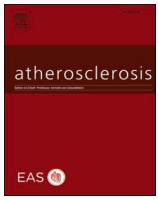
Atherosclerosis 398 (2024) 118591

Contents lists available at ScienceDirect

Atherosclerosis

journal homepage: www.elsevier.com/locate/atherosclerosis



Endothelial epoxyeicosatrienoic acid release is intact in aldosterone excess

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ARTICLE INFO

Keywords:
Aldosterone
Endothelial cells
Endothelial dysfunction
Primary aldosteronism
Vascular remodeling
Eicosanoids

ABSTRACT

Background and aims: Endothelial dysfunction (ED) is considered to be a major driver of the increased incidence of cardiovascular disease in primary aldosteronism (PA). The functionality of the epoxyeicosatrienoic acid (EET) pathway, involving the release of beneficial endothelium-derived lipid mediators, in PA is unknown. Evidence suggests this pathway to be disturbed in various models of experimental hypertension.
We therefore assessed EET production in primary human coronary artery endothelial cells exposed to aldosterone excess and measured circulating EET in patients with PA.
Methods: We used qPCR to investigate changes in the expression levels of essential genes for the synthesis and degradation of EET, calcium imaging to address the functional impact on overall endothelial function, as well as mass spectrometry to determine endothelial synthetic capacity to release EET upon stimulation. RNA-seq was performed to gain further mechanistic insights. Eicosanoid concentrations in patient's plasma were also determined by mass spectrometry.
Results: Aldosterone, while eliciting proinflammatory *VCAM1* expression and disturbed calcium response to acetylcholine, did not negatively affect stimulated release of endothelial EET. Likewise, no differences were observed in eicosanoid concentrations in plasma from patients with PA when compared to essential hypertensive controls.
However, an inhibitor of soluble epoxide hydrolase abrogated aldosterone-mediated *VCAM1* induction and led to a normalized endothelial calcium response probably by restoring expression of *CHRNE*.
Conclusion: EET release appears intact despite aldosterone excess. Epoxide hydrolase inhibition may revert aldosterone-induced functional changes in endothelial cells. These findings indicate a potential new therapeutic principle to address ED, which should be explored in future preclinical and clinical trials.

1. Introduction

Primary aldosteronism (PA) is the most common cause of endocrine hypertension. In recent years, it has been appreciated that in up to 12 % of patients arterial hypertension can be attributed to aldosterone (Aldo) excess [1]. It is of particular importance to address this condition adequately as high levels of Aldo cause a significantly greater prevalence of atherosclerotic sequelae as reflected by blood-pressure-independent detrimental effects on the cardiovascular system [2]. In particular, renal injury, atrial fibrillation, coronary artery disease and stroke, even when compared to essentially hypertensive control patients who had been matched for age, sex and blood pressure, are more abundant in

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https://doi.org/10.1016/j.atherosclerosis.2024.118591
Received 29 May 2024; Received in revised form 3 September 2024; Accepted 4 September 2024
Available online 5 September 2024

Table 1
Clinical characteristics of the patient cohort.

Patient characteristics (n = 48)	EH (n = 14)	PA (n = 46)	*p*
Age (years)	53 (38; 56)	49 (46; 54)	0.79
females (n, %)	8, 57	17, 50	0.76
Weight (kg)	79 (68; 90)	75 (66; 91)	0.87
Height (cm)	175 (165; 182)	172 (167; 177)	0.62
BMI (kg/m^2)	24.9 (23.5; 27.8)	26.2 (22.9; 29.4)	0.69
Systolic blood pressure (mmHg)	143 (134; 154)	149 (142; 160)	0.17
Diastolic blood pressure (mmHg)	91 (84; 100)	94 (85; 104)	0.54
Mean blood pressure (mmHg)	107 (102; 120)	112 (104; 124)	0.34
Pulse pressure (mmHg)	50 (45; 57)	56 (51; 62)	**0.0495**
Heart rate (min^{-1})	64 (60; 76)	69 (65; 80)	0.32
Duration of hypertension (months)	16 (5; 83)	36 (11; 86)	0.44
PROCAM score	30 (18; 39)	31 (24; 36)	0.87
Minimum serum K+ (mmol/L)	4.1 (4.0; 4.3)	3.9 (3.7; 4.0)	**0.0074**
Serum Na+ (mmol/L)	140 (139; 142)	141 (140; 143)	0.17
Serum cortisol prior to SIT or CCT (μg/dL)	8.3 (5.8; 10.9)	8.1 (6.1; 12.0)	0.80
SIT baseline aldosterone (pg/mL)	118 (70; 215)	125 (99; 177)	0.45
Post SIT aldosterone (pg/mL)	49 (45; 83)	82 (68; 110)	**0.0021**
SIT baseline active renin (mU/L)	7.3 (2.1; 17.0)	2.0 (2.0; 5.7)	**0.0048**
Post SIT active renin (mU/L)	3.6 (2.0; 8.5)	2.0 (2.0; 4.0)	**0.0146**
SIT baseline ARR (pg/mL:mU/L)	23.2 (11.4; 31.2)	48.5 (24.1; 73.3)	**0.0015**
CCT baseline Aldo min (pg/mL)	97 (75; 147)	113 (78; 192)	0.74
Post CCT aldosterone (pg/mL)	68 (51; 86)	100 (65; 159)	0.06
CCT baseline active renin (mU/L)	3.3 (2.0; 17.9)	2.2 (2.0; 4.1)	0.17
Post CCT active renin (mU/L)	4.4 (2.7; 29.7)	2.1 (2.0; 4.7)	**0.017**
HbA1c (%)	5.2 (5.1; 5.7)	5.2 (5.0; 5.5)	0.37
eGFR (mL/min)	96 (87; 111)	93 (84; 98)	0.17
24 h urinary Na + excretion (mmol/24 h)	103 (74; 164)	129 (85; 151)	0.41
Total cholesterol (mg/dL)	208 (170; 244)	186 (171; 215)	0.12
LDL cholesterol (mg/dL)	131 (99; 154)	113 (89; 134)	0.10
HDL cholesterol (mg/dL)	61 (52; 68)	56 (42; 75)	0.58
Non-HDL cholesterol (mg/dL)	153 (108; 173)	133 (100; 164)	0.21
Triglycerides (mg/dL)	79 (58; 136)	91 (63; 135)	0.72
NT-proBNP (pg/mL)	43 (32; 109)	100 (45; 173)	0.08
CrP (mg/dL)	0.1 (0.1; 0.2)	0.1 (0.1; 0.2)	0.65
IL-6 (pg/mL)	1.5 (1.1; 3.0)	2.3 (1.5; 3.1)	0.46

ARR, aldosterone-to-renin ratio; BMI, body mass index; CCT, captopril challenge test; CrP, C-reactive protein; eGFR, estimated glomerular filtration rate; EH, essential hypertension. HbA1c, glycated fraction of hemoglobin A; HDL, high density lipoprotein; IL-6, interleukin 6; LDL, low density lipoprotein; NT-proBNP, N-terminal pro-B-type natriuretic peptide; PA, primary aldosteronism; PROCAM, Prospective Cardiovascular Münster Study; SIT, saline infusion test; shown are median (25 % quintile; 75 % quintile). All *p* values are results of Mann Whitney test. Bold *p* values: $p < 0.05$.

patients with PA [2]. One of the earliest steps in the pathogenesis of atherosclerosis is endothelial dysfunction [3]. In line with previous observations indicating a high prevalence of atherosclerotic disease, endothelial dysfunction has been demonstrated in patients with PA [4–7]. Endothelial dysfunction is a descriptive term which does not allow for a mechanistic attribution. While experimental studies have linked Aldo-mediated endothelial dysfunction to a release of constrictive prostanoids [8] or endothelin-1 [9], most lines of explanation focus on impaired endothelial nitric oxide (NO) generation, decreased NO bioavailability or decreased smooth muscle response to NO [10–12].

However, the relative importance of NO as vasodilator decreases with ageing and sedentary life style [13]. Consequently, a shift to other endothelial mediators seems to occur. Other non-NO endothelial factors encompass substances like prostacyclin (PGI_2) and a heterogeneous group of mediators summarized as endothelium-derived hyperpolarizing factor (EDHF). Diverse mechanisms have been proposed to constitute molecular correlates of EDHF, such as localized increases in extracellular potassium via release from the endothelial cytosol, hyperpolarizing currents through myoendothelial gap junctions, release of hydrogen peroxide and arachidonic-acid-derived epoxyeicosatrienoic acids (EET) [14].

