UNIVERSIDADE FEDERAL DE MINAS GERAIS
FACULDADE DE FARMÁCIA

LUIZA OLIVEIRA PERUCCI

IMUNORREGULAÇÃO NA PRÉ-ECLÂMPSIA: ESTUDO DE MEDIADORES INFLAMATÓRIOS E PRÓ-RESOLUTIVOS

Belo Horizonte
2016

LUIZA OLIVEIRA PERUCCI

IMUNORREGULAÇÃO NA PRÉ-ECLÂMPSIA: ESTUDO DE MEDIADORES INFLAMATÓRIOS E PRÓ-RESOLUTIVOS

Tese apresentada ao Programa de Pós-Graduação em Análises Clínicas e Toxicológicas da Faculdade de Farmácia da Universidade Federal de Minas Gerais, como requisito parcial à obtenção de título de Doutora em Análises Clínicas e Toxicológicas.

Área de concentração: Análises Clínicas e Toxicológicas.

Orientadora: Profa. Dra. Lirlândia Pires de Sousa
Co-orientadoras: Profa. Dra. Karina Braga Gomes Borges e Profa. Dra. Luci Maria Sant'Ana Dusse

Belo Horizonte
2016

Perucci, Luiza Oliveira.
P471i Imunorregulação na pré-eclâmpsia: estudo de mediadores inflamatórios e pró-resolutivos / Luiza Oliveira Perucci. – 2016.

197 f. : il.

Orientadora: Lirlândia Pires de Sousa.

Co-orientadoras: Karina Braga Gomes Borges e Luci Maria Sant'Ana Dusse.

Tese (doutorado) – Universidade Federal de Minas Gerais, Faculdade de Farmácia, Programa de Pós-Graduação em Análises Clínicas e Toxicológicas.

1. Gravidez – Complicações – Teses. 2. Pré-eclampsia – Teses. 3. Inflamação – Teses. 4. Anexina A1 (AnxA1) – Teses. 5. Lipoxina A4 (LXA4) – Teses. 6. Proteína C reativa (PCR) – Teses. 7. Fator neurotrófico derivado do cérebro (BDNF) – Teses. I. Sousa, Lirlândia Pires de. II. Borges, Karina Braga Gomes. III. Dusse, Luci Maria Sant'Ana. IV. Universidade Federal de Minas Gerais. Faculdade de Farmácia. V. Título.

CDD: 618.36132

UNIVERSIDADE FEDERAL DE MINAS GERAIS

PROGRAMA DE PÓS-GRADUAÇÃO EM ANÁLISES CLÍNICAS E TOXICOLÓGICAS

UFMG

FOLHA DE APROVAÇÃO

IMUNORREGULAÇÃO NA PRÉ-ECLÂMPSIA: ESTUDO DE MEDIADORES INFLAMATÓRIOS E PRÓ-RESOLUTIVOS

LUIZA OLIVEIRA PERUCCI

Tese submetida à Banca Examinadora designada pelo Colegiado do Programa de Pós-Graduação em ANÁLISES CLÍNICAS E TOXICOLÓGICAS, como requisito para obtenção do grau de Doutor em ANÁLISES CLÍNICAS E TOXICOLÓGICAS, área de concentração ANÁLISES CLÍNICAS E TOXICOLÓGICAS.

Aprovada em 21 de outubro de 2016, pela banca constituída pelos membros:

Profa. Lirlândia Pires de Sousa - Orientadora
UFMG

Profa. Karina Braga Gomes Borges - Coorientadora
UFMG

Profa. Luci Maria Sant Ana Dusse - Coorientadora
UFMG

Profa. Eura Martins Lage
UFMG

Prof. Caio Tavares Fagundes
Universidade Federal de Minas Gerais

Profa. Danyelle Romana Alves Rios
UFSJ

Prof. Andre Talvani Pedrosa da Silva
UFOP

Belo Horizonte, 21 de outubro de 2016.

DEDICATÓRIA

Dedico este trabalho ao meu pai (*in memoriam*) que sempre valorizou os estudos e se esforçou para me proporcionar uma base acadêmica sólida.

AGRADECIMENTOS

À Deus por ter me abençoado com saúde, um lar estruturado e diversas oportunidades de crescimento acadêmico e profissional.

À minha mãe e irmã pelo apoio e amor incondicionais.

Ao Heron pelo companheirismo e por me tranquilizar nos momentos difíceis.

Aos integrantes da república Casinha da Vovó por terem me acolhido tão bem durante o final do meu doutorado.

Aos demais familiares e aos amigos pela torcida.

Às professoras Lirlândia Pires de Sousa, Luci Maria Sant'Anna Dusse e Karina Braga Gomes Borges, pela orientação, exemplo e paciência. Agradeço, em especial, por apoiarem o meu crescimento profissional.

Aos atuais coordenadores e ex-coordenadores do curso de Pós-Graduação em Análises Clínicas e Toxicológicas, professores Carlos Alberto Tagliati, Tânia Mara Pinto Dabés Guimarães e Vicente de Paulo Coelho Peixoto de Toledo; à funcionária Andréia e à ex-funcionária Ângela da Secretaria de Pós-Graduação.

Às participantes do estudo e às instituições colaboradoras.

Aos alunos, em especial à Fernanda Silva Carneiro, aos técnicos e aos professores do Laboratório de Sinalização na Inflamação da Faculdade de Farmácia da UFMG e do Laboratório Interdisciplinar de Investigação Médica da Faculdade de Medicina da UFMG.

Aos professores Ana Cristina Simões e Silva, Antônio Lúcio Teixeira, Mário Dias Corrêa e Regina Amélia Lopes Pessoa de Aguiar pela colaboração e incentivo.

À Universidade Federal de Ouro Preto pelo incentivo à minha qualificação.

Às agências financiadoras CAPES, FAPEMIG e CNPq.

RESUMO

A pré-eclâmpsia (PE) é uma doença caracterizada por uma resposta inflamatória materna excessiva. A anexina A1 (AnxA1) e a lipoxina A4 (LXA4) são moléculas anti-inflamatórias e pró-resolutivas, enquanto a proteína C reativa (PCR) e o receptor solúvel 1 do fator de necrose tumoral alfa (sTNF-R1) são marcadores pró-inflamatórios. Acredita-se que o fator neurotrófico derivado do cérebro (BDNF) também module a inflamação. Apesar dos mecanismos pró-inflamatórios terem sido extensivamente estudados nas últimas décadas, pouco se sabe sobre os mecanismos pró-resolutivos na PE. O estudo de moléculas pró-resolutivas na PE é importante para o melhor entendimento da sua etiopatogênese e para propor novos tratamentos farmacológicos para essa doença, cujo único tratamento efetivo é a realização do parto e a retirada completa da placenta. O objetivo principal deste estudo foi investigar os níveis circulantes de AnxA1, LXA4, PCR e BDNF na PE. Este estudo incluiu 133 mulheres, sendo: 41 não-gestantes, 39 gestantes normotensas e 53 gestantes com PE. Os níveis plasmáticos de AnxA1, LXA4 e BDNF foram dosados por ELISA, e os níveis de PCR por ensaio imunoturbidimétrico ultrassensível. A quantificação relativa da expressão do mRNA de AnxA1 em células mononucleares do sangue periférico (PBMCs) foi realizada por PCR em tempo real. As correlações entre os níveis dessas moléculas, do sTNF-R1 (avaliado em um estudo anterior), as características clínicas e os parâmetros laboratoriais das participantes também foram investigadas. Os níveis de AnxA1, LXA4 e PCR estavam aumentados nas gestantes com PE em comparação às mulheres não-gestantes. As gestantes com PE apresentaram níveis aumentados de AnxA1 e LXA4, níveis diminuídos de BDNF e níveis semelhantes de PCR em comparação às gestantes normotensas. Os níveis de PCR estavam mais elevados nas gestantes normotensas do que nas mulheres não-gestantes, porém não houve diferença em relação aos níveis de AnxA1 e LXA4. Além disso, os níveis de mRNA de AnxA1 em PBMCs foram similares nos três grupos. Correlações positivas foram detectadas entre AnxA1 e sTNF-R1, LXA4 e PCR, LXA4 e pressão arterial sistólica, LXA4 e pressão arterial diastólica, BDNF e pressão arterial diastólica, LXA4 e contagem global de leucócitos, BDNF e índice de massa corporal antes da gestação; e negativa entre BDNF e AnxA1. Conclui-se com esse estudo que os níveis plasmáticos aumentados de AnxA1 e LXA4 coincidem com um fenótipo pró-inflamatório em gestantes com PE, sugerindo possíveis falhas nos mecanismos

contra-regulatórios da resposta inflamatória. Níveis diminuídos de BDNF também podem contribuir para o controle inadequado da inflamação na doença.
Palavras-chave: Pré-eclâmpsia; inflamação; AnxA1; LXA4; PCR; BDNF.

ABSTRACT

Preeclampsia (PE) is a disease characterized by excessive maternal inflammatory response. Annexin A1 (AnxA1) and lipoxin A4 (LXA4) are anti-inflammatory and pro-resolving molecules, while C reactive protein (CRP) and soluble tumor necrosis factor alpha receptor 1(sTNF-R1) are pro-inflammatory markers. Brain-derived neurotrophic factor (BDNF) is also thought to modulate inflammation. Although the pro-inflammatory mechanisms have been extensively studied in the last decades, the pro-resolving mechanisms are poorly understood in PE. The study of pro-resolving molecules in PE is important to better understand its etiopathogenesis and to propose new pharmacological treatments for this disease, which delivery and complete removal of the placenta is the only effective treatment. The main objective of this study was to investigate the circulating levels of AnxA1, LXA4, CRP e BDNF in PE. This study included 133 women, as follows: 41 non-pregnant, 39 normotensive pregnant and 53 PE. AnxA1, LXA4 and BDNF plasma levels were measured by ELISA, and CRP levels by immunoturbidimetric assay. The relative quantification of AnxA1 mRNA expression in peripheral mononuclear cells (PBMCs) was performed using real time PCR. Correlation analyzes among the circulating levels of these molecules, sTNF-R1 (evaluated in a previous study), the clinical characteristics and the laboratory parameters of the participants were also investigated. AnxA1, LXA4 and CRP levels were higher in PE women when compared with non-pregnant women. PE women had higher levels of AnxA1 and LXA4, decreased levels of BDNF and similar levels of CRP when compared with normotensive pregnant women. CRP levels were higher in normotensive pregnant women than in non-pregnant women, but there was no difference regarding AnxA1 and LXA4 levels. Moreover, AnxA1 mRNA levels in PBMCs were similar in the three groups. Positive correlations were detected between AnxA1 and sTNF-R1, LXA4 and CRP, LXA4 and systolic blood pressure, LXA4 and diastolic blood pressure, BDNF and diastolic blood pressure, LXA4 and white blood cell count, BDNF and pre-pregnancy body mass index; and negative between BDNF and AnxA1. In conclusion, AnxA1 and LXA4 increased plasma levels coincide with a pro-inflammatory phenotype in PE women, suggesting possible failures in counter-regulatory mechanisms of the inflammatory response. BDNF decreased levels may also contribute to the inadequate regulation of inflammation in the disease.

Keywords: Preeclampsia; inflammation; AnxA1; LXA4; CRP; BDNF.

LISTA DE FIGURAS

LISTA DE ABREVIATURAS E SIGLAS

AAS	Ácido acetilsalicílico
ACOG	Colégio Americano de Obstetrícia e Ginecologia
ANOVA	Análise de variância
AnxA1	Anexina A1
AT_1	Receptor tipo 1 da angiotensina II
BDNF	Fator neurotrófico derivado do cérebro
Ca	Cálcio
cDNA	DNA complementar
CIUR	Crescimento intra-uterino retardado
Cols.	Colaboradores
COX	Ciclooxigenase
DAMP	Padrão molecular associado ao dano
DMSO	Dimetilsulfóxido
EDTA	Ácido etilenodiamino tetra-acético
ELISA	Ensaioimunoenzimático
FPR2	Receptor de peptídeo formilado 2
GAPDH	Gliceraldeído-3-fosfato desidrogenase
GC	Glicocorticóide
HELLP	Hemólise, níveis elevados de enzimas hepáticas e diminuição do número de plaquetas
HETE	Ácido hidroxieicosatetraenóico
HpETE	Ácido hidroperoxieicosatetraenóico
i.e.	Isto é
IG	Idade gestacional
IL	Interleucina
ILCs	Células linfóides inatas
IMC	Índice de massa corporal
KDa	Quilodalton
LOX	Lipoxigenase
LPS	Lipopolissacarídeo
LSD	*Least Significant Difference*
LT	Leucotrieno

LX	Lipoxina
mmHg	Milímetros de mercúrio
Mo.	Monócitos
Mres	Macrófago resolutivo
mRNA	RNA mensageiro
n	Tamanho amostral
Nº	Número
NK	*Natural Killer*
oxoETE	Ácido oxoeicosatetraenóico
PA	Pressão arterial
PBMCs	Células mononucleares do sangue periférico
PCR	Proteína C reativa/ Reação em cadeia da polimerase
PE	Pré-eclâmpsia/pré-eclâmptica
p.ex.	Por exemplo
PG	Prostaglandina
PLA2	Fosfolipase A2
PlGF	Fator de crescimento placentário
PMN	Polimorfonuclear
RNA	Ácido ribonucléico
ROS	Espécies reativas de oxigênio
RPMI	*Roswell Park Memorial Institute medium*
R_s	Coeficiente de correlação de *Spearman*
SFB	Soro fetal bovino
SPSS	*Statistical Package for Social Science*
sTNF-R1	Receptor solúvel 1 do TNF-α
sVEGFR-1	Receptor solúvel 1 do VEGF
Th	(Linfócitos) T auxiliares
TNF-α	Fator de necrose tumoral alfa
Treg	(Linfócitos) T reguladores
TrkB	Receptor tropomiosina cinase B
TX	Tromboxano
VEGF	Fator de crescimento do endotélio vascular

LISTA DE NOTAÇÕES OU SÍMBOLOS

α	Alfa
β	Beta
/	e, ou
ºC	Graus centígrados
>	Maior
≥	Maior ou igual
<	Menor
≤	Menor ou igual
%	Porcentagem
+	Positivo/cruz

SUMÁRIO

1 INTRODUÇÃO

1.1 Pré-eclâmpsia

As doenças hipertensivas da gestação acometem 5 a 10% das gestantes e são responsáveis por elevada morbi/mortalidade materna e fetal (Hutcheon *et al.*, 2011). De acordo com o Colégio Americano de Obstetrícia e Ginecologia (ACOG), esse grupo de doenças é composto pela hipertensão arterial crônica, pré-eclâmpsia (PE)/eclâmpsia, PE sobreposta à hipertensão arterial crônica e hipertensão gestacional (*Report of the National High Blood Pressure Education Program Working Group on High Blood Pressure in Pregnancy*, 2000). A PE é uma doença multissistêmica que, na sua forma pura, caracteriza-se pelo aparecimento de hipertensão e proteinúria após a vigésima semana de gestação em mulheres previamente normotensas. Na ausência de proteinúria, o diagnóstico da doença requer a presença de hipertensão associada a evidências de comprometimento sistêmico, como trombocitopenia, insuficiência hepática e renal, edema pulmonar e distúrbios visuais e/ou cerebrais (ACOG, 2013).

A PE tradicionalmente é classificada nas formas leve e grave, de acordo com os níveis pressóricos e de proteinúria (ACOG, 2002). A PE grave cursa com pressão arterial ≥160/100mmHg e sinais de comprometimento sistêmico. O grau de proteinuria foi recentemente retirado dessa classificação por não refletir adequadamente a gravidade da doença (ACOG, 2013). Nos últimos anos, uma classificação baseada na idade gestacional (IG) na qual surgem os sinais e sintomas da doença tem sido amplamente adotada. Dessa forma, a PE vem sendo classificada como precoce, quando os sintomas surgem antes da 34ª semana de gestação e tardia, quando surgem na 34ª semana ou depois (Von Dadelszen *et al.*, 2003). A PE precoce é clinicamente mais grave do que a PE tardia e admite-se que essas formas da doença tenham etiologias distintas (Raymond e Peterson, 2011).

A conduta clínica na PE consiste no controle da hipertensão e na prevenção de convulsões. O monitoramento do número de plaquetas circulantes, das funções hepática e renal e de sinais de hemólise é usualmente

feito. O único tratamento efetivo consiste na interrupção da gestação e na remoção completa da placenta. A adoção dessa medida deve sempre considerar as condições maternas e fetais. Sabe-se que os bebês prematuros têm menor sobrevida e maior morbidade (ACOG, 2013; Mol *et al.*, 2015). No entanto, nos casos graves de PE, como na hipertensão refratária ao tratamento farmacológico, a interrupção da gestação pode ser indicada antes de 34 semanas visando prevenir complicações maternas como a eclâmpsia e a síndrome HELLP (hemólise, níveis elevados de enzimas hepáticas e diminuição do número de plaquetas), e maior sofrimento fetal. Embora a etiologia da PE seja desconhecida, acredita-se que disfunções placentárias exerçam um papel central na sua patogênese (Chaiworapongsa *et al.*, 2014). A **Figura 1** resume o entendimento atual da fisiopatologia da PE.

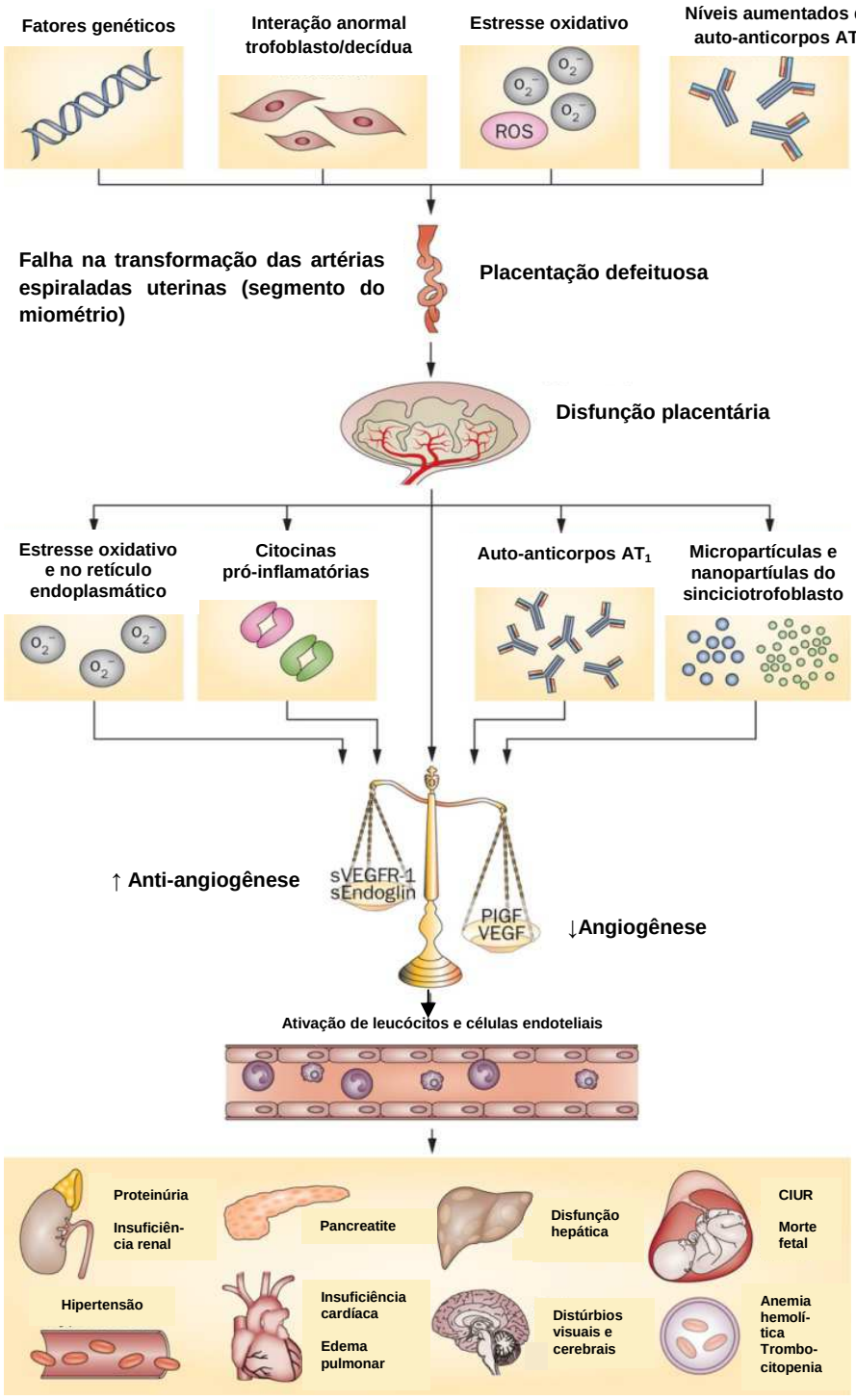


FIGURA 1 - Modelo integrado da fisiopatologia da pré-eclâmpsia. Múltiplos fatores genéticos e ambientais levam à falha na transformação das artérias espiraladas uterinas no início da gestação pré-eclâmptica que resulta em isquemia placentária. A placenta isquêmica libera diversos fatores solúveis para a circulação materna, como espécies reativas de oxigênio, citocinas pró-inflamatórias, auto-anticorpos AT1 e micropartículas/nanopartículas do sinciciotrofoblasto, os quais causam disfunção endotelial, devido a um aumento de moléculas anti-angiogênicas (p.ex.: sVEGFR-1 e endoglina solúvel - *sEndoglin*) e uma diminuição de moléculas pró-angiogênicas (p.ex.: PlGF e VEGF), além de inflamação intravascular e hipercoagulabilidade. Esses mecanismos, em conjunto, resultam em um comprometimento multissistêmico. Abreviações:AT_1, receptor tipo 1 da angiotensina II; CIUR, crescimento intra-uterino retardado; PlGF, fator de crescimento placentário; ROS, espécies reativas de oxigênio; sVEGFR-1, receptor solúvel 1 do VEGF; VEGF, fator de crescimento do endotélio vascular. Fonte: Adaptado de Chaiworapongsa *et al.* (2014).

Até o momento, não existem testes capazes de predizer a ocorrência da PE na prática clínica. No entanto, diversos fatores de risco contribuem para a ocorrência da doença, como: ser primigesta; ter tido PE em gestação anterior; ter história familiar de PE; estar nos extremos da faixa fértil; possuir comorbidades associadas à lesão endotelial, como diabetes mellitus e hipertensão arterial crônica; ter índice de massa corporal (IMC) maior que 30 antes da gestação; e ser de etnia negra (Trogstad *et al.*, 2011). Entretanto, na prática clínica, estes fatores predizem apenas 30% dos casos de PE (Leslie *et al.*, 2011).

Nenhum teste laboratorial é capaz de fornecer o diagnóstico precoce da PE, mas diversos marcadores biofísicos e bioquímicos, como índice de resistência/pulsatilidade das artérias uterinas e moléculas angiogênicas, têm sido propostos como testes de triagem no primeiro trimestre de gestação. No entanto, nenhum deles mostrou um potencial preditivo satisfatório em estudos multicêntricos (Mol *et al.*, 2015).

Como a fisiopatologia da PE não é bem compreendida, a sua prevenção ainda é empírica, incluindo modificações do estilo de vida e o uso de antioxidantes, baixas doses de ácido acetilsalicílico e suplementos de cálcio (Chaiworapongsa *et al.*, 2014).

Mulheres que tiveram PE parecem ter uma maior probabilidade de desenvolver doenças cardiovasculares e renais no futuro, provavelmente devido a fatores de riscos compartilhados entre essas doenças (Brown *et al.*, 2013; Lambert *et al.*, 2014). Por outro lado, mulheres que tiveram PE têm menor chance de desenvolver alguns tipos de câncer, como o de mama. Sugere-se que isso esteja associado a um estado anti-angiogênico persistente após a gestação (Gilbert *et al.*, 2012; Vatten *et al.*, 2009).

1.2 Inflamação

A inflamação é uma resposta fisiológica que pode ser desencadeada por vários estímulos, como infecção, lesão tecidual ou estresse/mau funcionamento tecidual. Dependendo do tipo de estímulo, a resposta inflamatória pode ter diferentes propósitos (p.ex.: *clearance* do patógeno),

porém todos visam à restauração da homeostase tecidual. Uma resposta inflamatória típica consiste em quatro componentes: um indutor ou estímulo inflamatório (p.ex.: lipopolissacarídeo - LPS), sensores que detectam esse indutor (p.ex.: receptores do tipo Toll-4), mediadores inflamatórios induzidos pelos sensores (p.ex.: citocinas, como o fator de necrose tumoral alfa - TNF-α) e o tecidos/órgãos afetados por esses mediadores. A inflamação também envolve componentes vasculares e apresenta como sinais clínicos característicos rubor, calor, edema, dor e perda de função (Medzhitov, 2008; Norling e Serhan, 2010; Sugimoto *et al.*, 2016).

A inflamação possui componentes das imunidades inata e adaptativa. A resposta imune inata é considerada a primeira linha de defesa do organismo contra agentes infecciosos ou estéreis e inclui mecanismos de defesa que estão, em geral, constitutivamente presentes, necessitando ser ativados em alguns casos. A imunidade inata consiste em barreiras físicas (p.ex.: epitélio), químicas (p.ex.: pH ácido estomacal), biológicas (p.ex.: microbiota normal do trato gastrointestinal), humorais (p.ex.: proteínas do sistema do complemento, citocinas) e celulares. As principais células efetoras da imunidade inata são os leucócitos polimorfonucleares (PMN), os macrófagos, as células dendríticas, as células *Natural Killer* (*NK*) e os mastócitos (Abbas, Lichtman e Pillai, 2010; Medzhitov e Janeway, 2000). Recentemente, foi descrita uma nova classe de células efetoras da imunidade inata: as células linfóides inatas (ILCs). As células NK foram as primeiras células descritas dessa família, a qual também é composta por ILCs RORγt-dependentes e por ILCs do tipo 2 (Hwang e Mckenzie, 2013). Os mecanismos da resposta imune inata atuam de forma inespecífica e imediata após detecção de indutores inflamatórios com o objetivo de remover patógenos e/ou *debris* celulares e de apresentar antígenos necessários para ativação e reconhecimento pelas células do sistema imune adaptativo (Abbas, Lichtman e Pillai, 2010; Medzhitov e Janeway, 2000).

Em contraste, a resposta imune adaptativa tem início tardio e maior duração, é específica e especializada, gera memória, e está envolvida no desenvolvimento de tolerância e auto-imunidade. Essa resposta é composta por elementos humorais (p.ex.: anticorpos) e celulares. Os linfócitos são as principais células efetoras da imunidade adaptativa, embora as células *NK*, os

macrófagos e os fibroblastos também exerçam papéis importantes. A resposta imune adaptativa visa à destruição e remoção do agente agressor, a recuperação tecidual e o restabelecimento funcional do tecido ou órgão (Abbas, Lichtman e Pillai, 2010; Medzhitov e Janeway, 2000).

A imunidade inata exerce um papel fundamental no controle de respostas imunes adaptativas. Dessa forma, as citocinas produzidas pelas células da imunidade inata induzem a diferenciação de linfócitos auxiliares (Th) *naïve* em linfócitos Th1, Th2, Th17 ou T reguladores (Treg) (Cosmi *et al.*, 2014; Iwasaki e Medzhitov, 2015). Os elementos da resposta imune inata predominam na fase aguda da inflamação, enquanto a imunidade adaptativa é característica da reposta inflamatória crônica, embora a resposta imune inata também exerça um papel importante na fase crônica (Libby, 2007). A resposta inflamatória aguda se torna crônica se o processo de resolução não ocorrer adequadamente (Nathan e Ding, 2010). A resolução da resposta inflamatória é um processo ativo, contínuo e altamente regulado, o qual é coordenado por células do sistema imunológico e por mediadores de natureza diversa, tais como proteínas, lipídios, gases, inibidores de proteases e neuromoduladores (Headland e Norling, 2015; Odaka *et al.*, 2003; Serhan *et al.*, 2007). Uma série de eventos envolve a resolução bem-sucedida de um processo inflamatório agudo (Alessandri *et al.*, 2013), como exemplificado na **Figura 2.**

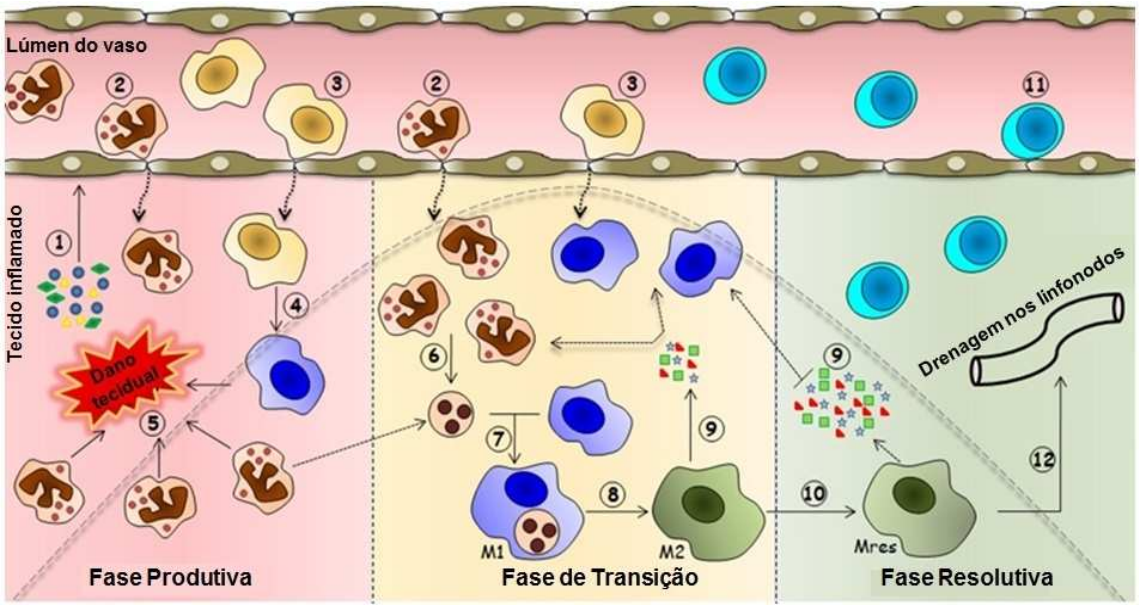


FIGURA 2 - Série orquestrada de eventos que levam à resolução bem-sucedida do processo inflamatório. A lesão tecidual estéril ou infecciosa leva ao reconhecimento de padrões moleculares pelas células residentes, as quais produzem rapidamente vários mediadores pró-inflamatórios (1). Na fase produtiva da inflamação, esses mediadores promovem a vasodilatação, o aumento da permeabilidade vascular e a ativação de células endoteliais, as quais passam a expressar moléculas de adesão e a produzir substâncias quimioatrativas para leucócitos. Os leucócitos PMN, principalmente os neutrófilos, são geralmente as primeiras células a serem recrutadas ao sítio inflamatório (2), seguido pelos monócitos (3). No tecido, os monócitos se transformam macrófagos (4). Com a progressão da resposta inflamatória, há um influxo intenso de leucócitos, os quais produzem diversos mediadores, como fatores de crescimento, citocinas, quimiocinas, mediadores lipídicos e espécies reativas de oxigênio, os quais podem lesar ainda mais o tecido (5). Na fase de transição da resposta inflamatória, mediadores pró-resolutivos induzem a apoptose dos PMN (6) que, em seguida, são fagocitados por macrófagos (eferocitose) (7). Durante a eferocitose, os macrófagos alteram o seu perfil pró-inflamatório (M1) para um perfil anti-inflamatório (M2) (8). Os macrófagos do tipo M2 têm alta capacidade de promover eferocitose e de produzir moléculas anti-inflamatórias e pró-resolutivas (9). Estes macrófagos podem, ainda, ser convertidos para o perfil do tipo resolutivo (Mres) na fase resolutiva da resposta inflamatória (10). Esta fase é caracterizada pela intensa produção de mediadores pró-resolutivos, anti-inflamatórios e anti-fibróticos pelos macrófagos Mres (9), pela repopulação de linfócitos (11) e pela drenagem de macrófagos nos linfonodos (12). Esses eventos levam, por fim, à resolução do processo inflamatório e à restauração da homeostase tecidual. Fonte: Adaptado de Alessandri *et al*. (2013).

Diversas moléculas estão envolvidas no controle da resposta inflamatória após uma lesão tecidual (infecciosa ou estéril), as quais possuem atividades anti-inflamatória e/ou pró-resolutiva, como a anexina A1 (AnxA1) e a lipoxina A4 (LXA4). Nesse sentido, faz-se necessário distinguir esses dois conceitos. Moléculas anti-inflamatórias inibem a fase inicial ou produtiva da resposta inflamatória. Por outro lado, moléculas com atividade pró-resolutiva modulam a resposta inflamatória já estabelecida visando a sua resolução e o retorno da homeostase tecidual (Headland e Norling, 2015; Serhan e Savill, 2005; Sugimoto *et al.*, 2016).

1.2.1 Anexina A1

A superfamília de anexinas em mamíferos é composta por 13 proteínas capazes de se ligar a fosfolípides de membrana com alta afinidade na presença de concentrações fisiológicas de Ca^{2+}(Gerke *et al.*, 2005). Estruturalmente, as anexinas são constituídas por dois domínios: uma extremidade N-terminal variável e uma extremidade carboxílica com maior grau de conservação entre os membros da superfamília (Gerke e Moss, 2002). A AnxA1 (37KDa), previamente identificada como lipocortina 1, foi a primeira proteína caracterizada desta superfamília (Flower e Blackwell, 1979).

A AnxA1 é altamente expressa em células da linhagem hematopoiética e a diferenciação celular, por exemplo de monócitos para macrófagos, está associada a uma maior expressão da proteína (Fava *et al.*, 1989; Gerke e Moss, 2002; Perretti e Flower, 1996). Os leucócitos PMN contêm grande quantidade de AnxA1, a qual representa 1-4% do seu conteúdo citoplasmático total. A AnxA1 é expressa em menor quantidade em mastócitos e linfócitos T, sendo a expressão nos linfócitos TCD4+ ligeiramente maior do que nos linfócitos TCD8+. Os linfócitos B e as plaquetas não expressam a proteína (Ernst *et al.*, 1990; Morand *et al.*, 1995; Spurr *et al.*, 2011). Em condições de repouso, a AnxA1 está localizada no citoplasma, porém a sua sublocalização depende do tipo celular. Por exemplo, a proteína está contida em grânulos de gelatinase dos neutrófilos e em grânulos α dos mastócitos (Oliani *et al.*, 2000; Perretti *et al.*, 2000). Após a ativação celular, por exemplo, através da adesão de leucócitos no endotélio vascular, a AnxA1 é prontamente mobilizada para a superfície celular, sendo em seguida secretada (Perretti *et al.*, 2000).

Os glicocorticóides (GCs), fármacos sintéticos análogos do hormônio endógeno cortisol, são utilizados com sucesso há várias décadas no tratamento de diversas doenças inflamatórias (Coutinho e Chapman, 2011). A AnxA1 foi descrita inicialmente como uma proteína induzida por GCs capaz de mediar alguns dos seus efeitos anti-inflamatórios, inicialmente pela inibição da enzima fosfolipase A2 (PLA2) (Flower e Blackwell, 1979). A inibição da atividade da PLA2 tem como consequência a inibição da formação de ácido araquidônico, com efeitos inibitórios sobre a geração de mediadores lipídicos

pró-inflamatórios, como prostaglandinas e leucotrienos **(Figura 3)** (Murakami e Kudo, 2002). A AnxA1 também inibe a expressão da enzima ciclooxigenase 2 (COX-2), o recrutamento de neutrófilos e a geração de mediadores pró-inflamatórios nos sítios inflamatórios, limitando, assim, a fase inicial (produtiva) da resposta inflamatória. Já foi demonstrado que AnxA1 também atua na fase resolutiva da inflamação, ao induzir a apoptose de neutrófilos e a eferocitose de neutrófilos apoptóticos por macrófagos (Perretti e D'acquisto, 2009). Recentemente, o nosso grupo de pesquisa demonstrou o participação da AnxA1 na resolução da resposta inflamatória natural e induzida pelo GC dexametasona em um modelo de pleurisia induzida por LPS (Vago *et al.*, 2012). A AnxA1 medeia os seus efeitos anti-inflamatórios e pró-resolutivos ao se ligar a um receptor transmembranar acoplado à proteína G denominado receptor de peptídeo formilado 2 (FPR2; também conhecido como ALX ou FPRL1 em humanos), o qual também se liga à LXA4 e à resolvina D1 (Perretti *et al.*, 2002).

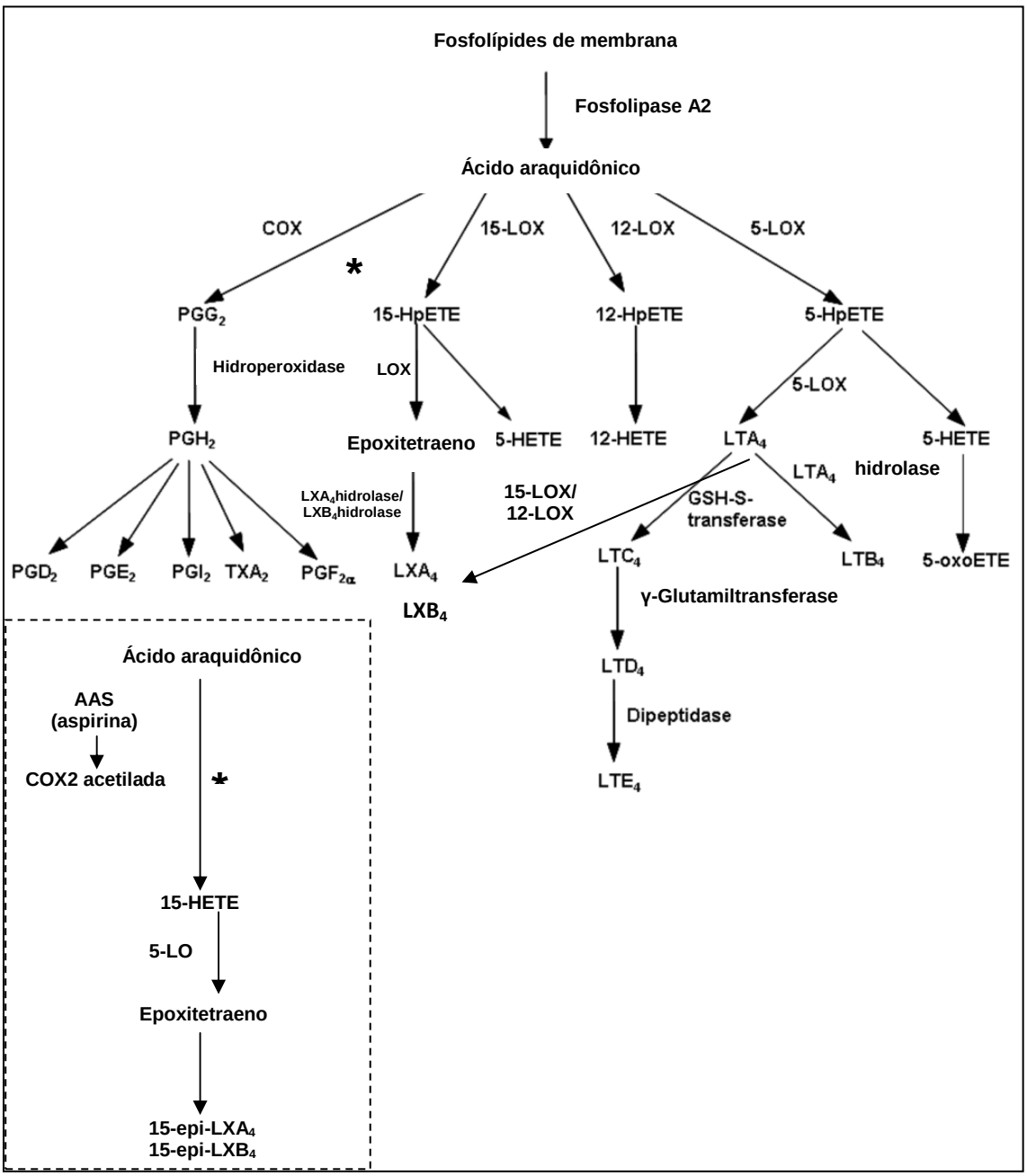


FIGURA 3 - Vias envolvidas na biossíntese de lipídios com propriedades pró-inflamatórias ou anti-inflamatórias/pró-resolutivas. COX, ciclooxigenase; HETE, ácido hidroxieicosatetraenóico; HpETE, ácido hidroperoxieicosatetraenóico; LOX, lipoxigenase; LT, leucotrieno; LX, lipoxina; oxoETE, ácido oxoeicosatetraenóico; PG, prostaglandina; TX, tromboxano. Fonte: Adaptado de Calder (2010).

Vários sítios de clivagem proteolítica foram identificados na extremidade N-terminal da AnxA1. A clivagem da AnxA1 por proteases, como a elastase de neutrófilos, é capaz de modificar as suas características físicas e funções biológicas (Rescher *et al.*, 2006). Formas clivadas da proteína, como a de 33KDa, são comumente encontradas em exsudados inflamatórios, os quais

são ricos em proteases, e parecem possuir efeitos pró-inflamatórios (Kwon *et al.*, 2012; Vishwanatha *et al.*, 1998). Williams e colaboradores mostraram que um produto de clivagem da AnxA1 promoveu o agrupamento (*clustering*) da molécula de adesão intercelular-1 ao redor de neutrófilos aderentes para ancorá-los em células endoteliais, facilitando a sua transmigração (Williams *et al.*, 2010). Corroborando com esses dados, o estudo de Ernst e colaboradores mostraram que um peptídeo derivado do domínio N-terminal da AnxA1 estimulou a quimiotaxia de leucócitos ao se ligar a receptores da família FPR (Ernst *et al.*, 2004).

A maior parte dos estudos na literatura focou nos efeitos da AnxA1 na imunidade inata e mostrou de forma contundente que ela medeia efeitos anti-inflamatórios e pró-resolutivos. Há evidências de que a AnxA1 também module a resposta imune adaptativa, mas o seu papel é menos consistente (Gavins e Hickey, 2012). Estudos iniciais de Hirata e Iwata sugeriram que a AnxA1 promove a geração e/ou maturação de células T “supressoras” (Hirata e Iwata, 1983). Em concordância, foi demonstrado posteriormente que a AnxA1 limitou a inflamação em modelos experimentais de doenças associadas à ativação do sistema imune adaptativo, como asma e artrite reumatóide (Ng *et al.*, 2011; Yang *et al.*, 2004). De forma oposta, D’Acquisto e colaboradores mostraram que a administração de AnxA1 humana recombinante em camundongos com artrite reumatóide exacerbou os sinais e sintomas da doença (D'acquisto *et al.*, 2007). Esse efeito pode ser explicado pelo fato de a AnxA1 promover a diferenciação de células Th1 e Th17, as quais têm um papel importante na patogênese de doenças auto-imunes e outras doenças inflamatórias crônicas. Por outro lado, a AnxA1 inibe a diferenciação de células Th2, envolvidas principalmente em respostas humorais alérgicas e contra parasitas extracelulares (Cosmi *et al.*, 2014; Gavins e Hickey, 2012).

1.2.2 Proteína C reativa

A proteína C reativa (PCR) é uma proteína de origem hepática que pertence à família das pentraxinas. A produção da PCR é regulada principalmente por citocinas pró-inflamatórias, como a interleucina (IL)-6 e a IL-

1β. Os seus níveis plasmáticos podem se elevar rapidamente após uma lesão tecidual infecciosa ou estéril, sendo considerada uma proteína de fase aguda e um marcador pró-inflamatório. A PCR participa da imunidade inata ao reconhecer componentes presentes na superfície de patógenos, levando à ativação do sistema do complemento, o que favorece a opsonização e a fagocitose dos patógenos. A PCR também é capaz de modular respostas imunes adaptativas ao interagir com receptores Fc-gamma (Ansar e Ghosh, 2013; Mortensen, 2001).

Indivíduos saudáveis apresentam níveis plasmáticos de PCR abaixo de 10mg/L, em geral (Clyne e Olshaker, 1999; Das *et al.*, 2003). A sua dosagem através do ensaio ultrassensível é frequentemente empregada na prática clínica para identificar pacientes com risco aumentado de doenças cardiovasculares (Kaptoge *et al.*, 2010). Como a PCR é um marcador pró-inflamatório, a sua dosagem também pode ser indicada para estimar a gravidade de doenças inflamatórias de uma forma geral. Sabe-se que a PCR se eleva de forma inespecífica em condições inflamatórias. Portanto, a interpretação da sua dosagem sérica em doenças inflamatórias crônicas deve ser feita com cautela, principalmente em situações em que há trauma tecidual e infecção (Ridker, 2016).

1.2.3 Lipoxina A4

Durante a fase inicial (produtiva) na resposta inflamatória, diversos mediadores lipídicos pró-inflamatórios são gerados, como as prostaglandinas. Esses mediadores promovem o aumento do fluxo sanguíneo e da permeabilidade vascular, eventos importantes para o influxo de leucócitos para o sítio inflamatório. No entanto, ocorre uma mudança no perfil dos mediadores lipídicos produzidos durante a fase resolutiva da resposta inflamatória. Nessa fase, diferentes vias biossintéticas convergem para a formação de lipídios com propriedades anti-inflamatórias e pró-resolutivas, como as lipoxinas (LXs), resolvinas, maresinas e protectinas, denominados coletivamente como *specialized pro-resolving lipid mediators* (Schwab e Serhan, 2006).

Serhan e colaboradores foram os primeiros pesquisadores a isolar e caracterizar as LXs (Serhan *et al.*, 1984a,b). As LXs são eicosanóides endógenos gerados a partir do ácido araquidônico através de biossíntese transcelular mediada por lipoxigenases. Essa classe de mediadores é composta pela LXA4 e por seu isômero, a LXB4 (Serhan, 2005). Os leucócitos e as plaquetas são as principais fontes endógenas de LXs (Lee, 1995). As LXs compartilham algumas ações biológicas, entretanto a LXB4 tem sido menos estudada por ser quimicamente e biologicamente menos estável do que a LXA4 (Maddox e Serhan, 1996; Papayianni *et al.*, 1996). Curiosamente, descobriu-se que o ácido acetilsalicílico (AAS) pode induzir a síntese de dois análogos de LXs, os quais são epímeros: 15-epi-LXA4 e 15-epi-LXB4 (Chiang e Serhan, 2006). A síntese das LXs e de seus análogos está representada na **Figura 3**.

Semelhante à AnxA1, a LXA4 e a 15-epi-LXA4 são capazes de inibir a ativação e a migração de neutrófilos para o sítio inflamatório, além de estimularem a eferocitose de neutrófilos apoptóticos (Serhan, 2014). A LXA4 também inibe a produção de citocinas pró-inflamatórias *in vitro* e *in vivo* (Hu *et al.*, 2015; Luo *et al.*, 2013). Após serem produzidas, as LXs são rapidamente inativadas enzimaticamente por reações de desidrogenação e redução. Desta forma, análogos estáveis têm sido preparados e utilizados em modelos experimentais para avaliar as funções da LXA4 (Serhan *et al.*, 1995). Diversos estudos têm demonstrado que esses análogos também atenuam a resposta inflamatória e são estáveis quando administrados por via oral, tópica, intraperitoneal e intravenosa (Serhan, 2005).

Tecidos linfóides, como o baço, produzem LXA4, sugerindo uma possível ação dessa molécula em linfócitos (Hong *et al.*, 2007). Semelhante à AnxA1, existem poucos estudos sobre o papel da LXA4 na resposta imune adaptativa. Feng e colaboradores foram uns dos primeiros pesquisadores a investigar os efeitos da LXA4 na função de linfócitos. Eles mostraram que a LXA4 inibiu a expressão do receptor de LTB4 em linfócitos T CD4+ de cobaias (Feng *et al.*, 1996). Posteriormente, Ariel e colaboradores mostraram que análogos de LXA4 foram capazes de bloquear a secreção de TNF-α por linfócitos T CD3+ purificados de células mononucleares do sangue periférico (PBMCs), efeito mediado através da ligação ao receptor FPR2 e inibição da via

de sinalização MEK/ERK (Ariel *et al.*, 2003). Paralelamente, Aliberti e colaboradores avaliaram o papel da LXA4 na resposta imunológica à infecção pelo parasita intracelular *Toxoplasma gondii.* Os resultados desse estudo sugerem que LXA4 modula a resposta imune adaptativa do tipo Th1, porém sem exercer efeito imunossupressor (Aliberti *et al.*, 2002). O efeito imunomodulador da LXA4 foi corroborado pelo estudo de Gao e colaboradores, no qual o tratamento com LXA4 diminuiu a população de linfócitos Th1 e Th17 e estimulou a produção de linfócitos Treg em linfonodos drenantes de camundongos em um modelo *in vivo* de doença do olho seco auto-imune (Gao *et al.*, 2015).

Coletivamente, os dados desses estudos apontam para a potencialidade do uso de análogos estáveis de LXA4 no tratamento de doenças inflamatórias humanas associadas à ativação dos sistemas imunes inato e adaptativo.

1.2.4 Fator neurotrófico derivado do cérebro

O fator neurotrófico derivado do cérebro (BDNF, do inglês *brain-derived neurotrophic factor*) é uma neurotrofina expressa abundantemente nos sistemas nervosos central e periférico. O precursor do BDNF (pro-BDNF) é clivado por proteases intracelulares ou extracelulares para gerar a forma madura do BDNF. O BDNF é conhecido por regular a sobrevivência neuronal e a plasticidade sináptica ao ativar o receptor tropomiosina cinase B (TrkB) (Hempstead, 2015). Níveis alterados dessa neurotrofina têm sido descritas em diversas doenças psiquiátricas e neurodegenerativas como o transtorno bipolar e a doença de Alzheimer (Fernandes *et al.*, 2015; Qin *et al.*, 2016). O BDNF também é expresso em tecidos não neuronais, principalmente no coração, timo e pulmões, sugerindo que essa neurotrofina tenha outras ações biológicas além da neuroprotetora (Ernfors *et al.*, 1990; Yamamoto *et al.*, 1996).

Há evidências de que a expressão de BDNF seja modulada pela inflamação em tecidos neuronais e não neuronais. Lapchak e colaboradores mostraram uma diminuição da expressão de BDNF no hipocampo de ratos após a administração sistêmica de LPS ou IL-1β (Lapchak *et al.*, 1993). Em

outro estudo, o tratamento de células endoteliais com TNF-α reduziu a expressão de BDNF, além de reduzir também a capacidade angiogênica dessas células, demonstrando a inter-relação entre inflamação e angiogênese (Xu *et al.*, 2015). De forma recíproca, sugere-se que o BDNF regule a resposta imunológica. Ji e colaboradores observaram que administração local de BDNF (lenti-BDNF) em sítios de lesão medular foi capaz de inibir a resposta inflamatória ao alterar o fenótipo de macrófagos M1 para M2 e induzir a síntese de IL-10 e IL-13 (Ji *et al.*, 2015). Além disso, o BDNF é capaz de inibir a expressão de moléculas de adesão em células endoteliais, reduzir a adesão de neutrófilos e a disfunção da barreira endotelial após o tratamento com IL-1β e TNF-α (Matsuda *et al.*, 2015; Takeda *et al.*, 2016). Esses resultados indicam uma relação complexa entre o BDNF e a reposta inflamatória, uma vez que o BDNF regula a inflamação e os seus níveis são influenciados pelo próprio micro-ambiente inflamatório.

Sugere-se que o BDNF também exerça um papel na resposta imune adaptativa. Segundo Besser e Wank, o receptor TrkB é expresso em linfócitos Th1, mas não é expresso em linfócitos Th2 ou Th0. Sendo assim, a expressão de TrkB requer a ativação celular (Besser e Wank, 1999). No entanto, ainda não está claro como o BDNF pode afetar a função de linfócitos Th1 e de outras subpopulações de linfócitos Th que ainda não foram investigadas. Curiosamente, Javeri e colaboradores mostraram que resposta Th1/Th17 é atenuada em camundongos $BDNF^{-/+}$ quando comparada aos camundongos selvagens na encefalopatia autoimune experimental (Javeri *et al.*, 2010). Esses dados sugerem que o BDNF possa ter efeitos contrários nas respostas imunes inata e adaptativa, porém mais estudos são necessários para esclarecer isso.

1.3 Inflamação na gestação normotensa e na pré-eclâmspia

Há evidências de que ativação do sistema imunológico materno seja essencial para uma gestação saudável. No entanto, a resposta inflamatória é modulada de forma diferencial ao longo da gestação normotensa (Nadeau-Vallée *et al.*, 2016; Schminkey e Groer, 2014).

A implantação embrionária e a placentação ocorrem durante o primeiro e o início do segundo trimestre de gestação. O blastocisto deve romper a barreira epitelial do endométrio durante o processo de implantação. No desenvolvimento placentário, as células trofoblásticas fetais invadem a decídua e parte do miométrio em direção às artérias espiraladas uterinas, transformando-as em vasos de grande calibre capazes de fornecer uma perfusão adequada ao desenvolvimento fetal. Durante esses processos, ocorre dano tecidual e células da interface materno-fetal liberam padrões moleculares associados ao dano (DAMPs ou alarminas) que agem em células da placenta, principalmente em trofoblastos, e em células mielóides maternas de forma a induzir uma resposta inflamatória estéril. Algumas substâncias consideradas alarminas são: as proteínas de alta mobilidade do grupo 1, o ácido úrico, a IL-1α e o DNA livre fetal. Esse ambiente inflamatório é necessário para que sejam ativados mecanismos de reparo tecidual (Alpoim *et al.*, 2013; Challis *et al.*, 2009; Mor *et al.*, 2011; Nadeau-Vallée *et al.*, 2016; Schminkey e Groer, 2014).

A metade e o final do segundo trimestre de gestação compreendem a segunda fase imunológica da gestação, em que há uma quiescência inflamatória. A imunossupressão durante essa fase é importante para prevenir a rejeição do aloenxerto fetal, garantindo que o seu crescimento e desenvolvimento ocorram adequadamente (Challis *et al.*, 2009; Mor *et al.*, 2011).

A terceira fase imunológica compreende o terceiro trimestre da gestação, em que o feto está totalmente formado e os seus órgãos amadurecem até o momento do parto. No final da gestação, ocorre um fluxo intenso de células imunes para o miométrio e a produção aumentada de mediadores inflamatórios, como as prostaglandinas. Esse ambiente pró-inflamatório é essencial para promover a contração do útero e a expulsão da placenta, além do *clearance* de debris celulares e o reparo tecidual após o parto (Challis *et al.*, 2009; Mor *et al.*, 2011).

A inflamação é considerada branda e controlada ao longo da gestação normotensa. Admite-se que a PE curse com um estado inflamatório de maior intensidade em relação à gestação normotensa (Redman *et al.*, 1999). Níveis circulantes de citocinas pró-inflamatórias, como o TNF-α e a IL-6, que já estão elevados nas gestantes normotensas, encontram-se em níveis

ainda mais altos nas gestantes com PE (Jahromi *et al.*, 2011; Lau *et al.*, 2013). Estudos do nosso grupo de pesquisa revelaram níveis plasmáticos elevados de marcadores pró-inflamatórios, como citocinas (TNF-α, IL-6, IL-8 e interferon gama) e o receptor solúvel 1 do TNF-α (sTNF-R1), na PE em comparação à gestação normotensa (Perucci *et al.*, 2014; Pinheiro *et al.*, 2013). Sabe-se que leucócitos, células endoteliais e trofoblastos da placenta podem secretar diversas moléculas inflamatórias para a circulação, apesar de não se saber ao certo em que extensão isso ocorre (Gu *et al.*, 2008; Keelan e Mitchell, 2007).

