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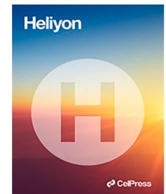

Research article

Lipid imbalance and inflammatory oxylipin cascade at the maternal-fetal interface in recurrent spontaneous abortion

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ABSTRACT

Background: Recurrent spontaneous abortion (RSA) is intricately linked to metabolic dysregulation at the maternal-fetal interface during early gestation. Abnormal levels of essential fatty acids and downstream oxylipins in decidua and chorionic villi have been identified as potential risk factors for RSA. Oxylipins have been linked to excessive inflammation, which might disrupt maternal-fetal immune tolerance, potentially contributing to RSA. Nonetheless, the exact fatty acid-oxylipin metabolic pathway at the matrernal-fetal interface in RSA occurrence remains unknown. Therefore, this research aimed to explore the effect of essential fatty acids, their transport, and downstream oxylipins at the maternal-fetal interface on RSA pathogenesis.
Methods: Plasma, chorionic villus, and decidual tissue samples from the first trimester were collected from healthy pregnant women undergoing elective pregnancy terminations, as well as from patients experiencing spontaneous abortion. The concentrations of essential fatty acids and their downstream oxylipins in the villi and decidua were quantified using gas chromatography-mass spectrometry (GC-MS) and liquid chromatography-tandem mass spectrometry (LC-MS).

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The expression of enzymes related to metabolic pathways was investigated by q-PCR. The ratios of M1/M2 macrophages were assessed by flow cytometry (FCM).

Results: This study found elevated concentrations of omega-6 fatty acids, encompassing arachidonic acid (AA), linoleic acid (LA), and dihomo-gamma-linolenic acid (DGLA) in maternal plasma and chorionic villi, whereas lower concentrations were observed in the decidua, than in samples from normal pregnancies. Further analysis revealed that the transport of these fatty acids was dysregulated at the maternal-fetal interface in RSA women, possibly due to the aberrant expression of the fatty acid translocase (FAT/CD36). In addition, this study revealed that RSA patients displayed higher levels of downstream oxylipins, such as prostaglandin F2a (PGF2a), prostaglandin E2 (PGE2), and leukotriene B4 (LTB4) in chorionic villi and decidua. These compounds may contribute to M1 inflammatory macrophage polarization in RSA, thereby forming a highly inflammatory environment and influencing immunomodulation at the maternal-fetal interface.

Conclusion: The study revealed alterations in omega-6 fatty acids, CD36 transport, and AA downstream oxylipins in RSA, which in turn promote M1 macrophage polarization. Thus, this research has established a foundation for identifying potential biomarkers for, and providing novel insights into, the diagnosis and pathophysiology of RSA.

1. Introduction

Spontaneous abortion is one of the most prevalent adverse outcomes of pregnancy, with an estimated 23 million cases annually worldwide [1]. Recurrent spontaneous abortion (RSA) is defined by the UK Royal College of Obstetricians and Gynaecologists as the loss of three or more successive pregnancies [2]. Notably, RSA can lead to perinatal complications, including an elevated risk of uterine infection, and placental adhesions [3,4]. RSA patients face an approximately 40 % risk of further pregnancy loss, significantly impacting their physical and mental well-being [2]. Although etiological studies have identified various factors contributing to RSA [5–7], the attributes of approximately 50 % of RSAs remain unknown.

The maternal-fetal interface, comprising the decidua and chorionic villi, serves as the border of direct contact between the embryo and mother. Current evidence suggests that malformation and/or malfunction of this interface during the first trimester could be a key factor in causing RSA [8–11]. Several studies have indicated that metabolic dysregulation in the decidua and chorionic villi leads to RSA [12]. Tsai et al. quantified metabolite levels, finding that the concentrations of amino acids, lipids, and organic acids were abnormal within RSA decidua [13]. Disruption of decidual metabolism, encompassing glycerophospholipid metabolism, sphingolipid metabolism, and the tricarboxylic acid (TCA) cycle, may contribute to RSA occurrence [14]. Abnormal levels of essential fatty acid and downstream oxylipins in decidua and chorionic villi have been identified as potential risk factors for RSA [15]. Oxylipins have been linked to excessive inflammation, which might disrupt maternal-fetal immune tolerance, potentially contributing to RSA [16–18]. In particular, an imbalance in the M1/M2 macrophage ratio in decidual tissue has been recognized as a factor contributing to RSA [19]. In addition, the maternal-fetal interface serves as the nexus for nutrient transfer from mother to fetus. Efficient transport of fatty acid across the placenta is crucial for normal fetal growth [20,21]. Several membrane proteins, including plasma membrane-associated fatty acid binding protein (FABPpm), fatty acid transport proteins (FATP 1–6), and fatty acid translocase (FAT/CD36), have been identified as important for fatty acid intake in the placenta [22–25]. Abnormalities of fatty acids transporters were believed to play a part in pregnancy complications, including intrauterine growth retardation and gestational diabetes mellitus [26,27]. However, the exact fatty acid-oxylipin metabolic pathway and fatty acid transport between decidua and chorionic villi in RSA remains unsolved.

Therefore, this research aims to investigate the influence of essential fatty acids, their transport, and downstream oxylipins are the maternal-fetal interface on RSA pathogenesis. Through an exploration of the relationship between metabolic alterations and pathological changes in decidua and chorionic villi, this study seeks to offer novel insights into the diagnosis and therapeutic interventions for RSA.

2. Methods

2.1. Study participants

Between April 2021 and September 2021, decidual tissues and chorionic villus from patients with RSA (n = 9) or with a normal pregnancy (n = 9) were collected from the Second Affiliated Hospital of Chongqing Medical University. Following the Declaration of Helsinki, the study implementation acquired approval from the Ethics Committee of the Second Affiliated Hospital, Chongqing Medical University (Chongqing, China) (2020150). All participants signed a written informed consent form prior participating in the research.

2.2. Sample collection and preservation

Exclusion criteria for this study included abortions resulting from infection, anatomical defects, or endocrine and chromosomal abnormalities. Plasma, decidual, villous tissues were collected from 9 pregnant women necessitating artificial abortion for nonmedical causes (maternal age: 20–40 years, BMI <24; gestational age: 5–10 weeks), and 9 women with RSA (maternal age: 20–40 years, BMI

<24; gestational age: 5–10 weeks). Trained nurses collected whole blood in ethylenediaminetetraacetic acid (EDTA) tubes. The collected blood underwent centrifugation at 2300 g for 10 min at 4 °C. Next, the supernatant was transferred to a 1.5 ml cryopreservation tube and stored at −80 °C before metabolite extraction. Immediately after the operation, decidua and chronic villi were collected under sterile conditions and sent to the university laboratory for further treatment. The tissues were relocated in pre-chilled phosphate buffered saline (PBS) and dissected into chorionic villus and decidua. Subsequently, the villous and decidual tissues were cleaned with cold PBS to eliminate contamination between the chorionic villus and decidua. For metabolite profiling, samples were immediately frozen in liquid nitrogen and stored at −80 °C until analysis.

2.3. Sample preparations for plasma and tissue

20 μL of a d4-alanine internal standard (10 mM, Sigma-Aldrich, USA) was added into aliquots of thawed plasma (150 μL). Cold methanol (400 μL, analytical grade, Adamas-beta, China) was added to precipitate protein from the plasma samples, and incubated at −20 °C for 30 min. Subsequently, the supernatant was isolated by centrifugation at 12,000 g for 15 min at 4 °C.

The tissue sample was prepared by dissecting 40 ± 0.5 mg and placing it in new tubes. Following the addition of an internal standard (20 μL, d4-alanine) and 400 μL of cold methanol, a TissueLyser II (QIAGEN, Germany) was used to homogenize tissue samples and centrifuged (10,000 g, 15 min, 4 °C) to isolate the supernatant. All supernatants were stored at −80 °C prior to derivitization.

2.4. Methyl chloroformate derivatization

The boiling points of the extracted metabolites were reduced through a methyl chloroformate (MCF) derivatization method, following the protocol published by Smart et al. [28]. In brief, 200 μL of sodium hydroxide (1 M, Sigma-Aldrich, USA) was added to the SpeedVac-dried samples. Methanol (167 μL) and 34 μL pyridine (analytical grade, Merck, Germany) were introduced as the methyl group donor and catalyst. The derivatization process commenced with the addition of 20 μL of MCF (analytical grade, Shandong Huayang Pesticide Chemical Industry Group, China).

Subsequently, an additional 20 μL of MCF was introduced and mixed for another 30 s using a vortex mixer. 400 μL of chloroform (analytical grade, Chuandong Chemical Industry, China) and 400 μL of sodium bicarbonate (50 mM, Sigma-Aldrich, USA) were added to the reaction mixture and vortexed for 10 s in order to separate the derivatized metabolites. Anhydrous sodium sulfate (Sigma-Aldrich, USA) was used to isolate the lower chloroform phase and eliminate extra water. Before samples were shifted to GC-MS vials, the aqueous layer was disposed of and any leftover water was extracted from the chloroform phase using more anhydrous sodium sulfate.

