.86 ±8.67
16,17-EDP + 16,17-DiHDPA	33.20 ±16.60	37.17 ±15.81	0.475	-3.97 ±18.59	27.83 ±14.51	21.75 ±9.18	0.182 #	6.09 ±14.80
19,20-EDP + 19,20-DiHDPA	24.20 ±10.57	26.26 ±10.68	0.48 #	-2.06 ±12.19	33.29 ±11.81	23.43 ±6.57	0.015 #	9.86 ±13.00
Ratio (9,10-DiHOME+12,13-DiHOME)/(9,10-EpOME+12,13-EpOME)	0.08 ±0.04	0.06 ±0.03	0.583 #	0.01 ±0.04	0.06 ±0.02	0.08 ±0.03	0.053	-0.02±0.03
Ratio(5,6-DHET+8,9-DHET+11,12-DHET+14,15-DHET)/(5,6-EET+8,9-EET +11,12 EET +14,15-EET)	0.04 ±0.02	0.04 ±0.01	0.53 #	0.00 ±0.02	0.03 ±0.01	0.03 ±0.01	0.51	0.00 ±0.01
Ratio(5,6-DiHETE+8,9-DiHETE+11,12-DiHETE+14,15-DiHETE+17,18-DiHETE)/(5,6-EEQ+8,9-EEQ+11,12-EEQ+14,15-EEQ+17,18-EEQ)	0.57 ±0.19	0.48 ±0.20	0.165	0.09 ±0.21	0.45 ±0.19	0.67 ±0.47	0.117 #	-0.22 ±0.46
Ratio(7,8-DiHDPA+10,11-DiHDPA+13,14-DiHDPA+16,17-DiHDPA+19,20-DiHDPA)/(7,8-EDP+10,11-EDP+13,14-EDP+16,17-EDP+19,20-EDP)	0.04 ±0.02	0.03 ±0.01	0.583 #	0.00 ±0.02	0.03 ±0.01	0.04 ±0.02	0.199	-0.01 ±0.01
9,10-DiHOME/9,10-EpOME	0.05 ±0.03	0.05 ±0.02	0.615	0.00 ±0.02	0.05 ±0.02	0.05 ±0.02	0.186	-0.01 ±0.02
12,13-DiHOME/12,13-EpOME	0.10 ±0.05	0.08 ±0.03	0.275	0.02 ±0.05	0.08 ±0.03	0.102 ±0.05	0.05 #	-0.02 ±0.04
5,6-DHET/5,6-EET	0.11 ±0.10	0.09 ±0.07	0.48 #	0.02 ±0.07	0.13 ±0.11	0.11 ±0.10	0.308 #	0.02 ±0.12
8,9-DHET/8,9-EET	0.06 ±0.04	0.06 ±0.03	0.638 #	0.01 ±0.02	0.05 ±0.02	0.05 ±0.03	0.875 #	-0.01 ±0.03
11,12-DHET/11,12-EET	0.031 ±0.01	0.02 ±0.01	0.638 #	0.00 ±0.01	0.02 ±0.01	0.02 ±0.01	0.995	0.00 ±0.01
14,15-DHET/14,15-EET	0.03 ±0.01	0.02 ±0.01	0.463	0.00 ±0.01	0.02 ±0.01	0.02 ±0.01	0.937	0.00 ±0.01
5,6-DiHETE/5,6-EEQ	1940.29 ±1425.66	1107.48 ±699.94	0.003 #	832.81 ±848.10	1242.35 ±1220.11	1551.99 ±1013.06	0.33	-309.64 ±1052.11
8,9-DiHETE/8,9-EEQ	0.08 ±0.03	0.07 ±0.02	0.384	0.01 ±0.03	0.06 ±0.02	0.08 ±0.03	0.042	-0.03 ±0.04
11,12-DiHETE/11,12-EEQ	1.15 ±0.97	1.17 ±0.98	0.388 #	-0.02 ±0.74	1.14 ±0.69	1.44 ±1.26	0.638 #	-0.30 ±1.36
14,15-DiHETE/14,15-EEQ	0.05 ±0.02	0.05 ±0.02	0.859	0.00 ±0.03	0.05 ±0.02	0.05 ±0.02	0.772	0.00 ±0.03
17,18-DiHETE/17,18-EEQ	0.14 ±0.08	0.12 ±0.06	0.324	0.02 ±0.06	0.09 ±0.06	0.14 ±0.08	0.032	-0.06 ±0.08
7,8-DiHDPA/7,8-EDP	0.04 ±0.02	0.04 ±0.01	0.638 #	0.00 ±0.01	0.03 ±0.01	0.03 ±0.02	0.308 #	0.00 ±0.01
10,11-DiHDPA/10,11-EDP	0.0477 ±0.0250	0.0421 ±0.0202	0.433 #	0.0056 ±0.0206	0.0342 ±0.0149	0.0427 ±0.0179	0.187	-0.0085 ±0.0208
13,14-DiHDPA/13,14-EDP	0.06 ±0.03		0.347 #		0.06 ±0.03	0.07 ±0.05	0.388 #	

