Aus der Klinik für Nephrologie und Intensivmedizin
der Medizinischen Fakultät Charité – Universitätsmedizin Berlin

DISSERTATION

Biotransformation of blood long-chain fatty acids and oxylipins in peripheral tissue during hemodialysis

Biotransformation von langkettigen Fettsäuren im Blut und Oxylipinen in peripheren Geweben während der Hämodialyse

zur Erlangung des akademischen Grades
Doctor medicinae (Dr. med.)

vorgelegt der Medizinischen Fakultät
Charité – Universitätsmedizin Berlin

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Datum der Promotion: 25.06.2023

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List of abbreviations

GBD	Global Burden of Disease
CVD	Cardiovascular disease
AV	Arterio-venous
CKD	Chronic kidney disease
ESRD	End-stage renal disease
HD	Hemodialysis
RAS	Renin-angiotensin System
AA	Arachidonic acid
COX	Cyclooxygenase
EDTA	Ethylenediaminetetra-acetic acid
FFA	Free fatty acid
HETE	Hydroxyeicosatetraenoic acid
LOX	Lipoxygenase
PUFAs	Polyunsaturated fatty acids
CYP	Cytochrome P450
EDPs	Epoxydocosapentaenoic acids
EEQs	Epoxyeicosatetraenoic acids
EETs	Epoxyeicosatrienoic acids
EpOMEs	Epoxyoctadecenoic acids
HDHA	Hydroxydocosahexaenoic acid
HEPE	Hydroxyeicosapentaenoic acid
HODE	Hydroxyoctadecadienoic acid
LOX	Lipoxygenase
sEH	Soluble epoxide hydrolase
EPA	Eicosapentaenoic acid
LA	Linolenic acid
DHA	Docosahexaenoic acid
DGLA	Dihomo-γ-linoleic acid

POA	Palmitoleic acid
PA	Palmitic acid
OA	Oleic acid
ALA	α-linoleic acid
SD	Standard deviation
PPAR	Peroxisome proliferator-activated receptor
Hb	Hemoglobin
DHETs	Dihydroxyeicosatrienoic acids
DiHOMEs	Dihydroxyctadecenoic acids
DiHDPAs	Dihydroxydocosapentaenoic acids
DiHETEs	Dihydroxyeicosatetraenoic acids
VLDL	Very low-density lipoproteins
LPS	Lipopolysaccharide
NaOH	Sodium hydroxide
SPE	Solid phase extraction
HPLC-MS/MS	High-performance liquid chromatography mass spectrometry
BKca	Calcium-activated potassium
PRRs	Pattern recognition receptors
LCFAs	Long-chain fatty acids
MUFAs	Monounsaturated fatty acids
SFAs	Saturated fatty acids
BHT	Butylated hydroxytoluene
ISTD	Internal standard
IQR	Interquartile range
Δ6D	Delta-6-desaturase
Δ5D	Delta-5-desaturase
Δ9D	Delta-9-desaturase
PD	Peritoneal dialysis
CRP	C-reactive protein
LDL	Low density lipoprotein

RCT	Randomized clinical trials
AKI	Acute kidney injury
CRP	C-reactive protein
I/R	Ischemia-reperfusion injury
BK_{Ca}	Calcium-dependent potassium channels
DN	Diabetic nephropathy

Abstract

Background: Cardiovascular disease (CVD) is the most common lethal complications in patients with end-stage renal disease (ESRD) undergoing hemodialysis (HD). The role of long-chain fatty acids (LCFAs) and their oxylipin derivatives is poorly understood in human cardiac and renal diseases. Based on previous scientific findings, there is evidence that many substance profiles of blood LCFAs and their oxylipin derivatives are altered in dialysis patients and influenced by extracorporeal circulation. In order to provide novel insights into biotransformation and/or bioaccumulation of these metabolites in peripheral tissue, we conducted a targeted lipidomic study and tested the hypothesis that arterio–venous (A–V) differences in blood LCFAs and oxylipins are present in vivo and sensitive to single HD treatment. **Methods:** We took arterial and venous blood samples specimens from ESRD patients (n=12) before and after HD and determined LCFAs and its derived epoxy and hydroxy metabolites in plasma and erythrocytes by high-performance liquid chromatography with mass spectrometry coupling (HPLC-MS). **Results**: Firstly, we found that the total amount of numerous saturated fatty acids (SFAs), monounsaturated fatty acids (MUFAs), and polyunsaturated fatty acids (PUFAs) in erythrocytes showed negative arteriovenous (AV) differences before HD, which disappeared after HD. The omega-3 index in erythrocytes did not show arteriovenous differences before and after HD. No significant findings were observed in LCFAs in plasma. Beyond that, all CYP epoxy metabolites in the plasma showed negative arteriovenous differences, mainly due to their significantly elevated levels in venous blood after dialysis. No changes were observed in LOX and LOX/CYP ω/(ω-1)-hydroxylase metabolites in the plasma before and after dialysis. This variation in epoxide metabolites may be attributed to a decrease in soluble epoxide hydrolase (sEH) activity. **Conclusions**: Our findings indicate that AV differences in LCFAs are present and active in mature red blood cells (RBCs) and that their bioaccumulation is susceptible to a single HD therapy [1]. HD treatment changes CYP epoxy metabolites from PUFAs in the plasma (accumulation), which may have deleterious effects on the circulation [2].

Zusammenfassung

Hintergrund: Herz-Kreislauf-Erkrankungen (CVD) sind eine der häufigsten tödlichen Komplikationen bei Patienten mit terminaler Niereninsuffizienz (ESRD), die sich einer Hämodialyse (HD) unterziehen. Die Rolle der langkettigen Fettsäuren (LCFAs) und ihrer Oxylipinderivate bei Herz- und Nierenerkrankungen des Menschen ist nur unzureichend geklärt. Ausgehend von früheren wissenschaftlichen Erkenntnissen gibt es Hinweise darauf, dass viele Substanzprofile der langkettigen Fettsäuren und ihrer Oxylipinderivate im Blut von Dialysepatienten Veränderungen aufweisen und durch den extrakorporalen Kreislauf beeinflusst werden. Um neue Erkenntnisse über die Biotransformation und/oder Bioakkumulation dieser Metaboliten im peripheren Gewebe zu gewinnen, haben wir eine gezielte Lipidomstudie durchgeführt und die Hypothese getestet, ob arterio-venöse (A-V) Unterschiede in den LCFAs und Oxylipinen im Blut in vivo vorherrschen, und ob sie auf eine einzelne HD-Behandlung reagieren. **Methoden:** Wir haben arterielle und venöse Blutproben von Patienten mit terminaler Niereninsuffizienz (n=12) vor und nach der Hämodialyse entnommen und LCFAs und davon abgeleitete Epoxy- und Hydroxy-Metaboliten im Plasma und in den Erythrozyten durch Hochleistungsflüssigkeitschromatographie mit Massenspektrometrie-Kopplung (HPLC-MS) bestimmt. **Ergebnisse:** Erstens stellten wir fest, dass die Gesamtmenge zahlreicher gesättigter Fettsäuren (SFAs), einfach ungesättigter Fettsäuren (MUFAs) und mehrfach ungesättigter Fettsäuren (PUFAs) in den Erythrozyten vor der HD negative arteriovenöse Unterschiede aufwiesen, die nach der HD verschwanden. Der Omega-3-Index in den Erythrozyten wies vor und nach der HD keine arteriovenösen Unterschiede auf. Bei den LCFAs im Plasma wurden keine signifikanten Ergebnisse beobachtet. Darüber hinaus wiesen alle CYP-Epoxid-Metaboliten im Plasma negative arteriovenöse Unterschiede auf, was hauptsächlich auf ihre signifikant erhöhten Werte im venösen Blut nach der Dialyse zurückzuführen ist. Bei den LOX- und LOX/CYP ω/(ω-1)-Hydroxylase-Metaboliten im Plasma wurden vor und nach der Dialyse keine Veränderungen beobachtet. Diese Veränderung der Epoxidmetaboliten kann auf eine Abnahme der Aktivität der löslichen Epoxidhydrolase (sEH) zurückgeführt werden. **Schlussfolgerungen:** Unsere Ergebnisse deuten darauf hin, dass A-V Unterschiede in LCFAs in reifen roten Blutkörperchen vorhanden und aktiv sind und dass ihre Bioakkumulation für eine einmalige HD Therapie anfällig ist [1]. Die HD Behandlung

verändert die CYP-Epoxy-Metaboliten von PUFAs im Plasma (Akkumulation), was sich nachteilig auf den Kreislauf auswirken kann [2].

1 Introduction

1.1 Current status of chronic kidney disease

1.1.1 Epidemiological survey of chronic kidney disease

The Global Burden of Disease (GBD) analysis demonstrated that 1.2 million people died of chronic kidney disease (CKD) worldwide in 2017 [3]. Between 1990 and 2017, the CKD mortality rate increased by 41.5%, while the prevalence rate increased by 29.3% [3]. Numerous factors contribute to the accelerated progression of CKD, including hyperglycemia, uncontrolled hypertension, and increased albuminuria [4]. Beyond that, CKD and cardiovascular disease (CVD) are risk factors for each other [5]. CKD is an important risk factor for CVD and all-cause mortality [6]. Correspondingly, when the disease progresses to end-stage renal disease (ESRD), hemodialysis (HD) is the primary option for most patients with CKD to prolong their lives. Conversely, most patients who undergo HD die from cardiovascular disease (CVD), which accounts for nearly 48% of total mortality [7].

1.1.2 Hemodialysis treatment

Many researchers worldwide have worked for decades to reduce CVD incidence during HD. Numerous studies have implicated that HD plays a role in hemodynamic instability, endothelial dysfunction, renin-angiotensin system (RAS) abnormalities, vascular calcification, and protein-energy metabolism irregularities, all of which may contribute to the development and progression of CVD [8]. For decades, clinicians have made tremendous efforts to optimize dialysis prescription, manage medications and diet, and work to decrease dialysis-associated adverse events in HD patients. Disappointingly, this has had little effect in mitigating dialysis-associated CVD events [9] [10]. In recent years, the role of lipids and their oxidative metabolites in dialysis has been proposed to uncover further the leading causes of CVD development and progression to improve the survival of dialysis patients [11] [12]. So far, numerous researches have shown that dialysis treatment affects lipid metabolites in blood and individual tissues and organs, especially epoxide metabolites derived from cytochrome P450 (CYP 450) [13] [14].

1.2 The role of long-chain fatty acids and their metabolites in the ESRD

Long-chain fatty acids (LCFAs) are recognized as one of the major organic compounds in biological organisms. They form the skeletal structure of cell membranes and are key substances for energy storage and the reception and transmission of signal molecules in the organism [15]. As far as current studies can concern, the most important LCFAs in the human body that maintain basic life activities and take part in disease onset, development, and regression are monounsaturated FAs (MUFAs), saturated FAs (SFAs), and polyunsaturated fatty acids (PUFAs), notably in the cardiovascular, neurological, and kidney diseases [16] [17].

Recent studies [18] have revealed that exogenous supplementation of MUFAs can inhibit non-programmed cell death caused by membrane lipid oxidation by promoting an ferroptosis-resistant cell state. It appears that the level and source of MUFAs are critical for maintaining homeostasis in the organism. Reducing dietary SFAs intake is thought to be associated with a reduced risk of atherosclerosis and CVD, perhaps by activating multiple pattern recognition receptors (PRRs) to induce inflammatory cytokine expression [19]. Abnormal levels and distribution of SFAs in serum have also been presented in ESRD patients, but the exact action mode remains controversial [20]. Historically, numerous researchers have noted that dietary PUFAs, primarily n-3 PUFAs, maybe the best alternative to SFAs since they are commonly believed to control cardiovascular risk events with a dietary supplement of n-3 PUFAs [21]. Whereas, several large-scale randomized clinical trials (RCT) recently revealed that combined supplementation with DHA-EPA in humans with high cardiovascular risk did not capture a substantial CVD benefit, while supplementation with purified EPA only was related to a lower CVD risk [22] [23] [24] [25].

Besides, the role of epoxy and hydroxy metabolites of PUFA have been described in many animal and human experiments. Although there is some controversy, their critical functions at the physiological and pathological levels are noteworthy. Linolenic acid (LA), arachidonic acid (AA), docosahexaenoic acid (DHA), and eicosapentaenoic acid (EPA) are the predominant n-6 and n-3 PUFAs in the body, which are metabolized by CYP450 to epoxy metabolites (epoxyoctadecenoic acids (EpOMEs), epoxyeicosatrienoic acids (EETs), epoxydocosapentaenoic acids (EDPs) and epoxyeicosatetraenoic acids (EEQs)), by LOX/ CYP ω/(ω-1)-hydroxylase to hydroxy

metabolites (hydroxyoctadecadienoic acid (HODEs), hydroxyeicosatetraenoic acids (HETEs), hydroxydocosahexaenoic acids (HDHA) and hydroxyeicosapentaenoic acids (HEPEs)), and by COX to prostaglandins and thromboxanes, etc. Among them, AA-derived oxylipins, mainly EETs, are engaged in crucial cellular processes such as apoptosis, metabolism, inflammatory processes, and regulation of endothelial cell function [26] [27]. EDPs are physiologically active metabolites of DHA that protect cardiac cells by improving and maintaining mitochondrial quality against lipopolysaccharide (LPS)-induced cell damage, as shown in a recent research [28]. 17,18-EEQ reduces endothelial cell activity and prevents the development of atherosclerosis in mice [29]. Additionally, most oxylipins biology is still unidentified, particularly those generated from the LOX/CYP ω/(ω-1) pathway. In general, soluble epoxide hydrolases (sEH) hydrolyze epoxide metabolites to less biologically active diols [30]. Alternatively, oxylipins can be esterified and incorporated into cell membrane phospholipids for temporary storage, and when the body is stimulated, these esterified oxylipins would be hydrolyzed to free oxylipins to participate in stress reaction [31]. Lipoprotein-bound oxylipins are another primary source of circulating esterified oxylipins, such as very low density lipoproteins (VLDL), which can bind to lipoprotein lipases on the cell membrane surface and drive cellular uptake on oxylipins, thereby affecting angiogenesis, mitosis, apoptosis, and peroxisome proliferator-activated receptor (PPAR)-activated gene expression [32]. In any case, esterification is essential for eliminating free oxylipins signaling and establishing the direct function of esterified oxylipins, but its exact mechanism of action is still in the exploratory stage, making it necessary to study these novel biomarkers. There is data that oxylipin profiles discriminate ESRD patients from normal controls and are influenced by renal replacement therapies [13]. In particular, all four subclasses of CYP epoxy metabolites are increased after the dialysis treatment in circulating arterial blood. Rather than resulting from altered sEH activity, the oxylipins were released and accumulated in the circulation. However, hemodialysis did not change the majority of LOX/CYP ω/(ω-1)-hydroxylase metabolites [13]. We also found that various CYP 450 epoxides and LOX/CYP ω/(ω-1)-hydroxylase products are increased in red blood cells of ESRD patients, compared to control subjects [33]. These products included metabolites of the following subclasses: EEQs, dihydroxyeicosatrienoic acids (DHETs), dihydroxydocosapentaenoic acids (DiHDPAs) and HETEs [33]. Furthermore, hemodialysis treatment did not affect most of the metabolites. However, interpretation of

data was difficult because there is no data on biotransformation of these lipid mediators in vivo, especially in terms of production, storage, or metabolism in upper limb muscle tissues.

1.3 Arteriovenous differences

It is well documented that arterial blood carries enormous amounts of nutrients and oxygen to peripheral tissues and organs, followed by substance exchange via capillaries, and ultimately by delivering metabolic wastes from peripheral tissues and organs to the venous system. Clinicians can determine the regional oxygenation status of specific tissues and organs by monitoring the arteriovenous oxygen difference [34]. Arteriovenous carbon dioxide differences can provide insight into the metabolism of the relevant tissues or organs [35]. There was a significant positive arteriovenous difference in plasma ammonia levels before HD in uremic patients, which disappeared after HD [36]. Similarly, based on the pathological or physiological state of the patient, non-esterified LCFAs may display positive (consumption) or negative (accumulation) A-V differences [37]. Besides that, the current study revealed arteriovenous differences in other laboratory indicators such as blood glucose, amino acids, nitric oxide, lactate, and catecholamines [38] [39] [40]. Our previous studies demonstrated the alterations of HD treatment on the LCFAs status and their oxidative metabolites in the arterial blood of ESRD patients [33] [41]. Nevertheless, it is unclear how HD treatment affects the bioaccumulation and/or biotransformation of LCFAs and oxylipins in vivo in peripheral tissues, particularly in upper limb muscles. Whether LCFAs and oxylipins in the blood are produced, degraded or stored during this period as they pass through the arteries into peripheral tissues and organs and then back into the venous circulatory system from the venous end of the capillaries [1] [2]. Hence, to better understand the biotransformation of these lipid mediators in ESRD patients treated with HD, we utilized large-scale lipidomics for the first time to measure the AV differences of LCFAs and their oxidative metabolites [1] [2].

1.4 Aims and Hypotheses

This study aimed to analyze the differences in arterial and venous levels of lipid mediators in ESRD patients before and after HD to see if and how the biotransformation of these lipid mediators is altered, especially in terms of production, storage, or

metabolism in upper limb muscle tissues. This will help us further understand whether changes in metabolites are responsible for the high incidence of CVD in ESRD patients undergoing HD and thus guide clinical treatment in the future.

Hypothesis #1:

We tested the hypothesis that hemodialysis would influence the arteriovenous differences LCFA metabolites of plasma and erythrocytes [1].

Hypothesis #2:

We examined the hypothesis that hemodialysis would influence the arteriovenous differences in plasma oxylipins [2].

2 Methods

2.1 Participants of the project

A total of nine men and three non-pregnant women were recruited, all of whom were diagnosed with ESRD and were receiving regular hemodialysis treatment three times a week. The main inclusion criteria were: i) stable hemodialysis treatment prescription; ii) dialysis is performed through a native fistula or gore-tex graft; iii) age over 18 years old. Exclusion criteria included: i) hemoglobin (Hb) levels below 8.0 g/dL; ii) active infection; iii) high hemodynamic fluctuations; iv) lack of strict compliance with hemodialysis prescriptions. The Ethics Institutional Review Board of the Charité University Medicine endorsed the study, and all participants signed an informed consent form prior to enrollment. The study was duly registered: (ClinicalTrials. gov, Identifier: NCT03857984) [1] [2].

2.2 Project Design

All subjects were treated with a Polyflux 170H dialyzer (PAES membrane, Gambro), and the ultrafiltration rate was maintained during the hemodialysis process. The treatment parameters were consistent for all patients during dialysis, i.e., the blood flow of 250 ml/min, dialysate flow of 500 ml/min, double-needle puncture technique, dialysis time of 4 h 15 min, and temperature. Arterial blood samples are captured on the fistula arm before the start of dialysis (pre-HD) and 5-15 minutes before the end of dialysis (post-HD). Peripheral venous blood samples were drawn by subcutaneous venipuncture

in the ipsilateral upper limb at the same time points [1] [2]. Results were compared with our previous findings [33].

2.3 Sample assessment

Clinically standardized parameters (body mass index, comorbidities) were recorded in a standardized manner during the research visit. Glucose, lipoproteins, and triglycerides were assessed in a standardized clinical laboratory using established procedures. All collected blood samples were immediately centrifuged at high speed, and whole blood samples were separated into plasma and red blood cells and stored separately in a freezer at -80° C for subsequent fatty acids and eicosanoids analysis [1] [2].

2.4 Sample pre-processing

To measure LCFAs, we first take 30μL of plasma or 30μg of erythrocytes and mix it with 100μL of distilled water. Total lipids were extracted from plasma or erythrocytes with 500 μL methanol containing 0.01% butylated hydroxytoluene (BHT). 300 μL of 10M sodium hydroxide (NaOH) was added to the lipid extract, and the mixture was alkaline hydrolyzed at 80°C for 2 h. Subsequently, 400 μL of 58% acetic acid was added to the hydrolyzed solution to neutralize the pH. 50 μL of the above mixture was taken into a new vial and mixed with 450 μL of methanol and 10 μL of internal standard (ISTD) for further high-performance liquid chromatography-mass spectrometry (HPLC-MS/MS) analysis, as described previously [1].

To measure total plasma and erythrocytes eicosanoids, we mixed 200 μL of plasma or 200μg of erythrocytes with 300 μL of distilled water, 5 μL of BHT, 10 μL of ISTD consisting of 15(S)-HETE-d8 (5 ng) and ± 8,9-EET-d11 (5 ng) and 1.5 mL of acetonitrile. The above mixture was then performed alkaline hydrolysis with NaOH. Subsequently, the pH of the hydrolyzed sample was regulated to 6 for solid-phase extraction (SPE), as previously described [2] [42] [43]. For measuring free plasma and erythrocytes eicosanoids, alkaline hydrolysis of the plasma sample is not required, and the supernatant of the centrifuged sample is used directly for SPE metabolite extraction [2] [33].

2.5 Extraction of eicosanoid profiles

The samples were extracted with a Varian Bond Elut Certify II column. The column was first pretreated with 3 mL of methanol. The column was then pretreated twice with 0.1 mol/L of phosphate buffer (pH=6) containing 5% methanol, 3 mL each time. Subsequently, the supernatant from the above treatment was loaded into the pretreated column for extraction. The column was washed with 3 ml of methanol/water (1:1, v/v) after spiking. Following this, the organic extracts were allowed to evaporate to dryness under negative pressure. The eicosanoids were further eluted with 2 ml of hexane/ethyl acetate (75:25, v/v) with 1% acetic acid. The eluate was vaporized to dryness on a heater at 40°C under a nitrogen flow, and then 100 µL of acetonitrile/water (60:40) was applied to dissolve the residue for detection by HPLC-MS/MS, as described previously [43] [2].

2.6 Parameter characteristics of LCFAs and eicosanoids

In total, we measured 21 LCFAs in plasma and erythrocytes, primarily consisting of SFAs, MUFAs, and PUFAs. To better understand the experimental results, we utilized γ-linoleic acid (C18:3 n-6) / linoleic acid (LA) (C18:2 n-6), AA (C20:4 n-6) / dihomo-γ-linoleic acid (DGLA) (C20:3 n-6), palmitoleic acid (POA) (C16:1 n-7) / palmitic acid (PA) (C16:0), oleic acid (OA) (C18:1 n-9) / stearic acid (C18:0), and DHA / DPA, to represent delta-6 desaturase (∆6D), delta-5 desaturase (∆5D), delta-9 desaturase (∆9D) and peroxisome functions, respectively [44] [11] [45] [46]. The n-3/n-6 and omega-3 quotients were calculated to reflect the relative proportions of n-3 and n-6 PUFAs [14] [47]. We also calculated the diols/epoxy metabolites ratios representing sEH activity, e.g., DiHOMEs/EpOMEs, DHETs/EETs, DiHDPA/EDPs, and DiHETEs/EEQs, as described previously [1] [2]. This approach has been developed in our previous study [33] for usage in the present study.

2.7 Statistical Analysis

Descriptive statistics were acquired, and the variables were checked for normality and chi-square to clarify whether the data conformed to a normal distribution. Paired t-tests were performed to compare pre-HD and post-HD values if the data fulfilled the conditions of both normality and chi-squareness. Otherwise, paired Wilcoxon tests were

run. A p-value less than 0.05 was regarded as statistically significant. Data that conformed to normal distribution were presented as mean ± standard deviation (SD). Otherwise, median and interquartile range (IQR) were presented. SPSS Statistics software was used to execute the statistical analysis (IBM Corporation, Armonk, NY, USA) [1] [2].

3. Results

3.1 Biotransformation of blood LCFAs in HD patients

3.1.1 Changes in plasma LCFAs during a single HD session

In the study on LCFAs, we first examined the AV differences in individual LCFAs in plasma before and after dialysis. Only docosanoic acid (C22:0) and lignocerine acid (C24:0) had statistically significant negative AV differences before HD, which disappeared after HD. Likewise, we observed no AV differences in total SFAs, MUFAs, and PUFAs in plasma before and after HD. Consistently, there were no AV differences in response to overall and individual n-3 and n-6 PUFA proportions, i.e., n-3/n-6, omega-3 quotient, EPA/AA, DHA/AA, and DHA/EPA, either before or after HD. ∆6D, ∆5D, ∆9D, and peroxisome functions are vital enzymes in FA metabolism [48], and parameters representing these desaturase activities did not display statistically significant differences before and after HD, i.e., C18:3 n-6 / C18:2 n-6, C20:4 n-6 / C20:3 n-6, C16:1 n-7 / C16:0, C18:1 n-9 / C18:0, and DHA/DPA ratios [1].

3.1.2 Changes in erythrocyte LCFAs during a single HD session

Secondly, we explored the role of HD on LCFAs biotransformation in erythrocytes. Surprisingly, C16:0, C18:2 n-6, C18:3 n-3 alpha, C20:1 n-9, C20:3 n-6, C20:4 n-6, C22:0, C22:1 n-9, C22:5 n-6 and C24:0 in erythrocytes all displayed negative AV differences before HD. Correspondingly, total SFAs, MUFAs, and n-6 PUFAs showed negative AV differences before HD. This was due to C16:0, C22:0, and C24:0 responding to total SFA change, C20:1 n-9, and C22:1 n-9 participating in total MUFA change, and C18:2 n-6, C20:3 n-6, C20:4 n-6, and C22:5 n-6 contributing to total n-6 PUFA change. However, HD made all the differences disappear. In keeping with the findings in plasma, the individual parameters in erythrocytes, n-3/n-6 ratio, omega-3 quotient, EPA/AA, DHA/AA, DHA/EPA ratios, C18:3 n-6/C18:2 n-6, C20:4 n-6 /C20:3 n-

6, C16:1 n-7/C16: 0, C18:1 n-9/C18:0, and DHA/DPA ratios, all remained unchanged before and after HD, i.e., no AV differences [1].

3.2 Biotransformation of epoxy and hydroxy metabolites derived from PUFAs during HD

In the study on plasma oxylipins, we first investigated the impact of HD on individual CYP epoxy and hydroxy metabolites derived from PUFAs. We observed only positive AV differences in 11-HETE and 13-HODE before HD. Excitingly, HD prompted an increase in all epoxy metabolites in the veins, showing significant negative AV differences, i.e., 9, 10/12, 13-EpOME, 5, 6/8, 9/11, 12/14, 15-EET, 5, 6/8, 9/11, 12/14, 15/17, 18-EEQ, and 7, 8/10, 11/13, 14/16, 17/19, 20-EDP after HD. In contrast, the LOX/CYP ω/(ω-1)-hydroxylase metabolites derived from PUFAs stayed unchanged before and after HD. As ESRD may lead to the rapid degradation of CYP epoxide metabolites to their diols, we next analyzed the sum of individual CYP epoxide metabolites and their corresponding diols, namely EETs+DHETs, EpOMEs+DiHOMEs, EEQs+DiHETEs, and EDPs+DiHDPAs, separately. We discovered that the sums were not different in arteries and veins in ESRD patients before HD, but presented a significant negative AV difference after HD, i.e., HD accumulated the epoxy metabolites and their diols in veins. Taken together, these findings demonstrate that hemodialysis treatment contributes to the onset of negative arteriovenous differences in CYP metabolites in plasma after HD by increasing all four CYP eicosanoids in the venous blood [2].

3.3 Hydrolysis of epoxy metabolites by circulating sEH during HD

CYP epoxy metabolites, such as EpOMEs, EETs, EEQs, and EDPs, are among the major substrates of sEH that can metabolize these epoxides into less biologically active or even toxic acting vicinal diols, such as dihydroxyctadecenoic acids (DiHOMEs), DHETs, dihydroxyeicosatetraenoic acids (DiHETEs), and DiHDPAs [49]. To elucidate the possible mechanisms of negative AV differences in epoxy metabolites in plasma after HD, we calculated the diols/epoxide metabolites ratios of all four class CYP eicosanoids. We found no arteriovenous differences in either individual or total DiHOMEs/EpOMEs, DHETs/EETs, DiHETEs/EEQs, and EDPs/DiHDPAs ratios before HD. Notably, these ratios showed a significant positive AV difference after HD, implying

that HD reduced the all diol/epoxide metabolite ratios, which was more obvious in the venous blood. Overall, reduced sEH activity may be responsible for the accumulation of plasma CYP epoxy metabolites in peripheral tissues during HD, especially in upper limb muscle [2].

3.4 Biotransformation of free plasma oxylipins during HD

Finally, the major free plasma CYP epoxy and LOX/CYP ω /(ω -1)-hydroxy metabolites did not present AV differences after HD. This finding implies that HD therapy has a more substantial effect on total plasma oxylipins, particularly total CYP epoxy metabolites, than on free plasma metabolites. In other words, the effect of HD on total plasma oxylipins is less likely to be caused by free plasma oxylipins, and it is more appropriate to consider the contribution of esterified oxylipins to this effect [2].

For detailed presentation of the results, please, see:

Liu T, Dogan I, Rothe M, Reichardt J, Knauf F, Gollasch M, Luft FC, Gollasch B. Bioaccumulation of Blood Long-Chain Fatty Acids during Hemodialysis. Metabolites. 2022;12(3):269.

Liu T, Dogan I, Rothe M, Kunz JV, Knauf F, Gollasch M, Luft FC, Gollasch B. Hemodialysis and Plasma Oxylipin Biotransformation in Peripheral Tissue. Metabolites. 2022; 12(1):34.

Gollasch B, Wu G, **Liu T**, Dogan I, Rothe M, Gollasch M, Luft FC. Hemodialysis and erythrocyte epoxy fatty acids. Physiol Rep. 2020 Oct;8(20):e14601.