Specifically, the role of EET in Aldo-mediated endothelial dysfunction has barely been investigated so far. EET comprise 4 bioactive regioisomers which are named according to the position of the epoxide group (5,6-, 8,9, 11,12- and 14,15-EET). They are hydrolyzed to inactive DHET (dihydroxyeicosatrienoic acids) by epoxide hydrolases. Clinical data indicate that levels of circulating 14,15-DHET, the breakdown product of 14,15-EET, are increased in patients with PA, which might point to increased breakdown of active EET [15]. Further, experimental hypertension induced by administration of the mineralocorticoid precursor deoxycorticosterone acetate (DOCA) was reversed after administration of an inhibitor of soluble epoxide hydrolase (sEH) [16], which in turn would increase the bioavailability of endothelium-derived EET. Plasma EET concentrations were also reported to increase in response to PA subtype-specific treatment [17]. These findings suggest a particular role of EET in the pathogenesis of mineralocorticoid-induced endothelial dysfunction and warrant further investigation.

Since the impact of Aldo excess on the EET pathway is unknown, we set out to explore for putative pathologic effects using a primary human coronary artery endothelial cell (EC) line. We specifically addressed EC capacity to synthesize and release EET. Findings were then compared to the results of eicosanoid measurements in patient plasma.

2. Patients and methods

The authors refer to the Supplementary data for a detailed list of compounds and procedures.

2.1. Patients

Patients were recruited during workup for potential secondary hypertension with elevated screening aldosterone-to-renin ratio (ARR). Clinical characteristics of the cohort are provided in Table 1.

The study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki. LMU Munich ethics committee approved the study protocol (project no. 18–468). Written informed consent was

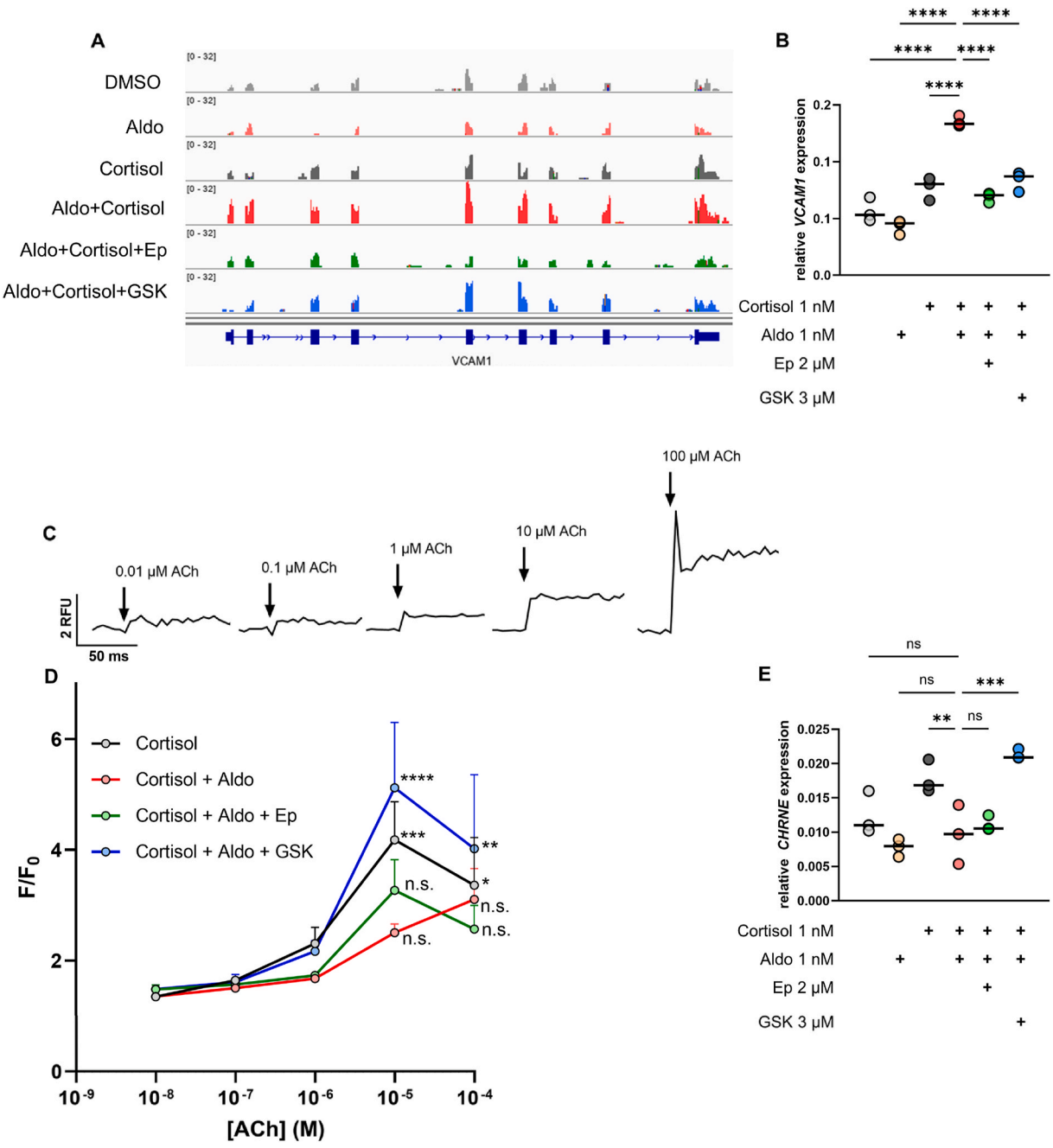
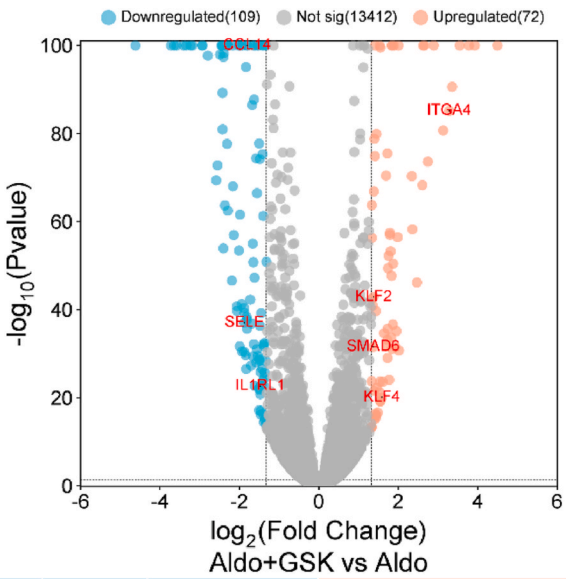


Fig. 1. Aldosterone induces endothelial dysfunction which is counteracted by inhibition of soluble epoxide hydrolase.
(A) Raw RNA-seq read counts of *VCAM1* in endothelial cells according to treatment. (B) Relative quantification of *VCAM1* read counts normalized to *EIF2B1* and *HPRT1*. One-way ANOVA, Holm-Šídák. ****, $p<0.0001$, n = 3 independent replicates per experimental group. (C) Example Fluo4 fluorescence traces of HCAEC in response to increasing acetylcholine (ACh) concentrations. RFU, relative fluorescence units. (D) Concentration-response curve to ACh after 48 h of culture under indicated conditions. Shown are mean+SEM. Two-way ANOVA, Dunnett, *, $p<0.05$, **$p<0.01$, ***$p<0.001$, ****$p<0.0001$ (F/F_0 after each ACh concentration within each group tested *versus* F/F_0 after 1 pM ACh), n = 11 per group. (E) Relative quantification of RNAseq reads for *CHRNE*. ACh, acetylcholine, Aldo, aldosterone, Ep, eplerenone, GSK, GSK2256294. One-way ANOVA, Dunnett. **, $p<0.01$; ***, $p<0.001$, n = 3 per group.

obtained from all participants included in the study. Data protection and privacy laws were adhered to.