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Table 3 (*continued*)

		0.05 ±0.03		0.01 ±0.03				-0.01 ±0.03
16,17-DiHDPA/16,17-EDP	0.02 ±0.02	0.02 ±0.01	0.48 #	0.00 ±0.02	0.03 ±0.02	0.03 ±0.02	0.853	0.00 ±0.02
19,20-DiHDPA/19,20-EDP	0.06±0.02	0.05 ±0.02	0.209 #	0.01 ±0.02	0.04 ±0.01	0.05 ±0.01	0.373	-0.01 ±0.02
Panel B: Free metabolite levels.								
11,12-EET + 11,12-DHET	0.88 ±0.30	0.66 ±0.25	0.099 #	0.22 ±0.41	1.26 ±0.61	1.00 ±0.39	0.28	0.26 ±0.80
14,15-EET + 14,15-DHET	0.95 ±0.32	0.76 ±0.32	0.136 #	0.19 ±0.44	1.50 ±0.63	1.10 ±0.33	0.091	0.40 ±0.74
5,6-EET + 5,6-DHET	0.70 ±0.32	0	0.388 #	0.11 ±0.44	1.04 ±0.49	0	0.272 #	0.21 ±0.58
8,9-EET + 8,9-DHET	1.46 ±0.76	0.12 ±0.08	0.83	-0.06 ±0.87	1.56 ±0.88	0.13 ±0.07	0.817	0.07 ±0.95
9,10-EpOME + 9,10-DiHOME	4.94 ±1.86	0.59 ±0.33	0.875 #	-2.11 ±9.18	5.08 ±2.40	0.83 ±0.32	0.739	-0.230 ±2.34
12,13-EpOME + 12,13-DiHOME	5.92 ±2.41	1.51 ±1.00	1 #	-2.85 ±11.33	5.97±2.50	1.49±0.68	0.754 #	-0.63 ±2.46
5,6-EEQ + 5,6-DiHETE	0	0.11 ±0.06	1 #	0	0	0.15 ±0.08	1 #	0
8,9-EEQ + 8,9-DiHETE	0.14 ±0.08	0.20 ±0.12	0.158 #	0.04 ±0.07	0.21 ±0.14	0.20 ±0.08	0.583 #	0.04 ±0.14
11,12-EEQ + 11,12-DiHETE	0.12 ±0.09	0.27 ±0.14	0.754 #	0.00 ±0.11	0.19 ±0.14	0.33 ±0.15	0.272 #	0.06 ±0.14
14,15-EEQ + 14,15-DiHETE	0.12 ±0.07	0.10 ±0.05	0.638 #	0.01 ±0.08	0.18 ±0.12	0.17 ±0.07	0.814 #	0.03 ±0.14
17,18-EEQ + 17,18-DiHETE	0.20 ±0.12	0.12 ±0.10	0.814 #	0.00 ±0.12	0.29 ±0.18	0.07 ±0.04	0.158 #	0.09 ±0.17
7,8-EDP + 7,8-DiHDPA	0.35 ±0.14	0.17 ±0.12	0.117 #	0.08 ±0.17	0.42 ±0.25	0.16 ±0.09	0.132	0.09 ±0.19
10,11-EDP + 10,11-DiHDPA	0.17 ±0.06	0.27 ±0.11	0.695 #	0.00 ±0.09	0.21 ±0.15	0.27 ±0.12	0.298	0.03 ±0.09
13,14-EDP + 13,14-DiHDPA	0.17 ±0.07	0.47 ±0.22	0.937 #	0.00 ±0.10	0.17 ±0.10	0.52 ±0.22	0.628	0.01 ±0.08
16,17-EDP + 16,17-DiHDPA	0.29 ±0.09	0.17 ±0.09	0.53 #	0.03 ±0.10	0.32 ±0.16	0.18 ±0.07	0.182 #	0.05 ±0.13
19,20-EDP + 19,20-DiHDPA	0.51 ±0.19	0.34 ±0.14	0.584	0.04 ±0.23	0.66 ±0.39	0.34 ±0.17	0.176	0.15 ±0.35