4. Discussion

In the present study, we conducted a targeted lipidomic study using LCFAs and oxylipins differences in arteries and veins for the first time to assess the impact of HD treatment on lipid mediators comprehensively, whether accumulated, consumed, or generated in ESRD patients during the blood circulation. Specifically, our study focuses on four major findings, as described in the following: (i) we detected negative AV differences mainly in SFAs, MUFAs, and n-6 PUFAs in erythrocytes before HD, which disappeared after HD. In plasma LCFAs, we only noticed that C22:0 and C24:0 in SFAs showed such alteration before HD. (ii) we identified negative AV differences after HD for the majority of CYP epoxy metabolites derived from PUFAs in plasma. This was mainly due to the increase of these epoxy metabolites in the venous blood after HD. However, HD had no obvious effect on the plasma hydroxy metabolites. (iii) these detected AV differences seem to be attributed to the renal replacement therapy itself, as we did not find AV differences in plasma CYP epoxy metabolites before HD. (iv) the accumulation of plasma CYP epoxide metabolites in peripheral tissues after HD probably correlated with the decrease in sEH activity. In brief, we conclude that AV differences in SFA, MUFA, and PUFA n-6 are present and active in mature erythrocytes and that this fatty acid status is sensitive to a single HD treatment [1]. In the meanwhile, alterations in plasma epoxy metabolites were consistent with the view that blood perfusion of peripheral tissues would induce an accumulation of epoxy metabolites. Hence, we speculate that plasma CYP epoxy metabolites probably have a facilitative effect on the blood flow response in the peripheral circulation during extracorporeal circulation therapy (hemodialysis) [2] .

4.1 LCFAs in the circulation

In our research, SFAs (C16:0, C22:0, and C24:0), MUFAs (eicosenoic acid C20:1 n-9 and erucic acid C22:1 n-9), n-3 PUFA [α-linoleic acid (ALA C18:3 n-3 alpha)] and n-6 PUFAs [C18:2 n-6, C20:3 n-6, C20:4 n-6, and docosapentaenoic acid ω-6 (DPA C22:5 n-6)] showed negative AV differences in erythrocytes passing through the upper limb before HD, which was affected by HD therapy. This demonstrates that LCFAs are presented and activated in erythrocytes and that their bioaccumulation effect in

peripheral tissues, i.e. upper limb muscle tissue, is influenced by renal replacement therapy [1].

Numerous studies have identified SFA as a pro-inflammatory and cellular damage factor and an independent risk factor for CVD development [50] [51]. Nevertheless, there are few clear reports on the effects of HD treatment on SFAs. We considered that bioaccumulation of Total-SFA in erythrocytes in ESRD patients was triggered and was influenced by HD treatment, which will provide new perspectives for following studies of dialysis-associated CVD. The anti-inflammatory action of MUFAs has been demonstrated in numerous studies [52]. In particular, OA can protect cells from SFA-induced damage by promoting β-oxidation of fatty acids [53]. However, high levels of MUFAs do not always have a favorable effect on the inflammatory state. A study in CKD showed that an increase in the circulating MUFA/SFA ratio was positively correlated with an increase in C-reactive protein (CRP) levels, indicating that the organism was in an inflammatory state [54]. A study by An WS et al. [55] identified elevated levels of MUFA in erythrocyte membranes in HD patients compared to healthy people and higher in patients on peritoneal dialysis (PD) than in HD patients. A systematic review from 53 literatures [56] revealed that there is still no standardized change pattern of LCFAs in plasma or erythrocytes of dialysis patients, with most of the studies reporting lower POA, LA, ALA, DHA, and total PUFA in erythrocytes in HD patients. Our results indicated that ALA, LA, DGLA, AA, and DPA were the primary n-3 and n-6 PUFA contributing to negative AV differences in Total-PUFA in ESRD patients. We summarized the altered erythrocyte n-3/ n-6 fatty acid profile in ESRD patients, whose status was implicated by HD therapy. Considering that HD has no effect on most LCFAs in plasma and the metabolism of these fatty acids, we suspect that direct endothelial-erythrocyte interactions play an essential role in the bioaccumulation of fatty acids in the erythrocyte membrane instead of taking up from the plasma [1].

To further understand the causes of the AV differences in LCFAs, we analyzed desaturase and peroxisome functions. The ∆5D enzyme converts DGLA to AA [11]. As a result, numerous studies applied the serum AA/DGLA ratio as an indirect signal for ∆5D activity. The AA/DGLA ratio is commonly elevated in ESRD patients undergoing HD and is a strong predictor of deleterious clinical outcomes [57]. The ∆6D, the rate-limiting enzyme for LA to AA conversion, has been involved in diabetes, breast cancer, metabolic syndrome, etc [58] [59] [60]. Higher ∆6D activity is associated with a higher risk of developing these diseases [58]. By catalyzing the synthesis of MUFAs, ∆9D may

be a potential therapeutic target for controlling the progression of diseases such as obesity, type 2 diabetes, and hepatic steatosis [61]. β-oxidation of the peroxisome is a key step in DHA synthesis and its dysfunction will lead to DHA deficiency in vivo [46] . Our findings serve as an inspiration and ideas for new research into desaturase and peroxisome roles in ESRD cardiovascular complications [1].

4.2 Epoxy-metabolites in plasma

We measured negative AV differences in the plasma level of 9, 10/12, 13-EpOME, 5, 6/8, 9/11, 12/14, 15-EET, 5, 6/8, 9/11, 12/14, 15/17, 18-EEQ, and 7, 8/10, 11/13, 14/16, 17/19, 20-EDP after HD, mainly attributing to their significantly elevated contents in venous blood after dialysis [2]. Although previous studies have proposed that reduced sEH activity in CKD/ESRD patients may lead to the accumulation of CYP epoxide metabolites[13] [62], the changes we observed may be associated with reduced sEH activity caused by HD therapy [2]. sEH is an enzyme that hydrolyzes specific epoxide metabolites to their counterpart diols with less biological activity [30]. We detected that all diols/epoxides ratios were markedly reduced in veins after HD. Nonetheless, researchers established that sEH is not the only enzyme capable of metabolizing epoxide and that COX can further metabolize epoxide [63]. Consequently, whether dialysis treatment can indeed inhibit the hydrolysis of epoxide metabolites by inhibiting sEH activity remains proven experimentally [2]. The results have provided mechanistic insights to better understand our earlier data [33].

Endothelial cells and erythrocytes are storage pools for EETs in vivo and can release EETs into the plasma in specific conditions [64] [65] [66]. However, the mechanisms of how EETs and other epoxide metabolites are released from tissues are largely unknown, making it difficult to interpret our findings. EETs can protect cardiorenal function from inflammation, fibrosis and apoptosis by activating TRPV4, calcium-dependent potassium channels (BKca) and signaling pathways when they are released [67]. Four regioisomers of EETs present their specific effects [68]. For instance, only exogenous supplementation of 8,9-EET produces a dose-dependent protective effect on glomerular function [69]. Although 11,12-EET and 14,15-EET are the most prevalent regional isomers in humans, only 14,15-EET is an optimal target for sEH, and only 11,12-EET suppresses the K+ channel in the renal cortical collecting duct [70] [71]. Our results support the view [72] that EETs are crucial vasodilator signaling molecules

with cardiovascular effects in ESRD/CKD that mitigate the vasoconstrictive reaction during dialysis [2]. sEH inhibitors are considered a new therapeutic approach that may enhance the beneficial biological effects of EETs and other epoxy metabolites [73]. However, it is postulated that higher concentrations of EETs in vivo may also have deleterious cardiovascular effects [74] [75] [2]. The extent to which the levels of EETs observed in our study have deleterious or beneficial biological effects is difficult to define [2].

Few studies described the exact role of EDPs and EEQs in HD patients. Most current studies regard EDPs as critical mediators that inhibit inflammation, angiogenesis, renal fibrosis, tumor growth, and metastasis and are involved in various chronic diseases, including hypertension, pain, and kidney disease, particularly 16, 17-EDP and 19, 20-EDP [76] [77] [78]. However, in a recent study in a mouse model of acute kidney injury (AKI), Deng et al. [76] demonstrated that 14,15-EET dose-dependently reversed apoptosis in mouse renal tubular epithelial cells caused by ischemia-reperfusion injury (I/R). Conversely, 19, 20-EDP dose-dependently induced apoptosis caused by I/R [76]. Meanwhile, the vasodilatory properties of 17,18-EEQ that hyperpolarize vascular smooth muscle cells mainly through activation of BKca have been demonstrated in mouse aorta, brain, and human pulmonary arteries [79] [80] [81]. Nevertheless, its specific function in ESRD patients is largely unknown. We conclude that EDPs and EEQs might be new vasoactive substances released by HD treatment stimulation to impact the hemodynamics in ESRD patients [2].

Previous studies [82] concluded that the leukotoxic effects of 9, 10-EpOME, and 12,13-DiHOME are dose-dependent. High doses of EpOMEs exert cardiovascular toxic effects mainly by reducing aortic flow and blood pressure and decreasing cardiac contractility [82]. In our study, plasma 9, 10/12, 13-EpOME was dramatically elevated in venous blood after HD in ESRD patients. HD promoted 9, 10/12, 13-EpOME bioaccumulation in peripheral tissues, but this influence cannot be exactly defined considering the dose-dependent status.

5. Conclusions

Our study demonstrates that blood flow perfusion in peripheral tissues, especially upper limb muscle tissue, affects erythrocyte fatty acid status by consuming numerous LCFAs in the erythrocyte membrane during HD [1]. Additionally, we observed that HD influences plasma oxylipins status by promoting the bioaccumulation of CYP epoxide metabolites, which is consistent with the view that dialysis blood perfusion peripheral tissue, especially the muscle, stimulates the accumulation and release of CYP epoxide metabolites [2]. Further, the decrease in the diol/epoxide metabolite ratio after HD may indirectly indicate a reduction in sEH activity, which is probably related to the accumulation of CYP epoxide metabolites in the circulation, at least in the upper limb circulation [2]. Owing to the limitations of clinical trials and the influence of multiple variables such as disease state and metabolite interactions, the specific biological effects that may result from the alterations in oxylipins and LCFAs detected in our study need to be proved rigorously designed through basic experiments.

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Statutory Declaration

I, Tong Liu, by personally signing this document in lieu of an oath, hereby affirm that I prepared the submitted dissertation on the topic *Biotransformation of blood long-chain fatty acids and oxylipins in peripheral tissue during hemodialysis / Biotransformation von langkettigen Fettsäuren im Blut und Oxylipinen in peripheren Geweben während der Hämodialyse* independently and without the support of third parties, and that I used no other sources and aids than those stated.

All parts which are based on the publications or presentations of other authors, either in letter or in spirit, are specified as such in accordance with the citing guidelines. The sections on methodology (in particular regarding practical work, laboratory regulations, statistical processing) and results (in particular regarding figures, charts and tables) are exclusively my responsibility.

Furthermore, I declare that I have correctly marked all of the data, the analyses, and the conclusions generated from data obtained in collaboration with other persons, and that I have correctly marked my own contribution and the contributions of other persons (cf. declaration of contribution). I have correctly marked all texts or parts of texts that were generated in collaboration with other persons.

My contributions to any publications to this dissertation correspond to those stated in the below joint declaration made together with the supervisor. All publications created within the scope of the dissertation comply with the guidelines of the ICMJE (International Committee of Medical Journal Editors; http://www.icmje.org) on authorship. In addition, I declare that I shall comply with the regulations of Charité – Universitätsmedizin Berlin on ensuring good scientific practice.

I declare that I have not yet submitted this dissertation in identical or similar form to another Faculty.

The significance of this statutory declaration and the consequences of a false statutory declaration under criminal law (Sections 156, 161 of the German Criminal Code) are known to me."

Date Signature

Declaration of your own contribution to the publications

Tong Liu contributed the following to the below listed publication:

Publication 1: **Liu T**, Dogan I, Rothe M, Reichardt J, Knauf F, Gollasch M, Luft FC, Gollasch B. Bioaccumulation of Blood Long-Chain Fatty Acids during Hemodialysis. *Metabolites*. 2022;12(3):269. **Impact Factor (2022): 4.93**

Contribution in detail: I contributed to sample collection, sample pre-processing, lipids profile analysis by LC-MS/MS tandem mass spectrometry, and collection and cleaning of lipids profile determination data. I performed statistical analysis of the data using SPSS statistics software, followed by further interpretation of the data. I created all the tables and Figure 1. I performed the literature search, interpreted the results, and drafted a first version of the manuscript.

Publication 2: **Liu T**, Dogan I, Rothe M, Kunz JV, Knauf F, Gollasch M, Luft FC, Gollasch B. Hemodialysis and Plasma Oxylipin Biotransformation in Peripheral Tissue. *Metabolites*. 2022; 12(1):34. **Impact Factor (2022): 4.93**

Contribution in detail: I contributed to sample collection, sample pre-processing, extraction of eicosanoid profiles by LC-MS/MS tandem mass spectrometry, and collection and cleaning of eicosanoid profile determination data. I performed statistical analysis of the data using SPSS statistics software, followed by further interpretation of the data. I created all the figures and tables except for Figure 1 and Figure 2. I performed the literature search, interpreted the results, and drafted a first version of the manuscript.

Publication 3: Gollasch B, Wu G, **Liu T**, Dogan I, Rothe M, Gollasch M, Luft FC. Hemodialysis and erythrocyte epoxy fatty acids. *Physiol Rep*. 2020 Oct;8(20):e14601. **Impact Factor (2022): 2.26**

Contribution in detail: I performed statistical analysis of the data using SPSS statistics software, followed by further interpretation of the data involved.

_____

Signature, date and stamp of first supervising university professor / lecturer

_____

Signature of doctoral candidate

Printing copies of the selected publications

Publication #1: Bioaccumulation of Blood Long-Chain Fatty Acids during Hemodialysis.

Liu T, Dogan I, Rothe M, Reichardt J, Knauf F, Gollasch M, Luft FC, Gollasch B. Bioaccumulation of Blood Long-Chain Fatty Acids during Hemodialysis. *Metabolites*. 2022;12(3):269.

https://doi.org/10.3390/metabo12030269

Publication #2: Hemodialysis and Plasma Oxylipin Biotransformation in Peripheral Tissue.

Liu T, Dogan I, Rothe M, Kunz JV, Knauf F, Gollasch M, Luft FC, Gollasch B. Hemodialysis and Plasma Oxylipin Biotransformation in Peripheral Tissue. *Metabolites*. 2022; 12(1):34.

https://doi.org/10.3390/metabo12010034

Excerpt from Journal Summary List

Journal Data Filtered By: **Selected JCR Year: 2020** Selected Editions: SCIE,SSCI Selected Categories: **"BIOCHEMISTRY and MOLECULAR BIOLOGY"** Selected Category Scheme: WoS

Gesamtanzahl: 297 Journale

Rank	Full Journal Title	Total Cites	Journal Impact Factor	Eigenfactor Score
1	NATURE MEDICINE	114,401	53.440	0.184050
2	CELL	320,407	41.582	0.526960
3	Molecular Cancer	24,931	27.401	0.030030
4	Annual Review of Biochemistry	24,394	23.643	0.021450
5	Signal Transduction and Targeted Therapy	3,848	18.187	0.005730
6	MOLECULAR CELL	86,299	17.970	0.161840
7	TRENDS IN MICROBIOLOGY	17,553	17.079	0.022820
8	NUCLEIC ACIDS RESEARCH	248,139	16.971	0.387070
9	MOLECULAR BIOLOGY AND EVOLUTION	61,557	16.240	0.082270
10	PROGRESS IN LIPID RESEARCH	7,328	16.195	0.004530
11	MOLECULAR PSYCHIATRY	28,622	15.992	0.046220
12	CELL DEATH AND DIFFERENTIATION	27,701	15.828	0.028730
13	NATURE STRUCTURAL & MOLECULAR BIOLOGY	32,038	15.369	0.051210
14	Nature Chemical Biology	27,428	15.040	0.047880
15	MOLECULAR ASPECTS OF MEDICINE	8,136	14.235	0.006640
16	TRENDS IN BIOCHEMICAL SCIENCES	22,003	13.807	0.025760
17	NATURAL PRODUCT REPORTS	13,293	13.423	0.011160
18	Molecular Plant	15,778	13.164	0.026860
19	Advances in Carbohydrate Chemistry and Biochemistry	752	12.200	0.000200
20	TRENDS IN MOLECULAR MEDICINE	13,213	11.951	0.014720

Rank	Full Journal Title	Total Cites	Journal Impact Factor	Eigenfactor Score
84	FASEB JOURNAL	54,279	5.191	0.044390
85	BIOCHIMICA ET BIOPHYSICA ACTA-MOLECULAR BASIS OF DISEASE	20,194	5.187	0.019800
86	JOURNAL OF BIOLOGICAL CHEMISTRY	397,453	5.157	0.155420
87	ACS Chemical Biology	16,023	5.100	0.029490
88	INTERNATIONAL JOURNAL OF BIOCHEMISTRY & CELL BIOLOGY	19,050	5.085	0.010120
89	JOURNAL OF ENZYME INHIBITION AND MEDICINAL CHEMISTRY	7,303	5.051	0.005690
90	MOLECULES AND CELLS	6,228	5.034	0.006380
91	STRUCTURE	17,041	5.006	0.022940
92	MACROMOLECULAR BIOSCIENCE	8,407	4.979	0.005540
93	RNA	14,914	4.942	0.017530
94	Metabolites	3,956	4.932	0.005970
95	Biomedical Journal	1,582	4.910	0.002220
96	Biomolecules	10,162	4.879	0.014390
97	MOLECULAR CARCINOGENESIS	7,350	4.784	0.007820
98	BMB Reports	3,847	4.778	0.005130
99	CURRENT OPINION IN LIPIDOLOGY	4,741	4.776	0.005120
100	BIOCONJUGATE CHEMISTRY	18,580	4.774	0.017200
101	BIOCHIMICA ET BIOPHYSICA ACTA-MOLECULAR CELL RESEARCH	20,873	4.739	0.017310
102	PLANT SCIENCE	20,176	4.729	0.013280
103	INSECT BIOCHEMISTRY AND MOLECULAR BIOLOGY	10,252	4.714	0.006770

Selected JCR Year: 2020; Selected Categories: "BIOCHEMISTRY and MOLECULAR BIOLOGY"

metabolites

MDPI

Article

Bioaccumulation of Blood Long-Chain Fatty Acids during Hemodialysis

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Abstract: Long-chain fatty acids (LCFAs) serve as energy sources, components of cell membranes, and precursors for signaling molecules. Uremia alters LCFA metabolism so that the risk of cardiovascular events in chronic kidney disease (CKD) is increased. End-stage renal disease (ESRD) patients undergoing dialysis are particularly affected and their hemodialysis (HD) treatment could influence blood LCFA bioaccumulation and transformation. We investigated blood LCFA in HD patients and studied LCFA profiles in vivo by analyzing arterio–venous (A–V) LFCA differences in upper limbs. We collected arterial and venous blood samples from 12 ESRD patients, before and after HD, and analyzed total LCFA levels in red blood cells (RBCs) and plasma by LC–MS/MS tandem mass spectrometry. We observed that differences in arterial and venous LFCA contents within RBCs (RBC LCFA A–V differences) were affected by HD treatment. Numerous saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), and polyunsaturated fatty acids (PUFA) n-6 showed negative A–V differences, accumulated during peripheral tissue perfusion of the upper limbs, in RBCs before HD. HD reduced these differences. The omega-3 quotient in the erythrocyte membranes was not affected by HD in either arterial or venous blood. Our data demonstrate that A–V differences in fatty acids status of LCFA are present and active in mature erythrocytes and their bioaccumulation is sensitive to single HD treatment.

Keywords: exercise; lipidomics; erythrocytes; fatty acids; chronic kidney disease; hemodialysis

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Citation: Liu, T.; Dogan, I.; Rothe, M.; Reichardt, J.; Knauf, F.; Gollasch, M.; Luft, F.C.; Gollasch, B. Bioaccumulation of Blood Long-Chain Fatty Acids during Hemodialysis. *Metabolites* **2022**, *12*, 269. https://doi.org/10.3390/metabo12030269

Academic Editor: Markus R. Meyer

Received: 20 February 2022
Accepted: 19 March 2022
Published: 21 March 2022

1. Introduction

Chronic kidney disease (CKD) is a major public health problem worldwide, with a prevalence of approximately 10–15%, and the incidence of CKD continues to rise [1,2]. End-stage renal disease (ESRD) is the final, permanent stage of CKD, necessitating renal replacement therapies, notably hemodialysis (HD). ESRD patients face excess risk of developing cardiovascular disease (CVD), accounting for approximately 48% of total mortality [3]. Obesity, diabetes, hypertension, and hypercholesterolemia do not fully explain, or even contradict, this phenomenon [4]. Furthermore, the HD treatment in and of itself is implicated in CVD progression. Thus, nontraditional putative CKD-related risk factors, particularly when related to HD, must be evaluated [5].

Long-chain fatty acids (LCFAs) are an essential source of energy and a major cell membrane component of cell membranes. They participate in signaling pathways, influencing cell membrane structure and fluidity, affecting receptor affinity and ion channels [6,7]. A

low omega-3 quotient in red blood cells (RBCs) is an independent risk factor for cardiovascular disease [8]. Eicosapentaenoic acid (EPA, C20:5 n-3) and docosahexaenoic acid (DHA, C22:6 n-3) are the two primary sources of dietary omega-3 fatty acids (FAs) derived from marine oil (Figure 1, orange font). Dietary supplementation with omega-3 FAs is effective in reducing cardiovascular risk in healthy people and ESRD patients [9,10], whereas studies that employed the combination EPA/DHA to standard of care therapy failed to derive any clinical benefit [11]. Several trials that tested purified EPA (JELIS, REDUCE-IT, EVAPORATE) were associated with reduced cardiovascular risk and the regression of atheroma coronary plaques [11]. Dietary EPA reduced ischemic events across the broad range of baseline eGFR categories [12]. Lower than normal blood polyunsaturated fatty acids (PUFA) n-3 levels [13] and increased blood monounsaturated FAs (MUFA) levels [10] are linked to increased cardiovascular risk in ESRD patients. Saturated FAs (SFA) may increase mortality by promoting vascular calcification in ESRD patients [14]. Dietary-induced changes in the FA composition of human plasma, platelet, and erythrocyte lipids follow a similar time course of several weeks [15–17]. Desaturases and elongases are involved in PUFA biotransformation [18] (Figure 1).

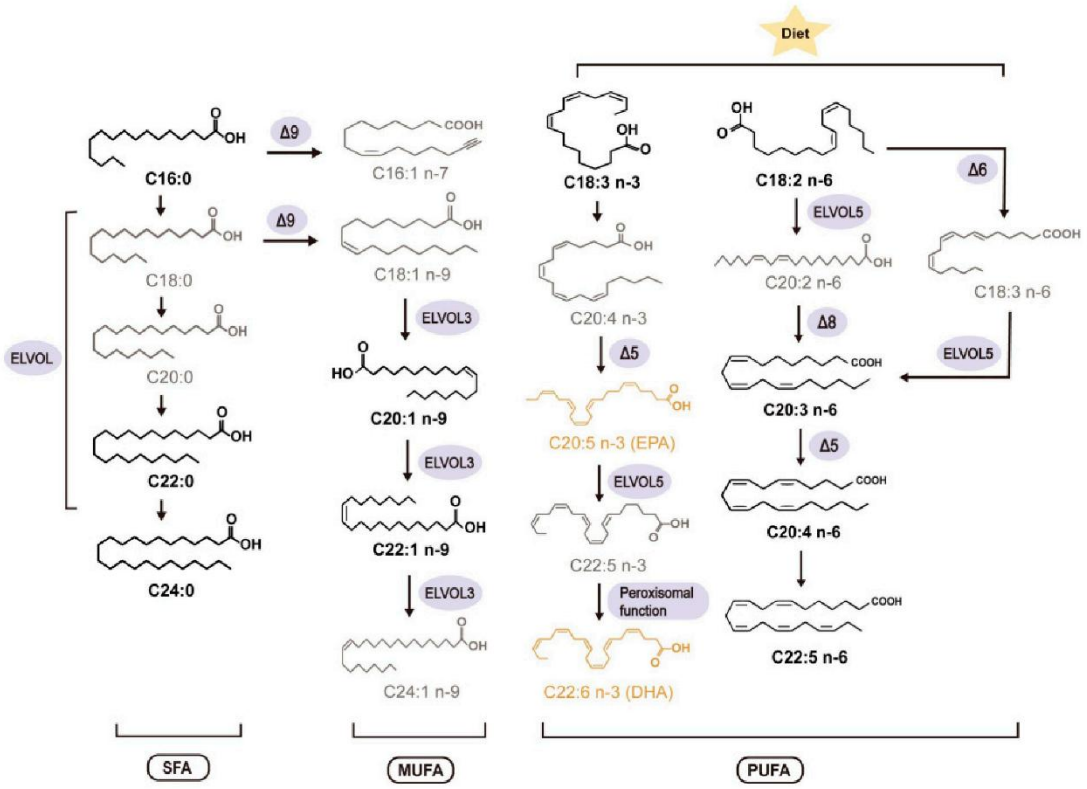

Figure 1. Fatty acid elongation and desaturation processes: SFA, saturated fatty acids; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acids; Δ6D, delta-6-desaturase; Δ5D, delta-5-desaturase; Δ9D, delta-9-desaturase. Eicosapentaenoic acid (C20:5 n-3, EPA) and docosahexaenoic acid (C22:6 n-3; DHA) (omega-3 quotient) are highlighted in orange font. Bold font FAs showed negative A–V differences (bioaccumulation) in RBCs after passage through upper limbs before dialysis treatment.

Our previous study [19] revealed that HD treatment alters the status of FAs and their oxidative metabolites, such as oxylipins, in arterial blood of ESRD patients. However, how HD treatment affects in vivo FA bioaccumulation and/or biotransformation in peripheral tissues, specifically whether FAs are produced, degraded, or stored in the blood as they pass through the arteries into peripheral tissues and organs and then from the venous end of the capillaries back into the venous circulatory system, is unclear. The arterio–venous (A–V) oxygen (O_2) difference is the difference in the blood O_2 content between the arterial blood and the venous blood. This difference shows how much O_2 is removed from the blood in capillaries as the blood circulates in the body. ESRD patients present, before HD, with high ammonia plasma levels in arterial blood with a significantly positive arterio–venous difference [20]. The A–V blood glucose gradient is related to the fact that the peripheral tissues, especially the muscles, either store or burn part of the traversing glucose [21]. Likewise, non-esterified long-chain FAs (LCFAs) can exhibit positive (decumulation, i.e., loss) or negative (accumulation) A–V differences depending on the patient's health or physical status and tissues perfused [22]. For example, negative differences in arterial and venous FAs within RBCs (RBC A–V differences) after passage through the upper limbs would be consistent with the bioaccumulation of those FAs in RBCs. In analogy, we tested the hypothesis that A–V differences in blood LCFAs are present in vivo and sensitive to single HD treatment. We collected arterial and venous blood samples of the upper limbs from ESRD patients, before and after HD, and measured the difference in the blood LCFAs content between the arterial blood and the venous blood by LC–MS/MS tandem mass spectrometry.

2. Results

2.1. Clinical Characteristics

The clinical features of the ESRD patients are shown in Table 1. The patients were diagnosed with FSGS (focal segmental glomerulosclerosis) (six patients), ADPKD (autosomal dominant polycystic kidney disease) (one patient), IgA nephropathy (one patient), hypertensive nephropathy (one patient), renal amyloidosis (one patient), drug-induced kidney injury (one patient), and cystic kidneys (one patient). All patients experienced macroangiopathic complications, including cardiovascular and cerebrovascular events and peripheral arterial disease. Table S1 shows that our patients did not have manifest diabetes but had hyperlipidemia.

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

	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 (ESRD)	
Focal segmental glomerulosclerosis (n)	6
IgA nephropathy (n)	1
Renal amyloidosis	1
Hypertension (n)	1
Drug induced (n)	1
ADPKD (n)	1
Cystic kidneys (n)	1
Complications	
Cardiovascular or Cerebrovascular (n)	12

Notes: Data are presented as mean + SD or frequencies. n, number.

2.2. Effects of Hemodialysis on Individual LCFAs in Plasma

The effects of hemodialysis treatment on individual FAs and their A–V differences are shown in Table 2. With the exception of C22:0 and C24:0, there were no A–V differences in plasma FAs levels before (pre-HD) and after HD (post-HD). Consistently, we did not detect

A–V differences in SFA, MUFA, and PUFA plasma levels pre-HD and post-HD (Table 3). The negative pre-HD A–V difference in plasma levels of C22:0 and C24:0 disappeared after HD (Table 2).

There were no A–V differences in the dietary n-3/n-6 ratio, omega-3 quotient (EPA + DHA)/AA, EPA/AA, DHA/AA, and EPA/DHA ratios pre-HD and post-HD (Tables 4 and S2). To see whether desaturase activities were affected by single HD treatment, we calculated the following ratios: C18:3 n-6/C18:2 n-6 ratio, representing Δ6D, C20:4 n-6/C20:3 n-6 ratio, representing Δ5D, C16:1 n-7/C16:0 and C18:1 n-9/C18:0 ratios, representing Δ9D and DHA/DPA peroxisome functions, respectively (Table 5). The results show that pre-HD or post-HD values were not statistically significant (Tables 5 and S3).

Table 2. Effects of hemodialysis on individual fatty acids in plasma and RBCs in the CKD patients before (pre-HD) and at cessation (post-HD) of hemodialysis ($n = 12$ each).