Citocinas pró-inflamatórias são capazes de ativar células endoteliais e interferir com o mecanismo de contração/relaxamento dos vasos sanguíneos, levando a alterações na integridade e no tônus vascular (Steyers e Miller, 2014). Acredita-se que níveis elevados de citocinas pró-inflamatórias participem da patogênese da PE, uma vez que as suas manifestações clínicas parecem resultar de ativação e disfunção endotelial sistêmica materna (Chaiworapongsa *et al.*, 2014). Como o endotélio é parte integrante da resposta inflamatória, o endotélio ativado pode ativar leucócitos circulantes, e vice-versa (Mantovani e Dejana, 1989). Neutrófilos ativados secretam espécies reativas de oxigênio e diversas proteases, como a elastase, as quais contribuem para a lesão endotelial. Tem sido descrita uma maior ativação de neutrófilos na PE, como evidenciado pelos níveis aumentados de elastase no plasma e na placenta dessas gestantes (Salama *et al.*, 2011). Além disso, existem evidências de que a neutrofilia, que se apresenta moderada na gestação normotensa, mostra-se exacerbada na PE, e que a redução da apoptose de neutrófilos seja o principal mecanismo associado a esse fenômeno (Canzoneri *et al.*, 2009; Von Dadelszen *et al.*, 1999).

Admite-se que a imunidade na gestação normotensa seja predominantemente do tipo Th2/Treg, a qual é importante para a tolerância materna aos aloantígenos do feto e para a regulação da resposta inflamatória ao longo da gestação. Por outro lado, a resposta do tipo Th1/Th17 está associada ao desenvolvimento de autoimunidade, doenças inflamatórias crônicas e complicações da gestação, como a PE e o aborto espontâneo recorrente (Saito *et al.*, 2010).

De forma análoga ao paradigma Th1/Th2/Th17/Treg, o conceito de polarização macrofágica foi recentemente proposto em doenças inflamatórias

humanas (Liu *et al.*, 2014). Conforme exemplificado na **Figura 2**, os macrófagos M1 estão envolvidos em respostas pró-inflamatórias, ao passo que os macrófagos M2 possuem perfil anti-inflamatório e participam da fase resolutiva da resposta inflamatória (Alessandri *et al.*, 2013). Na gestação normotensa, os macrófagos da decídua têm um perfil do tipo M2, caracterizado pela produção abundante de IL-10. Esse fenótipo favorece a tolerância materna aos aloantígenos do feto, semelhante à imunidade do tipo Th2/Treg. Além disso, os macrófagos participam do remodelamento das artérias espiraladas uterinas nas fases iniciais da gravidez humana ao produzirem proteases que degradam a matriz extracelular e removerem células apoptóticas (Nagamatsu e Schust, 2010). Resultados de experimentos *in vitro* sugerem que a PE esteja associada a um perfil do tipo M1 e que a atividade aberrante de macrófagos uterinos participe da patogênese da doença (Renaud *et al.*, 2007). Entretanto, não foram encontrados estudos que avaliaram a proporção de macrófagos M1/M2 na decídua de gestantes com PE.

Com base nos estudos mencionados anteriormente, sugere-se que a resposta inflamatória exacerbada na PE advém do somatório de uma imunidade inata super ativada e de uma disfunção na resposta imune adaptativa (Schminkey e Groer, 2014). Em um estudo recente, Fullerton e Gilroy propuseram que a resolução da resposta inflamatória funcione como uma ponte entre as imunidades inata e adaptativa (Fullerton e Gilroy, 2016). Sendo assim, a resolução incompleta de respostas inflamatórias características da imunidade inata poderia levar a respostas imunes adaptativas inadequadas, como ocorre na PE.

Embora as moléculas e os mecanismos pró-inflamatórios tenham sido extensivamente estudados na PE nas últimas décadas, pouco se sabe sobre os mecanismos pró-resolutivos nessa doença.

1.3.1 Níveis circulantes de AnxA1, PCR, LXA4 e BDNF em gestantes normotensas e em gestantes com pré-eclâmpsia

O presente estudo investigou, de forma inédita, os níveis plasmáticos de AnxA1 bem como sua expressão gênica em PBMCs de

gestantes com PE em comparação às gestantes normotensas e às mulheres não gestantes (**Capítulo 1**). Considerando o papel da AnxA1 na modulação da resposta inflamatória, a nossa hipótese inicial era a de que a sua concentração plasmática seria menor em gestantes com PE em comparação às gestantes normotensas e às mulheres não gestantes.

Também dosamos os níveis plasmáticos de PCR com o objetivo de avaliar o *status* inflamatório das participantes do estudo (**Capítulo 1**). A maior parte dos estudos na literatura relata que os níveis circulantes de PCR estão aumentados em gestantes normotensas quando comparado às mulheres não gestantes e que a sua concentração aumenta ao longo da gestação normotensa (Belo *et al.*, 2005; De Oliveira *et al.*, 2015; Farzadnia *et al.*, 2013; Saarelainen *et al.*, 2009). Esses dados estão em concordância com a premissa de que a gestação está fisiologicamente associada a um estado de inflamação e que a resposta inflamatória tem um pico no final da gestação (Borzychowski *et al.*, 2006; Redman *et al.*, 1999). Como a inflamação é mais intensa na PE, esperávamos encontrar níveis plasmáticos aumentados de PCR em gestantes com a doença, o que já foi relatado em vários estudos anteriores na literatura (Derzsy *et al.*, 2010; Mihu *et al.*, 2008; Paternoster *et al.*, 2006; Teran *et al.*, 2005).

Os níveis plasmáticos de LXA4 também foram investigados no presente estudo (**Capítulo 2**). Um estudo anterior relatou que os níveis circulantes de LXA4 foram menores nas mulheres não gestantes, quando comparado às gestantes normotensas e que os níveis circulantes de LXA4 aumentaram ao longo da gestação normotensa (Maldonado-Pérez *et al.*, 2011). Sugere-se que esse aumento dos níveis de LXA4 tenha um efeito protetor contra o aborto e a restrição do crescimento intra-uterino, e que esse efeito seja, em parte, atribuído à regulação da produção e da atividade de prostaglandinas no útero e na placenta (Lin *et al.*, 2013; Rinaldi *et al.*, 2015; Xu *et al.*, 2013, 2014). Como encontramos níveis plasmáticos aumentados de AnxA1 no **Capítulo 1,** e considerando que a AnxA1 e a LXA4 interagem com o mesmo receptor (FPR2), esperávamos encontrar níveis aumentados de LXA4 no plasma de gestantes com PE em comparação às gestantes normotensas. Os níveis circulantes de LXA4 na PE foram investigados em estudos prévios na

literatura e tanto níveis aumentados quanto diminuídos já foram relatados (Dong e Yin, 2014; Hu *et al.*, 2015; Huang *et al.*, 2014).

Os níveis plasmáticos de BDNF na PE também foram investigados neste trabalho (**Capítulo 3**). Vários estudos têm mostrado o papel do BDNF na regulação de processos fisiológicos relacionados à manutenção da gestação, incluindo a angiogênese, o desenvolvimento placentário e o crescimento fetal (Kawamura *et al.*, 2011; Kermani e Hempstead, 2007; Marosi e Mattson, 2014). Ainda não está claro como ocorre a regulação da produção de BDNF durante a gestação. Já foi observado tanto um aumento quanto uma diminuição dos níveis dos níveis de BDNF ao longo da gestação normotensa (Christian *et al.*, 2016; Garces *et al.*, 2014). Considerando o papel do BDNF em modular a resposta inflamatória, e que a sua produção pode ser inibida por moléculas inflamatórias, a nossa hipótese inicial era a de que os níveis plasmáticos de BDNF estariam diminuídos em gestantes com PE, nas quais a resposta inflamatória é intensa, quando comparado às gestantes normotensas (Ji *et al.*, 2015; Lapchak *et al.*, 1993; Matsuda *et al.*, 2015; Takeda *et al.*, 2016; Xu *et al.*, 2015). Estudos anteriores também investigaram possíveis alterações nos níveis circulantes de BDNF na PE, porém os resultados são bastante divergentes (Bienertova-Vasku *et al.*, 2013; D'souza *et al.*, 2014a,b; Fujita *et al.*, 2011).

O papel da AnxA1, da LXA4 e de outras moléculas pró-resolutivas na PE foi discutido no artigo de revisão do **Capítulo 4.** Evidências de estudos experimentais e em humanos sugerem que a AnxA1 e a LXA4 estejam envolvidas na fisiopatologia de outras doenças inflamatórias crônicas além da PE. O **Capítulo 5** desta tese, discute os estudos que avaliaram o papel da AnxA1 e dos mediadores lipídicos pró-resolutivos, incluindo a LXA4, em doenças crônicas cardiovasculares, respiratórias, intestinais, entre outras.

2 OBJETIVOS

2.1 Objetivo geral

Investigar os níveis circulantes de mediadores envolvidos com a inflamação e a sua resolução na PE.

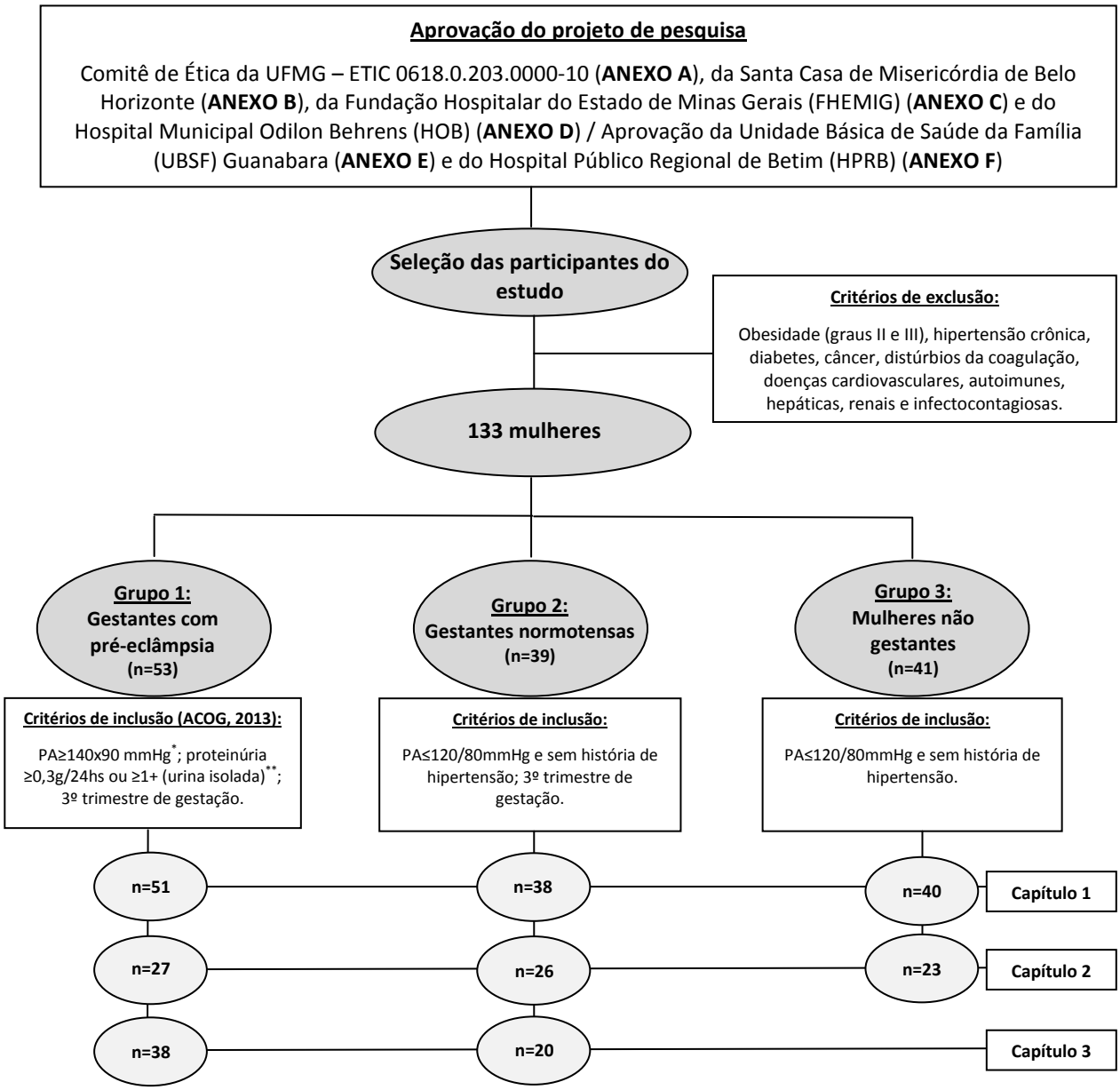
2.2 Objetivos específicos

- Determinar e comparar os níveis plasmáticos de AnxA1, além da expressão gênica de AnxA1 em PBMCs, em mulheres não gestantes, gestantes normotensas e gestantes com PE;
- Determinar e comparar os níveis plasmáticos de PCR eLXA4 em mulheres não gestantes, gestantes normotensas e gestantes com PE;
- Determinar e comparar os níveis plasmáticos de BDNF em gestantes normotensas e com PE;
- Estabelecer correlações entre os níveis das moléculas dosadas neste estudo (AnxA1, PCR, LXA4 e BDNF) e em um estudo anterior (sTNF-R1) e as características clínicas/parâmetros laboratoriais das participantes;
- Revisar e discutir os estudos existentes na literatura sobre mediadores pró-resolutivos na PE em específico, bem como em várias outras doenças inflamatórias humanas.

3 DELINEAMENTO EXPERIMENTAL

3.1 Casuística

Este foi um estudo transversal do tipo caso-controle. O esquema abaixo descreve de forma resumida como foi feito o delineamento experimental do estudo:



*Aferida no mínimo em duas medidas com intervalo de 4-6 horas.

**Na ausência de proteinúria, o diagnóstico da pré-eclâmpsia se deu pelo surgimento de hipertensão e de pelo menos um dos seguintes sinais e sintomas: trombocitopenia (contagem de plaquetas <100.000/mm^3); concentração sérica de creatinina superior a 1,1 mg/dL ou duplicação dos valores basais de creatinina na ausência de doença renal subjacente; disfunção hepática (duplicação das concentrações basais das transaminases hepáticas); edema pulmonar; sintomas visuais ou cerebrais.

As gestantes com PE (grupo 1) foram selecionadas no pré-parto da Santa Casa de Misericórdia de Belo Horizonte, da Maternidade Odete Valadares (rede FHEMIG), do Hospital Municipal Odilon Behrens e do Hospital Público Regional de Betim. As gestantes normotensas (grupo 2) foram selecionadas no pré-parto e durante a consulta de pré-natal na Unidade Básica de Saúde da Família Guanabara. As mulheres não gestantes (grupo 3) eram acompanhantes de pacientes ou estavam aguardando consulta médica para a avaliação de condições clínicas que não se enquadravam nos critérios de exclusão deste estudo, e foram recrutadas na Unidade Básica de Saúde da Família Guanabara e no Hospital Municipal Odilon Behrens.

Todas as participantes do estudo foram informadas sobre os objetivos da pesquisa no momento da coleta do sangue, utilizando-se linguagem clara, e assinaram o Termo de Consentimento Livre e Esclarecido – TCLE (**ANEXO G**). O protocolo da pesquisa não interferiu com as recomendações e prescrições médicas. Os dados clínicos e laboratoriais de interesse de cada uma das participantes do estudo foram coletados em fichas individuais padronizadas para cada grupo a partir do cartão de pré-natal e/ou prontuário médico e durante a entrevista presencial (**ANEXO H**).

O diagnóstico de PE foi feito pela equipe obstétrica dos hospitais e maternidades nas quais as pacientes foram selecionadas, e confirmado pela equipe de pesquisadores deste estudo com base nos critérios diagnósticos estabelecidos pela ACOG (2013). A data de coleta do sangue das gestantes com PE foi igual ou superior à data do diagnóstico da doença feita pela equipe obstétrica.

As gestantes com PE (grupo 1) foram distribuídas em dois subgrupos, de acordo com a idade gestacional na qual surgiram os sintomas da doença (Von Dadelszen *et al.*, 2003), sendo:

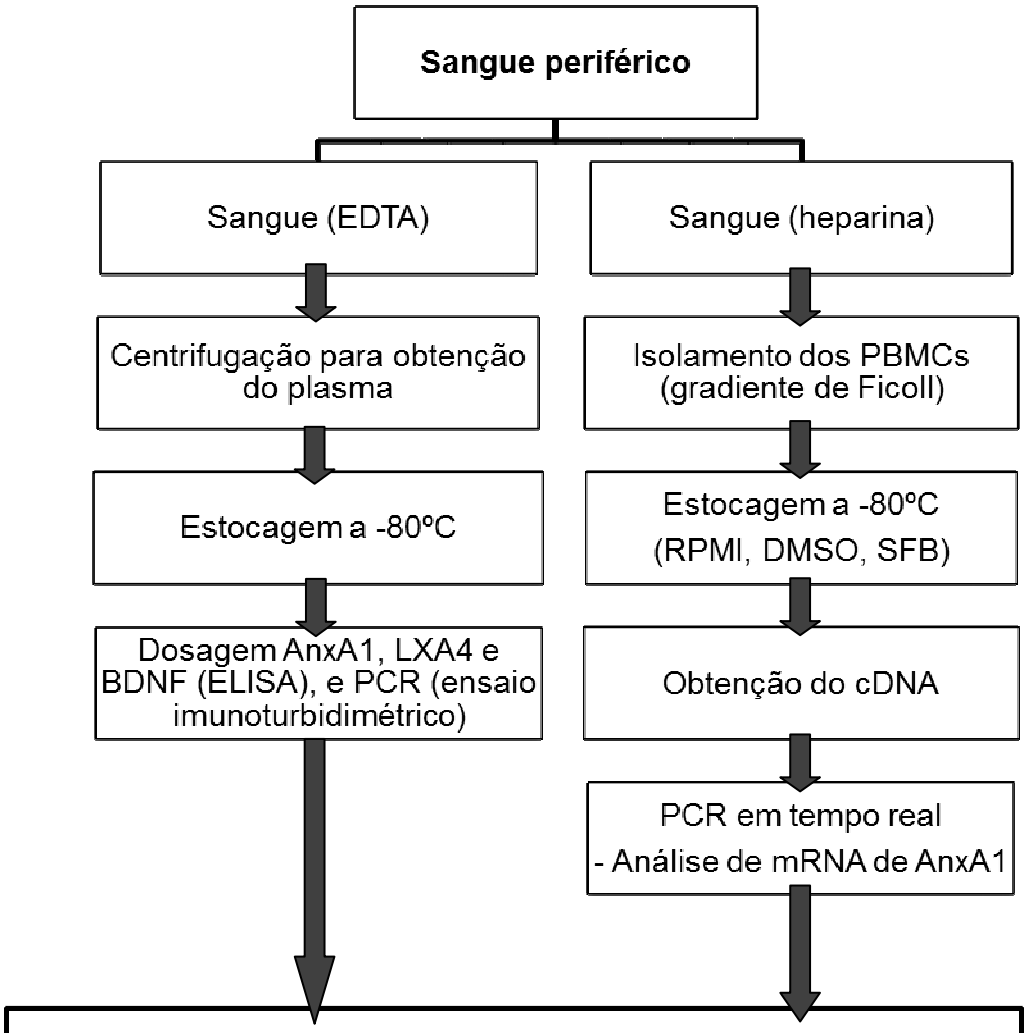
- PE precoce (n=23): Quando os sintomas surgiram antes da 34ª semana de gestação;
- PE tardia (n=30): Quando os sintomas surgiram na 34ª semana de gestação ou após esse período.

Dentre as gestantes com PE precoce, 91% apresentavam a forma grave de acordo com os critérios de gravidade estabelecidos pela ACOG (2013), em contraste com 73% das gestantes com a forma tardia.

As gestantes normotensas (grupo 2; Norm) também foram distribuídas em dois subgrupos de modo a parear os subgrupos de gestantes com PE precoce e de gestantes com PE tardia, tendo como ponto de corte 34 semanas de gestação (Von Dadelszen *et al.*, 2003), sendo:

- Norm < 34 semanas (n=15): Gestantes normotensas com menos de 34 semanas de gestação;
- Norm ≥ 34 semanas (n=24): Gestantes normotensas com 34 semanas ou mais de gestação.

3.2 Material, métodos e análise estatística



4 ARTIGOS RESULTANTES

4.1 CAPÍTULO 1 - “Annexin A1 Is Increased in the Plasma of Preeclamptic Women”

PLOS | ONE

RESEARCH ARTICLE

Annexin A1 Is Increased in the Plasma of Preeclamptic Women

Luiza O. Perucci[1,2], Fernanda S. Carneiro[1], Cláudia N. Ferreira[3], Michelle A. Sugimoto[1,4], Frederico M. Soriani[5], Gustavo G. Martins[6], Kátia M. Lima[1,7], Flávia L. Guimarães[1], Antônio L. Teixeira[8], Luci M. Dusse[1,2,4], Karina B. Gomes[1,2], Lirlândia P. Sousa[1,2,4]*

1 Departamento de Análises Clínicas e Toxicológicas, Faculdade de Farmácia, Universidade Federal de Minas Gerais, Belo Horizonte, Minas Gerais, Brazil, **2** Programa de Pós-Graduação em Análises Clínicas e Toxicológicas, Universidade Federal de Minas Gerais, Belo Horizonte, Minas Gerais, Brazil, **3** Colégio Técnico, Universidade Federal de Minas Gerais, Belo Horizonte, Minas Gerais, Brazil, **4** Programa de Pós-Graduação em Ciências Farmacêuticas, Universidade Federal de Minas Gerais, Belo Horizonte, Minas Gerais, Brazil, **5** Departamento de Biologia Geral, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais, Belo Horizonte, Brazil, **6** Hospital Municipal Odilon Behrens, Belo Horizonte, Minas Gerais, Brazil, **7** Programa de Pós-Graduação em Biologia Celular, Universidade Federal de Minas Gerais, Belo Horizonte, Minas Gerais, Brazil, **8** Laboratório Interdisciplinar de Investigação Médica, Faculdade de Medicina, Universidade Federal de Minas Gerais, Belo Horizonte, Brazil

* lipsousa72@gmail.com

Citation: Perucci LO, Carneiro FS, Ferreira CN, Sugimoto MA, Soriani FM, Martins GG, et al. (2015) Annexin A1 Is Increased in the Plasma of Preeclamptic Women. PLoS ONE 10(9): e0138475. doi:10.1371/journal.pone.0138475

Editor: Colette Kanellopoulos-Langevin, Xavier Bichat Medical School, INSERM-CNRS - Université Paris Diderot, FRANCE

Received: April 7, 2015

Accepted: August 31, 2015

Published: September 23, 2015

Data Availability Statement: All relevant data are within the paper and its Supporting Information files.

Funding: Fundação de Amparo à Pesquisa do Estado de Minas Gerais (APQ-00588-11; http://www.fapemig.br/) (LPS), Conselho Nacional de Desenvolvimento Científico e Tecnológico (http://www.cnpq.br/) (LPS), Pró-Reitoria de Pesquisa da UFMG (https://www.ufmg.br/prpq/) (LPS). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Abstract

Background

Preeclampsia (PE) is a pregnancy disease associated with exacerbated inflammatory response. Annexin A1 (AnxA1) is a glucocorticoid-regulated protein endowed with anti-inflammatory and proresolving properties that has been much studied in various animal models of inflammation but poorly studied in the context of human inflammatory diseases. The main objective of this study was to measure AnxA1 levels in PE women and to compare those levels in normotensive pregnant and non-pregnant women. We evaluated the association among AnxA1, ultrasensitive C reactive protein (us-CRP) and soluble tumor necrosis factor alpha receptor type 1 (sTNF-R1) plasma levels of the study participants.

Methods

This study included 40 non-pregnant, 38 normotensive pregnant and 51 PE women. PE women were stratified in early (N = 23) and late (N = 28) subgroups, according to gestational age (GA) at onset of clinical symptoms. Protein AnxA1 and us-CRP plasma levels were determined by ELISA and immunoturbidimetric assays, respectively. Transcript levels of AnxA1 in peripheral blood mononuclear cells (PBMC) were measured by real time RT-PCR.

Results

Increased levels of AnxA1 coincided with higher us-CRP levels in the plasma of PE women. Pregnant women with early PE had higher levels of AnxA1 and us-CRP than normotensive pregnant women with GA <34 weeks. No significant difference was found for AnxA1 and

Competing Interests: The authors have declared that no competing interests exist.

us-CRP, comparing late PE and normotensive pregnant women with GA ≥34 weeks. AnxA1 mRNA levels in PBMC were similar among the studied groups. AnxA1 was positively correlated with sTNF-R1, but not with us-CRP.

Conclusions

Our data show that increased AnxA1 levels were associated with a systemic inflammatory phenotype in PE, suggesting AnxA1 deregulation in PE pathogenesis. However, more studies are needed to clarify the role of AnxA1 and other proresolving molecules in the context of the systemic inflammatory response in this intriguing disease.

Introduction

Preeclampsia (PE) is a multisystem disease characterized by new-onset hypertension and proteinuria on or after 20 weeks of gestation [1]. PE affects 2–8% of all pregnancies and is often associated with adverse maternal and perinatal outcomes [2]. PE has been classified according the gestational age (GA) of clinical symptoms onset in early (GA<34 weeks) or late (GA≥34 weeks) [3]. Despite years of intense research, the etiopathogenesis of PE remains to be elucidated, although placental dysfunction is considered to play a central role in the development of the disease. It has been proposed that the ischemic placenta can release soluble factors into the maternal circulation that cause endothelial cell activation and/or dysfunction and a systemic inflammatory response [4].

Redman et al. initially proposed that the features of the systemic inflammatory response observed in normotensive pregnant women are also seen in PE women, but in a greater intensity [5]. Accordingly, several studies have described increased activation of circulating leukocytes, abnormal immune cell phenotype and higher pro-inflammatory markers levels, such as C-reactive protein (CRP), in PE compared to normotensive pregnancy [6–10].

Annexin A1 (AnxA1), previously named lipocortin-1, is a 37 kDa calcium-dependent phospholipid binding protein that regulates diverse cellular functions in various cellular types [11, 12]. AnxA1 was originally described as a glucocorticoid-regulated protein with anti-phospholipase activity, but the protein exhibits many other anti-inflammatory and proresolving properties, which include inhibition of neutrophils adhesion/transmigration through the endothelium and stimulation of macrophages phagocytic clearance of apoptotic neutrophils [13]. AnxA1 is also regulated by pro-inflammatory proteins, such as lipopolysaccharide (LPS) and interleukin (IL)-6, suggesting that it may act as a brake for controlling the inflammatory response [14, 15]. Given the large body of evidence describing the anti-inflammatory and proresolving actions of AnxA1, and knowing that PE is associated with an exacerbated inflammatory state, it is plausible to hypothesize that AnxA1 may be altered in PE women.

In the present study, we evaluated AnxA1 plasma levels and AnxA1 mRNA expression in peripheral blood mononuclear cells (PBMC) in preeclamptic, normotensive pregnant and non-pregnant women. The association between AnxA1 and ultrasensitive (us)-CRP levels was tested in order to investigate the potential role of AnxA1 in the context of inflammation. In a previous study of our group, soluble tumor necrosis factor alpha (TNF-α) receptor type 1 (sTNF-R1) plasma levels were higher in PE women than in normotensive pregnant women [16]. sTNF-R1 acts as an indirect marker of TNF-α release in the circulation [17]. Therefore, we also investigated whether AnxA1 plasma levels were correlated to sTNF-R1. To date, this is the first study that evaluates AnxA1 plasma levels and mRNA expression in PBMC in PE.

Materials and Methods

Study participants

This case control study enrolled Brazilian women. It was approved by Ethics Committees of Universidade Federal de Minas Gerais (Institutional Review Board Project #0618.0.203.000–10) and the participant hospitals. Written informed consent was obtained from all women. Blood samples were obtained from 129 women: 40 non-pregnant, 38 normotensive pregnant and 51 preeclamptic. All pregnant women were at the third trimester of gestation.

PE was defined as blood pressure $\geq$ 140/90 mmHg at least in two occasions, 6 or more hours apart, and proteinuria (at least 300 mg/day or 1+ on a urine dipstick) on or after 20 weeks of gestation. Headache, oliguria, visual disturbances, upper abdominal pain, high liver enzymes and thrombocytopenia were also considered in the disease diagnosis. No cases of chronic hypertension or superimposed PE were included in this study. Preeclamptic women were stratified in two subgroups according to gestational age at clinical symptoms onset: early PE (GA$<$34 weeks; N = 23) and late PE (GA$\geq$34 weeks, N = 28) [3]. The normotensive pregnant group included women with healthy pregnancies and was matched for gestational age at the time of blood sampling (GA$<$34 weeks: N = 14; GA$\geq$34 weeks: N = 24). The non-pregnant group included healthy women age matched. Exclusion criteria common for all groups were: obesity (grades II and III) [18]; diabetes mellitus; cancer; coagulation disorders; cardiovascular, autoimmune, hepatic, renal and inflammatory/infectious diseases.

Blood sampling and processing

Peripheral blood samples were collected from all women into EDTA anticoagulated tubes, which were centrifuged at 3000g for 15 minutes, room temperature. The aliquots of plasma were stored at -80°C until the analyzes for AnxA1 and us-CRP.

PBMC were isolated from the heparinized peripheral blood obtained from 12 non-pregnant, 16 normotensive pregnant and 17 PE women by Ficoll Diatrizoate gradient centrifugation (Ficoll-Paque Plus, GE Healthcare Life Sciences) at room temperature. Cells were collected, washed, resuspended in RPMI medium containing 10% of dimethyl sulfoxide and 20% of fetal bovine serum and stored at -80°C until RNA extraction.

ELISA and immunoturbidimetric assay

AnxA1 plasma levels were determined by a commercial ELISA kit (USCN Life Sciences Inc.). An immunoturbidimetric method (Wiener Lab) was used for us-CRP plasma levels determination. The assays were performed according to manufacturers' instructions. Samples from PE, normotensive pregnant and non-pregnant groups were run simultaneously and the assays were blinded for patient identification and disease status. The values are represented as AnxA1 μg/mL and us-CRP mg/L of plasma

Comparative real-time PCR

RNA was extracted from isolated PBMC using a commercial kit (RNeasy Mini Kit, Qiagen). An aliquot of RNA (130ng) was reverse transcribed into cDNA using reverse transcriptase (Superscript III, Invitrogen), following the manufacturers' protocols. Real-time PCR assay was carried out in duplicate in a 10μL volume using SYBR Green (Applied Biosystems) and the following primers: human annexin A1 (AnxA1) forward 5′ -ATCAGCGGTGAGCCCCTATC-3′ and reverse 5′ -TTCATCCAGGGGCTTTCCTG-3′ and human glyceraldehyde-3-phosphate dehydrogenase (GAPDH) forward 5′ -GGTCGGAGTCAACGGATTTG-3′ and reverse 5′ -ATGAGCCCCAGCCTTCTCCAT-3′ . Relative AnxA1 mRNA expression levels were calculated

from normalized ΔCT (cycle threshold) related to the housekeeping gene (GAPDH). For the detection of changes in gene expression in non-pregnant and PE groups the normalized ΔCT values for each sample were compared with the mean ΔCT level of the normotensive pregnant group and the change expression of AnxA1 gene (ΔΔCT) was calculated. The normotensive pregnant group was considered as the reference group because the main purpose of this analysis was to evaluate AnxA1 transcript levels in PE women in comparison to normotensive pregnant women. The obtained values were converted to a linear scale ($2^{-\Delta\Delta CT}$) and reported as the fold-change in expression (arbitrary units).

Statistical analysis

Statistical analysis was performed using SPSS 19.0 for Windows (Chicago, IL, USA). The normality of continuous variables was assessed using Shapiro-Wilk's W-test. Continuous variables did not follow a normal distribution and were analyzed by nonparametric Kruskal-Wallis test or Mann-Whitney U-test with Dunn-Bonferroni's post hoc correction. Comparison of categorical variables was performed by Pearson chi-square (X^2) test. Correlation analysis was evaluated by Spearman coefficients (Rs) and included all the participants of the study. All statistical analyzes were performed using a significance level of $\alpha = 0.05$.

Results

Clinical characteristics

Clinical characteristics of the studied participants are described in Table 1. There were no significant differences comparing age and body mass index among the three studied groups. PE women had higher gestational weight gain and increased percentage of primiparas compared to normotensive pregnant women. As expected, both systolic and diastolic blood pressures were significantly increased in PE than in the normotensive pregnant and non-pregnant women.

AnxA1 protein and mRNA levels

AnxA1 plasma levels were higher in PE women [median (25th–75th percentiles), 43.2 (30.8–57.8)μg/mL] comparing to non-pregnant women [25.9 (18.9–32.5)μg/mL] (P = 0.001). PE women also showed higher levels of AnxA1 compared to normotensive pregnant women [30.1 (19.0–35.7)μg/mL] (P = 0.026). No difference was found in AnxA1 levels when comparing non-pregnant and normotensive pregnant groups (Fig 1).

AnxA1 plasma levels were similar between normotensive pregnant women (GA<34 weeks) [32.6 (17.6–48.5)μg/mL] and normotensive pregnant women (GA≥34 weeks) [44.1 (28.6–53.4)μg/mL] (Fig 2A). No significant difference was found in AnxA1 plasma levels between early PE [43.5 (32.8–58.5)μg/mL] and late PE [44.3 (31.6–64.7)μg/mL] (Fig 2B). Pregnant women with early PE had higher AnxA1 levels compared to normotensive pregnant women (GA<34 weeks) (P = 0.020) (Fig 2C). However, AnxA1 levels were no significantly different between pregnant women with late PE and normotensive pregnant women (GA≥34 weeks) (Fig 2D).

Measurement of AnxA1 gene expression in PBMC from studied groups showed that AnxA1 mRNA levels were similar among them (Fig 3).

Us-CRP

Us-CRP plasma levels were higher in PE women [5.8 (3.6–15.0)mg/L] than in non-pregnant women [0.9 (0.2–2.4)mg/L] (P<0.001) and in normotensive pregnant women [3.9 (2.8–6.4)

Table 1. Clinical characteristics of the studied participants.

Variables	NP (N = 40)	Norm (N = 38)	PE (N = 51)	P value
Age (years)[a]	25 (22–30)	27 (21–30)	26 (21–30)	0.970[1]
BMI (Kg/m^2)[a]	21.4 (20.1–24.9)	22.8 (20.3–26.1)	23.5 (20.5–25.2)	0.1571
GWG (Kg)[a]	N/A	10.4 (7.4–13.5)	13.0 (9.6–20.9)	0.0051*
GA at blood draw (weeks) [a]	N/A	36 (31–39)	34 (31–37)	0.3531
Primiparas (%)[b]	N/A	12 (32)	27 (53)	0.0452*
SBP (mmHg)[a]	120 (110–120)	110 (100–110)	160 (160–170)	< 0.0011*
DBP (mmHg)[a]	80 (70–80)	70 (70–78)	110 (100–110)	<0.0011*

BMI (body mass index: before pregnancy), GWG (gestational weight gain), GA (gestational age), SBP (systolic blood pressure), DBP (diastolic blood pressure), NP (non-pregnant women), Norm (normotensive pregnant women), PE (preeclamptic women), N/A (not applicable).

[a]Data are presented as median (25th–75th percentiles).

[b]Data are presented as number (percentage).

[1]Kruskal-Wallis/Mann-Whitney test with Dunn-Bonferroni's correction

[2]Pearson chi-square (X^2) test.

*p<0.05.

doi:10.1371/journal.pone.0138475.t001

mg/L] compared to non-pregnant women (P<0.001). No significant difference was found in us-CRP levels between PE women and in normotensive pregnant women, without stratifying the groups according to gestational age (Fig 4).

us-CRP levels were higher in normotensive pregnant women (GA≥34 weeks) [5.7 (3.8–8.3) mg/L] comparing to normotensive pregnant women (GA<34 weeks) [3.2 (1.7–3.9)mg/L] (P = 0.002) (Fig 5A). Us-CRP levels were not significantly different between early PE [8.1 (2.8–13.6)mg/L] and late PE [5.1 (3.5–18.5)mg/L] (Fig 5B). Pregnant women with early PE had higher us-CRP levels compared to normotensive pregnant women (GA<34 weeks) (P = 0.018)

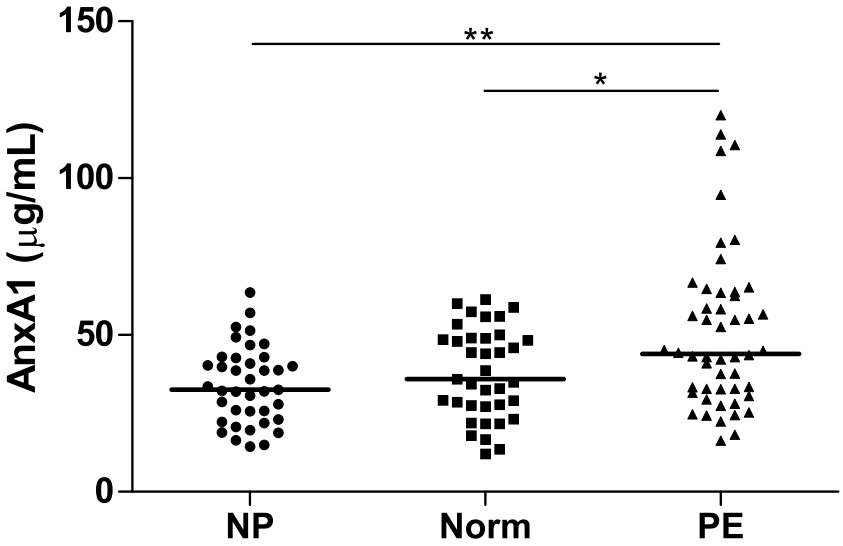


Fig 1. AnxA1 plasma levels in non-pregnant, normotensive pregnant and PE women. NP (non-pregnant women), Norm (normotensive pregnant women), PE (preeclamptic women). Horizontal bars represent median values for AnxA1 (micrograms/milliliter). **P<0.01, *P<0.05. Plasma levels of AnxA1 were higher in PE women than in normotensive pregnant and non-pregnant women. No significant differences were found comparing non-pregnant and normotensive pregnant women.

doi:10.1371/journal.pone.0138475.g001

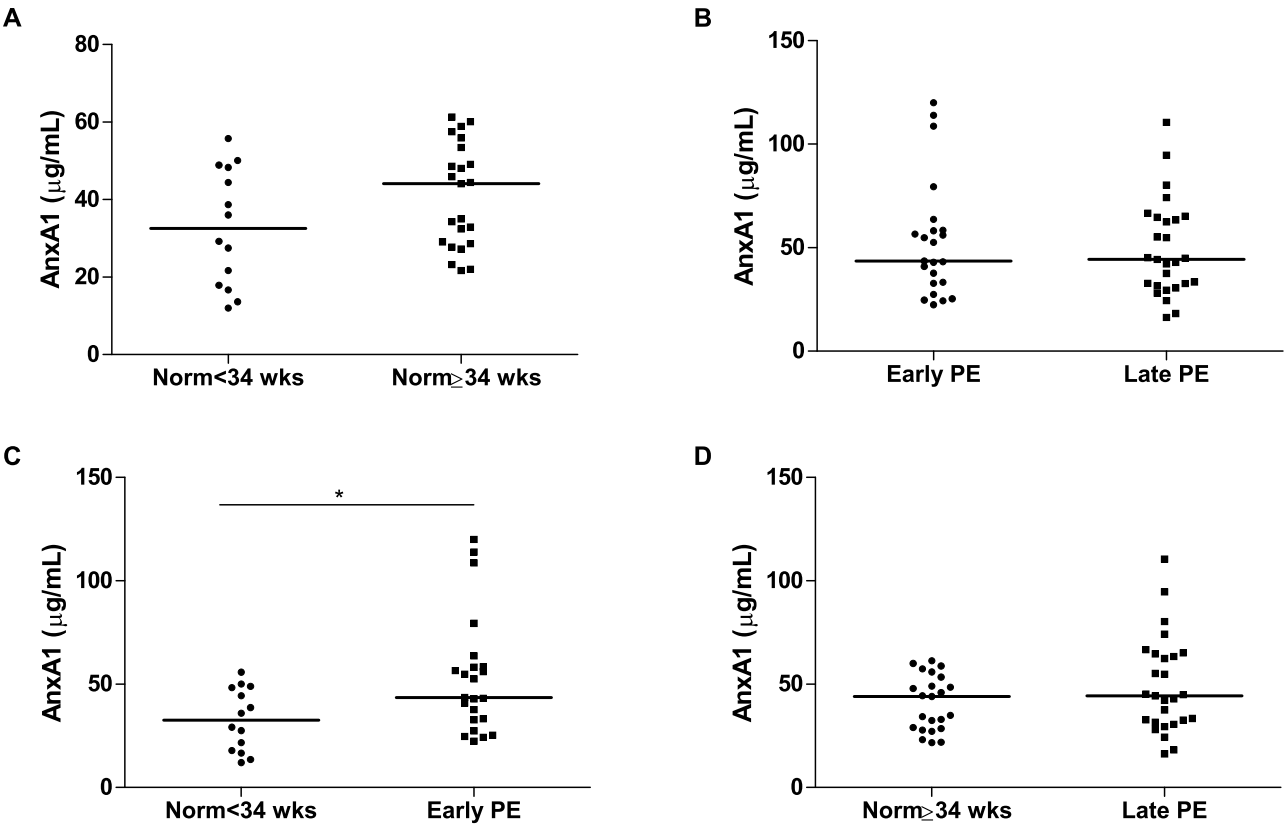


Fig 2. AnxA1 plasma levels in normotensive pregnant and PE women according to gestational age. Norm<34 wks (normotensive pregnant women with GA<34 weeks), Norm≥34 wks (normotensive pregnant women with GA≥34 weeks), PE (preeclampsia). Horizontal bars represent median for AnxA1 levels (micrograms/milliliter). *P<0.05. AnxA1 plasma levels were higher in pregnant women with early PE than in normotensive pregnant women with GA<34 weeks (C). No significant differences were detected between the normotensive pregnant subgroups (A), early and late PE (B) and normotensive pregnant women with GA≥34 weeks and pregnant women with late PE (D).

doi:10.1371/journal.pone.0138475.g002

(Fig 5C). However, us-CRP levels were similar between late PE and normotensive pregnant (GA≥34 weeks) (Fig 5D).

Correlations among AnxA1, us-CRP and sTNF-R1 plasma levels

In a prior study, we showed that sTNF-R1 plasma levels were higher in PE women [3479 (3182–4339) pg/mL) than in normotensive pregnant women [3028 (2468–3606) pg/mL] (P = 0.014) [16]. In this study we performed correlations among sTNF-R1, AnxA1 and us-CRP levels and found a positive correlation between AnxA1 and sTNF-R1 (R = 0.352, P = 0.002), and between us-CRP and sTNF-R1 (R = 0.555, P<0.001). On the other hand, AnxA1 and us-CRP plasma levels did not correlate.

Discussion

Our data showed that AnxA1, an anti-inflammatory and proresolving protein, is increased in preeclamptic women.

It is know that normotensive pregnancy is characterized by a state of mild systemic inflammation [5]. Consistent with this observation, we have found increased levels of us-CRP, a marker of systemic inflammatory response, in normotensive pregnancy compared to the non-pregnant state. Moreover, systemic inflammatory response strengthens as pregnancy advances,

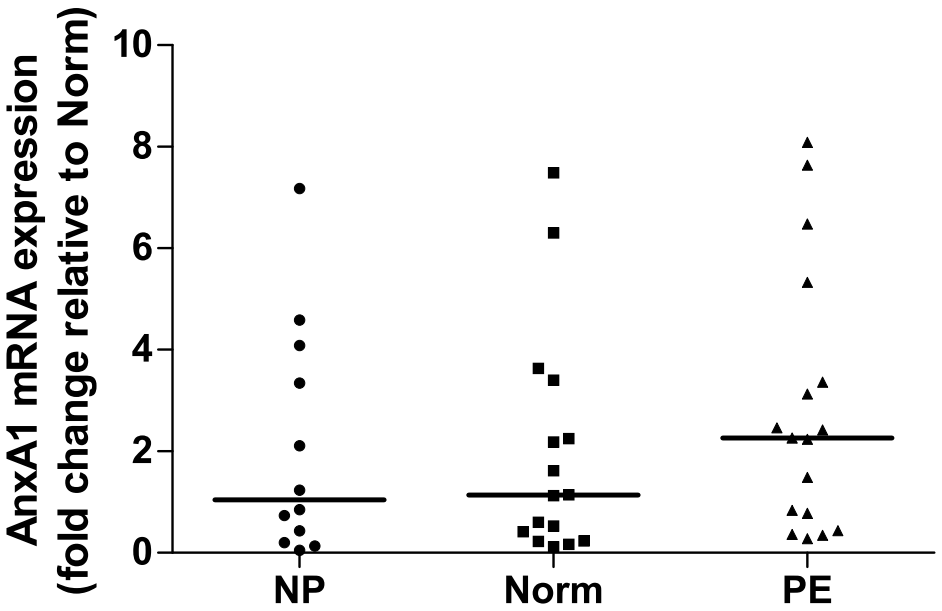


Fig 3. AnxA1 mRNA expression in PBMC of non-pregnant, normotensive pregnant and PE women. NP (non-pregnant women), Norm (normotensive pregnant women), PE (preeclamptic women). Horizontal bars represent median values for Anx1 mRNA fold change expression relative to the calibrator group (Norm). The transcript levels of AnxA1 were similar among the study groups.

doi:10.1371/journal.pone.0138475.g003

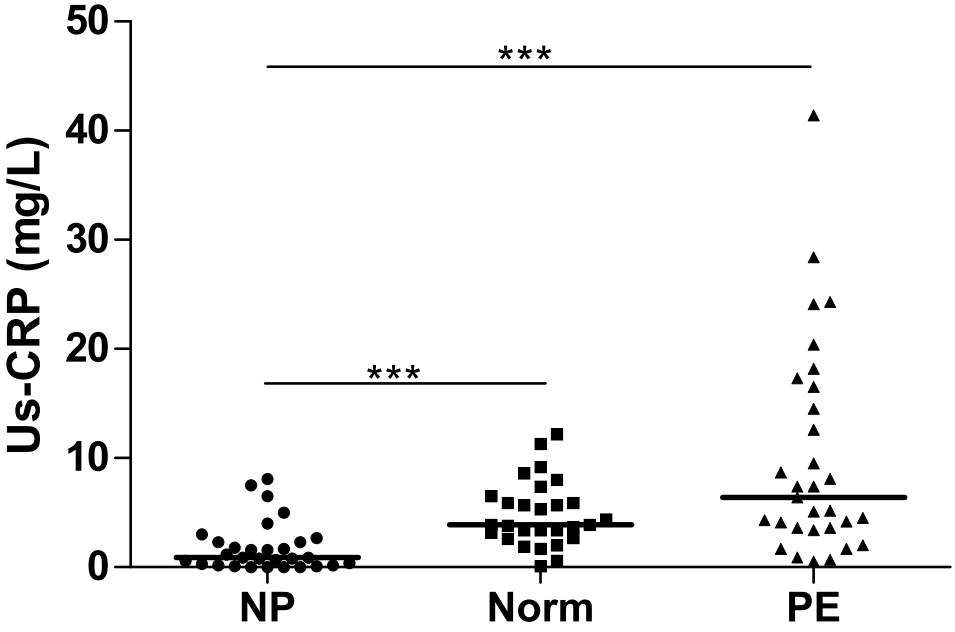


Fig 4. us-CRP plasma levels in non-pregnant, normotensive pregnant and PE women. NP (non-pregnant women), Norm (normotensive pregnant women), PE (preeclamptic women). Horizontal bars represent median values for us-CRP (milligrams/liter). ***P<0.001. Plasma levels of us-CRP were higher in normotensive pregnant women compared to non-pregnant women and in PE women than in non-pregnant women. No significant difference was detected between PE and normotensive pregnant women.

doi:10.1371/journal.pone.0138475.g004

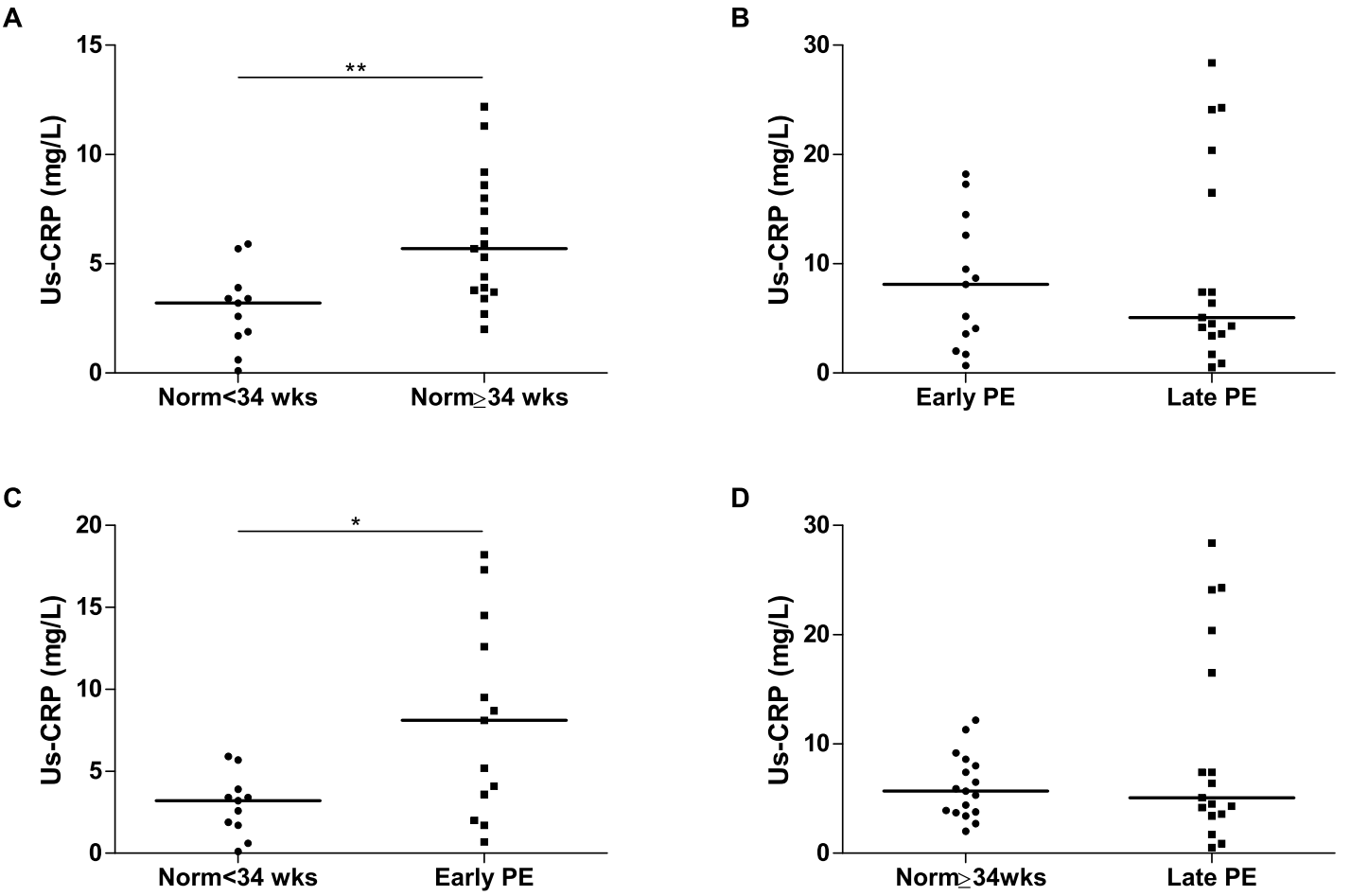


Fig 5. us-CRP plasma levels in normotensive pregnant and PE women according to gestational age. Norm<34 wks (normotensive pregnant women with GA<34 weeks), Norm≥34 wks (normotensive pregnant women with GA≥34 weeks), PE (preeclamptic women). Horizontal bars represent median us-CRP levels (milligrams/liter). *P<0.05. Plasma levels of us-CRP were higher in normotensive pregnant women (GA≥34 weeks) than in normotensive pregnant women (GA<34 weeks) (A). Pregnant women with early PE had higher levels of us-CRP than normotensive pregnant women with GA<34 weeks (C). No significant differences were found between pregnant women with early and late PE (B) and between pregnant women with late PE and normotensive pregnant women GA≥34 weeks (D).

doi:10.1371/journal.pone.0138475.g005

peaking during the third trimester of gestation [19]. Accordingly, in our study normotensive pregnant women (GA≥34 weeks) had higher us-CRP levels than normotensive pregnant women (GA<34 weeks).

PE has been associated to a greater inflammatory response [5]. In fact, us-CRP levels were increased in early PE than in normotensive pregnancy (GA<34 weeks). This result was not observed for the late PE subgroup. Strong evidence suggests that early PE is clinically a more severe disease than late PE [20, 21], consistent with our finding that us-CRP levels were different only when comparing early PE and the respective control.

It is well established that there is a physiological balance between pro-inflammatory and regulatory responses in normotensive pregnancies [9], which means that regulatory molecular pathways are sufficient to attenuate the mild inflammatory response. In PE, it has been proposed that this balance shifts toward a pro-inflammatory state and there is a failure to regulate the inflammation [5, 9]. AnxA1 is a protein that limits initial steps of inflammation and also acts on the resolution phase of the inflammatory response [13, 22, 23]. Our data may imply that Anx1 is increased in PE women in an attempt to attenuate the exacerbated inflammatory

response in these patients. Although AnxA1 and us-CRP did not correlate to each other, both factors were elevated in PE women. In addition, AnxA1 and sTNF-R1 (an inflammatory marker) levels were positively correlated. AnxA1 levels in normotensive pregnant women were comparable to those levels in non-pregnant women. Considering that inflammation is physiological in normotensive pregnancies, it is plausible to suggest that proresolving mechanisms are not increased during physiological conditions, but they arise when the inflammatory status increase, as in the case of pathological conditions such as PE. Other studies have shown increased levels of pro-inflammatory markers, such as tumor necrosis factor alpha (TNF-α), interleukin (IL)-6 and IL-1β, in early PE than in late PE and normotensive pregnancy [24,25]. Our findings corroborate the possible regulatory role of AnxA1 mainly in the severe clinical forms of the disease (i.e., early PE), in which the inflammatory response is exacerbated.

Chronic inflammation in PE suggests that the resolution of inflammation is dysfunctional. Accordingly, increased AnxA1 levels seem to be insufficient to resolve inflammation. Indeed, systemic levels of proresolving mediators are increased in others chronic inflammatory diseases such as inflammatory bowel disease and Alzheimer's disease [26, 27], suggesting that this resolution pathway is dysfunctional. Proresolving and anti-inflammatory actions of AnxA1 are mediated by a G-protein-couple receptor named formyl peptide receptor like-2 (FPR2)/lipoxin A4 receptor (ALXR), hereafter referred as "ALX" [28]. Cooray et al. [29] showed that ALX/ALX dimer signature is activated by AnxA1 trough p38/MAPKAPK/Hsp27/IL-10 pathway. These authors argue that AnxA1 up-regulation may be ineffective to resolve inflammation if ALX/ALX dimerization fails. Additionally, lower ALX expression level might be associated with reduced anti-inflammatory and proresolutive responses to AnxA1. Decreased ALX expression has been observed in patients with asthma, a chronic inflammatory disease [30]. Simieli et al. described a single nucleotide mutation (A/G) in the core promoter of ALX gene that reduced ~35–90% of its activity *in vitro*. ALX promoter activity may also be repressed by methylation [31]. These mechanisms might explain the apparent ineffectiveness of AnxA1 up-regulation in some human inflammatory/vascular diseases [32–34]. It remains to be investigated whether these dysfunctional mechanisms in AnxA1 resolution pathway are present in PE.