Ratio(5,6-DHET+8,9-DHET+11,12-DHET+14,15-DHET)/(5,6-EET+8,9-EET +11,12 EET +14,15-EET)	0.11 ±0.11	0.14 ±0.09	0.308 #	-0.01 ±0.07	0.07 ±0.05	0.08 ±0.04	0.695 #	0.00 ±0.05
Ratio(5,6-DiHETE+8,9-DiHETE+11,12-DiHETE+14,15-DiHETE+17,18-DiHETE)/(5,6-EEQ+ 8,9-EEQ+11,12-EEQ+14,15-EEQ+17,18-EEQ)	0.49±0.21	0.17±0.10	0.556	-0.06 ±0.33	0.34 ±0.13	0.09 ±0.04	0.638	0.02 ±0.14
Ratio (9,10-DiHOME+12,13-DiHOME)/(9,10-EpOME+12,13-EpOME)	0.29 ±0.12	0.55 ±0.33	0.305	-0.05 ±0.17	0.29 ±0.18	0.32 ±0.08	0.183	-0.05 ±0.11
Ratio(7,8-DiHDPA+10,11-DiHDPA +13,14-DiHDPA+16,17-DiHDPA+19,20-DiHDPA)/(7,8-EDP+10,11-EDP+13,14-EDP+16,17-EDP+19,20-EDP)	0.50 ±0.19	0.61 ±0.36	0.308 #	-0.11 ±0.34	0.70 ±0.60	0.56 ±0.39	0.583 #	0.14 ±0.71
9,10-DiHOME/9,10-EpOME	0.24 ±0.13	0.27 ±0.15	0.439	-0.03 ±0.13	0.21 ±0.13	0.24 ±0.12	0.164	-0.04 ±0.08
12,13-DiHOME/12,13-EpOME	0.34 ±0.1301	0.4102 ±0.1598	0.288	-0.0710 ±0.2201	0.3751 ±0.2404	0.4241 ±0.2253	0.308 #	-0.0490 ±0.1422
5,6-DHET/5,6-EET	0.07 ±0.05	0.07 ±0.04	0.48 #	0.00 ±0.05	0.04 ±0.03	0.04 ±0.03	0.583 #	0.00±0.02
8,9-DHET/8,9-EET	0.16 ±0.24	0.20 ±0.33	0.388 #	-0.04 ±0.32	0.10 ±0.13	0.09 ±0.10	1 #	0.01 ±0.06
11,12-DHET/11,12-EET	0.13 ±0.15	0.25 ±0.24	0.48 #	-0.01 ±0.11	0.08 ±0.07	0.12 ±0.08	0.388 #	0.00 ±0.07
14,15-DHET/14,15-EET	0.15 ±0.16	7.05 ±9.62	0.182 #	-0.02 ±0.13	0.08 ±0.07	5.31 ±3.03	0.433 #	-0.014 ±0.07
5,6-DiHETE/5,6-EEQ	0	0	1 #	0	0	0	1 #	0
8,9-DiHETE/8,9-EEQ	0.081 ±0.16	0.07 ±0.14	1 #	0.00 ±0.09	0.04 ±0.09	0.04 ±0.08	1 #	0.00±0.05
11,12-DiHETE/11,12-EEQ	0.29 ±0.19	0.26 ±0.28	0.638 #	0.03 ±0.28	0.21 ±0.26	0.13 ±0.05	0.239 #	0.08 ±0.25
14,15-DiHETE/14,15-EEQ	0.17 ±0.09	0.74 ±0.38	0.695 #	-0.07 ±0.26	0.12 ±0.06	0.70 ±0.43	0.937 #	0.00 ±0.11
17,18-DiHETE/17,18-EEQ	0.92 ±0.88	8.77 ±11.80	0.875 #	0.18 ±1.03	0.49 ±0.30	6.60 ±3.54	0.308 #	-0.22 ±0.55
7,8-DiHDPA/7,8-EDP	0.14 ±0.13	0.15 ±0.09	0.347 #	-0.01 ±0.13	0.11 ±0.08	0.14 ±0.07	0.182 #	-0.03 ±0.08
10,11-DiHDPA/10,11-EDP	0.23 ±0.25	0.24 ±0.17	0.158 #	-0.01 ±0.16	0.19 ±0.17	0.18 ±0.10	0.875 #	0.01 ±0.15