Fatty Acids	Chain	Pre-HD Median (IQR)				Post-HD Median (IQR)			
		Pre-HD Arterial	Pre-HD Venous	*p* Value, *t* Test (# Paired Wilcoxon test)	Pre-HD A–V Difference	Post-HD Arterial	Post-HD Venous	*p* Value, *t* Test (# Paired Wilcoxon Test)	Post-HD A–V Difference
Total-Plasma (μg/mL)									
Myristic Acid	C14:0	52.596 (34.455–74.978)	49.148 (35.486–61.274)	0.201	1.999 (−0.948–5.168)	69.936 (43.668–82.384)	55.294 (37.346–73.459)	0.05#	4.706 (2.599–11.296)
Myristolein acid	C14:1 n-5	3.234 (0.947–4.226)	3.043 (1.223–3.605)	0.328	0.054 (−0.289–0.518)	3.301 (1.639–5.112)	2.405 (1.255–4.273)	0.071#	0.114 (0.001–0.612)
Palmitic acid	C16:0	777.653 (573.133–955.245)	641.312 (546.790–1041.449)	0.259	38.589 (−7.998–83.389)	866.837 (608.336–1058.773)	757.319 (533.635–969.393)	0.081	81.436 (43.274–128.276)
Palmitoleic acid	C16:1 n-7	73.498 (49.472–93.766)	63.583 (50.81–93.068)	0.523	1.055 (−1.338–6.058)	66.312 (49.748–88.141)	56.138 (46.339–83.299)	0.16	5.162 (1.216–8.782)
Stearic acid	C18:0	127.090 (101.163–181.126)	133.632 (97.466–184.07)	0.92	6.2596 (−15.557–17.815)	153.798 (103.222–186.498)	127.594 (114.844–164.167)	0.222	11.989 (−6.372–44.691)
Oleic acid	C18:1 n-9	748.353 (613.3662–902.8834)	691.807 (586.015–991.742)	0.377	19.965 (−18.542–74.232)	825.9256 (638.758–986.897)	739.047 (587.658–929.974)	0.068	62.843 (38.444–85.592)
Linoleic acid	C18:2 n-6	562.592 (518.182–711.229)	581.052 (497.470–671.192)	0.397	9.3694 (−33.449–47.94)	630.349 (521.263–696.79)	590.649 (472.954–621.414)	0.05#	57.732 (24.256–71.841)
α-Linoleic acid	C18:3 n-3 alpha	13.945 (10.154–26.515)	15.015 (10.567–27.396)	0.719	0.893 (−1.754–0.565)	20.302 (13.724–27.295)	16.611 (13.909–26.029)	0.347#	1.159 (−0.614–2.801)
γ-Linoleic acid	C18:3 n-6 gamma	8.567 (6.489–14.347)	9.140 (6.723–14.17)	0.54	0.788 (−0.981–1.764)	13.178 (6.819–14.602)	8.862 (6.362–12.27)	0.233#	1.352 (−0.135–2.425)
Eicosenoic acid	C20:1 n-9	5.34 (4.860–7.483)	5.787 (4.983–7.009)	0.583#	−0.119 (−0.364–0.242)	6.550 (5.726–7.261)	6.151 (5.337–7.128)	0.272#	0.424 (−0.126–0.769)
Eicosa-dienoic acid	C20:2 n-6	5.238 (4.487–6.676)	5.987 (4.542–6.569)	0.951	0.127 (−0.811–0.583)	5.0493 (4.126–6.253)	5.199 (4.435–5.981)	0.81	0.127 (−0.443–0.551)
Dihomo-γ-Linoleic acid	C20:3 n-6	44.416 (39.639–47.495)	41.626 (36.919–47.670)	0.929	1.638 (−1.572–2.888)	41.933 (39.131–47.516)	38.733 (35.309–43.548)	0.084	2.743 (0.195–7.246)
Arachidonic acid	C20:4 n-6	159.677 (128.831–169.561)	146.238 (136.060–158.549)	0.308#	5.4890 (−5.1079–24.766)	154.717 (135.01–179.618)	156.270 (132.641–164.080)	0.209#	11.712 (−1.005–24.280)
Eicosapenta-enoic acid	C20:5 n-3	17.969 (11.385–24.730)	15.828 (10.826–22.529)	0.168	0.744 (−0.584–2.694)	15.567 (12.080–23.746)	13.6374 (11.4568–20.6670)	0.2	0.903 (0.330–3.0154)
Docosanoate	C22:0	**0.436 (0.243–0.803)**	**0.787 (0.526–1.066)**	**0.012#**	**−0.359 (−0.505–−0.146)**	0.5993 (0.4372–1.0188)	0.737 (0.253–0.853)	0.239#	0.213 (−0.192–0.346)
Erucic acid	C22:1 n-9	7.328 (6.898–8.415)	8.730 (7.581–9.637)	0.05#	−0.927 (−2.220–−0.385)	6.940 (6.582–7.573)	7.651 (7.203–8.111)	0.071#	−0.411 (−0.928–0.122)
Docosapentaenoic acid ω-3	C22:5 n-3	11.204 (8.975–13.927)	11.131 (8.707–13.017)	0.378	0.270 (−0.738–0.843)	11.945 (9.740–14.449)	11.412 (7.945–12.780)	0.267	0.782 (0.3697–1.1939)
Docosapentaenoic acid ω-6	C22:5 n-6	1.673 (1.401–2.283)	1.917 (1.604–2.483)	0.567	−0.034 (−0.214–0.094)	1.860 (1.471–2.430)	1.681 (1.504–2.419)	0.314	0.152 (−0.008–0.239)
Docosahexaenoic acid	C22:6 n-3	92.858 (74.276–116.778)	87.830 (81.820–99.411)	0.239#	2.672 (−3.136–10.778)	90.402 (83.206–119.631)	81.488 (76.371–105.830)	0.084#	5.538 (2.542–11.357)

Table 2. *Cont.*

Fatty Acids	Chain	Pre-HD Median (IQR)				Post-HD Median (IQR)			
		Pre-HD Arterial	Pre-HD Venous	*p* Value, *t* Test (# Paired Wilcoxon test)	Pre-HD A–V Difference	Post-HD Arterial	Post-HD Venous	*p* Value, *t* Test (# Paired Wilcoxon Test)	Post-HD A–V Difference
Lignocerine acid	C24:0	**0.406 (0.000–0.717)**	**1.685 (0.855–2.110)**	**0.009#**	**−1.066 (−1.910–0.285)**	0.350 (0.0003–0.594)	0.759 (0.043–0.993)	0.075#	−0.442 (−0.617–−0.042)
Nervonic acid	C24:1 n-9	2.918 (2.396–3.243)	3.070 (2.434–3.665)	0.568	−0.081 (−0.376–0.113)	2.732 (2.339–3.639)	2.701 (2.240–3.329)	0.497	0.190 (−0.460–0.450)
Total-RBC (μg/g)									
Myristic Acid	C14:0	22.836 (17.212–26.878)	26.270 (19.644–29.972)	0.084	2.478 (−6.704–0.204)	26.098 (19.788–31.595)	22.349 (20.057–36.542)	0.465	0.295 (−4.188–2.787)
Myristolein acid	C14:1 n-5	0.127 (0.000–0.316)	0.040 (0.000–0.265)	0.889#	0.000 (−0.140–0.067)	0.301 (0.001–0.531)	0.138 (0.054–0.606)	0.182#	−0.060 (−0.198–0.002)
Palmitic acid	C16:0	**338.150 (267.592–458.512)**	**400.180 (294.720–538.391)**	**0.032**	**−40.619 (−152.844–−0.799)**	367.433 (304.759–437.369)	343.501 (293.538–500.620)	0.308#	−5.556 (−78.396–30.194)
Palmitoleic acid	C16:1 n-7	16.349 (12.688–20.673)	20.828 (14.827–21.795)	0.091	−2.966 (−6.931–0.113)	17.388 (14.054–21.253)	17.995 (13.766–28.056)	0.083	−0.955 (−8.519–0.512)
Stearic acid	C18:0	244.115 (212.576–299.709)	236.321 (227.664–389.725)	0.05#	30.103 (−88.645–0.085)	229.340 (219.533–263.887)	233.455 (212.470–281.023)	0.583#	2.162 (−12.046–7.652)
Oleic acid	C18:1 n-9	281.487 (269.480–321.058)	340.480 (276.503–421.203)	0.06#	31.176 (−53.726–−1.625)	318.619 (299.014–371.419)	348.144 (292.854–372.476)	0.269	0.695 (−52.065–9.262)
Linoleic acid	C18:2 n-6	**224.086 (210.522–245.124)**	**283.474 (208.235–314.045)**	**0.041#**	**−27.407 (−62.497–−1.267)**	233.857 (224.404–307.236)	261.821 (220.0564–285.121)	0.48#	−3.814 (−58.30–14.185)
α-Linoleic acid	C18:3 n-3 alpha	**4.471 (2.936–5.837)**	**4.806 (3.553–9.541)**	**0.034#**	**−1.139 (−1.739–−0.271)**	4.794 (3.947–8.60)	5.397 (4.010–8.294)	0.53#	−0.200 (−0.8587–0.5433)
Eicosenoic acid	C20:1 n-9	**5.771 (4.362–7.056)**	**6.714 (5.836–7.281)**	**0.031**	**−0.594 (−2.049–0.093)**	5.786 (4.810–6.509)	6.068 (4.892–6.912)	0.084#	−0.558 (−0.909–0.081)
Eicosa-dienoic acid	C20:2 n-6	4.079 (3.372–4.719)	4.529 (4.208–5.115)	0.18	−0.194 (−0.890–0.052)	4.072 (3.703–4.422)	4.089 (3.970–4.395)	0.239#	−0.055 (−0.267–0.039)
Dihomo-γ-Linoleic acid	C20:3 n-6	**32.6767 (25.9329–36.5835)**	**39.1490 (34.5242–41.1978)**	**0.013**	**−4.2116 (−7.1576–0.5321)**	33.3307 (29.9956–37.4817)	33.2568 (27.5007–38.7330)	0.741	0.1820 (−4.7518–2.7220)
Arachidonic acid	C20:4 n-6	**269.517 (261.385–284.765)**	**280.996 (265.664–368.674)**	**0.015#**	**−16.985 (−64.073–7.210)**	298.174 (278.647–311.914)	292.519 (279.776–297.850)	0.281	15.277 (−13.874–23.519)
Eicosapenta-enoic acid	C20:5 n-3	12.140 (8.772–15.255)	13.310 (8.181–15.870)	0.738	1.152 (−3.982–−0.3250)	13.536 (9.583–15.274)	12.409 (9.473–14.470)	0.465	0.089 (−1.213–1.701)
Docosanoate	C22:0	**2.671 (2.251–4.065)**	**3.149 (2.482–6.082)**	**0.019#**	**−0.605 (−2.533–−0.079)**	2.843 (2.554–4.023)	3.065 (2.440–3.647)	0.638#	0.046 (−0.307–0.450)
Erucic acid	C22:1 n-9	**6.346 (6.018–7.581)**	**7.865 (7.411–10.785)**	**0.019#**	**−1.251 (−4.767–−0.811)**	6.286 (5.794–6.636)	6.751 (6.034–7.203)	0.308#	0.474 (−1.131–0.283)
Docosa-pentaenoic acid ω-3	C22:5 n-3	36.456 (34.872–39.675)	40.716 (37.685–51.770)	0.084#	−2.107 (−7.691–1.232)	39.810 (37.326–43.067)	38.287 (37.40–41.891)	0.399	1.194 (−1.344–2.491)
Docosapentaenoic acid ω-6	C22:5 n-6	**4.765 (3.293–5.372)**	**5.638 (4.832–6.071)**	**0.012**	**−0.802 (−1.363–−0.420)**	4.880 (3.968–5.815)	5.269 (4.577–5.580)	0.574	−0.086 (−0.339–0.283)

Table 2. *Cont.*

Fatty Acids	Chain	Pre-HD Median (IQR)				Post-HD Median (IQR)			
		Pre-HD Arterial	Pre-HD Venous	*p* Value, *t* Test (# Paired Wilcoxon test)	Pre-HD A–V Difference	Post-HD Arterial	Post-HD Venous	*p* Value, *t* Test (# Paired Wilcoxon Test)	Post-HD A–V Difference
Docosahexa-enoic acid	C22:6 n-3	197.746 (167.709–213.221)	200.355 (173.026–240.191)	0.088	−26.823 (−42.029—2.051)	192.054 (166.189–226.601)	190.915 (169.069–223.006)	0.59	2.531 (−10.018–9.290)
Lignocerine acid	C24:0	**5.304 (4.366–7.143)**	**6.621 (5.327–11.023)**	**0.032**	**−1.449 (−3.465–0.208)**	5.391 (4.744–6.764)	5.564 (4.536–7.146)	0.239#	0.623 (−0.427–1.058)
Nervonic acid	C24:1 n-9	10.635 (8.849–12.715)	12.092 (11.196–13.128)	0.112	0.149 (−3.933–0.275)	11.454 (9.683–13.231)	10.575 (9.739–13.225)	0.921	0.249 (−0.726–0.852)

Notes: A, arterial blood; V, venous blood. Median (IQR). A–V difference; arterio–venous difference.

Table 3. Effects of hemodialysis on total fatty acids in plasma and RBCs in the CKD patients before (pre-HD) and at cessation (post-HD) of hemodialysis (n = 12 each).

Fatty Acids	Pre-HD Median (IQR)				Post-HD Median (IQR)			
	Pre-HD Arterial	Pre-HD Venous	*p* Value, *t* Test (# Paired Wilcoxon Test)	Pre-HD A–V Difference	Post-HD Arterial	Post-HD Venous	*p* Value, *t* Test (# Paired Wilcoxon Test)	Post-HD A–V Difference
Total-Plasma (µg/mL)								
Total SFA	980.036 (709.890–1195.828)	819.448 (704.758–1313.913)	0.331	41.488 (−11.696–81.741)	1056.347 (772.398–1326.228)	927.555 (680.685–1202.097)	0.091	106.329 (39.294–178.681)
Total MUFA	844.511 (668.6085–1026.8815)	781.091 (650.605–1134.145)	0.452	20.134 (−21.531–82.456)	913.802 (712.350–1110.189)	809.196 (656.101–1045.294)	0.076	68.754 (42.861–95.648)
PUFA n-3	130.480 (109.134–195.917)	122.888 (118.934–168.358)	0.433#	2.373 (−5.795–14.639)	138.296 (124.327–205.347)	118.736 (109.134–160.080)	0.117#	8.880 (2.828–20.122)
PUFA n-6	744.004 (722.153–929.411)	798.519 (686.264–895.465)	0.433#	18.340 (−38.246–78.474)	821.264 (748.646–957.803)	807.937 (676.254–859.548)	0.094	79.758 (23.246–114.425)
Total-PUFA	857.990 (820.586–1136.295)	908.785 (782.665–1076.363)	0.433#	22.907 (−43.998–96.703)	947.742 (872.870–1160.028)	915.718 (784.884–1004.691)	0.103	89.139 (25.349–134.547)
Total-RBC (µg/g)								
Total SFA	**595.703 (513.040–757.739)**	**670.018 (553.384–972.688)**	**0.027**	**−56.325 (−277.577–2.217)**	624.941 (551.351–746.70)	591.402 (534.765–831.952)	0.182#	−28.462 (−94.268–36.130)
Total MUFA	**322.825 (305.306–380.602)**	**387.006 (329.542–476.590)**	**0.034#**	**−46.965 (−63.638–9.095)**	376.076 (332.646–409.804)	394.564 (330.508–432.247)	0.188	−5.169 (−62.982–7.602)
PUFA n-3	257.166 (218.402–273.419)	261.911 (235.961–312.850)	0.078	−37.862 (−48.270–4.691)	246.884 (223.435–303.050)	246.460 (218.649–284.657)	0.579	4.399 (−11.394–14.169)
PUFA n-6	**538.579 (527.422–560.087)**	**625.916 (544.230–707.882)**	**0.008#**	**−46.288 (−97.802–19.074)**	588.363 (554.219–649.017)	605.591 (539.772–637.355)	0.814#	15.263 (−80.730–38.335)
Total-PUFA	**784.818 (758.405–818.550)**	**905.273 (795.178–1041.732)**	**0.023#**	**−78.630 (−162.449–37.522)**	876.443 (778.130–892.759)	843.795 (801.30–888.677)	0.937#	33.365 (−92.123–67.535)

Notes: A, arterial blood; V, venous blood. Median (IQR). A–V difference; arterio–venous difference.

Table 4. Effect of hemodialysis on polyunsaturated fatty acid ratios in plasma and RBCs in the CKD patients before (pre-HD) and at cessation (post-HD) of hemodialysis (n = 12 each).

Ratio	Pre-HD Median (IQR)			Post-HD Median (IQR)		
	Pre-HD Arterial	Pre-HD Venous	p Value, t Test (# Paired Wilcoxon Test)	Post-HD Arterial	Post-HD Venous	p Value, t Test (# Paired Wilcoxon Test)
Total-Plasma						
DHA + EPA/AA	0.751 (0.627–0.875)	0.751 (0.627–0.875)	0.573	0.721 (0.610–0.873)	0.652 (0.595–0.817)	0.358
EPA/AA	0.105 (0.067–0.154)	0.098 (0.070–0.154)	0.3	0.101 (0.069–0.152)	0.081 (0.072–0.130)	0.61#
DHA/AA	0.620 (0.564–0.702)	0.636 (0.559–0.679)	0.754#	0.609 (0.534–0.695)	0.578 (0.512–0.661)	0.47
DHA/EPA	6.625 (4.853–7.133)	6.554 (5.180–7.437)	0.608	6.533 (5.242–7.165)	6.589 (5.856–7.350)	0.179
n-3/n-6	0.183 (0.151–0.212)	0.183 (0.151–0.212)	0.878	0.186 (0.151–0.210)	0.165 (0.154–0.218)	0.84
Total-RBC						
Omega-3 quotient	11.072 (9.634–13.067)	11.375 (7.710–12.171)	0.776	11.377 (9.737–12.382)	11.374 (8.304–12.544)	0.217
DHA + EPA/AA	0.759 (0.598–0.823)	0.655 (0.521–0.904)	0.835	0.738 (0.567–0.805)	0.703 (0.615–0.811)	0.851
EPA/AA	0.042 (0.030–0.058)	0.034 (0.030–0.058)	0.445	0.043 (0.032–0.055)	0.042 (0.034–0.051)	0.575
DHA/AA	0.710 (0.570–0.769)	0.620 (0.505–0.847)	0.908	0.695 (0.526–0.737)	0.661 (0.583–0.749)	0.759
DHA/EPA	16.161 (12.819–18.593)	15.261 (14.596–21.717)	0.814#	16.428 (12.26–18.764)	17.380 (13.466–20.360)	0.929
n-3/n-6	0.443 (0.404–0.493)	0.410 (0.352–0.505)	0.822	0.445 (0.361–0.485)	0.410 (0.388–0.462)	0.356

Notes: A, arterial blood; V, venous blood. Median (IQR).

Table 5. Effect of hemodialysis on the ratio of desaturase and peroxisome function in plasma and RBC in the CKD patients before (Pre-HD) and at cessation (Post-HD) of hemodialysis (n = 12 each).

Ratio	Pre-HD Median (IQR)			Post-HD Median (IQR)		
	Pre-HD Arterial	Pre-HD Venous	p Value, t Test (# Paired Wilcoxon Test)	Post-HD Arterial	Post-HD Venous	p Value, t Test (# Paired Wilcoxon Test)
Total-Plasma						
DHA/DPA	9.146 (6.889–11.009)	8.987 (7.0171–10.7715)	0.208	8.779 (6.518–11.679)	9.047 (6.545–13.840)	0.281
C20:4 n-6/C20:3 n-6 (Δ5 SCD)	3.663 (3.345–4.293)	3.573 (3.295–4.030)	0.201	3.799 (3.334–4.312)	3.906 (3.315–4.759)	0.849
C18:3 n-6/C18:2 n-6 (Δ6 SCD)	0.016 (0.010–0.019)	0.015 (0.010–0.019)	0.456#	0.017 (0.013–0.020)	0.017 (0.011–0.020)	0.223#
C16:1 n-7/C16:0 (Δ9 SCD)	0.095 (0.079–0.100)	0.088 (0.081–0.102)	0.79	0.084 (0.072–0.094)	0.086 (0.076–0.092)	0.314
C18:1 n-9/C18:0 (Δ9 SCD)	5.807 (5.315–6.423)	5.764 (4.642–7.018)	0.937#	6.290 (4.973–6.424)	6.005 (4.969–6.274)	0.484
Total-RBC						
DHA/DPA	5.283 (3.609–5.794)	5.015 (3.541–5.929)	0.813	5.110 (3.990–5.553)	4.985 (4.368–5.839)	0.859
C20:4 n-6/C20:3 n-6 (Δ5 SCD)	9.405 (7.575–10.389)	8.057 (6.667–10.316)	0.638#	8.659 (7.893–10.517)	8.145 (7.509–10.891)	0.44
C18:3 n-6/C18:2 n-6 (Δ6 SCD)	0.011 (0.006–0.013)	0.011 (0.007–0.013)	0.651	0.011 (0.008–0.013)	0.011 (0.007–0.014)	0.87
C16:1 n-7/C16:0 (Δ9 SCD)	0.042 (0.035–0.058)	0.044 (0.033–0.057)	0.903	0.043 (0.039–0.052)	0.048 (0.038–0.057)	0.143
C18:1 n-9/C18:0 (Δ9 SCD)	1.262 (1.068–1.500)	1.258 (0.914–1.523)	0.833	1.320 (1.184–1.593)	1.338 (1.177–1.484)	0.554

Notes: A, arterial blood; V, venous blood. Median (IQR).

2.3. *Effects of Hemodialysis on Individual LCFAs in RBCs*

Interestingly, most of the FAs in the erythrocyte membrane exhibited negative A–V differences before HD. These included C16:0, C18:2 n-6, C18:3 n-3 alpha, C20:1 n-9,

C20:3 n-6, C20:4 n-6, C22:0, C22:1 n-9, C22:5 n-6, and C24:0 (Table 2). Consistently, we detected negative A–V differences in SFA, MUFA, and PUFA n-6 in RBCs before HD (Table 3). HD reduced these differences (Table 3). The individual SFAs were C16:0, C22:0, and C24:0. The individual MUFAs were C20:1 n-9 and C22:1 n-9. The individual PUFAs were C18:2 n-6, C20:3 n-6, C20:4 n-6, and C22:5 n-6. Notably, the omega-3 and n-3/n-6 RBC quotients remained unchanged pre-HD and post-HD (Tables 4 and S2). Similarly, C18:3 n-6/C18:2 n-6, C20:4 n-6/C20:3 n-6, C16:1 n-7/C16:0 and C18:1 n-9/C18:0, and DHA/DPA ratios, representing Δ6D, Δ5D, Δ9D, and peroxisome functions, respectively, did not show differences pre-HD and post-HD (Tables 5 and S3).

3. Discussion

To our knowledge, our study is the first to study the A–V differences of blood FA content between the arterial blood and the venous blood. Venous blood was withdrawn from upper limbs. In this case, measurement of FA consumption by the specific organ system requires the determination of the FA content of venous blood draining from that organ [20–23]. We applied this approach to better understand bioaccumulation and biotransformation of FAs in vivo, particularly whether or not the peripheral tissues, especially the muscles in the upper limbs, either produce, store, or degrade part of the FAs that pass through them in response to dialysis treatment. We were particularly interested to understand how the FAs in RBCs and plasma are modified by hemodialysis treatment, which is known to cause oxidative stress, RBC-endothelial interactions, vascular damage, and inflammation. Our study identified negative A–V differences of a number of FAs in RBCs after passage through the upper limbs before dialysis treatment (pre-HD). This is consistent with the bioaccumulation of those FCAs in RBCs. These effects were caused by all four main LCFA modules in RBCs: SFAs [i.e., palmitic acid (PA) (C16:0), docosanoic acid (C22:0) and lignocerine acid (C24:0)], MUFAs [i.e., eicosenoic acid (C20:1 n-9), erucic acid (C22:1 n-9), ω-3 PUFA [alpha-linoleic acid (C18:3 n-3)], and ω-6 PUFAs [i.e., gamma-linoleic acid (C18:2 n-6), dihomo-γ-linoleic acid (C20:3 n-6), arachidonic acid (C20:4 n-6), docosapentaenoic acid omega 6 (C22:5 n-6)] (Figure 1, bold font) in RBCs. With the exception of the SFAs C22:0 and C24:0, these differences were not observed in plasma. We found that the A–V differences in RBC LCFAs were affected by HD treatment (post-HD). We conclude that A–V differences in the fatty acids status of SFA, MUFA, and PUFA n-6 are present and active in mature erythrocytes, and that this status is sensitive to single HD treatment. We have no evidence that these changes are caused by altered biotransformation (Figure 1, Tables 4 and 5). We found that the omega-3 quotient in erythrocyte membranes is not affected by HD in either arterial or venous blood, which aids clinical diagnostics of cardiovascular disease risk in healthy individuals and ESRD patients [24,25]. Furthermore, we observed that dialysis does not affect the dietary n-6/n-3 ratio (n-3/n-6) in both arterial and venous blood.

Previous studies have shown that RBC PUFAs reflect the phospholipid PUFA composition of major organs and could be used to monitor FA distribution in individual organs, for example, the heart [26–28]. In our study, we detected significant A–V differences in LCFAs in RBCs, i.e., numerous SFAs, MUFAs, PUFA (C18:3) n-3, and PUFAs n-6, which were affected by HD treatment. This indicates that LCFAs are present and active in mature erythrocytes and their bioaccumulation in peripheral tissues, namely the muscles in the upper limbs, is affected by renal replacement therapy. Consistent with other studies (for review see [10]), we previously detected decreased RBC EPA (C20:5 n-3) levels in the arterial blood of CKD patients compared with control subjects [19]. These changes were paralleled by decreased RBC C18:3 n-6 and C20:3 n-6 levels and decreased plasma levels of C18:2 n-6, C20:3 n-6, C20:4 n-6, C20:5 n-3, and C22:5 n-6 [10]. Together, the results indicate that there is an altered profile of n-3/n-6 fatty acids in ESRD patients, which is affected by hemodialysis treatment. With regard to the metabolism of these FAs (Figure 1) and our new findings in the present study, we speculate that direct endothelial–erythrocyte interactions may have contributed to this effect, rather than the uptake of fatty acids from plasma.

Finally, we investigated whether desaturase and peroxisome functions could be altered by HD treatment. Our data argue against a role of altered biotransformation of LCFA (Figure 1) in either plasma or RBCs by dialysis. A recent study used the alpha-linoleic acid/dihomo-γ-linoleic acid (AA/DGLA) ratio as a measure of Δ5D activity and found that a high AA/DGLA ratio is an independent predictor of cardiovascular risk and all-cause mortality in HD patients [29]. Δ9D is a rate-limiting enzyme for the conversion of SFA to MUFA [30]. Cho et al. found that Δ9D may exert cytoprotective effects by inhibiting the lipotoxic effects of excessive SFA accumulation [31]. An opposite effect was seen in a study in diabetes, which discovered that the risk of developing type 2 diabetes was associated with enhanced Δ6D and Δ9D activity in the erythrocyte membrane [31]. Our study provides motivation and suggestions for future studies on desaturase and peroxisome functions in cardiovascular complications of ESRD.

Our study has several limitations. First, the sample size of our study is small, and numerous FA levels pre-HD and post-HD showed only trends, which were not statistically significant. Second, the patient's diet was not strictly regulated, and different dietary habits may have led to individual differences in FAs. Finally, race, BMI, sex, and age might have had an impact on the results of our study.

4. Materials and Methods

4.1. Participants of the Study

The study was authorized by the Charité University Medicine's ethical committee, and signed informed consent was acquired. The research was appropriately registered: (ClinicalTrials.gov (accessed on 28 February 2019), Identifier: NCT03857984). Nine men and three non-pregnant women over the age of 18 were recruited if they had a history of renal failure demanding thrice-weekly HD. Patients had to have a stable HD prescription in order to participate in the trial. They had to be dialyzing by a native fistula or Gore-Tex® graft. Noncompliance with their dialysis prescription, anemia with hemoglobin (Hb) less than 8.0 g/dL, or an active infection were all exclusion criteria.

4.2. Assessment

All patients were treated while seated. Dialysis was performed on the subjects using a Polyflux 170H dialyzer (PAES membrane, Gambro, Lund, Sweden), with the ultrafiltration rate maintained constant throughout the HD sessions. All dialysis sessions had the same relevant dialysis treatment parameters (blood flow rate of 250 mL/min, dialysate flow rate of 500 mL/min, double needle puncture method, dialysis period of 4 h 15 min, and 37 °C dialysate temperature). Arterialized (shunt) blood samples were obtained on the fistula arm right before the start of dialysis (pre-HD) and at the end of dialysis (5–15 min before termination, post-HD). At the same time points, venous blood was taken on the ipsilateral limb through subcutaneous arm vein puncture to determine the arterio–venous (A–V) difference of the LCFAs. The A–V difference is noticeable since the peripheral tissues, particularly the muscles, generate, store, or decay a part of the LCFAs that circulate through them. All blood samples were collected via 4 °C precooled EDTA vacuum extraction tube systems. In a qualified clinical laboratory, glucose, lipoproteins, and triglycerides were measured with standardized techniques.

4.3. Plasma and RBC Membrane LCFAs Profile Analysis

The preparation and handling of samples, reference standards, and HPLC–MS measurements were performed as described elsewhere [32,33]. Plasma samples (200 µL), added with 300 µL of 10M sodium hydroxide (NaOH), were subjected to alkaline hydrolysis at 60 °C for 30 min. The sample pH was then adjusted to six using 300 µL 58% acetic acid. The prepared samples were then subjected to solid-phase extraction (SPE) using a Varian Bond Elut Certify II column. The extracted metabolites were evaluated by LC–MS/MS using an Agilent 6460 Triple Quad mass spectrometer (Agilent Technologies, Santa Clara, CA, USA) and an Agilent 1200 high-performance liquid chromatography (HPLC) system (degasser,

binary pump, well-plate sampler, thermostatic column chamber). A Phenomenex Kinetex column (150 mm 2.1 mm, 2.6 m; Phenomenex, Aschaffenburg, Germany) was used in the HPLC system. All samples were analyzed for total plasma and total RBC LCFAs.