We also investigated AnxA1 gene expression in PBMCs in order to evaluate one possible source of the protein in the circulation. No significant difference was detected among the studied groups, with a tendency of higher expression of AnxA1 mRNA in PE women. AnxA1 is found predominantly within differentiated cells, like neutrophils, monocytes/macrophages and mast cells, but the exact source of plasma AnxA1 has not been determined yet [35]. This protein is expressed in the placenta, predominantly in the synciciotrophoblast [36], therefore it may be another source of AnxA1 in the plasma. To date, no study has evaluated the differential expression of AnxA1 in the placenta of normotensive and preeclamptic women, a matter under investigation in our lab.

To the best of our knowledge this is the first work that shows higher AnxA1 levels in preeclamptic women. Moreover, patients with PE were stratified according to gestational age at clinical symptoms onset, which allowed evaluating AnxA1 in different mechanisms of disease. One limitation of this study is that AnxA1 transcript levels were not evaluated in all women and pregnant groups were not stratified according to the gestational age in this analysis due to the small sample size. Moreover, AnxA1 and us-CRP levels may be influenced by ethnicity and smoking. These parameters were not evaluated in our study due to the high genetic variability present in Brazilian population, and because it was difficult to obtain accurate information about women's smoking status. Unfortunately, we were unable to measure the exposure to cigarette smoking in this study using methods that evaluate nicotine metabolites, such as an ELISA assay for Cotinine, because a new collection of samples would be required for this

purpose, which would preclude the realization of correlation analyzes with AnxA1 and us-CRP levels for each participant of the study. The dosage of Cotinine, as well as other pro-resolving molecules, such as Lipoxin A4, will be a matter of future studies in our group. Finally, patients taking medications that could potentially impact the analyzes were not excluded in this study, since polytherapy is common in patients with PE.

Concluding, it is possible that AnxA1 participate in PE pathogenesis, but more studies are needed to clarify the role of AnxA1 and other proresolving molecules in the context of the systemic inflammatory response in this intriguing disease. The potential use of AnxA1 as a biomarker in PE should be better investigated in prospective studies.

Supporting Information

S1 File. Raw data from measurements of AnxA1 (protein and mRNA levels), us-CRP levels, sTNF-R1 levels, as well as clinical parameters (age, gestational age, number of pregnancies, body mass index, gestational weight gain, systolic and diastolic blood pressure) of the participants in this study.
(XLS)

Acknowledgments

The authors wish to thank the participants in this study.

Author Contributions

Conceived and designed the experiments: LPS KBG LMD. Performed the experiments: LOP FSC CNF GGM KML FLG. Analyzed the data: LPS KBG LMD ALT FMS. Contributed reagents/materials/analysis tools: LPS KBG LMD ALT FMS. Wrote the paper: LOP LPS KBG LMD ALT MAS.

References

1. Gynecologists ACoOa, Pregnancy TFoHi. Hypertension in pregnancy. Report of the American College of Obstetricians and Gynecologists' Task Force on Hypertension in Pregnancy. Obstet Gynecol. 2013; 122(5):1122–31. doi: 10.1097/01.AOG.0000437382.03963.88 PMID: 24150027.
2. Duley L. The global impact of pre-eclampsia and eclampsia. Semin Perinatol. 2009; 33(3):130–7. doi: 10.1053/j.semperi.2009.02.010 PMID: 19464502.
3. Dekker GA, de Vries JI, Doelitzsch PM, Huijgens PC, von Blomberg BM, Jakobs C, et al. Underlying disorders associated with severe early-onset preeclampsia. Am J Obstet Gynecol. 1995; 173(4):1042–8. PMID: 7485291.
4. Chaiworapongsa T, Chaemsaithong P, Yeo L, Romero R. Pre-eclampsia part 1: current understanding of its pathophysiology. Nat Rev Nephrol. 2014; 10(8):466–80. doi: 10.1038/nrneph.2014.102 PMID: 25003615.
5. Redman CW, Sacks GP, Sargent IL. Preeclampsia: an excessive maternal inflammatory response to pregnancy. Am J Obstet Gynecol. 1999; 180(2 Pt 1):499–506. PMID: 9988826.
6. Lau SY, Guild SJ, Barrett CJ, Chen Q, McCowan L, Jordan V, et al. Tumor necrosis factor-alpha, interleukin-6, and interleukin-10 levels are altered in preeclampsia: a systematic review and meta-analysis. Am J Reprod Immunol. 2013; 70(5):412–27. doi: 10.1111/aji.12138 PMID: 23790133.
7. Sacks GP, Studena K, Sargent K, Redman CW. Normal pregnancy and preeclampsia both produce inflammatory changes in peripheral blood leukocytes akin to those of sepsis. Am J Obstet Gynecol. 1998; 179(1):80–6. PMID: 9704769.
8. Luppi P, Tse H, Lain KY, Markovic N, Piganelli JD, DeLoia JA. Preeclampsia activates circulating immune cells with engagement of the NF-kappaB pathway. Am J Reprod Immunol. 2006; 56(2):135–44. doi: 10.1111/j.1600-0897.2006.00386.x PMID: 16836616.
9. Saito S. Th17 cells and regulatory T cells: new light on pathophysiology of preeclampsia. Immunol Cell Biol. 2010; 88(6):615–7. doi: 10.1038/icb.2010.68 PMID: 20498674.

10. Farzadnia M, Ayatollahi H, Hasan-Zade M, Rahimi HR. A comparative study of vascular cell adhesion molecule-1 and high-sensitive C-reactive protein in normal and preeclamptic pregnancies. Interv Med Appl Sci. 2013; 5(1):26–30. doi: 10.1556/IMAS.5.2013.1.5 PMID: 24265885; PubMed Central PMCID: PMCPMC3831793.
11. Flower RJ. Eleventh Gaddum memorial lecture. Lipocortin and the mechanism of action of the glucocorticoids. Br J Pharmacol. 1988; 94(4):987–1015. PMID: 2974738; PubMed Central PMCID: PMCPMC1854095.
12. Flower RJ, Rothwell NJ. Lipocortin-1: cellular mechanisms and clinical relevance. Trends Pharmacol Sci. 1994; 15(3):71–6. PMID: 8184489.
13. Parente L, Solito E. Annexin 1: more than an anti-phospholipase protein. Inflamm Res. 2004; 53 (4):125–32. doi: 10.1007/s00011-003-1235-z PMID: 15060718.
14. Vago JP, Nogueira CR, Tavares LP, Soriani FM, Lopes F, Russo RC, et al. Annexin A1 modulates natural and glucocorticoid-induced resolution of inflammation by enhancing neutrophil apoptosis. J Leukoc Biol. 2012; 92(2):249–58. doi: 10.1189/jlb.0112008 PMID: 22493082.
15. Solito E, de Coupade C, Parente L, Flower RJ, Russo-Marie F. IL-6 stimulates annexin 1 expression and translocation and suggests a new biological role as class II acute phase protein. Cytokine. 1998; 10(7):514–21. doi: 10.1006/cyto.1997.0325 PMID: 9702415.
16. Perucci LO, Gomes KB, Freitas LG, Godoi LC, Alpoim PN, Pinheiro MB, et al. Soluble endoglin, transforming growth factor-Beta 1 and soluble tumor necrosis factor alpha receptors in different clinical manifestations of preeclampsia. PLoS One. 2014; 9(5):e97632. doi: 10.1371/journal.pone.0097632 PMID: 24851923; PubMed Central PMCID: PMCPMC4031102.
17. Diez-Ruiz A, Tilz GP, Zangerle R, Baier-Bitterlich G, Wachter H, Fuchs D. Soluble receptors for tumour necrosis factor in clinical laboratory diagnosis. Eur J Haematol. 1995; 54(1):1–8. PMID: 7859870.
18. Obesity: preventing and managing the global epidemic. Report of a WHO consultation. World Health Organ Tech Rep Ser. 2000; 894:i–xii, 1–253. PMID: 11234459.
19. Borzychowski AM, Sargent IL, Redman CW. Inflammation and pre-eclampsia. Semin Fetal Neonatal Med. 2006; 11(5):309–16. doi: 10.1016/j.siny.2006.04.001 PMID: 16828580.
20. Hall DR, Odendaal HJ, Kirsten GF, Smith J, Grové D. Expectant management of early onset, severe pre-eclampsia: perinatal outcome. BJOG. 2000; 107(10):1258–64. PMID: 11028578.
21. MacKay AP, Berg CJ, Atrash HK. Pregnancy-related mortality from preeclampsia and eclampsia. Obstet Gynecol. 2001; 97(4):533–8. PMID: 11275024.
22. Perretti M, D'Acquisto F. Annexin A1 and glucocorticoids as effectors of the resolution of inflammation. Nat Rev Immunol. 2009; 9(1):62–70. doi: 10.1038/nri2470 PMID: 19104500.
23. Sousa LP, Alessandri AL, Pinho V, Teixeira MM. Pharmacological strategies to resolve acute inflammation. Curr Opin Pharmacol. 2013; 13(4):625–31. doi: 10.1016/j.coph.2013.03.007 PMID: 23578762.
24. Borges VT, Peraçoli JC, Romão M, Zanati S, Poiati JR, Weel IC, et al. Differentiation between early and late pre-eclampsia by inflammatory cytokines and the association between IL-1β and left cardiac hypertrophy. Pregnancy Hypertension. 2015; 5:53–156.
25. Zhang Z, Gao Y, Zhang L, Jia L, Wang P, Zhang L, et al. Alterations of IL-6, IL-6R and gp130 in early and late onset severe preeclampsia. Hypertens Pregnancy. 2013; 32(3):270–280. doi: 10.3109/10641955.2013.798332 PMID: 23782174.
26. Leoni G, Neumann PA, Kamaly N, Quiros M, Nishio H, Jones HR, et al. Annexin A1-containing extracellular vesicles and polymeric nanoparticles promote epithelial wound repair. J Clin Invest. 2015; 125 (3):1215–27. doi: 10.1172/JCI76693 PMID: 25664854.
27. Wang X, Zhu M, Hjorth E, Cortés-Toro V, Eyjolfsdottir H, Graff C, et al. Resolution of inflammation is altered in Alzheimer's disease. Alzheimers Dement. 2015; 11(1):40–50.e1–2. doi: 10.1016/j.jalz.2013.12.024 PMID: 24530025; PubMed Central PMCID: PMCPMC4275415.
28. Chiang N, Serhan CN, Dahlén SE, Drazen JM, Hay DW, Rovati GE, et al. The lipoxin receptor ALX: potent ligand-specific and stereoselective actions in vivo. Pharmacol Rev. 2006; 58(3):463–87. doi: 10.1124/pr.58.3.4 PMID: 16968948.
29. Cooray SN, Gobbetti T, Montero-Melendez T, McArthur S, Thompson D, Clark AJ, et al. Ligand-specific conformational change of the G-protein-coupled receptor ALX/FPR2 determines proresolving functional responses. Proc Natl Acad Sci U S A. 2013; 110(45):18232–7. doi: 10.1073/pnas.1308253110 PMID: 24108355; PubMed Central PMCID: PMCPMC3831442.
30. Planagumà A, Kazani S, Marigowda G, Haworth O, Mariani TJ, Israel E, et al. Airway lipoxin A4 generation and lipoxin A4 receptor expression are decreased in severe asthma. Am J Respir Crit Care Med. 2008; 178(6):574–82. doi: 10.1164/rccm.200801-061OC PMID: 18583575; PubMed Central PMCID: PMCPMC2542432.

31. Simiele F, Recchiuti A, Mattoscio D, De Luca A, Cianci E, Franchi S, et al. Transcriptional regulation of the human FPR2/ALX gene: evidence of a heritable genetic variant that impairs promoter activity. FASEB J. 2012; 26(3):1323–33. doi: 10.1096/fj.11-198069 PMID: 22131270.

32. Vong L, Ferraz JG, Dufton N, Panaccione R, Beck PL, Sherman PM, et al. Up-regulation of Annexin-A1 and lipoxin A(4) in individuals with ulcerative colitis may promote mucosal homeostasis. PLoS One. 2012; 7(6):e39244. doi: 10.1371/journal.pone.0039244 PMID: 22723974; PubMed Central PMCID: PMCPMC3377644.

33. Tsai WH, Li IT, Yu YB, Hsu HC, Shih CH. Serial changes in plasma annexin A1 and cortisol levels in sepsis patients. Chin J Physiol. 2014; 57(1):1–7. doi: 10.4077/CJP.2014.BAB193 PMID: 24621333.

34. Särndahl E, Bergström I, Nijm J, Forslund T, Perretti M, Jonasson L. Enhanced neutrophil expression of annexin-1 in coronary artery disease. Metabolism. 2010; 59(3):433–40. doi: 10.1016/j.metabol.2009.07.044 PMID: 19850308.

35. D'Acquisto F, Perretti M, Flower RJ. Annexin-A1: a pivotal regulator of the innate and adaptive immune systems. Br J Pharmacol. 2008; 155(2):152–69. doi: 10.1038/bjp.2008.252 PMID: 18641677; PubMed Central PMCID: PMCPMC2538690.

36. Sun M, Liu Y, Gibb W. Distribution of annexin I and II in term human fetal membranes, decidua and placenta. Placenta. 1996; 17(2–3):181–4. PMID: 8730889.

4.2 CAPÍTULO 2 - “Lipoxin A4 Is Increased in the Plasma of Preeclamptic Women”

American Journal of Hypertension Advance Access published May 13, 2016

ORIGINAL ARTICLE

Lipoxin A4 Is Increased in the Plasma of Preeclamptic Women

Luiza O. Perucci,[1,2] Patrícia C. Santos,[3] Lucas S. Ribeiro,[3] Danielle G. Souza,[3] Karina B. Gomes,[1,2] Luci M.S. Dusse,[1,2] and Lirlândia P. Sousa[1,2]

BACKGROUND
Excessive inflammation is involved in preeclampsia (PE) pathogenesis. Lipoxin A4 (LXA4) is an eicosanoid that counter-regulates inflammation. The main objective of this study was to determine LXA4 plasma levels in PE women. The correlations among LXA4 levels, ultrasensitive C-reactive protein (us-CRP) levels, and clinical/laboratory parameters of the studied participants were also investigated.

METHODS
LXA4 plasma levels were determined by ELISA in 23 nonpregnant, 26 normotensive pregnant, and 27 PE women (early PE (N = 10) and late PE (N = 17)), according to gestational age (GA) at clinical symptoms onset). The clinical/laboratory parameters included in Spearman's correlation analysis were: systolic and diastolic blood pressure (SBP and DBP, respectively), lactate dehydrogenase (LDH) activity, platelet count, proteinuria, and white blood cell count (WBC).

RESULTS
LXA4 levels were higher in PE women than in nonpregnant and normotensive pregnant women, and similar between nonpregnant and normotensive pregnant women. LXA4 plasma levels were higher in early PE vs. normotensive pregnancy (GA < 34 weeks) and in late PE vs. normotensive pregnancy (GA ≥ 34 weeks). No significant differences were detected between early and late PE. LXA4 levels were positively correlated with us-CRP levels, SBP, DBP, and WBC. No significant correlation was detected between LXA4 levels and the other laboratory parameters.

CONCLUSIONS
Chronic inflammation in PE, in spite of increased levels of LXA4, points to a possible failure in this regulatory pathway. Further studies are necessary to clarify this issue and to evaluate the role of LXA4 and other proresolving mediators of inflammation in the pathogenesis of PE.

Keywords : blood pressure; hypertension; inflammation; lipoxin A4; preeclampsia; resolution.

doi:10.1093/ajh/hpw053

Preeclampsia (PE) is a multisystem disorder of unknown cause that occurs at ≥20 weeks of gestation. This gestation-specific syndrome affects 2–8% of pregnancies and is associated with substantial maternal and perinatal morbidity and mortality worldwide.[1] Abnormal placentation and endothelial dysfunction are thought to play crucial roles in PE pathogenesis. These events may trigger an excessive activation of the inflammatory and haemostatic systems.[2] PE can be classified according to severity or onset-time of clinical symptoms.[3] The last classification has been more appreciated lately because it has been admitted that early and late PE have different etiopathogenesis, and it allows identifying PE women with worst prognosis.[4]

There are several evidences of systemic maternal inflammatory response in PE, such as altered cytokine levels and increased complement system and neutrophil activation.[5,6] Indeed, PE women show higher levels of ultrasensitive C-reactive protein (us-CRP) than normotensive pregnant women.[7–9] Although proinflammatory mechanisms have been extensively explored in this disease, pathways involved in the counter-regulation of inflammation are still poorly understood. Lipoxins (LXs) are endogenous lipid-based autacoids that are generated from arachidonic acid via lipoxygenase-mediated biosynthesis.[10,11] Among this lipid family, lipoxin A4 (LXA4) and its analogues (aspirin triggered LXs(ATL)) act as "braking signals" for inflammation. They evoke this action by modulating the onset of inflammation and by acting as an agonists on resolution phase of inflammation.[12]

Chronic inflammation in PE women indicates that the resolution of inflammation is dysfunctional. To investigate this hypothesis, we examined LXA4 levels in PE women

Correspondence: Lirlândia P. Sousa (lipsousa72@gmail.com).

Initially submitted January 31, 2016; date of first revision February 25, 2016; accepted for publication May 3, 2016.

[1]Departamento de Análises Clínicas e Toxicológicas, Faculdade de Farmácia, Universidade Federal de Minas Gerais, Belo Horizonte, Brazil; [2]Programa de Pós-Graduação em Análises Clínicas e Toxicológicas, Universidade Federal de Minas Gerais, Belo Horizonte, Brazil; [3]Departamento de Microbiologia, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais, Belo Horizonte, Brazil.

and compared with normotensive pregnant and nonpregnant women. We also investigated whether LXA4 levels were associated with us-CRP levels and clinical/laboratory parameters of the studied population.

METHODS

Subjects

This case–control study included 76 Brazilian women: 27 preeclamptic, 26 normotensive pregnant, and 23 nonpregnant. All pregnant women were at the third trimester of gestation at blood collection and were selected from public hospitals (Hospital Municipal Odilon Behrens and Maternidade Odete Valadares). Ethics approval was obtained from Universidade Federal de Minas Gerais (Institutional Review Board Project #0618.0.203.000-10) and from the participant hospitals (#0681.0.000.216-11 and CEP-FHEMIG 077/2008, respectively), and written informed consent was obtained from all enrolled women.

PE was defined as hypertension (i.e., blood pressure ≥ 140/90 mm Hg, at least on two occasions, 4 or more h apart) and proteinuria (≥300 mg in a 24-h urine collection or ≥1+ on a random urine sample) on or after 20 weeks of gestation. In the absence of proteinuria, PE was defined as new-onset hypertension plus new onset of any of the following features: thrombocytopenia, renal insufficiency, impaired liver function, pulmonary edema, cerebral, or visual disturbances.[3] No cases of chronic hypertension or superimposed PE were included in this study. Preeclamptic women were stratified in two subgroups according to gestational age (GA) at clinical symptoms onset: early PE (GA < 34 weeks; $N = 10$) and late PE (GA ≥ 34 weeks, $N = 17$).[13] Blood from PE women was collected after the time of diagnosis. The normotensive pregnant group included women with healthy pregnancies and it was matched for GA at the time of blood collection (GA < 34 weeks: $N = 10$; GA ≥ 34 weeks: $N = 16$) of the PE group. The nonpregnant group included healthy women age matched. Exclusion criteria common for all groups were: obesity (grades II and III)[14]; diabetes mellitus; cancer; coagulation disorders; cardiovascular, autoimmune, hepatic, renal, and inflammatory/infectious diseases. We did not exclude patients taking any kind of medication, since polytherapy is common in PE women. The clinical data were obtained from medical records and during the recruitment interview.

Sample collection and processing

Peripheral blood samples were obtained from all women into EDTA anticoagulant-coated tubes (BD Vacutainer). The plasma was separated by centrifugation spin at 3,000*g*, at room temperature, for 15 min and stored at −80 °C until LXA4 assay.

LXA4 measurement

LXA4 levels were measured in lipids extracted from plasma by a commercial ELISA kit (Neogen Corporation, Lexington, KY) according to the manufacturer's instructions. Samples from nonpregnant, normotensive pregnant and PE women were run simultaneously in the same plate. LXA4 levels are represented as pictogram per milliliter (pg/ml) of plasma. The assay is able to detect 100% of LXA4. Its cross-reactivity is 15-epi-lipoxin (24%), 5(S),6(R)-diHETE (5.0%), lipoxin B4 (1.0%), 15-HETE (0.10%), and 5-HETE (<0.01%). The assay range was 20–2,000 pg/ml. Both intra- and inter-assay coefficients were ≤10%.

Assessment of laboratory parameters

us-CRP levels were measured by an immunoturbidimetric assay, as described previously.[7] Lactate dehydrogenase (LDH) activity, platelet count, proteinuria, and white blood cell count (WBC) data were obtained from medical records at the time of blood collection and were only available for PE women. These parameters were assessed in this study because they have been traditionally correlated with clinical outcome in PE (LDH activity, platelet count and proteinuria) and with inflammation (WBC). The normal ranges considered in this study (82–524 U/L, 146–429 × 10^3/mm^3, <0.3 g/24 h urine specimen, 5.6–16.9 × 10^3/mm^3, respectively) were based on a population of healthy pregnant women in the third trimester of gestation.[15]

Statistical analysis

Statistical analysis was performed using Statistical Package for Social Sciences (SPSS) 19.0 software (SPSS IBM Corporation, Armonk, NY). The normality of continuous variables was assessed using Shapiro–Wilk's *W*-test. The comparison of continuous variables with normal distribution was performed by analysis of variance test with *post hoc* LSD test (three groups) or Student's *t*-test (two groups). The comparison of continuous variables not normally distributed was made by Kruskal–Wallis test with *post hoc* Dunn–Bonferroni's test (three groups) or Mann–Whitney *U*-test (two groups). Comparison of categorical variables was performed by Pearson chi-square (χ^2) test. The relationship between the variables which were significantly associated with Spearman coefficients was further evaluated with linear regression analysis and expressed as r^2. The correlations among LXA4 levels, us-CRP levels and the clinical parameters (systolic blood pressure (SBP) and diastolic blood pressure (DBP)) included all the participants of the study or the PE group, separately, while the correlations between LXA4 levels and the laboratory parameters (LDH activity, platelet count, proteinuria, and WBC) included only PE women. $P < 0.05$ was considered statistically significant.

A *t*-test for two independent groups was used in the sample size calculation (OpenEpi, version 3, Rollins School of Public Health, Emory University, USA), which was performed based on LXA4 values (mean ± SD) that were obtained from a similar study including normotensive pregnant women and PE women.[16] The calculated sample size for both groups was three participants (statistical power: 95%, confidence interval: 95%).

RESULTS

Clinical characteristics

The clinical characteristics of the studied participants are displayed in Tables 1 and 2. There were no significant differences in age and body mass index (BMI) before pregnancy

Table 1. | Clinical characteristics of the studied participants

Variables	NP (*N* = 23)	Norm (*N* = 26)	PE (*N* = 27)	*P* value
Age (years)[a]	26 ± 6	27 ± 6	26 ± 6	0.778[b]
BMI (kg/m^2)[c]	21.5 (20.1–23.9)	22.5 (20.6–26.1)	24.1 (20.6–26.3)	0.218[d]
GWG (kg)[c]	N/A	10.7 (7.0–13.6)	13.0 (10–20.6)	0.017[d]*
GA (weeks)[c]	N/A	36 (32–39)	34 (32–38)	0.574[d]
Primiparas (%)[e]	N/A	9 (35)	15 (56)	0.126[f]
SBP (mm Hg)[c]	120 (110–120)	110 (100–112.5)	160 (150–170)	<0.001[d]***
DBP (mm Hg)[c]	80 (70–80)	70 (68–80)	102 (100–110)	<0.001[d]***

Abbreviations: BMI, body mass index (before pregnancy for normotensive pregnant women and preeclamptic women); GWG, gestational weight gain; GA, gestational age at blood collection; SBP, systolic blood pressure; DBP, diastolic blood pressure; NP, nonpregnant women; Norm, normotensive pregnant women; PE, preeclamptic women; N/A, not applicable.

[a]Data are presented as mean ± standard deviation. [b]Analysis of variance with *post hoc* LSD. [c]Data are presented as median (25th–75th percentiles). [d]Kruskal–Wallis with *post hoc* Dunn–Bonferroni/Mann–Whitney test. [e]Data are presented as number (percentage). [f]Pearson chi-square (χ^2) test. *P < 0.05, ***P < 0.001.

Table 2 | Clinical characteristics of the subgroups of normotensive pregnant women and PE women

Variables	Norm < 34weeks (*N* = 10)	Norm ≥ 34weeks (*N* = 16)	*P* value	Early PE (*N* = 10)	Late PE (*N* = 17)	*P* value
Age (years)[a]	33 ± 7	30 ± 6	0.717[b]	29 ± 4	30 ± 7	0.787[b]
BMI (kg/m^2)[c]	22.3 (19.7–26.3)	22.5 (21.3–26.1)	0.660[d]	24.1 (21.1–26.3)	24.1 (20.4–26.3)	0.863[d]
GWG (kg)[c]	8.1 (6.0–11.1)	12.7 (9.0–15.7)	0.061[d]	12.0 (7.0–16.5)	16.4 (10.5–23)	0.049[d]*
GA (weeks)[c]	30 (29–32)	38 (36–39)	<0.001[d]***	31 (30–32)	37 (35–39)	<0.001[d]***
Primiparas (%)[e]	10 (50%)	4 (25)	0.234[f]	5 (50)	10 (59)	0.706[f]
SBP (mm Hg)[c]	105 (100–110)	110 (100–120)	0.279[d]	170 (160–180)	160 (150–170)	0.012[d]*
DBP (mm Hg)[c]	70 (63–78)	70 (70–80)	0.754[d]	110 (100–113)	100 (100–110)	0.186[d]

Abbreviations: BMI, body mass index (before pregnancy for normotensive pregnant women and preeclamptic women); GWG, gestational weight gain; GA, gestational age at blood collection; SBP, systolic blood pressure; DBP, diastolic blood pressure; NP, nonpregnant women; Norm, normotensive pregnant women; PE, preeclamptic women.

[a]Data are presented as mean ± standard deviation. [b]Student's *t*-test. [c]Data are presented as median (25th–75th percentiles). [d]Mann–Whitney test. [e]Data are presented as number (percentage). [f]Pearson chi-square (χ^2) test. *P < 0.05, ***P < 0.001.

among nonpregnant, normotensive pregnant and PE women. The GA at blood collection and number of primiparas were also similar between the pregnant groups. PE women had a greater gestational weight gain (GWG) than normotensive pregnant women (P = 0.017). As expected, both SBP and DBP were elevated in PE women compared with nonpregnant and normotensive pregnant women (all P < 0.001). In addition, normotensive pregnant women showed decreased SBP (P = 0.014) and DBP compared with nonpregnant women (P = 0.005).

Ten (37%) preeclamptic women were classified into early PE and 17 (63%) into late PE group. Early and late PE women had similar age, BMI, and DBP. Late PE women had a greater GWG than early PE women (P = 0.049), while SBP was higher in early PE (P = 0.012). The normotensive pregnant subgroups had similar age, BMI, GWG, SBP, and DBP. As expected, GA at blood collection was higher in late PE vs. early PE and in normotensive pregnancy with GA ≥34 weeks vs. normotensive pregnancy with GA <34 weeks (all P < 0.001). There were no cases of HELLP syndrome (hemolysis, elevated liver enzymes, and low platelet count) or eclampsia among PE women.

LXA4 plasma levels

Plasma levels of LXA4 were increased in PE women (median (25th–75th percentiles): 290 (110–430) pg/ml) than in nonpregnant women (70 (60–120) pg/ml) (P < 0.001) and in normotensive pregnant women (90 (70–130) pg/ml) (P = 0.001). Nonpregnant women and normotensive pregnant women showed similar plasma levels of LXA4 (Figure 1).

We also evaluated LXA4 plasma levels in PE women according to the onset-time of clinical symptoms and compared to those levels in normotensive pregnant women matched for gestational age at the time of blood collection. LXA4 levels were higher in early PE women (210 (110–380) pg/ml) than in normotensive pregnant women with GA <34 weeks (80 (50–130) pg/ml) (P = 0.029). Pregnant women with late PE (280 (90–440) pg/ml) also showed increased levels of LXA4 compared to normotensive pregnant women with GA ≥34 weeks (100 (80–130) pg/ml) (P = 0.026). There was no significant difference in LXA4 levels comparing early PE vs. late PE and normotensive pregnant women with GA <34 weeks vs. normotensive pregnant women with GA ≥34 weeks.

Laboratory parameters

LDH activity (median (25–75% percentiles): 425 (309–552) U/L), WBC (mean ± standard deviation: 10.6 ± 3.6 × 10^3/mm^3) and platelet count (208 ± 70 × 10^3/mm^3) values were within the normal range in the PE group (Methods section). As expected, proteinuria values were above the normal range (2.9 ± 2.4 g/24 h) in these women (Methods section). These parameters were analyzed in PE women according to the onset-time of clinical symptoms and no statistical differences were detected comparing early PE and late PE (data not shown).

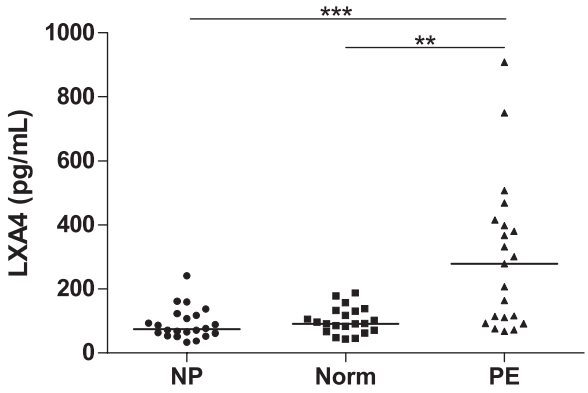


Figure 1. | LXA4 plasma levels in nonpregnant, normotensive pregnant and PE women. Horizontal bars represent median values for LXA4 (pg/ml). Plasma levels of LXA4 were higher in PE women than in normotensive pregnant and nonpregnant women. No significant differences were found comparing nonpregnant and normotensive pregnant women. Abbreviations: LXA4, lipoxin A4; NP, nonpregnant women; Norm, normotensive pregnant women; PE, preeclamptic women. $^{**}P < 0.01$, $^{***}P < 0.001$.

Correlations among LXA4, us-CRP, and clinical/laboratory parameters

In a prior study, we showed that us-CRP plasma levels were higher in PE women (5.8 (3.6–15.0) mg/L) than in nonpregnant women (0.9 (0.2–2.4) mg/L) ($P < 0.001$) and in early PE women (8.1 (2.8–13.6) mg/L) than in normotensive pregnant women with GA <34 weeks (3.2 (1.7–3.9) mg/L) ($P = 0.018$).[7] In the present study, we evaluated the correlations among LXA4, us-CRP, SBP, DBP, LDH, platelet count, proteinuria, and WBC of the studied participants. LXA4 levels showed a positive correlation with us-CRP levels, SBP and DBP only when all the participants were included in the analyses, but not in the PE group separately (Figure 2A–C). Moreover, LXA4 levels showed a positive correlation with WBC in PE women (Figure 2D). There were no statistical correlations between LXA4 levels and the other laboratory parameters analyzed in PE women.

DISCUSSION

Much evidence supports that the inflammatory response has a central role in PE pathogenesis.[5] Despite the intense investigation of proinflammatory mediators, the proresolving mechanisms have been poorly studied in the disease. In the present study, we showed that LXA4 concentration is

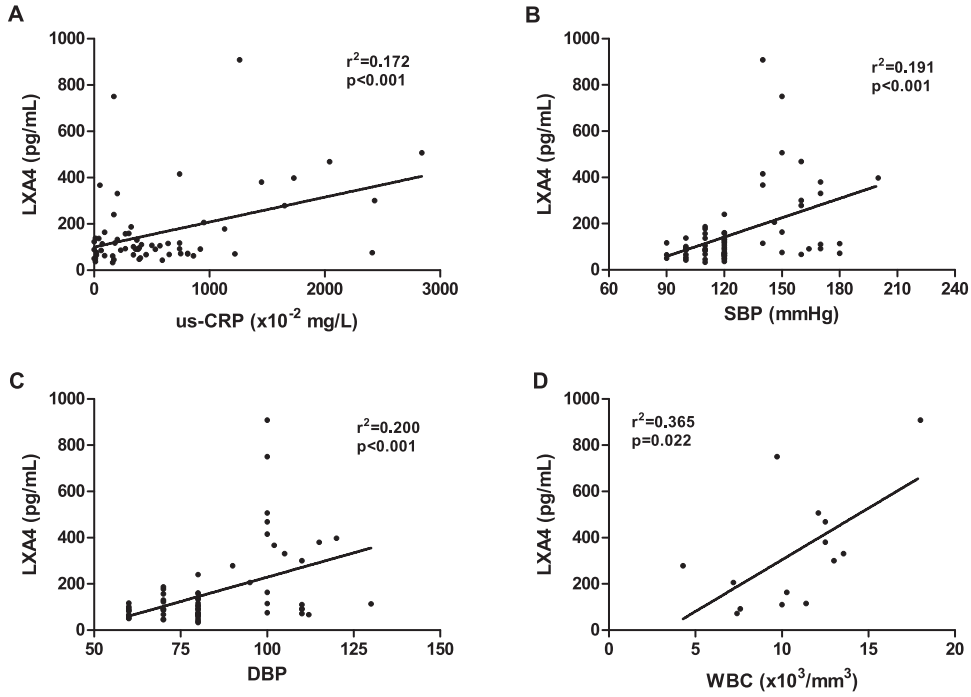


Figure 2. | Correlations among LXA4 levels, us-CRP levels, SBP, DBP, and WBC. The lines represent linear regression and the closed circles represent the participants of this study. LXA4 levels correlated positively with us-CRP levels (**A**), SBP (**B**), and DBP (**C**) when all the participants of the study were included in the analyses. LXA4 levels correlated positively with WBC in the PE group (**D**). Abbreviations: LXA4, lipoxin A4; us-CRP, ultrasensitive C-reactive protein; SBP, systolic blood pressure; DBP, diastolic blood pressure; WBC, white blood cell count.

increased in the plasma of PE women, regardless of the clinical form (early or late PE).

The inflammatory response is usually self-limiting and has a key role in maintaining tissue homeostasis, but it can progress to a chronic stage if the resolution process fails.[17] LXA4 is an endogenous eicosanoid that elicits counter-regulatory responses by interacting with its specific receptor, a G-protein-coupled receptor named lipoxin A4 receptor or formyl peptide receptor like-2 (ALX/FPR2, also known as FPRL1), hereafter referred as FPR2. LXA4 acts as an anti-inflammatory mediator by inhibiting cytokine and chemokine production, polymorphonuclear cell (PMN) activation and tissue infiltration. On the other hand, LXA4 prompts resolution of inflammation by increasing neutrophils apoptosis, non-phlogistic infiltration of monocytes and stimulating macrophage phagocytosis of apoptotic PMN.[12]

According to our review of the literature, two previous studies have shown increased circulating levels of LXA4 in PE women compared to normotensive pregnant women,[18,19] but an opposite finding was reported by Xu et al.[16] It is noteworthy that the studies involving proresolving molecules, like LXA4, in the context of PE are new, and aspects such as different populations analyzed, may affect the outcome of the proresolving response, due to different diet or hormones. Moreover, patients with diabetes, renal diseases and other co-morbidities were excluded in our study, but not in Xu et al. work, which may have biased their results. No difference in LXA4 levels was observed between nonpregnant and normotensive pregnant women in our study. However, Maldonado-Pérez *et al.*[20] reported decreased levels of LXA4 in nonpregnant women compared with normotensive pregnant women. LXA4 levels might be influenced by age and adiposity.[21,22] In Maldonado-Pérez *et al.* study, normotensive pregnant women had higher BMI than in our study (26.92 ± 2.12 vs. 22.5 (20.6–26.3) kg/m^2). In addition, nonpregnant and normotensive pregnant women seem diverge in age (25.33 ± 2.26 vs. 30.00 ± 2.67 years, respectively) and BMI (22.6 ± 0.55 vs. 26.99 ± 2.12 kg/m^2, respectively) in their work. Dong *et al.* and Huang *et al.* also measured LXA4 levels in mild and severe PE, but their results were contradictory, probably due to different GA at sample collection.[18,19] More studies with standardized methodologies and protocols of patient selection should be conducted in order to clarify these divergent findings.

To the best of our knowledge, no study has evaluated the differential expression of LXA4 in the placenta of normotensive and preeclamptic women. It would be also of great value the measurement of the enzymes involved in LXA4 synthesis such as 15-lipoxygenase-2. These issues are under investigation in our lab. We propose that LXA4 levels are increased in PE women in an attempt to attenuate the exacerbated inflammatory response in these women. Indeed, in our study LXA4 levels were positively correlated with WBC in PE women. In addition, there was a positive correlation between LXA4 and us-CRP, but this correlation failed to reach statistical significance in the PE group, probably due to its small sample size. An inverse correlation between LXA4 and CRP levels was reported in chronic heart failure and asthma.[23,24] Although PE, chronic heart failure and asthma are chronic inflammatory diseases, the mechanisms of immune regulation might differ among them.

Nevertheless, LXA4 concentration seems to be insufficient to attenuate inflammation in PE, because these women show features of systemic inflammatory response despite increased levels of LXA4. In a recent study, we showed that annexin A1 (AnxA1) levels are increased in PE women.[7] AnxA1 is a protein that is also endowed with anti-inflammatory and proresolving properties.[25] Chronic inflammation in PE, despite high levels of proresolving mediators, such as LXA4 and AnxA1, suggests a failure in the engagement of these resolution pathways, which might be a consequence of decreased expression of their receptors or defective engagement on them. Both LXA4 and AnxA1 activate and signal via FPR2 receptor.[26] In two previous studies, FPR2 mRNA placental expression was decreased in PE women compared with normotensive pregnant women,[16,19] although another study reported an opposite result.[18] Increased inactivation of LXA4 and AnxA1 could also interfere with their bioactions. LXA4 can be inactivated by dehydrogenases and oxidoreductases, while AnxA1 can be inactivated by proteolytic enzymes, such as proteinase 3 and neutrophil elastase, in an inflammatory milieu.[27–29] Indeed, placentas from PE women are rich of proteases, such as neutrophil elastase.[30] Based on these studies, we suggest that LXA4 and AnxA1 increase in the circulation of PE women could function as a compensatory mechanism to resolve inflammation, which may not be effective. Further studies are necessary to confirm whether and how these dysfunctional resolution mechanisms operate in PE.

Finally, LXA4 plasma levels were correlated positively with both SBP and DBP in the present study. However, these correlations failed to reach statistical significance when only the PE group was analyzed, probably due to the small sample size of this group. Hypertension, the main feature of PE, results from systemic endothelial dysfunction.[2] Besides its well-described immunomodulatory actions, LXA4 might also play an important role on vascular integrity. ATL suppress reactive oxygen species in endothelial cells and enhances oxide nitric generation.[31,32] Indeed, experimental PE rats treated with a synthetic analogue of LXA4 had their SBP and proinflammatory cytokines levels reduced.[33] Thus, LXA4 levels could be increased in PE in an attempt to attenuate not only inflammation, but also endothelial dysfunction. However, LXA4 increase seems to be insufficient to do that, probably due to inadequate LXA4 action, as previously discussed. LXA4 role in vascular integrity in PE should be better investigated in future studies. Although high LDH activity and low platelet count have been associated with a poor clinical outcome in PE, no differences were detected between early and late PE, and theses parameters were not correlated with LXA4 levels in our study. Proteinuria levels were not correlated with LXA4 levels and were also similar between early and late PE, reaffirming the weak association between proteinuria and PE prognosis.

The interpretation of our findings was limited by the relative small sample size of the study. Hence, the correlation analyses among LXA4, us-CRP, SBP, and DBP considering only PE women was probably prejudiced. Another limitation of this study was that the data about the laboratory parameters were only available for the PE group, which precluded us to evaluate the association between LXA4 levels and these

parameters in normotensive pregnant and nonpregnant women. In addition, we did not explore the potential causes (e.g., altered cellular expression of FPR2) underlying LXA4 apparently lack of effectiveness to resolve inflammation in PE women. This will be a matter of investigation in future studies of our research group.

We did not find any studies in the literature reporting the effects on LXA4 levels of the antihypertensives (nifedipine, methyldopa, and hydralazine) and anticonvulsivants (magnesium sulfate) used by PE women in our study. Glucocorticoids (GCs) may inhibit LXA4 synthesis.[34,35] GCs were prescribed only for PE women in our study, in 44% of them. LXA4 levels were analysed in PE women that had or not GC prescription and no difference was detected between them (data not shown). Moreover, LXA4 levels were higher in PE women in our study. Thus, we believe that GCs did not interfere significantly in this result.

Our data suggest that LXA4 may participate in PE pathogenesis. Increased LXA4 plasma levels in PE women, despite the exacerbated inflammatory response observed in these women, suggest a failure in the engagement of this resolution pathway. To the best of our knowledge, this is the first study that evaluated the association between LXA4 plasma levels and clinical/laboratory parameters of PE women. Moreover, this is the first report on LXA4 levels in preeclamptic Brazilian women. Further studies are necessary to investigate the role of LXA4 and other pro-resolving molecules in PE.

ACKNOWLEDGMENTS

This study was supported by grants from Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG).

DISCLOSURE

The authors declared no conflict of interest.

REFERENCES

1. Steegers EA, von Dadelszen P, Duvekot JJ, Pijnenborg R. Pre-eclampsia. *Lancet* 2010; 376:631–644.
2. Chaiworapongsa T, Chaemsaithong P, Yeo L, Romero R. Pre-eclampsia part 1: current understanding of its pathophysiology. *Nat Rev Nephrol* 2014; 10:466–480.
3. American College of Obstetricians and Gynecologists (ACOG). Hypertension in pregnancy. Report of the American College of Obstetricians and Gynecologists' Task Force on Hypertension in Pregnancy. *Obstet Gynecol*. 2013;122:1122–1131.
4. Soto E, Romero R, Kusanovic JP, Ogge G, Hussein Y, Yeo L, Hassan SS, Kim CJ, Chaiworapongsa T. Late-onset preeclampsia is associated with an imbalance of angiogenic and anti-angiogenic factors in patients with and without placental lesions consistent with maternal underperfusion. *J Matern Fetal Neonatal Med* 2012; 25:498–507.
5. Ramma W, Ahmed A. Is inflammation the cause of pre-eclampsia? *Biochem Soc Trans* 2011; 39:1619–1627.
6. Pinheiro MB, Martins-Filho OA, Mota AP, Alpoim PN, Godoi LC, Silveira AC, Teixeira-Carvalho A, Gomes KB, Dusse LM. Severe preeclampsia goes along with a cytokine network disturbance towards a systemic inflammatory state. *Cytokine* 2013; 62:165–173.
7. Perucci LO, Carneiro FS, Ferreira CN, Sugimoto MA, Soriani FM, Martins GG, Lima KM, Guimarães FL, Teixeira AL, Dusse LM, Gomes KB, Sousa LP. Annexin A1 is increased in the plasma of preeclamptic women. *PLoS One* 2015; 10:e0138475.
8. Farzadnia M, Ayatollahi H, Hasan-Zade M, Rahimi HR. A comparative study of serum level of vascular cell adhesion molecule-1 (sVCAM-1), intercellular adhesion molecule-1(ICAM-1) and high sensitive C-reactive protein (hs-CRP) in normal and pre-eclamptic pregnancies. *Iran J Basic Med Sci* 2013; 16:689–693.
9. Teran E, Escudero C, Moya W, Flores M, Vallance P, Lopez-Jaramillo P. Elevated C-reactive protein and pro-inflammatory cytokines in Andean women with pre-eclampsia. *Int J Gynaecol Obstet* 2001; 75:243–249.
10. Fiore S, Serhan CN. Formation of lipoxins and leukotrienes during receptor-mediated interactions of human platelets and recombinant human granulocyte/macrophage colony-stimulating factor-primed neutrophils. *J Exp Med* 1990; 172:1451–1457.
11. Serhan CN, Sheppard KA. Lipoxin formation during human neutrophil-platelet interactions. Evidence for the transformation of leukotriene A4 by platelet 12-lipoxygenase *in vitro*. *J Clin Invest* 1990; 85:772–780.
12. Schwab JM, Serhan CN. Lipoxins and new lipid mediators in the resolution of inflammation. *Curr Opin Pharmacol* 2006; 6:414–420.
13. von Dadelszen P, Magee LA, Roberts JM. Subclassification of preeclampsia. *Hypertens Pregnancy* 2003; 22:143–148.
14. Obesity: preventing and managing the global epidemic. Report of a WHO consultation. *World Health Organ Tech Rep Ser*. 2000;894:i-xii, 1–253.
15. Abbassi-Ghanavati M, Greer LG, Cunningham FG. Pregnancy and laboratory studies: a reference table for clinicians. *Obstet Gynecol* 2009; 114:1326–1331.
16. Xu Z, Zhao F, Lin F, Xiang H, Wang N, Ye D, Huang Y. Preeclampsia is associated with a deficiency of lipoxin A4, an endogenous anti-inflammatory mediator. *Fertil Steril* 2014; 102:282.e4–290.e4.
17. Serhan CN, Savill J. Resolution of inflammation: the beginning programs the end. *Nat Immunol* 2005; 6:1191–1197.
18. Dong W, Yin L. Expression of lipoxin A4, TNFα and IL-1β in maternal peripheral blood, umbilical cord blood and placenta, and their significance in pre-eclampsia. *Hypertens Pregnancy* 2014; 33:449–456.
19. Huang LL, Su S, Awale R, Zhang XY, Zhong LL, Tang H. Expression of anti-inflammatory mediator lipoxin A4 and inflammation responsive transcriptive factors NF-kappa B in patients with preeclampsia. *Clin Exp Obstet Gynecol* 2014; 41:561–566.
20. Maldonado-Pérez D, Golightly E, Denison FC, Jabbour HN, Norman JE. A role for lipoxin A4 as anti-inflammatory and proresolution mediator in human parturition. *FASEB J* 2011; 25:569–575.
21. Gangemi S, Pescara L, D'Urbano E, Basile G, Nicita-Mauro V, Davì G, Romano M. Aging is characterized by a profound reduction in anti-inflammatory lipoxin A4 levels. *Exp Gerontol* 2005; 40:612–614.
22. Yu D, Xu Z, Yin X, Zheng F, Lin X, Pan Q, Li H. Inverse relationship between serum lipoxin A4 level and the risk of metabolic syndrome in a middle-aged Chinese population. *PLoS One* 2015; 10:e0142848.
23. Reina-Couto M, Carvalho J, Valente MJ, Vale L, Afonso J, Carvalho F, Bettencourt P, Sousa T, Albino-Teixeira A. Impaired resolution of inflammation in human chronic heart failure. *Eur J Clin Invest* 2014; 44:527–538.
24. Wu SH, Yin PL, Zhang YM, Tao HX. Reversed changes of lipoxin A4 and leukotrienes in children with asthma in different severity degree. *Pediatr Pulmonol* 2010; 45:333–340.
25. D'Acquisto F, Perretti M, Flower RJ. Annexin-A1: a pivotal regulator of the innate and adaptive immune systems. *Br J Pharmacol* 2008; 155:152–169.
26. Perretti M, Chiang N, La M, Fierro IM, Marullo S, Getting SJ, Solito E, Serhan CN. Endogenous lipid- and peptide-derived anti-inflammatory pathways generated with glucocorticoid and aspirin treatment activate the lipoxin A4 receptor. *Nat Med* 2002; 8:1296–1302.
27. Maddox JF, Serhan CN. Lipoxin A4 and B4 are potent stimuli for human monocyte migration and adhesion: selective inactivation by dehydrogenation and reduction. *J Exp Med* 1996; 183:137–146.
28. Vong L, D'Acquisto F, Pederzoli-Ribeil M, Lavagno L, Flower RJ, Witko-Sarsat V, Perretti M. Annexin 1 cleavage in activated neutrophils: a pivotal role for proteinase 3. *J Biol Chem* 2007; 282:29998–30004.

29. Rescher U, Goebeler V, Wilbers A, Gerke V. Proteolytic cleavage of annexin 1 by human leukocyte elastase. *Biochim Biophys Acta* 2006; 1763:1320–1324.
30. Salama RH, Fathalla MM, Mekki AR, Elsadek Bel-K. Implication of umbilical cord in preeclampsia. *Med Princ Pract* 2011; 20:124–128.
31. Nascimento-Silva V, Arruda MA, Barja-Fidalgo C, Fierro IM. Aspirin-triggered lipoxin A4 blocks reactive oxygen species generation in endothelial cells: a novel antioxidative mechanism. *Thromb Haemost* 2007; 97:88–98.
32. Paul-Clark MJ, Van Cao T, Moradi-Bidhendi N, Cooper D, Gilroy DW. 15-Epi-lipoxin A4-mediated induction of nitric oxide explains how aspirin inhibits acute inflammation. *J Exp Med* 2004; 200:69–78.
33. Lin F, Zeng P, Xu Z, Ye D, Yu X, Wang N, Tang J, Zhou Y, Huang Y. Treatment of lipoxin A(4) and its analogue on low-dose endotoxin induced preeclampsia in rat and possible mechanisms. *Reprod Toxicol* 2012; 34:677–685.
34. Zhang D, Li Y, Peng H, Liu H, Cheng Q, Cheng X, Zeng P, Wu P, Chen H, Huang Y, Ye D. Glucocorticoids sensitize rat placental inflammatory responses via inhibiting lipoxin A4 biosynthesis. *Biol Reprod* 2014; 90:74.
35. Bhavsar PK, Levy BD, Hew MJ, Pfeffer MA, Kazani S, Israel E, Chung KF. Corticosteroid suppression of lipoxin A4 and leukotriene B4 from alveolar macrophages in severe asthma. *Respir Res* 2010; 11:71.

4.3 CAPÍTULO 3 - "Decreased plasma concentrations of brain derived neurotrophic factor in preeclampsia"

Clinica Chimica Acta 464 (2017) 142–147

Contents lists available at ScienceDirect

Clinica Chimica Acta

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

Decreased plasma concentrations of brain-derived neurotrophic factor in preeclampsia

Luiza Oliveira Perucci [a,b], Érica Leandro Marciano Vieira [c], Antônio Lúcio Teixeira [c], Karina Braga Gomes [a,b], Luci Maria Dusse [a,b], Lirlândia Pires Sousa [a,b,*]

[a] Departamento de Análises Clínicas e Toxicológicas, Faculdade de Farmácia, Universidade Federal de Minas Gerais, Belo Horizonte, Minas Gerais, Brazil
[b] Programa de Pós-Graduação em Análises Clínicas e Toxicológicas, Universidade Federal de Minas Gerais, Belo Horizonte, Minas Gerais, Brazil
[c] Laboratório Interdisciplinar de Investigação Médica, Faculdade de Medicina, Universidade Federal de Minas Gerais, Belo Horizonte, Minas Gerais, Brazil

ARTICLE INFO

Article history:
Received 27 September 2016
Received in revised form 21 November 2016
Accepted 21 November 2016
Available online 22 November 2016

Keywords:
Brain-derived neurotrophic factor
Annexin A1
Preeclampsia

ABSTRACT

Background: Preeclampsia (PE) is a disease characterized by excessive maternal inflammatory response. Early studies suggested that brain-derived neurotrophic factor (BDNF) modulates inflammation. The main objective of this study was to investigate BDNF plasma concentrations in PE women and to compare with BDNF concentrations from normotensive pregnant women. We also investigated the association among the plasma concentrations of BDNF and inflammatory mediators, and maternal clinical features.
Methods: BDNF plasma concentrations were measured by ELISA in 38 PE women (17 early onset and 21 late onset) and in 20 normotensive pregnant women (Norm) matched for gestational age (Norm < 34 weeks: $n = 8$; Norm ≥ 34 weeks: $n = 12$). Correlation analyses between laboratory parameters and clinical characteristics were evaluated through Spearman's coefficients.
Results: BDNF concentration was lower in PE women than in normotensive pregnant women, but no difference was detected between the subgroups of PE women and normotensive pregnant women. BDNF correlated negatively with annexin A1, and positively with body mass index and diastolic blood pressure. No correlation was significant in normotensive pregnant women.
Conclusions: Lower BDNF plasma concentrations and cross-talk between BDNF and AnxA1 signaling pathways might be involved in PE pathogenesis.

1. Introduction

Preeclampsia (PE) is a hypertensive and multi-system disease of pregnancy that represents one of the leading causes of maternal and fetal morbidity/mortality worldwide [1]. Although its pathophysiology is not fully elucidated, a series of evidence suggests that defective placentation is the initiating event which contributes to systemic endothelial dysfunction, oxidative stress and inflammation [2]. PE can be classified according to the gestational age (GA) of clinical symptoms development in early onset (GA < 34 weeks) and late onset (GA ≥ 34 weeks) [3].

Brain-derived neurotrophic factor (BDNF) is a growth factor that belongs to the neurotrophin family, and is abundantly expressed in the central and peripheral nervous systems. BDNF signals through tyrosine kinase B (TrkB) receptor to regulate neuronal development, function and plasticity [4]. BDNF is also expressed in non-neuronal tissues [5]. In addition to neuroprotective effects, BDNF stimulates angiogenesis, placental development and fetal growth [6,7]. It has also been shown that BDNF expression is modulated by oxidative stress and inflammation [8,9]. Conversely, BDNF is able to modulate inflammatory responses [10–12]. Therefore, altered concentrations of BDNF could contribute to PE pathogenesis.

Previous studies that evaluated BDNF circulating concentrations in PE women have reported either lower, higher or similar concentrations when compared with normotensive pregnant women [13–16]. We aimed to investigate BDNF plasma concentrations in women with early onset PE and late onset PE and in normotensive pregnant women matched for gestational age and socioeconomic background. We also analyzed the relationship among the concentration of BDNF, inflammatory molecules (soluble tumor necrosis factor receptor-1 - sTNF-R1 and annexin A1 - AnxA1) evaluated in previous studies [17,18] and maternal clinical features in order to better understand the role of BDNF in PE pathogenesis.

Abbreviations: AnxA1, annexin A1; BDNF, brain-derived neurotrophic factor; DBP, diastolic blood pressure; GA, gestational age; GWG, gestational weight gain; Norm, normotensive pregnant women; PE, preeclampsia/preeclamptic; SBP, systolic blood pressure; sTNF-R1, soluble tumor necrosis factor receptor-1; TNF-α, tumor necrosis factor alpha; TrkB, tyrosine kinase B.

* Corresponding author at: Av. Antonio Carlos, 6627 - Pampulha, 31270-901 Belo Horizonte, Minas Gerais, Brazil.
E-mail address: lipsousa72@gmail.com (L.P. Sousa).

http://dx.doi.org/10.1016/j.cca.2016.11.034
0009-8981/

2. Materials and methods

2.1. Ethics

The procedures in this study were in accordance with Ethics Committees of Universidade Federal de Minas Gerais and the participating hospitals (Santa Casa de Misericórdia de Belo Horizonte: Fundação Hospitalar do Estado de Minas Gerais; Hospital Municipal Odilon Behrens), and a written informed consent was obtained from each participant.

2.2. Patients

This study included 38 PE women and 20 normotensive pregnant women in the third trimester of pregnancy who were recruited from Brazilian public hospitals. PE women were stratified in early onset PE ($n = 17$) and in late onset PE ($n = 21$) subgroups [3]. Normotensive pregnant women were stratified in 2 subgroups considering the cut-off of 34 weeks (Norm < 34 weeks: $n = 8$; Norm ≥ 34 weeks: $n = 12$) to match the subgroups of PE women.

PE was defined by systolic and diastolic blood pressure ≥ 140/90-mm Hg after 20 weeks of gestation in a previously normotensive women, confirmed by 2 consecutive readings at least 4 h apart in association with proteinuria (≥300 mg/24 h or ≥1 + reading on dipstick in a random urine specimen) and/or evidence of end-organ dysfunction (thrombocytopenia, renal insufficiency, impaired liver function, pulmonary edema, cerebral or visual disturbances) [1]. Normotensive pregnant women had blood pressure < 120/80 mm Hg and no history of hypertension. All women were matched according to socioeconomic status. The exclusion criteria for both groups were: chronic hypertension, obesity (grades II and III) [19], diabetes, cancer, homeostatic abnormalities, infectious, cardiovascular, autoimmune, renal, hepatic, psychiatric and neurological diseases.