2.2. Cell culture and stimulation

Human coronary artery endothelial cells (HCAEC) were purchased from Lonza (Basel, CH) together with their specific growth media and growth factors and were cultured in specific growth-medium with supplements according to the manufacturer's recommendations. Cells were serum starved over night before treatment in EBM-2 as the confluence reached 85–90 %. Pharmacological treatment was performed in serum-free medium for an additional 48 h. Cells were either treated with DMSO (solvent, control condition), 1 nM aldosterone, 140 nM cortisol, 140 nM cortisol + 1 nM aldosterone, 140 nM cortisol + 1 nM aldosterone + 2 µM eplerenone, or 140 nM cortisol + 1 nM aldosterone + 3 µM GSK2256294.



GO Biological Process 2023	p-value	q-value	overlap genes
Cytokine-Mediated Signaling Pathway (GO:0019221)	0.000083	0.056616	*CCL14, EGR1, IL1RL1, IL15, IL3RA, CSF2RB, CX3CL1, BIRC3*
Positive Regulation Of Activated T Cell Proliferation (GO:0042104)	0.000143	0.056616	*IGFBP2, IGF2, IGF1*
Response To Heparin (GO:0071503)	0.000291	0.056616	*EGR1, GPIHBP1*

GO Biological Process 2023	p-value	q-value	overlap genes
Response To Laminar Fluid Shear Stress (GO:0034616)	0.000004	**0.002516**	*KLF4, SMAD6, KLF2*
Bradykinin Catabolic Process (GO:0010815)	0.000127	**0.036657**	*MME, CTSH*
Cellular Response To Laminar Fluid Shear Stress (GO:0071499)	0.000190	**0.036657**	*KLF4, KLF2*

Fig. 2. Volcano plot of RNAseq results of HCAEC after culture in the presence of Aldo and cortisol and Aldo, cortisol and GSK.
Blue dots indicate genes which are at least 2.5-fold downregulated in cells co-treated with Aldo, cortisol and GSK *versus* cells co-treated with Aldo and cortisol. Red dots indicate genes which are at least 2.5-fold upregulated. Genes represented by blue and red dots have q-values <0.05. Tables below indicate GO Biological Process clustering of genes. Aldo, aldosterone, GSK2256294. N = 3 independent RNAseq replicates per experimental group. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

2.3. Stimulation of endothelial EET release

Endothelial cells were synchronized under serum-free conditions, treated with the indicated pharmacological agents in serum-free medium for 48 h, loaded with 10 μM arachidonic acid and then stimulated with 100 μM acetylcholine in HEPES buffer to provoke the release of EET. Subsequently, supernatants were collected and stored at −80 °C until analysis.

2.4. Measurement of eicosanoids

Eicosanoids in both supernatants and patient plasma were determined using an established pipeline at the Helmholtz Center Munich as described in detail in the supplement. Concentrations in supernatants were normalized to total protein concentration of the respective experimental sample.

2.5. Data availability

2.6 All datasets analyzed during the current study are available from the corresponding author on reasonable request.

Part of the presented data have been published on a preprint server and are accessible under https://doi.org/10.1101/2021.02.04.429624.

2.6. Statistics

GraphPad Prism 10 (GraphPad Software, San Diego, CA, USA) was used for construction of graphs and computation of statistical significances. The testing strategy has been outlined in each figure caption. Volcano plot was drawn using SRplot [18]. Statistical significance levels were set to a two-sided *p*-value <0.05.

3. Results

Primary human coronary artery endothelial cells (EC) were treated for 48 h with aldosterone at a concentration of 1 nM [10,19] which is close to concentrations found in patients with PA [20]. In a subset of experiments, physiological concentrations of hydrocortisone (140 nM) were applied in addition to 1 nM aldosterone to simulate *in vivo* mineralocorticoid receptor (MR) activity of glucocorticoids. To validate the biological efficacy of this setup, we assessed endothelial pro-inflammatory *VCAM1* expression, a known transcriptional target of [21]. Aldosterone increased *VCAM1* expression with concomitant cortisol treatment in a mineralocorticoid receptor-dependent manner. Inhibition of sEH also antagonized the *VCAM1* induction (Fig. 1A and B).

In a subsequent series of experiments, we investigated endothelial calcium influx in response to stimulation with acetylcholine (ACh, Fig. 1C). In cells cultured in the presence of cortisol, 10 and 100 μM ACh elicited Fluo4 F/F_0 ratios which differed significantly from the response to the lowest concentration of ACh (1 pM). In contrast, in cells cultured in the presence of Aldo and cortisol none of the applied concentrations of ACh elicited a F/F_0 response that was significantly larger than the response to 1 pM ACh. Inhibition of MR with eplerenone (Ep) did not revert this phenotype. Inhibition of sEH, however, effectively counteracted Aldo as indicated by a F/F_0 response that was similar to values seen in cortisol-only treated cells (Fig. 1D).

RNAseq revealed that treatment with Aldo and cortisol reduced the expression of *CHRNE*, an essential ancillary subunit to nicotinic acetylcholine receptors. GSK2256294 (GSK) restored the expression levels back to levels of the cortisol-treated cells (Fig. 1E).

Gene ontology analysis showed that genes upregulated in cells treated with GSK, Aldo and cortisol clustered as “Response To Laminar Fluid Shear Stress”. Downregulated genes clustered under headings such

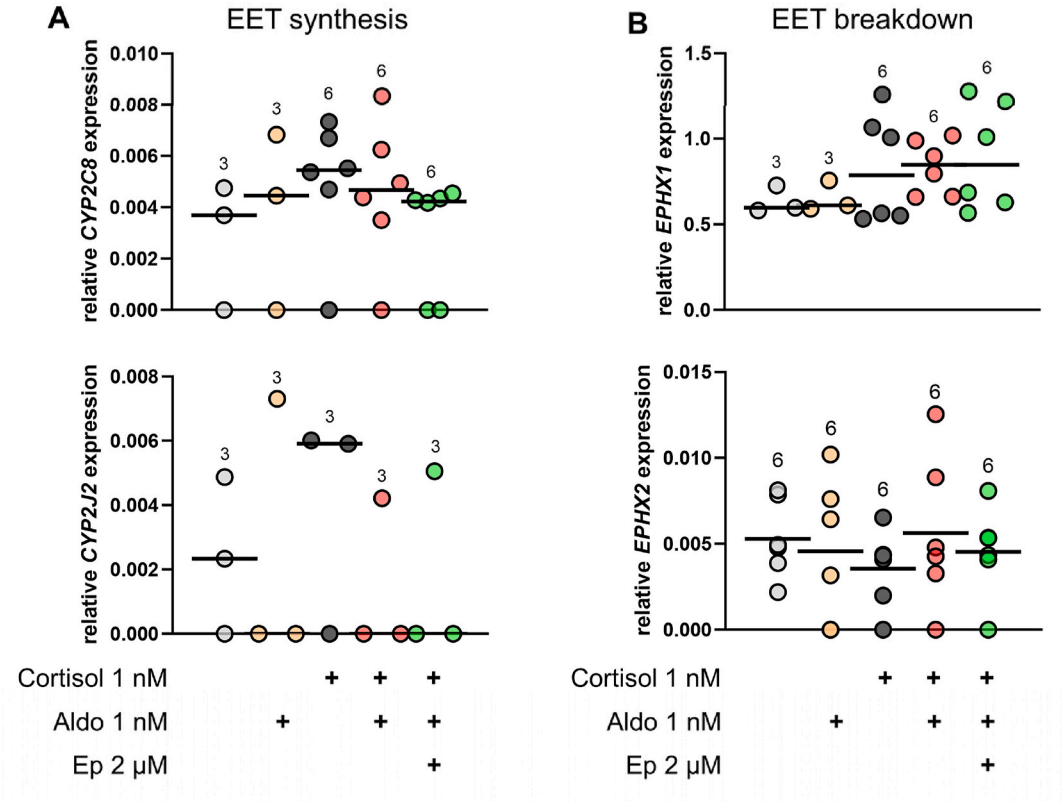


Fig. 3. HCAEC do not express relevant amounts of EET synthesis or enzymes as well as *EPHX2*.
(A) Relative expression (qPCR) of *CYP2C8* and *CYP2J2* which mediate EET synthesis in HCAEC. (B) Relative expression (qPCR) of *EPHX1* and *EPHX2,* which mediate EET breakdown. Horizontal lines indicate median values. Figures above data points indicate sample size. No significant differences between groups were detected. Aldo, aldosterone, Ep, eplerenone.