4.4. Plasma and RBC Membrane LCFAs Cluster

The LCFA cluster was composed of 21 FAs which represent major components of plasma and erythrocyte membrane lipids. There were four main modules: SFAs [e.g., palmitic acid (PA) (C16:0) and stearic acid (C18:0)], MUFAs [e.g., palmitoleic acid (C16:1 n-7) and oleic acid (C18:1 n-9)], ω-3 PUFAs [e.g., EPA (C20:5 n-3) and DHA (C22:6 n-3)], and ω-6 PUFAs [e.g., linolenic acid (LA) (C18:2 n-6), arachidonic acid (AA) (C20:4 n-6)] (Figure 1) [34]. Taking into account the different meanings represented by the proportions of different FAs, the following indices were calculated: dietary n-6/n-3 ratio (n-3/n-6) [35] and omega-3 quotient [(EPA + DHA)/total FAs] [36,37]. Meanwhile, the enzymatic index of the desaturases that mainly synthesize MUFA and PUFA was calculated by the product/precursor ratio of the FAs participated: delta-6-desaturase (Δ6D C18:3 n-6/C18:2 n-6), delta-5-desaturase (Δ5D C20:4 n-6/C20:3 n-6), delta-9-desaturase (Δ9D C16:1 n-7/C16:0, C18:1 n-9/C18:0) and peroxisome function [DHA/DPA (docosapentaenoic acid)] [38] (Figure 1).

4.5. Statistical Analysis

Descriptive statistics were obtained, and variables were checked for skewness and kurtosis to ensure that they met the normal distribution assumptions. To check if the data were normally distributed, we utilized the Shapiro–Wilk test. Levene's test was used to demonstrate variance homogeneity. To evaluate statistical significance, arterial vs. venous values were compared using the paired *t*-test or the paired Wilcoxon test. The statistical significance value was set at $p < 0.05$. All data are displayed as median and interquartile range (IQR) or standard deviation (SD). All statistical analyses were performed using SPSS Statistics software (IBM Corporation, Armonk, NY, USA).

5. Conclusions

Our data demonstrate that HD affects RBC status by decumulating numerous LCFAs during passage through peripheral tissues, at least in the upper limbs. These changes may contribute to the increased cardiovascular risk in ESRD patients. Interestingly, RBCs, EPA, and DHA levels are not affected by single HD treatment.

Supplementary Materials: The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/metabo12030269/s1: Table S1. Clinical parameters of patients (n = 12 each), Table S2. Effect of hemodialysis on polyunsaturated fatty acid ratios in venous plasma and RBCs in the CKD patients before (pre-HD) and at cessation (post-HD) of hemodialysis (n = 12 each). Median (IQR), Table S3. Effect of hemodialysis on the ratio of desaturase and peroxisome function in venous plasma and RBC in the CKD patients before (Pre-HD) and at cessation (Post-HD) of hemodialysis (n = 12 each). Median (IQR).

Author Contributions: B.G., M.G. and F.C.L. planned and designed the experimental studies. T.L., I.D. and M.R. performed the HPLC–MS spectrometry experiments. All authors contributed to the implementation and analyses of the experiments. T.L. and B.G. drafted the article, and all authors contributed to its completion. All authors have read and agreed to the published version of the manuscript.

Funding: The Deutsche Forschungsgemeinschaft (DFG) supported F.C.L. (LU 435/13-1). We express our thanks to the DFG for continuous support. We also acknowledge support from the Open Access Publication Fund of Charité—Universitätsmedizin Berlin.

Institutional Review Board Statement: The study was conducted according to the guidelines of the Declaration of Helsinki and approved by the Institutional Review Board (or Ethics Committee) of the ethical committee of the Charité—Universitätsmedizin Berlin (protocol code EA4/110/19; date of approval 4 September 2020).

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: Data is contained within the article or Supplementary Materials.

Acknowledgments: We wish to thank all patients for participating in this study. We thank Julius V. Kunz for helping us collect some blood samples.

Conflicts of Interest: The authors declare no conflict of interest.

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Supplementary Information

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Bioaccumulation of Blood Long-Chain Fatty Acids during Hemodialysis

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Table S1 Clinical parameters of patients (n=12).

Parameters	Patients
Glucose (60-110 mg/dL)	115.8 ± 36.7
Total cholesterol (< 200 mg/dL)	221.8 ± 181.2
LDL-cholesterol (< 130 mg/dL)	101.2 ± 30.7
HDL- cholesterol (>35 mg/dL)	42.4 ± 9.9
Triglycerides (<200 mg/dL)	151.0 ± 77.4

Notes: Data are presented as mean ± SD.

Table S2. Effect of hemodialysis on polyunsaturated fatty acid ratios in venous plasma and RBC in the CKD patients before (Pre-HD) and at cessation (Post-HD) of hemodialysis (n=12 each).

Ratio	pre-HD Venous	post-HD Venous	p value, t test (# paired Wilcoxon test)
Total-Plasma			
DHA+EPA/AA	0.7514 (0.6267 –0.8752)0	0.6516 (0.5946 –0.8166)0	0.127
EPA/AA	.0983 (0.0700 –0.1537)0.	.0806 (0.0715 –0.1303)0.	0.286#0
DHA/AA	6359 (0.5586 –0.6793)6.	5780 (0.5115 –0.6609)6.	.182#
DHA/EPA	5543 (5.1800 –7.4374)0.	5892 (5.8563 –7.3498)0.	0.793
n-3/n-6	1834 (0.1511 –0.2118)	1651 (0.1541 –0.2176)	0.81
Total-RBC			
Omega-3 quotient	11.3752 (7.7099 –12.1713)	11.3738 (8.3040 –12.5440)	0.676
DHA+EPA/AA	0.6547 (0.5208 –0.9044)	0.7027 (0.6151 –0.8107)	0.826
EPA/AA	0.0341 (0.0295 –0.0575)	0.0421 (0.0335 –0.0512)	0.404
DHA/AA	0.6202 (0.5050 –0.8469)	0.6605 (0.5833 –0.7487)	0.89
DHA/EPA	15.2611 (14.5957 – 21.7166)	17.3803 (13.4656 – 20.3596)	0.433#
n-3/n-6	0.4098 (0.3518 –0.5048)	0.4095 (0.3878 –0.4616)	0.701

Notes: Median (IQR)

Table S3. Effect of hemodialysis on the ratio of desaturase and peroxisome function in venous plasma and RBC in the CKD patients before (Pre-HD) and at cessation (Post-HD) of hemodialysis (n=12 each).

Ratio	pre-HD Venous	post-HD Venous	p value, t test (# paired Wilcoxon test)
Total-Plasma			
DHA/DPA	8.9873 (7.0171 –10.7715)	9.0468 (6.5445 –13.8399)	0.468
C20:4 n-6/C20:3 n-6 (Δ5 SCD)	3.5725 (3.2953 –4.0303)	3.9063 (3.3146 –4.7591)	0.088
C18:3 n-6/C18:2 n-6 (Δ6 SCD)	0.0149 (0.0102 –0.0185)	0.0167 (0.0112 –0.0204)	0.556#
C16:1 n-7/C16:0 (Δ9 SCD)	0.0880 (0.0808 –0.1015)	0.0859 (0.0763 –0.0921)	0.053
C18:1 n-9/C18:0 (Δ9 SCD)	5.7640 (4.6422 –7.0182)	6.0049 (4.9686 –6.2736)	0.599
Total-RBC			
DHA/DPA	5.0147 (3.5409 –5.9286)	4.9854 (4.3682 –5.8391)	0.877
C20:4 n-6/C20:3 n-6 (Δ5 SCD)	8.0572 (6.6672 –10.3164)	8.1449 (7.5088 –10.8905)	0.814#
C18:3 n-6/C18:2 n-6 (Δ6 SCD)	0.0108 (0.0067 –0.0131)	0.0110 (0.0068 –0.0136)	0.429
C16:1 n-7/C16:0 (Δ9 SCD)	0.0437 (0.0328 –0.0569)	0.0479 (0.0380 –0.0574)	0.371
C18:1 n-9/C18:0 (Δ9 SCD)	1.2582 (0.9138 –1.5227)	1.3379 (1.1765 –1.4844)	0.275

Notes: Median (IQR)

metabolites

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Article

Hemodialysis and Plasma Oxylipin Biotransformation in Peripheral Tissue

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Abstract: Factors causing the increased cardiovascular morbidity and mortality in hemodialysis (HD) patients are largely unknown. Oxylipins are a superclass of lipid mediators with potent bioactivities produced from oxygenation of polyunsaturated fatty acids. We previously assessed the impact of HD on oxylipins in arterial blood plasma and found that HD increases several oxylipins. To study the phenomenon further, we now evaluated the differences in arterial and venous blood oxylipins from patients undergoing HD. We collected arterial and venous blood samples in upper extremities from 12 end-stage renal disease (ESRD) patients before and after HD and measured oxylipins in plasma by LC-MS/MS tandem mass spectrometry. Comparison between cytochrome P450 (CYP), lipoxygenase (LOX), and LOX/CYP ω/(ω-1)-hydroxylase metabolites levels from arterial and venous blood showed no arteriovenous differences before HD but revealed arteriovenous differences in several CYP metabolites immediately after HD. These changes were explained by metabolites in the venous blood stream of the upper limb. Decreased soluble epoxide hydrolase (sEH) activity contributed to the release and accumulation of the CYP metabolites. However, HD did not affect arteriovenous differences of the majority of LOX and LOX/CYP ω/(ω-1)-hydroxylase metabolites. The HD treatment itself causes changes in CYP epoxy metabolites that could have deleterious effects in the circulation.

Keywords: hemodialysis; eicosanoids; lipidomics; oxylipins; erythrocyte; arterio-venous; biotransformation

Citation: Liu, T.; Dogan, I.; Rothe, M.; Kunz, J.V.; Knauf, F.; Gollasch, M.; Luft, F.C.; Gollasch, B. Hemodialysis and Plasma Oxylipin Biotransformation in Peripheral Tissue. *Metabolites* **2022**, *12*, 34. https://doi.org/10.3390/metabo12010034

Academic Editor: Manfredi Rizzo

Received: 15 December 2021
Accepted: 30 December 2021
Published: 4 January 2022

1. Introduction

Survival rates among end-stage renal disease (ESRD) hemodialysis (HD) patients are poor, and excess death rate is related to cardiovascular disease [1,2]. Lipids are essential for many functions in the body, where they serve as integral components for cellular membranes as well as energy storage and signaling molecules [3]. Polyunsaturated fatty acids (PUFA) are metabolized by different enzymes, mainly cytochromes P450 (CYP), monooxygenase, cyclooxygenase (COX), and lipoxygenase (LOX)/CYP ω/(ω-1)-hydroxylase pathways, which can produce a large superclass of biologically active substances, namely oxylipins (Figure 1).

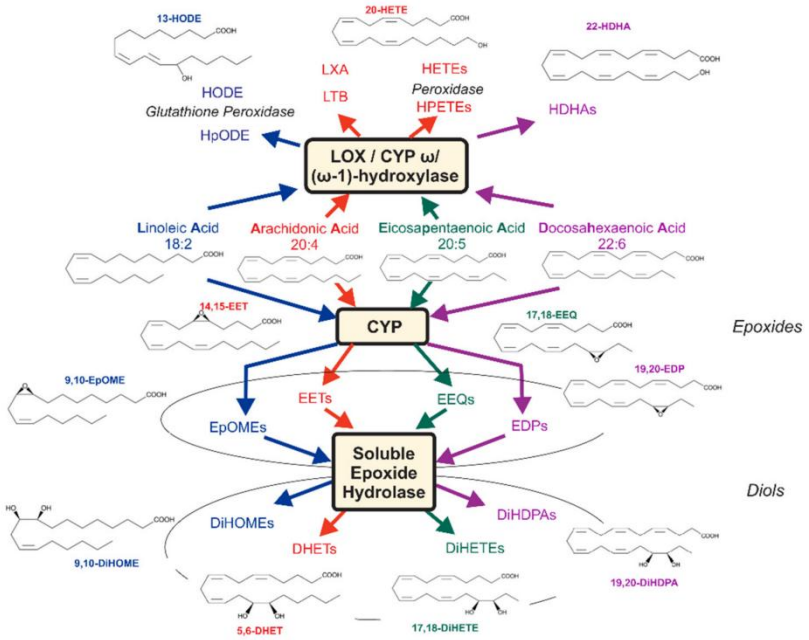

Figure 1. Assessment of cytochrome P450 epoxygenase (CYP) and 12- and 15-lipoxygenase (LOX)/CYP (omega-1)-hydroxylase pathways in response to hemodialysis treatment. Arachidonic (AA), linoleic (LA), eicosapentaenoic (EPA), and docosahexaenoic acids (DHA) are converted to epoxyoctadecenoic acids (EpOMEs, e.g., 9,10-EpOME), epoxyeicosatetraenoic acids (EEQs, e.g., 17,18-EEQ), epoxyeicosatrienoic acid (EETs, e.g., 5,6-EET), and epoxydocosapentaenoic acids (EDPs, e.g., 19,20-EDP) by CYP epoxygenase, respectively. EpOMEs, EETs, EEQs, and EDPs are primarily converted to dihydroxyctadecenoic acids (DiHOMEs), dihydroxyeicosatrienoic acids (DHETs, e.g., 5,6-DHET), dihydroxyeicosatetraenoic acids (DiHETEs, e.g., 17,18-DiHETE), and dihydroxydocosapentaenoic acids (DiHDPAs, e.g., 19,20-DiHDPA) by the soluble epoxide hydrolase (sEH). LA, EPA, AA, and DHA are converted to hydroperoxylinoleic acids (HpODEs), hydroxyoctadecadienoic acids (HODEs), leukotriene B (LTB), lipoxin A (LXA), hydroxydocosahexaenoic acids (HDHAs), hydroperoxyeicosatetraenoic acids (HPETEs), and hydroxyeicosatetraenoic acids (HETEs) by LOX, CYP omega/(omega-1)-hydroxylase, and peroxidase pathways. The metabolites measured in these metabolic pathways follow the changes observed in LA, AA, EPA, and DHA metabolism, respectively. Modified from [4].

Arachidonic acid (AA)-derived oxylipins, especially those derived from the CYP450 pathway, produce vasodilation and exhibit pro-angiogenic, anti-inflammatory, and cardioprotective effects [5]. Docosahexaenoic acid (DHA)-derived 19, 20-epoxydocosapentaenoic acid (EDP) and eicosapentaenoic acid (EPA)-derived 17, 18-epoxyeicosatetraenoic acid (EEQ) exert anti-arrhythmic effects by inhibiting Ca^{+} and isoproterenol-induced increase in cardiomyocyte contractility [6]. Beyond, many biological functions of this large group of widely still unknown lipids are undiscovered. Moreover, the main metabolic pathway of oxylipins is that a major part is hydrolyzed by soluble epoxide hydrolase (sEH) to biologically less-active diols [7]. The other part is re-esterified into phospholipids as a temporary storage pool of oxylipins that can be rapidly mobilized to exert biological effects when the organism receives a stimulus [8]. Another important source of circulating esterified oxylipins are lipoprotein-bound oxylipins, for example, very low-density lipoproteins (VLDL), which bind to cell surface lipoprotein lipases when they reach tissue cells [9]. In any case, esterification is important both in also removing free oxylipins signals and in

enabling direct effects of esterified oxylipins. Currently, their specific mechanisms of action are largely unknown.

In previous studies, we established the lipidomics approach for the analysis of oxylipins in human blood. We demonstrated that there are specific patterns of oxylipins profiles in peripheral blood released during short-term maximal cardiovascular stress (stress ergometry) that may influence cardiovascular function [10,11]. We also observed that hemodialysis treatment increased plasma levels of CYP epoxy-metabolites but did not change the majority of LOX/CYP ω/(ω-1)-hydroxylase metabolites [4]. The changes primarily affected esterified metabolites, whereas no significant differences were observed in free metabolites [4].

The arteriovenous oxygen difference is the difference in the oxygen content of the blood between the arterial blood and the venous blood. Knowing this difference aids in clinical diagnostics of how much oxygen is removed from the blood in capillaries as the blood circulates in the body. Uremic patients undergoing hemodialysis present pre-HD with high ammonia levels in arterial blood with a significantly positive arteriovenous difference [12]. The arteriovenous blood sugar content is due to the fact that the peripheral tissues, especially the muscles, either store or burn part of the glucose that passes through them [13]. The same is true for non-esterified fatty acids [14], which can develop positive or negative arteriovenous differences depending on patient's physical or health status and organs perfused [13,14]. Moreover, arteriovenous differences in plasma NO_2^- levels (as an index of endothelial nitric oxide (NO) formation) can detected in NO_2^- loading conditions and exercise [15].

To gain information on lipid biotransformation, we 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 (Figure 2). We performed the experiments to better understand biotransformation of the metabolites in vivo, particularly whether or not the peripheral tissues, especially the muscles in the upper limbs, either produce, store, or degrade part of the epoxy-metabolites that pass through them. We were particularly interested to clarify which arteriovenous relationships are present in plasma oxylipin profiles during hemodialysis and in what way they are modified by extracorporeal renal replacement therapy, which causes oxidative stress, chronic inflammation, and red blood cell–endothelial interactions (cf. circled arrows in Figure 2).

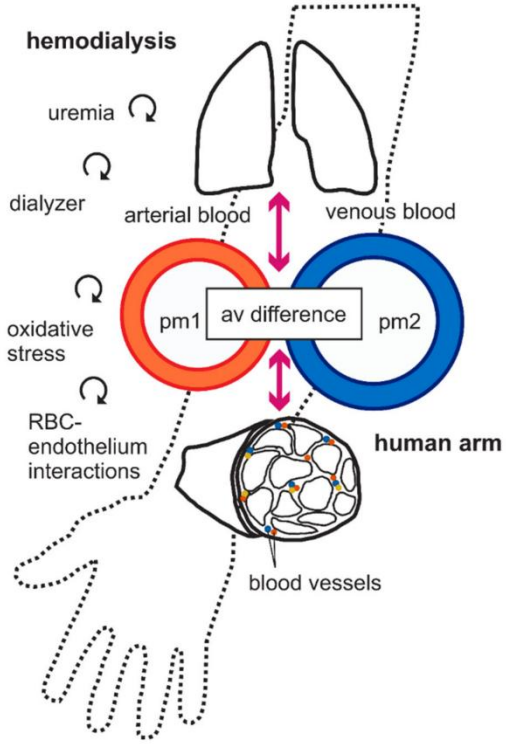

Figure 2. A simplified scheme of the relationship among different compartments. Central and peripheral compartments are shown. Central compartment is consisting of plasma, pm1 and pm2. Peripheral compartments are consisting of organ tissues, especially arm muscle, with extracellular fluid, red blood cells (RBCs), etc. The continuity between arterial and venous systems via pulmonary (top) and peripheral (bottom) arm muscle is illustrated in the diagram. Arterial and venous blood samples were taken before (pre-HD) and after HD (post-HD) treatment. Plasma oxylipins were measured in pm1 and pm2, i.e., arterial shunt and subcutaneous vein, respectively. It is obvious that the arteriovenous (av) difference is caused by the circumstance that the peripheral tissues, especially the muscles, either produce, store, or degrade part of the oxylipins that pass through them. The curved vector graphs represent the hypothetical influence of CKD and hemodialysis in conjunction with shear stress, dialyzer, red blood cell (RBC)-endothelial interactions, and oxidative stress affecting plasma oxylipin biotransformation levels in peripheral tissues.

2. Results

2.1. Clinical Characteristics

The clinical features of ESRD hemodialysis (HD) patients are summarized in Table 1. The patients had focal segmental glomerulosclerosis (six patients), ADPKD (autosomal dominant polycystic kidney disease) (one patient), IgA nephropathy (one patient), hypertensive nephropathy (one patient), renal amyloidosis (one patient), drug-induced kidney injury (one patient), and cystic kidneys (one patient). All patients had major cardiovascular complications, such as cardiovascular and cerebrovascular events, and/or peripheral artery disease. Table S1 shows that the patients were not diabetic but had hyperlipidemia.

Table 1. Clinical features 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
Race (n)	Caucasian = 12
Cause of end-stage renal disease	
Focal segmental glomerulosclerosis (n)	6
IgA nephropathy (n)	1
Renal amyloidosis (n)	1
Hypertension (n)	1
Drug induced (n)	1
ADPKD (n)	1
Cystic kidneys (n)	1
Complications	
Cardiovascular (n)	12

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

2.2. Effects of Hemodialysis

2.2.1. Pre-HD

The effects of hemodialysis treatment on plasma oxylipins in our patients are shown (Table 2 and Table S2). With exception of 11-HETE and 13-HODE, comparison between total CYP, LOX and LOX/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 CYP epoxy-metabolites were not significantly different in arterial vs. venous blood (Table 2). These metabolites included 5,6-EET, 8,9-EET, 11,12-EET, 14,-15-EET, 5,6-DHET, 8,9-DHET, 11,12-DHET, 14,15-DHET, 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, 9,10-EpOME, 12,13-EpOME, 9,10-DiHOME, 12,13-DiHOME, 5-HETE, 8-HETE, 9-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, 9-HODE, 16-HETE, 17-HETE, 18-HETE, 19-HETE, 20-HETE, 22-HDHA, and 20-HEPE (Table 2).

Figure 1 shows that the main pathway of EET, EpOME, EEQ, and EDP biotransformation in many cells is conversion to DHETs, DiHOMEs, DiHETEs, and DiHDPAs. This is achieved by the soluble epoxide hydrolase enzyme (sEH). Since ESRD might have caused EET, EpOME, EEQ, and EDP production rapidly degraded to their diols, we next analyzed the sums of the individual CYP epoxy-metabolites and their diols (Table 3). 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 metabolites (Table 3). Moreover, we calculated diol/epoxide ratios of the epoxy-metabolites (Table 3) and found that the four classes of epoxy-metabolites are unequally hydrolyzed to appear in the arterial circulation. We found that EEQs are better metabolized into their diols (ratio of DiHETEs/EEQs; 0.6541 ± 0.4424) than EETs, EDPs, and EPOMEs (ratios of those diols/epoxy-metabolites, 0.1104 ± 0.0878, 0.1369 ± 0.1095, and 0.2017 ± 0.0845, 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 individual metabolites in the venous blood, as shown (Table 3). Together, the findings indicate that CYP epoxy-metabolites are unequally hydrolyzed by sEH in arterial and venous

blood in vivo [4]. However, there is no arteriovenous difference in the epoxy-metabolites before hemodialysis.

2.2.2. Post-HD

Similar to our previous study [4], hemodialysis treatment caused an increase in a number of epoxy-metabolites in arterial blood, including 5,6-EET, 8,9-EET, 11,12-EET,14,15-EET, 8,9-EEQ, 11,12-EEQ, 9,10-EpOME, 12,13-EpOME, 9-HODE, and 13-HODE. The levels of other CYP, LOX, and LOX/CYP $\omega/(\omega-1)$-hydroxylase metabolites were unchanged (Table S2A).

However, the effects were more obvious in venous blood. Here, we first compared the individual CYP epoxy-metabolites and their diols (Table S2B). We observed that hemodialysis increased 5,6-EET, 8,9-EET, 11,12-EET, 14,15-EET, 5,6-DHET, 8,9-DHET, 7,8-EDP, 10,11-EDP, 13,14-EDP, 16,17-EDP, 19,20-EDP, 5,6-EEQ, 8,9-EEQ, 11,12-EEQ, 14,15-EEQ, 17,18-EEQ, 5,6-DiHETE, 9,10-EPOME, 12,13-EPOME, 9,10-DiHOME, 12,13-DiHOME, 5-HETE, 12-HETE, 12-HEPE, 9-HODE, 13-HODE, and 22-HDHA levels in venous blood (Table S2B). The levels of other CYP, LOX, and LOX/CYP $\omega/(\omega-1)$-hydroxylase metabolites were unchanged (Table S2B). As next step, we calculated the sums of the individual CYP epoxy-metabolites and their diols (Table 3) and compared the levels between arterial and venous blood (Table 3). The data show that the four classes of CYP metabolites (i.e., EET, EpOME, EEQ, and EDP plus their respective diols) were more prominently accumulated in venous blood compared to arterial blood (Table 3); i.e., we detected negative arteriovenous differences in 5,6-EET, 8,9-EET, 11,12-EET, 14,15-EET, 8,9-DHET, 11,12-DHET, 7,8-EDP, 10,11-EDP, 14,15-EDP, 16,17-EDP, 19,20-EDP, 7,8-DiHDPA, 10,11-DiHDPA, 5,6-EEQ, 8,9-EEQ, 11,12-EEQ, 14,15-EEQ, 17,18-EEQ, 9,10-EPOME, 12,13-EPOME, 12-HETE, and 17-HDHA (Table 2). This decrease was not seen for free epoxy-metabolites, with the exception of 5,6-DiHETE and 14,15-EET (Table 4). Thus, it is unlikely that the decrease in arteriovenous differences of total epoxy-metabolites is caused by metabolites in free state. The arteriovenous differences of other CYP, LOX, and LOX/CYP $\omega/(\omega-1)$-hydroxylase metabolites were unchanged (Table 2).

Together, the findings indicate that hemodialysis increases all four classes of CYP eicosanoids, particularly in venous blood, to cause negative arteriovenous differences of a number of CYP metabolites after the dialysis treatment (post-HD).

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

Amount ng/mL	Pre-HD A (Mean ± SD)	Pre-HD V (Mean ± SD)	p-Value, t-Test (# Paired Wilcoxon Test)	Pre-HD A-V Difference (Mean ± SD)	Post-HD A (Mean ± SD)	Post-HD V (Mean ± SD)	p-Value, t-Test (# Paired Wilcoxon Test)	Post-HD A-V Difference (Mean ± SD)
CYP epoxy-metabolites								
5,6-EET	25.2403 ± 22.8032	16.8013 ± 15.3681	0.325	8.1596 ± 22.0848	**37.0725 ± 16.6846**	**61.8386 ± 21.0905**	**0.003 #**	**−24.7661 ± 17.4189**
8,9-EET	10.5227 ± 10.8994	7.2706 ± 6.6772	0.806	3.1316 ± 11.4525	**13.3085 ± 5.6504**	**22.3969 ± 7.8620**	**0.002 #**	**−9.0884 ± 6.7881**
11,12-EET	9.9539 ± 10.0802	7.0358 ± 6.8420	0.806	2.7042 ± 10.2183	**14.2304 ± 6.6762**	**25.6937 ± 9.1845**	**0.002 #**	**−11.4633 ± 7.4968**
14,15-EET	12.5615 ± 12.5408	9.6655 ± 9.4944	0.538	2.6286 ± 12.4557	**19.0872 ± 9.2999**	**35.6871 ± 13.5842**	**0.002 #**	**−16.6000 ± 10.9006**
5,6-DHET	1.1450 ± 0.5549	1.0727 ± 0.5911	0.389	0.1175 ± 0.1770	1.3684 ± 0.5945	1.2782 ± 0.6163	0.177 #	0.0901 ± 0.2165
8,9-DHET	1.7434 ± 1.5788	1.6063 ± 1.3596	0.806	0.1581 ± 0.4252	**1.6086 ± 1.1218**	**1.2535 ± 0.9769**	**0.004 #**	**0.3550 ± 0.3980**
11,12-DHET	0.4366 ± 0.2207	0.4188 ± 0.1959	0.902	0.0311 ± 0.0878	**0.5221 ± 0.1967**	**0.4683 ± 0.1888**	**0.005**	**0.0538 ± 0.0534**
14,15-DHET	0.4159 ± 0.0996	0.3721 ± 0.0788	0.252	0.0480 ± 0.0902	0.4919 ± 0.1251	0.4212 ± 0.1045	0.132	0.0708 ± 0.1505
7,8-EDP	4.5236 ± 4.3719	3.2930 ± 3.1818	0.951	1.1887 ± 4.7923	**6.0064 ± 2.6897**	**10.4413 ± 3.5636**	**0.002 #**	**−4.4349 ± 2.2351**
10,11-EDP	4.4784 ± 4.4009	3.3370 ± 3.0863	0.806	1.0517 ± 4.5614	**6.6357 ± 3.0803**	**11.9332 ± 3.9274**	**<0.001**	**−5.2975 ± 2.5243**
13,14-EDP	3.3860 ± 3.4990	2.6621 ± 2.8208	0.806	0.6491 ± 3.6773	**4.8226 ± 2.2061**	**7.8177 ± 2.6177**	**<0.001**	**−2.9952 ± 1.9444**
16,17-EDP	2.9692 ± 2.8814	2.1926 ± 2.0367	0.806	0.7541 ± 2.8457	**3.8342 ± 1.6915**	**5.5352 ± 2.0151**	**0.008**	**−1.7010 ± 1.8424**
19,20-EDP	5.5558 ± 5.3859	3.9685 ± 3.1487	1	1.4739 ± 5.6060	**8.6761 ± 3.8647**	**16.3124 ± 6.2669**	**0.002 #**	**−7.6363 ± 3.7823**
7,8-DiHDPA	0.4489 ± 0.2473	0.4110 ± 0.2301	0.498	0.0405 ± 0.0429	**0.4905 ± 0.2287**	**0.4461 ± 0.2152**	**0.021**	**0.0444 ± 0.0571**
10,11-DiHDPA	0.1277 ± 0.0387	0.1210 ± 0.0346	0.664	0.0081 ± 0.0169	**0.1439 ± 0.0452**	**0.1291 ± 0.0539**	**0.031**	**0.0148 ± 0.0207**
13,14-DiHDPA	0.0892 ± 0.0274	0.0846 ± 0.0203	0.65	0.0051 ± 0.0114	0.0989 ± 0.0354	0.1040 ± 0.0439	0.55	−0.0051 ± 0.0285
16,17-DiHDPA	0.0926 ± 0.0361	0.0947 ± 0.0314	0.886	−0.0012 ± 0.0111	0.1160 ± 0.0464	0.1042 ± 0.0480	0.168	0.0118 ± 0.0277
19,20-DiHDPA	0.8379 ± 0.4051	0.8262 ± 0.3658	0.943	0.0383 ± 0.0710	0.9534 ± 0.4118	0.9134 ± 0.4345	0.393	0.0400 ± 0.1559
5,6-EEQ	0.0051 ± 0.0039	0.0034 ± 0.0026	0.23	−0.0064 ± 0.0048	**0.0084 ± 0.0052**	**0.0148 ± 0.0050**	**0.004 #**	**0.0012 ± 0.0042**
8,9-EEQ	1.4478 ± 1.2289	0.9972 ± 0.6972	0.498	0.4347 ± 1.3829	**2.6063 ± 1.7732**	**4.3902 ± 1.5836**	**0.008 #**	**−1.7839 ± 1.6485**
11,12-EEQ	0.9541 ± 0.8310	0.7197 ± 0.4820	0.806	0.2294 ± 0.9263	**1.8077 ± 1.2340**	**3.4951 ± 1.3735**	**0.003 #**	**−1.6874 ± 1.1790**
14,15-EEQ	0.9436 ± 0.7146	0.7047 ± 0.4467	0.343 #	0.2231 ± 0.8184	**1.7544 ± 1.2152**	**3.2728 ± 1.3192**	**0.004 #**	**−1.5184 ± 1.2985**
17,18-EEQ	1.6849 ± 1.3879	1.3915 ± 0.8490	0.543 #	0.2583 ± 1.4915	**2.9621 ± 2.1045**	**6.2605 ± 2.2755**	**0.002 #**	**−3.2984 ± 2.0268**
5,6-DiHETE	1.5299 ± 0.9299	1.3695 ± 0.7134	0.622 #	0.3200 ± 0.8708	2.0357 ± 1.4971	1.6895 ± 0.8335	0.53 #	0.3461 ± 1.2824
8,9-DiHETE	0.0908 ± 0.0378	0.0908 ± 0.0317	0.667 #	0.0061 ± 0.0374	0.1085 ± 0.0552	0.0960 ± 0.0386	0.36	0.0125 ± 0.0455
11,12-DiHETE	0.0476 ± 0.0379	0.0356 ± 0.0100	0.806	0.0128 ± 0.0418	0.0596 ± 0.0600	0.0372 ± 0.0119	0.099 #	0.0224 ± 0.0633
14,15-DiHETE	0.0528 ± 0.0201	0.0462 ± 0.0135	0.364 #	0.0075 ± 0.0205	0.0538 ± 0.0249	0.0487 ± 0.0203	0.49	0.0051 ± 0.0248
17,18-DiHETE	0.2149 ± 0.0825	0.2141 ± 0.0836	0.981 #	0.0030 ± 0.0659	0.2784 ± 0.1176	0.2454 ± 0.1082	0.253	0.0330 ± 0.0948
9,10-EpOME	33.0227 ± 31.0831	22.5987 ± 12.9602	0.806	9.9686 ± 32.0264	**51.7226 ± 18.8433**	**93.5874 ± 36.4954**	**0.002 #**	**−41.8648 ± 26.9869**
12,13-EpOME	30.9825 ± 26.7698	20.7024 ± 11.0768	0.46	9.7907 ± 27.7448	**46.9109 ± 15.0402**	**79.7224 ± 23.6841**	**<0.001**	**−32.8115 ± 15.3972**
9,10-DiHOME	4.1455 ± 1.7899	3.2993 ± 1.7108	0.085	0.7458 ± 0.9866	5.5991 ± 2.7877	5.6467 ± 2.4652	0.954 #	−0.0476 ± 2.7904
12,13-DiHOME	4.9809 ± 1.9437	3.8968 ± 1.6289	0.161 #	0.9906 ± 1.6559	6.9594 ± 3.7873	6.9340 ± 3.3774	0.875 #	0.0254 ± 2.8341