2.3. Sample collection, processing and storing

Five milliliters of maternal venous blood were collected in EDTA anticoagulant-coated tubes (BD Vacutainer). The blood was centrifuged at 3000*g* for 15 min at room temperature to separate the plasma. The plasma aliquots were stored at −80 °C until analyses.

2.4. BDNF measurement

BDNF plasma concentrations were measured by ELISA using a commercial available kit (R&D Systems) according to the manufacturer's instructions and were reported as pg/ml. The BDNF antibody used in this assay detects human BDNF in ELISA, and no cross-reactivity or interference was observed with recombinant human glial cell-derived neurotrophic factor, β-nerve growth factor, neurotrofin 3 or neurotrofin 4.

2.5. Statistical analysis

The data were analyzed using SPSS software ver 19.0. The normality of continuous variables was assessed using Shapiro-Wilk's W-test. Continuous variables not normally distributed were analyzed by Kruskal-Wallis test. When differences were detected among the groups, they were compared 2 × 2 with the Mann-Whitney *U* test or Mann-Whitney *U* test followed by Bonferroni's correction (4 groups). The comparison of continuous variables with normal distribution was performed by analysis of variance (ANOVA) test with *post hoc* LSD test (4 groups) or Student's *t*-test (2 groups). The comparison of categorical variables was performed by Pearson χ^2 test. Parametric data were expressed as mean ± SD, non-parametric data as median (25th–75th percentiles) and categorical variables as absolute number (percentage). Spearman's correlation coefficients (r_s) were used to investigate the possible correlations among the plasma concentrations of BDNF and inflammatory mediators evaluated in previous studies [17,18], and clinical parameters in PE women and in normotensive pregnant women. A *P*-value <0.05 denoted statistical significance.

3. Results

3.1. Clinical characteristics

Table 1 shows the clinical characteristics of the studied groups. No significant difference was detected in age, body mass index (BMI) before pregnancy, gestational weight gain (GWG) and GA at blood collection between normotensive pregnant women and PE women. PE group had lower number of gestations ($P = 0.009$) and higher number of primiparas ($P = 0.013$) than normotensive group. As expected, systolic and diastolic blood pressures (SBP and DBP, respectively) were significantly increased in PE women (all $P < 0.001$). There was no significant difference in educational degree between the groups.

The clinical characteristics of the subgroups of normotensive pregnant women and PE women are displayed in Table 2. No differences were found for age, BMI before pregnancy, number of gestations, number of primiparas and educational degree among the subgroups. Pregnant women with late onset PE had higher GWG than normotensive pregnant women with GA < 34 weeks ($P = 0.004$). As expected, GA at blood collection was higher in late onset PE when compared with early onset PE and normotensive pregnant women with GA < 34 weeks, and in normotensive pregnant women with GA ≥ 34 weeks when compared with early onset PE and normotensive pregnant women with GA < 34 weeks (all $P < 0.001$). In addition, SBP and DBP were higher in early onset PE and late onset PE when compared with normotensive pregnant women with GA < 34 weeks and normotensive pregnant women with GA ≥ 34 weeks (all $P < 0.001$). No participant in this study was illiterate or had completed higher education.

3.2. BDNF plasma concentrations

BDNF plasma concentrations were lower in PE women [2970 (2021–5403) pg/ml] than in normotensive pregnant women [4913 (2548–9551) pg/ml] ($P = 0.029$) (Fig. 1). No significant difference was

Table 1
Clinical characteristics of normotensive pregnant women and PE women.

Variables	Norm ($n = 20$)	PE ($n = 38$)	*P*
Age (y)[a]	23 (19–27)	26 (21–29)	0.325
BMI (kg/m^2)[b]	22.4 ± 3.5	23.5 ± 2.9	0.872
GWG (kg)[a]	10.4 (8.5–12.7)	12.5 (9.3–18.7)	0.062
GA (weeks)[a]	35 (30–39)	34 (32–38)	0.658
Parity			
Gravidity (n)[a]	2 (1–3)	1 (1–2)	0.009
Primiparas (%)[c]	5 (25)	23 (61)	0.013*
SBP (mm Hg)[a]	110 (100−110)	160 (150–170)	<0.001***
DBP (mm Hg)[a]	70 (70–70)	102 (100−111)	<0.001***
Education[c]			0.094††
Informed (%)	20 (100)	28 (74)	
Elementary school (%)†	1 (5)	4 (14)	
Middle school (%)†	11 (55)	7 (25)	
High school (%)†	8 (40)	17 (61)	
Not informed (%)	0 (0)	10 (26)	

Abbreviations: BMO before pregnancy; GWG, gestational weight gain; GA, gestational age at blood collection; n, number/sample size; SBP, systolic blood pressure; DBP, diastolic blood pressure; Norm, normotensive pregnant women; PE, preeclamptic women.
[a] Mann-Whitney *U* test; data are presented as median (25th–75th percentiles).
[b] Student's *t*-test; data are presented as mean ± SD.
[c] Pearson χ^2 test; data are presented as number (percentage).
* $P < 0.05$.
*** $P < 0.001$.
† The percentage of each educational variable was calculated considering the total of patients who informed their educational degree in each group.
†† The analysis of education considered only patients who informed their educational degree.

Table 2
Clinical characteristics of the subgroups of normotensive pregnant women and PE women.

Variables	Norm < 34 wks ($n = 8$)	Norm ≥ 34 wks ($n = 12$)	Early onset PE ($n = 17$)	Late onset PE ($n = 21$)
Age (y)[1]	22 (19–26)	24 (19–30)	24 (20 − 30)	26 (21–29)
BMI (kg/m^2)[2]	21.1 ± 2.4	23.4 ± 3.9	23.6 ± 2.7	23.4 ± 3.1
GWG (kg)[1]	9.4 (6.4–11.0)[a]	12.3 (9.0–15.7)	12.0 (7.5–16.2)	14.0 (10.3–22.8)
GA (weeks)[1]	30 (29–31)[a,b]	39 (36–40)[c]	31 (30 − 33)	37 (34–39)
Parity				
Gravidity (n)[1]	2 (1–3)	2 (2–3)	1 (1–2)	1 (1–2)
Primiparas (%)[3]	3 (38)	2 (17)	10 (59)	13 (62)
SBP (mm Hg)[1]	110 (100–110)[a,d]	110 (100–110)[c,e]	170 (160–180)	155 (140–170)
DBP (mm Hg)[1]	70 (70–70)[a,d]	70 (70–70)[c,e]	110 (100 − 120)	100 (100–110)
Education[3]				
Informed (%)	8 (100)	12 (100)	13 (76)	15 (71)
Elementary school (%)[+]	0 (0)	1(8)	1 (8)	3 (20)
Middle school (%)[+]	5 (63)	6 (50)	5 (38)	2 (13)
High school (%)[+]	3 (37)	5 (42)	7 (54)	10 (67)
Not informed (%)	0 (0)	0 (0)	4 (24)	6 (29)

Abbreviations: GWG, gestational weight gain; GA, gestational age at blood collection; n, number/sample size; SBP, systolic blood pressure; DBP, diastolic blood pressure; Norm, normotensive pregnant women; wks, weeks; PE, preeclamptic women.
[1] Kruskal-Wallis/Mann-Whitney *U* test with Bonferroni's correction; data are presented as median (25th–75th percentiles).
[2] ANOVA test with *post hoc* LSD test; data are presented as mean ± standard deviation.
[3] Pearson χ^2 test. Data are presented as number (percentage).
[a] $P < 0.0125$ (norm < 34 wks *vs.* late onset PE).
[b] $P < 0.0125$ (norm < 34 wks *vs.* norm ≥ 34 wks).
[c] $P < 0.0125$ (norm ≥ 34 wks *vs.* early onset PE).
[d] $P < 0.0125$ (norm < 34 wks *vs.* early onset PE).
[e] $P < 0.0125$ (norm ≥ 34 wks *vs.* late onset PE).
[+] The percentage of each educational variable was calculated considering the total of patients who informed their educational degree in each subgroup. The analysis of education considered only patients who informed their educational degree.

detected between the subgroups of early onset PE [3651 (2327–6575) pg/ml] *vs.* late onset PE [2548 (1748–5024) pg/ml], early onset PE *vs.* norm < 34 weeks [6803 (3543–9551) pg/ml], late onset PE *vs.* norm ≥ 34 weeks [4212 (2240–10,090) pg/ml] and norm < 34 weeks *vs.* norm ≥ 34 weeks.

3.3. Correlations among BDNF, sTNF-R1, AnxA1 and clinical characteristics

In previous studies from our group, PE women had higher plasma concentrations of sTNF-R1 and AnxA1 than normotensive pregnant women [17,18]. Considering the possible role of BDNF in modulating inflammatory responses [10–12], we evaluated the potential association between the plasma concentrations of BDNF and these 2 inflammatory molecules in PE women and in normotensive pregnant women. BDNF showed a negative correlation with AnxA1, but no significant correlation was detected between BDNF and sTNF-R1 in PE women. BDNF also correlated positively with BMI and DBP in these women. There were no further statistical correlations among BDNF and other clinical parameters in PE women. No correlation was significant in normotensive pregnant women. The significant correlations are shown in Fig. 2.

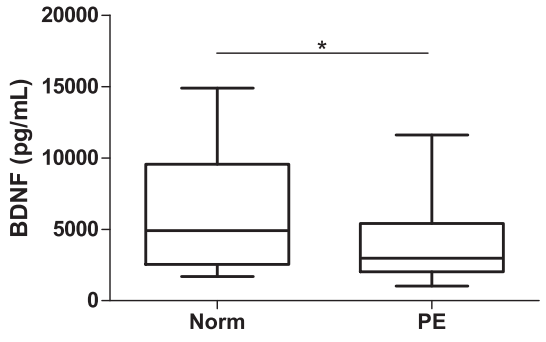


Fig. 1. BDNF plasma concentrations in normotensive pregnant women and in PE women. Lines within the boxes represent the median values for BDNF; top and bottom lines of the boxes represent 25th and 75th percentiles, and upper and lower bars outside the boxes represent maximum and minimum values, respectively. BDNF concentrations are expressed as pg/ml (picograms/ml). Plasma concentrations of BDNF were lower in PE women than in normotensive pregnant women. Abbreviations: BDNF, brain-derived neurotrophic factor; Norm, normotensive pregnant women; PE, preeclamptic women. *$P < 0.05$ (Mann-Whitney *U* test).

4. Discussion

We found lower plasma concentrations of BDNF in PE women when compared with normotensive pregnant women. These findings are in agreement with 2 previous studies [13,14], although higher or similar plasma concentrations of BDNF have also been reported [15,16]. It is noteworthy that BDNF circulating concentrations can be influenced by health-related lifestyle, like cigarettes and alcohol use [20–22]. The divergent results in the literature might also have been biased by differences in dietary habits of the studied populations, which are also known to influence BDNF concentrations.

Down-regulation of placental BDNF gene expression has been reported in PE women [14]. Decreased BDNF concentrations in PE women might be a consequence of defective immune and oxidant/antioxidant mechanisms along with decreased concentrations of endogenous omega-3 fatty acids [8,9,23]. Deficient BDNF concentrations might interfere with angiogenesis, placental development and fetal growth, therefore this neurotrophin has the potential to be involved in PE pathogenesis [6,7]. Moreover, Postma et al. suggested that persistent low BDNF circulating concentrations after pregnancy in women who had PE might be associated with maternal cognitive impairment later in life [24].

PE is a complex disease with variable clinical presentations and pathological features. The disease is classified based on the symptom severity (mild PE and severe PE) and, more recently, on the time of clinical symptoms onset (early onset PE *vs.* late onset PE; preterm PE: <37 weeks *vs.* term PE: ≥37 weeks) [1,3,25]. Much evidence suggests that the sooner the symptoms manifest, usually the worse is the prognosis, corroborating PE classification according to gestational age of clinical symptoms onset [26]. Nevertheless, BDNF plasma concentrations were not significantly different between early and late onset clinical forms in the current study. In addition, D'Souza and coworkers did not report differences in maternal BDNF plasma concentrations between PE women delivering preterm and term PE [13]. Sahay et al.

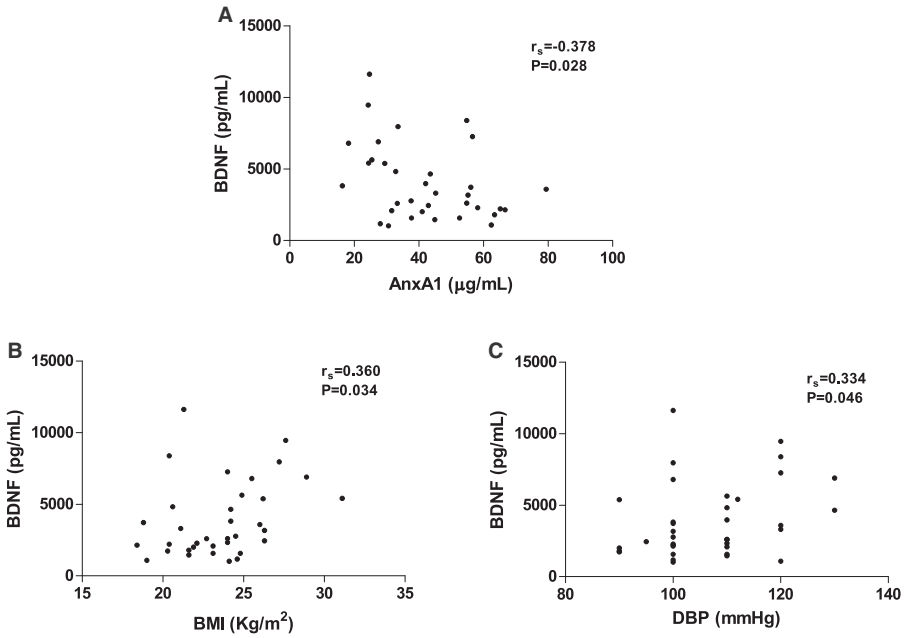


Fig. 2. Significant correlations among BDNF concentrations, AnxA1 concentrations, BMI and DBP in PE women. The closed circles represent the participants in this study. BDNF concentrations correlated negatively with AnxA1 concentrations (A) and positively with BMI before pregnancy (B) and DBP (C) in PE women. Correlation analyses were evaluated by Spearman's correlation coefficients (r_s). Abbreviations BDNF, brain-derived neurotrophic factor; AnxA1, annexin A1; BMI, body mass index; DBP, diastolic blood pressure.

evaluated BDNF protein expression in different regions of human placenta (central maternal, central fetal, peripheral maternal and peripheral fetal) and reported similar BDNF expression between preterm and term PE in each one of these regions. However, BDNF was up-regulated in central maternal region of placenta in preterm PE women when compared with normotensive pregnant women [27]. These data suggest that BDNF circulating concentrations may reflect its placental expression in term PE, but not in preterm PE, and that both placental and circulating BDNF cannot discriminate between early onset PE and late onset PE, as well as preterm PE and term PE.

It has been suggested that BNDF controls inflammation by modulating pro-inflammatory mediators production [28,29]. This is the first study evaluating the association among the plasma concentrations of BDNF, sTNF-R1 and AnxA1 in PE women. In previous studies from our group, PE women had higher plasma concentrations of sTNF-R1 [3479 (3182–4339) *vs.* 3028 (2468–3606) pg/ml, $P = 0.014$] and AnxA1 [43.2 (30.8–57.8) *vs.* 30.1 (19.0–35.7) μg/ml] ($P = 0.026$)] than normotensive pregnant women [17,18].

AnxA1 is a glucocorticoid (GC)-regulated protein endowed with anti-inflammatory actions and that promotes resolution of inflammation [30]. BDNF plasma concentrations correlated negatively with AnxA1 plasma concentrations in PE women in the current study. We hypothesized that AnxA1 plasma concentrations may be increased in PE women who have decreased BDNF plasma concentrations as a compensatory mechanism aiming to temper systemic inflammation. Nineteen (83%) early PE women had a prescription of GC prior to blood collection. We investigated AnxA1 concentrations between early PE women that had [median (25th–75th percentiles): 43.5 (33.0–61.1) μg/ml] or not [49.7 (38.8–56.6) μg/ml] GC prescription, but no significant difference was detected between them. Thus, AnxA1 plasma concentrations were not significantly influenced by GC administration in our study.

It is well established that TNF-R1 has the ability to bind to tumor necrosis factor alpha (TNF-α) and neutralize the effects of this pro-inflammatory cytokine. sTNF-R1 is regarded as an indirect marker of inflammation as it is usually increased in inflammatory conditions characterized by exaggerated TNF-α production, like PE [31,32]. Our group has previously demonstrated that TNF-α and sTNF-R1 plasma concentrations were increased in PE women, which was reinforced by other studies [17,32–34]. Considering that systemic inflammation is exacerbated in PE [2] and that PE women showed decreased BDNF and increased sTNF-R1 plasma concentrations in our studies [17], it was expected a negative correlation between sTNF-R1 and BDNF in the PE women. However, this correlation failed to reach statistical significance. As other pro-inflammatory molecules, like interleukin-1β and lipopolysaccharide, downregulate BDNF expression *in vivo* [35], it can inferred that distinct inflammatory mechanisms may regulate BDNF synthesis and sTNF-R1 concentrations in PE. However, more studies are necessary to clarify how inflammation regulates BDNF concentrations in PE women.

Despite being widely expressed in neurons of the central and peripheral nervous systems, BDNF is also expressed in tissues of gastrointestinal, cardiorespiratory and urogenital systems, especially in epithelial cells [5]. BDNF pattern of expression and ability to modulate synaptic transmission implicate this neurotrophin in regulating cardiovascular responses, such as blood pressure [36]. Indeed, results from *in vivo* experiments indicate that BDNF treatment increases arterial blood pressure by up-regulating angiotensin type-1 receptor and that aortic BDNF up-regulation precedes the development of hypertension in spontaneously hypertensive rats [37,38]. Accordingly, our data showed that among PE women, those with higher BDNF concentrations had higher DBP. By contrast, D'Souza et al. found no association between BDNF concentrations and blood pressure in PE women, while a negative correlation was found between BDNF concentrations and SBP in normotensive pregnant women [14]. These divergences can be explained by differences in blood pressure concentrations between the studied populations. For instance, D'Souza et al. evaluated PE women with lower blood pressure [mean arterial blood pressure at delivery = 110 ± 14 mm Hg] than in our study [121 (117–131) mm Hg]. The normotensive pregnant women in D'Souza et al.'s study also presented higher blood pressure (91 ± 7 mm Hg) comparing to normotensive pregnant women included in our study [83 (80–83) mm Hg].

Our data also showed that BDNF plasma concentrations correlated positively with pre-pregnancy BMI in PE women, but Bienertova-Vasku et al. did not find this correlation [16]. Several lines of evidence suggest that BDNF regulates energy homeostasis by modulating eating behavior and glucose metabolism in peripheral tissues [39]. Lebrun et

al. reported that BDNF treatment reduced food intake and impaired BDNF/TrkB signaling *in vivo* which was associated with hyperphagia and obesity [40]. Roth et al. revealed that BDNF concentrations were higher in patients with obesity than in normal weight subjects and correlated positively with BMI [41]. Altogether these data suggest a compensatory increase in BDNF concentrations in obesity aiming to regulate weight and food intake, probably due to impaired BDNF/TrkB signaling. A positive correlation between BDNF plasma concentrations and BMI has also been reported in patients with bipolar disorder, a disease characterized by low BDNF circulating concentrations [42,43]. Therefore, studies using experimental models of PE will be crucial to clarify the effect of BDNF in metabolic parameters.

Since it was difficult to obtain accurate information about women's smoking and drinking status, we did not evaluate their influence in BDNF plasma concentrations. Furthermore, we did not assess the dietary habits of the studied population, which could also have influenced BDNF plasma concentrations. Besides, BDNF concentrations were measured only in the third trimester of pregnancy, precluding us to conclude whether BDNF altered concentrations might predispose to PE or is a secondary effect of the disease.

5. Conclusions

Our data suggest that BDNF plasma concentrations are lower in PE women as compared to normotensive pregnant women. The significant negative association between BDNF and AnxA1 concentrations highlight the highly complex crosstalk among different signaling pathways in PE pathogenesis.

Acknowledgments

ALT, KBG, LMD and LPS are grateful to CNPq Research Fellowship (PQ). The authors would like to thank the funding agencies and the subjects for their participation in the study. This work was supported by Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG) (APQ-03318-15), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) (447452/2014-2) and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) (Ph.D. scholarship).

References

[1] American College of Obstetricians and Gynecologists, Hypertension in pregnancy. Report of the American College of Obstetricians and Gynecologists' Task Force on hypertension in pregnancy, Obstet. Gynecol. 122 (5) (2013) 1122.
[2] T. Chaiworapongsa, P. Chaemsaithong, L. Yeo, R. Romero, Pre-eclampsia part 1: current understanding of its pathophysiology, Nat. Rev. Nephrol. 10 (8) (2014) 466–480.
[3] P. von Dadelszen, L.A. Magee, J.M. Roberts, Subclassification of preeclampsia, Hypertens. Pregnancy 22 (2) (2003) 143–148.
[4] E.E. Benarroch, Brain-derived neurotrophic factor: regulation, effects, and potential clinical relevance, Neurology 84 (16) (2015) 1693–1704.
[5] M. Lommatzsch, A. Braun, A. Mannsfeldt, V.A. Botchkarev, N.V. Botchkareva, R. Paus, A. Fischer, G.R. Lewin, H. Renz, Abundant production of brain-derived neurotrophic factor by adult visceral epithelia. Implications for paracrine and target-derived Neurotrophic functions, Am. J. Pathol. 155 (4) (1999) 1183–1193.
[6] P. Kermani, B. Hempstead, Brain-derived neurotrophic factor: a newly described mediator of angiogenesis, Trends Cardiovasc. Med. 17 (4) (2007) 140–143.
[7] K. Kawamura, N. Kawamura, Y. Kumazawa, J. Kumagai, T. Fujimoto, T. Tanaka, Brain-derived neurotrophic factor/tyrosine kinase B signaling regulates human trophoblast growth in an in vivo animal model of ectopic pregnancy, Endocrinology 152 (3) (2011) 1090–1100.
[8] F. Calabrese, A.C. Rossetti, G. Racagni, P. Gass, M.A. Riva, R. Molteni, Brain-derived neurotrophic factor: a bridge between inflammation and neuroplasticity, Front. Cell. Neurosci. 8 (2014) 430.
[9] G. Hacioglu, A. Senturk, I. Ince, A. Alver, Assessment of oxidative stress parameters of brain-derived neurotrophic factor heterozygous mice in acute stress model, Iran J. Basic Med. Sci. 19 (4) (2016) 388–393.
[10] X.C. Ji, Y.Y. Dang, H.Y. Gao, Z.T. Wang, M. Gao, Y. Yang, H.T. Zhang, R.X. Xu, Local injection of Lenti-BDNF at the lesion site promotes M2 macrophage polarization and inhibits inflammatory response after spinal cord injury in mice, Cell. Mol. Neurobiol. 35 (6) (2015) 881–890.
[11] S. Matsuda, T. Fujita, M. Kajiya, K. Kashiwai, K. Takeda, H. Shiba, H. Kurihara, Brain-derived neurotrophic factor prevents the endothelial barrier dysfunction induced by interleukin-1β and tumor necrosis factor-α, J. Periodontal Res. 50 (4) (2015) 444–451.
[12] K. Takeda, Y. Obinata, A. Konishi, M. Kajiya, S. Matsuda, N. Mizuno, S. Sasaki, T. Fujita, H. Kurihara, Brain-derived neurotrophic factor inhibits intercellular adhesion molecule-1 expression in interleukin-1β-treated endothelial cells, Cell Biochem. Biophys. (2016).
[13] V.A. D'Souza, A.S. Kilari, A.A. Joshi, S.S. Mehendale, H.M. Pisal, S.R. Joshi, Differential regulation of brain-derived neurotrophic factor in term and preterm preeclampsia, Reprod. Sci. 21 (2) (2014) 230–235.
[14] V. D'Souza, V. Patil, H. Pisal, K. Randhir, A. Joshi, S. Mehendale, G. Wagh, S. Gupte, S. Joshi, Concentrations of brain derived neurotrophic factors across gestation in women with preeclampsia, Int. J. Dev. Neurosci. 37 (2014) 36–40.
[15] K. Fujita, K. Tatsumi, E. Kondoh, Y. Chigusa, H. Mogami, T. Fujii, S. Yura, K. Kakui, I. Konishi, Differential expression and the anti-apoptotic effect of human placental neurotrophins and their receptors, Placenta 32 (10) (2011) 737–744.
[16] J. Bienertova-Vasku, P. Bienert, F. Zlamal, Z. Splichal, J. Tomandl, M. Tomandlova, Z. Hodicka, P. Ventruba, A. Vasku, Brain-derived neurotrophic factor and ciliary neurotrophic factor in maternal plasma and umbilical cord blood from pre-eclamptic and physiological pregnancies, J. Obstet. Gynaecol. 33 (4) (2013) 359–363.
[17] L.O. Perucci, K.B. Gomes, L.G. Freitas, L.C. Godoi, P.N. Alpoim, M.B. Pinheiro, A.S. Miranda, A.L. Teixeira, L.M. Dusse, L.P. Sousa, Soluble endoglin, transforming growth factor-Beta 1 and soluble tumor necrosis factor alpha receptors in different clinical manifestations of preeclampsia, PLoS One 9 (5) (2014), e97632.
[18] L.O. Perucci, F.S. Carneiro, C.N. Ferreira, M.A. Sugimoto, F.M. Soriani, G.G. Martins, K.M. Lima, F.L. Guimarães, A.L. Teixeira, L.M. Dusse, K.B. Gomes, L.P. Sousa, Annexin A1 is increased in the plasma of preeclamptic women, PLoS One 10 (9) (2015), e0138475.
[19] World Health Organization, Obesity: preventing and managing the global epidemic. Report of a WHO consultation, World Health Organ. Tech. Rep. Ser. 894 (i-xii) (2000) 1–253.
[20] M. Jamal, W. Van der Does, B.M. Elzinga, M.L. Molendijk, B.W. Penninx, Association between smoking, nicotine dependence, and BDNF Val66Met polymorphism with BDNF concentrations in serum, Nicotine Tob. Res. 17 (3) (2015) 323–329.
[21] K.L. Chan, K.Y. Tong, S.P. Yip, Relationship of serum brain-derived neurotrophic factor (BDNF) and health-related lifestyle in healthy human subjects, Neurosci. Lett. 447 (2–3) (2008) 124–128.
[22] M.I. Davis, Ethanol-BDNF interactions: still more questions than answers, Pharmacol. Ther. 118 (1) (2008) 36–57.
[23] R.S. Rathod, A.A. Khaire, A.A. Kale, S.R. Joshi, Beneficial effects of omega-3 fatty acids and vitamin B12 supplementation on brain docosahexaenoic acid, brain derived neurotrophic factor, and cognitive performance in the second-generation Wistar rats, Biofactors 41 (4) (2015) 261–272.
[24] I.R. Postma, A. Bouma, I.F. Ankersmit, G.G. Zeeman, Neurocognitive functioning following preeclampsia and eclampsia: a long-term follow-up study, Am. J. Obstet. Gynecol. 211 (1) (2014) 37.e1–37.e9.
[25] B.M. Schroeder, ACOG practice bulletin on diagnosing and managing preeclampsia and eclampsia. American College of Obstetricians and Gynecologists, Am. Fam. Physician 66 (2) (2002) 330–331.
[26] Z.S. Khodzhaeva, Y.A. Kogan, R.G. Shmakov, N.I. Klimenchenko, A.S. Akatyeva, O.V. Vavina, A.M. Kholin, K.T. Muminova, G.T. Sukhikh, Clinical and pathogenetic features of early- and late-onset pre-eclampsia, J. Matern. Fetal Neonatal Med. 29 (18) (2016) 2980–2986.
[27] A.S. Sahay, D.P. Sundrani, G.N. Wagh, S.S. Mehendale, S.R. Joshi, Neurotrophin concentrations in different regions of the placenta and their association with birth outcome and blood pressure, Placenta 36 (8) (2015) 938–943.
[28] N. Noren Hooten, N. Ejiogu, A.B. Zonderman, M.K. Evans, Protective effects of BDNF against C-reactive protein-induced inflammation in women, Mediat. Inflamm. 2015 (2015) 516783.
[29] E.D. Papathanassoglou, P. Miltiadous, M.N. Karanikola, May BDNF be implicated in the exercise-mediated regulation of inflammation? Critical review and synthesis of evidence, Biol. Res. Nurs. 17 (5) (2015) 521–539.
[30] M. Perretti, F. D'Acquisto, Annexin A1 and glucocorticoids as effectors of the resolution of inflammation, Nat. Rev. Immunol. 9 (1) (2009) 62–70.
[31] K.J. Van Zee, T. Kohno, E. Fischer, C.S. Rock, L.L. Moldawer, S.F. Lowry, Tumor necrosis factor soluble receptors circulate during experimental and clinical inflammation and can protect against excessive tumor necrosis factor alpha in vitro and in vivo, Proc. Natl. Acad. Sci. U. S. A. 89 (11) (1992) 4845–4849.
[32] S.Y. Lau, S.J. Guild, C.J. Barrett, Q. Chen, L. McCowan, V. Jordan, L.W. Chamley, Tumor necrosis factor-alpha, interleukin-6, and interleukin-10 concentrations are altered in preeclampsia: a systematic review and meta-analysis, Am. J. Reprod. Immunol. 70 (5) (2013) 412–427.
[33] M.B. Pinheiro, O.A. Martins-Filho, A.P. Mota, P.N. Alpoim, L.C. Godoi, A.C. Silveira, A. Teixeira-Carvalho, K.B. Gomes, L.M. Dusse, Severe preeclampsia goes along with a cytokine network disturbance towards a systemic inflammatory state, Cytokine 62 (1) (2013) 165–173.
[34] G.S. Vince, P.M. Starkey, R. Austgulen, D. Kwiatkowski, C.W. Redman, Interleukin-6, tumour necrosis factor and soluble tumour necrosis factor receptors in women with pre-eclampsia, Br. J. Obstet. Gynaecol. 102 (1) (1995) 20–25.
[35] P.A. Lapchak, D.M. Araujo, F. Hefti, Systemic interleukin-1 beta decreases brain-derived neurotrophic factor messenger RNA expression in the rat hippocampal formation, Neuroscience 53 (2) (1993) 297–301.
[36] S.M. Rothman, K.J. Griffioen, R. Wan, M.P. Mattson, Brain-derived neurotrophic factor as a regulator of systemic and brain energy metabolism and cardiovascular health, Ann. N. Y. Acad. Sci. 1264 (2012) 49–63.

[37] S. Amoureux, L. Lorgis, P. Sicard, C. Girard, L. Rochette, C. Vergely, Vascular BDNF expression and oxidative stress during aging and the development of chronic hypertension, Fundam. Clin. Pharmacol. 26 (2) (2012) 227–234.
[38] B. Erdos, I. Backes, M.L. McCowan, L.F. Hayward, D.A. Scheuer, Brain-derived neurotrophic factor modulates angiotensin signaling in the hypothalamus to increase blood pressure in rats, Am. J. Physiol. Heart Circ. Physiol. 308 (6) (2015) H612–H622.
[39] K. Marosi, M.P. Mattson, BDNF mediates adaptive brain and body responses to energetic challenges, Trends Endocrinol. Metab. 25 (2) (2014) 89–98.
[40] B. Lebrun, B. Bariohay, E. Moyse, A. Jean, Brain-derived neurotrophic factor (BDNF) and food intake regulation: a minireview, Auton. Neurosci. 126-127 (2006) 30–38.
[41] C.L. Roth, C. Elfers, U. Gebhardt, H.L. Müller, T. Reinehr, Brain-derived neurotrophic factor and its relation to leptin in obese children before and after weight loss, Metabolism 62 (2) (2013) 226–234.
[42] S.Y. Lee, S.L. Chen, Y.H. Chang, P.S. Chen, S.Y. Huang, N.S. Tzeng, C.L. Wang, L.J. Wang, I.H. Lee, T.Y. Wang, K.C. Chen, Y.K. Yang, J.S. Hong, R.B. Lu, Correlation of plasma brain-derived neurotrophic factor and metabolic profiles in drug-naïve patients with bipolar II disorder after a twelve-week pharmacological intervention, Acta Psychiatr. Scand. 131 (2) (2015) 120–128.
[43] B.S. Fernandes, M.L. Molendijk, C.A. Köhler, J.C. Soares, C.M. Leite, R. Machado-Vieira, T.L. Ribeiro, J.C. Silva, P.M. Sales, J. Quevedo, V. Oertel-Knöchel, E. Vieta, A. González-Pinto, M. Berk, A.F. Carvalho, Peripheral brain-derived neurotrophic factor (BDNF) as a biomarker in bipolar disorder: a meta-analysis of 52 studies, BMC Med. 13 (2015) 289.

4.4 CAPÍTULO 4 - "Resolution of inflammation pathways in preeclampia – a narrative review"

(Artigo submetido na revista *Immunologic Research*)

RESOLUTION OF INFLAMMATION PATHWAYS IN PREECLAMPSIA – A NARRATIVE REVIEW

Luiza Oliveira Perucci[1,2]; Mário Dias Corrêa[3]; Luci Maria Dusse[1,2]; Karina Braga Gomes[1,2]; Lirlândia Pires Sousa[1,2]*

[1]Departamento de Análises Clínicas e Toxicológicas, Faculdade de Farmácia, Universidade Federal de Minas Gerais, Avenida Antônio Carlos, 6627, Pampulha, 31270-901, Belo Horizonte, Minas Gerais, Brazil. [2]Programa de Pós-Graduação em Análises Clínicas e Toxicológicas, Universidade Federal de Minas Gerais Avenida Antônio Carlos, 6627, Pampulha, 31270-901, Belo Horizonte, Minas Gerais, Brazil. [3]Departamento de Ginecologia e Obstetrícia, Faculdade de Medicina, Universidade Federal de Minas Gerais, Avenida Professor Alfredo Balena, 190, Santa Efigênia, 30130-100, Belo Horizonte, Minas Gerais, Brazil.

*Corresponding author

Lirlândia Pires Sousa – Email address: lipsousa72@gmail.com. Telephone: +55 31 34096883. Fax number: +55 31 3409 2651.

Acknowledgments: The authors are grateful to Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) Research Fellowship.

RESOLUTION OF INFLAMMATION PATHWAYS IN PREECLAMPSIA – A NARRATIVE REVIEW

ABSTRACT

Preeclampsia (PE) is one of the leading causes of maternal morbidity and mortality worldwide. This disease is believed to occur in two stages with placental dysfunction in early pregnancy leading to maternal clinical findings after 20 weeks of gestation, as consequence of systemic inflammation, oxidative stress and endothelial dysfunction. Much evidence suggests that PE women display an overshooting inflammatory response throughout pregnancy due to an unbalanced regulation of innate and adaptive immune responses. Recently, it has been suggested that dysregulation of endogenous protective pathways might be associated with PE etiopathogenesis. Resolution of inflammation is an active process coordinated by mediators from diverse nature that regulate key cellular events to restore tissue homeostasis. Inadequate or insufficient resolution of inflammation is believed to play an important role in the development of chronic inflammatory diseases, like PE. In this narrative review, we discuss possible pro-resolution pathways that might be compromised in PE women, which could be targets to novel therapeutic strategies in this disease.

Keywords: Preeclampsia; Inflammation; Resolution; Pro-resolving mediators.

1 INTRODUCTION

Preeclampsia (PE) has been defined as a new onset of hypertension and either proteinuria or end-organ dysfunction at gestational age ≥20 weeks as consequence of systemic inflammation, endothelial dysfunction and oxidative stress [1, 2]. Because PE is a heterogeneous disease, different classifications based on severity (mild PE/severe PE) and onset of clinical symptoms (early PE:<34weeks / late PE: ≥34weeks; preterm PE: <37weeks / term PE: ≥37weeks) have been proposed [3, 4]. It is widely accepted that early and late PE have different clinical features, prognosis and probably distinct etiopathogenesis [5, 6].

Traditionally, a "two-stage" theory of PE etiopathogenesis has been considered. According to this theory, an abnormal spiral artery remodeling in early pregnancy causes placental hypoxia (*stage 1*) and the ischemic placenta releases large amounts of soluble factors, such as reactive oxygen species, pro-inflammatory cytokines and anti-angiogenic factors, into the maternal circulation, which leads to the disease clinical manifestations and complications (*stage 2*) [7, 8]. Other paradigm has been recently proposed by Ashmed & Ramma [9], in which they use a metaphor to compare normotensive pregnancy as a car with accelerators and functioning brakes. The "accelerators" represent inflammation, oxidative stress and an anti-angiogenic state, while the "brakes" are the endogenous protective pathways. According to this theory, PE manifests when the braking systems fail and the "accelerators" cannot be stopped in early pregnancy. In their review, Ashmed & Ramma focused on the carbon monoxide, hydrogen sulphide and nitric oxide pathways. These gases have been associated with protective roles, such as regulation of uteroplacental perfusion and inhibition of oxidative stress and inflammation [10-12]. The new paradigm of dysregulated endogenous protective pathways can be combined with the traditional "two stage" theory of PE pathogenesis (*Figure 1*). Here, we raised the hypothesis of another protective pathway that may be compromised in PE women: the resolution of inflammation pathway.

1.1 Resolution of inflammation

Acute inflammation is usually a self-limited response that can be triggered by infectious and sterile injury and has the physiological purpose to restore tissue homeostasis [13]. Successful resolution of inflammation is an active and highly regulated process that evolves several cellular and biochemical events [14, 15]. During this process, the production of anti-inflammatory/pro-resolving factors prevails over the production of pro-inflammatory mediators. However, inflammation and resolution are not isolated events. In fact, they

continuously overlap because pro-inflammatory signals can induce anti-inflammatory and pro-resolving signals aiming to temper inflammation [16, 17].

In recent years, endogenous pro-resolution mediators from diverse nature have been identified, including proteins/peptides, specialized pro-resolving lipid mediators, gaseous mediators, protease inhibitors and neuromodulators [16, 18, 19]. They inhibit further leukocyte recruitment, induce neutrophil apoptosis and enhance efferocytosis of apoptotic neutrophils by macrophages, thus acting as “brakes” for inflammatory response. They are also able to switch macrophages from pro-inflammatory (M1) to anti-inflammatory and pro-resolving phenotypes (M2 and Mres), drain non-apoptotic leukocytes to lymph nodes and participate in tissue repair/healing mechanisms [14-16, 20]. The *Figure 2* shows the key steps of a successful resolution of inflammation.

Inflammation may become chronic and lead to further tissue damage if resolution process fails. Dysfunctional resolution of inflammation can occur due to decreased synthesis of pro-resolving mediators and receptors, altered receptors conformation, as well as increased inactivation of pro-resolving mediators. Inadequate amount or action of pro-resolving mediators can lead, for example, to persistent neutrophils recruitment and survival, failure in switch of macrophage phenotype and ineffective clearance of apoptotic neutrophils. In this sense, if the inflammatory stimulus is too high, it would be necessary a higher production of anti-inflammatory/pro-resolving molecules to neutralize the overshooting inflammation.

In a recent review, Fullerton & Gilroy proposed that resolution could be a bridge between innate and adaptive immunities. Therefore, unresolved inflammation could lead to maladaptive immune responses, which are commonly associated with chronic inflammatory diseases [21].

2 HYPOTHESIS

Embryo implantation, trophoblast invasion of uterine spiral arteries and labor are inflammatory events. Therefore, inflammation is necessary to successful reproduction [22]. Normotensive pregnancy is characterized by a state of mild/low grade inflammation, as demonstrated, for example, by increased levels of pro-inflammatory cytokines when compared to the non-pregnant state [23]. Innate immune responses are up-regulated in normotensive pregnant women, while adaptive immune responses are down-regulated and this balance is important to maintain maternal immune tolerance to the fetal allograph. By contrast, innate immune responses are even more activated and adaptive immune responses are not suppressed in PE [24]. Indeed, there is

a shift from T helper (Th)2/regulatory T cell responses in normotensive pregnant women to a predominant Th1/Th17 immunity in PE women [25]. Furthermore, there are evidences of placental M2 macrophage polarization in normotensive pregnancy and a predominant M1 phenotype in PE [26, 27]. Consequently, PE women display an overshooting inflammatory response throughout pregnancy [23].

Most studies in the literature have focused on the "accelerators" of the inflammatory response in PE. Here, we propose that the exaggerated inflammatory response seen in this disease may result from failures in a "breaking system" called resolution of inflammation. If so, unresolved inflammation may account for maladaptive immune responses in PE women.

3 METHODS

First, we performed a screening of database results through reading of titles and abstracts about pro-resolving mediators, previously described in general works [16, 18, 19], that were studied in the context of PE, as follows: Annexin A1, galectins, chemerin, lipoxin A4, nitric oxide, hydrogen sulfide, carbon monoxide, acetylcholine, netrin-1, protease inhibitors. Next, we used as key terms the specific pro-resolving mediator and preeclampsia to the search in PubMed database. The selection was based in complete read of each preselected article. Original research articles written in English language were included if they addressed these pro-resolving mediators in the context of PE or those that used animal models to study PE. General review articles were also included to provide a background on the role of these mediators and PE pathogenesis. Articles that not have focused on these issues were excluded.

4 RESULTS

This review included a total of 219 articles published between 1987 and 2016. The main conclusions obtained from them are described in the next subsections.

4.1 Annexin A1

Annexin A1 (AnxA1) is a 37KDa glucocorticoid-regulated protein that elicits anti-inflammatory/pro-resolving effects through binding to formyl peptide receptor type 2/lipoxin A4 receptor (FPR2/ALXR). These effects lie within AnxA1 N-terminal domain and include: inhibition of neutrophil migration to inflamed tissues,

induction of neutrophil apoptosis, stimulation of macrophage efferocytosis of apoptotic neutrophils and induction of macrophage reprogramming to a pro-resolving phenotype [28, 29]. AnxA1 was first recognized by its immunoregulatory actions by inhibiting phospholipase A2 and generation of eicosanoids, but subsequent studies revealed that this protein exerts a wider range of actions. It has been suggested that AnxA1 mediates part of neuroendocrine responses of the glucocorticoids, particularly in the hypothalamo-pituitary-adrenocortical axis. In addition, experimental data indicate that AnxA1 may be implicated in processes regulating pregnancy, lactation and fetal development [30, 31].

Altered AnxA1 synthesis might be involved in the pathogenesis of chronic inflammatory diseases, like asthma [32]. Of importance, intact AnxA1 (37kDa) can be cleaved in its N-terminal domain by proteases, such as neutrophil elastase, generating various fragments that are believed to be inactive or pro-inflammatory [33]. Indeed, increased levels of AnxA1 cleavage products (e.g. 33kDa) have been reported in inflammatory samples [34]. Moreover, FPR2/ALXR decreased expression or altered receptor conformation can impair AnxA1 to regulate inflammation [35, 36].

Previously, Perucci et al. investigated AnxA1 in PE and found increased plasma levels compared with normotensive pregnancy [37]. AnxA1 increased concentration, combined with an overwhelming inflammatory response, suggests a failure in this resolution pathway in PE, which could be a consequence of decreased FPR2/ALXR expression [38, 39] or presence of anti-AnxA1 auto-antibodies [40]. Considering that neutrophilia is a common feature in PE women and that neutrophil elastase is increased in their plasma and placenta [41-43], it is also plausible to hypothesize that AnxA1 cleavage could interfere with its actions. Although AnxA1 expression has been studied in placental tissues [44], the differential expression of its intact and cleaved forms in PE and normotensive pregnancy has not been determined, a matter under investigation in our group.

4.2 Galectins

Galectins are β-galactoside-binding proteins that were initially known to mediate developmental processes, including tissue organization and embryo implantation [45]. Further research indicated that galectins are secreted in response to inflammatory signals and cellular damage, acting as pattern recognition receptors, immunomodulators or damage-associated molecular patterns in innate and adaptive immune responses [46, 47]. Galectins are thought to modulate intracellular signaling pathways in immune cells due to their ability to induce the aggregation of specific cell-surface glycoreceptors [48, 49]. Emerging evidences also suggest that galectins

are capable of triggering platelet activation and inducing angiogenesis [50, 51]. Here, we give a general overview on the role of galectin-1 (Gal-1) and galectin-13 (Gal-13), the most studied galectins in PE.

4.2.1 Galectin 1

It has been suggested that Gal-1 plays a role in maternal-fetal tolerance, which is thought to be impaired in PE women. Blois et al. reported that Gal-1 deficient (*LGALS1*-/-) mice had increased fetal loss when compared with wild-type mice, an effect that was prevented by the treatment with recombinant Gal-1. According to their results, Gal-1 restored maternal immune tolerance by promoting the expansion of IL-10-secreting regulatory T cells [52]. This data was corroborated by van der Leij et al. study [53]. Gal-1 might also improve maternal-fetal tolerance by inducing the apoptosis of activated CD8+ T cells, Th1 and Th17 CD4+ cells [54]. Other immunomodulatory actions for Gal-1 have been proposed. For instance, Rostoker et al. showed that Gal-1 induced 12/15-lipoxygenase expression (lipoxin A4 synthetizing enzyme - see section 4.4.1) in murine macrophages and promoted their conversion into a pro-resolving phenotype [55].

Some works have demonstrated that Gal-1 gene expression was up-regulated in placenta of PE women compared with normotensive pregnant women [56-58]. Interestingly, *LGALS1*-knockout dams develop PE symptoms. However, when stratifying PE women according to clinical symptoms onset, early PE women show decreased Gal-1 placental expression than pregnant controls, while an opposite finding was reported for late PE women [58]. It has been proposed that Gal-1 decreased expression in early PE could be associated with placental dysfunction, whereas its overexpression might be a compensatory mechanism to attenuate inflammation in late PE [59]. In addition, Gal-1 circulating levels may reflect its placental expression in late PE, but not in early PE. Accordingly, Freitag et al. reported increased Gal-1 serum levels in late PE when compared with early PE and normotensive pregnancy, but no difference was found between early PE and normotensive pregnancy [58]. However, when both clinical forms were included in the same cohort, Gal-1 serum levels seemed to be similar between patients and controls [60]. Pregnant women in the second trimester of pregnancy who developed PE also showed decreased levels of Gal-1 than healthy pregnant women, indicating that Gal-1 might be an early predictor of PE [58].

Gal-1 seems to be differentially expressed in cells/tissues of PE women. Gal-1 is downregulated in T and natural killer cells in PE when compared with these cells in normotensive pregnancy, while no difference was detected in Gal-1 mRNA expression in decidua samples between these pregnant groups [58, 60]. Gal-1

decreased expression in these immune cells can be associated with maternal-fetal intolerance and exacerbated inflammatory response in PE women, as discussed above.

4.2.2 Galectin 13

Gal-13 is a galectin uniquely expressed in the placenta, mainly in the syncytiotrophoblast, and it is released from the placenta into the maternal circulation [61]. In vitro studies suggest that Gal-13 participates in the morfological differentiation from cytotrophoblast into syncytiotrophoblast [62, 63]. In addition, it has been demonstrated that Gal-13 is able to induce the apoptosis of activated human CD3+ T cells [64]. Interestingly, phagocytosed Gal-13 immunopositive deposits in immune cells coincided with zones of apoptotic and necrotic immune cells in Kliman et al. study [65]. These data indicate that Gal-13 might participate in placentation and in maternal adaptive immune responses at the maternal-fetal interface. Considering that these processes are impaired in PE women, it can be hypothesized that Gal-13 is involved in the disease pathogenesis.

Gal-13 placental-specific expression makes it a promising biomarker for PE early prediction. Indeed, Gal-13 protein and mRNA content in blood and placenta are decreased in the first trimester of gestation in women who developed PE, specially the early clinical form [66-71], and this could be associated with single nucleotide polymorphisms in the *LGALS13* gene [72]. Moreover, combining Gal-13 with background risk factors, other serum biomarkers and physical parameters increases the accuracy of predicting PE [73]. Gal-13 low levels in early gestation may lead to impaired placentation and maternal immune intolerance to the fetus [65, 74].

It has been demonstrated that Gal-13 serum levels increases throughout normotensive pregnancy and that preterm PE women have higher Gal-13 serum levels than preterm controls [66]. It was proposed that Gal-13 increased maternal serum concentration during the third trimester of gestation in PE women is a consequence of augmented placental shedding of microvesicles containing Gal-13 and this could be a compensatory mechanism aiming to restore homeostasis [66]. Nevertheless, both decreased and increased placental Gal-13 expression have been reported in PE women [66, 75].

4.3 Chemerin

Chemerin is an adipocyte-secreted protein originally identified as the natural ligand of chemR23 receptor, which is implicated in several biological processes, such as adipogenesis, glucose homeostasis and immune cell migration [76]. Fragments with distinct inflammatory actions can be generated after chemerin C-

terminal proteolytic processing, depending on the types of proteases predominating in the microenvironment [77, 78]. Some chemerin fragments can induce the chemotaxis of immune cells, in particular dendritic cells, macrophages and natural killer cells, toward inflammatory sites, thus contributing to the onset of inflammation. By contrast, other fragments can inhibit the synthesis of pro-inflammatory mediators. In addition, activation of the chemerin/chemR23 axis may increase the non-phlogistic phagocytosis of apoptotic cells by macrophages, and inhibit neutrophil activation and influx to inflammatory sites, thus promoting the resolution of inflammation. Therefore, chemerin-derived peptides may play a role both in initiation and in the resolution of the inflammatory response [77].

It has been suggested that chemerin is abundantly expressed in stromal cells and in extravillous trophoblast cells, but not in decidual endothelial cells in early pregnancy [79]. Moreover, chemerin may stimulate the angiogenesis and the accumulation of natural killer cells at maternal-fetal interface, and these immune cells have been implicated in uterine spiral artery remodeling [79-81]. Thus, chemerin can be involved in placental development, which is impaired in PE women. Chemerin also acts as a chemoattractant for dendritic cells [77]. Several lines of evidence indicate crucial roles for both natural killer and dendritc cells in the modulation of adaptive immune responses [82, 83]. Based on these data, it can be admitted that chemerin may contribute to maternal-fetal tolerance, but more studies are necessary to clarify this issue.

It has been shown that chemerin serum levels increases throughout normotensive pregnancy [84, 85]. Some studies have reported increased chemerin circulating levels, as well as increased mRNA and protein expression in the placenta of PE women when compared with normotensive pregnant women [86-88]. Higher chemerin levels were detected in the first trimester of gestation in women who developed PE, were associated with disease severity and remained significantly higher 6 months after delivery in former PE patients compared with controls [87-89]. Moreover, there is a positive correlation among chemerin levels, pro-inflammatory mediators and blood pressure [86-89]. However, these studies did not specify the chemerin-derived peptides types quantified. Hence, their role in PE pathogenesis remains unclear.

4.4 Specialized pro-resolving lipid mediators

Polyunsaturated fatty acids omega-6 and -3 are substrates for the biosynthesis of lipoxins (LXs), maresins, resolvins and protectins, which are collectively called *specialized pro-resolving lipid mediators* (SPMs). Prostaglandins and leukotrienes are lipid mediators that play pivotal roles in initiation of the inflammatory response, while SPMs attenuate inflammation and contribute to its timely resolution [90].

Curiously, aspirin induces the endogenous synthesis of LXs 15-epimers. Endogenous LXs and their epimers have been shown to counter-regulate inflammation in a variety of experimental models of inflammatory diseases. They downregulate pro-inflammatory mediators' synthesis (including prostaglandins and leukotrienes), inhibit neutrophil infiltration, induce macrophage efferocytosis of apoptotic neutrophils and stimulate interleukin (IL)-10 production [90, 91]. Furthermore, LXs may modulate other biological actions, such angiogenesis, airway smooth muscle function and activity of neuronal ion channels that convey nociceptive signals [92-94]. In this sense, LXs and other SPMs may contribute to resolution of both inflammation and pain [93].

4.4.1 Lipoxin A4

Lipoxin A4 (LXA4) is an eicosanoid synthesized from arachidonic acid, an omega-6 derivate, via lipoxygenase enzymes metabolism [95]. LXA4 interacts with FPR2/ALXR receptor, which also binds to AnxA1 [96]. An *in vitro* study showed that LXA4 inhibited IL-1β production by monocytes from severe PE women in a dose-dependent manner [97]. In another experiment, 15-epi-LXA4 reduced neutrophil-endothelium cell adhesion triggered by PE plasma [98]. Lin et al. administrated an LXA4 analogue in low-dose-endotoxin-treated pregnant rats and found that it attenuated inflammation and PE symptoms [99]. These experimental data suggest protective roles for LXA4 and its analogues in the disease.

Three works showed increased LXA4 circulating levels in PE women compared with normotensive pregnant women [39, 100, 101]. Interestingly, LXA4 plasma levels correlated with maternal blood pressure, white blood cell count and C-reactive protein levels in Perucci et al. study [101]. However, an opposite finding has also been reported [38]. Different studied populations or methodologies to quantify LXA4 might have contributed to these divergent results. Similar to AnxA1 discussion, LXA4 inefficiency to resolve inflammation could be a consequence, for example, to decreased FPR2/ALXR expression and/or increased LXA4 inactivation. However, these theories remain to be elucidated.

4.5 Gaseous mediators

Nitric oxide, hydrogen sulfide and carbon monoxide are the most studied gaseous mediators and, for many years, only their toxicity was known [102]. Recently, they have been implicated in key physiological functions, such as angiogenesis, inflammation and vascular tone regulation [103, 104]. They also participate in trophoblast invasion and spiral artery remodeling [105]. Experimental studies have demonstrated that these gases act as anti-inflammatory mediators at low concentrations, promoting resolution of inflammation, but exert pro-

inflammatory and damaging effects at high concentrations [12]. In line with this data, altered production or signaling of gaseous mediators has been reported in inflammatory diseases, like atherosclerosis and arthritis [106, 107].

4.5.1 Nitric oxide

Nitric oxide (NO) is synthesized by the conversion of L-arginine to L-citruline by one of the three isoforms of nitric oxide synthase (NOS): neuronal, endothelial (eNOS) or inducible (iNOS). NO acts as a vasodilatory molecule by inducing cyclic guanosine monophosphate (cGMP) synthesis [108]. However, NO can act by cGMP-independent pathways to regulate other mechanisms, such as leukocyte apoptosis [109, 110]. NO may have pro- or anti-inflammatory actions depending on the concentration used in the experiment, the delivery method and the system/disease model studied [111]. Low amounts of NO inhibit pro-inflammatory cytokines synthesis and reduce leukocyte-endothelium adhesion and transmigration to inflamed tissues, while high NO levels increase vascular permeability and leukocyte migration [112, 113]. NO might also play a role in resolution of inflammation since it induces neutrophil, but not macrophage, apoptosis [114].

In normal pregnancy, NO and cGMP biosynthesis are increased due to eNOS up-regulation. In addition, asymmetrical dimethylarginine (ADMA), a competitive inhibitor of NOS, is reduced. These events are important to regulate peripheral and placental bed vascular resistance, angiogenesis, platelet adhesion/aggregation and trophoblast invasion [115]. On the other hand, most studies have reported decreased iNOS and eNOS placental activity and increased ADMA levels in PE, but data on NO levels are inconsistent [116-118]. Moreover, increased ADMA levels in the first trimester of pregnancy may predict PE [119]. Additionally, ADMA increase seems to be more prominent in early than in late severe PE and eNOS polymorphisms may influence the disease onset-time [120, 121].

Other mechanisms may interfere with NO signaling in PE. It has been demonstrated that polymorphisms in the transcription factor STOX1 gene are associated with maternal susceptibility to PE and that overexpression of placental STOX1 induces a PE-like syndrome in mice [122, 123]. According to Doridot et al. study, placentas overexpressing STOX1 showed high concentration of reactive nitrogen species (RNS). They proposed that RNS could be rapidly generated in the placenta through NO association with reactive oxygen species (ROS) [124]. Therefore, overexpression of STOX1 could decrease NO bioavailability in endothelial cells, preventing the protective actions of this gaseous mediator in vascular tone and inflammation, and also contributing to oxidative and nitrosative stress in PE women [11]. Further evidence suggested a risk allele

(Y153H) in STOX1 gene that might be associated with a less invasive trophoblast phenotype [125]. Accordingly, a previous study showed that the ability of trophoblasts to remodel uteroplacental arteries depended on NO produced by extravillous trophoblasts in guinea pig pregnancy [126].