Table 2
Levels of eicosanoids in cell culture supernatant after stimulation with 100 µM acetylcholine.

Supernatant eicosanoid (ng/mg protein)	Cortisol (n = 3)	Cortisol + Aldo (n = 3)	Cortisol + Aldo + Ep (n = 2)	Cortisol + Aldo + GSK (n = 3)	*p* (overall)	*p* (Cortisol *vs* Cortisol + Aldo)
5,6-EET	0.111 (0.106; 0.118)	0.140 (0.032; 0.216)	0.099 (0.073; 0.125)	0.161 (0.027; 0.220)	0.87	0.70
5,6-DHET	1.5 (1.2; 1.7)	1.9 (0.4; 2.4)	1.1 (0.8; 1.4)	1.7 (0.3; 2.5)	0.83	0.70
8,9-EET	10.8 (9.6; 11.4)	13.7 (3.0; 18.7)	8.5 (6.2; 10.8)	13.4 (2.1; 15.6)	0.83	0.70
8,9-DHET	0.53 (0.42; 0.56)	0.62 (0.13; 0.75)	0.37 (0.27; 0.47)	0.54 (0.12; 0.91)	0.83	0.70
11,12-EET	14.7 (13.1; 16.2)	19.1 (4.2; 26.4)	11.6 (8.7; 14.6)	18.8 (3.0; 21.4)	0.79	0.70
11,12-DHET	1.8 (1.3; 1.8)	1.9 (0.4; 2.3)	1.1 (0.9; 1.3)	1.6 (0.4; 3.1)	0.76	0.70
14,15-EET	18.1 (14.3; 19.0)	20.2 (4.3; 29.8)	11.7 (10.0; 13.4)	18.1 (3.3; 29.2)	0.67	0.70
14,15-DHET	0.36 (0.30; 0.42)	0.51 (0.10; 0.61)	0.24 (0.19; 0.28)	0.33 (0.08; 0.61)	0.67	0.70
5,6-DHET/EET	13.5 (11.5; 14.3)	12.8 (11.1; 13.4)	10.9 (10.8; 11.0)	11.3 (10.3; 11.7)	0.11	0.40
8,9-DHET/EET	0.05 (0.04; 0.05)	0.04 (0.04; 0.05)	0.04 (0.04; 0.04)	0.06 (0.04; 0.06)	0.58	0.40
11,12-DHET/EET	0.11 (0.10; 0.12)	0.09 (0.09; 0.10)	0.09 (0.09; 0.10)	0.13 (0.08; 0.14)	0.51	0.20
14,15-DHET/EET	0.021 (0.019; 0.023)	0.024 (0.021; 0.025)	0.020 (0.019; 0.021)	0.021 (0.018; 0.024)	0.73	0.40
sum EET	43.7 (37.1; 46.8)	53.0 (11.5; 75.1)	31.9 (25.0; 38.9)	50.6 (8.3; 66.4)	0.79	0.70
sum DHET	4.2 (3.3; 4.4)	5.0 (1.0; 6.0)	2.8 (2.1; 3.4)	4.1 (0.9; 7.1)	0.83	0.70
sumDHET/sumEET	0.093 (0.088; 0.097)	0.088 (0.080; 0.093)	0.086 (0.085; 0.087)	0.107 (0.081; 0.107)	0.53	0.70
sumDHET + sumEET	47.9 (40.4; 51.1)	58.0 (12.5; 81.)	34.7 (27.1; 42.3)	54.6 (9.2; 73.5)	0.79	0.70

All raw concentrations in ng/mg total protein. Shown are median (25 % quantile; 75 % quantile). Overall *p* values: Kruskal Wallis test. *p* values Cortisol *vs* Cortisol + Aldo: Mann Whitney test. Aldo, aldosterone, Ep, eplerenone, GSK, GSK2256294.

as "Cytokine-Mediated Signaling Pathway" and "Positive Regulation Of Activated T Cell Proliferation", although in this type of analysis all of the suggested clusters closely missed statistical significance (q = 0.057 for the aforementioned GO terms) (Fig. 2).

With the above-mentioned results we concluded that the co-treatment of HCAEC with Aldo 1 nM plus cortisol 140 nM seems to adequately model the adverse biological activity in terms of inflammatory gene expression and impaired calcium influx and that sEH inhibition seems to antagonize these adverse effects.

In EC, EET are synthesized by CYP2C isoenzymes [22,23] as well as CYP2J2 [24], while the conversion of EET to inactive DHET is reported to depend on microsomal epoxide hydrolase (EPHX1) and soluble epoxide hydrolase (EPHX2), with the latter being more important [25].

In the above-mentioned RNAseq experiment, however, no signals for *CYP2C* or *CYP2J* and *EPHX2* could be detected. To exclude sensitivity issues of the RNAseq we conducted conventional qPCR. This revealed barely detectable mRNA of *CYP2C8* and *CYP2J2* (DMSO control: *CYP2C8* median Ct value = 38.4, *CYP2J2* = 38.4). *CYP2C9* mRNA could

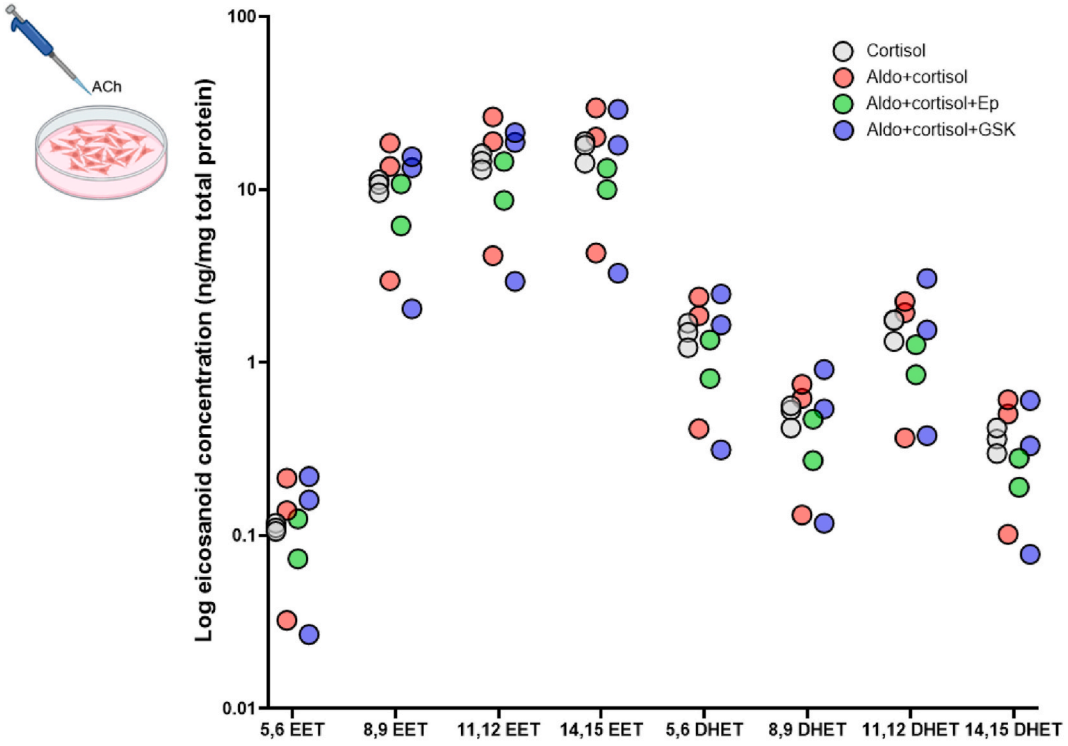


Fig. 4. Aldosterone excess has no impact on stimulated EET release *in vitro*.
Log-transformed concentrations of EET and DHET (normalized to protein content) in culture supernatant following acute stimulation with 100 μM acetylcholine. Groups are defined by preceding 48 h of culture under indicated conditions. No significant differences between groups were detected. Aldo, aldosterone, Ep, eplerenone, GSK, GSK2256294. Figure partly created with BioRender.com.