2.5. Gas chromatography-mass spectrometry (GC-MS) analysis

The derivatized specimens were analyzed using an Agilent 5977 A MSD system linked to an Agilent 7890B GC system. A RESTEK Rtx®− 2330column (90 % biscyanopropyl/10 % phenylcyanopropylpolysiloxane, 100 m, 0.25 mm ID, 0.2 μm df) was used to separate derivatized metabolites. Throughout the analysis, the inlet, which ran in split-less mode at 250 °C, received the injection of the sample (1 μL). The pressure of helium gas was maintained at a steady flow rate of 1 mL/min. The mass spectrometry parameters and oven temperature program were established in accordance with Smart et al. [28]. Automated mass spectral deconvolution and identification system (AMDIS) software was used to deconvolute and identify the GC-MS chromatographic peaks. By comparing the corresponding GC retention time and MS fragmentation patterns (mass-to-charge ratio and relative intensity of mass spectra to a reference ion) to an internal MS library created using chemical standards, the metabolites were identified. The relative quantification of identified metabolites was extracted using an XCMS-based R-script, by choosing the most abundant reference ion within an appropriate retention time bin. Only peaks detected in at least 40 % of the samples within a group were retained. Additionally, all peak heights were adjusted by subtracting the corresponding peaks found in blank samples, which were processed in the similar processing.

Table 1
Primers implicated in real-time RT-qPCR.

Sequence (5′-3′)		
Genes	Sense	Antisense
Gapdh	ATCTCTGCCCCCTCTGCTG	CATCACGCCACAGTTTCCC
CD36	CTTTGGCTTAATGAGACTGGGAC	GCAACAAACATCACCACACCA
Fabp1	CGGAAGAGCTCATCCAGAAG	TTGTCACCTTCCAACTGAACC
Fabp3	CACTCACCCACGGCACTGCA	TCCCGGTCAGTGGCACCTGA
COX-1	TGCGCTCCAACCTTATCCC	AGAGGGCAGAATACGAGTGTAA
COX-2	TAAGTGCGATTGTACCCGGAC	TTTGTAGCCATAGTCAGCATTGT
LOX-5	CTCAAGCAACACCGACGTAAA	CCTTGTGGCATTTGGCATCG
LOX12	ATGGCCCTCAAACGTGTTTAC	GCACTGGCGAACCTTCTCA
LOX15	GGGCAAGGAGACAGAACTCAA	CAGCGGTAACAAGGGAACCT
CYP2J2	TGGCTTGCCCTTAATCAAAGAA	GGCCACTTGACATAATCAATCCA

2.6. Quantitative real-time polymerase chain reaction (RT-qPCR)

RNA was extracted from human tissue samples with TRIzol Reagent (Accuratre Biology, China). Reverse transcription was conducted on 2 μg of total RNA utilizing the PrimeScript RT Master Mix (TaKaRa, Japan). For the reverse transcription of miRNA, the Mir-X miRNA First-Strand Synthesis Kit (TaKaRa, Japan) was employed. RT-qPCR was carried out using a SYBR Green Real-time PCR Master Mix kit (TaKaRa, Japan) under the following conditions: an initial pre-incubation at 95 °C for 30 s, followed by 40 cycles consisting of denaturation at 95 °C for 5 s and annealing/extension at 60 °C for 30 s. The data were normalized relative to the *Gapdh* expression level. All data were analyzed using QuantStudio 3 RealTime PCR system software (Agilent, USA). The primer sequences are provided in Table 1.

2.7. Oxylipin extraction and liquid chromatography-tandem mass spectrometry (LC-MS/MS) analysis

Oasis HLB cartridge columns (30 mg, 1 cc, Waters, UK) were used to extract oxylipins [29]. 6 mL of methanol (MeOH) were used to wash the column, and then 6 mL of 5 % MeOH and 0.1 % acetic acid (MS grade, Thermo Fisher Scientific, China) were used to equilibrate it. Following sample loading, contaminants were removed with a wash of 5 % MeOH and 0.1 % acetic acid. 4 mL of MeOH were then used to elute the desired metabolites. Before being reconstituted in 100 μL of MeOH/acetonitrile (ACN) (50:50, v/v) (MS grade, Thermo Fisher Scientific, China), the eluant was vacuum-dried [30]. With the exception of 9(10)-EpOME, prostaglandin F1α, and 11-deoxy prostaglandin E1, which were synthesized in MeOH, stock solutions of oxylipin chemical standards were made at a concentration of 100 ng/μL in ethanol (MS grade, Thermo Fisher Scientific, China). For all standard compounds, an intermediate stock solution with the 43 analytes was made at a concentration of 10 μg/μL. By diluting this intermediate stock solution with ethanol, calibrator working solutions were created, yielding concentrations of 0.01–0.05, 0.2–1, 5, and 20 ng/mL for each analyte. With the exception of PEG2-d4, which was prepared at 500 ng/μL, a mixture consisting of five working internal standard (IS) solutions was created by each individual stock of internal standard to yield a final concentration of 100 ng/mL in MeOH/ACN (1:1, v/v). Five deuterated compounds (PGE2-d4, PGF2α-d4, 13-HODE-d4, 20-HETE-d8, and 5-HETE-d8) were added to the specimen prior to sample preparation in order to mimic the extraction of endogenous compounds. Retention time and structural similarity were taken into consideration when choosing an IS for each analyte. On a triple quadrupole mass spectrometer (QqQ, Agilent 6460C, USA), samples were examined using electrospray ionization in conjunction with ultra-performance liquid chromatography (UPLC, Agilent 1260, USA). The autosampler was kept at 6 °C while a 10 μL aliquot of the extract was injected for analysis. Chromatographic separation was performed by an Agilent Poroshell EC-C18 column (3.0 × 150 mm; 1.9um; Agilent) with a flow rate of 0.5 mL/min at 40 °C using the solvents A (0.1 % acetic acid) and B (90:10 v/v ACN/isopropanol) over a 25 min gradient (0–3.5 min from 10 % B to 35 % B, 3.5–5.5 min B to 40 %, 5.5–7 min to 42 %B, 7–9 min to 50 %B, 9–15 min to 65 % B, 15–17 min to 75 % B, 17–18.5 min to 85 % B, 18.5–19.5 min to 95 % B, from 19.5 to 21 min to 10 % B, 21–25 min 10 % B). Using N_2 at a pressure of 35 psi for the nebulizer with a flow rate of 10 L/min and a temperature of 300 °C, electrospray ionization was carried out in the negative ion mode. A flow rate of 11 L/min and a sheath gas temperature of 350 °C were established. 3500 V was the capillary voltage and 1250 V was the nozzle voltage. For MS operation, the multiple reaction monitoring (MRM) scan mode was used.

2.8. Flow cytometry (FCM)

Human decidual stromal cells (DSCs) and decidual immune cells (DICs) were isolated from decidual tissues as previously described [31]. Subsequently, human decidual macrophages (dMφs) were isolated from DICs using an Anti-CD14 MicroBead Kit (Miltenyi, Germany). The purity of Vimentin$^+$ CD45$^-$ DSCs (above 98 %), CD45$^+$ CD14$^+$ dMφs (above 95 %), and CD45$^+$ DICs (above 98 %) was confirmed using FCM analysis. The isolated primary cells were cultured in RPMI 1640 (HyClone, USA) or DMEM/F12 (HyClone, USA) medium supplemented with 10 % fetal bovine serum (FBS, Gibco, Australia) in 5 % CO_2 at 37 °C. For FCM analyses, the cells were incubated with monoclonal antibodies as per the manufacturer's instructions (refer to Table 2).

Intracellular and intranuclear staining procedures were performed following fixation and permeabilization using a Transcription Factor Buffer Set (BD Pharmingen, USA) and Fixation/Permeabilization Solution Kit (BD Pharmingen, USA), respectively. FCM was performed using a Beckman-Coulter CyAN ADP Analyzer (Beckman-Coulter, USA) and analyzed with FlowJo software (version 10.0,

Table 2
Fluorescent antibodies used for flow cytometry.

Fluorescent antibodies	Clones	Suppliers	Identifiers	Type of staining
APC anti-human/mouse/rat Vimentin	280618	R&D Systems	Cat#IC2105A	Intracellular staining
BV510 anti-human CD45	HI30	BioLegend	RRID:AB_2561383	Cell surface staining
BV421 anti-human CD45	HI30	BioLegend	RRID:AB_2561357	Cell surface staining
Pacific Blue anti-mouse CD45	30-F11	BioLegend	RRID:AB_493535	Cell surface staining
FITC anti-human CD14	HCD14	BioLegend	RRID:AB_830677	Cell surface staining
BV421 anti-human CD14	HCD14	BioLegend	RRID:AB_2563296	Cell surface staining
APC anti-human TGF-β1	TW4-2F8	BioLegend	RRID:AB_10682896	Intranuclear staining
APC anti-human CD80	2D10	BioLegend	RRID:AB_2076147	Cell surface staining
APC anti-human CD209	9E9A8	BioLegend	RRID:AB_1134045	Cell surface staining
Alexa Fluor 647 anti-human IL-1β	JK1B-1	BioLegend	RRID:AB_604135	Cell surface staining

TreeStar).