Table 2. *Cont.*

Amount ng/mL	Pre-HD A (Mean ± SD)	Pre-HD V (Mean ± SD)	*p*-Value, *t*-Test (# Paired Wilcoxon Test)	Pre-HD A-V Difference (Mean ± SD)	Post-HD A (Mean ± SD)	Post-HD V (Mean ± SD)	*p*-Value, *t*-Test (# Paired Wilcoxon Test)	Post-HD A-V Difference (Mean ± SD)
LOX metabolites								
5-HETE	10.1945 ± 2.9568	8.9008 ± 2.5521	0.273 #	1.3211 ± 1.9205	11.6499 ± 3.0694	11.2376 ± 3.5321	0.53 #	0.4123 ± 3.1451
8-HETE	3.1627 ± 0.8646	2.9701 ± 0.9371	0.46 #	0.1954 ± 0.4666	3.5068 ± 1.3221	2.9656 ± 1.1078	0.185	0.5413 ± 1.3245
9-HETE	6.2378 ± 2.4916	5.0812 ± 2.0444	0.235 #	1.1362 ± 1.8189	6.6115 ± 2.5626	5.7522 ± 2.1829	0.209 #	0.8593 ± 2.1644
11-HETE	**4.3689 ± 1.3795**	**3.4094 ± 1.1668**	**0.027**	0.9124 ± 0.8519	4.5454 ± 1.4621	4.0433 ± 1.4575	0.071 #	0.5021 ± 1.5746
12-HETE	5.1343 ± 1.7200	5.8545 ± 2.6463	0.452 #	−0.7233 ± 1.8003	**6.0063 ± 2.3467**	**4.7972 ± 2.3597**	**0.023 #**	**1.2091 ± 1.8582**
15-HETE	6.1984 ± 1.9171	4.9051 ± 1.5388	0.085	1.2806 ± 1.1112	6.1869 ± 1.8919	5.7417 ± 3.2394	0.06 #	0.4452 ± 2.9529
4-HDHA	2.3149 ± 0.6472	2.1630 ± 0.5977	0.565 #	0.1653 ± 0.5413	2.4431 ± 0.9794	2.5609 ± 1.2943	0.875 #	−0.1178 ± 0.8863
7-HDHA	2.1060 ± 0.6072	1.7319 ± 0.5209	0.11	0.3892 ± 0.4553	2.1783 ± 0.8143	2.0061 ± 0.6765	0.259	0.1722 ± 0.5011
8-HDHA	1.2263 ± 0.4487	1.0900 ± 0.3821	0.44	0.1357 ± 0.1780	1.2990 ± 0.5943	1.1141 ± 0.4935	0.182 #	0.1849 ± 0.5362
10-HDHA	0.7721 ± 0.2512	0.6877 ± 0.2059	0.387	0.0793 ± 0.0990	0.7940 ± 0.3187	0.7020 ± 0.2701	0.196	0.0920 ± 0.2312
11-HDHA	1.0995 ± 0.3769	1.0493 ± 0.3586	0.902	0.0545 ± 0.2055	1.0862 ± 0.4291	1.0103 ± 0.3687	0.136 #	0.0758 ± 0.4079
13-HDHA	0.9168 ± 0.2861	0.8168 ± 0.2303	0.364	0.0963 ± 0.1441	0.9169 ± 0.3765	0.8015 ± 0.3238	0.259 #	0.1154 ± 0.3361
14-HDHA	1.1312 ± 0.4384	1.0678 ± 0.4203	0.727	0.0541 ± 0.2125	1.1712 ± 0.5441	0.9981 ± 0.5062	0.084 #	0.1732 ± 0.3398
16-HDHA	1.1430 ± 0.3468	0.9559 ± 0.3037	0.182	0.1782 ± 0.1994	1.1459 ± 0.4227	1.0653 ± 0.4675	0.117 #	0.0806 ± 0.4293
17-HDHA	1.4069 ± 0.4032	1.1633 ± 0.3601	0.141	0.2414 ± 0.2051	**1.4601 ± 0.5780**	**1.2773 ± 0.5100**	**0.041 #**	**0.1828 ± 0.5168**
20-HDHA	2.8698 ± 0.7707	2.4726 ± 0.7079	0.212 #	0.3802 ± 0.4872	2.9700 ± 0.9600	2.7939 ± 1.1603	0.084 #	0.1761 ± 0.9553
5-HEPE	1.4608 ± 0.5576	1.3111 ± 0.4406	0.481	0.1804 ± 0.3543	1.5968 ± 0.9534	1.4799 ± 0.6337	0.875 #	0.1170 ± 0.8054
8-HEPE	0.2118 ± 0.0669	0.1959 ± 0.0421	0.712	0.0193 ± 0.0428	0.2061 ± 0.1012	0.1817 ± 0.0752	0.329 #	0.0244 ± 0.0829
9-HEPE	0.4018 ± 0.1460	0.3661 ± 0.1209	0.528	0.0400 ± 0.1177	0.3664 ± 0.2280	0.3335 ± 0.1318	0.875 #	0.0329 ± 0.1871
11-HEPE	0.2752 ± 0.0907	0.2520 ± 0.0448	0.538	0.0217 ± 0.0697	0.2531 ± 0.1150	0.2364 ± 0.0928	0.388 #	0.0167 ± 0.1077
12-HEPE	0.5039 ± 0.1762	0.5213 ± 0.1704	0.812	−0.0162 ± 0.1369	0.4619 ± 0.2576	0.4038 ± 0.1731	0.638 #	0.0580 ± 0.2070
15-HEPE	0.3150 ± 0.1086	0.3142 ± 0.0634	0.983	0.0029 ± 0.0850	0.2890 ± 0.1524	0.2596 ± 0.1144	0.433 #	0.0294 ± 0.1342
18-HEPE	0.8580 ± 0.2946	0.8418 ± 0.2239	0.883 #	0.0156 ± 0.2704	0.7714 ± 0.3717	0.7502 ± 0.4476	0.53 #	0.0212 ± 0.3909
9-HODE	22.0631 ± 7.3185	17.1239 ± 6.0628	0.091 #	4.7896 ± 3.9253	36.2855 ± 21.5709	34.0942 ± 21.6186	0.071 #	2.1913 ± 11.7721
13-HODE	**17.7783 ± 6.0021**	**13.5081 ± 4.2496**	**0.012**	4.0845 ± 3.1626	25.0606 ± 10.8578	25.1650 ± 13.2212	0.48 #	−0.1044 ± 8.7534

Table 2. *Cont.*

Amount ng/mL	Pre-HD A (Mean ± SD)	Pre-HD V (Mean ± SD)	*p*-Value, *t*-Test (# Paired Wilcoxon Test)	Pre-HD A-V Difference (Mean ± SD)	Post-HD A (Mean ± SD)	Post-HD V (Mean ± SD)	*p*-Value, *t*-Test (# Paired Wilcoxon Test)	Post-HD A-V Difference (Mean ± SD)
CYP ω/(ω−1) metabolites								
16-HETE	0.2346 ± 0.0507	0.2175 ± 0.0583	0.462 #	0.0186 ± 0.0590	0.2194 ± 0.0562	0.2184 ± 0.0828	0.875 #	0.0010 ± 0.0660
17-HETE	0.0565 ± 0.0112	0.0560 ± 0.0145	0.922 #	0.0010 ± 0.0109	0.0602 ± 0.0182	0.0517 ± 0.0147	0.071 #	0.0085 ± 0.0161
18-HETE	0.1620 ± 0.0463	0.1499 ± 0.0282	0.538 #	0.0154 ± 0.0455	0.1651 ± 0.0450	0.1493 ± 0.0421	0.272 #	0.0158 ± 0.0416
19-HETE	0.1441 ± 0.0810	0.1354 ± 0.0411	0.854	0.0133 ± 0.0689	0.1599 ± 0.0533	0.1662 ± 0.0571	0.693	−0.0064 ± 0.0546
20-HETE	0.4738 ± 0.2011	0.3992 ± 0.2211	0.408 #	0.0787 ± 0.1668	0.4803 ± 0.1680	0.4787 ± 0.2014	0.958	0.0016 ± 0.1021
22-HDHA	0.0994 ± 0.0832	0.0763 ± 0.0603	0.325	0.0216 ± 0.0282	0.1067 ± 0.0758	0.1115 ± 0.0824	0.695 #	−0.0048 ± 0.0562
20-HEPE	0.1460 ± 0.0808	0.1343 ± 0.0692	0.713 #	0.0119 ± 0.0403	0.1550 ± 0.0963	0.1485 ± 0.0768	0.608	0.0065 ± 0.0426

Notes: A, arterial blood; V, venous blood; A-V difference, arteriovenous difference. Bold indicates significant difference.

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

Amount ng/ml	Pre-HD A (Mean ± SD)	Pre-HD V (Mean ± SD)	*p*-Value, *t*-Test (# Paired Wilcoxon test)	Pre-HD A-V Difference (Mean ± SD)	Post-HD A (Mean ± SD)	Post-HD V (Mean ± SD)	*p*-Value, *t*-test (# Paired Wilcoxon Test)	Pre-HD A-V Difference (Mean ± SD)
5,6-EET + 5,6-DHET	26.3853 ± 22.7488	17.8740 ± 15.3357	0.325 #	8.2771 ± 22.0947	**38.4409 ± 16.7998**	**63.1169 ± 21.0481**	**0.003 #**	**−24.6760 ± 17.5393**
8,9-EET + 8,9-DHET	12.2661 ± 10.8593	8.8769 ± 6.7288	0.758 #	3.2897 ± 11.3985	**14.9171 ± 6.2221**	**23.6504 ± 7.8856**	**0.003 #**	**−8.7333 ± 7.0409**
11,12-EET + 11,12-DHET	10.3905 ± 10.0509	7.4546 ± 6.8226	0.902 #	2.7353 ± 10.2061	**14.7525 ± 6.7118**	**26.1620 ± 9.1754**	**0.002 #**	**−11.4095 ± 7.5222**
14,15-EET + 14,15-DHET	12.9774 ± 12.5015	10.0376 ± 9.5061	0.667 #	2.6766 ± 12.4408	**19.5791 ± 9.2852**	**36.1083 ± 13.6146**	**0.002 #**	**−16.5292 ± 10.9098**
7,8-EDP + 7,8-DiHDPA	4.9725 ± 4.3744	3.7040 ± 3.1733	0.854 #	1.2292 ± 4.8036	**6.4969 ± 2.8052**	**10.8874 ± 3.5963**	**0.002 #**	**−4.3904 ± 2.2808**
10,11-EDP + 10,11-DiHDPA	4.6062 ± 4.3839	3.4580 ± 3.0889	0.806 #	1.0598 ± 4.5591	**6.7795 ± 3.0931**	**12.0623 ± 3.9527**	**<0.001**	**−5.2828 ± 2.5347**
13,14-EDP + 13,14-DiHDPA	3.4752 ± 3.4913	2.7467 ± 2.8205	0.854 #	0.6542 ± 3.6762	**4.9215 ± 2.2192**	**7.9218 ± 2.6423**	**<0.001**	**−3.0002 ± 1.9422**
16,17-EDP + 16,17-DiHDPA	3.0618 ± 2.8699	2.2873 ± 2.0344	0.712 #	0.7529 ± 2.8441	**3.9502 ± 1.7125**	**5.6394 ± 2.0354**	**0.009**	**−1.6892 ± 1.8365**
19,20-EDP + 19,20-DiHDPA	6.3937 ± 5.2944	4.7947 ± 3.1021	1 #	1.5121 ± 5.6027	**9.6295 ± 4.0652**	**17.2258 ± 6.4814**	**<0.001**	**−7.5963 ± 3.8630**
5,6-EEQ + 5,6-DiHETE	1.5350 ± 0.9296	1.3729 ± 0.7133	0.622 #	0.3217 ± 0.8716	2.0440 ± 1.5009	1.7043 ± 0.8338	0.388 #	0.3397 ± 1.2857

Table 3. *Cont.*

Amount ng/ml	Pre-HD A (Mean ± SD)	Pre-HD V (Mean ± SD)	*p*-Value, *t*-Test (# Paired Wilcoxon test)	Pre-HD A-V Difference (Mean ± SD)	Post-HD A (Mean ± SD)	Post-HD V (Mean ± SD)	*p*-Value, *t*-test (# Paired Wilcoxon Test)	Pre-HD A-V Difference (Mean ± SD)
8,9-EEQ + 8,9-DiHETE	1.5386 ± 1.2263	1.0880 ± 0.6966	0.285	0.4408 ± 1.3934	**2.7148 ± 1.8146**	**4.4861 ± 1.6000**	**0.008 #**	**−1.7714 ± 1.6800**
11,12-EEQ + 11,12-DiHETE	1.0017 ± 0.8179	0.7552 ± 0.4846	0.758 #	0.2422 ± 0.9263	**1.8673 ± 1.2279**	**3.5323 ± 1.3814**	**0.003 #**	**−1.6651 ± 1.1940**
14,15-EEQ + 14,15-DiHETE	0.9963 ± 0.7071	0.7508 ± 0.4514	0.328	0.2305 ± 0.8200	**1.8082 ± 1.2226**	**3.3214 ± 1.3339**	**0.004 #**	**−1.5133 ± 1.3083**
17,18-EEQ + 17,18-DiHETE	1.8998 ± 1.3591	1.6056 ± 0.8496	0.536	0.2613 ± 1.5047	**3.2405 ± 2.1560**	**6.5059 ± 2.3420**	**0.003 #**	**−3.2654 ± 2.0580**
9,10-EpOME + 9,10-DiHOME	37.1683 ± 31.3783	25.8981 ± 13.6496	0.712 #	10.7144 ± 32.1530	**57.3217 ± 18.9513**	**99.2342 ± 38.1881**	**0.002 #**	**−41.9125 ± 28.5066**
12,13-EpOME + 12,13-DiHOME	35.9634 ± 27.1475	24.5992 ± 12.1650	0.424 #	10.7813 ± 27.9218	**53.8703 ± 15.3169**	**86.6564 ± 25.6785**	**<0.001**	**−32.7861 ± 16.4280**
5,6-DHET/5,6-EET	0.0749 ± 0.0518	0.0864 ± 0.0565	0.617		**0.0431 ± 0.0261**	**0.0222 ± 0.0120**	**0.002 #**	
8,9-DHET/8,9-EET	0.2737 ± 0.2578	0.2858 ± 0.2519	0.854 #		**0.1253 ± 0.0630**	**0.0599 ± 0.0480**	**0.002 #**	
11,12-DHET/11,12-EET	0.0827 ± 0.0649	0.0874 ± 0.0650	0.926 #		**0.0426 ± 0.0205**	**0.0198 ± 0.0099**	**0.002 #**	
14,15-DHET/14,15-EET	0.0654 ± 0.0409	0.0550 ± 0.0284	0.485		**0.0326 ± 0.0210**	**0.0127 ± 0.0047**	**0.002 #**	
7,8-DiHDPA/7,8-EDP	0.1650 ± 0.1420	0.5745 ± 1.4719	0.622 #		**0.0892 ± 0.0353**	**0.0458 ± 0.0285**	**0.002 #**	
10,11-DiHDPA/10,11-EDP	0.0538 ± 0.0350	0.0514 ± 0.0285	0.859		**0.0256 ± 0.0118**	**0.0110 ± 0.0041**	**0.002 #**	
13,14-DiHDPA/13,14-EDP	0.0506 ± 0.0302	0.0484 ± 0.0269	0.855		**0.0237 ± 0.0101**	**0.0134 ± 0.0039**	**0.01 #**	
16,17-DiHDPA/16,17-EDP	0.0552 ± 0.0392	0.0601 ± 0.0343	0.755		**0.0335 ± 0.0139**	**0.0194 ± 0.0071**	**0.008**	
19,20-DiHDPA/19,20-EDP	0.2730 ± 0.2232	0.2905 ± 0.1983	0.806 #		**0.1212 ± 0.0480**	**0.0580 ± 0.0264**	**0.002 #**	
5,6-DiHETE/5,6-EEQ	483.3002 ± 348.2197	599.0058 ± 431.5977	0.49		**274.7175 ± 163.7055**	**121.8920 ± 63.0904**	**0.001**	
8,9-DiHETE/8,9-EEQ	0.1037 ± 0.0654	0.1332 ± 0.0872	0.372		**0.0502 ± 0.0261**	**0.0229 ± 0.0107**	**0.002 #**	
11,12-DiHETE/11,12-EEQ	0.1107 ± 0.1548	0.0634 ± 0.0260	0.806 #		**0.0596 ± 0.1111**	**0.0112 ± 0.0032**	**0.002 #**	
14,15-DiHETE/14,15-EEQ	0.1012 ± 0.0933	0.0817 ± 0.0318	0.854 #		**0.0436 ± 0.0466**	**0.0153 ± 0.0044**	**0.003 #**	

Table 3. *Cont.*

Amount ng/ml	Pre-HD A (Mean ± SD)	Pre-HD V (Mean ± SD)	*p*-Value, *t*-Test (# Paired Wilcoxon test)	Pre-HD A-V Difference (Mean ± SD)	Post-HD A (Mean ± SD)	Post-HD V (Mean ± SD)	*p*-Value, *t*-test (# Paired Wilcoxon Test)	Pre-HD A-V Difference (Mean ± SD)
17,18-DiHETE/17,18-EEQ	0.2320 ± 0.1551	0.2159 ± 0.1591	0.667 #		**0.1399 ± 0.1461**	**0.0397 ± 0.0133**	**0.002 #**	
9,10-DiHOME/9,10-EpOME	0.1799 ± 0.0860	0.1731 ± 0.1090	0.58 #		**0.1260 ± 0.0918**	**0.0617 ± 0.0222**	**0.01 #**	
12,13-DiHOME/12,13-EpOME	0.2255 ± 0.0981	0.2087 ± 0.0951	0.356 #		**0.1627 ± 0.1101**	**0.0877 ± 0.0327**	**0.008 #**	
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)	0.1104 ± 0.0878	0.1188 ± 0.0946	1 #		**0.0541 ± 0.0262**	**0.0253 ± 0.0143**	**0.002 #**	
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)	0.1369 ± 0.1095	0.1478 ± 0.1106	0.902 #		**0.0666 ± 0.0240**	**0.0336 ± 0.0143**	**0.002 #**	
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)	0.6541 ± 0.4424	0.6335 ± 0.4128	0.909		**0.3403 ± 0.2022**	**0.1315 ± 0.0660**	**0.002 #**	
Ratio (9,10-DiHOME+12,13-DiHOME) / (9,10-EpOME+12,13-EpOME)	0.2017 ± 0.0845	0.1904 ± 0.1007	0.58 #		**0.1445 ± 0.1017**	**0.0741 ± 0.0258**	**0.008 #**	

Notes: A, arterial blood; V, venous blood; A-V difference, arteriovenous difference. Bold indicates significant difference.

Table 4. Effects of hemodialysis on free oxylipins in the CKD patients at cessation (post-HD) of hemodialysis (n = 12 each).

	Post-HD A (Mean ± SD)	Post-HD V (Mean ± SD)	p-Value, t-Test (# Paired Wilcoxon Test)	Post-HD A-V Difference (Mean ± SD)
CYP epoxy-metabolites				
5,6-EET	0.2022 ± 0.0733	0.2317 ± 0.0812	0.303	−0.0295 ± 0.0945
8,9-EET	0.2672 ± 0.1586	0.2517 ± 0.1676	0.814 #	0.0155 ± 0.1947
11,12-EET	0.1754 ± 0.0675	0.2403 ± 0.1094	0.051	−0.0649 ± 0.1029
14,15-EET	0.2573 ± 0.1199	0.3791 ± 0.2067	0.049	−0.1219 ± 0.1913
5,6-DHET	0.0324 ± 0.0085	0.0321 ± 0.0104	0.814 #	0.0002 ± 0.0047
8,9-DHET	0.0910 ± 0.0281	0.0847 ± 0.0241	0.388 #	0.0063 ± 0.0207
11,12-DHET	0.1486 ± 0.0443	0.1454 ± 0.0379	0.631	0.0032 ± 0.0226
14,15-DHET	0.2038 ± 0.0569	0.2062 ± 0.0477	0.854	−0.0024 ± 0.0444
7,8-EDP	0.1852 ± 0.1197	0.2412 ± 0.1309	0.076	−0.0560 ± 0.0993
10,11-EDP	0.1358 ± 0.0948	0.1805 ± 0.1004	0.079	−0.0447 ± 0.0800
13,14-EDP	0.1273 ± 0.0808	0.1452 ± 0.0769	0.439	−0.0179 ± 0.0774
16,17-EDP	0.1922 ± 0.0843	0.2284 ± 0.0893	0.124	−0.0362 ± 0.0753
19,20-EDP	0.2899 ± 0.1669	0.3587 ± 0.1754	0.096	−0.0688 ± 0.1311
7,8-DiHDPA	0.0234 ± 0.0210	0.0330 ± 0.0115	0.388 #	−0.0097 ± 0.0267
10,11-DiHDPA	0.0581 ± 0.0148	0.0554 ± 0.0125	0.182 #	0.0027 ± 0.0067
13,14-DiHDPA	0.0696 ± 0.0222	0.0661 ± 0.0178	0.272 #	0.0035 ± 0.0090
16,17-DiHDPA	0.0865 ± 0.0293	0.0809 ± 0.0206	0.695 #	0.0056 ± 0.0148
19,20-DiHDPA	0.7042 ± 0.3142	0.6061 ± 0.2823	0.06 #	0.0981 ± 0.1753
8,9-EEQ	0.0663 ± 0.0419	0.0700 ± 0.0284	0.791	−0.0037 ± 0.0472
11,12-EEQ	0.0435 ± 0.0354	0.0490 ± 0.0252	0.388 #	−0.0055 ± 0.0418
14,15-EEQ	0.0607 ± 0.0349	0.0655 ± 0.0298	0.651	−0.0049 ± 0.0363
17,18-EEQ	0.0830 ± 0.0590	0.0936 ± 0.0518	0.602	−0.0106 ± 0.0684
5,6-DiHETE	0.0980 ± 0.0508	0.0799 ± 0.0403	0.01	0.0181 ± 0.0322
8,9-DiHETE	0.0233 ± 0.0063	0.0202 ± 0.0041	0.174	0.0031 ± 0.0074
11,12-DiHETE	0.0160 ± 0.0026	0.0155 ± 0.0023	0.461	0.0004 ± 0.0020
14,15-DiHETE	0.0181 ± 0.0080	0.0167 ± 0.0073	0.525	0.0015 ± 0.0078
17,18-DiHETE	0.1661 ± 0.0704	0.1476 ± 0.0603	0.337	0.0185 ± 0.0640
9,10-EpOME	4.6969 ± 3.1153	6.2952 ± 5.0363	0.182 #	−1.5984 ± 3.6422
12,13-EpOME	6.8441 ± 3.8513	8.4182 ± 6.5171	0.239 #	−1.5740 ± 4.1754
9,10-DiHOME	1.7695 ± 1.1345	1.7400 ± 1.1665	0.754 #	0.0295 ± 0.2442
12,13-DiHOME	3.3260 ± 2.0952	3.4845 ± 2.2350	0.347 #	−0.1585 ± 0.4498
LOX metabolites				
5-HETE	0.1100 ± 0.0465	0.1147 ± 0.0494	0.365	−0.0046 ± 0.0169
8-HETE	0.0672 ± 0.0281	0.0667 ± 0.0298	0.943	0.0006 ± 0.0273
9-HETE	0.1016 ± 0.0158	0.1058 ± 0.0218	0.31	−0.0042 ± 0.0136
11-HETE	0.0739 ± 0.0265	0.0800 ± 0.0305	0.262	−0.0061 ± 0.0180
12-HETE	0.5660 ± 0.3630	0.4319 ± 0.2449	0.094	0.1341 ± 0.2531
15-HETE	0.1789 ± 0.0583	0.1841 ± 0.0689	0.536	−0.0052 ± 0.0282
4-HDHA	0.0821 ± 0.0209	0.0844 ± 0.0239	0.695 #	−0.0023 ± 0.0109
7-HDHA	0.0852 ± 0.0189	0.0875 ± 0.0186	0.594	−0.0024 ± 0.0148
8-HDHA	0.0779 ± 0.0221	0.0763 ± 0.0241	0.584	0.0016 ± 0.0098
10-HDHA	0.0622 ± 0.0148	0.0636 ± 0.0173	0.658	−0.0014 ± 0.0105
11-HDHA	0.0722 ± 0.0107	0.0766 ± 0.0117	0.081	−0.0044 ± 0.0079
13-HDHA	0.0653 ± 0.0131	0.0671 ± 0.0143	0.272 #	−0.0018 ± 0.0065
14-HDHA	0.1165 ± 0.0638	0.1098 ± 0.0627	0.308 #	0.0067 ± 0.0218
16-HDHA	0.0589 ± 0.0142	0.0610 ± 0.0122	0.389	−0.0022 ± 0.0083
17-HDHA	0.1191 ± 0.0301	0.1196 ± 0.0325	0.924	−0.0005 ± 0.0174
20-HDHA	0.2074 ± 0.0539	0.2079 ± 0.0489	0.347 #	−0.0004 ± 0.0370
5-HEPE	0.1032 ± 0.0315	0.0962 ± 0.0336	0.171	0.0070 ± 0.0166
8-HEPE	0.0352 ± 0.0045	0.0327 ± 0.0051	0.128	0.0025 ± 0.0053
9-HEPE	0.0756 ± 0.0137	0.0703 ± 0.0095	0.155	0.0053 ± 0.0121
11-HEPE	0.0847 ± 0.0025	0.0856 ± 0.0035	0.875 #	−0.0008 ± 0.0045

Table 4. *Cont.*

	Post-HD A (Mean ± SD)	Post-HD V (Mean ± SD)	*p*-Value, *t*-Test (# Paired Wilcoxon Test)	Post-HD A-V Difference (Mean ± SD)
12-HEPE	0.1595 ± 0.0220	0.1540 ± 0.0228	0.136 #	0.0055 ± 0.0158
15-HEPE	0.1078 ± 0.0123	0.1036 ± 0.0105	0.084 #	0.0042 ± 0.0085
18-HEPE	0.1779 ± 0.0413	0.1596 ± 0.0278	0.07 #	0.0183 ± 0.0316
9-HODE	8.5190 ± 13.8940	8.3464 ± 12.8721	0.347 #	0.1726 ± 1.8733
13-HODE	4.3069 ± 3.0614	4.4488 ± 2.9851	0.53 #	−0.1419 ± 0.6334
CYP ω/(ω−1) metabolites				
16-HETE	0.0626 ± 0.0120	0.0631 ± 0.0114	0.772	−0.0006 ± 0.0069
17-HETE	0.0242 ± 0.0028	0.0240 ± 0.0029	0.713	0.0002 ± 0.0018
18-HETE	0.0456 ± 0.0082	0.0451 ± 0.0072	0.684	0.0005 ± 0.0044
19-HETE	0.0387 ± 0.0291	0.0392 ± 0.0269	0.638 #	−0.0005 ± 0.0216
20-HETE	0.1457 ± 0.1239	0.0709 ± 0.0885	0.084 #	0.0747 ± 0.1464
22-HDHA	0.0843 ± 0.0662	0.0754 ± 0.0632	0.177	0.0089 ± 0.0213
20-HEPE	0.0900 ± 0.0271	0.0856 ± 0.0284	0.638 #	0.0045 ± 0.0171

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

2.3. Diol/Epoxide Ratios and sEH Activity

To clarify possible mechanisms underlying this preferred increase in venous blood, we calculated diol/epoxide ratios of the eicosanoids (Table S3). Our analysis of sums of the individual CYP epoxy-metabolites and their diols demonstrated increased accumulation of all four classes of CYP epoxy-metabolites (5,6-EET + 5,6-DHET, 8,9-EET + 8,9-DHET, 11,12-EET + 11,12-DHET, 14,15-EET + 14,15-DHET, 7,8-EDP + 7,8-DiHDPA, 10,11-EDP + 10,11-DiHDPA, 13,14-EDP + 13,14-DiHDPA, 16,17-EDP + 16,17-DiHDPA, 19,20-EDP + 19,20-DiHDPA, 5,6-EEQ + 5,6-DiHETE, 8,9-EEQ + 8,9-DiHETE, 11,12-EEQ + 11,12-DiHETE, 14,15-EEQ + 14,15-DiHETE, 17,18-EEQ + 17,18-DiHETE, 9,10-EpOME + 9,10-DiHOME, 12,13-EpOME + 12,13-DiHOME, 5,6-DHET/5,6-EET, 8,9-DHET/8,9-EET, 11,12-DHET/11,12-EET, and 14,15-DHET/14,15-EET) in venous blood (Table S3). We found that ratios of diols/epoxides of all four subclasses were reduced by dialysis (Table S3). The effects were more obvious in venous compared to arterial blood (Table 3). Together, the results indicate that decreased sEH activity in peripheral tissue, especially the muscles, in vivo contributes to release and accumulation of CYP epoxy-metabolites in the peripheral upper limb circulation during hemodialysis.