Considering that NO interferes with several pathways that are known to be compromised in PE women, such as vascular tone, inflammation and oxidative/antioxidative status, impaired bioavailability and/or action of this gaseous mediator may be associated with the disease pathogenesis. In this sense, the therapeutical potential of drugs that enhance NO availability, inhibit cGMP degradation or reduce ADMA levels has been investigated *in vitro* and in experimental models of PE. PE was mimicked by inducing reduced uterine perfusion pressure (RUPP model) and the overexpression of the anti-angiogenic molecule soluble fms-like tyrosine kinase (sFlt1), and by administrating the NOS inhibitor N^G-nitro-L-arginine methyl ester (L-NAME) in rodents [127]. Although some of these findings seem promising, there is insufficient clinical evidence to use these drugs for PE treatment or prevention [127, 128].

4.5.2 Hydrogen sulfide

Endogenous hydrogen sulfide (H_2S) is primarily synthetized by the conversion of L-cysteine or homocysteine by two enzymes: cystathionine β-synthase (CBS) and cystathionine γ-lyase (CSE) [129]. H_2S is a vasodilatory mediator, an effect that can be mediated through eNOS activation and NO production [130]. H_2S exert anti-oxidant effects on cells and low concentrations seem to be cytoprotective, while higher H_2S exposure favors oxidative stress and cell apoptosis [131]. As for NO, H_2S role in inflammation is complex and not fully elucidated. H_2S might act as a pro-inflammatory mediator, as demonstrated in experimental sepsis [132, 133], or as an anti-inflammatory/pro-resolving molecule, for example, in gastrointestinal inflammation [134]. Evidences suggest that H_2S promotes resolution of inflammation by inducing neutrophil apoptosis, M2 macrophage polarization and clearance of apoptotic neutrophils by macrophages [135-137]. Recently, it has been suggested that part of H_2S anti-inflammatory/pro-resolving effects are mediated by AnxA1 [138].

H_2S has been implicated in placental vascular development and function, due to its pro-angiogenic and vasodilatory activities [130, 139]. Both CBS and CSE are expressed in human placenta during normal pregnancies [140], but the studies on their expression in PE have conflicting results. Wang et al. reported CSE mRNA and protein down-regulation in the placenta of PE women [141]. Moreover, abnormal Doppler placenta have increased microRNA-21 expression, which negatively regulates CSE expression [142]. By contrast, in Holwerda et al. study, placental CSE mRNA levels were unchanged, while CBS mRNA levels were decreased in

early PE [143]. These divergent results might be attributed to differences in the studied PE clinical forms [127]. Furthermore, Wang et al. reported decreased H_2S plasma levels in PE women [141]. Abnormal H_2S synthesis could contribute to endothelial dysfunction, oxidative stress and overwhelming inflammation observed in PE women [127].

H_2S-based therapies have been studied in animal models of PE and in human disease. The administration of a slow-releasing H_2S-generating compound (GYY4137) ameliorated PE-like symptoms induced by the treatment with an inhibitor of H_2S synthesis (DL-propargylglycine) [141]. However, oral administration of an H_2S donor (N-acetylcysteine) to severe early PE women did not improve maternal outcomes [144]. More studies should be conducted in order to evaluate the therapeutical potential of H_2S-releasing compounds in PE.

4.5.3 Carbon monoxide

Heme oxygenase (HO) enzymes convert heme to biliverdin, free iron and carbon monoxide (CO) in the endoplasmatic reticulum. HO enzymes exist as inducible (HO-1) and constitutive (HO-2) isoforms [145]. HO-CO system regulates many biological processes, such as vascular tone, oxidant-antioxidant status and platelet aggregation. Further, CO acts as a signaling molecule in the neuronal system, where it regulates neurotransmitters release. Like NO and H_2S, CO is toxic at high concentrations, but has cytoprotective actions at low concentrations [146, 147]. It has been suggested that part of the protective and deleterious effects of CO are due to its ability to regulate different types of ion channels [148]. Most studies have reported contra-regulatory actions for CO in inflammatory responses. Low CO exposure inhibits neutrophil-endothelial adhesion and transmigration to inflamed tissues, suppresses pro-inflammatory cytokine production, promotes neutrophil apoptosis and enhances macrophage efferocytosis of apoptotic neutrophils [149-152]. Moreover, CO accelerates resolution of inflammation by shifting the lipid profile in the inflammatory milieu [152].

Both HO-1 and HO-2 are expressed in human placenta [153]. During pregnancy, CO regulates the perfusion and oxidant-antioxidant status within placental tissues, as well as spiral artery transformation [153-155]. HO adequate expression might also be important to maintain maternal-fetal tolerance [156]. Considering the importance of CO in regulating multiple processes during pregnancy, it would be expected alterations in the HO-CO system in PE. Indeed, PE women seem to have reduced CO breath levels and carboxyhemoglobin concentration in the umbilical cord blood than normotensive pregnant women [157, 158]. These data are in line with the observation that CO exposure in cigarette smoke decreases the risk of developing PE [159, 160].

However, HO placental expression during PE is not clear. Either decreased, increased or unchanged HO-1 and HO-2 placental expression have been reported in PE women [161-165].

Compounds that induce HO expression have been studied in experimental and human PE [127]. A recent work showed that pravastatin treatment stabilized blood pressure, proteinuria and serum uric acid levels in severe PE women [166]. These effects seem to be partially mediated by up-regulating HO-1 placental expression [167]. McCarthy et al. work studied rosiglitazone effects using the RUPP rat model of PE and found that this drug prevented disease-like symptoms development via HO-dependent pathway [168]. Moreover, CO application at low doses prevented hypertension and proteinuria in adenovirus sFlt1 PE-like mouse model [169]. In conclusion, CO or HO-inducing agents administration might be beneficial for treating or preventing PE, but further investigation is necessary.

4.6 Neuromodulators

4.6.1 Acetylcholine

Cholinergic neurons release acetylcholine (ACh), a neurotransmitter known to regulate skeletal, smooth and cardiac muscle contractions. ACh also acts as neuromodulator in the central nervous system, where it alters neural excitability, synaptic transmission and plasticity, thus interfering with learning, memory and mood [170, 171]. Studies about the role of neural reflexes in inflammation and immunity are recent. It has been demonstrated that ACh binding to α7-nicotinic receptors in macrophages inhibit the synthesis and release of pro-inflammatory cytokines [172-175]. Alternative anti-inflammatory cholinergic mechanisms have been proposed. For instance, nicotine (a cholinergic agonist drug) attenuates inflammation by up-regulating HO-1 expression in macrophages [176, 177]. Other anti-inflammatory and pro-resolving effects of nicotine include inhibition of neutrophil migration and stimulation of its apoptosis [178, 179]. Moreover, nicotinic ACh receptors activation enhances macrophage phagocytosis and protects M2 macrophages from apoptosis [180, 181]. The role of this neural pathway in controlling inflammatory responses was further confirmed by studies showing that vagus nerve lesions enhance pro-inflammatory cytokine production and are associated with non-resolving inflammation [173, 182]. Accordingly, chronic inflammatory conditions, such as inflammatory bowel disease, have decreased vagus nerve function [173].

Yang et al. reported reduced vagus nerve function in PE women [183]. Thus, ACh synthesis might be reduced in these women and contribute to excessive inflammation. Accordingly, nicotine binding to ACh

receptor supresses *ex vivo* placental cytokine production [184]. These data corroborate with the theory that nicotine, and also CO, in cigarette smoke might protect from PE [159, 160]. Some studies have also shown that nicotinic ACh receptors are up-regulated in PE women [185, 186], which could be a compensatory mechanism to decreased ACh levels.

4.6.2 Netrin-1

Netrin-1 was originally described as a laminin-related protein that guides axonal trajectories during central nervous system development, by repulsing/abolishing the attraction of neuronal cells expressing the UNC5b receptor [187]. Subsequently, it was implicated in regulation of various biological processes, including angiogenesis and, recently, inflammation. Netrin-1 suppresses neutrophil trafficking, probably as consequence of UNC5b receptor strong expression in these cells [188, 189]. It also inhibits prostaglandin E2 synthesis, suppresses Th1/Th2/Th17 cytokine production, induces M2 polarization, increases apoptotic polymorfonuclear (PMN) cells efferocytosis and stimulates SPM endogenous biosynthesis [190-193]. Accordingly, *in vivo* studies have reported protective functions of netrin-1 in inflammatory conditions [193-196]. Yang et al. investigated netrin-1 placental expression and found that it was down-regulated in severe PE women [197]. More studies are needed to understand the association between netrin-1 and inflammation in PE.

4.7 Protease inhibitors

Proteases are enzymes that hydrolyze peptide bonds of proteins, releasing polypeptides or free amino acids. They regulate the activity and localization of several proteins, modulate the interactions among them and participate in cellular signaling events. Currently, proteases are classified based on their mechanisms of catalysis into four classes: serine proteases, metalloproteases, aspartic proteases and cysteine proteases. Their activities are tempered by proteases inhibitors or antiproteases [198, 199]. Proteases are usually up-regulated in inflammatory conditions and defective antiproteolytic control mechanisms may participate in the pathogenesis of chronic inflammatory diseases, like cystic fibrosis [200, 201]. Thus, protease inhibitors have the potential to be developed as new therapeutic agents for these diseases.

4.7.1 Metalloproteinases inhibitors

Metalloproteinases are proteolytic enzymes that hydrolyze extracellular matrix components, playing important roles on tissue repair. They participate in extracellular matrix remodeling during trophoblast invasion

and in uterine spiral arteries transformation. This family of enzymes comprises, among other members, matrix metalloproteinases (MMPs) and membrane-anchored disintegrin metalloproteinases (ADAMs) [202, 203]. Activated metalloproteinases can be regulated by general or specific protease inhibitors (tissue inhibitors of metalloproteinases-TIMPs) [204].

Several non-matrix substrates for metalloproteinases have been identified, including cytokines, chemokines and their receptors. Metalloproteinases cleave these substrates in short fragments, altering their bioactions and, in case of receptors, interfering with their responsiveness and downstream signaling. Metalloproteinases modulate additional aspects of inflammation, such as integrity of physical barriers, leukocytes transmigration and survival [205, 206]. Metalloproteinases may have pro-inflammatory or anti-inflammatory/pro-resolving actions. For instance, ADAM17, also known as also known as tumor necrosis factor alpha (TNF-α) converting enzyme, releases the membrane-bound TNF-α, increasing the bioavailability of this pro-inflammatory cytokine. By contrast, ADAM17 sheds TNF-α soluble receptors (sTNFRs) in the circulation, which sequester TNF-α, neutralizing its systemic effects [207]. ADAM17 also prevents neutrophil transmigration trough the endothelium by shedding L-selectin from them, without altering monocyte recruitment. In addition, ADAM17 induces neutrophil apoptosis [208, 209].

There is increasing evidence of metalloproteinases/TIMPs imbalance in inflammatory diseases, like inflammatory bowel diseases [210]. Metalloproteinases and their inhibitors may also participate in PE pathogenesis. Ma et al. reported that ADAM17 was up-regulated in the placenta of PE women and induced TNF-α production by placental trophoblasts [211]. Later, they showed that TIMP3 (ADAM17 inhibitor) placental levels were decreased in PE women and that TIMP3 down-regulation increased TNF-α production by placental trophoblasts [212]. Further reports on increased circulating levels of TNF-α and sTNFRs in PE women corroborates with these findings [213-215], since increased ADAM 17 levels and decreased TIMP3 levels may induce TNF-α release and the consequent shedding of neutralizing sTNFRs receptors in the circulation of PE women. Decreased, increased or similar levels of other metalloproteinases and TIMPs have been described in PE [216]. These discrepancies are probably due to differences in the types of specimens analyzed, gestational age of specimen collection and quantification methodologies. Therefore, the role of metalloproteinases and their inhibitors in PE pathogenesis requires further investigation.

5 CONCLUDING REMARKS

Several evidences support that there is a balance of pro-inflammatory and anti-inflammatory/pro-resolving pathways in normotensive pregnant women as consequence of functioning mechanisms of resolution of inflammation, leading to a state of controlled inflammatory response in these women. On the other hand, inflammation is overwhelming in PE women, probably because of dysregulated resolution of inflammation mechanisms (*Figure 3*). Moreover, pro-inflammatory and anti-inflammatory/pro-resolving mediators from diverse nature might be increased, decreased or unchanged in PE women compared with normotensive pregnant women, reinforcing the complex regulation of resolution pathways.

The apparently contradictory findings regarding the measurement of pro-resolving mediators in PE can be a mirror of the biological sample tested (serum/plasma - systemic *vs.* placenta - local) and moment (onset *vs.* stablished inflammation) in which such mediators were measured. It is known that some of the pro-resolving mediators may have dual activities during the inflammatory response, i.e., they can be pro-inflammatory at the beginning of inflammation to assure proper activation of the immunologic system and, as inflammation progresses, they can be pro-resolving, acting as "brakes" for the inflammatory response. In addition, the activity of some mediators may be influenced by several factors, such as molecule structure (e.g., AnxA1 cleavage generates short peptides believed to have pro-inflammatory activities), the cell type in which they act (e.g., LXA4 induces apoptosis of neutrophils while rescue macrophage from death), or concentration (e.g., NO and H_2S have anti-inflammatory actions at low concentrations but pro-inflammatory actions at high concentrations) [33, 112, 113, 132-134, 217, 218].

There are few mechanistic studies in the literature for most of the pro-resolving mediators described in this work in the context of PE. Further investigation about the role of pro-resolving mediators in PE pathogenesis is warranted, for example using knockout animals and therapeutic strategies in animal models. However, none of the available PE animal models can mimic the full spectrum of the human disease [219]. Prospective studies with standardized methodologies would also be valuable to assess whether altered levels and/or actions of pro-resolving mediators in PE women are causes or consequences of the disease. The knowledge acquired from these studies will provide a basis for future clinical trials about novel therapies targeting pro-resolving mechanisms in PE.

Funding: This study was funded by Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG; APQ-03318-15), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq; Research Fellowship) and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES; Ph.D. scholarship).

Conflict of Interest: The authors declare that they have no conflict of interest.

REFERENCES

1. American College of Obstetricians and Gynecologists. Hypertension in pregnancy. Report of the American College of Obstetricians and Gynecologists' Task Force on Hypertension in Pregnancy. Obstet Gynecol. 2013;122(5):1122-31.

2. Chaiworapongsa T, Chaemsaithong P, Yeo L, Romero R. Pre-eclampsia part 1: current understanding of its pathophysiology. Nat Rev Nephrol. 2014;10(8):466-80.

3. American College of Obstetricians and Gynecologists. ACOG practice bulletin. Diagnosis and management of preeclampsia and eclampsia. Number 33, January 2002. Obstet Gynecol. 2002;99(1):159-67.

4. von Dadelszen P, Magee LA, Roberts JM. Subclassification of preeclampsia. Hypertens Pregnancy. 2003;22(2):143-8.

5. Steegers EA, von Dadelszen P, Duvekot JJ, Pijnenborg R. Pre-eclampsia. Lancet. 2010;376(9741):631-44.

6. Raymond D, Peterson E. A critical review of early-onset and late-onset preeclampsia. Obstet Gynecol Surv. 2011;66(8):497-506.

7. Roberts JM, Hubel CA. The two stage model of preeclampsia: variations on the theme. Placenta. 2009;30 Suppl A:S32-7.

8. Lam C, Lim KH, Karumanchi SA. Circulating angiogenic factors in the pathogenesis and prediction of preeclampsia. Hypertension. 2005;46(5):1077-85.

9. Ahmed A, Ramma W. Unravelling the theories of pre-eclampsia: are the protective pathways the new paradigm? Br J Pharmacol. 2015;172(6):1574-86.

10. Cotechini T, Komisarenko M, Sperou A, Macdonald-Goodfellow S, Adams MA, Graham CH. Inflammation in rat pregnancy inhibits spiral artery remodeling leading to fetal growth restriction and features of preeclampsia. J Exp Med. 2014;211(1):165-79.

11. Matsubara K, Higaki T, Matsubara Y, Nawa A. Nitric oxide and reactive oxygen species in the pathogenesis of preeclampsia. Int J Mol Sci. 2015;16(3):4600-14.

12. Wallace JL, Ianaro A, Flannigan KL, Cirino G. Gaseous mediators in resolution of inflammation. Semin Immunol. 2015;27(3):227-33.

13. Chovatiya R, Medzhitov R. Stress, inflammation, and defense of homeostasis. Mol Cell. 2014;54(2):281-8.

14. Serhan CN, Savill J. Resolution of inflammation: the beginning programs the end. Nat Immunol. 2005;6(12):1191-7.

15. Serhan CN, Brain SD, Buckley CD, Gilroy DW, Haslett C, O'Neill LA, et al. Resolution of inflammation: state of the art, definitions and terms. FASEB J. 2007;21(2):325-32.

16. Headland SE, Norling LV. The resolution of inflammation: Principles and challenges. Semin Immunol. 2015;27(3):149-60.

17. Sugimoto M, Sousa L, Pinho V, Perretti M, Teixeira M. Resolution of inflammation: what Controls its Onset? . Frontiers in Immunology. 2016;160(7). p. 1-18.

18. Vago JP, Tavares LP, Sugimoto MA, Lima GL, Galvão I, de Caux TR, et al. Proresolving Actions of Synthetic and Natural Protease Inhibitors Are Mediated by Annexin A1. J Immunol. 2016;196(4):1922-32.

19. Odaka C, Mizuochi T, Yang J, Ding A. Murine macrophages produce secretory leukocyte protease inhibitor during clearance of apoptotic cells: implications for resolution of the inflammatory response. J Immunol. 2003;171(3):1507-14.

20. Titos E, Rius B, González-Périz A, López-Vicario C, Morán-Salvador E, Martínez-Clemente M, et al. Resolvin D1 and its precursor docosahexaenoic acid promote resolution of adipose tissue inflammation by eliciting macrophage polarization toward an M2-like phenotype. J Immunol. 2011;187(10):5408-18.

21. Fullerton JN, Gilroy DW. Resolution of inflammation: a new therapeutic frontier. Nat Rev Drug Discov. 2016;15(8):551-67.

22. Orsi NM. Cytokine networks in the establishment and maintenance of pregnancy. Hum Fertil (Camb). 2008;11(4):222-30.

23. Redman CW, Sacks GP, Sargent IL. Preeclampsia: an excessive maternal inflammatory response to pregnancy. Am J Obstet Gynecol. 1999;180(2 Pt 1):499-506.

24. Schminkey DL, Groer M. Imitating a stress response: a new hypothesis about the innate immune system's role in pregnancy. Med Hypotheses. 2014;82(6):721-9.

25. Saito S, Nakashima A, Shima T, Ito M. Th1/Th2/Th17 and regulatory T-cell paradigm in pregnancy. Am J Reprod Immunol. 2010;63(6):601-10.

26. Li M, Piao L, Chen CP, Wu X, Yeh CC, Masch R, et al. Modulation of Decidual Macrophage Polarization by Macrophage Colony-Stimulating Factor Derived from First-Trimester Decidual Cells: Implication in Preeclampsia. Am J Pathol. 2016;186(5):1258-66.

27. Lee CL, Guo Y, So KH, Vijayan M, Wong VH, Yao Y, et al. Soluble human leukocyte antigen G5 polarizes differentiation of macrophages toward a decidual macrophage-like phenotype. Hum Reprod. 2015;30(10):2263-74.

28. Perretti M, D'Acquisto F. Annexin A1 and glucocorticoids as effectors of the resolution of inflammation. Nat Rev Immunol. 2009;9(1):62-70.

29. Sugimoto MA, Vago JP, Teixeira MM, Sousa LP. Annexin A1 and the Resolution of Inflammation: Modulation of Neutrophil Recruitment, Apoptosis, and Clearance. J Immunol Res. 2016;2016:8239258.

30. John CD, Gavins FN, Buss NA, Cover PO, Buckingham JC. Annexin A1 and the formyl peptide receptor family: neuroendocrine and metabolic aspects. Curr Opin Pharmacol. 2008;8(6):765-76.

31. John CD, Christian HC, Morris JF, Flower RJ, Solito E, Buckingham JC. Annexin 1 and the regulation of endocrine function. Trends Endocrinol Metab. 2004;15(3):103-9.

32. Eke Gungor H, Tahan F, Gokahmetoglu S, Saraymen B. Decreased levels of lipoxin A4 and annexin A1 in wheezy infants. Int Arch Allergy Immunol. 2014;163(3):193-7.

33. Williams SL, Milne IR, Bagley CJ, Gamble JR, Vadas MA, Pitson SM, et al. A proinflammatory role for proteolytically cleaved annexin A1 in neutrophil transendothelial migration. J Immunol. 2010;185(5):3057-63.

34. Tsao FH, Meyer KC, Chen X, Rosenthal NS, Hu J. Degradation of annexin I in bronchoalveolar lavage fluid from patients with cystic fibrosis. Am J Respir Cell Mol Biol. 1998;18(1):120-8.

35. Cooray SN, Gobbetti T, Montero-Melendez T, McArthur S, Thompson D, Clark AJ, et al. Ligand-specific conformational change of the G-protein-coupled receptor ALX/FPR2 determines proresolving functional responses. Proc Natl Acad Sci U S A. 2013;110(45):18232-7.

36. Planagumà A, Kazani S, Marigowda G, Haworth O, Mariani TJ, Israel E, et al. Airway lipoxin A4 generation and lipoxin A4 receptor expression are decreased in severe asthma. Am J Respir Crit Care Med. 2008;178(6):574-82.

37. Perucci LO, Carneiro FS, Ferreira CN, Sugimoto MA, Soriani FM, Martins GG, et al. Annexin A1 Is Increased in the Plasma of Preeclamptic Women. PLoS One. 2015;10(9):e0138475.

38. Xu Z, Zhao F, Lin F, Xiang H, Wang N, Ye D, et al. Preeclampsia is associated with a deficiency of lipoxin A4, an endogenous anti-inflammatory mediator. Fertil Steril. 2014;102(1):282-90.e4.

39. Dong W, Yin L. Expression of lipoxin A4, TNFα and IL-1β in maternal peripheral blood, umbilical cord blood and placenta, and their significance in pre-eclampsia. Hypertens Pregnancy. 2014;33(4):449-56.

40. Behrouz GF, Farzaneh GS, Leila J, Jaleh Z, Eskandar KS. Presence of auto-antibody against two placental proteins, annexin A1 and vitamin D binding protein, in sera of women with pre-eclampsia. J Reprod Immunol. 2013;99(1-2):10-6.

41. Canzoneri BJ, Lewis DF, Groome L, Wang Y. Increased neutrophil numbers account for leukocytosis in women with preeclampsia. Am J Perinatol. 2009;26(10):729-32.

42. Gupta AK, Gebhardt S, Hillermann R, Holzgreve W, Hahn S. Analysis of plasma elastase levels in early and late onset preeclampsia. Arch Gynecol Obstet. 2006;273(4):239-42.

43. Salama RH, Fathalla MM, Mekki AR, Elsadek Bl-K. Implication of umbilical cord in preeclampsia. Med Princ Pract. 2011;20(2):124-8.

44. Sun M, Liu Y, Gibb W. Distribution of annexin I and II in term human fetal membranes, decidua and placenta. Placenta. 1996;17(2-3):181-4.

45. Leffler H, Carlsson S, Hedlund M, Qian Y, Poirier F. Introduction to galectins. Glycoconj J. 2004;19(7-9):433-40.

46. Sato S, St-Pierre C, Bhaumik P, Nieminen J. Galectins in innate immunity: dual functions of host soluble beta-galactoside-binding lectins as damage-associated molecular patterns (DAMPs) and as receptors for pathogen-associated molecular patterns (PAMPs). Immunol Rev. 2009;230(1):172-87.

47. Rubinstein N, Ilarregui JM, Toscano MA, Rabinovich GA. The role of galectins in the initiation, amplification and resolution of the inflammatory response. Tissue Antigens. 2004;64(1):1-12.

48. Arikawa T, Simamura E, Shimada H, Nakamura T, Hatta T, Shoji H. Significance of sugar chain recognition by galectins and its involvement in disease-associated glycosylation. Congenit Anom (Kyoto). 2014;54(2):77-81.

49. Vasta GR. Galectins as pattern recognition receptors: structure, function, and evolution. Adv Exp Med Biol. 2012;946:21-36.

50. Romaniuk MA, Negrotto S, Campetella O, Rabinovich GA, Schattner M. Identification of galectins as novel regulators of platelet signaling and function. IUBMB Life. 2011;63(7):521-7.

51. Blois SM, Conrad ML, Freitag N, Barrientos G. Galectins in angiogenesis: consequences for gestation. J Reprod Immunol. 2015;108:33-41.

52. Blois SM, Ilarregui JM, Tometten M, Garcia M, Orsal AS, Cordo-Russo R, et al. A pivotal role for galectin-1 in fetomaternal tolerance. Nat Med. 2007;13(12):1450-7.

53. van der Leij J, van den Berg A, Blokzijl T, Harms G, van Goor H, Zwiers P, et al. Dimeric galectin-1 induces IL-10 production in T-lymphocytes: an important tool in the regulation of the immune response. J Pathol. 2004;204(5):511-8.

54. Kopcow HD, Rosetti F, Leung Y, Allan DS, Kutok JL, Strominger JL. T cell apoptosis at the maternal-fetal interface in early human pregnancy, involvement of galectin-1. Proc Natl Acad Sci U S A. 2008;105(47):18472-7.

55. Rostoker R, Yaseen H, Schif-Zuck S, Lichtenstein RG, Rabinovich GA, Ariel A. Galectin-1 induces 12/15-lipoxygenase expression in murine macrophages and favors their conversion toward a pro-resolving phenotype. Prostaglandins Other Lipid Mediat. 2013;107:85-94.

56. Jeschke U, Mayr D, Schiessl B, Mylonas I, Schulze S, Kuhn C, et al. Expression of galectin-1, -3 (gal-1, gal-3) and the Thomsen-Friedenreich (TF) antigen in normal, IUGR, preeclamptic and HELLP placentas. Placenta. 2007;28(11-12):1165-73.

57. Than NG, Erez O, Wildman DE, Tarca AL, Edwin SS, Abbas A, et al. Severe preeclampsia is characterized by increased placental expression of galectin-1. J Matern Fetal Neonatal Med. 2008;21(7):429-42.

58. Freitag N, Tirado-González I, Barrientos G, Herse F, Thijssen VL, Weedon-Fekjær SM, et al. Interfering with Gal-1-mediated angiogenesis contributes to the pathogenesis of preeclampsia. Proc Natl Acad Sci U S A. 2013;110(28):11451-6.

59. Blois SM, Dechend R, Barrientos G, Staff AC. A potential pathophysiological role for galectins and the renin-angiotensin system in preeclampsia. Cell Mol Life Sci. 2015;72(1):39-50.

60. Molvarec A, Blois SM, Stenczer B, Toldi G, Tirado-Gonzalez I, Ito M, et al. Peripheral blood galectin-1-expressing T and natural killer cells in normal pregnancy and preeclampsia. Clin Immunol. 2011;139(1):48-56.

61. Than NG, Pick E, Bellyei S, Szigeti A, Burger O, Berente Z, et al. Functional analyses of placental protein 13/galectin-13. Eur J Biochem. 2004;271(6):1065-78.

62. Orendi K, Gauster M, Moser G, Meiri H, Huppertz B. The choriocarcinoma cell line BeWo: syncytial fusion and expression of syncytium-specific proteins. Reproduction. 2010;140(5):759-66.

63. Than NG, Romero R, Xu Y, Erez O, Xu Z, Bhatti G, et al. Evolutionary origins of the placental expression of chromosome 19 cluster galectins and their complex dysregulation in preeclampsia. Placenta. 2014;35(11):855-65.

64. Than NG, Romero R, Goodman M, Weckle A, Xing J, Dong Z, et al. A primate subfamily of galectins expressed at the maternal-fetal interface that promote immune cell death. Proc Natl Acad Sci U S A. 2009;106(24):9731-6.

65. Kliman HJ, Sammar M, Grimpel YI, Lynch SK, Milano KM, Pick E, et al. Placental protein 13 and decidual zones of necrosis: an immunologic diversion that may be linked to preeclampsia. Reprod Sci. 2012;19(1):16-30.

66. Than NG, Abdul Rahman O, Magenheim R, Nagy B, Fule T, Hargitai B, et al. Placental protein 13 (galectin-13) has decreased placental expression but increased shedding and maternal serum concentrations in patients presenting with preterm pre-eclampsia and HELLP syndrome. Virchows Arch. 2008;453(4):387-400.

67. Huppertz B, Sammar M, Chefetz I, Neumaier-Wagner P, Bartz C, Meiri H. Longitudinal determination of serum placental protein 13 during development of preeclampsia. Fetal Diagn Ther. 2008;24(3):230-6.

68. Gonen R, Shahar R, Grimpel YI, Chefetz I, Sammar M, Meiri H, et al. Placental protein 13 as an early marker for pre-eclampsia: a prospective longitudinal study. BJOG. 2008;115(12):1465-72.

69. Sekizawa A, Purwosunu Y, Yoshimura S, Nakamura M, Shimizu H, Okai T, et al. PP13 mRNA expression in trophoblasts from preeclamptic placentas. Reprod Sci. 2009;16(4):408-13.

70. Shimizu H, Sekizawa A, Purwosunu Y, Nakamura M, Farina A, Rizzo N, et al. PP13 mRNA expression in the cellular component of maternal blood as a marker for preeclampsia. Prenat Diagn. 2009;29(13):1231-6.

71. Farina A, Zucchini C, Sekizawa A, Purwosunu Y, de Sanctis P, Santarsiero G, et al. Performance of messenger RNAs circulating in maternal blood in the prediction of preeclampsia at 10-14 weeks. Am J Obstet Gynecol. 2010;203(6):575.e1-7.

72. Gebhardt S, Bruiners N, Hillermann R. A novel exonic variant (221delT) in the LGALS13 gene encoding placental protein 13 (PP13) is associated with preterm labour in a low risk population. J Reprod Immunol. 2009;82(2):166-73.

73. Than NG, Balogh A, Romero R, Kárpáti E, Erez O, Szilágyi A, et al. Placental Protein 13 (PP13) - A Placental Immunoregulatory Galectin Protecting Pregnancy. Front Immunol. 2014;5:348.

74. Huppertz B, Meiri H, Gizurarson S, Osol G, Sammar M. Placental protein 13 (PP13): a new biological target shifting individualized risk assessment to personalized drug design combating pre-eclampsia. Hum Reprod Update. 2013;19(4):391-405.

75. Sammar M, Nisemblat S, Fleischfarb Z, Golan A, Sadan O, Meiri H, et al. Placenta-bound and body fluid PP13 and its mRNA in normal pregnancy compared to preeclampsia, HELLP and preterm delivery. Placenta. 2011;32 Suppl:S30-6.

76. Zabel BA, Kwitniewski M, Banas M, Zabieglo K, Murzyn K, Cichy J. Chemerin regulation and role in host defense. Am J Clin Exp Immunol. 2014;3(1):1-19.

77. Mariani F, Roncucci L. Chemerin/chemR23 axis in inflammation onset and resolution. Inflamm Res. 2015;64(2):85-95.

78. Zabel BA, Allen SJ, Kulig P, Allen JA, Cichy J, Handel TM, et al. Chemerin activation by serine proteases of the coagulation, fibrinolytic, and inflammatory cascades. J Biol Chem. 2005;280(41):34661-6.

79. Carlino C, Trotta E, Stabile H, Morrone S, Bulla R, Soriani A, et al. Chemerin regulates NK cell accumulation and endothelial cell morphogenesis in the decidua during early pregnancy. J Clin Endocrinol Metab. 2012;97(10):3603-12.

80. Tessier DR, Yockell-Lelievre J, Gruslin A. Uterine Spiral Artery Remodeling: The Role of Uterine Natural Killer Cells and Extravillous Trophoblasts in Normal and High-Risk Human Pregnancies. Am J Reprod Immunol. 2015;74(1):1-11.

81. Kaur J, Adya R, Tan BK, Chen J, Randeva HS. Identification of chemerin receptor (ChemR23) in human endothelial cells: chemerin-induced endothelial angiogenesis. Biochem Biophys Res Commun. 2010;391(4):1762-8.

82. Moretta A, Marcenaro E, Parolini S, Ferlazzo G, Moretta L. NK cells at the interface between innate and adaptive immunity. Cell Death Differ. 2008;15(2):226-33.

83. Hespel C, Moser M. Role of inflammatory dendritic cells in innate and adaptive immunity. Eur J Immunol. 2012;42(10):2535-43.

84. Garces MF, Sanchez E, Ruiz-Parra AI, Rubio-Romero JA, Angel-Muller E, Suarez MA, et al. Serum chemerin levels during normal human pregnancy. Peptides. 2013;42:138-43.

85. Kasher-Meron M, Mazaki-Tovi S, Barhod E, Hemi R, Haas J, Gat I, et al. Chemerin concentrations in maternal and fetal compartments: implications for metabolic adaptations to normal human pregnancy. J Perinat Med. 2014;42(3):371-8.

86. Wang L, Yang T, Ding Y, Zhong Y, Yu L, Peng M. Chemerin plays a protective role by regulating human umbilical vein endothelial cell-induced nitric oxide signaling in preeclampsia. Endocrine. 2015;48(1):299-308.

87. Duan DM, Niu JM, Lei Q, Lin XH, Chen X. Serum levels of the adipokine chemerin in preeclampsia. J Perinat Med. 2012;40(2):121-7.

88. Stepan H, Philipp A, Roth I, Kralisch S, Jank A, Schaarschmidt W, et al. Serum levels of the adipokine chemerin are increased in preeclampsia during and 6 months after pregnancy. Regul Pept. 2011;168(1-3):69-72.

89. Xu QL, Zhu M, Jin Y, Wang N, Xu HX, Quan LM, et al. The predictive value of the first-trimester maternal serum chemerin level for pre-eclampsia. Peptides. 2014;62:150-4.

90. Serhan CN. Pro-resolving lipid mediators are leads for resolution physiology. Nature. 2014;510(7503):92-101.

91. Serhan CN. Lipoxins and aspirin-triggered 15-epi-lipoxins are the first lipid mediators of endogenous anti-inflammation and resolution. Prostaglandins Leukot Essent Fatty Acids. 2005;73(3-4):141-62.

92. Fierro IM. Angiogenesis and lipoxins. Prostaglandins Leukot Essent Fatty Acids. 2005;73(3-4):271-5.

93. Choi G, Hwang SW. Modulation of the Activities of Neuronal Ion Channels by Fatty Acid-Derived Pro-Resolvents. Front Physiol. 2016;7:523.

94. Levy BD, Serhan CN. Exploring new approaches to the treatment of asthma: potential roles for lipoxins and aspirin-triggered lipid mediators. Drugs Today (Barc). 2003;39(5):373-84.

95. Lee TH. Lipoxin A4: a novel anti-inflammatory molecule? Thorax. 1995;50(2):111-2.

96. Gavins FN, Sawmynaden P, Chatterjee BE, Perretti M. A twist in anti-inflammation: annexin 1 acts via the lipoxin A4 receptor. Prostaglandins Leukot Essent Fatty Acids. 2005;73(3-4):211-9.

97. Wang J, Huang Y, Zhou J, Liu X. Effect of lipoxin A_4 on IL-1β production of monocytes and its possible mechanism in severe preeclampsia. J Huazhong Univ Sci Technolog Med Sci. 2010;30(6):767-70.

98. Gil-Villa AM, Norling LV, Serhan CN, Cordero D, Rojas M, Cadavid A. Aspirin triggered-lipoxin A4 reduces the adhesion of human polymorphonuclear neutrophils to endothelial cells initiated by preeclamptic plasma. Prostaglandins Leukot Essent Fatty Acids. 2012;87(4-5):127-34.

99. Lin F, Zeng P, Xu Z, Ye D, Yu X, Wang N, et al. Treatment of Lipoxin A(4) and its analogue on low-dose endotoxin induced preeclampsia in rat and possible mechanisms. Reprod Toxicol. 2012;34(4):677-85.

100. Huang LL, Su S, Awale R, Zhang XY, Zhong LL, Tang H. Expression of anti-inflammatory mediator lipoxin A4 and inflammation responsive transcriptive factors NF-kappa B in patients with preeclampsia. Clin Exp Obstet Gynecol. 2014;41(5):561-6.

101. Perucci LO, Santos PC, Ribeiro LS, Souza DG, Gomes KB, Dusse LM, et al. Lipoxin A4 Is Increased in the Plasma of Preeclamptic Women. Am J Hypertens. 2016;29(10):1179-85.

102. Cooper CE, Brown GC. The inhibition of mitochondrial cytochrome oxidase by the gases carbon monoxide, nitric oxide, hydrogen cyanide and hydrogen sulfide: chemical mechanism and physiological significance. J Bioenerg Biomembr. 2008;40(5):533-9.

103. Wang R. Two's company, three's a crowd: can H2S be the third endogenous gaseous transmitter? FASEB J. 2002;16(13):1792-8.

104. Kajimura M, Fukuda R, Bateman RM, Yamamoto T, Suematsu M. Interactions of multiple gas-transducing systems: hallmarks and uncertainties of CO, NO, and H2S gas biology. Antioxid Redox Signal. 2010;13(2):157-92.

105. Lyall F. Development of the utero-placental circulation: the role of carbon monoxide and nitric oxide in trophoblast invasion and spiral artery transformation. Microsc Res Tech. 2003;60(4):402-11.

106. Li H, Horke S, Förstermann U. Vascular oxidative stress, nitric oxide and atherosclerosis. Atherosclerosis. 2014;237(1):208-19.

107. Nagy G, Koncz A, Telarico T, Fernandez D, Ersek B, Buzás E, et al. Central role of nitric oxide in the pathogenesis of rheumatoid arthritis and systemic lupus erythematosus. Arthritis Res Ther. 2010;12(3):210.

108. Ignarro LJ, Buga GM, Wood KS, Byrns RE, Chaudhuri G. Endothelium-derived relaxing factor produced and released from artery and vein is nitric oxide. Proc Natl Acad Sci U S A. 1987;84(24):9265-9.

109. Sogo N, Magid KS, Shaw CA, Webb DJ, Megson IL. Inhibition of human platelet aggregation by nitric oxide donor drugs: relative contribution of cGMP-independent mechanisms. Biochem Biophys Res Commun. 2000;279(2):412-9.

110. Ward C, Wong TH, Murray J, Rahman I, Haslett C, Chilvers ER, et al. Induction of human neutrophil apoptosis by nitric oxide donors: evidence for a caspase-dependent, cyclic-GMP-independent, mechanism. Biochem Pharmacol. 2000;59(3):305-14.

111. Lo Faro ML, Fox B, Whatmore JL, Winyard PG, Whiteman M. Hydrogen sulfide and nitric oxide interactions in inflammation. Nitric Oxide. 2014;41:38-47.

112. Laroux FS, Lefer DJ, Kawachi S, Scalia R, Cockrell AS, Gray L, et al. Role of nitric oxide in the regulation of acute and chronic inflammation. Antioxid Redox Signal. 2000;2(3):391-6.

113. Guzik TJ, Korbut R, Adamek-Guzik T. Nitric oxide and superoxide in inflammation and immune regulation. J Physiol Pharmacol. 2003;54(4):469-87.

114. Shaw CA, Taylor EL, Fox S, Megson IL, Rossi AG. Differential susceptibility to nitric oxide-evoked apoptosis in human inflammatory cells. Free Radic Biol Med. 2011;50(1):93-101.

115. Huang LT, Hsieh CS, Chang KA, Tain YL. Roles of nitric oxide and asymmetric dimethylarginine in pregnancy and fetal programming. Int J Mol Sci. 2012;13(11):14606-22.

116. Morris NH, Sooranna SR, Learmont JG, Poston L, Ramsey B, Pearson JD, et al. Nitric oxide synthase activities in placental tissue from normotensive, pre-eclamptic and growth retarded pregnancies. Br J Obstet Gynaecol. 1995;102(9):711-4.

117. Böger RH, Diemert A, Schwedhelm E, Lüneburg N, Maas R, Hecher K. The role of nitric oxide synthase inhibition by asymmetric dimethylarginine in the pathophysiology of preeclampsia. Gynecol Obstet Invest. 2010;69(1):1-13.

118. López-Jaramillo P, Arenas WD, García RG, Rincon MY, López M. The role of the L-arginine-nitric oxide pathway in preeclampsia. Ther Adv Cardiovasc Dis. 2008;2(4):261-75.

119. Bian Z, Shixia C, Duan T. First-Trimester Maternal Serum Levels of sFLT1, PGF and ADMA Predict Preeclampsia. PLoS One. 2015;10(4):e0124684.

120. Alpoim PN, Godoi LC, Freitas LG, Gomes KB, Dusse LM. Assessment of L-arginine asymmetric 1 dimethyl (ADMA) in early-onset and late-onset (severe) preeclampsia. Nitric Oxide. 2013;33:81-2.

121. Alpoim PN, Gomes KB, Pinheiro MeB, Godoi LC, Jardim LL, Muniz LG, et al. Polymorphisms in endothelial nitric oxide synthase gene in early and late severe preeclampsia. Nitric Oxide. 2014;42:19-23.

122. Doridot L, Passet B, Mehats C, Rigourd V, Barbaux S, Ducat A, et al. Preeclampsia-like symptoms induced in mice by fetoplacental expression of STOX1 are reversed by aspirin treatment. Hypertension. 2013;61(3):662-8.

123. van Dijk M, Mulders J, Poutsma A, Konst AA, Lachmeijer AM, Dekker GA, et al. Maternal segregation of the Dutch preeclampsia locus at 10q22 with a new member of the winged helix gene family. Nat Genet. 2005;37(5):514-9.

124. Doridot L, Chatre L, Ducat A, Vilotte JL, Lombes A, Mehats C, et al. Nitroso-redox balance and mitochondrial homeostasis are regulated by STOX1, a pre-eclampsia-associated gene. Antioxid Redox Signal. 2014;21(6):819-34.

125. van Dijk M, van Bezu J, van Abel D, Dunk C, Blankenstein MA, Oudejans CB, et al. The STOX1 genotype associated with pre-eclampsia leads to a reduction of trophoblast invasion by alpha-T-catenin upregulation. Hum Mol Genet. 2010;19(13):2658-67.

126. Nanaev A, Chwalisz K, Frank HG, Kohnen G, Hegele-Hartung C, Kaufmann P. Physiological dilation of uteroplacental arteries in the guinea pig depends on nitric oxide synthase activity of extravillous trophoblast. Cell Tissue Res. 1995;282(3):407-21.

127. Holwerda KM, Faas MM, van Goor H, Lely AT. Gasotransmitters: a solution for the therapeutic dilemma in preeclampsia? Hypertension. 2013;62(4):653-9.

128. Meher S, Duley L. Nitric oxide for preventing pre-eclampsia and its complications. Cochrane Database Syst Rev. 2007(2):CD006490.

129. Olas B. Hydrogen sulfide in signaling pathways. Clin Chim Acta. 2015;439:212-8.

130. Kida M, Sugiyama T, Yoshimoto T, Ogawa Y. Hydrogen sulfide increases nitric oxide production with calcium-dependent activation of endothelial nitric oxide synthase in endothelial cells. Eur J Pharm Sci. 2013;48(1-2):211-5.

131. Szabó C. Hydrogen sulphide and its therapeutic potential. Nat Rev Drug Discov. 2007;6(11):917-35.

132. Collin M, Anuar FB, Murch O, Bhatia M, Moore PK, Thiemermann C. Inhibition of endogenous hydrogen sulfide formation reduces the organ injury caused by endotoxemia. Br J Pharmacol. 2005;146(4):498-505.

133. Zhang H, Zhi L, Moochhala S, Moore PK, Bhatia M. Hydrogen sulfide acts as an inflammatory mediator in cecal ligation and puncture-induced sepsis in mice by upregulating the production of cytokines and chemokines via NF-kappaB. Am J Physiol Lung Cell Mol Physiol. 2007;292(4):L960-71.

134. Wallace JL, Vong L, McKnight W, Dicay M, Martin GR. Endogenous and exogenous hydrogen sulfide promotes resolution of colitis in rats. Gastroenterology. 2009;137(2):569-78, 78.e1.

135. Mariggio MA, Minunno V, Riccardi S, Santacroce R, De Rinaldis P, Fumarulo R. Sulfide enhancement of PMN apoptosis. Immunopharmacol Immunotoxicol. 1998;20(3):399-408.

136. Miao L, Shen X, Whiteman M, Xin H, Shen Y, Xin X, et al. Hydrogen Sulfide Mitigates Myocardial Infarction via Promotion of Mitochondrial Biogenesis-Dependent M2 Polarization of Macrophages. Antioxid Redox Signal. 2016;25(5):268-81.

137. Dufton N, Natividad J, Verdu EF, Wallace JL. Hydrogen sulfide and resolution of acute inflammation: A comparative study utilizing a novel fluorescent probe. Sci Rep. 2012;2:499.

138. Brancaleone V, Mitidieri E, Flower RJ, Cirino G, Perretti M. Annexin A1 mediates hydrogen sulfide properties in the control of inflammation. J Pharmacol Exp Ther. 2014;351(1):96-104.

139. Papapetropoulos A, Pyriochou A, Altaany Z, Yang G, Marazioti A, Zhou Z, et al. Hydrogen sulfide is an endogenous stimulator of angiogenesis. Proc Natl Acad Sci U S A. 2009;106(51):21972-7.

140. Patel P, Vatish M, Heptinstall J, Wang R, Carson RJ. The endogenous production of hydrogen sulphide in intrauterine tissues. Reprod Biol Endocrinol. 2009;7:10.

141. Wang K, Ahmad S, Cai M, Rennie J, Fujisawa T, Crispi F, et al. Dysregulation of hydrogen sulfide producing enzyme cystathionine γ-lyase contributes to maternal hypertension and placental abnormalities in preeclampsia. Circulation. 2013;127(25):2514-22.

142. Cindrova-Davies T, Herrera EA, Niu Y, Kingdom J, Giussani DA, Burton GJ. Reduced cystathionine γ-lyase and increased miR-21 expression are associated with increased vascular resistance in growth-restricted pregnancies: hydrogen sulfide as a placental vasodilator. Am J Pathol. 2013;182(4):1448-58.

143. Holwerda KM, Bos EM, Rajakumar A, Ris-Stalpers C, van Pampus MG, Timmer A, et al. Hydrogen sulfide producing enzymes in pregnancy and preeclampsia. Placenta. 2012;33(6):518-21.

144. Roes EM, Raijmakers MT, Boo TM, Zusterzeel PL, Merkus HM, Peters WH, et al. Oral N-acetylcysteine administration does not stabilise the process of established severe preeclampsia. Eur J Obstet Gynecol Reprod Biol. 2006;127(1):61-7.

145. Ryter SW, Choi AM. Heme oxygenase-1/carbon monoxide: from metabolism to molecular therapy. Am J Respir Cell Mol Biol. 2009;41(3):251-60.

146. Wu L, Wang R. Carbon monoxide: endogenous production, physiological functions, and pharmacological applications. Pharmacol Rev. 2005;57(4):585-630.

147. Bilban M, Haschemi A, Wegiel B, Chin BY, Wagner O, Otterbein LE. Heme oxygenase and carbon monoxide initiate homeostatic signaling. J Mol Med (Berl). 2008;86(3):267-79.

148. Peers C, Boyle JP, Scragg JL, Dallas ML, Al-Owais MM, Hettiarachichi NT, et al. Diverse mechanisms underlying the regulation of ion channels by carbon monoxide. Br J Pharmacol. 2015;172(6):1546-56.

149. Urquhart P, Rosignoli G, Cooper D, Motterlini R, Perretti M. Carbon monoxide-releasing molecules modulate leukocyte-endothelial interactions under flow. J Pharmacol Exp Ther. 2007;321(2):656-62.

150. Morse D, Pischke SE, Zhou Z, Davis RJ, Flavell RA, Loop T, et al. Suppression of inflammatory cytokine production by carbon monoxide involves the JNK pathway and AP-1. J Biol Chem. 2003;278(39):36993-8.

151. Otterbein LE, May A, Chin BY. Carbon monoxide increases macrophage bacterial clearance through Toll-like receptor (TLR)4 expression. Cell Mol Biol (Noisy-le-grand). 2005;51(5):433-40.

152. Chiang N, Shinohara M, Dalli J, Mirakaj V, Kibi M, Choi AM, et al. Inhaled carbon monoxide accelerates resolution of inflammation via unique proresolving mediator-heme oxygenase-1 circuits. J Immunol. 2013;190(12):6378-88.

153. Lyall F, Barber A, Myatt L, Bulmer JN, Robson SC. Hemeoxygenase expression in human placenta and placental bed implies a role in regulation of trophoblast invasion and placental function. FASEB J. 2000;14(1):208-19.

154. Bainbridge SA, Smith GN. HO in pregnancy. Free Radic Biol Med. 2005;38(8):979-88.

155. McCaig D, Lyall F. Inhibitors of heme oxygenase reduce invasion of human primary cytotrophoblast cells in vitro. Placenta. 2009;30(6):536-8.

156. Sollwedel A, Bertoja AZ, Zenclussen ML, Gerlof K, Lisewski U, Wafula P, et al. Protection from abortion by heme oxygenase-1 up-regulation is associated with increased levels of Bag-1 and neuropilin-1 at the fetal-maternal interface. J Immunol. 2005;175(8):4875-85.

157. Baum M, Schiff E, Kreiser D, Dennery PA, Stevenson DK, Rosenthal T, et al. End-tidal carbon monoxide measurements in women with pregnancy-induced hypertension and preeclampsia. Am J Obstet Gynecol. 2000;183(4):900-3.

158. Yusuf K, Kamaluddeen M, Wilson RD, Akierman A. Carboxyhemoglobin levels in umbilical cord blood of women with pre-eclampsia and intrauterine growth restriction. J Perinat Med. 2012;40(6):619-24.

159. Zhai D, Guo Y, Smith G, Krewski D, Walker M, Wen SW. Maternal exposure to moderate ambient carbon monoxide is associated with decreased risk of preeclampsia. Am J Obstet Gynecol. 2012;207(1):57.e1-9.

160. Wikström AK, Stephansson O, Cnattingius S. Tobacco use during pregnancy and preeclampsia risk: effects of cigarette smoking and snuff. Hypertension. 2010;55(5):1254-9.

161. Maebayashi Asanuma A, Yamamoto T, Azuma H, Kato E, Yamamoto N, Murase T, et al. Expression of placenta growth factor, soluble fms-like tyrosine kinase-1, metal-responsive transcription factor-1, heme

oxygenase 1 and hypoxia inducible factor-1α mRNAs in pre-eclampsia placenta and the effect of pre-eclampsia sera on their expression of choriocarcinoma cells. J Obstet Gynaecol Res. 2014;40(10):2095-103.

162. Zenclussen AC, Lim E, Knoeller S, Knackstedt M, Hertwig K, Hagen E, et al. Heme oxygenases in pregnancy II: HO-2 is downregulated in human pathologic pregnancies. Am J Reprod Immunol. 2003;50(1):66-76.

163. Barber A, Robson SC, Myatt L, Bulmer JN, Lyall F. Heme oxygenase expression in human placenta and placental bed: reduced expression of placenta endothelial HO-2 in preeclampsia and fetal growth restriction. FASEB J. 2001;15(7):1158-68.

164. Eide IP, Isaksen CV, Salvesen KA, Langaas M, Schønberg SA, Austgulen R. Decidual expression and maternal serum levels of heme oxygenase 1 are increased in pre-eclampsia. Acta Obstet Gynecol Scand. 2008;87(3):272-9.

165. Tong S, Kaitu'u-Lino TJ, Onda K, Beard S, Hastie R, Binder NK, et al. Heme Oxygenase-1 Is Not Decreased in Preeclamptic Placenta and Does Not Negatively Regulate Placental Soluble fms-Like Tyrosine Kinase-1 or Soluble Endoglin Secretion. Hypertension. 2015;66(5):1073-81.

166. Brownfoot FC, Tong S, Hannan NJ, Binder NK, Walker SP, Cannon P, et al. Effects of Pravastatin on Human Placenta, Endothelium, and Women With Severe Preeclampsia. Hypertension. 2015;66(3):687-97; discussion 445.

167. Ramma W, Ahmed A. Therapeutic potential of statins and the induction of heme oxygenase-1 in preeclampsia. J Reprod Immunol. 2014;101-102:153-60.

168. McCarthy FP, Drewlo S, Kingdom J, Johns EJ, Walsh SK, Kenny LC. Peroxisome proliferator-activated receptor-γ as a potential therapeutic target in the treatment of preeclampsia. Hypertension. 2011;58(2):280-6.

169. Venditti CC, Casselman R, Young I, Karumanchi SA, Smith GN. Carbon monoxide prevents hypertension and proteinuria in an adenovirus sFlt-1 preeclampsia-like mouse model. PLoS One. 2014;9(9):e106502.

170. Picciotto MR, Higley MJ, Mineur YS. Acetylcholine as a neuromodulator: cholinergic signaling shapes nervous system function and behavior. Neuron. 2012;76(1):116-29.

171. Prado MA, Reis RA, Prado VF, de Mello MC, Gomez MV, de Mello FG. Regulation of acetylcholine synthesis and storage. Neurochem Int. 2002;41(5):291-9.

172. Andersson U, Tracey KJ. Reflex principles of immunological homeostasis. Annu Rev Immunol. 2012;30:313-35.

173. Andersson U, Tracey KJ. Neural reflexes in inflammation and immunity. J Exp Med. 2012;209(6):1057-68.

174. Sundman E, Olofsson PS. Neural control of the immune system. Adv Physiol Educ. 2014;38(2):135-9.

175. Báez-Pagán CA, Delgado-Vélez M, Lasalde-Dominicci JA. Activation of the Macrophage α7 Nicotinic Acetylcholine Receptor and Control of Inflammation. J Neuroimmune Pharmacol. 2015;10(3):468-76.

176. Tsoyi K, Jang HJ, Kim JW, Chang HK, Lee YS, Pae HO, et al. Stimulation of alpha7 nicotinic acetylcholine receptor by nicotine attenuates inflammatory response in macrophages and improves survival in experimental model of sepsis through heme oxygenase-1 induction. Antioxid Redox Signal. 2011;14(11):2057-70.

177. Maldifassi MC, Atienza G, Arnalich F, López-Collazo E, Cedillo JL, Martín-Sánchez C, et al. A new IRAK-M-mediated mechanism implicated in the anti-inflammatory effect of nicotine via α7 nicotinic receptors in human macrophages. PLoS One. 2014;9(9):e108397.

178. Huston JM, Rosas-Ballina M, Xue X, Dowling O, Ochani K, Ochani M, et al. Cholinergic neural signals to the spleen down-regulate leukocyte trafficking via CD11b. J Immunol. 2009;183(1):552-9.

179. Mariggiò MA, Guida L, Laforgia A, Santacroce R, Curci E, Montemurro P, et al. Nicotine effects on polymorphonuclear cell apoptosis and lipopolysaccharide-induced monocyte functions. A possible role in periodontal disease? J Periodontal Res. 2001;36(1):32-9.

180. van der Zanden EP, Snoek SA, Heinsbroek SE, Stanisor OI, Verseijden C, Boeckxstaens GE, et al. Vagus nerve activity augments intestinal macrophage phagocytosis via nicotinic acetylcholine receptor alpha4beta2. Gastroenterology. 2009;137(3):1029-39, 39.e1-4.

181. Lee RH, Vazquez G. Evidence for a prosurvival role of alpha-7 nicotinic acetylcholine receptor in alternatively (M2)-activated macrophages. Physiol Rep. 2013;1(7):e00189.