2.9. Data normalization and statistical analysis

To improve quantitative robustness and reduce human and instrumental variability, the metabolite levels were first normalized using several internal standards (nonadecanoic acid and tridecanoic acid), depending on their connection with metabolites in the quality control (QC) samples. Then, a calibrator was applied using blank samples to remove contaminants and any carryover from identified metabolites. Student's t-test, non-parametric Mann-Whitney *U* test, Chi-square test, and Fisher's exact test were performed in R to compare maternal and gestational clinical characteristics. To understand the overall metabolic changes between samples in each group and the degree of variability within the group, orthogonal partial least squares discriminatory analysis (OPLS-DA) with leave-one-out cross validation (LOOCV) was performed. Heatmaps were generated with the ggplot2 R package [32].

3. Results

3.1. Clinical characteristics of participants

There were no significant disparities between the normal pregnancy and RSA groups in baseline clinical characteristics, including maternal age, gestational age, crown-rump length, and BMI (Table 3).

3.2. Metabolite profiles of chorionic villus and decidual tissues from RSA and normal pregnancy participants

In this study, GC-MS based metabolomics revealed over 150 chromatographic peaks. This analysis identified 90, 103, and 101 metabolites in plasam, decidua, chorionic villus samples, respectively (Supplemental Tables 1–3). Applying orthogonal partial least squares discriminatory analysis (OPLS-DA), we successfully discriminated between RSA and normal pregnancy groups for plasma (Fig. 1A), decidua (Fig. 1B), and chorionic villus samples (Fig. 1C). These results suggested that plasma, chorionic villi (fetal tissues), and decidua (maternal tissues) show metabolic perturbation during early abortion. Moreover, univariate analysis t-tests and FDR revealed that eleven plasma metabolites, eighteen decidual metabolites, and four chorionic villus metabolites had statistically significant different concentrations (both *P*-value and *q*-value <0.05, and VIP value > 1) in RSA and normal pregnancy samples (Fig. 1 D). Notably, plasma from RSA participants exhibited overall higher concentrations of metabolites, while RSA decidua displayed overall lower concentrations. In chorionic villi from RSA participants, the two metabolites dihomo-arachidonic acid (AA) and gamma-linolenic acid (DGLA) were at lower concentrations, whereas two metabolites (11-eicosenoic acid, and nervonic acid) displayed higher concentrations than samples from normal pregnancies. Remarkably, omega-6 fatty acids were the most common metabolites with significantly altered concentrations across the three comparisons. Absolute quantification of omega-6 fatty acids including linoleic acid (LA), AA, gamma-linolenic acid (GLA), and DGLA revealed the highest concentration in plasma, followed by chorionic villi, and the lowest concentration in decidua. These results suggest that dysregulated metabolism of omega-6 fatty acids is associated with RSA occurrence.

3.3. Relative expression of fatty acid transporters

Given the varied patterns of omega-6 fatty acid concentrations observed in the decidua and chorionic villi samples from RSA patients, we assessed the omega-6 fatty acid ratios among chorionic villi and decidua in samples from both normal pregnancies and RSA. The results revealed a higher proportion of omega-6 fatty acids in RSA samples than in samples from normal pregnancies (Fig. 2A). Considering the elevated concentrations of omega-6 fatty acids in chorionic villi compared to decidua (refer to Fig. 1E), we hypothesized that there was an upregulated transport of omega-6 fatty acids from decidua to chorionic villi under RSA conditions. Recognizing that trophoblast uptake of fatty acids largely depends on proteins attached to the microvillous plasma membrane [33], we measured the mRNA levels of the fatty acid transporter *CD36* and *FABPs* in chorionic villi and decidua from both RSA and normal pregnancies. We found that the expression of *CD36* mRNA was higher, while *FABP1* and *FABP3* mRNA levels were lower in RSA than in normal pregnancies (Fig. 2B).

Table 3
Demographic details of the study participants.

	Normal pregnancy (n = 9)	RSA (n = 9)	*P* value
Maternal age, years	31.0 (26.5, 32.0)	32.0 (30.5, 33.5)	0.099
Gestational age, weeks	7.1 (6.4, 8.1)	8.6 (7.2, 9.0)	0.131
Crown-rump length, mm	4.0 (3.0, 5.5)	3.0 (1.0, 4.5)	0.141
Pre-pregnancy body mass index, kg/m^2	21.7 (20.0, 23.3)	21.3 (19.3, 21.8)	0.387

All continuous variables are displayed as medians (25th percentile, 75th percentile). *P* values, determined by the Student's t-test or Wilcoxon test, were implicated for pairwise comparisons of continuous variables. RSA, recurrent spontaneous abortion.

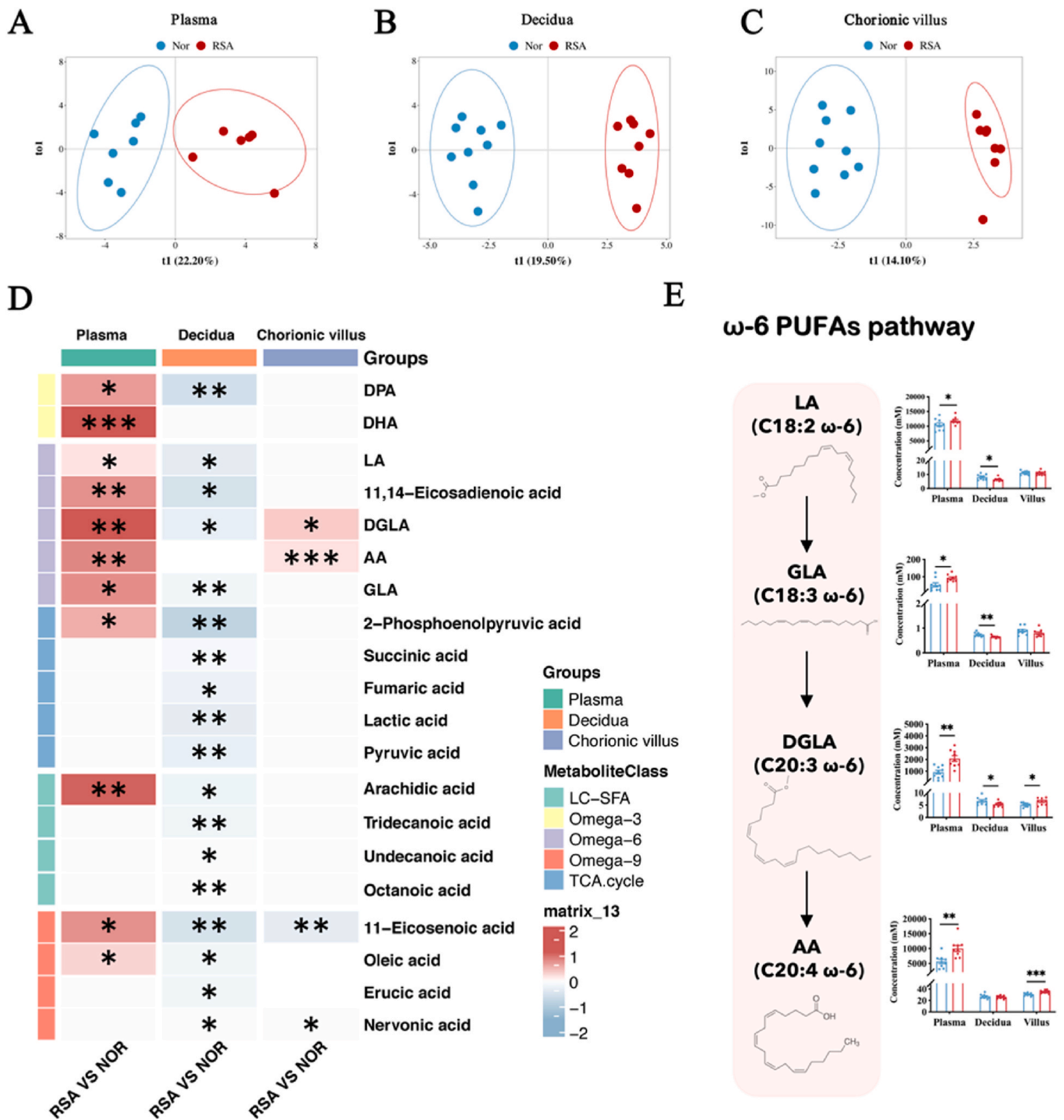

Fig. 1. Metabolite analysis of chorionic villus and decidual tissues from RSA and normal pregnancy participants. (A–C) Orthogonal partial least squares discriminatory analysis (OPLS-DA) score plots of identified metabolites. Circular points in the plot represent an individual participant. Blue points are samples from normal pregnancies and red points are samples from RSA. (D) Heatmap of the metabolite concentrations in different tissues. Red colors signify elevated metabolite concentrations, while blue colors represent lower metabolite concentrations in the RSA group than in the normal group. The relative level of metabolites was illustrated using a log2 scale. (E) The level of omega-6 fatty acids in the plasma, decidua, and chorionic villus samples from normal pregnancy and RSA patients. The blue and red bars represent metabolite concentrations measured in normal pregnancy and RSA patients, respectively. The statistically significant differences in metabolite concentrations are denoted with asterisks (**P*-values$<$ 0.05, ***P*-values $<$0.01, ****P*-values $<$0.001). Abbreviations: RSA, recurrent spontaneous abortion; Nor, normal pregnancy; DGLA, dihomo-gamma-linolenic acid; AA, arachidonic acid; LA, linoleic acid; GLA, gamma-linolenic acid; DPA, Diphenylamine; DHA, Docosahexaenoic acid.