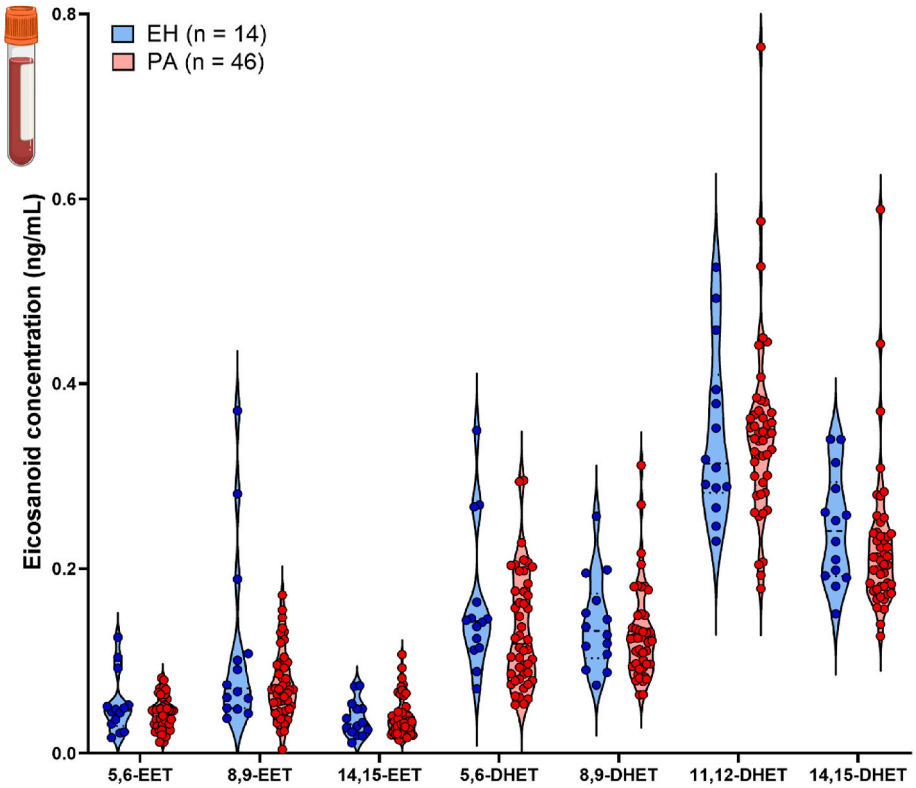


Fig. 5. No differences in circulating plasma EETs between patients with essential hypertension (EH) and primary aldosteronism (PA).
Violin plots of plasma concentrations of EET and DHET as measured by mass spectrometry in patient plasma. No significant differences were detected between the two groups. Figure partly created with BioRender.com.

Table 3
Levels of circulating plasma eicosanoids.

Plasma eicosanoid (ng/mL)	EH (n = 14)	PA (n = 46)	*p*
5,6-EET	0.05 (0.03; 0.06)	0.05 (0.03; 0.05)	0.54
5,6-DHET	0.14 (0.11; 0.19)	0.12 (0.08; 0.18)	0.26
8,9-EET	0.07 (0.05; 0.13)	0.07 (0.05; 0.10)	0.42
8,9-DHET	0.13 (0.10; 0.17)	0.12 (0.09; 0.15)	0.36
11,12-DHET	0.31 (0.28; 0.41)	0.34 (0.29; 0.37)	0.83
14,15-EET	0.03 (0.02; 0.05)	0.03 (0.02; 0.05)	0.70
14,15-DHET	0.24 (0.19; 0.29)	0.21 (0.18; 0.24)	0.16
5,6-DHET/EET	3.17 (2.58; 3.84)	2.95 (2.45; 4.20)	0.60
8,9-DHET/EET	1.77 (1.21; 2.26)	1.78 (1.46; 2.25)	0.34
14,15-DHET/EET	7.66 (4.76; 8.95)	6.03 (4.98; 7.75)	0.32
sum EET	0.15 (0.10; 0.24)	0.15 (0.10; 0.21)	0.67
sum DHET	0.81 (0.70; 1.12)	0.79 (0.68; 0.92)	0.52
sumDHET/sumEET	5.41 (3.60; 6.61)	5.35 (4.79; 6.65)	0.59
sumDHET + sumEET	0.99 (0.79; 1.35)	0.91 (0.80; 1.15)	0.56

All raw concentrations in ng/mL. Shown are median (25 % quantile; 75 % quantile). All *p* values are results of Mann Whitney test.

not be detected. Neither *CYP2J2* nor *CYP2C8* mRNA showed significant changes upon challenge with Aldo, with or without co-stimulation by cortisol (Fig. 3A).

Microsomal epoxide hydrolase (*EPHX1*) and soluble epoxide hydrolase (sEH, *EPHX2*) were not dysregulated in Aldo-treated ECs (Fig. 3B) when compared to control. *EPHX2* was also expressed at barely detectable levels (DMSO control: median Ct value = 38.6).

We concluded that HCAEC do not express relevant amounts of *CYP2C8*, *CYP2C9*, *CYP2J2* and *EPHX2* as the mRNA levels were either close to or below the limit of detection.

Next, we directly assessed the concentrations of endothelial EET released upon acute stimulation after an exposure to Aldo and cortisol for 48 h. Cells were stimulated with 100 μM ACh [26–28] and EET concentration were determined in supernatant by high pressure liquid chromatography tandem mass spectrometry (LC-MS/MS).

We were able to detect and quantify all 4 EET regioisomers and their respective DHET epoxide hydrolase products. 11,12-EET and 14,15-EET were found to be the most abundant regioisomers. ACh-stimulated secretion of EET was unaltered when compared to cells only treated with cortisol. Likewise, culture in the presence of an MR antagonist (eplerenone, 2 μM) or an inhibitor of sEH (GSK, 3 μM) on top of Aldo and cortisol did not result in detectable changes. Individual concentrations of eicosanoids and derived measures are provided in Table 2. Fig. 4 provides a graphical overview of all EET and DHET concentrations.

We then analyzed plasma samples from a human cohort of 14 patients with essential hypertension and 46 patients with primary aldosteronism with the same LC-MS/MS approach. Groups were defined using strict biochemical criteria. All participants underwent at least one confirmatory test for PA. Of note, patients were recruited prospectively whilst confirmatory test results for PA were still pending, which lowered the risk of bias introduction.

Patient characteristics are summarized in Table 1. Virtually no imbalances, especially not in aggregate measures of cardiovascular risk (PROCAM score) were detected. In addition to the pre-defined biochemical parameters, the two groups only differed in pulse pressure and serum K+ concentration.

In this well-matched cohort, determination of plasma eicosanoid concentrations did not reveal any differences between the two subgroups (Fig. 5). Individual concentrations can be found in Table 3. Levels of 11,12-EET were too low for reliable quantification.

4. Discussion

In the present study, we investigated whether Aldo concentrations, comparable to plasma concentrations in patients with PA, impair HCAEC synthetic capacity to release EET. We deliberately chose ECs of coronary origin since the effects of EET have been extensively validated in coronary arteries from a variety of species, including bovine [29] and human arteries [30]. Plasma EET concentrations were also assessed in a well-matched patient cohort with PA compared to essentially hypertensive patients.

At 1 nM, Aldo showed adverse biological activity as reflected by inflammatory *VCAM1* expression and impaired calcium response to acetylcholine. Aldo induces the expression of vascular cell adhesion molecule 1 (*VCAM1*) in endothelial cells in a mineralocorticoid receptor-dependent manner [21]. Aldo neither altered expression levels of the investigated CYP-epoxygenases nor the epoxide hydrolases.

Of note, a recent single-cell resource of cardiac endothelial cells challenged by *in vivo* administration of Aldo essentially confirmed our findings:

Data mining of the published resource revealed that in cardiac endothelial cells, *Ephx2* expression was virtually absent, and according to mRNA levels the main epoxide hydrolase was *Ephx1*. Moreover, *Vcam1* was validated as Aldo-induced gene [31].

In our study, the stimulated release of eicosanoids from endothelial cells was independent from pretreatment with Aldo. Furthermore, none of the classically reported CYP epoxygenases seemed to mediate EET synthesis in HCAEC.