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Table 3 (*continued*)

13,14-DiHDPA/13,14-EDP	0.53 ±0.44	0.61 ±0.45	0.638 #	-0.08 ±0.62	0.46 ±0.43	0.44 ±0.22	0.308 #	0.02 ±0.44
16,17-DiHDPA/16,17-EDP	0.26 ±0.15	0.30 ±0.13	0.308 #	-0.05 ±0.15	0.23 ±0.12	0.29 ±0.18	0.48 #	-0.06 ±0.21
19,20-DiHDPA/19,20-EDP	1.81 ±1.15	1.97 ±1.29	0.53 #	-0.16 ±0.95	3.53 ±3.36	1.73 ±1.35	0.084 #	1.80 ±3.37

Notes: Ratios were estimated using total concentrations of epoxides and diols in RBCs. A, arterial blood; V, venous blood. A-V difference; arteriovenous difference. Mean±SD.

which are particularly vulnerable in hemodialysis. Our results indicate that RBCs could represent a reservoir for PUFA hydroxy metabolites, which on release may act at the blood-endothelial interface to affect cardiovascular responses in hemodialysis patients.

4.1. Arteriovenous difference

The arteriovenous oxygen difference is the difference in the oxygen content of blood in the arterial *vs.* the venous blood. Knowing this difference aids in clinical diagnostics of how much oxygen is taken up from the blood by the tissues. ESRD patients present before hemodialysis with high ammonia levels in circulating arterial blood with a significantly positive arteriovenous difference [19]. The arteriovenous blood sugar content results from the fact that the peripheral tissues, especially the muscles, either store or burn part of the glucose that passes through them [20]. Venous blood can be withdrawn from specific organs, e.g. upper and lower legs or the mesentery. In this case, measurement of oxygen consumption by the specific organ system requires determination of the oxygen content of venous blood draining that organ [19–22]. We applied this approach (i.e. determining AV differences in content of oxylipins in RBCs) to better understand biotransformation of oxylipins in RBCs *in vivo*, particularly whether or not the peripheral tissues, especially the muscles in the upper limbs, either produce, store or degrade part of the hydroxy- and epoxy-metabolites in RBCs that pass through them. We were interested on how hemodialysis treatment affects the arteriovenous difference of these erythro-metabolites.