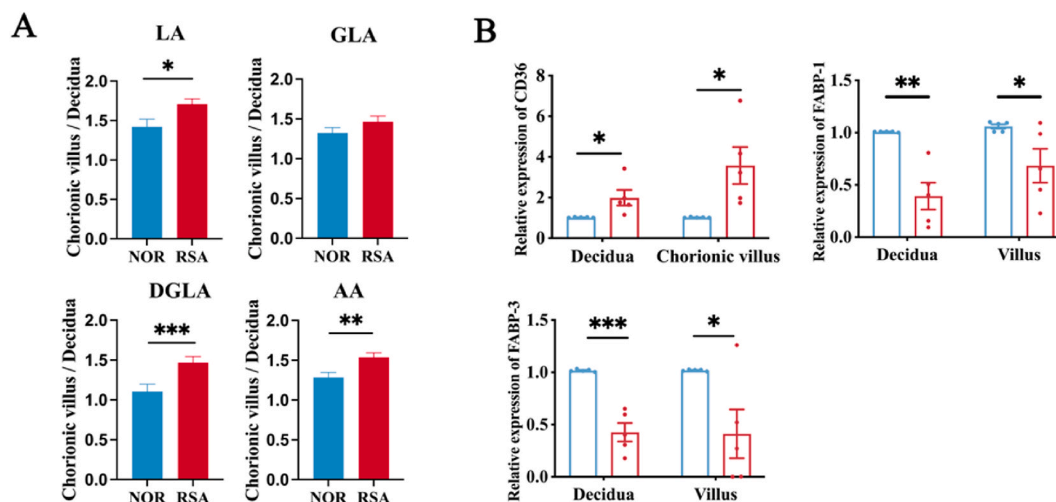

Fig. 2. Evidence for transport of omega-6 fatty acids in human decidua and chorionic villus. (A) The omega-6 fatty acids ratio (chorionic villus/ decidua) in RSA and normal pregnancy samples. (B) mRNA expression of *CD36*, *FABP1*, and *FABP3* in chorionic villus and decidua using qPCR. Blue bars are samples from normal pregnancies and red bars are samples from RSA. $*P < 0.05$; $**P < 0.01$; $***P < 0.001$. Abbreviations: FAT/CD36, fatty acid translocase; FABP, Fatty acid binding protein.

3.4. The metabolism of oxylipins derived from omega-6 fatty acids

After identifying omega-6 fatty acids as potential metabolites associated with RSA, we investigated the metabolism of downstream oxylipins derived from omega-6 fatty acids in RSA. Initial assessments focused on the mRNA levels of downstream enzymes related to fatty acid peroxidation, namely cyclooxygenase-1 (COX1), cyclooxygenase-2 (COX2), lipoxygenase-12 (LOX12), lipoxygenase-15 (LOX15), lipoxygenase-5 (LOX5), and cytochrome p450 epoxygenase 2J2 (CYP2J2), and we found higher expression of these enzymes at the RSA maternal-fetal interface than in samples from normal pregnancies. Moreover, the concentrations of most AA -and LA-derived oxylipin were elevated in both decidua and chorionic villi from RSA cases related to those from normal pregnancies (Fig. 3A–D). Specifically, thirteen, six, and one oxylipin derived from AA, LA, and DGLA, respectively, exhibited significantly higher concentrations in the chorionic villi samples except for AA-derived PGF2a, leukotriene E4 (LTE4), and 18 hydroxy-eicosatetraenoic acid (18-HETE), which were at higher concentrations in the decidua samples. A Sankey diagram revealed that many of these oxylipins were associated with inflammation (Fig. 3E). We further described the profiles of macrophages in the early decidua of RSA and normal pregnancy. In cases of spontaneous abortion, dMφs had a greater M1/M2 ratio, indicating higher expression of M1-associated markers (CD80, CD163, and IL-1β) and lower expression of M2-associated markers (CD209 and TGF-β1) (Fig. 3F). Higher AA-derived oxylipins may cause M1-like polarization of dMφs, perhaps contributing to early spontaneous miscarriage.

4. Discussion

The pathogenesis of RSA is intricately linked to metabolic dysregulation at the maternal-fetal junction in early pregnancy. This study systematically explored the metabolic characteristics of plasma, decidua, and chorionic villi collected from normal pregnancies and RSA patients in the first trimester. Significantly elevated levels of omega-6 fatty acids, such as AA, DGLA, and LA, were observed in maternal plasma and chronic villi, while lower concentrations were found in the decidua of RSA than in normal pregnancy samples. This study also found greater expression of the fatty acid transporter CD36 in the decidua and chorionic villi of RSA samples than in normal pregnancy samples, suggesting that this transporter may be responsible for the altered omega-6 fatty acid levels in RSA. Subsequently, higher levels of the downstream oxylipin derivatives of AA, DGLA, and LA were observed in the maternal-fetal interface of RSA samples, including PGE2, PGF2a, and LTB4, which may contribute to M1 inflammatory macrophage polarization in RSA. Thus, this study proposed that omega-6 fatty acids and their downstream lipid peroxidation oxylipins could significantly impact the pathophysiology of RSA by forming a highly inflammatory environment and influencing immunomodulation at the maternal-fetal interface.

Omega-6 fatty acids have been reported to participant an essential role in early pregnancy [34]. This investigation revealed that omega-6 fatty acids, namely AA, DGLA, and LA, were present in higher concentrations in the plasma and chorionic villi of RSA patients than in samples from normal pregnancies (Fig. 1). Previous studies have shown that dysregulation of omega-6 acid levels impairs placental development [35,36]. In particular, AA increases placental oxidative stress [37], potentially contributing to RSA. Li et al. demonstrated a significantly higher AA concentration in women with uterine functional defects than in a control group [38]. Consistently, previous research has found higher AA levels in RSA plasma and identified the AA downstream peroxidation pathway as a potential target for RSA [39]. In addition, the maternal plasma profiles of LA and AA exhibit dynamic changes throughout pregnancy, underscoring the importance of regulating their homeostasis during gestation [40]. A previous *in vitro* study found that high LA levels