One possible explanation could be an alternative EET synthesizing enzyme. Rat Cyp1a1 was reported to harbor synthetic capacity for EET [32]. Studies with the human isoform revealed that CYP1A1 converts ca. 7 % of arachidonic acid to 14,15-EET [33]. We consistently detected *CYP1A1* expression in HCAECs (Supplemental Fig. 1). On the other hand, despite treatment with GSK, which resulted in significantly lower expression levels of *CYP1A1*, we did not observe changes in EET, DHET or the sum of EET and DHET (EET and their breakdown products summed up to represent a time integral of EET synthesis). In conclusion, our results neither support a major role for *CYP1A1* as arachidonic acid expoxygenase.

Another possibility may be that HCAEC did not produce EET themselves, but rather incorporated EET from fetal calf serum into their plasma lipids. Kinetic studies showed that incorporated EET are cleared from endothelial cells with a plateau of ca. 30 % at 4 h, which is maintained until 6 h [34]. This leaves the possibility that our cells may still have retained a relevant amount of incorporated EET throughout the starvation period preceding the acute stimulation. Taken together, our data indicate that HCAEC do not actively synthesize EET but rather incorporate them from serum contained in cell culture medium. Aldo excess does not seem to interfere with stimulated release of these EET.

With the expression of *EPHX2* being virtually absent, the precise site of action of GSK remains to be determined. RNAseq revealed that GSK antagonized the Aldo-mediated decrease in *CHRNE* expression. This subunit of nicotinergic acetylcholine receptors is vital for the function of the whole receptor, as indicated by the early postnatal lethality of global loss-of function mutants [35]. While endothelium-derived relaxation to acetylcholine in healthy states is mediated by muscarinergic acetylcholine receptors, the importance of nicotinergic receptors increases in hypertensive animals [36]. Although our experiments were not designed to uncover off-target modes of action of GSK and, therefore, are by no means definitive, they allow to speculate that an increase in *CHRNE* expression may have increased the number of signal transduction sites for acetylcholine, resulting in a recovery of the intracellular calcium response. Of note, eplerenone did not achieve this normalization, suggesting that GSK may even be superior as an endothelial protective agent in settings of Aldo excess.

Gene ontology analysis revealed that GSK treatment induces responses that are involved in processes generally associated with functional integrity of endothelial cells, such as laminar shear stress and anti-inflammatory effects.

The cell culture findings were essentially replicated in the patient cohort, where no differences in circulating EET or DHET were detected in patients with PA when compared to patients with EH.

In patients with PA, so far only two studies have been conducted

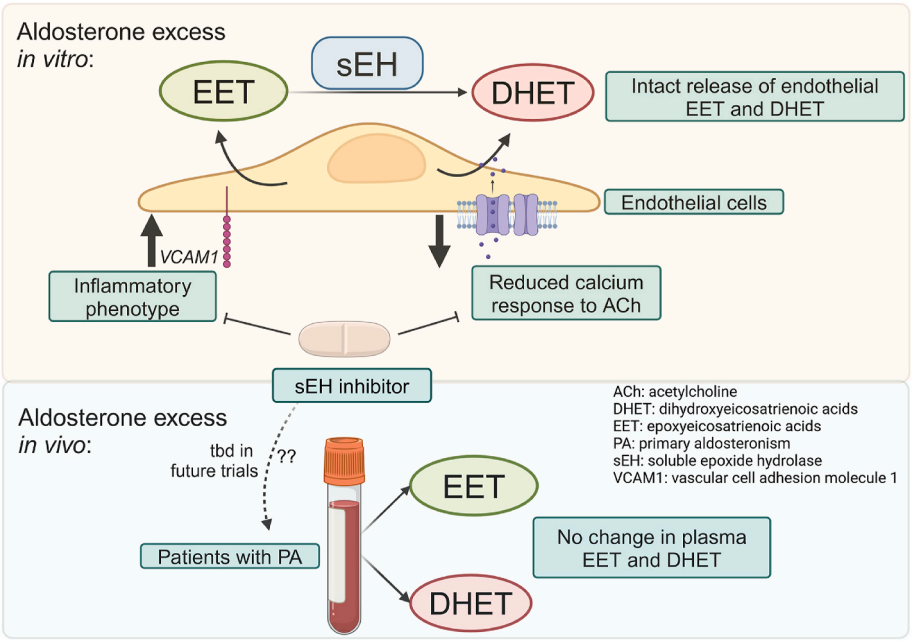


Fig. 6. Graphical abstract.

which analyzed plasma eicosanoids: Liu and colleagues found a positive correlation between 14,15-DHET and abdominal aortic calcification [15] in patients with PA. We could not reproduce the reported PA-associated increase in 14,15-DHET which may be related to differences in cohort characteristics. The largest differences between both cohorts are the duration of hypertension in the PA cohort (5 years vs. 3 years in our study) and the biochemical severity of PA (plasma Aldo 392 pg/mL vs. 125 pg/mL in our study). It is also possible that the discrepancies of 14,15-DHET levels between the studies is based on different quantification methods (ELISA in the study of Liu and colleagues vs. LC-MS/MS in our approach).

Luther et al. described that subtype-specific treatment of PA results in increased total EET, an effect that was mainly driven by an increase in 14,15-EET concentrations [17]. Of note, no changes in 14,15-DHET, other DHET or the sum of DHET were found in patients after treatment when compared to concentrations before treatment. The study indirectly suggested an increased EET synthesis by specific treatment of PA. It also described a negative linear correlation between plasma Aldo concentration and 11,12-EET, 14,15-EET and sum of EET.

We could not reproduce the association between plasma EET and plasma Aldo concentration (Supplemental Fig. 2). Due to case definition of PA, patients in the study by Luther and colleagues again had substantially higher Aldo concentrations (332 ng/mL vs 125 ng/mL in our study). It is possible that the negative correlation of plasma levels of Aldo and EET only becomes apparent in individuals exposed to very high concentrations of Aldo.

Luther et al. [20] further reported that Aldo infusion over 3 days resulted in increased sEH expression in adipose tissue of mice. The fact that sEH expression remained unchanged in our endothelial cells may reflect cell-type specific responses to Aldo yielding different transcriptional effects in adipocytes vs endothelial cells.

Our study shows that Aldo-mediated endothelial dysfunction does not seem to influence the stimulated endothelial eicosanoid release or circulating eicosanoids in patients with PA. Experimental trials have shown that soluble epoxide hydrolase inhibition was able to reverse DOCA-induced hypertension and endothelial dysfunction [16]. Our transcriptomic results point to the same direction. Thus, although soluble epoxide hydrolase inhibition probably cannot be simply understood as a reversal of the underlying pathophysiological processes, its benefits in conditions of mineralocorticoid excess should be further investigated.

The main limitation of our study is that the *in vitro* part could not be complemented by directly assessing the effects of endothelial EET on smooth muscle contraction in isolated arteries and is, thus, limited to cultured cells. The clinical part suffers from a limited sample size.

On the other hand, we believe that a particular point of strength is the consistency of observations in the cell culture system and the clinical cohort.

A substantial proportion of patients with PA still suffers from hypertension even after unilateral adrenalectomy and biochemical cure from Aldo excess [37]. Moreover, medication side effects of MRA hamper therapeutic adherence of patients with bilateral disease. There is, thus, an urgent need for novel pharmacological strategies targeting the downstream effector cells of mineralocorticoid excess. Based on our results, it is therefore tempting to propose that the EET-EDHF axis might be intact in patients with PA. Therefore, therapeutic modulation of EET half-lives with epoxide hydrolase inhibitors in conditions of mineralocorticoid excess should be investigated in future experiments, since the structures responsible for signal transduction in endothelial cells seem to remain intact (Fig. 6).

Conflict of interest

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

Financial support

H. Schneider was supported by the Clinician Scientist PRogram In Vascular MEdicine (PRIME) funded by the Deutsche Forschungsgemeinschaft (DFG, German Research Foundation) - project number MA 2186/14-1. The study received further support through the European Research Council (ERC) under the European Union's Horizon 2020 research and innovation programme (grant agreement No [694913] to M. Reincke) and through the Deutsche Forschungsgemeinschaft (DFG) project number 314061271 to H. Schneider, M. Mederos y Schnitzler, T. Gudermann, F. Beuschlein and M. Reincke, and the Else Kröner-Fresenius Stiftung in support of the German Conn Registry-Else-Kröner Hyperaldosteronism Registry

(2013_A182 and 2015_A171 to M. Reincke). This work was also supported by the Clinical Research Priority Program of the University of Zurich for the CRPP HYRENE to F. Beuschlein.