4.2. LOX/CYP ω/(ω-1)-hydroxylase metabolites

We found that hemodialysis caused positive arteriovenous differences in a wide range of RBC LOX metabolites. The changes were particularly prominent in the free RBC compartment as compared with the total RBC compartment. The metabolites included 8-HETE, 9-HETE, 11-HETE, 12-HETE, 15-HETE, 20-HETE, 4-HDHA, 7-HDHA, 8-HDHA, 10-HDHA, 11-HDHA, 13-HDHA, 14-HDHA, 16-HDHA, 17-HDHA, 20-HDHA, 5-HEPE, 8-HEPE, 9-HEPE, 11-HEPE, 12-HEPE, 15-HEPE, 18-HEPE and 13-HODE. Little is known about the (patho)physiologial functions of those metabolites. HETEs are involved in many chronic diseases such as inflammation, cardiovascular disease, kidney disease, and cancer, for review see [23]. 20-HETE is pro-inflammatory and has largely detrimental functions, for example in hypertension, promoting systemic vasoconstriction [24], in neovascularization [25] and ischemia-reperfusion injury [26,27]. Up-regulation of 20-HETE contributes to oxidative stress, immune responses, endothelial dysfunction and peripheral vascular resistance [28]. 5-HETE and 15-HETE activate neutrophils and monocytes [29]. There is an accumulation of 15-HETE in human carotid plaques, and this is believed to play a role in the induction of atherothrombotic events by increasing platelet aggregation and thrombin generation [30]. 6-HETE inhibits neutrophil adhesion and high 19-HETE concentrations have been correlated with cardiovascular events [31]. 4-HDHA mediates antiangiogenic effects of n-3 PUFAs [32]. 5-HEPE promotes neutrophil chemotaxis and enhances the induction of regulatory T cells (Tregs) that modulate the immune system [33]. 13-HODE inhibits platelets [34] and plays a role in redox and inflammatory responses [35,36]. 18-HEPE is released by macrophages to inhibit cardiac fibrosis and inflammation [37]. 18-HEPE can down-regulate pro-inflammatory and pro-proliferative effects, possibly *via* conversion to E-series resolvins [32]. Furthermore, resolvin E1 and resolvin D1, a DHA metabolite synthesized via 17-HDHA [38], have been reported to switch macrophage polarity towards the M2 phenotype [39], which may explain effects of HDHAs in inflammation [40]. 9-HODE and 13-HODE are believed to be atherogenic through the induction of pro-inflammatory cytokines and by formation of foam cells from macrophages by activation of PPARs and other receptors [36]. Nonetheless, it is unknown whether RBCs are capable of liberating PUFA hydroxy metabolites into blood or tissues. Previous studies detected small changes in plasma levels of prostaglandin E2 (PGE2), prostaglandin F2a (PGF2a) [41,42] and 5/12-HETE [43] after hemodialysis. Our data demonstrate that LOX metabolites are exhausted (positive arteriovenous difference) in RBCs that pass through the peripheral tissues, specifically the muscles in the upper limbs, during hemodialysis. The mechanism by which CKD and hemodialysis raises the arteriovenous differences in the levels of those erythro-metabolites is not known. Since those metabolites can be synthesized endogenously by hemoglobin's (quasi-) pseudo-lipoxygenase activity [10,11], this mechanism may eventually have led to the changes of lipid hydroperoxides in the RBCs (Fig. 1). Alternatively, accelerated release into and uptake from plasma could be a possible explanation. The more pronounced changes observed in free metabolite levels within the RBCs, as compared with the total RBC compartment, indicate that free erythro-LOX products should be considered more dynamic or vulnerable in CKD and hemodialysis. Our data indicate that RBC-hydroxy metabolites are novel candidates potentially released in peripheral tissues in hemodialysis, which could have deleterious effects in the circulation. Nevertheless, the impact of those hydroxy metabolites has yet to be integrated into a (patho)physiological context.

4.3. EETs and other PUFA metabolites

RBCs have been proposed to serve as reservoir of EETs which on release may act in a vasoregulatory capacity [44,45]. In addition to serving as carriers of oxygen, RBCs can regulate the microvascular perfusion by production of adenosine triphosphate (ATP) and EETs *via* a mechanism involving the RBC membrane P2×7 receptor upon exposure to a low oxygen environment [45–47]. Erythro-EETs are produced by direct oxidation of arachidonic acid (AA) and the monooxygenase-like activity of hemoglobin [44,45,48]. On release, EETs and their diols (DHETs) are potent vasodilators [49,50]. Moreover, these products are pro-fibrinolytic and anti-inflammatory [44,45,48]. DHETs are pro-inflammatory in general and exhaustive exercise increases 5, 6-DHET levels in arterial blood [16]. In this study, we were unable to demonstrate that peripheral tissues, specifically the muscles in the upper limbs, either produce, store or degrade the EETs and other CYP epoxides before and after hemodialysis. Although, RBCs possess a soluble hydroxide hydrolase (sEH) [15,16], we were unable to detect biotransformation of EETs by erythro-sEH *in vivo,* i.e. in peripheral perfusion of upper limbs before and after hemodialysis. Together, the data indicate that hemodialysis does not influence RBCs in the peripheral circulation to liberate erythro-EETs/DHETs into the blood and/or tissues in ESRD patients. The data also argue against an important role of RBC sEH in the peripheral circulation *in vivo*. In line, we observed changes in only few erythro-CYP metabolites (10,11-EDP, 19,20-EDP, 8,9-EEQ, 11,12-EEQ,

14,14-EEQ and 14,15-DiHETE). These metabolites deserve further attention. EDPs and EEQs are bioactive [14,51–54] and exhibit anti-angiogenic [55], anti-fibrotic and anti-inflammatory cardiovascular effects [55,56].