182. Tracey KJ. Physiology and immunology of the cholinergic antiinflammatory pathway. J Clin Invest. 2007;117(2):289-96.

183. Yang CC, Chao TC, Kuo TB, Yin CS, Chen HI. Preeclamptic pregnancy is associated with increased sympathetic and decreased parasympathetic control of HR. Am J Physiol Heart Circ Physiol. 2000;278(4):H1269-73.

184. Dowling O, Rochelson B, Way K, Al-Abed Y, Metz CN. Nicotine inhibits cytokine production by placenta cells via NFkappaB: potential role in pregnancy-induced hypertension. Mol Med. 2007;13(11-12):576-83.

185. Machaalani R, Ghazavi E, David RV, Hinton T, Makris A, Hennessy A. Nicotinic acetylcholine receptors (nAChR) are increased in the pre-eclamptic placenta. Hypertens Pregnancy. 2015;34(2):227-40.

186. Kwon JY, Kim YH, Kim SH, Kang MH, Maeng YS, Lee KY, et al. Difference in the expression of alpha 7 nicotinic receptors in the placenta in normal versus severe preeclampsia pregnancies. Eur J Obstet Gynecol Reprod Biol. 2007;132(1):35-9.

187. Bradford D, Cole SJ, Cooper HM. Netrin-1: diversity in development. Int J Biochem Cell Biol. 2009;41(3):487-93.

188. Ly NP, Komatsuzaki K, Fraser IP, Tseng AA, Prodhan P, Moore KJ, et al. Netrin-1 inhibits leukocyte migration in vitro and in vivo. Proc Natl Acad Sci U S A. 2005;102(41):14729-34.

189. Aherne CM, Collins CB, Masterson JC, Tizzano M, Boyle TA, Westrich JA, et al. Neuronal guidance molecule netrin-1 attenuates inflammatory cell trafficking during acute experimental colitis. Gut. 2012;61(5):695-705.

190. Ranganathan PV, Jayakumar C, Mohamed R, Dong Z, Ramesh G. Netrin-1 regulates the inflammatory response of neutrophils and macrophages, and suppresses ischemic acute kidney injury by inhibiting COX-2-mediated PGE2 production. Kidney Int. 2013;83(6):1087-98.

191. Tadagavadi RK, Wang W, Ramesh G. Netrin-1 regulates Th1/Th2/Th17 cytokine production and inflammation through UNC5B receptor and protects kidney against ischemia-reperfusion injury. J Immunol. 2010;185(6):3750-8.

192. Ranganathan PV, Jayakumar C, Ramesh G. Netrin-1-treated macrophages protect the kidney against ischemia-reperfusion injury and suppress inflammation by inducing M2 polarization. Am J Physiol Renal Physiol. 2013;304(7):F948-57.

193. Schlegel M, Köhler D, Körner A, Granja T, Straub A, Giera M, et al. The neuroimmune guidance cue netrin-1 controls resolution programs and promotes liver regeneration. Hepatology. 2015;63(5):1689-705.

194. Carney EF. Diabetic nephropathy: Netrin-1 expression in proximal tubular epithelial cells protects against kidney inflammation and injury. Nat Rev Nephrol. 2012;8(12):681.

195. Mirakaj V, Gatidou D, Pötzsch C, König K, Rosenberger P. Netrin-1 signaling dampens inflammatory peritonitis. J Immunol. 2011;186(1):549-55.

196. Podjaski C, Alvarez JI, Bourbonniere L, Larouche S, Terouz S, Bin JM, et al. Netrin 1 regulates blood-brain barrier function and neuroinflammation. Brain. 2015;138(Pt 6):1598-612.

197. Yang Y, Zou L, Xu KS. [Expression of netrin-1 in placenta from patients with pre-eclampsia and the relation to placental angiogenesis]. Zhonghua Fu Chan Ke Za Zhi. 2006;41(9):597-600.

198. López-Novoa JM, Bernabeu C. The physiological role of endoglin in the cardiovascular system. Am J Physiol Heart Circ Physiol. 2010;299(4):H959-74.

199. Powers JC, Odake S, Oleksyszyn J, Hori H, Ueda T, Boduszek B, et al. Proteases--structures, mechanism and inhibitors. Agents Actions Suppl. 1993;42:3-18.

200. Mancek-Keber M. Inflammation-mediating proteases: structure, function in (patho) physiology and inhibition. Protein Pept Lett. 2014;21(12):1209-29.

201. Greene CM, McElvaney NG. Proteases and antiproteases in chronic neutrophilic lung disease - relevance to drug discovery. Br J Pharmacol. 2009;158(4):1048-58.

202. Apte SS, Parks WC. Metalloproteinases: A parade of functions in matrix biology and an outlook for the future. Matrix Biol. 2015;44-46:1-6.

203. Cohen M, Meisser A, Bischof P. Metalloproteinases and human placental invasiveness. Placenta. 2006;27(8):783-93.

204. Murphy G. Tissue inhibitors of metalloproteinases. Genome Biol. 2011;12(11):233.

205. Khokha R, Murthy A, Weiss A. Metalloproteinases and their natural inhibitors in inflammation and immunity. Nat Rev Immunol. 2013;13(9):649-65.

206. Nissinen L, Kähäri VM. Matrix metalloproteinases in inflammation. Biochim Biophys Acta. 2014;1840(8):2571-80.

207. Black RA, Doedens JR, Mahimkar R, Johnson R, Guo L, Wallace A, et al. Substrate specificity and inducibility of TACE (tumour necrosis factor alpha-converting enzyme) revisited: the Ala-Val preference, and induced intrinsic activity. Biochem Soc Symp. 2003(70):39-52.

208. Tang J, Zarbock A, Gomez I, Wilson CL, Lefort CT, Stadtmann A, et al. Adam17-dependent shedding limits early neutrophil influx but does not alter early monocyte recruitment to inflammatory sites. Blood. 2011;118(3):786-94.

209. Wang Y, Robertson JD, Walcheck B. Different signaling pathways stimulate a disintegrin and metalloprotease-17 (ADAM17) in neutrophils during apoptosis and activation. J Biol Chem. 2011;286(45):38980-8.

210. Lakatos G, Hritz I, Varga MZ, Juhász M, Miheller P, Cierny G, et al. The impact of matrix metalloproteinases and their tissue inhibitors in inflammatory bowel diseases. Dig Dis. 2012;30(3):289-95.

211. Ma R, Gu Y, Groome LJ, Wang Y. ADAM17 regulates TNFα production by placental trophoblasts. Placenta. 2011;32(12):975-80.

212. Ma R, Gu B, Gu Y, Groome LJ, Wang Y. Down-regulation of TIMP3 leads to increase in TACE expression and TNFα production by placental trophoblast cells. Am J Reprod Immunol. 2014;71(5):427-33.

213. Perucci LO, Gomes KB, Freitas LG, Godoi LC, Alpoim PN, Pinheiro MB, et al. Soluble endoglin, transforming growth factor-Beta 1 and soluble tumor necrosis factor alpha receptors in different clinical manifestations of preeclampsia. PLoS One. 2014;9(5):e97632.

214. Pinheiro MB, Martins-Filho OA, Mota AP, Alpoim PN, Godoi LC, Silveira AC, et al. Severe preeclampsia goes along with a cytokine network disturbance towards a systemic inflammatory state. Cytokine. 2013;62(1):165-73.

215. Vince GS, Starkey PM, Austgulen R, Kwiatkowski D, Redman CW. Interleukin-6, tumour necrosis factor and soluble tumour necrosis factor receptors in women with pre-eclampsia. Br J Obstet Gynaecol. 1995;102(1):20-5.

216. Palei AC, Granger JP, Tanus-Santos JE. Matrix metalloproteinases as drug targets in preeclampsia. Curr Drug Targets. 2013;14(3):325-34.

217. Prieto P, Cuenca J, Traves PG, Fernandez-Velasco M, Martin-Sanz P, Bosca L. Lipoxin A4 impairment of apoptotic signaling in macrophages: implication of the PI3K/Akt and the ERK/Nrf-2 defense pathways. Cell Death Differ. 2010;17(7):1179-88.

218. El Kebir D, Jozsef L, Pan W, Wang L, Petasis NA, Serhan CN, et al. 15-epi-lipoxin A4 inhibits myeloperoxidase signaling and enhances resolution of acute lung injury. Am J Respir Crit Care Med. 2009;180(4):311-9.

219. Sunderland N, Hennessy A, Makris A. Animal models of pre-eclampsia. Am J Reprod Immunol. 2011;65(6):533-41.

Figure captions

Fig.1 The combination of "the two stage" and "the accelerator and brake" theories might explain PE etiopathogenesis

This schematic diagram illustrates the sequential events involved in PE etiopathogenesis. Genetic and environmental factors disrupt endogenous protective pathways, leading to inadequate invasion of uterine spiral arteries by placental trophoblasts and a failure of physiological transformation of uterine spiral arteries. This results in placental hypoxia/ischemia. The dysfunctional placenta releases large amounts of soluble factors into the maternal circulation, which lead to generalized inflammation, oxidative stress/nitrosative stress and endothelial dysfunction, events that are interconnected and precede PE clinical symptoms and complications. Alternatively, dysregulation of endogenous protective pathways can directly cause inflammation, oxidative stress and endothelial dysfunction.

Fig.2 Success and failure in resolution of inflammation

The productive phase of the inflammatory response is characterized by significant influx of PMN cells to the inflamed tissue and increased generation of pro-inflammatory mediators by endothelial cells, migrated and resident immune cells. As the inflammatory response progresses (transition phase), there is a switch of pro-inflammatory to anti-inflammatory mediators and a reduction of PMN cells migration that parallel an increase in mononuclear cells influx. In addition, pro-inflammatory signals induce PMN cells apoptosis and macrophage phagocytosis of apoptotic PMN cells (efferocytosis). During this process, pro-inflammatory macrophages (M1) alter their phenotype to anti-inflammatory macrophages (M2). M2 macrophages have greater efferocytosis capacity and produce anti-inflammatory/pro-resolving mediators. During the resolution phase of inflammation, the influx of monocytes prevails over the influx of PMN, the synthesis of anti-inflammatory/pro-resolving mediators is increased while pro-inflammatory mediators levels are decreased. Moreover, M2 macrophages are converted into a pro-resolving phenotype (Mres), with greater ability to produce anti-inflammatory/pro-resolving mediators, and lymphocytes repopulate the affected tissue. Collectively, these events lead to successful resolution of acute inflammation. On the other hand, failures in resolution of inflammation pathways lead to persistent inflammation and maladaptive immune responses.

Fig.3 Schematic representation of inflammatory and contrarregulatory mechanisms in non-pregnant women, normotensive pregnant women and PE women

Healthy non-pregnant women have basal levels of anti-inflammatory/pro-resolving mediators and pro-inflammatory mediators, which are in a state of equilibrium due to functioning resolution of inflammation mechanisms. Normotensive pregnant women show higher levels of pro-inflammatory mediators than non-pregnant women, but the inflammatory response is mild and controlled, because resolution of inflammation mechanisms are able to adjust properly to this physiological state (increased gear symbol). By contrast, failures in pro-resolving mechanisms probably lead to an exacerbated inflammatory response in PE women, despite the up-regulation of some anti-inflammatory/pro-resolving mediators.

Figure 1

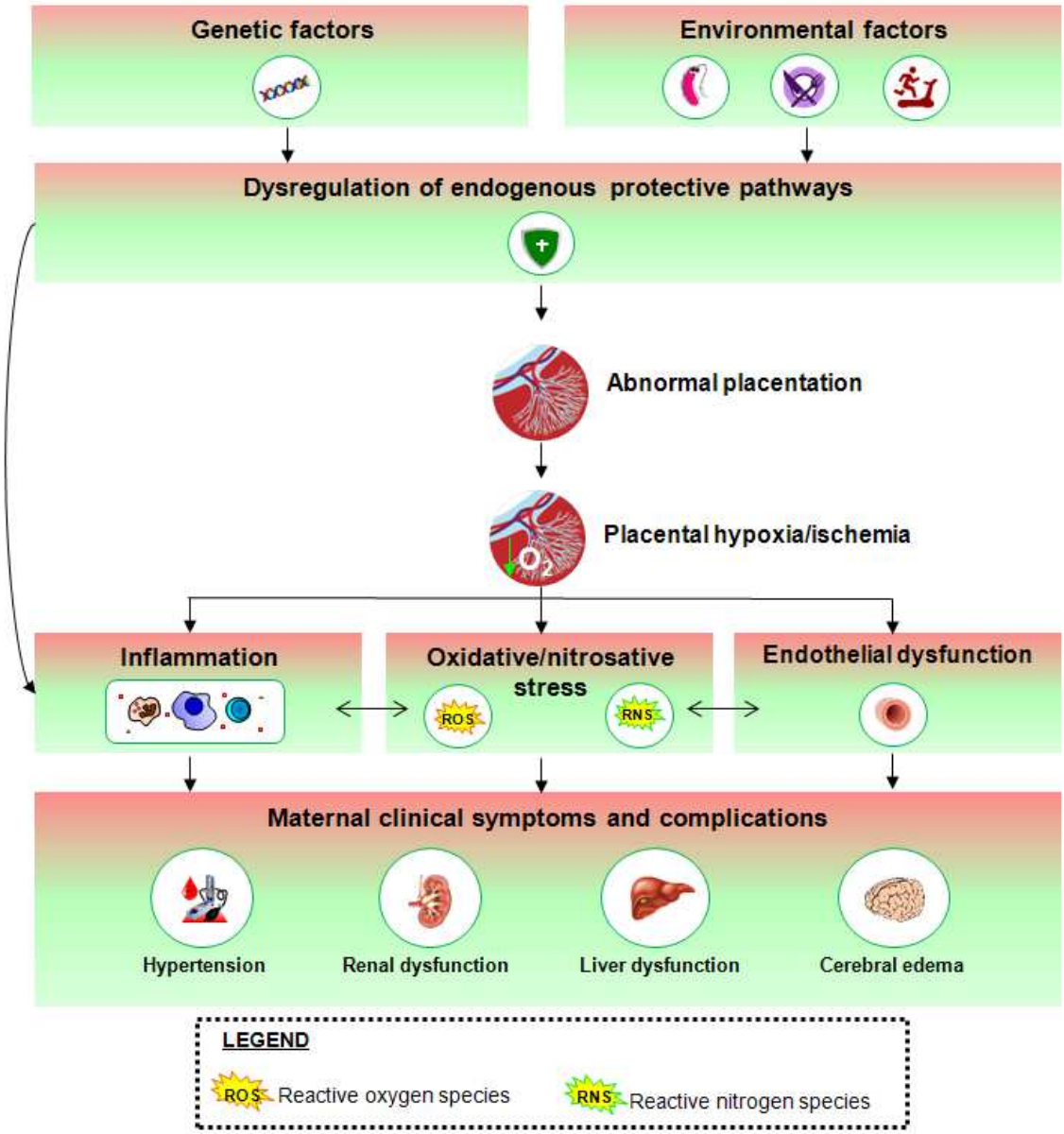

Genetic factors
Environmental factors
Dysregulation of endogenous protective pathways
Abnormal placentation
Placental hypoxia/ischemia
Inflammation
Oxidative/nitrosative stress
ROS
RNS
Endothelial dysfunction
Maternal clinical symptoms and complications
Hypertension
Renal dysfunction
Liver dysfunction
Cerebral edema
LEGEND
ROS Reactive oxygen species
RNS Reactive nitrogen species

Figure 2

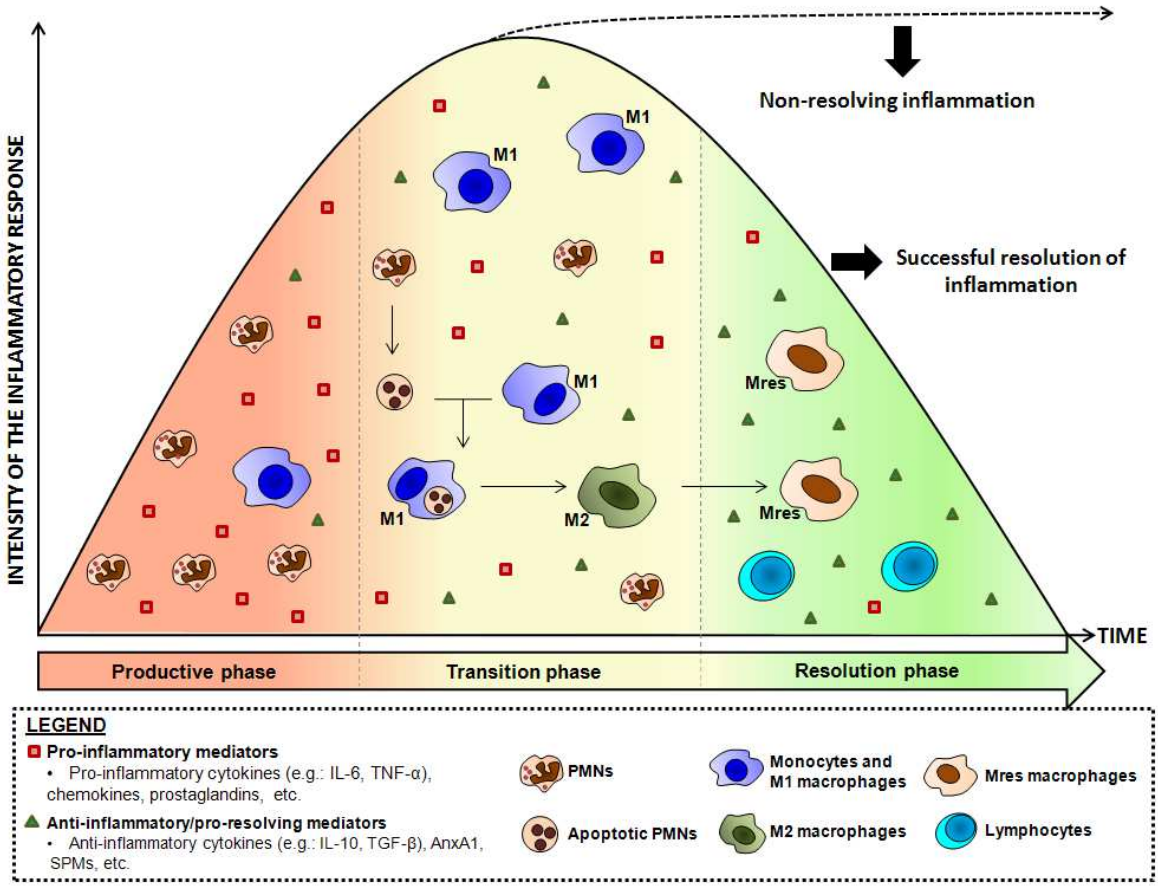
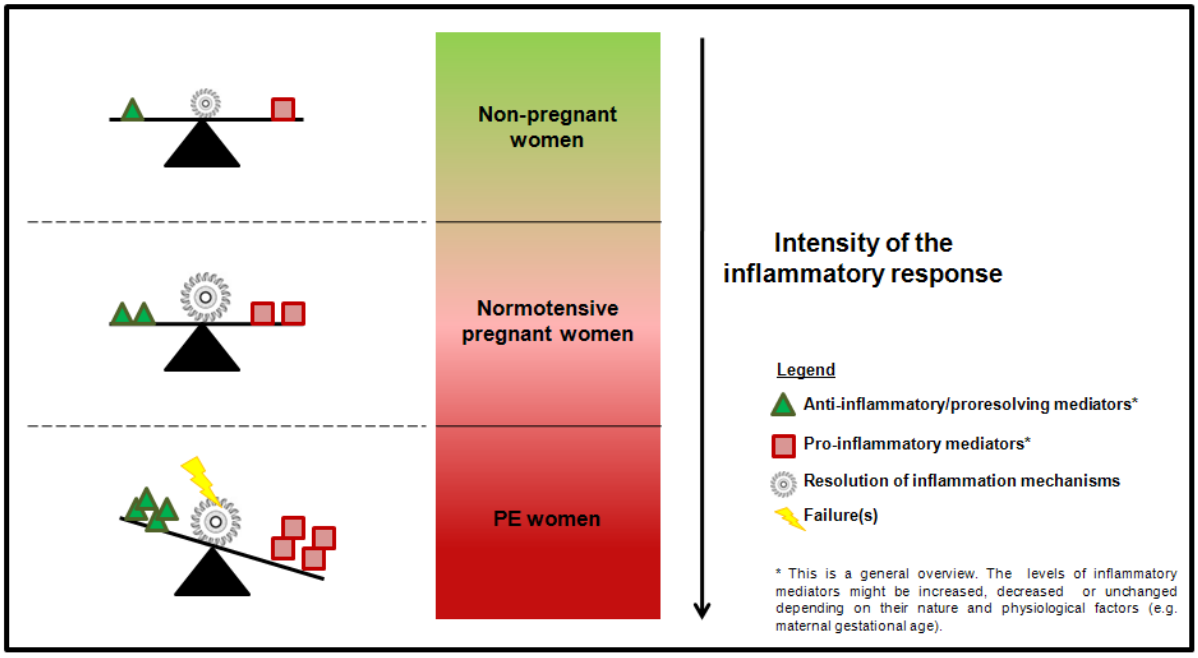

INTENSITY OF THE INFLAMMATORY RESPONSE
Non-resolving inflammation
Successful resolution of inflammation
M1
M1
M1
M1
M2
Mres
Mres
TIME
Productive phase
Transition phase
Resolution phase
LEGEND
Pro-inflammatory mediators
• Pro-inflammatory cytokines (e.g.: IL-6, TNF-α), chemokines, prostaglandins, etc.
Anti-inflammatory/pro-resolving mediators
• Anti-inflammatory cytokines (e.g.: IL-10, TGF-β), AnxA1, SPMs, etc.
PMNs
Monocytes and M1 macrophages
Mres macrophages
Apoptotic PMNs
M2 macrophages
Lymphocytes

Figure 3


Non-pregnant women
Normotensive pregnant women
PE women
Intensity of the inflammatory response
Legend
Anti-inflammatory/proresolving mediators*
Pro-inflammatory mediators*
Resolution of inflammation mechanisms
Failure(s)
* This is a general overview. The levels of inflammatory mediators might be increased, decreased or unchanged depending on their nature and physiological factors (e.g. maternal gestational age).

4.5 CAPÍTULO 5- "Annexin A1 and specialized pro-resolving lipid mediators in human inflammatory diseases"

(Artigo em fase de submissão)

Annexin A1 and Specialized Pro-Resolving Lipid Mediators in Human Inflammatory Diseases

Luiza Oliveira Perucci[1,2], Karina Braga Gomes[1,2], Luci Maria Dusse[1,2], Mauro Martins Teixeira[3]; Lirlândia Pires Sousa[1,2*]

[1]Departamento de Análises Clínicas e Toxicológicas, Faculdade de Farmácia, Universidade Federal de Minas Gerais, Belo Horizonte, Minas Gerais, Brazil;

[2]Programa de Pós-Graduação em Análises Clínicas e Toxicológicas, Universidade Federal de Minas Gerais, Belo Horizonte, Minas Gerais, Brazil;

[3]Departamento de Bioquímica e Imunologia, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais, Belo Horizonte, Minas Gerais, Brazil.

*Corresponding author

Lirlândia P. Sousa - Av. Antonio Carlos, 6627 - Pampulha, 31270-901 - Belo Horizonte, MG, Brazil. Phone: +55 31 3409 6883. FAX: +55 31 3409 2651.

Email: lipsousa72@gmail.com

ABSTRACT

Acute inflammation has the physiological purpose to protect the host against infection and sterile injury and its timely resolution is essential to restore tissue homeostasis. Resolution of inflammation is an active process modulated by proteins and specialized pro-resolving lipid mediators (SPMs), such as annexin A1 (AnxA1), lipoxin A4, resolvins, protectins and maresins. If unresolved, inflammation can lead to further tissue damage and gives rise to chronic inflammatory diseases. The anti-inflammatory and pro-resolving effects of AnxA1 and SPMs have been much explored *in vitro* and *in vivo* by using pre-clinical inflammatory models in mice. However, studies investigating the role of these molecules in human diseases are just emerging. This review aims to highlight the recent advances on the role of these mediators in human inflammatory diseases.

Keywords: annexin A1; specialized pro-resolving lipid mediators; inflammation; resolution; chronic diseases.

Abbreviations: ASA, acetylsalicylic acid; AD, Alzheimer's disease; Aβ, amyloid-beta; AnxA1, annexin A1; ASD, autism spectrum disorder; BAL, bronchoalveolar lavage; CSF, cerebrospinal fluid; ChemR23, chemerin receptor 23; CD, Crohn's disease; CF, cystic fibrosis; DN, diabetic nephropathy; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; EV, extracellular vesicles; FPR2, formyl peptide receptor like-2; GCs, glucocorticoids; IBD, inflammatory bowel diseases; KO, knockout; LT, leukotriene; LXA4, lipoxin A4; LOX, lipoxygenase; MaR1, maresin1; MCI, mild cognitive impairment; PD, Parkinson's disease; PD1, protectin D1; PE, preeclampsia;

PMN, polymorfonuclear; RvD, resolvins of the D series; RvE, resolvins of E-series; SPMs, specialized pro-resolving lipid mediators; SCI, subjective cognitive impairment; TNF-α, tumor necrosis factor alpha; T2D, type 2 diabetes mellitus; UC, ulcerative colitis; WT, wild-type.

INTRODUCTION

Acute inflammation

The acute inflammatory response is an event that can be triggered by infectious or sterile injurious stimuli. Molecules derived from these episodes are recognized by specific receptors (e.g. toll like receptors) in resident cells (e.g. macrophages), activating downstream signaling pathways (e.g. nuclear factor kappa beta pathway) to produce soluble pro-inflammatory mediators, such as cytokines, chemokines and eicosanoids. Concomitantly, polymorphonuclear (PMN) cells migrate from the blood to the affected tissue following chemokines' gradients to phagocyte and eliminate microorganisms and cellular debris. This process can occur trough extracellular or intracellular mechanisms, for example, involving superoxide radicals or neutrophil extracellular traps, respectively. Increased migration of PMN cells is a consequence of increased expression of adhesion molecules in these cells and in endothelial cells combined with vasodilatation and increased vascular permeability induced by pro-inflammatory mediators [1, 2].

Resolution of acute inflammation

In the past, resolution of acute inflammation was considered a passive process, which consisted in reduction of pro-inflammatory cytokines, leukocyte chemoattractants and inflammatory cells after the end of the damaging stimuli. Contrarily, recent studies suggest that resolution of inflammation is an active and programmed response coordinated by immune cells and soluble mediators that promotes tissue repair and adaptive immunity. The transition from acute inflammation to successful resolution, and the consequent restoration of tissue homeostasis,

requires the following steps: 1) elimination of the damaging stimulus; 2) switch in the production of pro-inflammatory to anti-inflammatory / pro-resolving mediators, stopping further PMN recruitment; 3) PMN death (preferably by apoptosis); 4) non-phlogistic monocyte recruitment to the affected tissue; 5) clearance of apoptotic PMN by monocyte-derived macrophages (efferocytosis) and macrophage switch from M1 to M2 and Mres phenotypes (with higher efferocytosis activity and higher capacity to produce anti-inflammatory / pro-resolving mediators); 6) Lymphatic drain of macrophages and lymphocyte repopulation of the affected area; 7) Tissue regeneration and repair [2-4].

The acute inflammatory response is usually protective to the host and self-limiting. However, if the resolution process fails, inflammation can lead to further tissue damage and loss of organ function [3, 4]. In a recent work, Fullerton & Gilroy have proposed that complete resolution of innate immune responses is important to build an adequate adaptive immunity. Thus, non-resolving inflammation could impair adaptive immune responses, leading, for example, to autoimmunity [2]. “Frustrated” resolution of inflammation can occur due to inadequate synthesis or function of pro-resolving agonists and their responders (cellular receptors and signaling pathways) [3]. Arising from this knowledge, it has been proposed that defective or “frustrated” resolution of inflammation represents the underlying basis for chronic inflammatory diseases.

Understanding the role of pro-resolving pathways in human chronic inflammatory diseases is important to elucidate their etiopathogenesis and to provide novel pharmacological strategies. In fact, clinical trials have been conducted in order to evaluate the efficacy and the safety of therapies based in proresolving analogues [5]. In this sense, it is important to clarify the difference between anti-inflammatory

and pro-resolving therapies. The former include pharmacological strategies that inhibit the initial events of the inflammatory response by decreasing the synthesis or blocking the action of pro-inflammatory mediators and by inhibiting PMN cells influx (e.g. non-steroidal anti-inflammatory drugs and anti-TNF-α therapies). By contrast, pro-resolving strategies interfere with the disease process after it has been established, by targeting molecular pathways acting as endogenous agonist that accelerate resolution of inflammation [2].

Annexin A1

Glucocorticoids (GCs) are drugs widely used for the treatment of inflammatory diseases. GCs evoke both anti-inflammatory and pro-resolving actions and that is why they are so effective in controlling inflammatory responses [6]. Consistent evidences indicate that annexin A1 (AnxA1) mediates part of the anti-inflammatory and pro-resolving effects of endogenous and synthetic GCs [6]. AnxA1 is a Ca^{2+} and phospholipid binding protein originally described as a phospholipase A_2-inhibitory protein [7]. Several studies using models of inflammation have shown that AnxA1 or the bioactive peptides that comprises its N-terminal region (e.g., Ac2-26) modulates production of pro-inflammatory cytokines, decrease PMN accumulation, induce neutrophils apoptosis and efferocytosis and increase interleukin (IL)-10 production [8-11].

The intact form of AnxA1 (37KDa) exert its anti-inflammatory and pro-resolving actions through binding to the formyl peptide receptor like-2 (FPR2), whose acronym is FPR2/ALX, since it also conveys signals induced by lipoxin A4 (LXA4) and resolving D1 (RvD1) [12, 13]. The biological activity of intact AnxA1 is attributed to its N-terminal domain [14]. This domain can be cleaved by proteases such as elastase,

which are abundant in inflammatory sites [15]. It is believed that proteolysis in the N-terminal domain reduces AnxA1 biological activity [16]. Indeed, AnxA1 cleavage-resistant mutants [17, 18] or elastase inhibitors [19] can be used as pharmacological strategies. In addition, AnxA1 cleaved forms, like the 33KDa product, may show pro-inflammatory properties *in vitro* [20] and are found associated with increased neutrophil influx in mice [9, 19] and neutrophil necrosis in inflamed lung from patients with cystic fibrosis [21].

Specialized pro-resolving lipid mediators

Omega-3 and omega-6 poly-unsaturated fatty acids, which are found in fish oils and vegetable oils, respectively, have long been associated with protective properties to human health [22]. Recent studies revealed that the resolution of inflammation is mediated by omega-3 and omega-6 derivates called *specialized pro-resolving lipid mediators* (SPMs) [23]. Lipoxins, the first described SPMs, are endogenous eicosanoids generated from arachidonic acid (an omega-6 derivate) via lipoxygenase (LOX)-mediated transcellular biosynthesis. Among them, LXA4 and its analogues (aspirin-triggered lipoxins) are thought to act as “stop signals of inflammation” [24]. Novel SPMs that are generated from the omega-3 fatty acids eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) have been discovered: resolvins of E-series (RvE), if derived from EPA; and resolvins of the D series (RvD), protectin D1 (PD1) and maresin 1 (MaR1), if generated from DHA. RvD epimers (aspirin-triggered RvD) can also be generated in vivo after acetylsalicylic acid (ASA) metabolization [25]. SPMs regulate several critical cellular events of the inflammatory response, including inhibition of neutrophils migration and activation, inhibition of the synthesis and actions of pro-inflammatory mediators, non-phlogistic

recruitment of monocytes and the induction of efferocytosis of apoptotic neutrophils [26, 27].

As mentioned before, LXA4 and RvD1 bind to FPR2 receptor to evoke anti-inflammatory and pro-resolving responses. LXA4 also interacts with cysteinyl leukotriene receptor 1, leukotriene B4 receptor, as well as with cytokine, chemokine and growth factor receptors, contributing to regulate inflammation [28, 29]. Moreover, RvD1 binds to G protein-coupled receptor 32, while RvE1 binds to chemerin receptor 23 (ChemR23) and to leukotriene B4 receptor [30-32]. Other SPMs receptors have also been proposed. A more detailed data about SPMs receptors was described by Serhan et al. (2011) [29].

The aim of this review was to describe studies regarding AnxA1 and SPMs role in the context of human chronic inflammatory diseases, through gathering data suggesting that dysregulation in pro-resolving mechanisms might be involved the pathogenesis of these diseases.

AnxA1 and SPMs in human inflammatory diseases

Table 1 summarizes the findings about the pro-resolving mediators AnxA1 and SPMs in human inflammatory diseases.

Table 1 – Summary of pro-resolving mediators' findings in human inflammatory diseases

Disease	Findings	References
Obesity	↓AnxA (plasma) ↑AnxA1 (serum and adipose tissue); ↑cleaved AnxA1 and ↓intact AnxA1 (adipose tissue) ↑ LXA4 (plasma)	[36] [38], [Pietrani et al. 2016, submitted manuscript] [41]
Type 2 diabetes mellitus	**Type 2 diabetes**	

and complications	↓AnxA (adipose tissue)	[46]
	AnxA1 (serum) - not changed	[Pietrani et al. 2016, submitted manuscript]
	+ Dry eye syndrome: ↑ AnxA1	[47]
	+ Diabetic nephropathy:	
	↓ AnxA1 (plasma)	[Pietrani et al. 2016, submitted manuscript]
	↑AnxA1 (urine)	[57]
Cardiovascular diseases		
Atherosclerosis	↑AnxA1 (atherosclerotic plaques and plaque derived smooth cells) of asymptomatic patients in comparison to symptomatic patients	[64, 65]
Coronary artery disease	↑AnxA1 (neutrophils)	[67]
Chronic heart failure	↓LXA4 (plasma) in severe disease in comparison to mild-to-moderate disease and ↓15-epi-LXA4 (urine)	[68]
Sepsis	↑AnxA1 (plasma) over a 7-day period after hospital admission	[74]
	↓AnxA1 and LXA4 (plasma) at hospital admission	[75]
	Similar AnxA1 levels between survivors and nonsurvivors	[74], [75]
Respiratory diseases		
Asthma	↓LXA4 and AnxA1 (plasma) in wheezy infants	[89]
	↓LXA4 (blood, BAL fluids and sputum) in severe disease than in moderate disease	[92-96]
	↓FPR2 mRNA and protein expression (blood granulocytes) in severe disease	[94]
	↓15-LOX (blood and BAL cells) in severe disease	[94], [96]
Cystic fibrosis	↓AnxA1 (nasal epithelial cells) in patients bearing Y122X and 489delC mutations	[105]
	↑cleaved AnxA1 and ↓intact AnxA1 (BAL fluids and peripheral blood neutrophils)	[106], [21]
	↓LXA4/LTB4 ratio (BAL fluids), ↓15-LOX-2 mRNA (BAL fluids) and ↓LXA4 (platelets)	[110]
Neurological diseases		
Alzheimer's disease	Rs2811226 polymorphism in AnxA1 gene	[118]
	↑AnxA1 (superior frontal gyrus and periventricular	[119], [120]

	white matter lesions)	
	↓LXA4 (CSF and hippocampus)	[121]
	↓MaR1 (hippocampus)	[121]
	RvD1 (CSF and hippocampus) – not changed	[121]
	↑FPR2 and ↑ChemR23 (hippocampus)	[121]
	↑15-LOX-2 (hippocampus)	[121]
Parkinson's disease	↑AnxA1 (microglial regions of substantia nigra containing fragmented neurons)	[128]
Autism spectrum disorders	↓LXA4 (plasma) and negative correlation with disease severity	[135]
	Recurrent tandem duplication in AnxA1 gene	[136]
Inflammatory bowel diseases		
Crohn's Disease	↓AnxA1 protein (plasma and gut mucosa) and mRNA (peripheral blood mononuclear cells)	[139]
	↑AnxA1 (gut mucosa) in clinical responders to infliximab therapy	[139]
	↑AnxA1-containing EV (serum) in active disease	[149]
	↑AnxA1 auto-antibodies (serum)	[145]
Ulcerative colitis	↑AnxA1 (colon)	[141], [142]
	↑AnxA1-containing EV (serum) in active disease	[149]
	↑AnxA1 auto-antibodies (serum)	[145]
	↑LXA4 (colon) in patients with clinical remission	[142]
Pregnancy diseases		
Preeclampsia	↑AnxA1 (plasma)	[157]
	↑AnxA1 auto-antibodies (serum)	[158]
	↑LXA4 (circulation)	[159], [160], [161]
	↓LXA4 (circulation)	[162]
	↓FPR2 mRNA (placenta)	[161], [162]
	↑FPR2 mRNA (placenta)	[160]

Abbreviations: AnxA1, annexin A1; BAL, bronchoalveolar lavage; ChemR23, chemerin receptor 23; CSF, cerebrospinal fluid; EV, extracellular vesicles; FPR2, formyl peptide receptor like-2, LTB4, leucotriene B4; LOX, lipoxygenase; LXA4, lipoxin A4.

Obesity

Obesity is largely associated with a state of low chronic inflammation in the adipose tissue, particularly in the visceral compartment. The visceral adipose tissue synthesizes and secretes adipokines and pro-inflammatory mediators that might contribute to endothelium dysfunction and insulin resistance [33, 34]. Thus, understanding the potential targets for modulating the inflammatory response in obesity could be important for the development of novel pharmacological strategies in the broader context of metabolic diseases [35].

Kosicka et al. investigated AnxA1 plasma levels in obese individuals and found that they were decreased when compared with non-obese individuals [36]. This data is in line with the increased adiposity in AnxA1 KO mice observed in Akasheh et al. study [37]. By contrast, Henegar evaluated the transcriptomic signature of human adipose tissue and found that AnxA1 was up-regulated in obese individuals [38]. The latter findings parallel the data from our laboratory demonstrating that AnxA1 serum concentration was increased in obese than in overweight and lean individuals (Pietrani et al. 2016, submitted manuscript). It should be highlighted that gender and age differences among the studied populations or the methodologies to quantify AnxA1 levels could have influenced the results of the aforementioned works. Thus, further investigations with standardized methodologies are necessary to elucidate these controversial results.

AnxA1 can be secreted by immune cells [39], but there might be other sources of the circulating protein in the body, such as the adipose tissue [36]. Interestingly, both AnxA1 gene expression and protein were up-regulated during adipogenesis in a human adipocyte cell line in Kosicka's study [36]. However, AnxA1 in adipose tissue seems to be insufficient to attenuate the local inflammatory response. It has been

admitted that full-length AnxA1 (37-kDa) is endowed with anti-inflammatory activity, while its cleaved form (33-kDa) is inactive or may has a pro-inflammatory role [20]. In our study, we have observed higher levels of cleaved AnxA1 in human adipose tissue of obese individuals compared to non-obese individuals suggesting a more complex regulation of this protein (Pietrani et al. 2016, submitted manuscript). Our hypothesis is that AnxA1 fails to regulated inflammation in obese individuals since the biologically active protein is cleaved in the adipose tissue.

The adipose tissue is an important storage site of omega-3 and omega-6 fatty acids [40]. In a recent study, levels of LXA4 (an omega-6 SPM) were found to be increased in the plasma of obese individuals [41]. The authors suggest that LXA4 might be produced through leukotriene B4 (LTB4) conversion in these individuals, since they observed higher 5- and 15-hydroxyeicosatetraenoic acid levels, which implicates increased 5- and 15-LOX activities. LXA4 could be increased in obese individuals in an attempt to attenuate adipose inflammation. In fact, LXA4 attenuated TNF-α expression, promoted a macrophage M1-to-M2 switch and increased AnxA1 levels in the adipose tissue of mice with high-fat diet-induced obesity [42]. Adipose tissue inflammation in obese individuals, in spite of LXA4 increased levels, points to possible failures in this resolution pathway, which should be investigated in future studies.

Other SPMs have also been investigated in obesity using *in vivo* experimental models. Neuhofer et al. reported decreased RvD1 and PD1 levels in the adipose tissue of obese mice. Moreover, dietary treatment with omega-3 polyunsaturated fatty acids (EPA and DHA) attenuated adipose tissue inflammation in these mice [43]. Interestingly, RvD1, which is derived from DHA, attenuated adipose tissue inflammation in high-fat diet-induced obese mice by stimulating macrophage

polarization towards an M2 phenotype [43]. The role of these SPMs in human obesity remains to be evaluated.

Type 2 diabetes mellitus and complications

Type 2 diabetes mellitus (T2D) is a chronic metabolic disorder resulting from defects in insulin secretion and insulin resistance [44]. As observed in other metabolic disorders, chronic low grade inflammation and activation of the immune system are linked to T2D pathogenesis [33, 45].

A recent work reported that pre-obese patients with T2D had decreased levels of AnxA1 in the visceral adipose tissue than pre-obese subjects with normal glucose tolerance [46]. This result indicates that AnxA1 deficiency in the adipose tissue might be associated with the progression from pre-obesity to diabetes. However, results from our research group revealed that AnxA1 plasma levels were similar between T2D patients and controls with median body mass indexes in the overweight range (Pietrani et al. 2016, submitted manuscript). In another study, patients with diabetes and dry eye syndrome had increased expression of AnxA1 in tears compared with healthy controls [47]. It can be hypothesized that AnxA1 concentration in peripheral blood of diabetic patients does not reflect its levels in adipose tissue and tears.

It has been reported that patients with T2D have decreased levels of arachidonic acid (an omega-6 derivate), omega-3 and DHA (an omega-3 derivate) when compared to controls [48]. In another study, the combination of omega-3 treatment with a low fat/high carbohydrate weight-loss program resulted in greater benefits in insulin sensitivity than weight-loss alone in insulin-resistant women [49]. Accordingly, dietary treatment with EPA and DHA improved insulin sensitivity in obese mice in Neuhofer et al. study [43]. This effect is probably mediated by LXA4, RvE1, RvD1 and PD1 synthesis [50-52]. In addition, SPMs treatment may promote

wound healing and innervation impaired by diabetes, by restoring macrophage capacity to phagocyte apoptotic cells, macrophage-dependent production of pro-angiogenic factors and kertinocytes' activity [53, 54].

Diabetic nephropathy (DN), the most common cause of end-stage renal disease, is characterized by glomerular infiltration of macrophages and increased expression of pro-inflammatory cytokines, adhesion molecules and chemokines in the renal tissue of diabetic patients [55]. In a recent study published by our research group, AnxA1 plasma levels were lower in patients with T2D with nephropathy than in T2D patients without this complication [56]. By contrast, urinary AnxA1 levels were increased in DN patients than in healthy controls in a study of Ka et al. [57]. It is important to highlight that the studied populations and biological samples were different in these two studies. The fist study evaluated plasma AnxA1 and did not include a healthy control group, while the second study measured urinary AnxA1 and did not include patients with T2D diabetes without nephropathy. Moreover, there might be a more prominent urinary loss of circulating AnxA1 in DN patients than in controls. Similar to T2D, patients with DN also show decreased circulating levels of arachidonic acid, omega-3 and DHA compared to normal controls [48]. SPMs potential benefits in DN have been investigated in studies using *in vivo* experimental models, such as unilateral ureteral obstruction UUO-induced kidney injury, which mimics common features of chronic renal diseases, like DN. According to these studies, SPMs may present anti-fibrotic and anti-inflammatory actions. They reduce collagen deposition, decrease pro-inflammatory cytokines (e.g. tumor necrosis factor alpha - TNF-α) levels and increase the concentration of interleukin 10, an anti-inflammatory cytokine, and induce a macrophage M2c reparative phenotype [58].

Cardiovascular diseases

Cardiovascular diseases represent one of the leading causes of mortality worldwide (approximately 31% of all deaths) and include coronary heart disease, cerebrovascular disease, rheumatic heart disease and other disorders [59]. Atherosclerosis, the major cause of cardiovascular diseases, is a chronic inflammatory condition. The atherosclerotic plaques are full of immune cells that produce pro-inflammatory cytokines. Furthermore, oxidized low density lipoprotein, which are abundant in these plaques, have pro-inflammatory properties [60].

In vivo experimental studies have reported protective actions of the AnxA1 mimetic peptide Ac2-26 against atherosclerosis and myocardial injury [61-63]. These effects are mediated by limiting neutrophil infiltration, suppressing oxidative stress and preserving cardiomyocyte viability and contractile function [61, 63]. AnxA1 might also exert cardioprotective effects in humans. AnxA1 is up-regulated in atherosclerotic plaques and in plaque-derived smooth muscle cells of asymptomatic patients in comparison to symptomatic patients with atherosclerosis, suggesting that this protein may has a stabilizing effect and prevent plaque complications [64, 65]. Indeed, Kusters et al showed that AnxA1 attenuated progression of existing plaques of aortic arch and subclavian artery in LDLR knockout (KO) mice [66]. Furthermore, neutrophils expression of AnxA1 was significantly increased in patients with coronary artery disease compared to controls in a study conducted by Särndahl et al. The authors of this last work suggest that AnxA1 might be up-regulated in neutrophils in an attempt to attenuate disease-related immune activation [67].

Reina-Couto et al. investigated the LXA4 plasma levels in human chronic heart failure and found that the patients with severe disease had reduced levels of LXA4 than those with mild-to-moderate disease. They also reported that the urinary

excretion of aspirin triggered 15-epi-LXA4 was reduced in severe chronic heart failure [68]. These data suggest that cardiovascular diseases may be associated with altered levels of SPMs. Accordingly, several evidences support that the EPA and DHA are cardioprotective to humans. Unsaturated fatty acids from dietary and endogenous sources lower triglycerides levels and favor cardiac diastolic filling and arterial compliance [69]. Results with animals indicate that 12/15-lipoxygenase expression, and the subsequent production of LXA4, RvD1, and PD1, protect mice against atherosclerosis [70]. Therefore, pro-resolving lipid mediators probable mediate EPA and DHA cardioprotective effects and may be new targets for treatment of cardiovascular diseases.

Sepsis

Sepsis is an uncontrolled inflammatory and pro-coagulant response that follows bacterial infection [71]. Patients with severe sepsis can develop acute disseminated intravascular coagulation, which contributes to multiple organ failure [72]. In the past two decades, it has been proposed that an initial pro-inflammatory phase is followed by a compensatory anti-inflammatory response in this disease [71]. However, recent studies have demonstrated that both pro-inflammatory and anti-inflammatory responses are regulated simultaneously in septic patients, although not necessarily with the same time courses [73]. Thus, understanding the controlling mechanisms of inflammation in sepsis is essential to clarify its pathogenesis and to develop more effective pharmacological therapies in this condition.

Tsai et al. measured AnxA1 plasma levels from healthy controls and septic patients over a period of 7 days after admission in an intensive care unit. AnxA1 levels were increased in 56% of sepsis patients over the observation period [74]. In

another study from the same research group, AnxA1 plasma levels were decreased in septic patients selected during hospital admission when compared to controls [75]. In both studies, AnxA1 levels were similar between survivors and non-survivors, indicating that this protein is not a good marker for prognosis in sepsis cases. The results divergences of these studies could be attributed to the time-point of blood collection of septic patients. The first study performed several measurements of AnxA1 during one-week period, while the second study evaluated AnxA1 levels only at hospital admission. Probably AnxA1 levels increase during the progression of sepsis in an attempt to control the over exuberant inflammation. Really, AnxA1 gene is activated by lipopolysaccharide (LPS), a pathogen-associated molecular pattern, in several cellular types that play a role in experimental endotoxemia, including neutrophils and epithelial cells [76]. AnxA1 role in controlling the inflammatory response in sepsis was further confirmed by other studies. Gavins et al. observed that, after LPS intraperitoneal administration, AnxA1 KO mice exhibited increased pro-inflammatory markers and higher leukocyte rolling and adhesion in cerebral vases when compared with wild-type (WT) mice [77]. Accordingly, Gobbetti et al. reported higher levels of inflammatory markers, increased granulocyte/monocyte ratio in peritoneal lavages and worse disease clinical symptoms in FPR2-KO mice than in WT mice in cecal ligation and puncture experimental model [78]. Moreover, AnxA1 mimetic peptide Ac2-26 reduced leukocyte adhesion in both WT and AnxA1 KO mice when compared with saline control animals [77].

Tsai et al. also reported decreased LXA4 levels in septic patients on hospital admission than in controls. LXA4 deficiency in patients with sepsis may contribute to the overwhelming inflammatory response in this disease [75]. Recent data indicate that low doses of ASA attenuate inflammatory and haemostatic disturbances in

sepsis, and are strongly associated with patient survival [79, 80]. The benefits of ASA can be attributed not only to its antiplatelet actions, but also to the metabolic formation of 15-epi-lipoxin A4, which shares anti-inflammatory and pro-resolving actions with endogenous LXA4 [81]. Ueda et al. investigated the direct action of 15-epi-lipoxin A4 in *E. coli*-infected mice and found that the combination therapy of 15-epi-lipoxin A4 with antibiotics attenuated the systemic inflammatory response, decreased bacterial load in the serum and improved survival rates when compared with antibiotics therapy alone [82]. Endogenous LXA4 and 15-epi-lipoxin A4 may mediate these effects by increasing macrophage recruitment, reducing neutrophils migration and increasing neutrophils phagocytic ability without excessive free radical production [83, 84].

In another study, RvD1 decreased the levels of pro-inflammatory cytokines and the number of peritoneal neutrophils in an endotoxin shock model, indicating that other SPMs-based therapies might be useful in sepsis [85]. Accordingly, Chiang et al. observed that RvD1 and RvD5 reduced bacterial load in blood and peritoneal lavage and increased survival of mice infected by *E. coli.* In addition, RvD1, RvD5 and PD1 enhanced phagocytosis of *E. coli* by human macrophages. This study also showed that, as with LXA4, the combined treatment of RvD1 and antibiotics accelerated resolution of inflammation in infected mice.

Taken together, these data suggest that LXA4 deficiency is associated with the pathogenesis of sepsis and that SPMs analogues may be beneficial in the treatment of septic patients.

Respiratory diseases

Asthma

Asthma is a respiratory disease characterized by persistent airways inflammation that manifests clinically with recurring cough, shortness of breath, wheezing and chest retraction [86]. In children, persistent wheezing may progress to asthma [87]. There is growing interest to investigate if alterations in endogenous pro-resolving pathways are associated with the progression of asthma and with the disease severity [88].

In a recent study, AnxA1 plasma levels were decreased in wheezy infants than in the control group, indicating that alteration in the levels of pro-resolving molecules could be an early event in asthma progression [89]. The control of the inflammatory response in asthmatic patients might also depend on AnxA1 actions. Ng et al. showed that AnxA1 KO mice exposed to ovalbumin (an allergen) had exacerbated features of allergic asthma when compared with WT mice [90]. The possible anti-asthmatic mechanisms of AnxA1 were investigated in Wang et al. work, in which AnxA1 mimetic peptide Ac2-26 suppressed eosinophils accumulation in airways and reduced both prostaglandin D_2 (PGD_2) levels and CRTH2 expression (PGD_2 receptor) in bronchoalveolar lavage (BAL) fluids [91].

As with AnxA1, wheezing children showed decreased LXA4 plasma levels than controls, suggesting that LXA4 might be a marker of disease progression [89]. Moreover, severe asthma is associated with decreased levels of LXA4 in blood, BAL fluids and sputum compared with moderate asthma [92-96]. The decreased of LXA4 airway levels in patients with severe disease coincides with a down regulation of FPR2 (RNA and protein) in blood granulocytes [94]. Diminished expression of 15-

LOX enzyme in blood and BAL cells of severe asthma patients has also been described [94, 96]. Other SPMs pathways have also been investigated in asthma. Of interest, PD1 levels are lower in exhaled breath condensates and in eosinophils from patients with severe asthma [97, 98]. Collectively, these data indicate that SPMs deficient production and signaling could have a role in asthma pathogenesis and progression. Moreover, SPMs compounds could provide novel therapeutic approaches for the treatment of asthmatic patients. Indeed, RvD1 (and aspirin-triggered RvD1), PD1 and MaR1 decreased eosinophilia, inhibited pro-inflammatory mediators production, induced regulatory T cells generation, enhanced macrophage clearance of allergens from the airways of sensitized mice, accelerating resolution of allergic airway inflammation [97, 99, 100].

Cystic fibrosis

Cystic fibrosis (CF) is a human genetic disease characterized by chronic inflammation and multiple organ dysfunctions. Pulmonary disease is the main cause of morbidity and mortality in CF patients [101, 102]. Several mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene have been identified in these patients, being F508del the most common [103]. However, these mutations solely cannot explain all the disease clinical manifestations [104].

Bensalem et al. showed that AnxA1 was down-regulated in nasal epithelial cells from CF patients caring Y122X and 489delC mutations than in healthy controls [105]. Accordingly, AnxA1 is absent in lungs and pancreas of CFTR KO mice, tissues that normally express this protein [105], and the administration of human recombinant AnxA1 attenuates the inflammatory response associated with CFTR deficiency [106]. It has been suggested that AnxA1 defective action, combined with

its decreased synthesis, contribute to the inflammatory phenotype characteristic of this disease. Indeed, the intact form of AnxA1 is decreased, while the cleaved form of AnxA1 (33 kDa) is increased, in BAL fluids and neutrophils from peripheral blood of CF patients than in healthy controls [21, 107]. CF is characterized by intense neutrophilic inflammation in the airways [108]. It is well known that neutrophils that undergo necrosis release large amounts proteases, such as elastase, which are able to cleave intact AnxA1 [15, 109]. Tsao et al. proposed that neutrophils elastase released by necrotic neutrophils is involved in AnxA1 degradation and lack of biological activity in inflamed lung tissues from CF patients [21].

The role of LXA4 in CF pathogenesis has also been investigated. The conversion of leukotrienes into lipoxins mediated by 15-LOX-2 enzyme is an important step in the resolution of inflammation. Ringholz et al. recently reported that children with CF had decreased ratio of LXA4/LTB4 and reduced 15-LOX-2 transcript levels in BAL fluids than pediatric controls. Moreover, platelets from CF patients express less LXA4 than platelets from health individuals [110]. These results points to a possible dysfunction in lipid mediators “class switching” in the lungs of CF patients, which might be associated with persistent tissue inflammation [111]. Thus, administration of LXA4 stable analogues could compensate the deficiency of endogenous LXA4 generation in CF patients. Accordingly, Karp et al. showed that LXA4 stable analogues attenuated pulmonary inflammation and bacterial load, as well as disease severity in a mouse model of CF [112]. Besides controlling inflammation and infection, LXA_4 might regulate airway physiological functions, such as bronchial epithelium ion transport, airway surface liquid layer height and epithelial barrier integrity [113]. Interestingly, Yang et al. performed a metabolic profiling of sputum and observed that CF patients with detectable levels of RvE1 presented a

better lung function compared with those patients with undetectable levels of this proresolving mediator, indicating that other SPMs might have protective roles in CF [114].

Neurological diseases

Alzheimer's disease

Alzheimer's disease (AD) is the most common type of dementia. The pathological hallmarks of this chronic neurodegenerative disease are the senile plaques and neurofibrillary tangles [115]. The senile plaques are extracellular deposits of amyloid-beta (Aβ) in brain's grey matter. Aβ is able to active microglia, triggering an inflammatory response, which can damage the brain tissue and shorten neuronal survival [116]. Indeed, several studies have reported increased levels of pro-inflammatory mediators in the brain and cerebrospinal fluid (CSF) of AD patients [117].

A genome-wide association study identified one single nucleotide polymorphism (rs2811226) in AnxA1 gene involved in the regulation of programmed cell death that might contribute to AD susceptibility [118]. Of importance, AnxA1 is up-regulated in the superior frontal gyrus and in periventricular white matter lesions of AD patients compared with non-demented controls [119, 120]. It has been also demonstrated that microglial-derived AnxA1 promotes the phagocytic removal of apoptotic neuron-like cells and suppresses microglial activation *in vitro* [120]. However, remains still unknown why AnxA1 fails to contra-regulate inflammation in AD brains *in vivo,* despite its increased expression, and whether rs2811226 polymorphism is associated with this event.