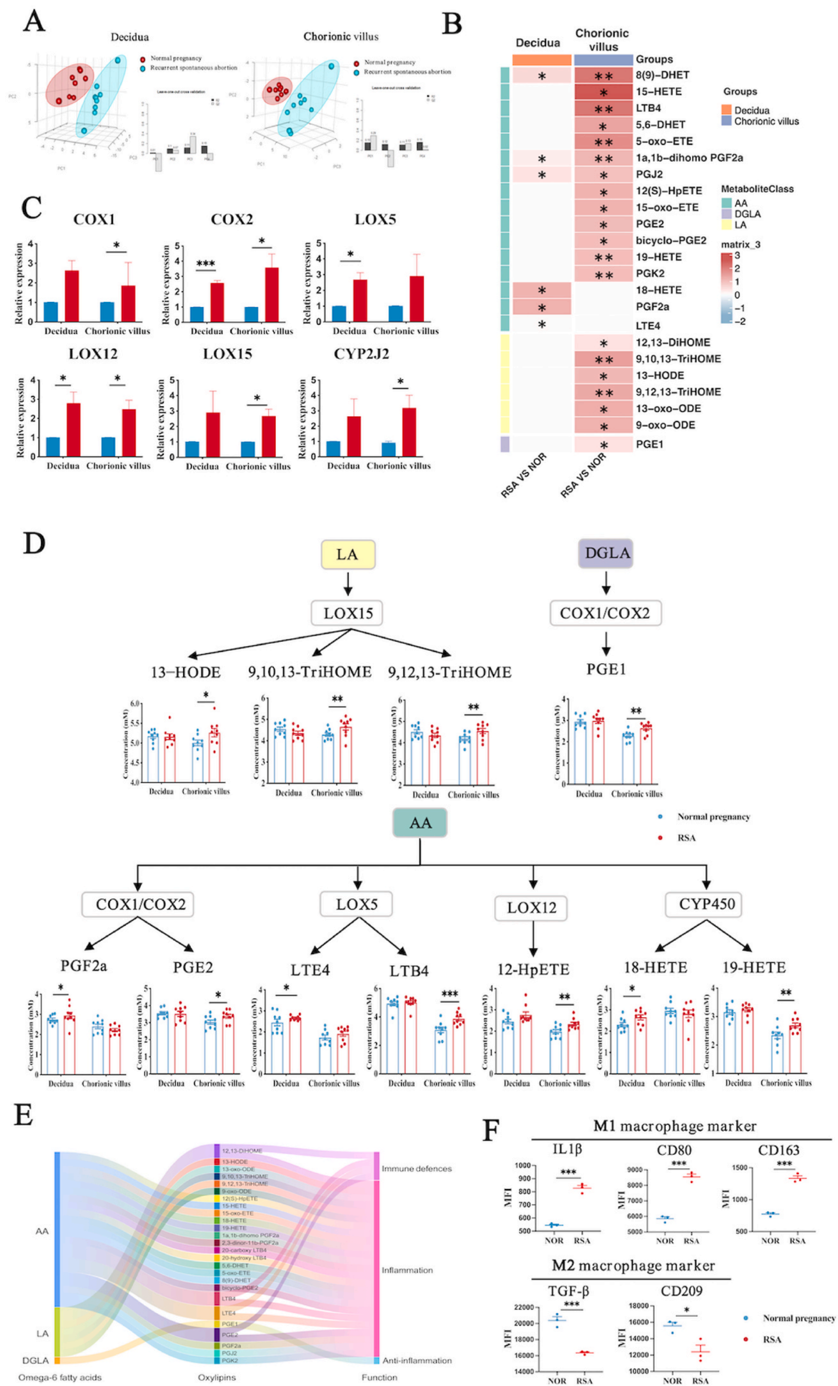

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Fig. 3. The metabolism of oxylipins derived from omega-6 fatty acids in normal and RSA samples. (A) Partial least squares discriminant analysis (PLS-DA) analysis of oxylipin profiles in decidua and chorionic villus samples. (B) Heatmap illustrating oxylipin concentrations in decidua and chorionic villus. Red colors signify elevated metabolite concentrations, while blue colors mean lower metabolite levels in the RSA group than in the normal group, plotted using a $\log_2$ scale. (C) mRNA expression analysis of COX1, COX2, LOX5, LOX12, LOX15, and CYP2J2 in decidua and chorionic villus using qPCR. Blue bars are samples from normal pregnancies and red bars are samples from RSA. (D) The absolute concentrations of oxylipins derived from LA, DGLA, and AA in decidua and chorionic villus samples. Blue bars are samples from normal pregnancies and red bars are samples from RSA. (E) A Sankey diagram indicating that a significantly higher concentration of oxylipins from decidua and chorionic villus are involved in immune defences, inflammation, and anti-inflammation. The omega-6 fatty acids, oxylipins, and their function are positioned on the left, middle, and right respectively. (F) Flow cytometry (FCM) analysis of immune profiles, including the protein levels of CD80, CD209, CD163, IL-1β, and TGF-β1 in decidual macrophages (dMφs) from normal pregnancy and RSA samples. $*P < 0.05$; $**P < 0.01$; $***P < 0.001$. Abbreviations: PF2a, prostaglandin F2a; PGE2, prostaglandin E2; LTB4, COX-2, cyclooxygenase-2; LOX-5, leukotriene B4; lipoxygenase-5, FAT/CD36, fatty acid translocase.

compromised mouse embryo development [41]. In contrast, however, a prior clinical study in the USA reported no correlation between omega fatty acid serum levels and miscarriage risk [42]. These conflicting results may be attributed to differences in the ethnicity of participants. Nevertheless, little research has been reported on the effect of DGLA on RSA. Hence, these findings highlight the prominence of AA and LA, prompting further investigations into their transport mechanisms in maternal-fetal interphase and downstream metabolic effects to understand the influence of omega-6 fatty acids on RSA pathogenesis.

Appropriate FA transport through the placenta is required to develop a healthy fetus [43,44]. It is believed that fatty acid uptake by the trophoblasts relies largely on active transporters located in the microvillous plasma membrane [45–47]. This research found a higher chorionic villus/decidua ratio of LA, DGLA, and AA in RSA than in normal pregnancy, indicating a potential dysregulation in their transport at the RSA maternal-fetal interface. Interestingly, our study observed a higher expression of transporter CD36 in both decidual and chorionic villus tissues of RSA women than in normal pregnancy samples, whereas the expression of fatty acid binding proteins FABP1 and FABP3 was downregulated. Previous findings have shown that the enhanced placental CD36 expression was related to increased fatty acid uptake [48,49]. While the regulation of placental CD36 transport remains unclear, several proposed mechanisms have been reported. Feng et al. proposed that the elevated placental expression of CD36 might be attributed to higher levels of fatty acids in women, suggesting that placental fatty acid transporter expression may be influenced, at least partially, by the accumulation of fatty acids [50,51]. Additional studies have found that fatty acids may enhance CD36 ubiquitination which regulates its transport function in the placenta [52,53]. Furthermore, studies have demonstrated that FABP3 regulates the transport of omega-6 PUFA in rodent trophoblast cells [43,54], whereas Daoud et al. found no correlation between FABP1/FABP3 expressions and LA uptake in human trophoblasts [55]. Thus, the influence of FABPs on fatty acid transportation in trophoblasts remains controversial. Based on these findings, our study suggests that increased expression of CD36 might play a crucial role in fatty acid transportation and

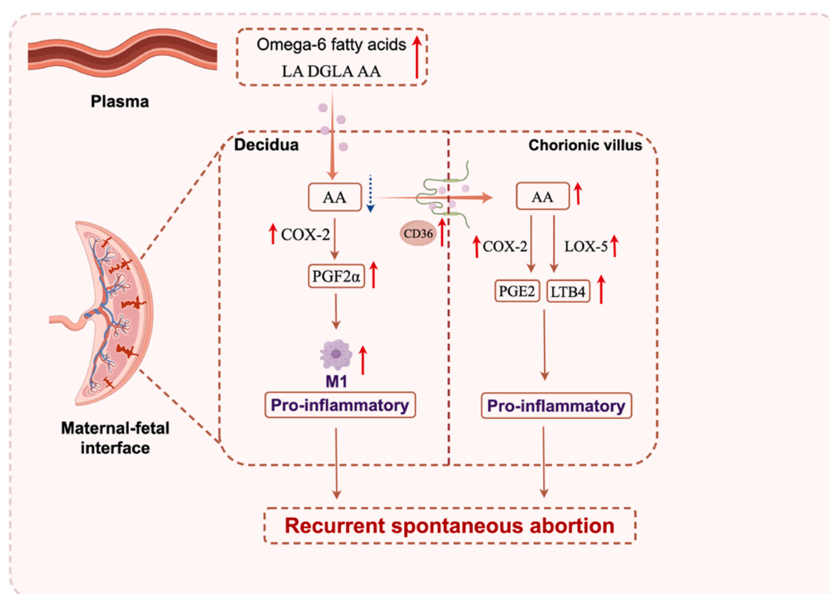

Fig. 4. Proposed metabolic changes in the decidua and chorionic villi of RSA cases. Abnormal increases in the concentrations of omega-6 fatty acids, including AA, were observed in RSA patients. At the maternal-fetal interface, there were upregulated concentrations of AA-derived oxylipins, such as PGE2, PGF2a, and LTB4, along with increased expression of LOX-5 and COX-2 oxygenase enzymes. The CD36 transporter plays a crucial role in transporting omega-6 fatty acids from the decidua and chorionic villi. Ultimately, these changes lead to the polarization of pro-inflammatory M1 macrophages, which contributes to the pathogenesis of RSA. The red arrow represents significantly elevated concentration/upregulation. The blue dotted arrow means reduced concentration/downregulation, although with no statistically significant difference.

in the pathogenesis of RSA, but the underlying mechanisms will require further investigation.

Subsequently, it is demonstrated higher AA-derived oxylipins may be involved in early spontaneous abortion by promoting the M1-like deviation of dMφs. During early pregnancy, excessive inflammation could compromise maternal-fetal immune tolerance and contribute to pregnancy complications, including spontaneous abortion [18]. We found that the majority of LA, DGLA, and AA-derived oxylipins and related COX-2 and 12-LOX oxygenases were upregulated at the RSA maternal-fetal interface. Interestingly, fifteen eicosanoids derived from AA (Fig. 3), including PGF2α, PGE2, LTB4 and 12(S)-HPETE, have been associated with the formation of an inflammatory microenvironment in RSA [33,56–58]. Animal studies have shown elevated PGF2α concentrations in the uteri of rats with RSA, with consistent results observed at the mRNA level [59,60]. Moreover, incorporating PGF2α in the culture medium reduced both *in vitro* and *in vivo* development of embryos in various animal models [53,61,62]. In human clinical studies, significantly elevated levels of PGF2α and PGE2 were found in the amniotic fluid of abortion patients [63]. Noticeably, COX-2 (PG oxygenase) involves a vital role in the vasculoinflammatory response and may lead to abortion in early pregnancy [64,65]. In accordance with our mRNA results, previous researches have revealed that COX-2 was upregulated at the protein level in the decidua and chorionic villus of patients with RSA [66,67]. Thus, these results support a role for PGF2α and PGE2 in RSA. Additionally, our study revealed remarkably higher expression of M1 polarization markers and reduced expression of M2 polarization markers in RSA decidua. Similarly, an elevated population of M1 macrophages and a decreased population of M2 macrophages were found in the uteri of RSA patients by immunohistochemistry and immunofluorescence [68]. Several studies have demonstrated that PGEs and LTs promote the generation of pro-inflammatory M1 macrophages [69–71]. Nelson et al. specifically linked the M1 phenotype to PGE2 and LTB4 exposure [72]. Thus, these findings suggest that PGE2 and LTB4 may promote the occurrence of RSA by influencing macrophage polarization. Furthermore, 12(S)-HPETE, an AA product generated by the 12-LOX oxygenase, has been recognized as a potent inflammatory compound [73,74]. The 12-LOX oxygenase has also been reported to activate proinflammatory cytokine expression in macrophages [75]. Overall, our study provides evidence that elevated AA-derived eicosanoids may promote the generation of pro-inflammatroy M1 macrophages at the maternal-fetal interface, advancing to RSA pathogenetsis (Fig. 4).