5. Conclusions

Our results demonstrate profound arteriovenous differences in LOX pathway metabolites in RBCs after hemodialysis, including a wide range of HETEs, HDHAs and HEPEs. We detected more pronounced changes in free metabolites in RBCs after HD, as compared with the total RBC compartment. Hemodialysis treatment did not affect the majority of CYP and CYP ω/(ω-1)-hydroxylase products in RBCs. Our data indicate that erythro-metabolites of the LOX pathway are influenced by renal-replacement therapies, which could contribute to the cardiovascular risk. More research is needed to determine the contribution of RBC hydroxy- and epoxy-metabolites to cardiovascular morbidity and mortality.

CRediT authorship contribution statement

BG, MG and FCL planned and designed the experimental studies. TL, ID and MR performed the HPLC-MS spectrometry experiments. TL performed the statistics. All authors contributed to the implementation and analyses of the experiments. TL and BG drafted the article, and all authors, contributed to its completion.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.plefa.2022.102453.

References

[1] M. Cozzolino, et al., Cardiovascular disease in dialysis patients, Nephrol Dial Transplant 33 (suppl_3) (2018) iii28-iii34.
[2] A. Karaboyas, et al., Low hemoglobin at hemodialysis initiation: an international study of anemia management and mortality in the early dialysis period, Clin Kidney J 13 (3) (2020) 425–433.
[3] A. Besarab, et al., The effects of normal as compared with low hematocrit values in patients with cardiac disease who are receiving hemodialysis and epoetin, N Engl J Med 339 (9) (1998) 584–590.
[4] A.K. Singh, et al., Correction of anemia with epoetin alfa in chronic kidney disease, N Engl J Med 355 (20) (2006) 2085–2098.
[5] T.B. Drueke, et al., Normalization of hemoglobin level in patients with chronic kidney disease and anemia, N Engl J Med 355 (20) (2006) 2071–2084.
[6] M.A. Pfeffer, et al., A trial of darbepoetin alfa in type 2 diabetes and chronic kidney disease, N Engl J Med 361 (21) (2009) 2019–2032.
[7] K.U. Eckardt, et al., Safety and efficacy of vadadustat for anemia in patients undergoing dialysis, N Engl J Med 384 (17) (2021) 1601–1612.
[8] H. Jiang, et al., Hydrolysis of cis- and trans-epoxyeicosatrienoic acids by rat red blood cells, J Pharmacol Exp Ther 326 (1) (2008) 330–337.
[9] D.W. Starke, K.S. Blisard, J.J. Mieyal, Substrate specificity of the monooxygenase activity of hemoglobin, Mol Pharmacol 25 (3) (1984) 467–475.
[10] H. Kuhn, et al., Quasi-lipoxygenase activity of haemoglobin. A model for lipoxygenases, Eur J Biochem 120 (1) (1981) 161–168.
[11] C.G. Hover, A.P. Kulkarni, A simple and efficient method for hemoglobin removal from mammalian tissue cytosol by zinc sulfate and its application to the study of lipoxygenase, Prostaglandins Leukot Essent Fatty Acids 62 (2) (2000) 97–105.
[12] T. Nakamura, D.L. Bratton, R.C. Murphy, Analysis of epoxyeicosatrienoic and monohydroxyeicosatetraenoic acids esterified to phospholipids in human red blood cells by electrospray tandem mass spectrometry, J Mass Spectrom 32 (8) (1997) 888–896.
[13] J. McGee, F. Fitzpatrick, Enzymatic hydration of leukotriene A4. Purification and characterization of a novel epoxide hydrolase from human erythrocytes, J Biol Chem 260 (23) (1985) 12832–12837.
[14] B. Gollasch, et al., Hemodialysis and erythrocyte epoxy fatty acids, Physiol Rep 8 (20) (2020) e14601.
[15] R. Fischer, et al., Dietary omega-3 fatty acids modulate the eicosanoid profile in man primarily via the CYP-epoxygenase pathway, J Lipid Res 55 (6) (2014) 1150–1164.
[16] B. Gollasch, et al., Maximal exercise and plasma cytochrome P450 and lipoxygenase mediators: a lipidomics study, Physiol Rep 7 (13) (2019) e14165.
[17] B. Gollasch, et al., Maximal exercise and erythrocyte epoxy fatty acids: a lipidomics study, Physiol Rep 7 (22) (2019) e14275.
[18] B. Gollasch, et al., Effects of hemodialysis on plasma oxylipins, Physiol Rep 8 (12) (2020) e14447.