3. Discussion

To gain further insight into oxylipin metabolism, we evaluated the arteriovenous differences of oxylipins levels in uremic patients treated by HD treatment. Our major findings are three-fold, as follows: (1) We detected negative arteriovenous differences of a number of CYP metabolites (EETs, EDPs, EEQs, EPOMEs, and their diols) after the dialysis treatment (post-HD). These were primarily due to exuberant increases of the metabolites in the venous blood stream of the arm. However, hemodialysis did not change arteriovenous differences of the majority of LOX and LOX/CYP ω/(ω-1)- hydroxylase metabolites. (2) The observed arteriovenous differences were caused by the dialysis treatment itself since we did not detect arteriovenous differences between the CYP metabolite levels before HD treatment (pre-HD). (3) Decreased soluble epoxide hydrolase (sEH) activity contributed to the release and accumulation of the CYP metabolites after HD. Together, our data indicate that CYP epoxy-metabolites are influenced by renal-replacement therapies and are consistent with the notion that blood perfusing peripheral tissue, especially the muscle, acts as a stimulus for release and accumulation of CYP epoxy-metabolites in the upper limb during dialysis treatment. Based on our data, we suggest that CYP epoxy-metabolites may be a contributing factor to the blood flow response in the peripheral circulation

during hemodialysis. Future studies can clarify whether the identified metabolites exhibit beneficial or detrimental cardiovascular effects, possibly in metabolite-interacting networks.

Fick first capitalized on the arteriovenous oxygen difference to determine cardiac output and thereby to determine oxygen delivery and extraction. His insights permitted measuring the amount of oxygen taken up from the blood by the individual tissues. Usually, the arterial oxygen concentration is measured in blood from the radial, femoral, or brachial artery, and the oxygen content of mixed venous blood is measured from blood collected from the right heart (i.e., pulmonary artery). The mixed venous oxygen content represents the weighted average of oxygen content in venous blood from all organ systems. However, venous blood can also be withdrawn from specific organs, e.g., legs, arms, or the mesentery. In this case, measurement of oxygen consumption by the specific organ system requires withdrawal of venous blood draining that organ [12–15]. We applied this approach to better understand biotransformation of oxylipins in vivo, particularly whether or not the peripheral tissues, especially the muscles in the upper limbs, either produce, store, or degrade part of the epoxy-metabolites that pass through them in response to dialysis treatment. We were particularly interested to understand how the plasma oxylipins are modified by extracorporeal renal replacement therapy, which is known to cause oxidative stress, chronic inflammation, and red blood cell–endothelial interactions. Our study identified negative arteriovenous differences of a number of CYP metabolites in upper extremities, especially the muscles, after the dialysis treatment (post-HD). These changes were caused by exuberant increases of all four subclasses of the CYP epoxy-metabolites (i.e., EET, EDP, EEQ, EpOME) in the venous blood stream of the upper limbs.

We found that the following CYP epoxy-metabolites were increased in venous blood after HD: 5,6-EET, 8,9-EET, 11,12-EET, 14,15-EET, 7,8-EDP, 10,11-EDP, 14,15-EDP, 16,17-EDP, 19,20-EDP, 5,6-EEQ, 8,9-EEQ, 11,12-EEQ, 14,15-EEQ, 17,18-EEQ, 9,10-EPOME, and 12,13-EPOME. Although reduced in-vivo sEH activity in CKD/ESRD [4,16] may have contributed to the increased accumulation of the four eicosanoid classes itself, our data indicate that the observed changes are likely related to reduced sEH activity by HD treatment. Accordingly, we found that ratios of diols/epoxides of all four subclasses were reduced by dialysis. The sEH is an enzyme that converts specific epoxides to less biologically effective diols [7]. In plasma, we were able to observe that all ratios of diols/epoxides were reduced in the venous plasma after hemodialysis. Going further, we compared the differences in arterial and venous levels before and after dialysis and found that almost all the differences in metabolite ratios were due to their decrease in venous plasma after dialysis. Nevertheless, whether or not dialysis treatment inhibits the hydrolysis of epoxide metabolites to diols by sEH remains to be experimentally verified.

Although CYP450 can oxidize AA to four regional isomers, their content is unevenly distributed, with 11,12- and 14,15-EET being the predominant regional isomers in mammals, accounting for approximately 67–80% of all EETs [17,18]. Endothelial and red blood cells store EETs and can release these epoxides into plasma [19–22]. The mechanisms of how EETs and other epoxy-metabolites are released from the tissues are largely unknown, making our findings difficult to explain. The anti-inflammatory effects of EETs in the cardiovascular system, in the kidney, and in the brain are well established, and their main vascular effect is the diminution of the NF-kappaB-dependent inflammatory response [23,24]. 5,6-EET can produce vasodilation in various vascular beds [25,26]. 8,9-EET inhibits NF-kappaB nuclear translocation in mouse B lymphocytes activated by lipopolysaccharide, causing a reduction in basal and activation-induced antibody production [27]. As a result of suppressing LOX-1 receptor upregulation and NF-kappaB activation, 11,12 and 14,15-EET reduce the oxidized low density lipoprotein (LDL)-related inflammation in the vasculature [28]. 5,6-EET is the only one of these regional isomers that induces a bidirectional effect. In a study on the pulmonary vasculature [29], 5,6-EET was found to cause sustained vasoconstriction in the lung during hypoxia. Our findings are consistent with the idea that EETs are relevant vasodilatory signaling molecules for exhibiting cardiovascular effects in ESRD/CKD [30], which could counteract vasoconstrictor responses during dialysis. Pharmaceutical sEH

inhibition is viewed as a novel treatment for enhancing the beneficial biological effects of EETs and other epoxy-metabolites [5]. However, presumably higher levels of EETs in blood and tissue in vivo may have also detrimental cardiovascular effects [31–33]. The extent to which the EETs increase observed in our study has detrimental or beneficial biological effects should be investigated.

We detected increases in 7,8-EDP, 10,11-EDP, 14,15-EDP, 16,17-EDP, 19,20-EDP, 5,6-EEQ, 8,9-EEQ, 11,12-EEQ, 14,15-EEQ, and 17,18-EEQ during dialysis. Little is known about the (patho) physiological functions of EEQs and EDPs. 16,17-EDP and 19,20-EDP are potent vasodilators in the peripheral circulation. They decrease blood pressure and can protect the heart by preservation of mitochondrial function [19,34]. EEQs and EDPs have a significant role in anti-cardiac fibrosis, improving post-ischemic reperfusion injury in the heart and reducing inflammation and pain, especially 19,20-EDPs [35] and 17,18-EEQs [36]. EDPs protect cardiac cells by improving and maintaining mitochondrial function against LPS-induced cell damage, as shown in a recent study [37]. A recent mouse ex vivo experiment [35] found that 19, 20-EDP could exert cardioprotective effects by inhibiting NLR Family Pyrin Domain Containing 3 (NLRP3) inflammatory vesicles compared to 17,18-EEQ. 17,18-EEQ decreases endothelial activity and prevents atherosclerosis [19]. 17,18-EEQ has been identified to activate Ca^{2+}-activated potassium channels to produce vasodilation, an effect that is even more effective than for EETs [38,39]. 17,18-EEQ also inhibits Ca^{+} and isoproterenol-induced increases in cardiomyocytes contractility, suggesting that 17,18-EEQ could be used as a potential antiarrhythmic agent [6]. Our findings implicate that both EEQs and EDPs are novel candidates for vasoactive substances potentially released by hemodialysis to affect hemodynamics in these conditions.

We observed pronounced increases in venous plasma levels of 9,10-EpOME and 12,13-EpOME after hemodialysis. Recent findings suggest that EpOMEs exhibit cardio-inhibitory effects [40–42] and vasodilation [43]. Furthermore, they can cause vasoconstrictor effects in ischemic heart disease [40,44]. Since we observed increases in 9,10-EpOME and 12,13-EpOME after hemodialysis, our findings suggest the notion that increases in EpOMEs could play a detrimental role in cardiac ischemia and hemodynamics in ESRD patients.

We believe that our findings could have clinical relevance. We were able to analyze arteriovenous relationships in plasma oxylipin profiles in ESRD patients and the extent to which these are altered by renal replacement therapies. Our data indicate that CYP epoxy-metabolites are influenced by the hemodialysis treatment and are consistent with the notion that dialyzed blood perfusing peripheral tissue, especially the muscle, acts as a stimulus for release and accumulation of CYP epoxy-metabolites. Our analysis allowed an overall assessment of the biotransformation of plasma oxylipins in peripheral tissue, specifically upper extremity muscle, in vivo. The results provided new insights into local metabolic and hemodynamic formation processes of oxylipins in peripheral tissues in vivo. The extent to which the observed effects in oxylipins cause detrimental or beneficial cardiovascular effects, possibly in metabolite-interacting networks, or are influenced by specific underlying renal diseases or patient phenotypes should be explored in future studies.

4. Materials and Methods

4.1. Participants

The ethical committee of the Charité University Medicine approved the study. Written informed consent was obtained from all participants. The study was duly registered on the ClinicalTrials.gov website (Identifier: NCT03857984). Nine men and three women were recruited. To be included in the study, age over 18 years and presence of CKD requiring hemodialysis (three times a week) with stable hemodialysis prescriptions were defined as inclusion criteria. They had to have been dialyzed through a native fistula or a gore-tex graft. Exclusion criteria included age less than 18 years, pregnancy, inability to follow simple instructions, non-compliance with their dialysis prescription, and an anemia with hemoglobin (Hb) below 8.0 g/dL or an active infection.

4.2. Assessment

All patients were treated in sitting position. Subjects underwent dialysis treatments with a Polyflux 170H dialyzer (PAES membrane, Gambro); the ultrafiltration rate was unchanged during the hemodialysis treatment. Relevant dialysis treatment conditions (blood flow rate with ~250 mL/min, dialysate flow rate ~500 mL/min, double needle puncture technique, and dialysis time on average 4 h 15 min) were identical in all dialysis sessions. Arterial (shunt) blood samples were withdrawn on the fistula arm right before beginning of the dialysis (pre-HD) and at the end of the dialysis (5–15 min before cessation, post-HD). Venous blood was collected on the ipsilateral extremity by subcutaneous arm vein puncture at same time points to determine the arteriovenous difference of the epoxy-metabolites (Figure 2). The arteriovenous difference is caused by the fact that the peripheral tissues, specially the muscles, either produce, store, or degrade part of the epoxy-metabolites that perfuse them. All blood samples were obtained by 4 °C precooled EDTA vacuum extraction tube systems. Glucose, cholesterol, and triglycerides were determined in a certified clinical laboratory.

4.3. Determination of Eicosanoid Profiles

For the detection of total plasma oxylipins, we took a plasma sample (200 µL), added 300 µL of 10M sodium hydroxide (NaOH), and subjected it to alkaline hydrolysis at 60 °C for 30 min. The sample pH was then adjusted to 6 using 300 µL 58% acetic acid. The prepared samples were then subjected to solid phase extraction (SPE) using a Varian Bond Elut Certify II column. Specific experimental steps were described as previously [11,45]. For the detection of free plasma oxylipins, SPE extraction was performed directly after pH adjustment without prior alkaline hydrolysis.

The extracted metabolites were evaluated by LC-MS/MS using an Agilent 6460 Triple Quad mass spectrometer (Agilent Technologies, Santa Clara, CA, USA) and an Agilent 1200 high-performance liquid chromatography (HPLC) system (degasser, binary pump, well plate sampler, thermostatic column chamber). A Phenomenex Kinetex column (150 mm 2.1 mm, 2.6 m; Phenomenex, Aschaffenburg, Germany) was used in the HPLC system. The specific analysis process has been described previously [45]. Free and total plasma oxylipins were measured in the blood samples.

4.4. Statistical Analysis

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 to compare pre-HD vs. post-HD values. The significance level (p) of 0.05 was selected. All data are provided as mean ± SD. The statistics was performed using SPSS Statistics software (IBM Corporation).

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/metabo12010034/s1, Table S1. Clinical parameters of hemodialysis (HD) patients (n = 12 each), Table S2. Effects of hemodialysis on total plasma oxylipins in the CKD patients before (pre-HD) and at cessation (post-HD) of hemodialysis (n = 12 each); Table S3. Effects of hemodialysis on total plasma oxylipins and their ratios in venous blood of the CKD patients before (pre-HD) and at cessation (post-HD) of hemodialysis (n = 12 each).

Author Contributions: B.G., M.G., and F.C.L. planned and designed the experimental studies. T.L., I.D. and M.R. performed the HPLC-MS spectrometry experiments. All authors contributed to the implementation and analyses of the experiments. T.L. and B.G. drafted the article, and all authors contributed to its completion. All authors have read and agreed to the published version of the manuscript.

Funding: The Deutsche Forschungsgemeinschaft (DFG) supported FCL (LU 435/13-1). We express our thanks to the DFG for continuous support. We also acknowledge support from the Open Access Publication Fund of Charité—Universitätsmedizin Berlin.

Institutional Review Board Statement: The study was conducted according to the guidelines of the Declaration of Helsinki and approved by the Institutional Review Board (or Ethics Committee) of the ethical committee of the Charité—Universitätsmedizin Berlin (protocol code EA4/110/19; date of approval 4 September 2020).

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: No new data were created or analyzed in this study. Data sharing is not applicable to this article.

Acknowledgments: We wish to thank all patients for participating in this study. We thank Jana Reichardt for helping us collect some blood samples.

Conflicts of Interest: The authors declare no conflict of interest.

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Supplementary Information

metabolites

MDPI

Article

Hemodialysis and Plasma Oxylipin Biotransformation in Peripheral Tissue

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Table S1. Clinical parameters of hemodialysis (HD) patients (n=12 each)

Parameters	Patients
Glucose (60-110 mg/dl)	115.8 ± 36.7
Total cholesterol (< 200 mg/dl)	221.8 ± 181.2
LDL-cholesterol (< 130 mg/dl)	101.2 ± 30.7
HDL- cholesterol (>35 mg/dl)	42.4 ± 9.9
Triglycerides (<200 mg/dl)	151.0 ± 77.4

Data are presented as mean + SD

Table S2. Effects of hemodialysis on total plasma oxylipins in the CKD patients before (Pre-HD) and at cessation (Post-HD) of hemodialysis (n=12 each)

Amount ng/ml	pre-HD A	post-HD A	p value, t test (# paired Wilcoxon test)	pre-HD V	post-HD V	p value, t test (# paired Wilcoxon test)
	Panel A			Panel B		
CYP epoxy-metabolites						
5,6-EET	**25.2403 ±22.8032**	**37.0725 ±16.6846**	**0.042 #**	**16.8013 ±15.3681**	**61.8386 ±21.0905**	**0.002 #**
8,9-EET	**10.5227 ±10.8994**	**13.3085 ±5.6504**	**0.042 #**	**7.2706 ±6.6772**	**22.3969 ±7.8620**	**0.002 #**
11,12-EET	**9.9539 ±10.0802**	**14.2304 ±6.6762**	**0.036 #**	**7.0358 ±6.8420**	**25.6937 ±9.1845**	**0.002 #**
14,15-EET	**12.5615 ±12.5408**	**19.0872 ±9.2999**	**0.031 #**	**9.6655 ±9.4944**	**35.6871 ±13.5842**	**0.002 #**
5,6-DHET	1.1450 ±0.5549	1.3684 ±0.5945	0.176 #	**1.0727 ±0.5911**	**1.2782 ±0.6163**	**0.012 #**
8,9-DHET	1.7434 ±1.5788	1.6086 ±1.1218	0.902 #	**1.6063 ±1.3596**	**1.2535 ±0.9769**	**0.012 #**
11,12-DHET	0.4366 ±0.2207	0.5221 ±0.1967	0.176 #	0.4188 ±0.1959	0.4683 ±0.1888	0.056
14,15-DHET	0.4159 ±0.0996	0.4919 ±0.1251	0.124	0.3721 ±0.0788	0.4212 ±0.1045	0.07
7,8-EDP	4.5236 ±4.3719	6.0064 ±2.6897	0.097 #	**3.2930 ±3.1818**	**10.4413 ±3.5636**	**0.002 #**
10,11-EDP	4.4784 ±4.4009	6.6357 ±3.0803	0.056 #	**3.3370 ±3.0863**	**11.9332 ±3.9274**	**0.002 #**
13,14-EDP	3.3860 ±3.4990	4.8226 ±2.2061	0.097 #	**2.6621 ±2.8208**	**7.8177 ±2.6177**	**0.002 #**
16,17-EDP	2.9692 ±2.8814	3.8342 ±1.6915	0.085 #	**2.1926 ±2.0367**	**5.5352 ±2.0151**	**0.002 #**
19,20-EDP	5.5558 ±5.3859	8.6761 ±3.8647	0.056 #	**3.9685 ±3.1487**	**16.3124 ±6.2669**	**0.002 #**
7,8-DiHDPA	0.4489 ±0.2473	0.4905 ±0.2287	0.58 #	0.4110 ±0.2301	0.4461 ±0.2152	0.158 #
10,11-DiHDPA	0.1277 ±0.0387	0.1439 ±0.0452	0.371	0.1210 ±0.0346	0.1291 ±0.0539	0.475
13,14-DiHDPA	0.0892 ±0.0274	0.0989 ±0.0354	0.473	0.0846 ±0.0203	0.1040 ±0.0439	0.054
16,17-DiHDPA	0.0926 ±0.0361	0.1160 ±0.0464	0.195	0.0947 ±0.0314	0.1042 ±0.0480	0.313
19,20-DiHDPA	0.8379 ±0.4051	0.9534 ±0.4118	0.506	0.8262 ±0.3658	0.9134 ±0.4345	0.25
5,6-EEQ	0.0051 ± 0.0039	0.0084 ± 0.0052	0.069 #	**0.0034 ± 0.0026**	**0.0148 ± 0.0050**	**0.002 #**
8,9-EEQ	**1.4478 ±1.2289**	**2.6063 ±1.7732**	**0.036 #**	**0.9972 ±0.6972**	**4.3902 ±1.5836**	**<0,001**
11,12-EEQ	**0.9541 ±0.8310**	**1.8077 ±1.2340**	**0.042 #**	**0.7197 ±0.4820**	**3.4951 ±1.3735**	**0.002 #**

14,15-EEQ	0.9436 ±0.7146	1.7544 ±1.2152	0.065 #	**0.7047 ±0.4467**	**3.2728 ±1.3192**	**0.002 #**
17,18-EEQ	1.6849 ±1.3879	2.9621 ±2.1045	0.085 #	**1.3915 ±0.8490**	**6.2605 ±2.2755**	**0.002 #**
5,6-DiHETE	1.5299 ±0.9299	2.0357 ±1.4971	0.356 #	**1.3695 ±0.7134**	**1.6895 ±0.8335**	**<0,001**
8,9-DiHETE	0.0908 ±0.0378	0.1085 ±0.0552	0.389 #	0.0908 ±0.0317	0.0960 ±0.0386	0.376
11,12-DiHETE	0.0476 ±0.0379	0.0596 ±0.0600	0.255 #	0.0356 ±0.0100	0.0372 ±0.0119	0.451
14,15-DiHETE	0.0528 ±0.0201	0.0538 ±0.0249	0.916	0.0462 ±0.0135	0.0487 ±0.0203	0.458
17,18-DiHETE	0.2149 ±0.0825	0.2784 ±0.1176	0.152	0.2141 ±0.0836	0.2454 ±0.1082	0.129
9,10-EpOME	**33.0227 ±31.0831**	**51.7226 ±18.8433**	**0.023 #**	**22.5987 ±12.9602**	**93.5874 ±36.4954**	**0.002 #**
12,13-EpOME	**30.9825 ±26.7698**	**46.9109 ±15.0402**	**0.023 #**	**20.7024 ±11.0768**	**79.7224 ±23.6841**	**0.002 #**
9,10-DiHOME	4.1455 ±1.7899	5.5991 ±2.7877	0.176 #	**3.2993 ±1.7108**	**5.6467 ±2.4652**	**0.006 #**
12,13-DiHOME	4.9809 ±1.9437	6.9594 ±3.7873	0.135	**3.8968 ±1.6289**	**6.9340 ±3.3774**	**0.01 #**
LOX metabolites						
5-HETE	10.1945 ±2.9568	11.6499 ±3.0694	0.261	**8.9008 ±2.5521**	**11.2376 ±3.5321**	**0.01 #**
8-HETE	3.1627 ±0.8646	3.5068 ±1.3221	0.806 #	2.9701 ±0.9371	2.9656 ±1.1078	0.985
9-HETE	6.2378 ±2.4916	6.6115 ±2.5626	0.58 #	5.0812 ±2.0444	5.7522 ±2.1829	0.158 #
11-HETE	4.3689 ±1.3795	4.5454 ±1.4621	0.622 #	3.4094 ±1.1668	4.0433 ±1.4575	0.084 #
12-HETE	5.1343 ±1.7200	6.0063 ±2.3467	0.325	**5.8545 ±2.6463**	**4.7972 ±2.3597**	**0.023 #**
15-HETE	6.1984 ±1.9171	6.1869 ±1.8919	0.758 #	4.9051 ±1.5388	5.7417 ±3.2394	0.48 #
4-HDHA	2.3149 ±0.6472	2.4431 ±0.9794	0.902 #	2.1630 ±0.5977	2.5609 ±1.2943	0.388 #
7-HDHA	2.1060 ±0.6072	2.1783 ±0.8143	0.951 #	1.7319 ±0.5209	2.0061 ±0.6765	0.137
8-HDHA	1.2263 ±0.4487	1.2990 ±0.5943	0.975 #	1.0900 ±0.3821	1.1141 ±0.4935	0.875 #
10-HDHA	0.7721 ±0.2512	0.7940 ±0.3187	0.857	0.6877 ±0.2059	0.7020 ±0.2701	0.801
11-HDHA	1.0995 ±0.3769	1.0862 ±0.4291	0.758 #	1.0493 ±0.3586	1.0103 ±0.3687	0.48 #
13-HDHA	0.9168 ±0.2861	0.9169 ±0.3765	0.622 #	0.8168 ±0.2303	0.8015 ±0.3238	0.855
14-HDHA	1.1312 ±0.4384	1.1712 ±0.5441	0.849	1.0678 ±0.4203	0.9981 ±0.5062	0.158 #
16-HDHA	1.1430 ±0.3468	1.1459 ±0.4227	0.986	0.9559 ±0.3037	1.0653 ±0.4675	0.48 #
17-HDHA	1.4069 ±0.4032	1.4601 ±0.5780	0.802	1.1633 ±0.3601	1.2773 ±0.5100	0.308 #

20-HDHA	2.8698 ±0.7707	2.9700 ±0.9600	0.786	2.4726 ±0.7079	2.7939 ±1.1603	0.239 #
5-HEPE	1.4608 ±0.5576	1.5968 ±0.9534	0.902 #	1.3111 ±0.4406	1.4799 ±0.6337	0.114
8-HEPE	0.2118 ±0.0669	0.2061 ±0.1012	0.498 #	0.1959 ±0.0421	0.1817 ±0.0752	0.358
9-HEPE	0.4018 ±0.1460	0.3664 ±0.2280	0.268 #	0.3661 ±0.1209	0.3335 ±0.1318	0.182 #
11-HEPE	0.2752 ±0.0907	0.2531 ±0.1150	0.616	0.2520 ±0.0448	0.2364 ±0.0928	0.099 #
12-HEPE	0.5039 ±0.1762	0.4619 ±0.2576	0.268 #	**0.5213 ±0.1704**	**0.4038 ±0.1731**	**0.001**
15-HEPE	0.3150 ±0.1086	0.2890 ±0.1524	0.356 #	0.3142 ±0.0634	0.2596 ±0.1144	0.061
18-HEPE	0.8580 ±0.2946	0.7714 ±0.3717	0.325 #	0.8418 ±0.2239	0.7502 ±0.4476	0.117 #
9-HODE	**22.0631 ±7.3185**	**36.2855 ±21.5709**	**0.019 #**	**17.1239 ±6.0628**	**34.0942 ±21.6186**	**0.015 #**
13-HODE	**17.7783 ±6.0021**	**25.0606 ±10.8578**	**0.016 #**	**13.5081 ±4.2496**	**25.1650 ±13.2212**	**0.006 #**
CYP ω/(ω−1) metabolites						
16-HETE	0.2346 ±0.0507	0.2194 ±0.0562	0.505	0.2175 ±0.0583	0.2184 ±0.0828	0.695 #
17-HETE	0.0565 ±0.0112	0.0602 ±0.0182	0.951 #	0.0560 ±0.0145	0.0517 ±0.0147	0.198
18-HETE	0.1620 ±0.0463	0.1651 ±0.0450	0.758 #	0.1499 ±0.0282	0.1493 ±0.0421	0.952
19-HETE	0.1441 ±0.0810	0.1599 ±0.0533	0.585	0.1354 ±0.0411	0.1662 ±0.0571	0.103
20-HETE	0.4738 ±0.2011	0.4803 ±0.1680	0.934	0.3992 ±0.2211	0.4787 ±0.2014	0.138
22-HDHA	0.0994 ±0.0832	0.1067 ±0.0758	0.58 #	**0.0763 ±0.0603**	**0.1115 ±0.0824**	**0.028 #**
20-HEPE	0.1460 ±0.0808	0.1550 ±0.0963	0.81	0.1343 ±0.0692	0.1485 ±0.0768	0.413

Notes: Mean+SD. Panel A: Arterial blood. Panel B: Venous blood.