The data on SPMs seem to differ from those of AnxA1 in AD. Wang et al. investigated the pro-resolving lipids LXA4, RvD1 and MaR1 and the receptors FPR2 (AnxA1, LXA4 and RvD1 receptor) and ChemR23 (RvE1 receptor) in patients with AD, mild cognitive impairment (MCI) and subjective cognitive impairment (SCI). They showed that LXA4 levels were reduced in the CSF of AD compared to MCI and SCI. LXA4 and MaR1 levels in postmortem hippocampal tissue were also reduced in AD patients than in healthy controls. However, RvD1 levels were similar between these groups of patients in CSF and hippocampus. Interestingly, both FPR2 and ChemR23 receptors were up-regulated in the brains of AD patients. In addition, patients with AD had higher levels of 15-LOX-2 enzyme in hippocampal tissue [121]. These results suggest that SPMs deficiency may be associated with AD progression. Accordingly, one *in vitro* study showed that RvD1 promotes the phagocytosis of Aβ by macrophages isolated from peripheral blood of AD patients [122]. Furthermore, the up-regulation of their receptors, combined with increased levels of their synthesizing enzymes could be compensatory mechanisms to attenuate this deficiency. However, as the proresolving agonists are in low levels, maybe the signals are not properly triggered and this may explain, at least in part, why neuroinflammation persists in AD patients despite these compensatory mechanisms. Moreover, LXA4 can be inactivated by dehydrogenases and oxidoreductases in inflammatory sites [123]. Thus, metabolic inactivation of SPMs could also partially explain their inefficiency to resolve inflammation in chronic inflammatory diseases such as AD. This mechanism should be better investigated in future studies.

In conclusion, AnxA1 and SPMs altered levels or action may play a role in the AD pathogenesis, but it is still unclear how failures in these pro-resolving pathways contribute to chronic inflammation in AD patients.

Parkinson's Disease

Parkinson's Disease (PD) is characterized by the progressive degeneration of dopaminergic neurons in the substantia nigra and neuro inflammation is considered an important contributor to the disease pathogenesis [124]. There is experimental evidence of microglial activation in the substantia nigra in PD [125]. When the brain is injured, as observed in PD, microglia is activated and become cytotoxic [126].

AnxA1 is abundantly present in microglia and suppresses microglial activation *in vitro* [120]. Moreover, a recent work demonstrated that AnxA1 is able to polarize microglia to an anti-inflammatory M2 phenotype that protects neurons from ischemia-like injury [127]. Interestingly, Knott et al. showed that microglial regions of substantia nigra that contained fragmented neurons had a higher intensity of AnxA1 than regions containing intact neurons in human PD. Additionally, there was no difference in AnxA1 levels in regions that contained intact neurons between PD patients and controls in this study [128]. Collectively, these data suggest that AnxA1 up-regulation in the microglia of PD patients may be contra-regulatory mechanism to attenuate neuroinflammation and brain injury.

Results from pre-clinical studies indicate that omega-3 poly-unsaturated fatty acids have neuroprotective effects in PD [129]. For instance, it has been demonstrated that DHA (an omega-3 poly-unsaturated fatty acid) prevents dopaminergic neurons cell death [130]. RvD2 is able to attenuate neuronal injury in the LPS-induced rat model of PD by reducing the expression of pro-inflammatory cytokines in the brain tissue [131]. Thus, RvD2 might mediate part of the neuroprotective effects of omega-3 poly-unsaturated fatty acids, although the exact mechanisms underlying these effects are unclear.

Autism spectrum disorders

Autism spectrum disorders (ASD) are a set o neurodevelopment disorders characterized by neurological and behavioral deficits. Genetic and environmental factors play a role in ASD development [132, 133]. Accumulating evidence indicate that immune deregulation participates in ASD pathogenesis and correlates with severity of behavior impairment. Indeed, many studies have reported abnormal cytokine levels in ASD patients that shifts towards a pro-inflammatory profile [134].

Although pro-inflammatory molecules have been extensively investigated over the last years in ASD, studies on the role of pro-resolving mediators are still insipient. Recently, Correa et al. reported decreased LXA4 plasma levels in autistic children than in normal children and that LXA4 levels inversely correlated with disease severity [135]. Another recent study has identified arecurrent tandem duplication in the AnxA1 gene in autistic patients that was not identified in controls. Many parents and siblings carrying this duplication presented a broader autism phenotype and co-morbidities. It was proposed that AnxA1 gene duplication could be one more risk factor for ASD [136].

Inflammatory bowel diseases

The inflammatory bowel diseases (IBDs) Crohn's Disease (CD) and ulcerative colitis (UC) are chronic disorders associated with gastrointestinal inflammation in response to the dysregulated immune system in genetically predisposed individuals. Despite sharing various pathological features, CD can affect any part of the gastrointestinal tract, while UC is limited to the colon [137, 138].

The engagement of AnxA1 pathway might differ according to the type of IBD. Sena et al showed that AnxA1 plasma levels were decreased in CD patients

compared with healthy controls. This finding coincided with reduced AnxA1 mRNA levels in peripheral blood mononuclear cells and reduced AnxA1 protein levels in gut mucosa of CD patients. Moreover, AnxA1 was up-regulated in clinical positive responders than in negative responders to infliximab therapy [139]. These results indicate that AnxA1 deficiency might be involved in CD pathogenesis and that measuring AnxA1 levels could be valuable in the evaluation of infliximab therapy efficiency. Since patients with CD have deficient levels of endogenous AnxA1, they could benefit with AnxA1 analogues-based therapies. Indeed, mice with experimental colitis had colonic inflammation reversed after treatment with MC-12, an AnxA1-based tripeptide [140].

By contrast, Vergnolle et al. reported that endogenous AnxA1 was secreted in the colon of patients with severe UC but not by biopsies from patients with slight or moderate UC, nor by biopsies from healthy colons [141]. In accordance, Vong et al. showed that AnxA1 expression was increased in biopsies from UC patients, with active disease or clinical remission, in comparison to healthy controls [142]. These findings were corroborated by studies reporting AnxA1 overexpression and increased secretion, notably by neutrophils, in colonic tissues of rats with experimental colitis [143, 144]. Increased AnxA1 levels seem to be insufficient to resolve inflammation in UC patients, particularly in those with severe disease. Interestingly, IBD patients not taking corticosteroids have increased levels of AnxA1 auto-antibodies than controls, which could impair AnxA1 actions [145]. Other mechanisms, such as proteolytic cleavage of AnxA1 N-terminal portion by proteases, which are abundant in the inflamed gut [146], could contribute to AnxA1 failure in resolving inflammation in these clinical conditions. Furthermore, there was a difference in AnxA1 localization between the patient groups in Vong et al. study. AnxA1 expression was associated

with neutrophils in biopsies of patients with active UC disease, and with macrophages in those with clinical remission, as evidenced by co-localization in double-staining experiments with markers of neutrophil (neutrophil elastase) and macrophage (CD68) expression. These results indicated that AnxA1 pattern of secretion depends on the inflammation status and that this protein might exert different actions according to the cell type [142].

Endogenous AnxA1 can be released in the circulation as a component of extracellular vesicles (EVs) derived from neutrophils and intestinal epithelial cells [147-149]. Patients with active IBD (UC or CD) have increased serum levels of secreted AnxA1-containing EVs than in patients with milder disease activity, indicating that intestinal mucosal inflammation stimulates AnxA1-containing EVs releasing to the circulation as a contrarregulatory mechanism of inflammation. Moreover, these data suggest that EVs could be potential biomarkers of disease severity [149]. Increased release of AnxA1 as a component of EVs in IBDs corroborates to Vergnolle et al. and Vong et al. findings, but cannot explain the results obtained by Sena et al. Therefore, more studies are necessary to understand AnxA1 role in the different types of IBD.

Vong et al. also evaluated LXA4 mucosal expression in UC patients and found that it increased only in patients with clinical remission. Thus, LXA4 overexpression might attenuate gut inflammation and promote mucosal healing [142]. Accordingly, LXA4 levels correlated negatively with disease progression in a experimental model of colitis [150]. *In vivo* studies have also shown that LXA4 analogues and omega-3 derivates, including 17(R)-hydroxy docosahexaenoic acid and RvD2, as well as aspirin triggered-RvD1, reduces colitis severity by inhibiting pro-inflammatory mediators production and increasing the phagocytic activity of macrophages [151-

153]. Based on these findings, SPMs analogues and ASA have the potential to be used for IBD treatment.

Pregnancy diseases

Preeclampsia

Preeclampsia (PE) is a pregnancy disease characterized by new-onset hypertension plus proteinuria or end-organ dysfunction at ≥20 weeks of gestation. Deficient uteroplacental perfusion, endothelial dysfunction and overwhelming inflammation are considered central features in PE pathogenesis [154]. It has been proposed that normotensive pregnancy is characterized by low grade/controlled inflammation and maternal immune tolerance to the allogenic fetus, as consequences of activated innate immunity and decreased adaptive immune responses. By contrast, both innate and adaptive immune systems are activated in PE women, leading to exacerbated inflammatory response and maladaptive immunity, which has been associated with maternal immune intolerance to the fetus [155, 156]. According to Fullerton & Gilroy, “frustrated” resolution of inflammation might cause maladaptive immune responses in chronic inflammatory diseases [2], leading us to hypothesize that dysfunctional mechanisms of resolution could be an underlying pathological mechanism in PE.

Our group first found that AnxA1 plasma levels were increased in PE women with early onset disease than in normotensive pregnant women matched for gestational age [157]. AnxA1 increase in PE women points to possible failures in AnxA1 actions. A recent study identified AnxA1 as a possible target protein expressed in the placenta that may induce the production of auto-antibodies in PE

[158]. The presence of anti-AnxA1 auto-antibodies in the PE women circulation may contribute to the failure in regulating systemic inflammation. Our group previously reported increased LXA4 plasma levels in PE women [159], which is in accordance with two other studies [160, 161]. However, Xu et al. found decreased levels in PE women than in normotensive pregnant women [162]. Other mechanisms, such as downregulation of FPR2 and receptor dimerization failure, may account for AnxA1 and LXA4 ineffectiveness to attenuate inflammation [163, 164]. Indeed, FPR2 mRNA placental expression was downregulated in women with PE in two studies [161, 162]. However, an opposite finding has also been described [160]. Moreover, an increased inactivation of these pro-resolving mediators in the inflamed tissues might interfere with their actions. For instance, it has been demonstrated that PE placenta is rich of proteases, like neutrophil elastase, which could cleave AnxA1 bioactive N-terminal region, interfering with its anti-inflammatory and pro-resolving effects [15, 16, 165]. However, these possible dysfunctional mechanisms of resolution should be clarified in the context of PE.

CONCLUDING REMARKS

Taken togheter, these studies suggest that altered levels of pro-resolving mediators, such as AnxA1 and SPMs, may be involved in the pathogenesis of human chronic inflammatory diseases. Moreover, increased metabolic inactivation of pro-resolving mediators and/or downregulation of their receptors might explain their inefficacy to resolve inflammation in these conditions. Understanding the role of these molecules in chronic inflammatory diseases is a challenging due to their

complexity and different etiopathogenesis. More studies are necessary to clarify this issue and to evaluate potential therapies targeting pro-resolving mechanisms.

ACKNOWLEGMENTS

KBG, LMD and LPS are grateful to Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and Fundação de Amparo a Pesquisa do Estado de Minas Gerais (FAPEMIG) fellowship.

REFERENCES

[1] R. Chovatiya, R. Medzhitov, Stress, inflammation, and defense of homeostasis, Mol Cell 54(2) (2014) 281-8.

[2] J.N. Fullerton, D.W. Gilroy, Resolution of inflammation: a new therapeutic frontier, Nat Rev Drug Discov (2016).

[3] C.N. Serhan, J. Savill, Resolution of inflammation: the beginning programs the end, Nat Immunol 6(12) (2005) 1191-7.

[4] C.N. Serhan, S.D. Brain, C.D. Buckley, D.W. Gilroy, C. Haslett, L.A. O'Neill, M. Perretti, A.G. Rossi, J.L. Wallace, Resolution of inflammation: state of the art, definitions and terms, FASEB J 21(2) (2007) 325-32.

[5] M. Perretti, X. Leroy, E.J. Bland, T. Montero-Melendez, Resolution Pharmacology: Opportunities for Therapeutic Innovation in Inflammation, Trends Pharmacol Sci 36(11) (2015) 737-55.

[6] M. Perretti, F. D'Acquisto, Annexin A1 and glucocorticoids as effectors of the resolution of inflammation, Nat Rev Immunol 9(1) (2009) 62-70.

[7] R.J. Flower, Eleventh Gaddum memorial lecture. Lipocortin and the mechanism of action of the glucocorticoids, Br J Pharmacol 94(4) (1988) 987-1015.

[8] D.G. Souza, C.T. Fagundes, F.A. Amaral, D. Cisalpino, L.P. Sousa, A.T. Vieira, V. Pinho, J.R. Nicoli, L.Q. Vieira, I.M. Fierro, M.M. Teixeira, The required role of endogenously produced lipoxin A4 and annexin-1 for the production of IL-10 and inflammatory hyporesponsiveness in mice, J Immunol 179(12) (2007) 8533-43.

[9] J.P. Vago, C.R. Nogueira, L.P. Tavares, F.M. Soriani, F. Lopes, R.C. Russo, V. Pinho, M.M. Teixeira, L.P. Sousa, Annexin A1 modulates natural and glucocorticoid-induced resolution of inflammation by enhancing neutrophil apoptosis, J Leukoc Biol 92(2) (2012) 249-58.

[10] H.B. Patel, K.N. Kornerup, A.L. Sampaio, F. D'Acquisto, M.P. Seed, A.P. Girol, M. Gray, C. Pitzalis, S.M. Oliani, M. Perretti, The impact of endogenous annexin A1 on glucocorticoid control of inflammatory arthritis, Ann Rheum Dis 71(11) (2012) 1872-80.

[11] B.E. Chatterjee, S. Yona, G. Rosignoli, R.E. Young, S. Nourshargh, R.J. Flower, M. Perretti, Annexin 1-deficient neutrophils exhibit enhanced transmigration in vivo and increased responsiveness in vitro, J Leukoc Biol 78(3) (2005) 639-46.

[12] A. Walther, K. Riehemann, V. Gerke, A novel ligand of the formyl peptide receptor: annexin I regulates neutrophil extravasation by interacting with the FPR, Mol Cell 5(5) (2000) 831-40.

[13] Y. Le, J.M. Wang, X. Liu, Y. Kong, X. Hou, L. Ruan, H. Mou, Biologically active peptides interacting with the G protein-coupled formylpeptide receptor, Protein Pept Lett 14(9) (2007) 846-53.

[14] P. Raynal, H.B. Pollard, Annexins: the problem of assessing the biological role for a gene family of multifunctional calcium- and phospholipid-binding proteins, Biochim Biophys Acta 1197(1) (1994) 63-93.

[15] U. Rescher, V. Goebeler, A. Wilbers, V. Gerke, Proteolytic cleavage of annexin 1 by human leukocyte elastase, Biochim Biophys Acta 1763(11) (2006) 1320-4.

[16] K.S. Huang, P. McGray, R.J. Mattaliano, C. Burne, E.P. Chow, L.K. Sinclair, R.B. Pepinsky, Purification and characterization of proteolytic fragments of lipocortin I that inhibit phospholipase A2, J Biol Chem 262(16) (1987) 7639-45.

[17] J. Dalli, A.P. Consalvo, V. Ray, C. Di Filippo, M. D'Amico, N. Mehta, M. Perretti, Proresolving and tissue-protective actions of annexin A1-based cleavage-resistant peptides are mediated by formyl peptide receptor 2/lipoxin A4 receptor, J Immunol 190(12) (2013) 6478-87.

[18] M. Pederzoli-Ribeil, F. Maione, D. Cooper, A. Al-Kashi, J. Dalli, M. Perretti, F. D'Acquisto, Design and characterization of a cleavage-resistant Annexin A1 mutant to control inflammation in the microvasculature, Blood 116(20) (2010) 4288-96.

[19] J.P. Vago, L.P. Tavares, M.A. Sugimoto, G.L. Lima, I. Galvão, T.R. de Caux, K.M. Lima, A.L. Ribeiro, F.S. Carneiro, F.F. Nunes, V. Pinho, M. Perretti, M.M. Teixeira, L.P. Sousa, Proresolving Actions of Synthetic and Natural Protease Inhibitors Are Mediated by Annexin A1, J Immunol (2016).

[20] S.L. Williams, I.R. Milne, C.J. Bagley, J.R. Gamble, M.A. Vadas, S.M. Pitson, Y. Khew-Goodall, A proinflammatory role for proteolytically cleaved annexin A1 in neutrophil transendothelial migration, J Immunol 185(5) (2010) 3057-63.

[21] F.H. Tsao, Z. Xiang, A. Abbasi, K.C. Meyer, Neutrophil necrosis and annexin 1 degradation associated with airway inflammation in lung transplant recipients with cystic fibrosis, BMC Pulm Med 12 (2012) 44.

[22] M. de Lorgeril, P. Salen, New insights into the health effects of dietary saturated and omega-6 and omega-3 polyunsaturated fatty acids, BMC Med 10 (2012) 50.

[23] G. Bannenberg, C.N. Serhan, Specialized pro-resolving lipid mediators in the inflammatory response: An update, Biochim Biophys Acta 1801(12) (2010) 1260-73.

[24] J.M. Schwab, C.N. Serhan, Lipoxins and new lipid mediators in the resolution of inflammation, Curr Opin Pharmacol 6(4) (2006) 414-20.

[25] C.N. Serhan, M. Arita, S. Hong, K. Gotlinger, Resolvins, docosatrienes, and neuroprotectins, novel omega-3-derived mediators, and their endogenous aspirin-triggered epimers, Lipids 39(11) (2004) 1125-32.

[26] C.N. Serhan, Pro-resolving lipid mediators are leads for resolution physiology, Nature 510(7503) (2014) 92-101.

[27] C.N. Serhan, J. Dalli, R.A. Colas, J.W. Winkler, N. Chiang, Protectins and maresins: New pro-resolving families of mediators in acute inflammation and resolution bioactive metabolome, Biochim Biophys Acta 1851(4) (2015) 397-413.

[28] C.N. Serhan, Lipoxins and aspirin-triggered 15-epi-lipoxins are the first lipid mediators of endogenous anti-inflammation and resolution, Prostaglandins Leukot Essent Fatty Acids 73(3-4) (2005) 141-62.

[29] C.N. Serhan, S. Krishnamoorthy, A. Recchiuti, N. Chiang, Novel anti-inflammatory--pro-resolving mediators and their receptors, Curr Top Med Chem 11(6) (2011) 629-47.

[30] M. Arita, F. Bianchini, J. Aliberti, A. Sher, N. Chiang, S. Hong, R. Yang, N.A. Petasis, C.N. Serhan, Stereochemical assignment, antiinflammatory properties, and receptor for the omega-3 lipid mediator resolvin E1, J Exp Med 201(5) (2005) 713-22.

[31] D. El Kebir, P. Gjorstrup, J.G. Filep, Resolvin E1 promotes phagocytosis-induced neutrophil apoptosis and accelerates resolution of pulmonary inflammation, Proc Natl Acad Sci U S A 109(37) (2012) 14983-8.

[32] L.V. Norling, J. Dalli, R.J. Flower, C.N. Serhan, M. Perretti, Resolvin D1 limits polymorphonuclear leukocyte recruitment to inflammatory loci: receptor-dependent actions, Arterioscler Thromb Vasc Biol 32(8) (2012) 1970-8.

[33] J.S. Yudkin, Inflammation, obesity, and the metabolic syndrome, Horm Metab Res 39(10) (2007) 707-9.

[34] M. Masoodi, O. Kuda, M. Rossmeisl, P. Flachs, J. Kopecky, Lipid signaling in adipose tissue: Connecting inflammation & metabolism, Biochim Biophys Acta (2014).

[35] R.T. Akasheh, J. Pang, J.M. York, G. Fantuzzi, New pathways to control inflammatory responses in adipose tissue, Curr Opin Pharmacol 13(4) (2013) 613-7.

[36] A. Kosicka, A.D. Cunliffe, R. Mackenzie, M.G. Zariwala, M. Perretti, R.J. Flower, D. Renshaw, Attenuation of plasma annexin A1 in human obesity, FASEB J 27(1) (2013) 368-78.

[37] R.T. Akasheh, M. Pini, J. Pang, G. Fantuzzi, Increased adiposity in annexin A1-deficient mice, PLoS One 8(12) (2013) e82608.

[38] C. Henegar, J. Tordjman, V. Achard, D. Lacasa, I. Cremer, M. Guerre-Millo, C. Poitou, A. Basdevant, V. Stich, N. Viguerie, D. Langin, P. Bedossa, J.D. Zucker, K. Clement, Adipose tissue transcriptomic signature highlights the pathological relevance of extracellular matrix in human obesity, Genome Biol 9(1) (2008) R14.

[39] F. D'Acquisto, M. Perretti, R.J. Flower, Annexin-A1: a pivotal regulator of the innate and adaptive immune systems, Br J Pharmacol 155(2) (2008) 152-69.

[40] D.S. Lin, W.E. Conner, Are the n-3 fatty acids from dietary fish oil deposited in the triglyceride stores of adipose tissue?, Am J Clin Nutr 51(4) (1990) 535-9.

[41] C.A. Pickens, L.M. Sordillo, S.S. Comstock, W.S. Harris, K. Hortos, B. Kovan, J.I. Fenton, Plasma phospholipids, non-esterified plasma polyunsaturated fatty acids and oxylipids are associated with BMI, Prostaglandins Leukot Essent Fatty Acids 95 (2015) 31-40.

[42] E. Börgeson, A.M. Johnson, Y.S. Lee, A. Till, G.H. Syed, S.T. Ali-Shah, P.J. Guiry, J. Dalli, R.A. Colas, C.N. Serhan, K. Sharma, C. Godson, Lipoxin A4 Attenuates Obesity-Induced Adipose Inflammation and Associated Liver and Kidney Disease, Cell Metab 22(1) (2015) 125-37.

[43] A. Neuhofer, M. Zeyda, D. Mascher, B.K. Itariu, I. Murano, L. Leitner, E.E. Hochbrugger, P. Fraisl, S. Cinti, C.N. Serhan, T.M. Stulnig, Impaired local production of proresolving lipid mediators in obesity and 17-HDHA as a potential treatment for obesity-associated inflammation, Diabetes 62(6) (2013) 1945-56.

[44] W.H. Organization, Definition, Diagnosis and Classification of Diabetes Mellitus and its Complications. Part 1: Diagnosis and Classification of Diabetes Mellitus, 1999.

[45] N. Esser, S. Legrand-Poels, J. Piette, A.J. Scheen, N. Paquot, Inflammation as a link between obesity, metabolic syndrome and type 2 diabetes, Diabetes Res Clin Pract 105(2) (2014) 141-50.

[46] M. Murri, M. Insenser, M.R. Bernal-Lopez, P. Perez-Martinez, H.F. Escobar-Morreale, F.J. Tinahones, Proteomic analysis of visceral adipose tissue in pre-obese patients with type 2 diabetes, Mol Cell Endocrinol 376(1-2) (2013) 99-106.

[47] B. Li, M. Sheng, L. Xie, F. Liu, G. Yan, W. Wang, A. Lin, F. Zhao, Y. Chen, Tear proteomic analysis of patients with type 2 diabetes and dry eye syndrome by two-

dimensional nano-liquid chromatography coupled with tandem mass spectrometry, Invest Ophthalmol Vis Sci 55(1) (2014) 177-86.

[48] U.N. Das, Essential fatty acid metabolism in patients with essential hypertension, diabetes mellitus and coronary heart disease, Prostaglandins Leukot Essent Fatty Acids 52(6) (1995) 387-91.

[49] J.D. Krebs, L.M. Browning, N.K. McLean, J.L. Rothwell, G.D. Mishra, C.S. Moore, S.A. Jebb, Additive benefits of long-chain n-3 polyunsaturated fatty acids and weight-loss in the management of cardiovascular disease risk in overweight hyperinsulinaemic women, Int J Obes (Lond) 30(10) (2006) 1535-44.

[50] E. Börgeson, F.C. McGillicuddy, K.A. Harford, N. Corrigan, D.F. Higgins, P. Maderna, H.M. Roche, C. Godson, Lipoxin A4 attenuates adipose inflammation, FASEB J 26(10) (2012) 4287-94.

[51] A. González-Périz, R. Horrillo, N. Ferré, K. Gronert, B. Dong, E. Morán-Salvador, E. Titos, M. Martínez-Clemente, M. López-Parra, V. Arroyo, J. Clària, Obesity-induced insulin resistance and hepatic steatosis are alleviated by omega-3 fatty acids: a role for resolvins and protectins, FASEB J 23(6) (2009) 1946-57.

[52] J. Hellmann, Y. Tang, M. Kosuri, A. Bhatnagar, M. Spite, Resolvin D1 decreases adipose tissue macrophage accumulation and improves insulin sensitivity in obese-diabetic mice, FASEB J 25(7) (2011) 2399-407.

[53] J. Hellmann, Y. Tang, M. Spite, Proresolving lipid mediators and diabetic wound healing, Curr Opin Endocrinol Diabetes Obes 19(2) (2012) 104-8.

[54] S. Hong, H. Tian, Y. Lu, J.M. Laborde, F.A. Muhale, Q. Wang, B.V. Alapure, C.N. Serhan, N.G. Bazan, Neuroprotectin/protectin D1: endogenous biosynthesis and actions on diabetic macrophages in promoting wound healing and innervation impaired by diabetes, Am J Physiol Cell Physiol 307(11) (2014) C1058-67.

[55] F. Barutta, G. Bruno, S. Grimaldi, G. Gruden, Inflammation in diabetic nephropathy: moving toward clinical biomarkers and targets for treatment, Endocrine (2014).

[56] N.T. Pietrani, C.N. Ferreira, K.F. Rodrigues, A.A. Bosco, M.C. Oliveira, A.L. Teixeira, A.V. Ferreira, L.P. Sousa, K.B. Gomes, Annexin A1 concentrations is decreased in patients with diabetes type 2 and nephropathy, Clin Chim Acta 436 (2014) 181-2.

[57] S.M. Ka, P.Y. Tsai, T.K. Chao, S.M. Yang, Y.J. Hung, J.S. Chen, H.A. Shui, A. Chen, Urine annexin A1 as an index for glomerular injury in patients, Dis Markers 2014 (2014) 854163.

[58] E. Börgeson, C. Godson, Resolution of inflammation: therapeutic potential of pro-resolving lipids in type 2 diabetes mellitus and associated renal complications, Front Immunol 3 (2012) 318.

[59] W.H. Organization, Cardiovascular diseases, 2016.

[60] J. Frostegård, Immunity, atherosclerosis and cardiovascular disease, BMC Med 11 (2013) 117.

[61] C. Qin, Y.H. Yang, L. May, X. Gao, A.G. Stewart, Y. Tu, O.L. Woodman, R.H. Ritchie, Cardioprotective potential of annexin-A1 mimetics in myocardial infarction, Pharmacol Ther 148 (2015) 47-65.

[62] R.H. Ritchie, J.M. Gordon, O.L. Woodman, A.H. Cao, G.J. Dusting, Annexin-1 peptide Anx-1(2-26) protects adult rat cardiac myocytes from cellular injury induced by simulated ischaemia, Br J Pharmacol 145(4) (2005) 495-502.

[63] G. Fredman, N. Kamaly, S. Spolitu, J. Milton, D. Ghorpade, R. Chiasson, G. Kuriakose, M. Perretti, O. Farokhzad, O. Farokzhad, I. Tabas, Targeted nanoparticles

containing the proresolving peptide Ac2-26 protect against advanced atherosclerosis in hypercholesterolemic mice, Sci Transl Med 7(275) (2015) 275ra20.

[64] B.L. Cheuk, S.W. Cheng, Annexin A1 expression in atherosclerotic carotid plaques and its relationship with plaque characteristics, Eur J Vasc Endovasc Surg 41(3) (2011) 364-71.

[65] L.E. Viiri, L.E. Full, T.J. Navin, S. Begum, A. Didangelos, N. Astola, R.K. Berge, I. Seppälä, J. Shalhoub, I.J. Franklin, M. Perretti, T. Lehtimäki, A.H. Davies, R. Wait, C. Monaco, Smooth muscle cells in human atherosclerosis: proteomic profiling reveals differences in expression of Annexin A1 and mitochondrial proteins in carotid disease, J Mol Cell Cardiol 54 (2013) 65-72.

[66] D.H. Kusters, M.L. Chatrou, B.A. Willems, M. De Saint-Hubert, M. Bauwens, E. van der Vorst, S. Bena, E.A. Biessen, M. Perretti, L.J. Schurgers, C.P. Reutelingsperger, Pharmacological Treatment with Annexin A1 Reduces Atherosclerotic Plaque Burden in LDLR-/- Mice on Western Type Diet, PLoS One 10(6) (2015) e0130484.

[67] E. Särndahl, I. Bergström, J. Nijm, T. Forslund, M. Perretti, L. Jonasson, Enhanced neutrophil expression of annexin-1 in coronary artery disease, Metabolism 59(3) (2010) 433-40.

[68] M. Reina-Couto, J. Carvalho, M.J. Valente, L. Vale, J. Afonso, F. Carvalho, P. Bettencourt, T. Sousa, A. Albino-Teixeira, Impaired resolution of inflammation in human chronic heart failure, Eur J Clin Invest 44(6) (2014) 527-38.

[69] D. Mozaffarian, J.H. Wu, (n-3) fatty acids and cardiovascular health: are effects of EPA and DHA shared or complementary?, J Nutr 142(3) (2012) 614S-625S.

[70] A.J. Merched, K. Ko, K.H. Gotlinger, C.N. Serhan, L. Chan, Atherosclerosis: evidence for impairment of resolution of vascular inflammation governed by specific lipid mediators, FASEB J 22(10) (2008) 3595-606.

[71] M. Adib-Conquy, J.M. Cavaillon, Compensatory anti-inflammatory response syndrome, Thromb Haemost 101(1) (2009) 36-47.

[72] M. Levi, T. Poll, Coagulation in patients with severe sepsis, Semin Thromb Hemost 41(1) (2015) 9-15.

[73] R.S. Hotchkiss, G. Monneret, D. Payen, Immunosuppression in sepsis: a novel understanding of the disorder and a new therapeutic approach, Lancet Infect Dis 13(3) (2013) 260-8.

[74] W.H. Tsai, I.T. Li, Y.B. Yu, H.C. Hsu, C.H. Shih, Serial changes in plasma annexin A1 and cortisol levels in sepsis patients, Chin J Physiol 57(1) (2014) 1-7.

[75] W.H. Tsai, C.H. Shih, Y.B. Yu, H.C. Hsu, Plasma levels in sepsis patients of annexin A1, lipoxin A4, macrophage inflammatory protein-3a, and neutrophil gelatinase-associated lipocalin, J Chin Med Assoc 76(9) (2013) 486-90.

[76] A.S. Damazo, S. Yona, F. D'Acquisto, R.J. Flower, S.M. Oliani, M. Perretti, Critical protective role for annexin 1 gene expression in the endotoxemic murine microcirculation, Am J Pathol 166(6) (2005) 1607-17.

[77] F.N. Gavins, E.L. Hughes, N.A. Buss, P.M. Holloway, S.J. Getting, J.C. Buckingham, Leukocyte recruitment in the brain in sepsis: involvement of the annexin 1-FPR2/ALX anti-inflammatory system, FASEB J 26(12) (2012) 4977-89.

[78] T. Gobbetti, S.M. Coldewey, J. Chen, S. McArthur, P. le Faouder, N. Cenac, R.J. Flower, C. Thiemermann, M. Perretti, Nonredundant protective properties of FPR2/ALX in polymicrobial murine sepsis, Proc Natl Acad Sci U S A 111(52) (2014) 18685-90.

[79] M.A. Sanchez, C.B. Thomas, H.R. O'Neal, Do aspirin and statins prevent severe sepsis?, Curr Opin Infect Dis 25(3) (2012) 345-50.

[80] D.P. Eisen, D. Reid, E.S. McBryde, Acetyl salicylic acid usage and mortality in critically ill patients with the systemic inflammatory response syndrome and sepsis, Crit Care Med 40(6) (2012) 1761-7.

[81] G.P. Otto, M. Sossdorf, J. Boettel, B. Kabisch, H. Breuel, J. Winning, W. Lösche, Effects of low-dose acetylsalicylic acid and atherosclerotic vascular diseases on the outcome in patients with severe sepsis or septic shock, Platelets 24(6) (2013) 480-5.

[82] T. Ueda, K. Fukunaga, H. Seki, J. Miyata, M. Arita, T. Miyasho, T. Obata, K. Asano, T. Betsuyaku, J. Takeda, Combination therapy of 15-epi-lipoxin A4 with antibiotics protects mice from Escherichia coli-induced sepsis*, Crit Care Med 42(4) (2014) e288-95.

[83] B. Wu, J. Walker, B. Spur, A. Rodriguez, K. Yin, Effects of Lipoxin A4 on antimicrobial actions of neutrophils in sepsis, Prostaglandins Leukot Essent Fatty Acids 94 (2015) 55-64.

[84] J. Walker, E. Dichter, G. Lacorte, D. Kerner, B. Spur, A. Rodriguez, K. Yin, Lipoxin a4 increases survival by decreasing systemic inflammation and bacterial load in sepsis, Shock 36(4) (2011) 410-6.

[85] T. Murakami, K. Suzuki, H. Tamura, I. Nagaoka, Suppressive action of resolvin D1 on the production and release of septic mediators in D-galactosamine-sensitized endotoxin shock mice, Exp Ther Med 2(1) (2011) 57-61.

[86] M.C. McCormack, P.L. Enright, Making the diagnosis of asthma, Respir Care 53(5) (2008) 583-90; discussion 590-2.

[87] J.M. Bhatt, A.R. Smyth, The management of pre-school wheeze, Paediatr Respir Rev 12(1) (2011) 70-7.

[88] O. Haworth, B.D. Levy, Lipoxins, resolvins and protectins: new leads for the treatment of asthma, Expert Opin Drug Discov 3(10) (2008) 1209-22.

[89] H. Eke Gungor, F. Tahan, S. Gokahmetoglu, B. Saraymen, Decreased levels of lipoxin A4 and annexin A1 in wheezy infants, Int Arch Allergy Immunol 163(3) (2014) 193-7.

[90] F.S. Ng, K.Y. Wong, S.P. Guan, F.B. Mustafa, T.S. Kajiji, P. Bist, S.K. Biswas, W.S. Wong, L.H. Lim, Annexin-1-deficient mice exhibit spontaneous airway hyperresponsiveness and exacerbated allergen-specific antibody responses in a mouse model of asthma, Clin Exp Allergy 41(12) (2011) 1793-803.

[91] L.M. Wang, W.H. Li, Y.C. Xu, Q. Wei, H. Zhao, X.F. Jiang, Annexin 1-derived peptide Ac2-26 inhibits eosinophil recruitment in vivo via decreasing prostaglandin D_2, Int Arch Allergy Immunol 154(2) (2011) 137-48.

[92] B.D. Levy, C. Bonnans, E.S. Silverman, L.J. Palmer, G. Marigowda, E. Israel, N.H. Severe Asthma Research Program, L.ng, and Blood Institute, Diminished lipoxin biosynthesis in severe asthma, Am J Respir Crit Care Med 172(7) (2005) 824-30.

[93] G.E. Celik, F.O. Erkekol, Z. Misirligil, M. Melli, Lipoxin A4 levels in asthma: relation with disease severity and aspirin sensitivity, Clin Exp Allergy 37(10) (2007) 1494-501.

[94] A. Planagumà, S. Kazani, G. Marigowda, O. Haworth, T.J. Mariani, E. Israel, E.R. Bleecker, D. Curran-Everett, S.C. Erzurum, W.J. Calhoun, M. Castro, K.F. Chung, B. Gaston, N.N. Jarjour, W.W. Busse, S.E. Wenzel, B.D. Levy, Airway lipoxin A4 generation and lipoxin A4 receptor expression are decreased in severe asthma, Am J Respir Crit Care Med 178(6) (2008) 574-82.

[95] I. Vachier, C. Bonnans, C. Chavis, M. Farce, P. Godard, J. Bousquet, P. Chanez, Severe asthma is associated with a loss of LX4, an endogenous anti-inflammatory compound, J Allergy Clin Immunol 115(1) (2005) 55-60.

[96] S.H. Wu, P.L. Yin, Y.M. Zhang, H.X. Tao, Reversed changes of lipoxin A4 and leukotrienes in children with asthma in different severity degree, Pediatr Pulmonol 45(4) (2010) 333-40.

[97] B.D. Levy, P. Kohli, K. Gotlinger, O. Haworth, S. Hong, S. Kazani, E. Israel, K.J. Haley, C.N. Serhan, Protectin D1 is generated in asthma and dampens airway inflammation and hyperresponsiveness, J Immunol 178(1) (2007) 496-502.

[98] J. Miyata, K. Fukunaga, R. Iwamoto, Y. Isobe, K. Niimi, R. Takamiya, T. Takihara, K. Tomomatsu, Y. Suzuki, T. Oguma, K. Sayama, H. Arai, T. Betsuyaku, M. Arita, K. Asano, Dysregulated synthesis of protectin D1 in eosinophils from patients with severe asthma, J Allergy Clin Immunol 131(2) (2013) 353-60.e1-2.

[99] A.P. Rogerio, O. Haworth, R. Croze, S.F. Oh, M. Uddin, T. Carlo, M.A. Pfeffer, R. Priluck, C.N. Serhan, B.D. Levy, Resolvin D1 and aspirin-triggered resolvin D1 promote resolution of allergic airways responses, J Immunol 189(4) (2012) 1983-91.

[100] N. Krishnamoorthy, P.R. Burkett, J. Dalli, R.E. Abdulnour, R. Colas, S. Ramon, R.P. Phipps, N.A. Petasis, V.K. Kuchroo, C.N. Serhan, B.D. Levy, Cutting edge: maresin-1 engages regulatory T cells to limit type 2 innate lymphoid cell activation and promote resolution of lung inflammation, J Immunol 194(3) (2015) 863-7.

[101] J.C. Davies, A.M. Ebdon, C. Orchard, Recent advances in the management of cystic fibrosis, Arch Dis Child 99(11) (2014) 1033-6.

[102] S. Kumar, A. Tana, A. Shankar, Cystic fibrosis--what are the prospects for a cure?, Eur J Intern Med 25(9) (2014) 803-7.

[103] G.R. Cutting, Cystic fibrosis genetics: from molecular understanding to clinical application, Nat Rev Genet 16(1) (2015) 45-56.

[104] Correlation between genotype and phenotype in patients with cystic fibrosis. The Cystic Fibrosis Genotype-Phenotype Consortium, N Engl J Med 329(18) (1993) 1308-13.

[105] N. Bensalem, A.P. Ventura, B. Vallée, J. Lipecka, D. Tondelier, N. Davezac, A. Dos Santos, M. Perretti, A. Fajac, I. Sermet-Gaudelus, M. Renouil, J.F. Lesure, F. Halgand, O. Laprévote, A. Edelman, Down-regulation of the anti-inflammatory protein annexin A1 in cystic fibrosis knock-out mice and patients, Mol Cell Proteomics 4(10) (2005) 1591-601.

[106] J. Dalli, G. Rosignoli, R.P. Hayhoe, A. Edelman, M. Perretti, CFTR inhibition provokes an inflammatory response associated with an imbalance of the annexin A1 pathway, Am J Pathol 177(1) (2010) 176-86.

[107] F.H. Tsao, K.C. Meyer, X. Chen, N.S. Rosenthal, J. Hu, Degradation of annexin I in bronchoalveolar lavage fluid from patients with cystic fibrosis, Am J Respir Cell Mol Biol 18(1) (1998) 120-8.

[108] A.M. Gifford, J.D. Chalmers, The role of neutrophils in cystic fibrosis, Curr Opin Hematol 21(1) (2014) 16-22.

[109] C. Haslett, Granulocyte apoptosis and its role in the resolution and control of lung inflammation, Am J Respir Crit Care Med 160(5 Pt 2) (1999) S5-11.

[110] D. Mattoscio, V. Evangelista, R. De Cristofaro, A. Recchiuti, A. Pandolfi, S. Di Silvestre, S. Manarini, N. Martelli, B. Rocca, G. Petrucci, D.F. Angelini, L. Battistini, I. Robuffo, T. Pensabene, L. Pieroni, M.L. Furnari, F. Pardo, S. Quattrucci, S. Lancellotti, G. Davì, M. Romano, Cystic fibrosis transmembrane conductance

regulator (CFTR) expression in human platelets: impact on mediators and mechanisms of the inflammatory response, FASEB J 24(10) (2010) 3970-80.

[111] F.C. Ringholz, P.J. Buchanan, D.T. Clarke, R.G. Millar, M. McDermott, B. Linnane, B.J. Harvey, P. McNally, V. Urbach, Reduced 15-lipoxygenase 2 and lipoxin A4/leukotriene B4 ratio in children with cystic fibrosis, Eur Respir J 44(2) (2014) 394-404.

[112] C.L. Karp, L.M. Flick, K.W. Park, S. Softic, T.M. Greer, R. Keledjian, R. Yang, J. Uddin, W.B. Guggino, S.F. Atabani, Y. Belkaid, Y. Xu, J.A. Whitsett, F.J. Accurso, M. Wills-Karp, N.A. Petasis, Defective lipoxin-mediated anti-inflammatory activity in the cystic fibrosis airway, Nat Immunol 5(4) (2004) 388-92.

[113] V. Urbach, G. Higgins, P. Buchanan, F. Ringholz, The role of Lipoxin A4 in Cystic Fibrosis Lung Disease, Comput Struct Biotechnol J 6 (2013) e201303018.

[114] J. Yang, J.P. Eiserich, C.E. Cross, B.M. Morrissey, B.D. Hammock, Metabolomic profiling of regulatory lipid mediators in sputum from adult cystic fibrosis patients, Free Radic Biol Med 53(1) (2012) 160-71.

[115] V.J. De-Paula, M. Radanovic, B.S. Diniz, O.V. Forlenza, Alzheimer's disease, Subcell Biochem 65 (2012) 329-52.

[116] E. Solito, M. Sastre, Microglia function in Alzheimer's disease, Front Pharmacol 3 (2012) 14.

[117] M.T. Heneka, M.J. Carson, J.E. Khoury, G.E. Landreth, F. Brosseron, D.L. Feinstein, A.H. Jacobs, T. Wyss-Coray, J. Vitorica, R.M. Ransohoff, K. Herrup, S.A. Frautschy, B. Finsen, G.C. Brown, A. Verkhratsky, K. Yamanaka, J. Koistinaho, E. Latz, A. Halle, G.C. Petzold, T. Town, D. Morgan, M.L. Shinohara, V.H. Perry, C. Holmes, N.G. Bazan, D.J. Brooks, S. Hunot, B. Joseph, N. Deigendesch, O. Garaschuk, E. Boddeke, C.A. Dinarello, J.C. Breitner, G.M. Cole, D.T. Golenbock,

M.P. Kummer, Neuroinflammation in Alzheimer's disease, Lancet Neurol 14(4) (2015) 388-405.

[118] Y.H. Lee, G.G. Song, Genome-wide pathway analysis of a genome-wide association study on Alzheimer's disease, Neurol Sci 36(1) (2015) 53-9.

[119] E.M. Castaño, C.L. Maarouf, T. Wu, M.C. Leal, C.M. Whiteside, L.F. Lue, T.A. Kokjohn, M.N. Sabbagh, T.G. Beach, A.E. Roher, Alzheimer disease periventricular white matter lesions exhibit specific proteomic profile alterations, Neurochem Int 62(2) (2013) 145-56.

[120] S. McArthur, E. Cristante, M. Paterno, H. Christian, F. Roncaroli, G.E. Gillies, E. Solito, Annexin A1: a central player in the anti-inflammatory and neuroprotective role of microglia, J Immunol 185(10) (2010) 6317-28.

[121] X. Wang, M. Zhu, E. Hjorth, V. Cortés-Toro, H. Eyjolfsdottir, C. Graff, I. Nennesmo, J. Palmblad, M. Eriksdotter, K. Sambamurti, J.M. Fitzgerald, C.N. Serhan, A.C. Granholm, M. Schultzberg, Resolution of inflammation is altered in Alzheimer's disease, Alzheimers Dement 11(1) (2015) 40-50.e1-2.

[122] M.T. Mizwicki, G. Liu, M. Fiala, L. Magpantay, J. Sayre, A. Siani, M. Mahanian, R. Weitzman, E.Y. Hayden, M.J. Rosenthal, I. Nemere, J. Ringman, D.B. Teplow, 1α,25-dihydroxyvitamin D3 and resolvin D1 retune the balance between amyloid-β phagocytosis and inflammation in Alzheimer's disease patients, J Alzheimers Dis 34(1) (2013) 155-70.

[123] J.F. Maddox, C.N. Serhan, Lipoxin A4 and B4 are potent stimuli for human monocyte migration and adhesion: selective inactivation by dehydrogenation and reduction, J Exp Med 183(1) (1996) 137-46.

[124] J. Yan, Q. Fu, L. Cheng, M. Zhai, W. Wu, L. Huang, G. Du, Inflammatory response in Parkinson's disease (Review), Mol Med Rep 10(5) (2014) 2223-33.

[125] C. Barcia, A. Sánchez Bahillo, E. Fernández-Villalba, V. Bautista, M. Poza Y Poza, A. Fernández-Barreiro, E.C. Hirsch, M.T. Herrero, Evidence of active microglia in substantia nigra pars compacta of parkinsonian monkeys 1 year after MPTP exposure, Glia 46(4) (2004) 402-9.

[126] K. Nakajima, S. Kohsaka, Microglia: activation and their significance in the central nervous system, J Biochem 130(2) (2001) 169-75.

[127] Z.Z. Luo, Y. Gao, N. Sun, Y. Zhao, J. Wang, B. Tian, J. Shi, Enhancing the interaction between annexin-1 and formyl peptide receptors regulates microglial activation to protect neurons from ischemia-like injury, J Neuroimmunol 276(1-2) (2014) 24-36.

[128] C. Knott, G. Stern, G.P. Wilkin, Inflammatory regulators in Parkinson's disease: iNOS, lipocortin-1, and cyclooxygenases-1 and -2, Mol Cell Neurosci 16(6) (2000) 724-39.

[129] M.O. Trépanier, K.E. Hopperton, S.K. Orr, R.P. Bazinet, N-3 polyunsaturated fatty acids in animal models with neuroinflammation: An update, Eur J Pharmacol (2015).

[130] G. Hacioglu, Y. Seval-Celik, G. Tanriover, O. Ozsoy, E. Saka-Topcuoglu, S. Balkan, A. Agar, Docosahexaenoic acid provides protective mechanism in bilaterally MPTP-lesioned rat model of Parkinson's disease, Folia Histochem Cytobiol 50(2) (2012) 228-38.

[131] Y. Tian, Y. Zhang, R. Zhang, S. Qiao, J. Fan, Resolvin D2 recovers neural injury by suppressing inflammatory mediators expression in lipopolysaccharide-induced Parkinson's disease rat model, Biochem Biophys Res Commun 460(3) (2015) 799-805.

[132] M. Fakhoury, Autistic spectrum disorders: A review of clinical features, theories and diagnosis, Int J Dev Neurosci (2015).

[133] S. Bhat, U.R. Acharya, H. Adeli, G.M. Bairy, A. Adeli, Autism: cause factors, early diagnosis and therapies, Rev Neurosci 25(6) (2014) 841-50.

[134] A. Masi, D.S. Quintana, N. Glozier, A.R. Lloyd, I.B. Hickie, A.J. Guastella, Cytokine aberrations in autism spectrum disorder: a systematic review and meta-analysis, Mol Psychiatry 20(4) (2015) 440-6.

[135] C.L. Yan, J. Zhang, Y. Hou, Decreased plasma levels of lipoxin A4 in children with autism spectrum disorders, Neuroreport 26(6) (2015) 341-5.

[136] C.T. Correia, I.C. Conceição, B. Oliveira, J. Coelho, I. Sousa, A.F. Sequeira, J. Almeida, C. Café, F. Duque, S. Mouga, W. Roberts, K. Gao, J.K. Lowe, B. Thiruvahindrapuram, S. Walker, C.R. Marshall, D. Pinto, J.I. Nurnberger, S.W. Scherer, D.H. Geschwind, G. Oliveira, A.M. Vicente, Recurrent duplications of the annexin A1 gene (ANXA1) in autism spectrum disorders, Mol Autism 5(1) (2014) 28.

[137] D.Q. Shih, S.R. Targan, Insights into IBD Pathogenesis, Curr Gastroenterol Rep 11(6) (2009) 473-80.

[138] A. Bousvaros, D.A. Antonioli, R.B. Colletti, M.C. Dubinsky, J.N. Glickman, B.D. Gold, A.M. Griffiths, G.P. Jevon, L.M. Higuchi, J.S. Hyams, B.S. Kirschner, S. Kugathasan, R.N. Baldassano, P.A. Russo, H.p. North American Society for Pediatric Gastroenterology, and Nutrition, C.F.o. America, Differentiating ulcerative colitis from Crohn disease in children and young adults: report of a working group of the North American Society for Pediatric Gastroenterology, Hepatology, and Nutrition and the Crohn's and Colitis Foundation of America, J Pediatr Gastroenterol Nutr 44(5) (2007) 653-74.

[139] A. Sena, I. Grishina, A. Thai, L. Goulart, M. Macal, A. Fenton, J. Li, T. Prindiville, S.M. Oliani, S. Dandekar, S. Sankaran-Walters, Dysregulation of anti-inflammatory annexin A1 expression in progressive crohns disease, PLoS One 8(10) (2013) e76969.

[140] N. Ouyang, C. Zhu, D. Zhou, T. Nie, M.F. Go, R.J. Richards, B. Rigas, MC-12, an annexin A1-based peptide, is effective in the treatment of experimental colitis, PLoS One 7(7) (2012) e41585.

[141] N. Vergnolle, P. Pagès, R. Guimbaud, S. Chaussade, L. Buéno, J. Escourrou, C. Coméra, Annexin 1 is secreted in situ during ulcerative colitis in humans, Inflamm Bowel Dis 10(5) (2004) 584-92.

[142] L. Vong, J.G. Ferraz, N. Dufton, R. Panaccione, P.L. Beck, P.M. Sherman, M. Perretti, J.L. Wallace, Up-regulation of Annexin-A1 and lipoxin A(4) in individuals with ulcerative colitis may promote mucosal homeostasis, PLoS One 7(6) (2012) e39244.

[143] N. Vergnolle, C. Coméra, L. Buéno, Annexin 1 is overexpressed and specifically secreted during experimentally induced colitis in rats, Eur J Biochem 232(2) (1995) 603-10.

[144] C. Coméra, P. Brousset, J. Moré, N. Vergnolle, L. Buéno, Inflammatory neutrophils secrete annexin 1 during experimentally induced colitis in rats, Dig Dis Sci 44(7) (1999) 1448-57.

[145] T.R. Stevens, S.F. Smith, D.S. Rampton, Antibodies to human recombinant lipocortin-I in inflammatory bowel disease, Clin Sci (Lond) 84(4) (1993) 381-6.

[146] N.R. O'Shea, A.M. Smith, Matrix metalloproteases role in bowel inflammation and inflammatory bowel disease: an up to date review, Inflamm Bowel Dis 20(12) (2014) 2379-93.

[147] J. Dalli, L.V. Norling, D. Renshaw, D. Cooper, K.Y. Leung, M. Perretti, Annexin 1 mediates the rapid anti-inflammatory effects of neutrophil-derived microparticles, Blood 112(6) (2008) 2512-9.

[148] J. Dalli, C.N. Serhan, Specific lipid mediator signatures of human phagocytes: microparticles stimulate macrophage efferocytosis and pro-resolving mediators, Blood 120(15) (2012) e60-72.

[149] G. Leoni, P.A. Neumann, N. Kamaly, M. Quiros, H. Nishio, H.R. Jones, R. Sumagin, R.S. Hilgarth, A. Alam, G. Fredman, I. Argyris, E. Rijcken, D. Kusters, C. Reutelingsperger, M. Perretti, C.A. Parkos, O.C. Farokhzad, A.S. Neish, A. Nusrat, Annexin A1-containing extracellular vesicles and polymeric nanoparticles promote epithelial wound repair, J Clin Invest 125(3) (2015) 1215-27.

[150] E.R. Ağış, B. Savaş, M. Melli, Impact of colonic mucosal lipoxin A4 synthesis capacity on healing in rats with dextran sodium sulfate-induced colitis, Prostaglandins Other Lipid Mediat 121(Pt A) (2015) 63-9.

[151] T. Köhnke, B. Gomolka, S. Bilal, X. Zhou, Y. Sun, M. Rothe, D.C. Baumgart, K.H. Weylandt, Acetylsalicylic Acid reduces the severity of dextran sodium sulfate-induced colitis and increases the formation of anti-inflammatory lipid mediators, Biomed Res Int 2013 (2013) 748160.

[152] A.T. Gewirtz, L.S. Collier-Hyams, A.N. Young, T. Kucharzik, W.J. Guilford, J.F. Parkinson, I.R. Williams, A.S. Neish, J.L. Madara, Lipoxin a4 analogs attenuate induction of intestinal epithelial proinflammatory gene expression and reduce the severity of dextran sodium sulfate-induced colitis, J Immunol 168(10) (2002) 5260-7.

[153] A.F. Bento, R.F. Claudino, R.C. Dutra, R. Marcon, J.B. Calixto, Omega-3 fatty acid-derived mediators 17(R)-hydroxy docosahexaenoic acid, aspirin-triggered

resolvin D1 and resolvin D2 prevent experimental colitis in mice, J Immunol 187(4) (2011) 1957-69.

[154] T. Chaiworapongsa, P. Chaemsaithong, L. Yeo, R. Romero, Pre-eclampsia part 1: current understanding of its pathophysiology, Nat Rev Nephrol 10(8) (2014) 466-80.

[155] C.W. Redman, G.P. Sacks, I.L. Sargent, Preeclampsia: an excessive maternal inflammatory response to pregnancy, Am J Obstet Gynecol 180(2 Pt 1) (1999) 499-506.

[156] D.L. Schminkey, M. Groer, Imitating a stress response: a new hypothesis about the innate immune system's role in pregnancy, Med Hypotheses 82(6) (2014) 721-9.

[157] L.O. Perucci, F.S. Carneiro, C.N. Ferreira, M.A. Sugimoto, F.M. Soriani, G.G. Martins, K.M. Lima, F.L. Guimarães, A.L. Teixeira, L.M. Dusse, K.B. Gomes, L.P. Sousa, Annexin A1 Is Increased in the Plasma of Preeclamptic Women, PLoS One 10(9) (2015) e0138475.

[158] G.F. Behrouz, G.S. Farzaneh, J. Leila, Z. Jaleh, K.S. Eskandar, Presence of auto-antibody against two placental proteins, annexin A1 and vitamin D binding protein, in sera of women with pre-eclampsia, J Reprod Immunol 99(1-2) (2013) 10-6.

[159] L.O. Perucci, P.C. Santos, L.S. Ribeiro, D.G. Souza, K.B. Gomes, L.M. Dusse, L.P. Sousa, Lipoxin A4 Is Increased in the Plasma of Preeclamptic Women, Am J Hypertens (2016).

[160] W. Dong, L. Yin, Expression of lipoxin A4, TNFα and IL-1β in maternal peripheral blood, umbilical cord blood and placenta, and their significance in pre-eclampsia, Hypertens Pregnancy 33(4) (2014) 449-56.

[161] L.L. Huang, S. Su, R. Awale, X.Y. Zhang, L.L. Zhong, H. Tang, Expression of anti-inflammatory mediator lipoxin A4 and inflammation responsive transcriptive

factors NF-kappa B in patients with preeclampsia, Clin Exp Obstet Gynecol 41(5) (2014) 561-6.

[162] Z. Xu, F. Zhao, F. Lin, H. Xiang, N. Wang, D. Ye, Y. Huang, Preeclampsia is associated with a deficiency of lipoxin A4, an endogenous anti-inflammatory mediator, Fertil Steril 102(1) (2014) 282-290.e4.