[19] B. Agroyannis, et al., Arteriovenous difference of blood ammonia in uremic patients under hemodialysis, Artif Organs 22 (8) (1998) 703–705.
[20] B.Y. Glassberg, The arteriovenous difference on blood sugar content, Arch Intern Med (Chic) 46 (4) (1930) 605–609.
[21] A. Konttinen, et al., Arteriovenous relationship of non-esterified fatty acids, triglycerides, cholesterol and phospholipids in exercise, Ann Med Exp Biol Fenn 40 (1962) 250–256.
[22] T. Ishibashi, et al., Arteriovenous differences in NO2- kinetics in anesthetized rabbits, Biol Pharm Bull 32 (3) (2009) 399–404.
[23] M. Gabbs, et al., Advances in our understanding of oxylipins derived from dietary PUFAs, Adv Nutr 6 (5) (2015) 513–540.
[24] S.L. Hoopes, et al., Vascular actions of 20-HETE, Prostaglandins Other Lipid Mediat 120 (2015) 9–16.
[25] L. Chen, et al., CYP4A/20-HETE regulates ischemia-induced neovascularization via its actions on endothelial progenitor and preexisting endothelial cells, Am J Physiol Heart Circ Physiol 316 (6) (2019) H1468–H1479.
[26] U. Hoff, et al., Inhibition of 20-HETE synthesis and action protects the kidney from ischemia/reperfusion injury, Kidney Int 79 (1) (2011) 57–65.
[27] Y. Han, et al., 20-Hydroxyeicosatetraenoic acid mediates isolated heart ischemia/reperfusion injury by increasing NADPH oxidase-derived reactive oxygen species production, Circ J 77 (7) (2013) 1807–1816.
[28] M. Waldman, et al., The role of 20-HETE in cardiovascular diseases and its risk factors, Prostaglandins Other Lipid Mediat 125 (2016) 108–117.
[29] R.J. Smith, et al., Arachidonic acid and 15(S)-hydroxy-5,8,11-cis-13-trans-eicosatetraenoic acid modulate human polymorphonuclear neutrophil activation by monocyte derived neutrophil activating factor, Biochem Biophys Res Commun 148 (2) (1987) 636–645.
[30] C. Vijil, et al., Arachidonate 15-lipoxygenase enzyme products increase platelet aggregation and thrombin generation, PLoS One 9 (2) (2014) e88546.
[31] C.C. Huang, et al., Association of arachidonic acid-derived lipid mediators with subsequent onset of acute myocardial infarction in patients with coronary artery disease, Sci Rep 10 (1) (2020) 8105.
[32] P. Sapieha, et al., 5-Lipoxygenase metabolite 4-HDHA is a mediator of the antiangiogenic effect of omega-3 polyunsaturated fatty acids, Sci Transl Med 3 (69) (2011) 69ra12.
[33] J.R. Heidel, et al., In vivo chemotaxis of bovine neutrophils induced by 5-lipoxygenase metabolites of arachidonic and eicosapentaenoic acid, Am J Pathol 134 (3) (1989) 671–676.
[34] M.R. Buchanan, et al., 13-Hydroxyoctadecadienoic acid is the vessel wall chemorepellant factor, LOX, J Biol Chem 260 (30) (1985) 16056–16059.
[35] A. Pecorelli, et al., 13-HODE, 9-HODE and ALOX15 as potential players in Rett syndrome OxInflammation, Free Radic Biol Med 134 (2019) 598–603.
[36] V. Vangaveti, et al., 9- and 13-HODE regulate fatty acid binding protein-4 in human macrophages, but does not involve HODE/GPR132 axis in PPAR-gamma regulation of FABP4, Ther Adv Endocrinol Metab 9 (5) (2018) 137–150.
[37] J. Endo, et al., 18-HEPE, an n-3 fatty acid metabolite released by macrophages, prevents pressure overload-induced maladaptive cardiac remodeling, J Exp Med 211 (8) (2014) 1673–1687.
[38] P.B. Derogis, et al., The development of a specific and sensitive LC-MS-based method for the detection and quantification of hydroperoxy- and hydroxydocosahexaenoic acids as a tool for lipidomic analysis, PLoS One 8 (10) (2013) e77561.
[39] M. Herova, et al., ChemR23, the receptor for chemerin and resolvin E1, is expressed and functional on M1 but not on M2 macrophages, J Immunol 194 (5) (2015) 2330–2337.
[40] O. Kuda, et al., Docosahexaenoic Acid-Derived Fatty Acid Esters of Hydroxy Fatty Acids (FAHFAs) With Anti-inflammatory Properties, Diabetes 65 (9) (2016) 2580–2590.
[41] G. Schultze, et al., Prostaglandin E2 promotes hypotension on low-sodium hemodialysis, Nephron 37 (4) (1984) 250–256.