Table S3. Effects of hemodialysis on total plasma oxylipins and their ratios in venous blood of the CKD patients before (Pre-HD) and at cessation (Post-HD) of hemodialysis (n=12 each)

Amount ng/ml	pre-HD Venous	post-HD Venous	p value, t test (# paired Wilcoxon test)
5,6-EET + 5,6-DHET	17.8740 ±15.3357	63.1169 ±21.0481	0.002 #
8,9-EET + 8,9-DHET	8.8769 ±6.7288	23.6504 ±7.8856	0.002 #
11,12-EET + 11,12-DHET	7.4546 ±6.8226	26.1620 ±9.1754	0.002 #
14,15-EET + 14,15-DHET	10.0376 ±9.5061	36.1083 ±13.6146	0.002 #
7,8-EDP + 7,8-DiHDPA	3.7040 ±3.1733	10.8874 ±3.5963	0.002 #
10,11-EDP + 10,11-DiHDPA	3.4580 ±3.0889	12.0623 ±3.9527	0.002 #
13,14-EDP + 13,14-DiHDPA	2.7467 ±2.8205	7.9218 ±2.6423	0.002 #
16,17-EDP + 16,17-DiHDPA	2.2873 ±2.0344	5.6394 ±2.0354	0.002 #
19,20-EDP + 19,20-DiHDPA	4.7947 ±3.1021	17.2258 ±6.4814	0.002 #
5,6-EEQ + 5,6-DiHETE	1.3729 ±0.7133	1.7043 ±0.8338	<0,001
8,9-EEQ + 8,9-DiHETE	1.0880 ±0.6966	4.4861 ±1.6000	<0,001
11,12-EEQ + 11,12-DiHETE	0.7552 ±0.4846	3.5323 ±1.3814	0.002 #
14,15-EEQ + 14,15-DiHETE	0.7508 ±0.4514	3.3214 ±1.3339	0.002 #
17,18-EEQ + 17,18-DiHETE	1.6056 ±0.8496	6.5059 ±2.3420	0.002 #
9,10-EpOME + 9,10-DiHOME	25.8981 ±13.6496	99.2342 ±38.1881	0.002 #
12,13-EpOME + 12,13-DiHOME	24.5992 ±12.1650	86.6564 ±25.6785	0.002 #
5,6-DHET/5,6-EET	0.0864 ±0.0565	0.0222 ±0.0120	0.002 #
8,9-DHET/8,9-EET	0.2858 ±0.2519	0.0599 ±0.0480	0.002 #
11,12-DHET/11,12-EET	0.0874 ±0.0650	0.0198 ±0.0099	0.002 #
14,15-DHET/14,15-EET	0.0550 ±0.0284	0.0127 ±0.0047	0.002 #
7,8-DiHDPA/7,8-EDP	0.5745 ±1.4719	0.0458 ±0.0285	0.002 #
10,11-DiHDPA/10,11-EDP	0.0514 ±0.0285	0.0110 ±0.0041	0.002 #
13,14-DiHDPA/13,14-EDP	0.0484 ±0.0269	0.0134 ±0.0039	0.002 #
16,17-DiHDPA/16,17-EDP	0.0601 ±0.0343	0.0194 ±0.0071	0.003 #
19,20-DiHDPA/19,20-EDP	0.2905 ±0.1983	0.0580 ±0.0264	0.002 #
5,6-DiHETE/5,6-EEQ	599.0058 ±431.5977	121.8920 ±63.0904	0.002 #
8,9-DiHETE/8,9-EEQ	0.1332 ±0.0872	0.0229 ±0.0107	0.002 #
11,12-DiHETE/11,12-EEQ	0.0634 ±0.0260	0.0112 ±0.0032	0.002 #
14,15-DiHETE/14,15-EEQ	0.0817 ±0.0318	0.0153 ±0.0044	0.002 #
17,18-DiHETE/17,18-EEQ	0.2159 ±0.1591	0.0397 ±0.0133	0.002 #
9,10-DiHOME/9,10-EpOME	0.1731 ±0.1090	0.0617 ±0.0222	0.003 #
12,13-DiHOME/12,13-EpOME	0.2087 ±0.0951	0.0877 ±0.0327	0.003 #
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)	0.1188 ±0.0946	0.0253 ±0.0143	0.002 #

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)	**0.1478 ±0.1106**	**0.0336 ±0.0143**	**0.002 #**
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)	**0.6335 ±0.4128**	**0.1315 ±0.0660**	**0.002 #**
Ratio (9,10-DiHOME+12,13-DiHOME) / (9,10-EpOME+12,13-EpOME)	**0.1904 ±0.1007**	**0.0741 ±0.0258**	**0.003 #**

Notes: Mean+SD

Publication #3: Hemodialysis and erythrocyte epoxy fatty acids.

Gollasch B, Wu G, **Liu T**, Dogan I, Rothe M, Gollasch M, Luft FC. Hemodialysis and erythrocyte epoxy fatty acids. *Physiol Rep*. 2020 Oct;8(20):e14601.

https://doi.org/10.14814/phy2.14601

Not included in SCIE and SSCI.

Physiological Reports is a peer-reviewed open access online only scientific journal

covering original research in all areas of physiology. It is published by Wiley-Blackwell on

behalf of The Physiological Society and the American Physiological Society.

DOI: 10.14814/phy2.14601

ORIGINAL RESEARCH

Physiological Reports

Hemodialysis and erythrocyte epoxy fatty acids

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Funding information
We thank the Deutsche Forschungsgemeinschaft (DFG) for continuous support, which enabled us to pursue this exciting research direction. The DFG supported Friedrich C. Luft (LU 435/13-1). We acknowledge support from the DFG and the Open Access Publication Fund of Charité—Universitätsmedizin Berlin.

Abstract

Fatty acid products derived from cytochromes P450 (CYP) monooxygenase and lipoxygenase (LOX)/CYP ω/(ω-1)-hydroxylase pathways are a superclass of lipid mediators with potent bioactivities. Whether or not the chronic kidney disease (CKD) and hemodialysis treatments performed on end-stage renal disease (ESRD) patients affect RBC epoxy fatty acids profiles remains unknown. Measuring the products solely in plasma is suboptimal. Since such determinations invariably ignore red blood cells (RBCs) that make up 3 kg of the circulating blood. RBCs are potential reservoirs for epoxy fatty acids that regulate cardiovascular function. We studied 15 healthy persons and 15 ESRD patients undergoing regular hemodialysis treatments. We measured epoxides derived from CYP monooxygenase and metabolites derived from LOX/CYP ω/(ω-1)-hydroxylase pathways in RBCs by LC–MS/MS tandem mass spectrometry. Our data demonstrate that various CYP epoxides and LOX/CYP ω/(ω-1)-hydroxylase products are increased in RBCs of ESRD patients, compared to control subjects, including dihydroxyeicosatrienoic acids (DHETs), epoxyeicosatetraenoic acids (EEQs), dihydroxydocosapentaenoic acids (DiHDPAs), and hydroxyeicosatetraenoic acids (HETEs). Hemodialysis treatment did not affect the majority of those metabolites. Nevertheless, we detected more pronounced changes in free metabolite levels in RBCs after dialysis, as compared with the total RBC compartment. These findings indicate that free RBC eicosanoids should be considered more dynamic or vulnerable in CKD.

KEYWORDS

chronic kidney disease (CKD), dialysis, erythrocytes, fatty acids, lipidomics

Abbreviations: AA, arachidonic acid, C20:4; CYP, cytochrome P450; DHA, docosahexaenoic acid, C22:6 n-3; DHET, dihydroxyeicosatrienoic acid; DiHDHA, dihydroxydocosahexaenoic acid; DiHDPA, dihydroxydocosapentaenoic acid; DiHETE, dihydroxyeicosatetraenoic acid; DiHOME, dihydroxyctadecenoic acid; EDHF, endothelium-derived hyperpolarizing factor; EDP, epoxydocosapentaenoic acid; EEQ, epoxyeicosatetraenoic acid; EET, epoxyeicosatrienoic acid; EPA, eicosapentaenoic acid, C20:5 n-3; EpOME, epoxyoctadecenoic acid; HDHA, hydroxydocosahexaenoic acid; HEPE, hydroxyeicosapentaenoic acid; HETE, hydroxyeicosatetraenoic acid; HODE, hydroxyoctadecadienoic acid; HPETE, hydroperoxyeicosatetraenoic acid; HpODE, hydroperoxylinoleic acid; LA, linoleic acid, C18:2; LOX, lipoxygenase; PUFA, polyunsaturated fatty acid.

1 | INTRODUCTION

Chronic kidney disease (CKD) is a risk factor for the composite outcome of all-cause mortality and cardiovascular disease (Weiner et al., 2004). Although mortality and cardiovascular disease burden have decreased for end-stage renal disease (ESRD) hemodialysis patients in the United States, the 5-year mortality is still ~50% (McGill et al., 2019), Most of these deaths are related to cardiovascular disease (CVD) (Felasa | Federation for Laboratory Animal Science Associations, 2012; Luft, 2000). Dietary omega-3 (n-3) fatty acid intake is associated with a reduced CVD risk (Harris et al., 2008; Huang et al., 2011; InterAct Consortium et al., 2011). Erythrocyte red-blood-cell (RBC) n-3 fatty-acid status is inversely related to cardiovascular events, such as cardiac arrhythmias, myocardial infarction, and sudden cardiac death (Bucher et al., 2002).

Epoxides and hydro(pero)xy fatty acids (or oxylipins) are lipid peroxidation products of polyunsaturated fatty acids (PUFA), including C18:2 linoleic (LA), C20:0 arachidonic (AA), C20:5 n-3 eicosapentaenoic (EPA), and C22:5 n-3 docosahexaenoic acids (DHA). These products are derived from CYP monooxygenase, cyclooxygenase (COX), and LOX/CYP ω/(ω-1)-hydroxylase pathways, which catalyze the production in a highly tissue-dependent and regioisomer-specific manner (Figure 1). The resulting products are epoxyoctadecenoic acids (EpOMEs), epoxyeicosatrienoic acid (EETs), epoxyeicosatetraenoic acids (EEQs), epoxydocosapentaenoic acids (EDPs), hydroperoxylinoleic acids (HpODEs), hydroxyoctadecadienoic acids (HODEs), hydroxydocosahexaenoic acids (HDHAs), hydroperoxyeicosatetraenoic acids (HPETEs), and hydroxyeicosatetraenoic acids (HETEs) (Figure 1). EpOMEs, EETs, EEQs, and EDPs are converted depending on cell type, into secondary eicosanoids and their metabolites. The major metabolic route of CYP epoxides is incorporation into phospholipids and hydrolysis to corresponding diols by the enzyme soluble epoxide hydrolase (sEH) (Spector & Kim, 2015). CYP-derived EETs and other epoxides, such as 17,18-EEQ, serve as endothelium-derived hyperpolarizing factors (EDHFs) to cause vasodilation (Campbell et al., 1996; Hercule et al., 2007; Hu & Kim, 1993). Recently, RBCs (~3 kg in human body) have been identified as a reservoir for CYP epoxides, in particular EETs, which on release may act in a vasoregulatory capacity (Jiang et al., 2010, 2011). Maximal exercise has been found to increase such erythro-epoxides in RBCs, including 9,10-EpOME, 12,13-EpOME, 5,6-EET, 11,12-EET, 14,15-EET, 16,17-EDP, and 19,20-EDP (Gollasch et al., 2019). Furthermore, sEH in the RBC and the resulting increase in EETs presumably contribute to a greater degree on regional blood flow than sEH inhibition localized in the arterial wall (Jiang et al., 2011; Yu et al., 2004). Nonetheless, the impact of epoxy and hydroxy fatty acids measurements in the RBCs for the prediction of CVD and mortality have not been previously elucidated. Whether or not CKD or hemodialysis treatment itself affect RBC-epoxids and hydroxy metabolites remains unknown. We tested the hypotheses that CKD and hemodialysis treatments performed on end-stage renal disease (ESRD) patients affect RBC epoxy fatty acids profiles.

2 | METHODS

The Charité University Medicine Institutional Review Board approved this duly registered study (ClinicalTrials.gov, Identifier: NCT03857984). Recruitment was primarily *via* person-to-person interview. Prior to participation in the study, 15 healthy volunteers (6 male and 9 female) and 15 CKD patients (7 male and 8 female) undergoing regular hemodialysis treatment signed informed consent forms which outlined the treatments to be taken and the possible risks involved. All healthy control subjects were not taking medications. Venous blood was collected in each healthy subject by subcutaneous arm vein puncture in the sitting position. In the group of dialyzed patients (CKD group), all the blood samples were collected on the fistula arm right before beginning of the dialysis (starting of the HD, pre-HD) and at the end of the dialysis (5–15 min before termination, post-HD). Patients underwent thrice-weekly dialysis, which lasted from 3 hr 45 min to 5 hr, based on high flux AK 200 dialyzers (Gambro GmbH, Hechingen, Germany). All samples were analyzed for RBC lipids. All blood samples were obtained by 4°C precooled EDTA vacuum extraction tube systems. Cells were separated from plasma by centrifugation for 10 min at 1,000–2,000 *g* using a refrigerated centrifuge RBCs were separated from EDTA blood by centrifugation as previously described (Gollasch, et al., 2020). RBC lipidomics was performed using LC–MS/MS tandem mass spectrometry as described in (Fischer et al., 2014; Gollasch et al., 2019; Gollasch et al., 2019). Concentrations are given in nanogram/g.

Descriptive statistics were calculated and variables were examined for meeting assumptions of normal distribution without skewness and kurtosis. In order to determine statistical significance, *t* test or Mann–Whitney test was used to compare the values of CKD versus control groups. Paired *t*-test or paired Wilcoxon test were used to compare pre-HD versus post-HD values. In order to determine statistical significance between the four classes of epoxy-metabolites hydrolyzed to appear in the circulation, Friedman's test followed by applying Dunn's multiple comparison test was used. In order to determine statistical significance between the four classes of epoxy-metabolites hydrolyzed to appear in the circulation, Friedman's test followed by applying Dunn's multiple comparison test was used. The analysis included Mauchly's test of sphericity followed by applying the test of within-subjects effects with Greenhouse–Geisser correction to ensure sphericity assumption (Gollasch et al., 2019; Gollasch et al., 2019). The .05 level of significance (p) was chosen. All data are

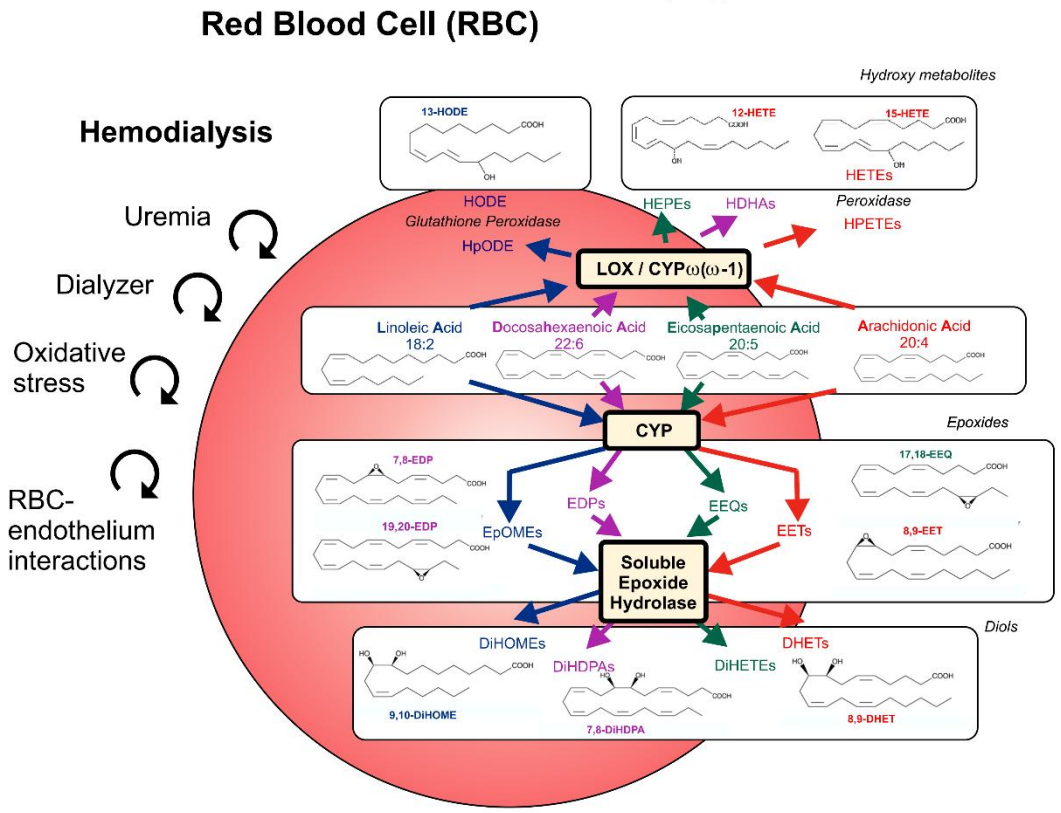

FIGURE 1 Hypothetic influence of CKD and hemodialysis associated with shear stress, red blood cell (RBC)-dialyzer interactions, red blood cell (RBC)-endothelial interactions, and oxidative stress affecting the content of cytochrome P450 epoxygenase (CYP) and 12- and 15-lipoxygenase (LOX)/CYP omega-hydroxylase metabolites in RBCs. The scheme illustrates the epoxide and hydroxy metabolites pathways studied. Linoleic (LA), arachidonic (AA), eicosapentaenoic (EPA), and docosahexaenoic acids (DHA) are converted to epoxyoctadecenoic acids (EpOMEs, e.g., 9,10-EpOME), epoxyeicosatrienoic acid (EETs, e.g., 8,9-EET), epoxyeicosatetraenoic acids (EEQs, e.g., 17,18-EEQ), and epoxydocosapentaenoic acids (EDPs, e.g., 17,18-EDP and 19,20-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., 8,9-DHET), dihydroxyeicosatetraenoic acids (DiHETEs), and dihydroxydocosapentaenoic acids (DiHDPAs, e.g., 7,8-DiHDPA), respectively, by the soluble epoxide hydrolase (sEH) enzyme. LA, AA, EPA, and DHA are converted to hydroperoxylinoleic acids (HpODEs), hydroxyoctadecadienoic acids (HODEs, e.g., 13-HODE), hydroxydocosahexaenoic acids (HDHAs), hydroperoxyeicosatetraenoic acids (HPETEs), and hydroxyeicosatetraenoic acids (HETEs, e.g., 12-HETE and 15-HETE) by LOX, CYP omega/(omega-1)-hydroxylase and peroxidase pathways. The metabolites measured within these pathways track the changes observed. Arrows demarcate metabolic pathways evaluated

presented as mean ± *SD*. All statistical analyses were performed using SPSS Statistics software (IBM Corporation) or All-Therapy statistics beta (AICBT Ltd).

3 | RESULTS

3.1 | Clinical characteristics

The age between ESRD patients and the healthy subjects was not different (50 ± 18 years vs. 47 ± 12 years, respectively, $p > .05$, $n = 15$ each). The body mass indices between the two groups were also not different (24.8 ± 3.4 kg/m^2 and 24.7 ± 4.6 kg/m^2, respectively, $p > .05$, $n = 15$ each). The patients in the group CKD were diagnosed for the following conditions: diabetes mellitus ($n = 4$ patients), hypertension ($n = 3$), membranous glomerulonephritis ($n = 2$), autosomal dominant polycystic kidney disease ($n = 1$), other or unknown ($n = 5$). Major cardiovascular complications in the CKD group included peripheral artery disease ($n = 3$), cardiovascular ($n = 2$) and cerebrovascular ($n = 1$) events. Subjects were Caucasians, with the exception of one Black

patient in the CKD group and one Asian subject in the control group.

3.2 | RBC epoxy and hydroxy metabolites in CKD

We first determined the total levels of various CYP epoxides and LOX/CYP ω/(ω-1)-hydroxylase products in RBCs of the HD patients (Table 1) and compared the results with the healthy control subjects. Total CYP epoxides were analyzed for each member (Table 1A) and together within the four subclasses (Table 2A). RBCs of hemodialysis patients showed increased total levels of various individual CYP epoxides, namely 8,9-DHET, 14,15-DHET, 5,6-EEQ, 11,12-EEQ, 14,15-EEQ, 17,18-EEQ, 7,8-DiHDPA, 10,11-DiHDPA, 13,14-DiHDPA, and 16,17-DiHDPA in the RBCs (Table 1A). EpOMEs, DiHOMEs, EETs, EDPs (with exception of 19,20-EDP), and DiHETEs were not different between both groups (Table 1A). Free CYP epoxides in the RBCs were also not different or only slightly decreased (8,9-EET, 14,15-EET, and 5,6-EEQ) in RBCs of hemodialysis patients. Nonetheless, our analysis of the four CYP epoxide classes demonstrates that ESRD patients can be discriminated from controls by characteristic increases in three epoxide classes, that is, signatures, namely increased levels of total DHETs, EEQs, and DiHDPAs in the RBCs, that is, 5,6-DHET+8,9-DHET+11,12-DHET+14,15-DHET, 5,6-EEQ+8,9-EEQ+11,12-EEQ+14,15-EEQ+17,18-EEQ, and 7,8-DiHDPA+10,11-DiHDPA+13,14-DiHDPA+16,17-DiHDPA+19,20-DiHDPA (Table 2A). We next inspected the total levels of various LOX/CYP ω/(ω-1)-hydroxylase products in RBCs of the HD patients (Table 1A). We found that 5-HETE, 8-HETE, 9-HETE, 11-HETE, 12-HETE, 15-HETE, and 19-HETE levels were increased in the hemodialysis patients, whereas 13-HODE, 16-HETE, 17-HETE, 18-HETE, 20-HETE, 12 -HpETE, 5-HEPE, 8-HEPE, 9-HEPE, 12-HEPE, 15-HEPE, 18-HEPE, 19-HEPE, 20-HEPE, 4-HDHA, 7-HDHA, 8-HDHA, 10-HDHA, 11-HDHA, 13-HDHA, 14-HDHA, 16-HDHA, 17-HDHA, 20-HDHA, 21-HDHA, and 22-HDHA levels, were normal or nondetectable (Table 1A). Of note, free LOX/CYP ω/(ω-1)-hydroxylase products were generally increased in RBCs of hemodialysis patients, with exception of 17-HETE, 18-HETE, 19-HETE, 20-HETE, 12-HpETE, 19-HEPE, 20-HEPE, and 20-HDHA which were normal or non-detectable (Table 1B). Together, the findings indicate that ESRD patients show an altered RBC fatty acid metabolite status, that is, individual signature, which shows the accumulation of three CYP epoxide classes (DHETs, EEQs, and DiHDPAs) and various HETEs and other LOX/CYP ω/(ω-1) metabolites in RBCs, the latter mostly accumulated in free state.

3.3 | Ratios

The main route of EpOMEs, EETs, EEQs, and EDPs metabolism in many cells is conversion into DiHOMEs, DHETs, dihydroxyeicosatetraenoic acids (DiHETEs), and dihydroxydocosapentaenoic acids (DiHDPAs) by the sEH, respectively (Figure 1). To provide possible insights into the nature of the observed accumulation of DHETs, EEQs, and DiHDPAs in RBCs of ESRD patients, we calculated diol/epoxide ratios in RBCs and compared the results with the control subjects (Table 2B). We found that the four classes of epoxy-metabolites are unequally hydrolyzed and appear in the RBCs (Table 2B for controls). Compared to EETs and EEQs (ratios diols/epoxy-metabolites, 0.0096 ± 0.0017 vs. 0.0042 ± 0.00012, Dunn's multiple comparison test, $p > .05$), EpOMEs and EDPs (ratios diols/epoxy-metabolites, 0.1628 ± 0.0658 vs. 0.0244 ± 0.0053, Dunn's multiple comparison test, $p > .05$) are preferentially metabolized into their diols. In fact, the following order of ratios was identified: DiHOMEs/EpOMEs=DiHDPA/EDPs>DHETs/EETs=DiHETEs/EEQs (Dunn's multiple comparison test, $p < .05$). ESRD patients showed increased ratios for DHET/EET and DiHDPA/EDP, which indicates that increased sEH activity preferred for EET and EDP substrate classes in vivo may have caused the observed accumulation of 8,9-DHET, 14,15-DHET, 7,8-DiHDPA, 10,11-DiHDPA, 13,14-DiHDPA, and 16,17-DiHDPA in the RBCs in ESRD. The observed accumulation of EEQs is unlikely to result from changes in sEH activity (Table 2B) or accumulation of eicosapentaenoic acid (EPA) as EPA levels are not increased in RBCs of our patients (Gollasch et al., 2020) (Figure 1).

3.4 | Effects of hemodialysis

With the exception of 7,8-DiHDPA, the data (Table 3) demonstrate no change of total CYP epoxides and LOX/CYP ω/(ω-1)-hydroxylase metabolites in response to a single dialysis (Table 3A). Accordingly, the diol/epoxide ratios were not altered (Table 4). However, hemodialysis treatment increased several CYP epoxides and LOX/CYP ω/(ω-1)-hydroxylase metabolites in free state, such as 11,12-DHET, 13-HODE, 5-HETE, 8-HETE, 9-HETE, 11-HETE, 15-HETE, 5-HEPE, 8-HDHA, 10-HDHA, 13-HDHA, 16-HDHA, and 17-HDHA (Table 3B).

TABLE 1 Comparison of epoxy- and hydroxy-metabolites between control subjects versus CKD patients before hemodialysis (HD) ($n = 15$ each)

Amount (ng/g)	Control (Mean ± *SD*)	HD (mean ± *SD*)	*p* value, *t* test (#Mann-Whitney test)
(A) Total metabolites in RBCs			
CYP epoxy-metabolites			
(a) EpOMEs/DiHOMES			
9,10-EpOME	29.36 ± 12.01	25.48 ± 6.59	.267#
12,13-EpOME	13.67 ± 9.22	10.62 ± 6.16	.305#
9,10-DiHOME	4.12 ± 1.30	5.13 ± 1.92	.081#
12,13-DiHOME	2.26 ± 0.90	2.92 ± 1.45	.161#
(b) EETs/DiHOMEs			
5,6-EET	170.67 ± 29.90	148.54 ± 44.94	.124
8,9-EET	39.03 ± 6.25	39.90 ± 9.00	.761
11,12-EET	39.46 ± 5.51	37.86 ± 11.98	.644
14,15-EET	66.17 ± 11.64	59.58 ± 22.69	.328
5,6-DHET	0.89 ± 0.17	0.98 ± 0.43	.457
8,9-DHET	**1.07 ± 0.23**	**2.03 ± 1.81**	**.001#**
11,12-DHET	0.62 ± 0.14	0.96 ± 0.61	.081#
14,15-DHET	**0.40 ± 0.05**	**0.51 ± 0.16**	**.030**
(c) EEQs/DiHETEs			
5,6-EEQ	**41.54 ± 13.39**	**51.78 ± 98.53**	**.019#**
8,9-EEQ	2.48 ± 0.89	3.51 ± 6.41	.126#
11,12-EEQ	**2.09 ± 0.68**	**2.56 ± 4.74**	**.016#**
14,15-EEQ	**1.44 ± 0.48**	**1.91 ± 3.57**	**.041#**
17,18-EEQ	**3.25 ± 1.03**	**3.90 ± 7.31**	**.021#**
5,6-DiHETE	0.21 ± 0.10	0.28 ± 0.49	.202#
8,9-DiHETE	0.01 ± 0.01	0.01 ± 0.01	.776#
11,12-DiHETE	0.01 ± 0.01	0.01 ± 0.01	.677#
14,15-DiHETE	0.01 ± 0.01	0.01 ± 0.01	.697#
17,18-DiHETE	0.01 ± 0.01	0.01 ± 0.01	.787#
(d) EDPs/DiHDPAs			
7,8-EDP	15.58 ± 4.55	18.16 ± 12.19	.838#
10,11-EDP	1.22 ± 0.43	1.35 ± 0.47	.463
13,14-EDP	0.39 ± 0.25	0.44 ± 0.15	.158#
16,17-EDP	4.49 ± 1.34	4.72 ± 1.78	.967#
19,20-EDP	**6.72 ± 4.26**	**4.22 ± 1.52**	**.026#**
7,8-DiHDPA	**0.21 ± 0.10**	**0.40 ± 0.30**	**.041#**
10,11-DiHDPA	**0.50 ± 0.20**	**0.09 ± 0.05**	**.007**
13,14-DiHDPA	**0.08 ± 0.02**	**0.11 ± 0.04**	**.037#**
16,17-DiHDPA	**0.14 ± 0.03**	**0.19 ± 0.06**	**.022**
19,20-DiHDPA	0.20 ± 0.07	0.26 ± 0.16	.187#
LOX/CYP ω/(ω−1) metabolites			
13-HODE	69.46 ± 19.97	77.47 ± 18.89	.098#
5-HETE	**38.43 ± 7.90**	**53.45 ± 14.83**	**.002**
8-HETE	**27.30 ± 5.72**	**35.11 ± 10.20**	**.015**
9-HETE	**27.49 ± 4.72**	**37.84 ± 9.77**	**.001**
11-HETE	**41.90 ± 7.00**	**54.16 ± 14.84**	**.009**
12-HETE	**32.71 ± 5.66**	**43.47 ± 12.68**	**.007**

(Continues)

TABLE 1 (Continued)

Amount (ng/g)	Control (Mean ± *SD*)	HD (mean ± *SD*)	*p* value, *t* test (#Mann-Whitney test)
15-HETE	**74.29 ± 14.38**	**93.95 ± 24.59**	**.012**
16-HETE	4.60 ± 0.82	4.91 ± 1.43	.461
17-HETE	0.18 ± 0.03	0.22 ± 0.10	.512#
18-HETE	0.24 ± 0.05	0.32 ± 0.21	.461#
19-HETE	**0.26 ± 0.11**	**0.42 ± 0.11**	**.001#**
20-HETE	0.59 ± 0.09	0.62 ± 0.08	.371
12-HpETE	n.d.	n.d.	n/a
5-HEPE	1.47 ± 0.51	2.05 ± 2.64	.838#
8-HEPE	0.75 ± 0.31	1.15 ± 1.55	.744#
9-HEPE	0.93 ± 0.37	1.35 ± 1.64	.744#
12-HEPE	1.38 ± 0.52	2.15 ± 3.12	.935#
15-HEPE	1.18 ± 0.41	2.06 ± 2.74	.345#
18-HEPE	3.19 ± 1.30	5.28 ± 7.10	.567#
19-HEPE	1.32 ± 0.50	1.89 ± 2.80	.902#
20-HEPE	n.d.	n.d.	n/a
4-HDHA	9.11 ± 2.99	11.20 ± 4.61	.267#
7-HDHA	4.56 ± 1.36	5.90 ± 2.69	.137#
8-HDHA	5.27 ± 1.77	7.16 ± 3.11	.061#
10-HDHA	6.39 ± 1.99	8.05 ± 3.79	.148
11-HDHA	7.38 ± 2.41	9.43 ± 4.47	.217#
13-HDHA	9.35 ± 2.80	10.43 ± 4.20	.414
14-HDHA	5.41 ± 1.75	6.82 ± 3.38	.345#
16-HDHA	8.79 ± 2.69	9.80 ± 3.88	.486#
17-HDHA	12.98 ± 3.97	15.55 ± 6.92	.227
20-HDHA	19.16 ± 5.89	22.57 ± 9.88	.261
21-HDHA	3.04 ± 1.18	3.76 ± 1.70	.184
22-HDHA	n.d.	n.d.	n/a
(B) Free metabolites in RBCs			
CYP epoxy-metabolites			
(a) EpOMEs/DiHOMES			
9,10-EpOME	1.42 ± 0.59	1.79 ± 1.00	.367#
12,13-EpOME	1.22 ± 0.63	1.25 ± 0.91	.624#
9,10-DiHOME	0.43 ± 0.29	0.52 ± 0.34	.595#
12,13-DiHOME	1.70 ± 0.96	2.20 ± 1.52	.412#
(b) EETs/DiHOMEs			
5,6-EET	0.55 ± 0.21	0.45 ± 0.19	.170
8,9-EET	**0.12 ± 0.06**	**0.06 ± 0.04**	**.013#**
11,12-EET	0.24 ± 0.07	0.20 ± 0.08	.100
14,15-EET	**1.08 ± 0.40**	**0.74 ± 0.36**	**.015#**
5,6-DHET	n.d.	n.d.	n/a
8,9-DHET	n.d.	n.d.	n/a
11,12-DHET	0.01 ± 0.01	0.01 ± 0.01	.467
14,15-DHET	**0.01 ± 0.01**	**0.01 ± 0.01**	**.074#**
(c) EEQs/DiHETEs			
5,6-EEQ	**1.29 ± 1.14**	**0.90 ± 3.39**	**.010#**
8,9-EEQ	0.22 ± 0.12	0.31 ± 0.50	.351#