4.1. *Strengths and limitations*

At present, there are few human metabolomics studies on RSA, and the present study systematically characterizes the metabolic characteristics of plasma, decidua and chorionic villi in RSA patients. For the first time, this study explored the role of maternal-fetal interface fatty acid transporters in the pathogenesis of RSA. In addition, immunoassays on decidual macrophages were combined with metabolomic analyses to allow a preliminary analysis of the main mechanisms by which oxidized lipids downstream of omega-6 fatty acids affect the occurrence of RSA. Despite the promising results, this investigation has several limitations. Firstly, the tissue sample size obtained was moderately small, and precluded extensive protein and immunohistochemical analyses. Future studies should obtain larger samples to enable protein expression level verification and M1 macrophage immunohistochemistry. Secondly, considering RSA occurred during sample collection, further investigation is needed to determine if the detected differential metabolite concentrations and metabolic pathway fluxes are causative of, or consequential to, RSA. Thirdly, *in vivo* animal studies are needed to establish an experimental basis for RSA treatment.

5. Conclusion

In conclusion, abnormally elevated concentrations of AA and AA-derived oxylipins, along with the increased expression of COX-2 and LOX-5 oxygenase enzymes, were found in RSA. Increased expression of the CD36 transporter is crucial for transporting omega-6 fatty acids from the decidua and chorionic villi. Ultimately, these changes lead to the polarization of pro-inflammatory M1 macrophages, contributing to the pathogenesis of RSA. Thus, this study provides evidence that elevated AA and AA-derived eicosanoids may lead to the occurrence of RSA by promoting an excessive inflammatory response at the maternal-fetal interface. These findings highlight the potential of AA and its derived oxylipins as diagnostic biomarkers of RSA and could serve as promising therapeutic targets for RSA invention. Future studies will need to consider the role of Omega-6 fatty acids and AA oxylipins in women with recurrent miscarriage due to anti-phospholipid syndrome.

CRediT authorship contribution statement

Hao Liu: Writing – original draft, Investigation, Formal analysis. **Huijia Chen:** Investigation, Data curation. **Ting Han:** Methodology, Investigation. **Xin Wang:** Methodology. **Jingcong Dai:** Investigation. **Xiaojia Yang:** Methodology. **ShanAn Chan:** Methodology. **Richard D. Cannon:** Writing – review & editing. **Yang Yang:** Methodology, Data curation. **Hatem Mousa:** Writing – review & editing. **Shufang Chang:** Funding acquisition. **Ruiqi Chang:** Writing – review & editing, Visualization, Validation, Supervision, Resources, Project administration, Funding acquisition. **Ting-Li Han:** Writing – review & editing, Validation, Supervision, Software, Resources, Project administration, Funding acquisition.

Data availability statement

Any inquiries regarding the data should be directed to the corresponding authors.

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Declaration of competing interest

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

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e40515.

References

[1] S. Quenby, I.D. Gallos, R.K. Dhillon-Smith, M. Podesek, M.D. Stephenson, J. Fisher, J.J. Brosens, J. Brewin, R. Ramhorst, E.S. Lucas, R.C. McCoy, R. Anderson, S. Daher, L. Regan, M. Al-Memar, T. Bourne, D.A. MacIntyre, R. Rai, O.B. Christiansen, M. Sugiura-Ogasawara, J. Odendaal, A.J. Devall, P.R. Bennett, S. Petrou, A. Coomarasamy, 42-Miscarriage matters: the epidemiological, physical, psychological, and economic costs of early pregnancy loss, Lancet 397 (2021) 1658–1667, https://doi.org/10.1016/S0140-6736(21)00682-6.

[2] N.A. Du Fossé, M.-L.P. Van Der Hoorn, J.M.M. Van Lith, S. Le Cessie, E.E.L.O. Lashley, Advanced paternal age is associated with an increased risk of spontaneous miscarriage: a systematic review and meta-analysis, Hum. Reprod. Update 26 (2020) 650–669, https://doi.org/10.1093/humupd/dmaa010.

[3] J. Gunnarsdottir, O. Stephansson, S. Cnattingius, H. Åkerud, A.-K. Wikström, Risk of placental dysfunction disorders after prior miscarriages: a population-based study, Am. J. Obstet. Gynecol. 211 (2014) 34, https://doi.org/10.1016/j.ajog.2014.01.041, e1-34.e8.

[4] M. Sugiura-Ogasawara, T. Ebara, Y. Yamada, N. Shoji, T. Matsuki, H. Kano, T. Kurihara, T. Omori, M. Tomizawa, M. Miyata, M. Kamijima, S. Saitoh, the Japan Environment, Children's Study (JECS) Group, Adverse pregnancy and perinatal outcome in patients with recurrent pregnancy loss: multiple imputation analyses with propensity score adjustment applied to a large-scale birth cohort of the Japan Environment and Children's Study, Am. J. Reprod. Immunol. 81 (2019) e13072, https://doi.org/10.1111/aji.13072.

[5] M. Dahl, T.V.F. Hviid, Human leucocyte antigen class Ib molecules in pregnancy success and early pregnancy loss, Hum. Reprod. Update 18 (2012) 92–109, https://doi.org/10.1093/humupd/dmr043.

[6] M.Á. Martínez-Zamora, R. Cervera, J. Balasch, Recurrent miscarriage, antiphospholipid antibodies and the risk of thromboembolic disease, Clin. Rev. Allergy Immunol. 43 (2012) 265–274, https://doi.org/10.1007/s12016-012-8316-0.

[7] G. Teklenburg, M. Salker, C. Heijnen, N.S. Macklon, J.J. Brosens, The molecular basis of recurrent pregnancy loss: impaired natural embryo selection, Mol. Hum. Reprod. 16 (2010) 886–895, https://doi.org/10.1093/molehr/gaq079.

[8] Y. Cui, W. Wang, N. Dong, J. Lou, D.K. Srinivasan, W. Cheng, X. Huang, M. Liu, C. Fang, J. Peng, S. Chen, S. Wu, Z. Liu, L. Dong, Y. Zhou, Q. Wu, Role of corin in trophoblast invasion and uterine spiral artery remodelling in pregnancy, Nature 484 (2012) 246–250, https://doi.org/10.1038/nature10897.

[9] J. Hustin, E. Jauniaux, J.P. Schaaps, Histological study of the materno-embryonic interface in spontaneous abortion, Placenta 11 (1990) 477–486, https://doi.org/10.1016/s0143-4004(05)80193-6.

[10] T.Y. Khong, H.S. Liddell, W.B. Robertson, Defective haemochorial placentation as a cause of miscarriage; a preliminary study, BJOG An Int. J. Obstet. Gynaecol. 94 (1987) 649–655, https://doi.org/10.1111/j.1471-0528.1987.tb03169.x.

[11] R. Romero, J.P. Kusanovic, T. Chaiworapongsa, S.S. Hassan, Placental bed disorders in preterm labor, preterm PROM, spontaneous abortion and abruptio placentae, Best Pract. Res. Clin. Obstet. Gynaecol. 25 (2011) 313–327, https://doi.org/10.1016/j.bpobgyn.2011.02.006.

[12] J. Li, L. Wang, J. Ding, Y. Cheng, L. Diao, L. Li, Y. Zhang, T. Yin, Multiomics studies investigating recurrent pregnancy loss: an effective tool for mechanism exploration, Front. Immunol. 13 (2022) 826198, https://doi.org/10.3389/fimmu.2022.826198.

[13] J.-H. Tsai, M.M.-Y. Chi, M.B. Schulte, K.H. Moley, The fatty acid beta-oxidation pathway is important for decidualization of endometrial stromal cells in both humans and Mice1, Biol. Reprod. 90 (2014), https://doi.org/10.1095/biolreprod.113.113217.

[14] B. Wang, L. Wu, J. Chen, L. Dong, C. Chen, Z. Wen, J. Hu, I. Fleming, D.W. Wang, Metabolism pathways of arachidonic acids: mechanisms and potential therapeutic targets, Signal Transduct. Targeted Ther. 6 (2021) 94, https://doi.org/10.1038/s41392-020-00443-w.

[15] K. Li, X. Zhang, G. Chen, L. Pei, H. Xiao, J. Jiang, J. Li, X. Zheng, D. Li, Association of fatty acids and lipids metabolism in placenta with early spontaneous pregnancy loss in Chinese women, Food Funct. 9 (2018) 1179–1186, https://doi.org/10.1039/C7FO01545C.

[16] P.C. Calder, n-3 polyunsaturated fatty acids, inflammation, and inflammatory diseases, Am. J. Clin. Nutr. 83 (2006) 1505S–1519S, https://doi.org/10.1093/ajcn/83.6.1505S.

[17] K.I. Williams, G.A. Higgs, Eicosanoids and inflammation, J. Pathol. 156 (1988) 101–110, https://doi.org/10.1002/path.1711560204.

[18] J.S. Cuffe, Z.C. Xu, A.V. Perkins, Biomarkers of oxidative stress in pregnancy complications, Biomarkers Med. 11 (2017) 295–306, https://doi.org/10.2217/bmm-2016-0250.

[19] N. Yu, J. Yang, Y. Guo, J. Fang, T. Yin, J. Luo, X. Li, W. Li, Q. Zhao, Y. Zou, W. Xu, Intrauterine administration of peripheral blood mononuclear cells (PBMCs) improves endometrial receptivity in mice with embryonic implantation dysfunction, Am. J. Reprod. Immunol. N. Y. N 71 (2014) 24–33, https://doi.org/10.1111/aji.12150, 1989.