CRediT authorship contribution statement

Yao Meng: Methodology, Investigation, Formal analysis. **Aynur Bilyal:** Methodology, Investigation, Data curation. **Li Chen:** Investigation, Writing – review & editing. **Michael Mederos y Schnitzler:** Methodology, Writing – review & editing. **Julien Kocabiyik:** Methodology, Writing – review & editing. **Thomas Gudermann:** Writing – review & editing. **Fabien Riols:** Methodology, Investigation, Formal analysis, Writing – review & editing. **Mark Haid:** Methodology, Investigation, Formal analysis, Writing – review & editing. **Jair G. Marques:** Methodology, Writing – review & editing. **Jeannie Horak:** Methodology, Writing – review & editing. **Berthold Koletzko:** Methodology, Writing – review & editing. **Jing Sun:** Investigation, Formal analysis. **Felix Beuschlein:** Conceptualization, Writing – review & editing. **Daniel A. Heinrich:** Conceptualization, Writing – review & editing. **Christian Adolf:** Conceptualization, Writing – review & editing. **Martin Reincke:** Funding acquisition, Conceptualization, Supervision, Writing – review & editing. **Holger Schneider:** Conceptualization, Funding acquisition, Supervision, Data curation, Formal analysis, Project administration, Writing – original draft, Writing – review & editing.

Acknowledgements

We are indebted to Michaela Höhne for the excellent technical assistance.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.atherosclerosis.2024.118591.

References

[1] S. Monticone, J. Burrello, D. Tizzani, C. Bertello, A. Viola, F. Buffolo, L. Gabetti, G. Mengozzi, T.A. Williams, F. Rabbia, F. Veglio, P. Mulatero, Prevalence and clinical manifestations of primary aldosteronism encountered in primary care practice, J. Am. Coll. Cardiol. 69 (2017) 1811–1820, https://doi.org/10.1016/j.jacc.2017.01.052.

[2] Y. Ohno, M. Sone, N. Inagaki, T. Yamasaki, O. Ogawa, Y. Takeda, I. Kurihara, H. Itoh, H. Umakoshi, M. Tsuiki, T. Ichijo, T. Katabami, Y. Tanaka, N. Wada, Y. Shibayama, T. Yoshimoto, Y. Ogawa, J. Kawashima, K. Takahashi, M. Fujita, M. Watanabe, Y. Matsuda, H. Kobayashi, H. Shibata, K. Kamemura, M. Otsuki, Y. Fujii, K. Yamamoto, A. Ogo, S. Okamura, S. Miyauchi, T. Fukuoka, S. Izawa, T. Yoneda, S. Hashimoto, T. Yanase, T. Suzuki, T. Kawamura, Y. Tabara, M. Naruse, Nagahama Study, JPAS Study Group, Prevalence of cardiovascular disease and its risk factors in primary aldosteronism: a multicenter study in Japan, Hypertension 71 (2018) 530–537, https://doi.org/10.1161/HYPERTENSIONAHA.117.10263.

[3] P. Hopkins, Molecular biology of atherosclerosis, Physiol. Rev. 93 (2013) 1317–1542, https://doi.org/10.1007/978-3-642-37078-6_5.

[4] K. Tsuchiya, T. Yoshimoto, Y. Hirata, Endothelial dysfunction is related to aldosterone excess and raised blood pressure, Endocr. J. 56 (2009) 553–559, https://doi.org/10.1507/endocrj.K09E-014.

[5] T. Matsumoto, K. Oki, M. Kajikawa, A. Nakashima, T. Maruhashi, Y. Iwamoto, A. Iwamoto, N. Oda, T. Hidaka, Y. Kihara, N. Kohno, K. Chayama, C. Goto, Y. Aibara, K. Noma, J.K. Liao, Y. Higashi, Effect of aldosterone-producing adenoma on endothelial function and rho-associated kinase activity in patients with primary aldosteronism, Hypertension 65 (2015) 841–848, https://doi.org/10.1161/HYPERTENSIONAHA.114.

[6] S. Kishimoto, K. Oki, T. Maruhashi, M. Kajikawa, S. Matsui, H. Hashimoto, Y. Takaeko, Y. Kihara, K. Chayama, C. Goto, Y. Aibara, F.M. Yusoff, A. Nakashima, K. Noma, J.K. Liao, Y. Higashi, Eplerenone improves endothelial function and arterial stiffness and inhibits Rho-associated kinase activity in patients with idiopathic hyperaldosteronism, J. Hypertens. 1 (2018), https://doi.org/10.1097/HJH.0000000000001989.

[7] D. Watanabe, S. Morimoto, N. Morishima, A. Ichihara, Clinical impacts of endothelium-dependent flow-mediated vasodilation assessment on primary aldosteronism, Endocrinol. Conn. 10 (2021) 578–587, https://doi.org/10.1530/EC-21-0057.

[8] J. Blanco-Rivero, V. Cachofeiro, V. Lahera, R. Aras-Lopez, I. Márquez-Rodas, M. Salaices, F.E. Xavier, M. Ferrer, G. Balfagón, Participation of prostacyclin in endothelial dysfunction induced by aldosterone in normotensive and hypertensive rats, Hypertension 46 (2005) 107–112, https://doi.org/10.1161/01.HYP.0000171479.36880.17.

[9] R. Lariviere, G. Thibault, E.L. Schiffrin, Increased endothelin-1 content in blood vessels of deoxycorticosterone acetate-salt hypertensive but not in spontaneously hypertensive rats, Hypertension 21 (1993) 294–300, https://doi.org/10.1161/01.HYP.21.3.294.

[10] J.A. Leopold, A. Dam, B.A. Maron, A.W. Scribner, R. Liao, D.E. Handy, R. C. Stanton, B. Pitt, J. Loscalzo, Aldosterone impairs vascular reactivity by decreasing glucose-6-phosphate dehydrogenase activity, Nat. Med. 13 (2007) 189–197, https://doi.org/10.1038/nm1545.

[11] D. Nagata, M. Takahashi, K. Sawai, T. Tagami, T. Usui, A. Shimatsu, Y. Hirata, M. Naruse, Molecular mechanism of the inhibitory effect of aldosterone on endothelial NO synthase activity, Hypertension 48 (2006) 165–171, https://doi.org/10.1161/01.HYP.0000226054.53527.bb.

[12] A. Garnier, J.K. Bendall, S. Fuchs, B. Escoubet, F. Rochais, J. Hoerter, J. Nehme, M. L. Ambroisine, N. De Angelis, G. Morineau, P. D'Estienne, R. Fischmeister, C. Heymes, F. Pinet, C. Delcayre, Cardiac specific increase in aldosterone production induces coronary dysfunction in aldosterone synthase-transgenic mice, Circulation 110 (2004) 1819–1825, https://doi.org/10.1161/01.CIR.0000142858.44680.27.

[13] S. Taddei, F. Galetta, A. Virdis, L. Ghiadoni, G. Salvetti, F. Franzoni, C. Giusti, A. Salvetti, Physical activity prevents age-related impairment in nitric oxide availability in elderly athletes, Circulation 101 (2000) 2896–2901, https://doi.org/10.1161/01.CIR.101.25.2896.

[14] M. Félétou, Endothelium-dependent hyperpolarization and endothelial dysfunction, J. Cardiovasc. Pharmacol. 67 (2016) 373–387, https://doi.org/10.1097/FJC.0000000000000346.

[15] P. Liu, S. Zhang, J. Gao, Y. Lin, G. Shi, W. He, R.M. Touyz, L. Yan, H. Huang, Downregulated serum 14, 15-epoxyeicosatrienoic acid is associated with abdominal aortic calcification in patients with primary aldosteronism, Hypertension 71 (2018) 592–598, https://doi.org/10.1161/HYPERTENSIONAHA.117.10644.

[16] D. Loch, A. Hoey, C. Morisseau, B.O. Hammock, L. Brown, Prevention of hypertension in DOCA-salt rats by an inhibitor of soluble epoxide hydrolase, Cell Biochem. Biophys. 47 (2007) 87–98. CBB:47:1:87 [pii].