(Continues)

TABLE 1 (Continued)

Amount (ng/g)	Control (Mean ± *SD*)	HD (mean ± *SD*)	*p* value, *t* test (#Mann-Whitney test)
11,12-EEQ	0.06 ± 0.04	0.07 ± 0.14	.116#
14,15-EEQ	0.14 ± 0.10	0.20 ± 0.23	.851#
17,18-EEQ	0.39 ± 0.19	0.53 ± 1.02	.217#
5,6-DiHETE	n.d.	n.d.	n/a
8,9-DiHETE	n.d.	n.d.	n/a
11,12-DiHETE	n.d.	n.d.	n/a
14,15-DiHETE	0.01 ± 0.01	0.01 ± 0.04	.285#
17,18-DiHETE	0.04 ± 0.02	0.11 ± 0.23	.902#
(d) EDPs/DiHDPAs			
7,8-EDP	0.12 ± 0.05	0.17 ± 0.17	.539#
10,11-EDP	0.01 ± 0.01	0.01 ± 0.01	.222#
13,14-EDP	n.d.	n.d.	n/a
16,17-EDP	n.d.	n.d.	n/a
19,20-EDP	0.06 ± 0.05	0.11 ± 0.22	.505#
7,8-DiHDPA	n.d.	n.d.	n/a
10,11-DiHDPA	n.d.	n.d.	n/a
13,14-DiHDPA	n.d.	n.d.	n/a
16,17-DiHDPA	0.01 ± 0.01	0.02 ± 0.01	.461#
19,20-DiHDPA	0.12 ± 0.06	0.15 ± 0.14	.744#
LOX/CYP ω/(ω−1) metabolites			
13-HODE	**8.96 ± 4.64**	**36.76 ± 31.23**	**<.001#**
5-HETE	**0.21 ± 0.07**	**0.60 ± 0.37**	**<.001#**
8-HETE	**0.28 ± 0.14**	**0.90 ± 0.59**	**<.001#**
9-HETE	**0.55 ± 0.32**	**1.85 ± 1.46**	**<.001#**
11-HETE	**0.84 ± 0.32**	**2.66 ± 1.64**	**<.001#**
12-HETE	**4.23 ± 2.53**	**28.11 ± 33.78**	**<.001#**
15-HETE	**0.65 ± 0.25**	**2.15 ± 1.05**	**<.001**
16-HETE	**0.10 ± 0.03**	**0.15 ± 0.06**	**.003**
17-HETE	n.d.	n.d.	n/a
18-HETE	n.d.	n.d.	n/a
19-HETE	n.d.	n.d.	n/a
20-HETE	0.10 ± 0.05	0.10 ± 0.04	.877
12-HpETE	n.d.	n.d.	n/a
5-HEPE	**0.03 ± 0.02**	**0.14 ± 0.34**	**.021#**
8-HEPE	**0.04 ± 0.03**	**0.32 ± 0.87**	**<.001#**
9-HEPE	**0.05 ± 0.04**	**0.35 ± 0.96**	**.003#**
12-HEPE	**0.97 ± 0.52**	**8.06 ± 14.72**	**.006#**
15-HEPE	**0.06 ± 0.04**	**0.70 ± 1.82**	**<.001#**
18-HEPE	**0.12 ± 0.06**	**1.52 ± 3.96**	**<.001#**
19-HEPE	0.03 ± 0.02	0.22 ± 0.69	.367#
20-HEPE	n.d.	n.d.	n/a
4-HDHA	**0.03 ± 0.02**	**0.18 ± 0.32**	**.001#**
7-HDHA	**0.02 ± 0.01**	**0.11 ± 0.05**	**.001#**
8-HDHA	**0.04 ± 0.02**	**0.22 ± 0.35**	**<.001#**
10-HDHA	**0.06 ± 0.03**	**0.63 ± 1.07**	**<.001#**
11-HDHA	**0.19 ± 0.08**	**0.87 ± 1.32**	**<.001#**

(Continues)

TABLE 1 (Continued)

Amount (ng/g)	Control (Mean ± *SD*)	HD (mean ± *SD*)	*p* value, *t* test (#Mann-Whitney test)
13-HDHA	**0.08 ± 0.04**	**0.44 ± 0.61**	**<.001#**
14-HDHA	**0.35 ± 0.17**	**2.81 ± 3.60**	**<.001#**
16-HDHA	**0.07 ± 0.03**	**0.37 ± 0.63**	**<.001#**
17-HDHA	**0.42 ± 0.15**	**2.59 ± 4.22**	**<.001#**
20-HDHA	0.27 ± 0.09	0.67 ± 1.00	.050#
21-HDHA	**0.11 ± 0.05**	**0.42 ± 0.59**	**.002#**
22-HDHA	**0.72 ± 0.29**	**1.27 ± 0.71**	**.013**

Note: Bold font indicates statistical significance.

Abbreviations: n.d., not detected; n/a, not applicable.

TABLE 2 Comparison of epoxy-metabolites and their ratios between control subjects versus CKD patients before hemodialysis (HD) ($n = 15$ each)

(A) Concentrations of individual total epoxides together or their respective diols in RBCs			
Epoxides or Diols (ng/g)	**Control (Mean ± *SD*)**	**HD (Mean ± *SD*)**	***p*-value, Mann-Whitney test**
9,10-EpOME+12,13-EpOME	43.03 ± 21.07	36.10 ± 10.60	.3195
9,10-DiHOME+12,13-DiHOME	6.377 ± 2.104	8.049 ± 3.178	.0971
5,6-EET+8,9-EET+11,12 EET+14,15-EET	315.3 ± 51.27	285.9 ± 86.25	.2998
5,6-DHET+8,9-DHET+11,12-DHET+14,15-DHET	**2.986 ± 0.5208**	**4.477 ± 2.789**	**.0421**
5,6-EEQ+8,9-EEQ+11,12-EEQ+14,15-EEQ+17,18-EEQ	**50.81 ± 16.35**	**63.65 ± 120.5**	**.0225**
5,6-DiHETE+8,9-DiHETE+11,12-DiHETE+14,15-DiHETE+17,18-DiHETE	0.2153 ± 0.1021	0.3420 ± 0.7263	.1835
7,8-EDP+10,11-EDP+13,14-EDP+16,17-EDP+19,20-EDP	28.40 ± 9.805	28.86 ± 14.26	.6187
7,8-DiHDPA+10,11-DiHDPA+13,14-DiHDPA+16,17-DiHDPA+19,20-DiHDPA	**0.6813 ± 0.2123**	**1.039 ± 0.5678**	**.0464**
(B) Ratios estimated using total concentrations of epoxides and diols in RBCs			
Ratios	**Control (Mean ± *SD*)**	**HD (Mean ± *SD*)**	***p*-value, Mann-Whitney test**
Ratio (9,10-DiHOME+12,13-DiHOME)/(9,10-EpOME+12,13-EpOME)	0.1628 ± 0.06583	0.2425 ± 0.1255	.0564
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)	**0.0096 ± 0.001705**	**0.01652 ± 0.009067**	**.0279**
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)	0.00416 ± 0.001188	0.005927 ± 0.004070	.2627
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)	**0.02445 ± 0.005347**	**0.03765 ± 0.01382**	**.0025**

Note: Bold font indicates statistical significance.

TABLE 3 Effects of hemodialysis on epoxy- and hydroxy-metabolites in the CKD patients before (pre-HD) and at cessation (post-HD) of hemodialysis ($n = 15$ each)

Amount, (ng/g)	Pre-HD (Mean ± *SD*)	Post-HD (mean ± *SD*)	*p* value, paired *t* test (#paired Wilcoxon test)
(A) Total metabolites in RBCs			
CYP epoxy-metabolites			
(a) EpOMEs/DiHOMES			
9,10-EpOME	25.48 ± 6.59	25.91 ± 5.94	.802
12,13-EpOME	10.62 ± 6.16	11.52 ± 7.93	.307#
9,10-DiHOME	5.13 ± 1.92	5.27 ± 1.42	.623
12,13-DiHOME	2.92 ± 1.45	3.00 ± 0.90	.914
(b) EETs/DiHOMEs			
5,6-EET	148.54 ± 44.94	162.71 ± 46.95	.198
8,9-EET	39.90 ± 9.00	43.76 ± 8.50	.134
11,12-EET	37.86 ± 11.98	41.54 ± 11.54	.112
14,15-EET	59.58 ± 22.69	63.97 ± 21.75	.162
5,6-DHET	0.98 ± 0.43	1.06 ± 0.43	.117
8,9-DHET	2.03 ± 1.81	2.13 ± 1.67	.112#
11,12-DHET	0.96 ± 0.61	0.99 ± 0.50	.334#
14,15-DHET	0.51 ± 0.16	0.53 ± 0.12	.148
(c) EEQs/DiHETEs			
8,9-EEQ	3.51 ± 6.41	3.39 ± 5.75	1.000#
5,6-EEQ	51.78 ± 98.53	45.89 ± 69.79	.650#
11,12-EEQ	2.56 ± 4.74	2.40 ± 3.49	.125#
14,15-EEQ	1.91 ± 3.5	1.66 ± 2.55	.910#
17,18-EEQ	3.90 ± 7.31	3.66 ± 5.81	.460#
5,6-DiHETE	0.28 ± 0.49	0.24 ± 0.32	.733#
8,9-DiHETE	n.d.	n.d.	n/a
11,12-DiHETE	n.d.	n.d.	n/a
14,15-DiHETE	n.d.	n.d.	n/a
17,18-DiHETE	n.d.	n.d.	n/a
(d) EDPs/DiHDPAs			
7,8-EDP	18.16 ± 12.19	19.48 ± 12.59	.307#
10,11-EDP	1.35 ± 0.47	1.50 ± 0.73	.427#
13,14-EDP	0.44 ± 0.15	0.52 ± 0.31	.551#
16,17-EDP	4.72 ± 1.78	5.46 ± 2.50	.078#
19,20-EDP	4.22 ± 1.52	5.14 ± 2.84	.109
7,8-DiHDPA	**0.40 ± 0.30**	**0.48 ± 0.42**	**.036#**
10,11-DiHDPA	0.09 ± 0.05	0.10 ± 0.07	.256#
13,14-DiHDPA	0.11 ± 0.04	0.12 ± 0.04	.363#
16,17-DiHDPA	0.19 ± 0.06	0.20 ± 0.07	.124
19,20-DiHDPA	0.26 ± 0.16	0.27 ± 0.14	.173#
LOX/CYP ω/(ω−1) metabolites			
13-HODE	77.47 ± 18,89	82.00 ± 18.35	.391
5-HETE	53.45 ± 14.83	56.62 ± 10.08	.295
8-HETE	35.11 ± 10.20	36.63 ± 7.23	.379

(Continues)

TABLE 3 (Continued)

Amount, (ng/g)	Pre-HD (Mean ± *SD*)	Post-HD (mean ± *SD*)	*p* value, paired *t* test (#paired Wilcoxon test)
9-HETE	37.84 ± 9.77	39.89 ± 7.07	.268
11-HETE	54.16 ± 14.84	56.92 ± 10.96	.323
12-HETE	43.47 ± 12.68	45.33 ± 8.36	.466
15-HETE	93.95 ± 24.59	99.31 ± 18.34	.281
16-HETE	4.91 ± 1.43	5.14 ± 1.08	.412
17-HETE	0.22 ± 0.10	0.22 ± 0.08	.363#
18-HETE	0.32 ± 0.21	0.34 ± 0.23	.112#
19-HETE	0.42 ± 0.11	0.49 ± 0.17	.085
20-HETE	0.62 ± 0.08	0.65 ± 0.23	.602
12-HpETE	n.d.	n.d.	
5-HEPE	2.05 ± 2.64	2.30 ± 3.25	.281#
8-HEPE	1.15 ± 1.55	1.26 ± 1.88	.363#
9-HEPE	1.35 ± 1.64	1.51 ± 2.11	.281#
12-HEPE	2.15 ± 3.12	2.29 ± 3.38	.307#
15-HEPE	2.06 ± 2.74	2.23 ± 2.95	.053#
18-HEPE	5.28 ± 7.10	5.63 ± 7.64	.140#
19-HEPE	1.89 ± 2.80	1.80 ± 2.32	.910#
20-HEPE	n.d.	n.d.	
4-HDHA	11.20 ± 4.61	12.71 ± 5.81	.140#
7-HDHA	5.90 ± 2.69	6.33 ± 2.85	.233#
8-HDHA	7.16 ± 3.11	7.74 ± 3.35	.112#
10-HDHA	8.05 ± 3.79	8.57 ± 3.93	.334#
11-HDHA	9.43 ± 4.47	10.03 ± 4.90	.140#
13-HDHA	10.43 ± 4.20	11.22 ± 4.95	.173#
14-HDHA	6.82 ± 3.38	7.41 ± 3.41	.156#
16-HDHA	9.80 ± 3.88	10.55 ± 4.14	.112#
17-HDHA	15.55 ± 6.92	16.83 ± 7.47	.078#
20-HDHA	22.57 ± 9.88	24.53 ± 10.60	.112#
21-HDHA	3.76 ± 1.70	3.71 ± 1.32	.790
22-HDHA	n.d.	n.d.	n/a
(B) Free metabolites in RBCs			
CYP epoxy-metabolites			
(a) EpOMEs/DiHOMES			
9,10-EpOME	1.79 ± 1.00	2.08 ± 0.48	.156#
12,13-EpOME	1.25 ± 0.91	1.89 ± 0.88	.053#
9,10-DiHOME	0.52 ± 0.34	0.65 ± 0.29	.147
12,13-DiHOME	2.20 ± 1.52	2.91 ± 1.87	.256#
(b) EETs/DiHOMEs			
5,6-EET	0.45 ± 0.19	0.54 ± 0.21	.114
8,9-EET	0.06 ± 0.04	0.07 ± 0.10	.480#
11,12-EET	0.20 ± 0.08	0.21 ± 0.06	.654
14,15-EET	0.74 ± 0.36	0.93 ± 0.35	.100#
5,6-DHET	<0.01 ± 0.01	<0.01 ± 0.01	n/a

(Continues)

TABLE 3 (Continued)

Amount, (ng/g)	Pre-HD (Mean ± *SD*)	Post-HD (mean ± *SD*)	*p* value, paired *t* test (#paired Wilcoxon test)
8,9-DHET	0.02 ± 0.01	0.03 ± 0.03	.131#
11,12-DHET	**<0.02 ± 0.01**	**0.02 ± 0.01**	**.005**
14,15-DHET	0.01 ± 0.01	0.02 ± 0.01	.427#
(c) EEQs/DiHETEs			
5,6-EEQ	0.90 ± 3.39	1.08 ± 3.52	.068#
8,9-EEQ	0.31 ± 0.50	0.20 ± 0.55	.128#
11,12-EEQ	0.07 ± 0.14	0.07 ± 0.09	.424#
14,15-EEQ	0.20 ± 0.23	0.17 ± 0.24	.477#
17,18-EEQ	0.53 ± 1.02	0.49 ± 0.80	.955#
5,6-DiHETE	<0.01 ± 0.01	<0.01 ± 0.01	.477
8,9-DiHETE	<0.01	<0.01	n/a
11,12-DiHETE	<0.01	<0.01	n/a
14,15-DiHETE	0.01 ± 0.04	0.02 ± 0.04	.394#
17,18-DiHETE	0.11 ± 0.23	0.16 ± 0.38	.394#
(d) EDPs/DiHDPAs			
7,8-EDP	0.17 ± 0.17	0.22 ± 0.18	.112#
10,11-EDP	0.01 ± 0.01	0.01 ± 0.01	.463#
13,14-EDP	n.d.	n.d.	n/a
16,17-EDP	n.d.	n.d.	n/a
19,20-EDP	0.11 ± 0.22	0.09 + 0.09	.507#
7,8-DiHDPA	n.d.	n.d.	n/a
10,11-DiHDPA	<0.01 ± 0.01	<0.01 ± 0.01	n/a
13,14-DiHDPA	<0.01 ± 0.01	0.01 ± 0.01	.465#
16,17-DiHDPA	0.02 ± 0.01	0.03 ± 0.02	.140#
19,20-DiHDPA	0.15 ± 0.14	0.18 ± 0.18	.334#
LOX/CYP ω/(ω−1) metabolites			
13-HODE	**36.76 ± 31.23**	**45.70 ± 31.56**	**.031#**
5-HETE	**0.60 ± 0.37**	**0.85 ± 0.53**	**.023#**
8-HETE	**0.90 ± 0.59**	**1.24 ± 0.83**	**.008#**
9-HETE	**1.85 ± 1.46**	**2.51 ± 1.84**	**.031#**
11-HETE	**2.66 ± 1.64**	**3.37 ± 2.16**	**.017#**
12-HETE	28.11 ± 33.78	34.20 ± 33.78	.334#
15-HETE	**2.15 ± 1.05**	**2.78 ± 1.54**	**.008#**
16-HETE	0.15 ± 0.06	0.15 ± 0.04	.999
17-HETE	n.d.	n.d.	n/a
18-HETE	n.d.	n.d.	n/a
19-HETE	n.d.	n.d.	n/a
20-HETE	0.10 + 0.04	0.12 ± 0.06	.155
12-HpETE	n.d.	n.d.	n/a
5-HEPE	**0.14 ± 0.34**	**0.18 ± 0.45**	**.031#**
8-HEPE	0.32 ± 0.87	0.35 ± 0.98	.394#
9-HEPE	0.35 ± 0.96	0.42 ± 1.14	.112#
12-HEPE	8.06 ± 14.72	10.61 ± 21.49	.191#
15-HEPE	0.70 ± 1.82	0.75 ± 1.96	.307#

(Continues)

TABLE 3 (Continued)

Amount, (ng/g)	Pre-HD (Mean ± *SD*)	Post-HD (mean ± *SD*)	*p* value, paired *t* test (#paired Wilcoxon test)
18-HEPE	1.52 ± 3.96	1.53 ± 3.96	.776#
19-HEPE	0.22 ± 0.69	0.21 ± 0.64	.955#
20-HEPE	n.d.	n.d.	n/a
4-HDHA	0.18 ± 0.32	0.25 ± 0.45	.061#
7-HDHA	0.11 ± 0.05	0.15 ± 0.28	.112#
8-HDHA	**0.22 ± 0.35**	**0.31 ± 0.49**	**.031#**
10-HDHA	**0.63 ± 1.07**	**0.79 ± 1.39**	**.023#**
11-HDHA	0.87 ± 1.32	1.07 ± 1.60	.100#
13-HDHA	**0.44 ± 0.61**	**0.55 ± 0.74**	**.036#**
14-HDHA	2.81 ± 3.60	3.40 ± 4.40	.078#
16-HDHA	**0.37 ± 0.63**	**0.50 ± 0.93**	**.012#**
17-HDHA	**2.59 ± 4.22**	**3.35 ± 5.24**	**.031#**
20-HDHA	0.67 ± 1.00	0.83 ± 1.37	.112#
21-HDHA	0.42 ± 0.59	0.48 ± 0.68	.256#
22-HDHA	1.27 ± 0.71	1.30 ± 0.78	.837

Note: Bold font indicates statistical significance.

TABLE 4 Effects of hemodialysis on epoxide and their respective diol ratios in the CKD patients before (pre-HD) and at cessation (post-HD) of hemodialysis ($n = 15$ each). Ratios were estimated using total concentrations of epoxides and diols in RBCs

Ratios	Pre-HD (Mean ± *SD*)	Post-HD (Mean ± *SD*)	*p*-value, Paired Wilcoxon test)
Ratio (9,10-DiHOME+12,13-DiHOME)/(9,10-EpOME+12,13-EpOME)	0.2425 ± 0.1255	0.2435 ± 0.1043	.8904
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)	0.01652 ± 0.009067	0.01623 ± 0.008816	.8647
Ratio (5,6-DiHETE+8,9-DiHETE+14,15-DiHETE+17,18-DiHETE)/(5,6-EEQ+8,9-EEQ+11,12-EEQ+14,15-EEQ+17,18-EEQ)	0.005927 ± 0.004070	0.005647 ± 0.003565	.4896
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)	0.03765 ± 0.01382	0.03873 ± 0.01658	.4887

4 | DISCUSSION

Our data demonstrate that RBCs of ESRD patients accumulated three CYP epoxide classes (DHETs, EEQs, and DiHDPAs) and various HETEs, including 5-HETE, 8-HETE, 9-HETE, 11-HETE, 12-HETE, 15-HETE, and 19-HETE, compared to control subjects. Furthermore, hemodialysis treatment is insufficient to change the total concentrations of these and other LOX/CYP metabolites in RBCs of ESRD patients. Since the four subclasses of CYP epoxy metabolites increase in plasma after the dialysis treatment (Gollasch et al., 2020), we suggest that total CYP metabolites in RBCs are relatively invulnerable in CKD and hemodialysis (possibly due to slow exchange). Of note, ESRD is associated with increased levels of several free CYP epoxides and LOX/CYP ω/(ω-1)-hydroxylase metabolites in RBCs. Since several of those mediators are also increased by hemodialysis treatment itself, we suggest that free RBC eicosanoids constitute a fraction of lipid mediators, which are particularly vulnerable in CKD and hemodialysis. The extent to which the RBC eicosanoids exhibit beneficial or detrimental cardiovascular effects in CKD, possibly in comprehensive lipidomic (patho)physiological networks, remains to be explored. Nonetheless, our results indicate that RBCs could represent a reservoir for PUFA CYP epoxy-metabolites and LOX/CYP hydroxy metabolites, which on release may act in a

vasoregulatory capacity to affect cardiovascular responses in hemodialysis patients.

4.1 | EETs

RBCs are reservoir of EETs which on release may act in a vasoregulatory capacity (Jiang et al., 2010, 2011). In addition to serving as carriers of O_2, RBCs are known to regulate the microvascular perfusion by liberating adenosine triphosphate (ATP) and EETs upon exposure to a low O_2 environment (Jiang et al., 2010; Sprague et al., 2010). The release of EETs is activated by $P2X_7$ receptor stimulation *via* ATP to cause the circulatory response (Jiang et al., 2007). RBCs are believed to serve as a source of plasma EETs, which are esterified to the phospholipids of lipoproteins. Therefore, levels of free EETs in plasma are found to be low (~3% of circulating EETs) (Jiang et al., 2010, 2011). Erythro-EETs are produced by direct oxidation of AA and the monooxygenase-like activity of hemoglobin (Jiang et al., 2010, 2011, 2012). On release, EETs and their diols (DHETs) produce vasodilation (Hercule et al., 2009; Lu et al., 2001), are pro-fibrinolytic and reduce inflammation (Jiang et al., 2010, 2011, 2012). Exhaustive exercise increases the circulating levels of 5,6-DHET (Gollasch et al., 2019). In this study, we were able to demonstrate that RBCs of ESRD patients show increased accumulation of total DHETs. In particular, we observed increases in total concentrations of 8,9-DHET and 14,15-DHET in the RBCs. Hemodialysis did not affect this accumulation. It remains unknown whether RBCs are capable of liberating erythro-DHETs into the blood and/or tissues in kidney patients. Our results indicate that CKD affects the RBC reservoir for DHETs, but not EETs, which on release may affect the cardiovascular response.

4.2 | Other PUFA metabolites

We observed increases in total concentrations of EEQs (5,6-EEQ, 11,12-EEQ, 14,15-EEQ, 17,18-EEQ) and EDP/DiHDPAs (19,20-EDP, 7,8-DiHDPA, 10,11- DiHDPA, 13,14-DiHDPA, 16,17-DiHDPA) and HETEs (5-HETE, 8-HETE, 9-HETE, 11-HETE, 12-HETE, 15-HETE, 19-HETE) in RBCs of our ESRD patients. Little is known about the functions of EEQs and EDPs. Both EEQs and EDPs are potent vasodilators (Hercule et al., 2007; Lauterbach et al., 2002; Morin et al., 2011; Ulu et al., 2014). EDPs have antiangiogenic (McDougle et al., 2017), anti-fibrotic (Sharma et al., 2016) and protective effects in post-ischemic functional recovery, at least in particular by maintaining mitochondrial function and reducing inflammatory responses (Arnold et al., 2010; Darwesh et al., 2019). It is possible that their diols (DiHDPAs) are also biologically active and may exert beneficial effects in cardiac arrhythmias (Zhang et al., 2016). DiHDPAs dilate coronary microvessels with similar potency to EEQ isomers in canine and porcine models (Zhang et al., 2001) and inhibit human platelet aggregation with moderately lower potency to EDPs and EEQs (VanRollins, 1995). Specific 17,18-EEQ analogs are in development to serve as novel antiarrhythmic agents (Adebesin et al., 2019). HETEs are involved in many chronic diseases such as inflammation, obesity, cardiovascular disease, kidney disease, and cancer, for review see (Gabbs et al., 2015). Nonetheless, it remains unknown whether RBCs are capable of liberating EEQs, DiHDPAs, or HETEs into blood or tissues. Our data indicate that both metabolite classes are novel candidates potentially released by RBCs to exhibit cardiovascular effects in health and CKD.

Surprisingly, we did detect increases in various free CYP epoxides and LOX/CYP ω/(ω-1)-hydroxylase metabolites in RBCs in ESRD, which were augmented by hemodialysis. The mechanism by which CKD and hemodialysis raises the levels of those erythro-metabolites is not known. Since those metabolites cannot be synthesized endogenously in appreciable amounts, 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-eicosanoids should be considered more dynamic or vulnerable with respect to metabolite flux. The design of our study does not differentiate between patient groups undergoing long-term dialysis therapy with regard to the specific underlying renal disease. Nevertheless, the impact of those epoxides and hydroxy metabolites has yet to be integrated into a (patho)physiological context.

5 | CONCLUSIONS

Our results show that CKD affects the levels of numerous CYP epoxides and hydroxy metabolites (DHETs, EEQs, DiHDPAs, and HETEs) in circulating RBCs compared to control subjects, which on release may act in a vasoregulatory capacity. Although hemodialysis treatment was insufficient to change the majority of those total metabolites, we detected pronounced changes in free metabolite levels within the ESRD RBCs and in response to hemodialysis, indicating that free erythro-epoxides could also contribute to the cardiovascular risk, for example, in diabetes or hypertension. More research is needed to determine the contribution of RBC epoxy- and hydroxy-metabolites to cardiac performance and blood pressure regulation in health, cardiovascular, and specific kidney diseases.

ACKNOWLEDGMENTS

We express our thanks to all volunteers and patients for participating in our study. We are thankful to Christina Eichhorn for support in statistics. Open access funding enabled and organized by Projekt DEAL. [Correction added on 6 November 2020, after first online publication: Projekt Deal funding statement has been added.]

CONFLICT OF INTEREST

None.

AUTHOR CONTRIBUTIONS

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

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How to cite this article: Gollasch B, Wu G, Liu T, et al. Hemodialysis and erythrocyte epoxy fatty acids. *Physiol Rep*. 2020;8:e14601. https://doi.org/10.14814/phy2.14601

Curriculum Vitae

My curriculum vitae does not appear in the electronic version of my paper for reasons of data protection.

Complete list of publications

Liu T, Dogan I, Rothe M, et al. Bioaccumulation of Blood Long-Chain Fatty Acids during Hemodialysis. *Metabolites*. 2022;12(3):269. **Impact Factor (2022): 4.93**

Liu T, Dogan I, Rothe M, Kunz JV, Knauf F, Gollasch M, Luft FC, Gollasch B. Hemodialysis and Plasma Oxylipin Biotransformation in Peripheral Tissue. *Metabolites*. 2022; 12(1):34. **Impact Factor (2022): 4.93**

Gollasch B, Wu G, **Liu T**, Dogan I, Rothe M, Gollasch M, Luft FC. Hemodialysis and erythrocyte epoxy fatty acids. *Physiol Rep.* 2020 Oct;8(20):e14601. **Impact Factor (2022): 2.26**

Acknowledgments

In the midst of the epidemic, the white building standing on the banks of the Spree River is my home-school, Charite Medical University. This was the first foreign institution I chose, and three years have passed since then. I have transformed from a fresh researcher abroad to a medical doctor-to-be who can research independently, think independently, and stand up for myself.

In this process, I would first like to thank Prof. Dr. med. Dr. rer. nat. Maik Gollasch, who gave me the opportunity to study at the Charité and live in Germany. With his keen scientific awareness and highly responsible researcher's mentality, he guided me in my MD project, which helped me to a great extent to complete my thesis. No words can express my heartfelt gratitude to him. Please accept my deepest thanks.

Next, I would like to thank Dr. Benjamin Gollasch for his patience, professional support, and recognition of my abilities during the program. I would like to say thank you to this kind, humble and intelligent person, and I hope all the good things in the world will come to you.

Likewise, I would like to thank those partners who have helped me during my research work, in particular for support, training and companionship: Prof. Friedrich C. Luft, Dr. Michael Rothe, Inci Dogan, Heike Zweers, Dr. Felix Knauf, Dr. Julius V. Kunz, Dr. Jana Reichardt, Yoland-Marie Anistan, Zhihuang Zheng, Yibin Wang, Dr. Mario Kaßmann, Dr. Dmitry Tsvetkov

I would also like to thank my friends in Berlin, I had a great three years with you all.

Finally, I would like to thank my parents and my brother. They have given me a strong moral support during these three years. As I am about to graduate, I would like to thank my dearest people in this world.

Lastly, thanks to myself for working hard, the future will be better!