[20] A. Islam, Y. Kagawa, K. Sharifi, M. Ebrahimi, H. Miyazaki, Y. Yasumoto, S. Kawamura, Y. Yamamoto, S. Sakaguti, T. Sawada, N. Tokuda, N. Sugino, R. Suzuki, Y. Owada, Fatty acid binding protein 3 is involved in n–3 and n–6 PUFA transport in mouse trophoblasts, J. Nutr. 144 (2014) 1509–1516, https://doi.org/10.3945/jn.114.197202.

[21] I. Cetin, F. Parisi, C. Berti, C. Mandò, G. Desoye, Placental fatty acid transport in maternal obesity, J. Dev. Orig. Health Dis 3 (2012) 409–414, https://doi.org/10.1017/S2040174412000414.

[22] W. Stremmel, G. Strohmeyer, F. Borchard, S. Kochwa, P.D. Berk, Isolation and partial characterization of a fatty acid binding protein in rat liver plasma membranes, Proc. Natl. Acad. Sci. U.S.A. 82 (1985) 4–8, https://doi.org/10.1073/pnas.82.1.4.

[23] N.A. Abumrad, M.R. el-Maghrabi, E.Z. Amri, E. Lopez, P.A. Grimaldi, Cloning of a rat adipocyte membrane protein implicated in binding or transport of long-chain fatty acids that is induced during preadipocyte differentiation. Homology with human CD36, J. Biol. Chem. 268 (1993) 17665–17668.
[24] D. Hirsch, A. Stahl, H.F. Lodish, A family of fatty acid transporters conserved from mycobacterium to man, Proc. Natl. Acad. Sci. USA 95 (1998) 8625–8629, https://doi.org/10.1073/pnas.95.15.8625.
[25] R.E. Gimeno, A.M. Ortegon, S. Patel, S. Punreddy, P. Ge, Y. Sun, H.F. Lodish, A. Stahl, Characterization of a heart-specific fatty acid transport protein, J. Biol. Chem. 278 (2003) 16039–16044, https://doi.org/10.1074/jbc.M211412200.
[26] L. Zhu, H. Chen, M. Liu, Y. Yuan, Z. Wang, Y. Chen, J. Wei, F. Su, J. Zhang, Treg/Th17 cell imbalance and IL-6 profile in patients with unexplained recurrent spontaneous abortion, Reprod. Sci. 24 (2017) 882–890, https://doi.org/10.1177/1933719116670517.
[27] A.L. Magnusson, I.J. Waterman, M. Wennergren, T. Jansson, T.L. Powell, Triglyceride hydrolase activities and expression of fatty acid binding proteins in the human placenta in pregnancies complicated by intrauterine growth restriction and diabetes, J. Clin. Endocrinol. Metab. 89 (2004) 4607–4614, https://doi.org/10.1210/jc.2003-032234.
[28] K.F. Smart, R.B.M. Aggio, J.R. Van Houtte, S.G. Villas-Bôas, Analytical platform for metabolome analysis of microbial cells using methyl chloroformate derivatization followed by gas chromatography–mass spectrometry, Nat. Protoc. 5 (2010) 1709–1729, https://doi.org/10.1038/nprot.2010.108.
[29] F. Xia, C. He, M. Ren, F.-G. Xu, J.-B. Wan, Quantitative profiling of eicosanoids derived from n-6 and n-3 polyunsaturated fatty acids by twin derivatization strategy combined with LC-MS/MS in patients with type 2 diabetes mellitus, Anal. Chim. Acta 1120 (2020) 24–35, https://doi.org/10.1016/j.aca.2020.04.064.
[30] Y. Wang, A.M. Armando, O. Quehenberger, C. Yan, E.A. Dennis, Comprehensive ultra-performance liquid chromatographic separation and mass spectrometric analysis of eicosanoid metabolites in human samples, J. Chromatogr. A 1359 (2014) 60–69, https://doi.org/10.1016/j.chroma.2014.07.006.
[31] P.-F. Guo, M.-R. Du, H.-X. Wu, Y. Lin, L.-P. Jin, D.-J. Li, Thymic stromal lymphopoietin from trophoblasts induces dendritic cell–mediated regulatory TH2 bias in the decidua during early gestation in humans, Blood 116 (2010) 2061–2069, https://doi.org/10.1182/blood-2009-11-252940.
[32] V. Gómez-Rubio, ggplot2 - elegant graphics for data analysis, J. Stat. Software 77 (2017), https://doi.org/10.18637/jss.v077.b02, 2nd Edition.
[33] A.A. Daak, M.A. Lopez-Toledano, M.M. Heeney, Biochemical and therapeutic effects of Omega-3 fatty acids in sickle cell disease, Compl. Ther. Med. 52 (2020) 102482, https://doi.org/10.1016/j.ctim.2020.102482.
[34] D.C. Wathes, D.R.E. Abayasekara, R.J. Aitken, Polyunsaturated fatty acids in male and female reproduction, Biol. Reprod. 77 (2007) 190–201, https://doi.org/10.1095/biolreprod.107.060558.
[35] I. Cetin, G. Alvino, Intrauterine growth restriction: implications for placental metabolism and transport. A review, Placenta 30 (Suppl A) (2009) S77–S82, https://doi.org/10.1016/j.placenta.2008.12.006.
[36] I. Cetin, N. Giovannini, G. Alvino, C. Agostoni, E. Riva, M. Giovannini, G. Pardi, Intrauterine growth restriction is associated with changes in polyunsaturated fatty acid fetal-maternal relationships, Pediatr. Res. 52 (2002) 750–755, https://doi.org/10.1203/00006450-200211000-00023.
[37] M. Haghiac, X. Yang, L. Presley, S. Smith, S. Dettelback, J. Minium, M.A. Belury, P.M. Catalano, S. Hauguel-de Mouzon, Dietary omega-3 fatty acid supplementation reduces inflammation in obese pregnant women: a randomized double-blind controlled clinical trial, PLoS One 10 (2015) e0137309, https://doi.org/10.1371/journal.pone.0137309.
[38] J. Li, L. Guan, H. Zhang, Y. Gao, J. Sun, X. Gong, D. Li, P. Chen, X. Liang, M. Huang, H. Bi, Endometrium metabolomic profiling reveals potential biomarkers for diagnosis of endometriosis at minimal-mild stages, Reprod. Biol. Endocrinol. 16 (2018) 42, https://doi.org/10.1186/s12958-018-0360-z.
[39] M. Li, Y. Haixia, M. Kang, P. An, X. Wu, H. Dang, X. Xu, The arachidonic acid metabolism mechanism based on UPLC-MS/MS metabolomics in recurrent spontaneous abortion rats, Front. Endocrinol. 12 (2021) 652807, https://doi.org/10.3389/fendo.2021.652807.
[40] E. Aparicio, C. Martín-Grau, C. Hernández-Martinez, N. Voltas, J. Canals, V. Arija, Changes in fatty acid levels (saturated, monounsaturated and polyunsaturated) during pregnancy, BMC Pregnancy Childbirth 21 (2021) 778, https://doi.org/10.1186/s12884-021-04251-0.
[41] T. Nonogaki, Y. Noda, Y. Goto, J. Kishi, T. Mori, Developmental blockage of mouse embryos caused by fatty acids, J. Assist. Reprod. Genet. 11 (1994) 482–488, https://doi.org/10.1007/BF02215713.
[42] J. Stanhiser, A.M.Z. Jukic, A.Z. Steiner, Serum omega-3 and omega-6 fatty acid concentrations and natural fertility, Hum. Reprod. 35 (2020) 950–957, https://doi.org/10.1093/humrep/dez305.
[43] A. Islam, Y. Kagawa, K. Sharifi, M. Ebrahimi, H. Miyazaki, Y. Yasumoto, S. Kawamura, Y. Yamamoto, S. Sakaguti, T. Sawada, N. Tokuda, N. Sugino, R. Suzuki, Y. Owada, Fatty acid binding protein 3 is involved in n–3 and n–6 PUFA transport in mouse trophoblasts, J. Nutr. 144 (2014) 1509–1516, https://doi.org/10.3945/jn.114.197202.
[44] I. Cetin, F. Parisi, C. Berti, C. Mandò, G. Desoye, Placental fatty acid transport in maternal obesity, J. Dev. Orig. Health Dis 3 (2012) 409–414, https://doi.org/10.1017/S2040174412000414.
[45] F.M. Campbell, M.J. Gordon, A.K. Dutta-Roy, Preferential uptake of long chain polyunsaturated fatty acids by isolated human placental membranes, Mol. Cell. Biochem. 155 (1996) 77–83, https://doi.org/10.1007/BF00714336.
[46] F.M. Campbell, P.G. Bush, J.H. Veerkamp, A.K. Dutta-Roy, Detection and cellular localization of plasma membrane-associated and cytoplasmic fatty acid-binding proteins in human placenta, Placenta 19 (1998) 409–415, https://doi.org/10.1016/S0143-4004(98)90081-9.
[47] D.P.Y. Koonen, J.F.C. Glatz, A. Bonen, J.J.F.P. Luiken, Long-chain fatty acid uptake and FAT/CD36 translocation in heart and skeletal muscle, Biochim. Biophys. Acta 1736 (2005) 163–180, https://doi.org/10.1016/j.bbalip.2005.08.018.
[48] I.J. Goldberg, R.H. Eckel, N.A. Abumrad, Regulation of fatty acid uptake into tissues: lipoprotein lipase- and CD36-mediated pathways, J. Lipid Res. 50 (2009) S86–S90, https://doi.org/10.1194/jlr.R800085-JLR200.
[49] G. Endemann, L.W. Stanton, K.S. Madden, C.M. Bryant, R.T. White, A.A. Protter, CD36 is a receptor for oxidized low density lipoprotein, J. Biol. Chem. 268 (1993) 11811–11816.
[50] X. Feng, Y. Jiang, P. Meltzer, P.M. Yen, Thyroid hormone regulation of hepatic genes in vivo detected by complementary DNA microarray, Mol. Endocrinol. 14 (2000) 947–955, https://doi.org/10.1210/mend.14.7.0470.
[51] L. Tian, S.S. Dong, J. Hu, J.J. Yao, P.S. Yan, The effect of maternal obesity on fatty acid transporter expression and lipid metabolism in the full-term placenta of lean breed swine, J. Anim. Physiol. Anim. Nutr. 102 (2018), https://doi.org/10.1111/jpn.12735.
[52] J. Smith, X. Su, R. El-Maghrabi, P.D. Stahl, N.A. Abumrad, Opposite regulation of CD36 ubiquitination by fatty acids and insulin, J. Biol. Chem. 283 (2008) 13578–13585, https://doi.org/10.1074/jbc.M800008200.
[53] M. Miranda, A. Sorkin, Regulation of receptors and transporters by ubiquitination: new insights into surprisingly similar mechanisms, Mol. Interv. 7 (2007) 157–167, https://doi.org/10.1124/mi.7.3.7.
[54] T. Hanhoff, C. Lücke, F. Spener, Insights into binding of fatty acids by fatty acid binding proteins, Mol. Cell. Biochem. 239 (2002) 45–54.
[55] G. Daoud, L. Simoneau, A. Masse, E. Rassart, J. Lafond, Expression of cFABP and PPAR in trophoblast cells: effect of PPAR ligands on linoleic acid uptake and differentiation, Biochim. Biophys. Acta BBA - Mol. Cell Biol. Lipids 1687 (2005) 181–194, https://doi.org/10.1016/j.bbalip.2004.11.017.
[56] A. Franczak, G. Kotwica, B. Kurowicka, A. Oponowicz, I. Wocławek-Potocka, B.K. Petroff, Expression of enzymes of cyclooxygenase pathway and secretion of prostaglandin E2 and F2α by porcine myometrium during luteolysis and early pregnancy, Theriogenology 66 (2006) 1049–1056, https://doi.org/10.1016/j.theriogenology.2006.03.001.
[57] F. Hertelendy, T. Zakár, Prostaglandins and the myometrium and cervix, prostaglandins leukot. Essent. Fatty, Acids 70 (2004) 207–222, https://doi.org/10.1016/j.plefa.2003.04.009.
[58] S.M. Innis, Essential fatty acid transfer and fetal development, Placenta 26 (2005) S70–S75, https://doi.org/10.1016/j.placenta.2005.01.005.
[59] M. Li, Y. Haixia, M. Kang, P. An, X. Wu, H. Dang, X. Xu, The arachidonic acid metabolism mechanism based on UPLC-MS/MS metabolomics in recurrent spontaneous abortion rats, Front. Endocrinol. 12 (2021) 652807, https://doi.org/10.3389/fendo.2021.652807.
[60] G. Liggins, T. Wilson, Phospholipases in the control of human parturition, Am. J. Perinatol. 6 (1989) 153–158, https://doi.org/10.1055/s-2007-999567.
[61] K.F. Breuel, P.E. Lewis, F.N. Schrick, A.W. Lishman, E.K. Inskeep, R.L. Butcher, Factors affecting fertility in the postpartum cow: role of the oocyte and follicle in conception Rate1, Biol. Reprod. 48 (1993) 655–661, https://doi.org/10.1095/biolreprod48.3.655.