[17] J.M. Luther, D.S. Wei, K. Ghoshal, D. Peng, G.K. Adler, A.F. Turcu, H. Nian, C. Yu, C.C. Solorzano, A. Pozzi, N.J. Brown, Treatment of primary aldosteronism increases plasma epoxyeicosatrienoic acids, Hypertension (2021) 1–9, https://doi.org/10.1161/HYPERTENSIONAHA.120.14808.

[18] D. Tang, M. Chen, X. Huang, G. Zhang, L. Zeng, G. Zhang, S. Wu, Y. Wang, SRplot: a free online platform for data visualization and graphing, PLoS One 18 (2023) 1–8, https://doi.org/10.1371/journal.pone.0294236.

[19] Y. Jeong, D.F. Chaupin, K. Matsushita, M. Yamakuchi, S.J. Cameron, C.N. Morrell, C.J. Lowenstein, Aldosterone activates endothelial exocytosis, Proc. Natl. Acad. Sci. U. S. A 106 (2009) 3782–3787, https://doi.org/10.1073/pnas.0804037106.

[20] D.A. Heinrich, C. Adolf, L.C. Rump, I. Quack, M. Quinkler, S. Hahner, A. Januszewicz, J. Seufert, H.S. Willenberg, N. Nirschl, L. Sturm, F. Beuschlein, M. Reincke, Primary aldosteronism : key characteristics at diagnosis : a trend toward milder forms, Eur. J. Endocrinol. 178 (2018) 605–611.

[21] M. Moser, M. Huck, J.S. Esser, L. Deng, L. Hein, A. Lother, J. Kowalski, D. Fürst, C. Bode, Endothelial cell mineralocorticoid receptors oppose VEGF-induced gene expression and angiogenesis, J. Endocrinol. 240 (2018) 15–26, https://doi.org/10.1530/joe-18-0494.

[22] S.-S. Bolz, B. Fisslthaler, S. Pieperhoff, C. de Wit, I. Fleming, R. Busse, U. Pohl, Antisense oligonucleotides against cytochrome P450 2C8 attenuate EDHF-mediated Ca 2+ changes and dilation in isolated resistance arteries, Faseb. J. 14 (2000) 255–260.

[23] B. Fisslthaler, R. Popp, L. Kiss, M. Potente, D.R. Harder, I. Fleming, R. Busse, Cytochrome P450 2C is an EDHF synthase in coronary arteries, Nature 401 (1999) 493–497, https://doi.org/10.1038/46816.

[24] K. Node, Y. Huo, X. Ruan, B. Yang, M. Spiecker, K. Ley, D.C. Zeldin, J.K. Liao, Anti-inflammatory properties of derived eicosanoids, Science 285 (1999) 1276–1280.

[25] M.L. Edin, B.G. Hamedani, A. Gruzdev, J.P. Graves, F.B. Lih, S.J. Arbes, R. Singh, A. C.O. Leon, J.A. Bradbury, L.M. DeGraff, S.L. Hoopes, M. Arand, D.C. Zeldin, Epoxide hydrolase 1 (EPHX1) hydrolyzes epoxyeicosanoids and impairs cardiac recovery after ischemia, J. Biol. Chem. 293 (2018) 3281–3292, https://doi.org/10.1074/jbc.RA117.000298.

[26] V.E. Rand, C.J. Garland, Endothelium-dependent relaxation to acetylcholine in the rabbit basilar artery: importance of membrane hyperpolarization, Br. J. Pharmacol. 106 (1992) 143–150, https://doi.org/10.1111/j.1476-5381.1992.tb14307.x.

[27] T. Huang, T. Chu, H. Chen, C.J. Jen, Heterogeneity of [Ca 2+]i signaling in intact rat aortic endothelium, Faseb. J. 14 (2000) 797–804, https://doi.org/10.1096/fasebj.14.5.797.

[28] E. Zuccolo, U. Laforenza, S. Negri, L. Botta, R. Berra-Romani, P. Faris, G. Scarpellino, G. Forcaia, G. Pellavio, G. Sancini, F. Moccia, Muscarinic M5 receptors trigger acetylcholine-induced Ca 2+ signals and nitric oxide release in human brain microvascular endothelial cells, J. Cell. Physiol. 234 (2019) 4540–4562, https://doi.org/10.1002/jcp.27234.

[29] K.M. Gauthier, E.M. Edwards, J.R. Falck, D.S. Reddy, W.B. Campbell, 14,15-Epoxyeicosatrienoic acid represents a transferable endothelium-dependent relaxing factor in bovine coronary arteries, Hypertension 45 (2005) 666–671, https://doi.org/10.1161/01.HYP.0000153462.06604.5d.

[30] B.T. Larsen, H. Miura, O.A. Hatoum, W.B. Campbell, B.D. Hammock, D.C. Zeldin, J. R. Falck, D.D. Gutterman, Epoxyeicosatrienoic and dihydroxyeicosatrienoic acids dilate human coronary arterioles via BKCa channels: implications for soluble

epoxide hydrolase inhibition, Am. J. Physiol. Heart Circ. Physiol. 290 (2006) 491–499, https://doi.org/10.1152/ajpheart.00927.2005.
[31] L. Deng, L. Pollmeier, R. Bednarz, C. Cao, P. Laurette, L. Wirth, A. Mamazhakypov, C. Bode, L. Hein, R. Gilsbach, A. Lother, Atlas of cardiac endothelial cell enhancer elements linking the mineralocorticoid receptor to pathological gene expression, Sci. Adv. 10 (2024), https://doi.org/10.1126/sciadv.adj5101.
[32] J.H. Capdevila, A. Karara, D.J. Waxman, M.V. Martin, J.R. Falck, F. Peter Guengerich, Cytochrome P-450 enzyme-specific control of the regio- and enantiofacial selectivity of the microsomal arachidonic acid epoxygenase, J. Biol. Chem. 265 (1990) 10865–10871, https://doi.org/10.1016/s0021-9258(19)38526-6.
[33] D. Schwarz, P. Kisselev, S.S. Ericksen, G.D. Szklarz, A. Chernogolov, H. Honeck, W. H. Schunck, I. Roots, Arachidonic and eicosapentaenoic acid metabolism by human CYP1A1: highly stereoselective formation of 17(R),18(S)-epoxyeicosatetraenoic acid, Biochem. Pharmacol. 67 (2004) 1445–1457, https://doi.org/10.1016/j.bcp.2003.12.023.
[34] M. VanRollins, T.L. Kaduce, H.R. Knapp, A.A. Spector, 14,15-Epoxyeicosatrienoic acid metabolism in endothelial cells, J. Lipid Res. 34 (1993) 1931–1942, https://doi.org/10.1016/s0022-2275(20)35111-7.
[35] A.C. Missias, J. Mudd, J.M. Cunningham, J.H. Steinbach, J.P. Merlie, J.R. Sanes, Deficient development and maintenance of postsynaptic specializations in mutant mice lacking an “adult” acetylcholine receptor subunit, Development (Cambridge, U. K.) 124 (1997) 5075–5086, https://doi.org/10.1242/dev.124.24.5075.
[36] Q. Zou, S.W.S. Leung, P.M. Vanhoutte, Activation of nicotinic receptors can contribute to endothelium-dependent relaxations to acetylcholine in the rat aorta, J. Pharmacol. Exp. Therapeut. 341 (2012) 756–763, https://doi.org/10.1124/jpet.112.192229.
[37] T.A. Williams, J.W.M. Lenders, P. Mulatero, J. Burrello, M. Rottenkolber, C. Adolf, F. Satoh, L. Amar, M. Quinkler, J. Deinum, F. Beuschlein, K.K. Kitamoto, U. Pham, R. Morimoto, H. Umakoshi, A. Prejbisz, T. Kocjan, M. Naruse, M. Stowasser, T. Nishikawa, W.F. Young, C.E. Gomez-Sanchez, J.W. Funder, M. Reincke, Outcomes after adrenalectomy for unilateral primary aldosteronism: an international consensus on outcome measures and analysis of remission rates in an international cohort, Lancet Diabetes Endocrinol. 5 (2017) 689–699, https://doi.org/10.1016/S2213-8587(17)30135-3.