[42] G. Losonczy, et al., Changes of arterial prostaglandin E2 during haemodialysis, Nephrol Dial Transplant 5 (11) (1990) 937–941.
[43] B. Dolegowska, et al., Presence of glucose in dialyzing fluid and synthesis of selected lipoxygenase-derived eicosanoids during hemodialysis, Int Urol Nephrol 44 (6) (2012) 1799–1804.
[44] H. Jiang, et al., Increases in plasma trans-EETs and blood pressure reduction in spontaneously hypertensive rats, Am J Physiol Heart Circ Physiol 300 (6) (2011) H1990–H1996.
[45] H. Jiang, G.D. Anderson, J.C. McGiff, Red blood cells (RBCs), epoxyeicosatrienoic acids (EETs) and adenosine triphosphate (ATP), Pharmacol Rep 62 (3) (2010) 468–474.
[46] H. Jiang, et al., Stimulation of rat erythrocyte P2×7 receptor induces the release of epoxyeicosatrienoic acids, Br J Pharmacol 151 (7) (2007) 1033–1040.
[47] R.S. Sprague, et al., Divergent effects of low-O(2) tension and iloprost on ATP release from erythrocytes of humans with type 2 diabetes: implications for O(2) supply to skeletal muscle, Am J Physiol Heart Circ Physiol 299 (2) (2010) H566–H573.
[48] H. Jiang, G.D. Anderson, J.C. McGiff, The red blood cell participates in regulation of the circulation by producing and releasing epoxyeicosatrienoic acids, Prostaglandins Other Lipid Mediat 98 (3-4) (2012) 91–93.
[49] H.C. Hercule, et al., Interaction between P450 eicosanoids and nitric oxide in the control of arterial tone in mice, Arterioscler Thromb Vasc Biol 29 (1) (2009) 54–60.
[50] T. Lu, et al., Dihydroxyeicosatrienoic acids are potent activators of Ca(2+)-activated K(+) channels in isolated rat coronary arterial myocytes, J Physiol 534 (Pt 3) (2001) 651–667.
[51] H.C. Hercule, et al., The vasodilator 17,18-epoxyeicosatetraenoic acid targets the pore-forming BK alpha channel subunit in rodents, Exp Physiol 92 (6) (2007) 1067–1076.
[52] B. Lauterbach, et al., Cytochrome P450-dependent eicosapentaenoic acid metabolites are novel BK channel activators, Hypertension 39 (2 Pt 2) (2002) 609–613.
[53] A. Ulu, et al., An omega-3 epoxide of docosahexaenoic acid lowers blood pressure in angiotensin-II-dependent hypertension, J Cardiovasc Pharmacol 64 (1) (2014) 87–99.
[54] C. Morin, S. Fortin, E. Rousseau, 19,20-EpDPE, a bioactive CYP450 metabolite of DHA monoacyglyceride, decreases Ca(2)(+) sensitivity in human pulmonary arteries, Am J Physiol Heart Circ Physiol 301 (4) (2011) H1311–H1318.
[55] D.R. McDougle, et al., Anti-inflammatory omega-3 endocannabinoid epoxides, Proc Natl Acad Sci U S A 114 (30) (2017) E6034–E6043.
[56] A. Sharma, et al., Novel omega-3 fatty acid epoxygenase metabolite reduces kidney fibrosis, Int J Mol Sci 17 (5) (2016).