[62] F.N. Scenna, J.L. Edwards, N.R. Rohrbach, M.E. Hockett, A.M. Saxton, F.N. Schrick, Detrimental effects of prostaglandin F2α on preimplantation bovine embryos, Prostag. Other Lipid Mediat. 73 (2004) 215–226, https://doi.org/10.1016/j.prostaglandins.2004.02.001.
[63] A. Zarei, M. Mahboubi, M.E. Parsanezhad, S. Alborzi, M. Younesi, G. Madadi, Effects of piroxicam administration on pregnancy outcome in intrauterine insemination (IUI) cycles: a randomized clinical trial, Clin. Exp. Obstet. Gynecol. 43 (2016) 225–229.
[64] M. Vogt, A.W. Sallum, J.G. Cecatti, S.S. Morais, Periodontal disease and some adverse perinatal outcomes in a cohort of low risk pregnant women, Reprod. Health 7 (2010) 29, https://doi.org/10.1186/1742-4755-7-29.
[65] T. Ohnishi, M. Muroi, K. Tanamoto, The lipopolysaccharide-recognition mechanism in cells expressing TLR4 and CD14 but lacking MD-2, FEMS Immunol. Med. Microbiol. 51 (2007) 84–91, https://doi.org/10.1111/j.1574-695X.2007.00281.x.
[66] X.-J. Yin, W. Hong, F.-J. Tian, X.-C. Li, Proteomic analysis of decidua in patients with recurrent pregnancy loss (RPL) reveals mitochondrial oxidative stress dysfunction, Clin. Proteonomics 18 (2021) 9, https://doi.org/10.1186/s12014-021-09312-2.
[67] J. Wu, A.D. Lu, L.P. Zhang, Y.X. Zuo, Y.P. Jia, [Study of clinical outcome and prognosis in pediatric core binding factor-acute myeloid leukemia, Zhonghua Xue Ye Xue Za Zhi Zhonghua Xueyexue Zazhi 40 (2019) 52–57, https://doi.org/10.3760/cma.j.issn.0253-2727.2019.01.010.
[68] J. Ding, T. Yin, N. Yan, Y. Cheng, J. Yang, FasL on decidual macrophages mediates trophoblast apoptosis: a potential cause of recurrent miscarriage, Int. J. Mol. Med. (2019), https://doi.org/10.3892/ijmm.2019.4146.
[69] C. Bi, Y. Fu, Z. Zhang, B. Li, Prostaglandin E2 confers protection against diabetic coronary atherosclerosis by stimulating M2 macrophage polarization via the activation of the CREB/BDNF/TrkB signaling pathway, Faseb. J. 34 (2020) 7360–7371, https://doi.org/10.1096/fj.201902055R.
[70] R.L. Simões, N.M. De-Brito, H. Cunha-Costa, V. Morandi, I.M. Fierro, I.M. Roitt, C. Barja-Fidalgo, Lipoxin A 4 selectively programs the profile of M2 tumor-associated macrophages which favour control of tumor progression, Int. J. Cancer 140 (2017) 346–357, https://doi.org/10.1002/ijc.30424.
[71] J. Dalli, M. Zhu, N.A. Vlasenko, B. Deng, J.Z. Haeggström, N.A. Petasis, C.N. Serhan, The novel 13 S ,14 S -epoxy-maresin is converted by human macrophages to maresin 1 (MaR1), inhibits leukotriene A 4 hydrolase (LTA 4 H), and shifts macrophage phenotype, Faseb. J. 27 (2013) 2573–2583, https://doi.org/10.1096/fj.13-227728.
[72] A.J. Nelson, D.J. Stephenson, C.L. Cardona, X. Lei, A. Almutairi, T.D. White, Y.G. Tusing, M.A. Park, S.E. Barbour, C.E. Chalfant, S. Ramanadham, Macrophage polarization is linked to Ca2+-independent phospholipase A2β-derived lipids and cross-cell signaling in mice, J. Lipid Res. 61 (2020) 143–158, https://doi.org/10.1194/jlr.RA119000281.
[73] R. Natarajan, J.L. Nadler, Lipid inflammatory mediators in diabetic vascular disease, Arterioscler. Thromb. Vasc. Biol. 24 (2004) 1542–1548, https://doi.org/10.1161/01.ATV.0000133606.69732.4c.
[74] Y. Huo, L. Zhao, M.C. Hyman, P. Shashkin, B.L. Harry, T. Burcin, S.B. Forlow, M.A. Stark, D.F. Smith, S. Clarke, S. Srinivasan, C.C. Hedrick, D. Praticò, J. L. Witztum, J.L. Nadler, C.D. Funk, K. Ley, Critical role of macrophage 12/15-lipoxygenase for atherosclerosis in apolipoprotein E-deficient mice, Circulation 110 (2004) 2024–2031, https://doi.org/10.1161/01.CIR.0000143628.37680.F6.
[75] Y. Wen, J. Gu, S.K. Chakrabarti, K. Aylor, J. Marshall, Y. Takahashi, T. Yoshimoto, J.L. Nadler, The role of 12/15-lipoxygenase in the expression of interleukin-6 and tumor necrosis factor-alpha in macrophages, Endocrinology 148 (2007) 1313–1322, https://doi.org/10.1210/en.2006-0665.