[163] S.N. Cooray, T. Gobbetti, T. Montero-Melendez, S. McArthur, D. Thompson, A.J. Clark, R.J. Flower, M. Perretti, Ligand-specific conformational change of the G-protein-coupled receptor ALX/FPR2 determines proresolving functional responses, Proc Natl Acad Sci U S A 110(45) (2013) 18232-7.

[164] F. Simiele, A. Recchiuti, D. Mattoscio, A. De Luca, E. Cianci, S. Franchi, V. Gatta, A. Parolari, J.P. Werba, M. Camera, B. Favaloro, M. Romano, Transcriptional regulation of the human FPR2/ALX gene: evidence of a heritable genetic variant that impairs promoter activity, FASEB J 26(3) (2012) 1323-33.

[165] R.H. Salama, M.M. Fathalla, A.R. Mekki, B.l.-K. Elsadek, Implication of umbilical cord in preeclampsia, Med Princ Pract 20(2) (2011) 124-8.

5 DISCUSSÃO INTEGRADORA DOS RESULTADOS

5.1 Características clínicas dos grupos avaliados

Ao analisarmos as características clínicas de todas as participantes do estudo incluídas na tese, não foi observada diferença significativa em relação à idade, ao IMC antes da gestação e à idade gestacional no momento da coleta de sangue, comparando-se os três grupos de participantes.

O ganho de peso gestacional foi maior nas gestantes com PE em comparação às gestantes normotensas (P=0,009). As gestantes com PE geralmente têm um maior ganho de peso ao longo da gestação devido à retenção hídrica, a qual pode ser evidenciada pelo edema. Apesar de grande parte das gestantes com PE apresentar edema, esse parâmetro foi retirado dos critérios diagnósticos da doença devido ao seu caráter inespecífico (Davison, 1997; *Report of the National High Blood Pressure Education Program Working Group on High Blood Pressure in Pregnancy*, 2000).

Houve maior frequência de primigestas no grupo de gestantes com PE em relação ao de gestantes normotensas (P=0,042). Sabe-se que a PE é mais comum na primeira gestação. As gestantes que já tiveram filhos e que trocam de parceiros também têm uma maior probabilidade de desenvolverem a doença (Trogstad *et al.*, 2011). Para que uma gestação seja bem-sucedida, o sistema imunológico materno deve tolerar os aloantígenos paternos expressos na placenta. A adaptação do sistema imunológico materno parece não ser tão efetiva na primeira gestação ou quando há troca do parceiro, devido a um menor tempo de exposição aos antígenos paternos presentes no esperma, condição que poderia predispor à PE (Redman e Sargent, 2010).

Como esperado, as medidas de pressão arterial sistólica e diastólica foram superiores nas gestantes com PE em relação às gestantes normotensas (P<0,001), e nas gestantes com PE quando comparado às mulheres não gestantes (P<0,001). Além disso, as gestantes normotensas apresentaram pressões arteriais sistólica e diastólica inferiores em comparação às mulheres não gestantes (P=0,006 e P=0,020, respectivamente), resultado que está em concordância com estudos anteriores, os quais mostram que, fisiologicamente, a pressão arterial diminui até a

metade da gestação e retorna aos valores anteriores ao da gestação no período pós-termo (Macdonald-Wallis *et al.*, 2015; Macgillivray *et al.*, 1969). A redução dos níveis pressóricos durante a gestação pode decorrer da diminuição da resistência vascular sistêmica. Consequentemente há um aumento do fluxo sanguíneo, evento importante para o desenvolvimento da gestação, e da filtração glomerular (Delascio e El-Kadre, 1983).

A terapia anti-hipertensiva foi administrada a 41 (77%) das gestantes com PE, sendo que 19 (46%) destas fizeram uso de mais de um anti-hipertensivo simultaneamente. Os anti-hipertensivos administrados foram nifedipina, metildopa e hidralazina. Além disso, 18 (34%) das gestantes com PE utilizaram sulfato de magnésio para prevenir a ocorrência de convulsões e 22 (42%) utilizaram corticosteróide (betametasona ou dexametasona) para induzir o amadurecimento pulmonar do feto.

5.2 Avaliação dos níveis circulantes de AnxA1, PCR, LXA4 e BDNF

No presente estudo, os níveis de PCR estavam aumentados nas gestantes normotensas, em comparação às mulheres não gestantes, e níveis ainda maiores foram obtidos nas gestantes com PE. Ressalta-se que em um estudo anterior do nosso grupo de pesquisa, foram avaliados os níveis plasmáticos de TNF-R1, um marcador indireto da liberação de TNF-α, e as diferenças entre os grupos foi idêntica à obtida para PCR no presente estudo, corroborando com a premissa de inflamação branda na gestação normotensa e exacerbada na PE (Lau *et al.*, 2013; Perucci *et al.*, 2014; Redman *et al.*, 1999). Esses dados estão em concordância com a teoria inicialmente proposta por Redman, Sacks e Sargent de que a PE não é uma condição clínica distinta, mas o extremo de um *continuum* de respostas inflamatórias sistêmicas causadas pela própria gestação (Redman *et al.*, 1999). Além disso, sabe-se que resposta inflamatória é crescente ao longo da gestação (Borzychowski *et al.*, 2006). No presente estudo, de fato, os níveis de PCR foram superiores nas gestantes normotensas com IG≥34 semanas do que nas gestantes normotensas com IG<34 semanas. Considerando que a resposta inflamatória é controlada nas gestantes normotensas, e exacerbada nas gestantes com PE, é possível inferir que

os mecanismos de regulação da resposta inflamatória não estejam funcionando adequadamente nestas.

As gestantes com PE apresentaram níveis plasmáticos aumentados de AnxA1 (somente PE precoce) e LXA4 (PE precoce e tardia) em comparação às gestantes normotensas pareadas de acordo com a IG, no presente estudo. Os níveis elevados desses dois mediadores anti-inflamatórios e pró-resolutivos coincidiram com um fenótipo pró-inflamatório, como demonstrado pela correlação positiva entre os níveis de AnxA1 e sTNF-R1, LXA4 e PCR, e LXA4 e contagem global de leucócitos. Por outro lado, as concentrações plasmáticas de AnxA1 e LXA4 foram similares entre as gestantes normotensas e as mulheres não gestantes. Adicionalmente, não foram encontradas diferenças significativas em relação aos níveis de mRNA de AnxA1 em PBMCs de gestantes com PE, gestantes normotensas e mulheres não-gestantes, provavelmente devido ao pequeno tamanho amostral dos grupos. Esses dados sugerem que o aumento dos níveis circulantes de AnxA1 e de LXA4 nas gestantes com PE constitui uma tentativa de controlar a resposta inflamatória exacerbada. Nesse raciocínio, a não obtenção de diferença dos níveis de AnxA1 e a LXA4 entre gestantes normotensas e mulheres não gestantes sugere que os mecanismos contrarreguladores da resposta inflamatória na gestação normotensa estão funcionando adequadamente, não havendo, desta forma, um estímulo para a produção aumentada de AnxA1 e LXA4.

Apesar dos níveis plasmáticos de AnxA1 e LXA4 estarem aumentados na PE, os mesmos não parecem ser suficientes para controlar a inflamação sistêmica. Uma provável explicação é a de que os mediadores pró-inflamatórios estão em uma concentração superior em relação aos mediadores anti-inflamatórios/pró-resolutivos, como a AnxA1 e a LXA4, de modo que a elevação destes não seja suficiente para controlar a inflamação. Resultados não publicados deste estudo sugerem que a razão PCR/AnxA1 foi maior nas gestantes com PE quando comparado às mulheres não gestantes ($P<0,001$). No entanto, não foi observada diferença estatística entre gestantes com PE e gestantes normotensas. Além disso, a razão PCR/AnxA1 foi maior nas gestantes normotensas em comparação às mulheres não gestantes ($P<0,001$). Também foi feita a análise da razão PCR/LXA4, porém não foi observada diferença estatística entre os três grupos após a correção de Bonferroni (dados não publicados). Adicionalmente, a razão sTNR-R1/AnxA1 foi maior nas gestantes normotensas em comparação às mulheres não gestantes ($P=0,009$). Não foram

detectadas diferenças significativas para esse parâmetro entre gestantes normotensas e gestantes com PE, e entre gestantes com PE e mulheres não gestantes (dados não publicados).

Falhas nas ações dos mediadores anti-inflamatórios/pró-resolutivos também podem contribuir para um controle inadequado na resposta inflamatória na PE. Isso pode ocorrer devido à inativação enzimática dessas moléculas, por exemplo, por proteases que são comuns em ambientes inflamatórios, e pela expressão diminuída ou alterações conformacionais dos seus receptores, como a falha na dimerização do FPR2 (Maddox e Serhan, 1996; Rescher *et al.*, 2006; Vong *et al.*, 2007). Corroborando com esta hipótese, dois estudos relataram menor expressão de FPR2 na placenta de gestantes com PE em comparação às gestantes normotensas. No entanto, a expressão diminuída de FPR2 também já foi descrita na PE (Dong e Yin, 2014; Huang *et al.*, 2014; Xu *et al.*, 2014).

Outra possível causa que favoreceria o estado inflamatório na PE seria a produção de auto-anticorpos direcionados contra os mediadores anti-inflamatórios/pró-resolutivos. Behrouz e colaboradores identificaram a presença de auto-anticorpos anti-AnxA1 no soro de gestantes com PE os quais poderiam ser direcionados contra AnxA1 expressa na placenta (Behrouz *et al.*, 2013). Esses mecanismos de falha da ação da AnxA1 e da LXA4 devem ser melhor investigados no contexto da PE. Cumpre ressaltar que outras moléculas com atividade anti-inflamatórias e pró-resolutivas têm seus níveis alterados em gestantes com PE, como discutido no **Capítulo 4**, e podem contribuir para o controle inadequado da resposta inflamatória nessa doença.

No presente estudo, foram obtidos níveis plasmáticos diminuídos de BDNF em gestantes com PE, quando comparado às gestantes normotensas. No entanto, não houve diferença significativa entre gestantes com PE precoce (n=17) e gestantes com IG<34 semanas (n=21), e entre gestantes com PE tardia (n=8) e gestantes normotensas com IG≥34 semanas (n=12). O pequeno tamanho amostral nesses subgrupos poderia justificar a não obtenção de diferença. Estudos *in vivo* mostram que moléculas pró-inflamatórias, como o TNF-α e a IL-1β, são capazes de inibir a expressão de BDNF em células neuronais e não-neuronais (Lapchak *et al.*, 1993; Xu *et al.*, 2015). Dessa forma, é plausível inferir que os níveis aumentados de mediadores pró-inflamatórios modulam de forma negativa a expressão de BDNF na PE. De forma recíproca, concentrações diminuídas de BDNF podem contribuir para

a exacerbação da resposta inflamatória na PE, uma vez essa neurotrofina apresenta atividades anti-inflamatórias e pró-resolutivas *in vivo* e *in vitro* (Ji *et al.*, 2015; Matsuda *et al.*, 2015; Takeda *et al.*, 2016). Foi observada uma correlação negativa entre os níveis plasmáticos de BDNF e AnxA1 na PE, o que permite inferir que a AnxA1 esteja aumentada em gestantes com PE que têm níveis diminuídos de BDNF como um mecanismo compensador, visando atenuar a resposta inflamatória sistêmica nessas gestantes.

Os níveis plasmáticos de LXA4 mostraram correlação positiva com a pressão arterial sistólica e diastólica somente quando todas as participantes foram incluídas nas análises, enquanto os níveis de BDNF se correlacionaram positivamente com a pressão arterial diastólica em gestantes com PE. Esses resultados sugerem um papel dessas moléculas no controle da pressão arterial. De fato, estudos experimentais indicam uma ação protetora vascular da LXA4 (Nascimento-Silva *et al.*, 2007; Paul-Clark *et al.*, 2004), o que permite inferir que os níveis aumentados de LXA4 poderiam exercer um efeito protetor para o endotélio nas gestantes com pressão arterial elevada. Por outro lado, Erdos e colaboradores demonstraram que o tratamento com BDNF induziu um aumento dos níveis pressóricos (Erdos *et al.*, 2015), e já foi descrito que níveis elevados de BDNF precedem o desenvolvimento de hipertensão *in vivo* (Amoureux *et al.*, 2012). De fato, as gestantes com PE que apresentaram níveis elevados de BDNF também apresentaram pressão arterial diastólica aumentada.

Uma correlação positiva entre os níveis plasmáticos de BDNF e o IMC antes da gestação foi obtida para as gestantes com PE, o que está de acordo com o papel do BDNF na regulação do metabolismo energético proposto em estudos anteriores (Lebrun *et al.*, 2006; Marosi e Mattson, 2014).

A comparação dos níveis plasmáticos de AnxA1, PCR, LXA4 e BDNF entre as gestantes com PE precoce e PE tardia não revelou diferenças significativas, sugerindo que essas moléculas não seriam úteis para distinguir essas formas clínicas da doença. No entanto, cumpre ressaltar que as determinações AnxA1, PCR, LXA4 e BDNF foram feitas em um momento tardio da gestação (terceiro trimestre). Pode ser que os níveis de AnxA1, PCR, LXA4 e BDNF fossem diferentes no primeiro e/ou segundo trimestres de gestação entre gestantes que desenvolveram as formas precoce e tardia, mas se igualaram no final da gestação, quando a resposta inflamatória se mostra mais intensa. Corroborando com essa

hipótese, Cheng e colaboradores encontraram níveis aumentados de PCR no primeiro trimestre de gestação em gestantes que desenvolveram PE precoce em comparação às gestantes que desenvolveram a forma tardia da doença (Cheng *et al.*, 2016). No entanto, Balcı Ekmekçi e colaboradores revelaram que os níveis de PCR foram similares entre as gestantes com PE precoce e tardia no terceiro trimestre de gestação (Balcı Ekmekçi *et al.*, 2015). D'Souza e colaboradores encontraram níveis diminuídos de BDNF em gestantes com PE entre 16 e 20 semanas de gestação, mas esse estudo não diferenciou as gestantes com as formas precoce e tardia (D'souza *et al.*, 2014). Os dados obtidos por Cheng e colaboradores e por D'Souza e colaboradores também sugerem que alterações nos níveis de CRP e o BDNF podem ter um papel na etiologia da PE. Não foram encontrados estudos que avaliaram os níveis circulantes de AnxA1 e LXA4 em gestantes com PE de forma prospectiva.

Cumpre ressaltar o caráter inédito do presente estudo, ao avaliar os níveis plasmáticos de AnxA1 na PE, a associação entre a LXA4 e as características clínicas/parâmetros laboratoriais dessas gestantes e a associação entre os níveis de BDNF, sTNF-R1 e AnxA1 na PE.

5.3 Limitações do estudo

Não se pode descartar a influência da etnia nos níveis plasmáticos de AnxA1, PCR, LXA4 e BDNF neste estudo. A etnia das participantes não foi avaliada devido à elevada miscigenação da população brasileira. A dieta também pode ter influenciado os níveis de moléculas inflamatórias. No entanto, essa variável não foi controlada por não fazer parte do protocolo inicial da pesquisa. Em adição, dados acurados sobre tabagismo, alcoolismo e prática de atividade física entre as participantes do estudo não foram obtidos, o que impossibilitou a avaliação adequada de possíveis interferências nos níveis dos marcadores estudados. Além disso, não foi possível excluir pacientes em uso de medicamentos que poderiam potencialmente interferir nas análises, considerando que a politerapia é comum em pacientes com PE.

O desenho experimental do estudo não permitiu distinguir se as alterações nos níveis das moléculas avaliadas antecedem o desenvolvimento da PE

ou são consequências da doença. Estudos prospectivos devem ser desenvolvidos nesse sentido. Além disso, como as participantes não foram acompanhadas até o final da gestação, não é possível descartar a possibilidade de que algumas gestantes que foram classificadas como normotensas, principalmente àquelas com IG<34 semanas, tenham desenvolvido PE posteriormente.

Por fim, não foi possível realizar uma análise de regressão logística multivariada incluindo os níveis de AnxA1, PCR, LXA4 e BDNF, pois nem todas as moléculas foram dosadas em todas as participantes do estudo.

6 CONCLUSÕES

Os resultados deste estudo sugerem que os níveis plasmáticos de AnxA1, PCR e LXA4 estão aumentados, e os de BDNF estão diminuídos em gestantes com PE, e que níveis alterados ou a dificuldade de sinalização dessas moléculas inflamatórias podem contribuir para a patogênese da doença, como exemplificado na **Figura 4**.

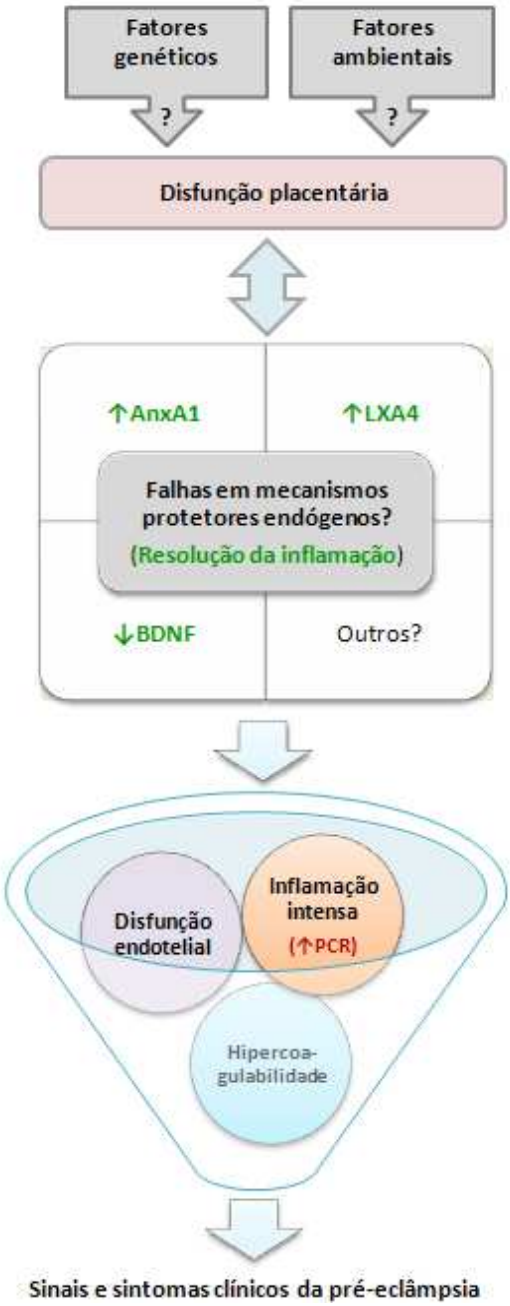


FIGURA 4 - Resumo dos resultados do presente estudo integrado ao modelo proposto da fisiopatologia da pré-eclâmpsia.

REFERÊNCIAS BIBLIOGRÁFICAS

ABBAS, A. K.; LICHTMAN, A. H.; PILLAI, S. **Cellular and molecular immunology**. Updated 6. ed. Philadelphia: Saunders/Elsevier, 2010.

ALESSANDRI, A. L. *et al.* Resolution of inflammation: mechanisms and opportunity for drug development. **Pharmacol Ther,** v. 139, n. 2, p. 189-212, ago. 2013.

ALIBERTI, J.; SERHAN, C.; SHER, A. Parasite-induced lipoxin A4 is an endogenous regulator of IL-12 production and immunopathology in Toxoplasma gondii infection. **J Exp Med,** v. 196, n. 9, p. 1253-62, nov. 2002.

ALPOIM, P. N. *et al.* **Pré-eclâmpsia: o que há de anômalo na placentação?** Preeclampsia: what is wrong with placentation?: Femina. v.41, n. 2, p. 99-105, mar./abr. 2013.

AMERICAN COLLEGE OF OBSTETRICIANS AND GYNECOLOGISTS. ACOG practice bulletin on diagnosing and managing preeclampsia and eclampsia. American College of Obstetricians and Gynecologists. **Am Fam Physician,** v. 66, n. 2, p. 330-1, jul. 2002.

AMERICAN COLLEGE OF OBSTETRICIANS AND GYNECOLOGISTS. Hypertension in pregnancy. Report of the American College of Obstetricians and Gynecologists' Task Force on Hypertension in Pregnancy. **Obstet Gynecol,** v. 122, n. 5, p. 1122-31, nov. 2013.

AMOUREUX, S. *et al.* Vascular BDNF expression and oxidative stress during aging and the development of chronic hypertension. **Fundam Clin Pharmacol,** v. 26, n. 2, p. 227-34, abr. 2012.

ANSAR, W.; GHOSH, S. C-reactive protein and the biology of disease. **Immunol Res,** v. 56, n. 1, p. 131-42, maio 2013.

ARIEL, A. *et al.* Aspirin-triggered lipoxin A4 and B4 analogs block extracellular signal-regulated kinase-dependent TNF-alpha secretion from human T cells. **J Immunol,** v. 170, n. 12, p. 6266-72, jun. 2003.

BALCı EKMEKÇI, Ö. *et al.* Evaluation of Lp-PLA2 mass, vitronectin and PAI-1 activity levels in patients with preeclampsia. **Arch Gynecol Obstet,** v. 292, n. 1, p. 53-8, jul. 2015.

BEHROUZ, G. F. *et al.* Presence of auto-antibody against two placental proteins, annexin A1 and vitamin D binding protein, in sera of women with pre-eclampsia. **J Reprod Immunol,** v. 99, n. 1-2, p. 10-6, set. 2013.

BELO, L. *et al.* Fluctuations in C-reactive protein concentration and neutrophil activation during normal human pregnancy. **Eur J Obstet Gynecol Reprod Biol,** v. 123, n. 1, p. 46-51, nov. 2005.

BESSER, M.; WANK, R. Cutting edge: clonally restricted production of the neurotrophins brain-derived neurotrophic factor and neurotrophin-3 mRNA by human immune cells and Th1/Th2-polarized expression of their receptors. **J Immunol,** v. 162, n. 11, p. 6303-6, jun. 1999.

BIENERTOVA-VASKU, J. *et al.* Brain-derived neurotrophic factor and ciliary neurotrophic factor in maternal plasma and umbilical cord blood from pre-eclamptic and physiological pregnancies. **J Obstet Gynaecol,** v. 33, n. 4, p. 359-63, maio 2013.

BORZYCHOWSKI, A. M.; SARGENT, I. L.; REDMAN, C. W. Inflammation and pre-eclampsia. **Semin Fetal Neonatal Med,** v. 11, n. 5, p. 309-16, out. 2006.

BROWN, M. C. *et al.* Cardiovascular disease risk in women with pre-eclampsia: systematic review and meta-analysis. **Eur J Epidemiol,** v. 28, n. 1, p. 1-19, jan. 2013.

CALDER, P. C. Omega-3 fatty acids and inflammatory processes. **Nutrients,** v. 2, n. 3, p. 355-74, mar. 2010.

CANZONERI, B. J. *et al.* Increased neutrophil numbers account for leukocytosis in women with preeclampsia. **Am J Perinatol,** v. 26, n. 10, p. 729-32, nov. 2009.

CHAIWORAPONGSA, T. *et al.* Pre-eclampsia part 1: current understanding of its pathophysiology. **Nat Rev Nephrol,** v. 10, n. 8, p. 466-80, ago. 2014.

CHAIWORAPONGSA, T. *et al.* Pre-eclampsia part 2: prediction, prevention and management. **Nat Rev Nephrol,** v. 10, n. 9, p. 531-40, set. 2014.

CHALLIS, J. R. *et al.* Inflammation and pregnancy. **Reprod Sci,** v. 16, n. 2, p. 206-15, fev. 2009.

CHIANG, N.; SERHAN, C. N. New mechanism for an old drug: aspirin triggers anti-inflammatory lipid mediators with gender implications. **Compr Ther,** v. 32, n. 3, p. 150-7, outono 2006.

CHRISTIAN, L. M. *et al.* Serum brain-derived neurotrophic factor (BDNF) across pregnancy and postpartum: Associations with race, depressive symptoms, and low birth weight. **Psychoneuroendocrinology,** v. 74, p. 69-76, ago. 2016.

CLYNE, B.; OLSHAKER, J. S. The C-reactive protein. **J Emerg Med,** v. 17, n. 6, p. 1019-25, nov./dez. 1999.

COSMI, L. *et al.* T helper cells plasticity in inflammation. **Cytometry A,** v. 85, n. 1, p. 36-42, jan. 2014.

COUTINHO, A. E.; CHAPMAN, K. E. The anti-inflammatory and immunosuppressive effects of glucocorticoids, recent developments and mechanistic insights. **Mol Cell Endocrinol,** v. 335, n. 1, p. 2-13, mar. 2011.

D'ACQUISTO, F. *et al.* Annexin-1 modulates T-cell activation and differentiation. **Blood,** v. 109, n. 3, p. 1095-102, fev. 2007.

D'SOUZA, V. A. *et al.* Differential regulation of brain-derived neurotrophic factor in term and preterm preeclampsia. **Reprod Sci,** v. 21, n. 2, p. 230-5, fev. 2014,a.

_____. Levels of brain derived neurotrophic factors across gestation in women with preeclampsia. **Int J Dev Neurosci,** v. 37, p. 36-40, out. 2014,b.

DAS, T. *et al.* Induction of glycosylation in human C-reactive protein under different pathological conditions. **Biochem J,** v. 373, n. Pt 2, p. 345-55, jul. 2003.

DAVISON, J. M. Edema in pregnancy. **Kidney Int Suppl,** v. 59, p. S90-6, jun. 1997.

DE OLIVEIRA, L. C. *et al.* Factors associated with maternal serum C-reactive protein throughout pregnancy: A longitudinal study in women of Rio de Janeiro, Brazil. **Nutrition,** v. 31, n. 9, p. 1103-8, set. 2015.

DELASCIO, D.; EL-KADRE, D. **Hipertensão na gravidez**. 2. ed. Sao Paulo: Savier, 1983. 169 p.

DERZSY, Z. *et al.* Activation of the complement system in normal pregnancy and preeclampsia. **Mol Immunol,** v. 47, n. 7-8, p. 1500-6, abr. 2010.

DONG, W.; YIN, L. Expression of lipoxin A4, TNFα and IL-1β in maternal peripheral blood, umbilical cord blood and placenta, and their significance in pre-eclampsia. **Hypertens Pregnancy,** v. 33, n. 4, p. 449-56, nov. 2014.

ERDOS, B. *et al.* Brain-derived neurotrophic factor modulates angiotensin signaling in the hypothalamus to increase blood pressure in rats. **Am J Physiol Heart Circ Physiol,** v. 308, n. 6, p. H612-22, mar. 2015.

ERNFORS, P. *et al.* Identification of cells in rat brain and peripheral tissues expressing mRNA for members of the nerve growth factor family. **Neuron,** v. 5, n. 4, p. 511-26, out. 1990.

ERNST, J. D. *et al.* Purification and characterization of an abundant cytosolic protein from human neutrophils that promotes Ca2(+)-dependent aggregation of isolated specific granules. **J Clin Invest,** v. 85, n. 4, p. 1065-71, abr. 1990.

ERNST, S. *et al.* An annexin 1 N-terminal peptide activates leukocytes by triggering different members of the formyl peptide receptor family. **J Immunol,** v. 172, n. 12, p. 7669-76, jun. 2004.

FARZADNIA, M. *et al.* A comparative study of vascular cell adhesion molecule-1 and high-sensitive C-reactive protein in normal and preeclamptic pregnancies. **Interv Med Appl Sci,** v. 5, n. 1, p. 26-30, mar. 2013.

FAVA, R. A.; MCKANNA, J.; COHEN, S. Lipocortin I (p35) is abundant in a restricted number of differentiated cell types in adult organs. **J Cell Physiol,** v. 141, n. 2, p. 284-93, nov. 1989.

FENG, Z. *et al.* Leukotriene B4 modulates in vivo expression of delayed-type hypersensitivity by a receptor-mediated mechanism: regulation by lipoxin A4. **J Pharmacol Exp Ther,** v. 278, n. 2, p. 950-6, ago. 1996.

FERNANDES, B. S. *et al.* Peripheral brain-derived neurotrophic factor (BDNF) as a biomarker in bipolar disorder: a meta-analysis of 52 studies. **BMC Med,** v. 13, p. 289, nov. 2015.

FLOWER, R. J.; BLACKWELL, G. J. Anti-inflammatory steroids induce biosynthesis of a phospholipase A2 inhibitor which prevents prostaglandin generation. **Nature,** v. 278, n. 5703, p. 456-9, mar. 1979.

FUJITA, K. *et al.* Differential expression and the anti-apoptotic effect of human placental neurotrophins and their receptors. **Placenta,** v. 32, n. 10, p. 737-44, out. 2011.

FULLERTON, J. N.; GILROY, D. W. Resolution of inflammation: a new therapeutic frontier. **Nat Rev Drug Discov**, v. 15, n. 8, p. 551-67, mar. 2016.

GAO, Y. *et al.* Female-Specific Downregulation of Tissue Polymorphonuclear Neutrophils Drives Impaired Regulatory T Cell and Amplified Effector T Cell Responses in Autoimmune Dry Eye Disease. **J Immunol,** v. 195, n. 7, p. 3086-99, out. 2015.

GARCES, M. F. *et al.* Brain-derived neurotrophic factor is expressed in rat and human placenta and its serum levels are similarly regulated throughout pregnancy in both species. **Clin Endocrinol (Oxf),** v. 81, n. 1, p. 141-51, jul. 2014.

GAVINS, F. N.; HICKEY, M. J. Annexin A1 and the regulation of innate and adaptive immunity. **Front Immunol,** v. 3, n. 354, p. 1-11, nov. 2012.

GERKE, V.; CREUTZ, C. E.; MOSS, S. E. Annexins: linking Ca2+ signalling to membrane dynamics. **Nat Rev Mol Cell Biol,** v. 6, n. 6, p. 449-61, jun. 2005.

GERKE, V.; MOSS, S. E. Annexins: from structure to function. **Physiol Rev,** v. 82, n. 2, p. 331-71, abr. 2002.

GILBERT, J. S. *et al.* The opposing roles of anti-angiogenic factors in cancer and preeclampsia. **Front Biosci (Elite Ed),** v. 4, p. 2752-69, jun. 2012.

GU, Y. *et al.* Elevated maternal IL-16 levels, enhanced IL-16 expressions in endothelium and leukocytes, and increased IL-16 production by placental trophoblasts in women with preeclampsia. **J Immunol,** v. 181, n. 6, p. 4418-22, set. 2008.

HEADLAND, S. E.; NORLING, L. V. The resolution of inflammation: Principles and challenges. **Semin Immunol,** v. 27, n. 3, p. 149-60, maio 2015.

HEMPSTEAD, B. L. Brain-Derived Neurotrophic Factor: Three Ligands, Many Actions. **Trans Am Clin Climatol Assoc,** v. 126, p. 9-19, 2015.

HIRATA, F.; IWATA, M. Role of lipomodulin, a phospholipase inhibitory protein, in immunoregulation by thymocytes. **J Immunol,** v. 130, n. 4, p. 1930-6, abr. 1983.

HONG, S. *et al.* Resolvin D1, protectin D1, and related docosahexaenoic acid-derived products: Analysis via electrospray/low energy tandem mass spectrometry based on spectra and fragmentation mechanisms. **J Am Soc Mass Spectrom,** v. 18, n. 1, p. 128-44, jan. 2007.

HU, F. *et al.* Lipoxin A4 inhibits proliferation and inflammatory cytokine/chemokine production of human epidermal keratinocytes associated with the ERK1/2 and NF-κB pathways. **J Dermatol Sci,** v. 78, n. 3, p. 181-8, jun. 2015.

HUANG, L. L. *et al.* Expression of anti-inflammatory mediator lipoxin A4 and inflammation responsive transcriptive factors NF-kappa B in patients with preeclampsia. **Clin Exp Obstet Gynecol,** v. 41, n. 5, p. 561-6, 2014.

HUTCHEON, J. A.; LISONKOVA, S.; JOSEPH, K. S. Epidemiology of pre-eclampsia and the other hypertensive disorders of pregnancy. **Best Pract Res Clin Obstet Gynaecol,** v. 25, n. 4, p. 391-403, ago. 2011.

HWANG, Y. Y.; MCKENZIE, A. N. Innate lymphoid cells in immunity and disease. **Adv Exp Med Biol,** v. 785, p. 9-26, 2013.

IWASAKI, A.; MEDZHITOV, R. Control of adaptive immunity by the innate immune system. **Nat Immunol,** v. 16, n. 4, p. 343-53, abr. 2015.

JAHROMI, A. S.; ZAREIAN, P.; MADANI, A. Association of Insulin Resistance with Serum Interleukin-6 and TNF-α Levels During Normal Pregnancy. **Biomark Insights,** v. 6, p. 1-6, 2011.

JAVERI, S. *et al.* Involvement of brain-derived neurotrophic factor (BDNF) in MP4-induced autoimmune encephalomyelitis. **Clin Immunol,** v. 137, n. 2, p. 181-9, nov. 2010.

JI, X. C. *et al.* Local Injection of Lenti-BDNF at the Lesion Site Promotes M2 Macrophage Polarization and Inhibits Inflammatory Response After Spinal Cord Injury in Mice. **Cell Mol Neurobiol,** v. 35, n. 6, p. 881-90, ago. 2015.

KAPTOGE, S. *et al.* C-reactive protein concentration and risk of coronary heart disease, stroke, and mortality: an individual participant meta-analysis. **Lancet,** v. 375, n. 9709, p. 132-40, jan. 2010.

KAWAMURA, K. *et al.* Brain-derived neurotrophic factor/tyrosine kinase B signaling regulates human trophoblast growth in an in vivo animal model of ectopic pregnancy. **Endocrinology,** v. 152, n. 3, p. 1090-100, mar. 2011.

KEELAN, J. A.; MITCHELL, M. D. Placental cytokines and preeclampsia. **Front Biosci,** v. 12, p. 2706-27, 2007.

KERMANI, P.; HEMPSTEAD, B. Brain-derived neurotrophic factor: a newly described mediator of angiogenesis. **Trends Cardiovasc Med,** v. 17, n. 4, p. 140-3, maio 2007.

KWON, J. H. *et al.* Regulation of cytosolic phospholipase A2 phosphorylation by proteolytic cleavage of annexin A1 in activated mast cells. **J Immunol,** v. 188, n. 11, p. 5665-73, jun. 2012.

LAMBERT, G. *et al.* Preeclampsia: an update. **Acta Anaesthesiol Belg,** v. 65, n. 4, p. 137-49, 2014.

LAPCHAK, P. A.; ARAUJO, D. M.; HEFTI, F. Systemic interleukin-1 beta decreases brain-derived neurotrophic factor messenger RNA expression in the rat hippocampal formation. **Neuroscience,** v. 53, n. 2, p. 297-301, mar. 1993.

LAU, S. Y. *et al.* Tumor Necrosis Factor-Alpha, Interleukin-6, and Interleukin-10 Levels are Altered in Preeclampsia: A Systematic Review and Meta-Analysis. **Am J Reprod Immunol,** v. 70, n. 5, p. 412-27, nov. 2013.

LEBRUN, B. *et al.* Brain-derived neurotrophic factor (BDNF) and food intake regulation: a minireview. **Auton Neurosci,** v. 126-127, p. 30-8, jun. 2006.

LEE, T. H. Lipoxin A4: a novel anti-inflammatory molecule? **Thorax,** v. 50, n. 2, p. 111-2, fev. 1995.

LESLIE, K.; THILAGANATHAN, B.; PAPAGEORGHIOU, A. Early prediction and prevention of pre-eclampsia. **Best Pract Res Clin Obstet Gynaecol,** v. 25, n. 3, p. 343-54, jun. 2011.

LIBBY, P. Inflammatory mechanisms: the molecular basis of inflammation and disease. **Nutr Rev,** v. 65, n. 12 Pt 2, p. S140-6, dez. 2007.

LIN, F. *et al.* A synthetic analog of lipoxin A4 partially alleviates dexamethasone-induced fetal growth restriction in rats. **Placenta,** v. 34, n. 10, p. 941-8, out. 2013.

LIU, Y. C. *et al.* Macrophage polarization in inflammatory diseases. **Int J Biol Sci,** v. 10, n. 5, p. 520-9, 2014.

LUO, C. L. *et al.* Lipoxin A4 attenuates brain damage and downregulates the production of pro-inflammatory cytokines and phosphorylated mitogen-activated protein kinases in a mouse model of traumatic brain injury. **Brain Res,** v. 1502, p. 1-10, mar. 2013.

MACDONALD-WALLIS, C. *et al.* Gestational-age-specific reference ranges for blood pressure in pregnancy: findings from a prospective cohort. **J Hypertens,** v. 33, n. 1, p. 96-105, jan. 2015.

MACGILLIVRAY, I.; ROSE, G. A.; ROWE, B. Blood pressure survey in pregnancy. **Clin Sci,** v. 37, n. 2, p. 395-407, out. 1969.

MADDOX, J. F.; SERHAN, C. N. Lipoxin A4 and B4 are potent stimuli for human monocyte migration and adhesion: selective inactivation by dehydrogenation and reduction. **J Exp Med,** v. 183, n. 1, p. 137-46, jan. 1996.

MALDONADO-PÉREZ, D. *et al.* A role for lipoxin A4 as anti-inflammatory and proresolution mediator in human parturition. **FASEB J,** v. 25, n. 2, p. 569-75, fev. 2011.

MANTOVANI, A.; DEJANA, E. Cytokines as communication signals between leukocytes and endothelial cells. **Immunol Today,** v. 10, n. 11, p. 370-5, nov. 1989.

MAROSI, K.; MATTSON, M. P. BDNF mediates adaptive brain and body responses to energetic challenges. **Trends Endocrinol Metab,** v. 25, n. 2, p. 89-98, fev. 2014.

MATSUDA, S. *et al.* Brain-derived neurotrophic factor prevents the endothelial barrier dysfunction induced by interleukin-1β and tumor necrosis factor-α. **J Periodontal Res,** v. 50, n. 4, p. 444-51, ago. 2015.

MEDZHITOV, R. Origin and physiological roles of inflammation. **Nature,** v. 454, n. 7203, p. 428-35, jul. 2008.

MEDZHITOV, R.; JANEWAY, C. Innate immunity. **N Engl J Med,** v. 343, n. 5, p. 338-44, ago. 2000.

MIHU, D. *et al.* C-reactive protein, marker for evaluation of systemic inflammatory response in preeclampsia. **Rev Med Chir Soc Med Nat Iasi,** v. 112, n. 4, p. 1019-25, out.-dez. 2008.

MOL, B. W. *et al.* Pre-eclampsia. **Lancet**, v. 5, n. 387(10022), p. 999-1011, mar. 2016.

MOR, G. *et al.* Inflammation and pregnancy: the role of the immune system at the implantation site. **Ann N Y Acad Sci,** v. 1221, p. 80-7, mar. 2011.

MORAND, E. F. *et al.* Detection of intracellular lipocortin 1 in human leukocyte subsets. **Clin Immunol Immunopathol,** v. 76, n. 2, p. 195-202, ago. 1995.

MORTENSEN, R. F. C-reactive protein, inflammation, and innate immunity. **Immunol Res,** v. 24, n. 2, p. 163-76, 2001.

MURAKAMI, M.; KUDO, I. Phospholipase A2. **J Biochem,** v. 131, n. 3, p. 285-92, mar. 2002.

NADEAU-VALLÉE, M. *et al.* Sterile inflammation and pregnancy complications: a review. **Reproduction,** v. 152, n. 6, p. R277-R292, dez. 2016.

NAGAMATSU, T.; SCHUST, D. J. The immunomodulatory roles of macrophages at the maternal-fetal interface. **Reprod Sci,** v. 17, n. 3, p. 209-18, mar. 2010.

NASCIMENTO-SILVA, V. *et al.* Aspirin-triggered lipoxin A4 blocks reactive oxygen species generation in endothelial cells: a novel antioxidative mechanism. **Thromb Haemost,** v. 97, n. 1, p. 88-98, jan. 2007.

NATHAN, C.; DING, A. Nonresolving inflammation. **Cell,** v. 140, n. 6, p. 871-82, mar. 2010.

NG, F. S. *et al.* Annexin-1-deficient mice exhibit spontaneous airway hyperresponsiveness and exacerbated allergen-specific antibody responses in a mouse model of asthma. **Clin Exp Allergy,** v. 41, n. 12, p. 1793-803, dez. 2011.

NORLING, L. V.; SERHAN, C. N. Profiling in resolving inflammatory exudates identifies novel anti-inflammatory and pro-resolving mediators and signals for termination. **J Intern Med,** v. 268, n. 1, p. 15-24, jul. 2010.

ODAKA, C. *et al.* Murine macrophages produce secretory leukocyte protease inhibitor during clearance of apoptotic cells: implications for resolution of the inflammatory response. **J Immunol,** v. 171, n. 3, p. 1507-14, ago. 2003.

OLIANI, S. M. *et al.* An immunocytochemical and in situ hybridization analysis of annexin 1 expression in rat mast cells: modulation by inflammation and dexamethasone. **Lab Invest,** v. 80, n. 9, p. 1429-38, set. 2000.

PAPAYIANNI, A.; SERHAN, C. N.; BRADY, H. R. Lipoxin A4 and B4 inhibit leukotriene-stimulated interactions of human neutrophils and endothelial cells. **J Immunol,** v. 156, n. 6, p. 2264-72, mar. 1996.

PATERNOSTER, D. M. *et al.* C-reactive protein in hypertensive disorders in pregnancy. **Clin Appl Thromb Hemost,** v. 12, n. 3, p. 330-7, jul. 2006.

PAUL-CLARK, M. J. *et al.* 15-epi-lipoxin A4-mediated induction of nitric oxide explains how aspirin inhibits acute inflammation. **J Exp Med,** v. 200, n. 1, p. 69-78, jul. 2004.

PERRETTI, M. *et al.* Annexin I is stored within gelatinase granules of human neutrophil and mobilized on the cell surface upon adhesion but not phagocytosis. **Cell Biol Int,** v. 24, n. 3, p. 163-74, 2000.

PERRETTI, M. *et al.* Endogenous lipid- and peptide-derived anti-inflammatory pathways generated with glucocorticoid and aspirin treatment activate the lipoxin A4 receptor. **Nat Med,** v. 8, n. 11, p. 1296-302, nov. 2002.

PERRETTI, M.; D'ACQUISTO, F. Annexin A1 and glucocorticoids as effectors of the resolution of inflammation. **Nat Rev Immunol,** v. 9, n. 1, p. 62-70, jan. 2009.

PERRETTI, M.; FLOWER, R. J. Measurement of lipocortin 1 levels in murine peripheral blood leukocytes by flow cytometry: modulation by glucocorticoids and inflammation. **Br J Pharmacol,** v. 118, n. 3, p. 605-10, jun. 1996.

PERUCCI, L. O. *et al.* Soluble endoglin, transforming growth factor-Beta 1 and soluble tumor necrosis factor alpha receptors in different clinical manifestations of preeclampsia. **PLoS One,** v. 9, n. 5, p. e97632, maio. 2014.

PINHEIRO, M. B. *et al.* Severe preeclampsia goes along with a cytokine network disturbance towards a systemic inflammatory state. **Cytokine,** v. 62, n. 1, p. 165-73, abr. 2013.

QIN, X. Y. *et al.* Decreased peripheral brain-derived neurotrophic factor levels in Alzheimer's disease: a meta-analysis study (N=7277). **Mol Psychiatry**, abr. 2016 (artigo publicado eletronicamente antes da versão impressa).

RAYMOND, D.; PETERSON, E. A critical review of early-onset and late-onset preeclampsia. **Obstet Gynecol Surv,** v. 66, n. 8, p. 497-506, ago. 2011.

REDMAN, C. W.; SACKS, G. P.; SARGENT, I. L. Preeclampsia: an excessive maternal inflammatory response to pregnancy. **Am J Obstet Gynecol,** v. 180, n. 2 Pt 1, p. 499-506, fev. 1999.

REDMAN, C. W.; SARGENT, I. L. Immunology of pre-eclampsia. **Am J Reprod Immunol,** v. 63, n. 6, p. 534-43, jun. 2010.

RENAUD, S. J.; MACDONALD-GOODFELLOW, S. K.; GRAHAM, C. H. Coordinated regulation of human trophoblast invasiveness by macrophages and interleukin 10. **Biol Reprod,** v. 76, n. 3, p. 448-54, mar. 2007.

REPORT OF THE NATIONAL HIGH BLOOD PRESSURE EDUCATION PROGRAM WORKING GROUP ON HIGH BLOOD PRESSURE IN PREGNANCY. **Am J Obstet Gynecol,** v. 183, n. 1, p. S1-S22, jul. 2000.

RESCHER, U. *et al.* Proteolytic cleavage of annexin 1 by human leukocyte elastase. **Biochim Biophys Acta,** v. 1763, n. 11, p. 1320-4, nov. 2006.

RIDKER, P. M. A Test in Context: High-Sensitivity C-Reactive Protein. **J Am Coll Cardiol,** v. 67, n. 6, p. 712-23, fev. 2016.

RINALDI, S. F. *et al.* 15-epi-lipoxin A4 reduces the mortality of prematurely born pups in a mouse model of infection-induced preterm birth. **Mol Hum Reprod,** v. 21, n. 4, p. 359-68, abr. 2015.

SAARELAINEN, H. *et al.* Flow mediated vasodilation and circulating concentrations of high sensitive C-reactive protein, interleukin-6 and tumor necrosis factor-alpha in normal pregnancy--The Cardiovascular Risk in Young Finns Study. **Clin Physiol Funct Imaging,** v. 29, n. 5, p. 347-52, set. 2009.

SAITO, S. *et al.* Th1/Th2/Th17 and regulatory T-cell paradigm in pregnancy. **Am J Reprod Immunol,** v. 63, n. 6, p. 601-10, jun. 2010.

SALAMA, R. H. *et al.* Implication of umbilical cord in preeclampsia. **Med Princ Pract,** v. 20, n. 2, p. 124-8, 2011.

SCHMINKEY, D. L.; GROER, M. Imitating a stress response: a new hypothesis about the innate immune system's role in pregnancy. **Med Hypotheses,** v. 82, n. 6, p. 721-9, jun. 2014.

SCHWAB, J. M.; SERHAN, C. N. Lipoxins and new lipid mediators in the resolution of inflammation. **Curr Opin Pharmacol,** v. 6, n. 4, p. 414-20, ago. 2006.

SERHAN, C. N. Lipoxins and aspirin-triggered 15-epi-lipoxins are the first lipid mediators of endogenous anti-inflammation and resolution. **Prostaglandins Leukot Essent Fatty Acids,** v. 73, n. 3-4, p. 141-62, set./out. 2005.

_____. Pro-resolving lipid mediators are leads for resolution physiology. **Nature,** v. 510, n. 7503, p. 92-101, jun. 2014.

SERHAN, C. N. *et al.* Design of lipoxin A4 stable analogs that block transmigration and adhesion of human neutrophils. **Biochemistry,** v. 34, n. 44, p. 14609-15, nov. 1995.

SERHAN, C. N. *et al.* Resolution of inflammation: state of the art, definitions and terms. **FASEB J,** v. 21, n. 2, p. 325-32, fev. 2007.

SERHAN, C. N.; HAMBERG, M.; SAMUELSSON, B. Lipoxins: novel series of biologically active compounds formed from arachidonic acid in human leukocytes. **Proc Natl Acad Sci U S A,** v. 81, n. 17, p. 5335-9, set. 1984a.

_____. Trihydroxytetraenes: a novel series of compounds formed from arachidonic acid in human leukocytes. **Biochem Biophys Res Commun,** v. 118, n. 3, p. 943-9, fev. 1984b.

SERHAN, C. N.; SAVILL, J. Resolution of inflammation: the beginning programs the end. **Nat Immunol,** v. 6, n. 12, p. 1191-7, dez. 2005.

SPURR, L. *et al.* Comparative analysis of Annexin A1-formyl peptide receptor 2/ALX expression in human leukocyte subsets. **Int Immunopharmacol,** v. 11, n. 1, p. 55-66, jan. 2011.

STEYERS, C. M.; MILLER, F. J. Endothelial dysfunction in chronic inflammatory diseases. **Int J Mol Sci,** v. 15, n. 7, p. 11324-49, 2014.

SUGIMOTO, M. *et al.* **Resolution of inflammation: what Controls its Onset?** Frontiers in Immunology. v. 7, n. 160, p. 1-18, abr. 2016.

TAKEDA, K. *et al.* Brain-Derived Neurotrophic Factor Inhibits Intercellular Adhesion Molecule-1 Expression in Interleukin-1β-Treated Endothelial Cells. **Cell Biochem Biophys**, jun. 2016.

TERAN, E.; ESCUDERO, C.; CALLE, A. C-reactive protein during normal pregnancy and preeclampsia. **Int J Gynaecol Obstet,** v. 89, n. 3, p. 299-300, jun. 2005.

TROGSTAD, L.; MAGNUS, P.; STOLTENBERG, C. Pre-eclampsia: Risk factors and causal models. **Best Pract Res Clin Obstet Gynaecol,** v. 25, n. 3, p. 329-42, jun. 2011.

VAGO, J. P. *et al.* Annexin A1 modulates natural and glucocorticoid-induced resolution of inflammation by enhancing neutrophil apoptosis. **J Leukoc Biol,** v. 92, n. 2, p. 249-58, ago. 2012.

VATTEN, L. J. *et al.* Angiogenic balance in pregnancy and subsequent breast cancer risk and survival: a population study. **Cancer Epidemiol Biomarkers Prev,** v. 18, n. 7, p. 2074-8, jul. 2009.

VISHWANATHA, J. K. *et al.* Annexin I degradation in bronchoalveolar lavage fluids from healthy smokers: a possible mechanism of inflammation. **Clin Cancer Res,** v. 4, n. 10, p. 2559-64, out. 1998.

VON DADELSZEN, P. *et al.* Maternal neutrophil apoptosis in normal pregnancy, preeclampsia, and normotensive intrauterine growth restriction. **Am J Obstet Gynecol,** v. 181, n. 2, p. 408-14, ago. 1999.

VON DADELSZEN, P.; MAGEE, L. A.; ROBERTS, J. M. Subclassification of preeclampsia. **Hypertens Pregnancy,** v. 22, n. 2, p. 143-8, 2003.

VONG, L. *et al.* Annexin 1 cleavage in activated neutrophils: a pivotal role for proteinase 3. **J Biol Chem,** v. 282, n. 41, p. 29998-30004, out. 2007.

WILLIAMS, S. L. *et al.* A proinflammatory role for proteolytically cleaved annexin A1 in neutrophil transendothelial migration. **J Immunol,** v. 185, n. 5, p. 3057-63, set. 2010.

XU, H. *et al.* Downregulation of BDNF Expression by PKC and by TNF-α in Human Endothelial Cells. **Pharmacology,** v. 96, n. 1-2, p. 1-10, 2015.

XU, Z. *et al.* Spontaneous miscarriages are explained by the stress / glucocorticoid / lipoxin A4 axis. **J Immunol,** v. 190, n. 12, p. 6051-8, jun. 2013.

XU, Z. *et al.* Preeclampsia is associated with a deficiency of lipoxin A4, an endogenous anti-inflammatory mediator. **Fertil Steril,** v. 102, n. 1, p. 282-290.e4, jul. 2014.

XU, Z. *et al.* Lipoxin A4: The double-edge sword in mice pregnancy. **Am J Reprod Immunol,** v. 72, n. 1, p. 57-64, jul. 2014.

YAMAMOTO, M. *et al.* Expression of mRNAs for neurotrophic factors (NGF, BDNF, NT-3, and GDNF) and their receptors (p75NGFR, trkA, trkB, and trkC) in the adult human peripheral nervous system and nonneural tissues. **Neurochem Res,** v. 21, n. 8, p. 929-38, ago. 1996.

YANG, Y. H. *et al.* Modulation of inflammation and response to dexamethasone by Annexin 1 in antigen-induced arthritis. **Arthritis Rheum,** v. 50, n. 3, p. 976-84, mar. 2004.

ANEXO A – Parecer do Comitê de Ética em Pesquisa da Universidade Federal de Minas Gerais (UFMG)

UNIVERSIDADE FEDERAL DE MINAS GERAIS
COMITÊ DE ÉTICA EM PESQUISA - COEP

Parecer nº. ETIC 0618.0.203.000-10

Interessado(a): Profa. Luci Maria Sant'Ana Dusse
Departamento de Análises Clínicas e Toxicológicas
Faculdade de Farmácia - UFMG

DECISÃO

O Comitê de Ética em Pesquisa da UFMG – COEP aprovou, no dia 26 de abril de 2011, após atendidas as solicitações de diligência, o projeto de pesquisa intitulado **"Pré-eclâmpsia: inter-relação dos sistemas hemostático e inflamatório"** bem como o Termo de Consentimento Livre e Esclarecido.

O relatório final ou parcial deverá ser encaminhado ao COEP um ano após o início do projeto.

Profa. Maria Teresa Marques Amaral
Coordenadora do COEP-UFMG

Av. Pres. Antonio Carlos, 6627 – Unidade Administrativa II - 2° andar – Sala 2005 – Cep:31270-901 – BH-MG
Telefax: (031) 3409-4592 - e-mail: coep@prpq.ufmg.br

ANEXO B – Parecer do Comitê de Ética em Pesquisa da Santa Casa de Misericórdia de Belo Horizonte

Registro CEP: 035/2009 (Este número deve ser citado nas correspondências referentes a este projeto)

Belo Horizonte, 27 de abril de 2009.

Interessada: Valeria Cristina Sandrim

Parecer:

O Comitê de Ética em Pesquisa (CEP) da Santa Casa de Misericórdia de Belo Horizonte, em reunião do dia 24 de abril de 2009 analisou e **APROVOU** o protocolo de pesquisa **"Estudo haplotípico de novos variantes da eNOS em gestantes brancas e negras com pré-eclâmpsia: impacto sobre resposta terapêutica anti-hipertensiva."**, registrado neste CEP sob número 035/2009, no qual V. Sa. figura como pesquisadora responsável.

OBS.:
Após o início da pesquisa, o pesquisador responsável deverá enviar ao CEP relatórios semestrais e final (para o primeiro semestre o prazo é de 30 de junho; para o segundo semestre é 31 de dezembro).

Dr. Francisco das Chagas Lima e Silva
Coordenador do CEP

ANEXO C – Parecer do Comitê de Ética em Pesquisa da Fundação Hospitalar do Estado de Minas Gerais (FHEMIG)

FHEMIG
FUNDAÇÃO HOSPITALAR DO ESTADO DE MINAS GERAIS

01/11/15/17/11/2	21/10/2010/16
RUBRICA:	DATA: 21/01/2010
ÓRGÃO / ENTIDADE: FHEMIG - Fundação Hospitalar do Estado de Minas Gerais	

FUNDAÇÃO HOSPITALAR DO ESTADO DE MINAS GERAIS

COMITÊ DE ÉTICA EM PESQUISA

Solicitação de Emenda

O CEP-FHEMIG recebeu, em 23 de Junho de 2010, solicitações de emenda ao Projeto: **"Pré-eclampsia e polimorfismos nos genes do fator VII e do receptor de estrogênio"**, enviados pela Pesquisadora Karina Braga Gomes Borges.

EMENDAS SOLICITADAS:

- Metodologia: inclusão de exames para a identificação de novos marcadores biológicos, importantes no diagnóstico e prognóstico da pré-eclampsia.
- Impacto ao sujeito da pesquisa: obtenção de coleta de 10 mL de sangue além do já utilizado.
- Alterações nos TCLE: (1) Para os grupos de gestantes com pré-eclâmpsia, (2) Para os grupos de gestantes controles, (3) Para o grupo de mulheres não gestantes.

CONSIDERAÇÕES:

- O projeto já foi apresentado e aprovado neste CEP em 2008.
- As alterações metodológicas são pertinentes e ampliam a qualidade do trabalho.
- O impacto aos participantes da pesquisa é mínimo e está devidamente previsto no TCLE.
- Os pesquisadores garantem o cumprimento da Resolução 196/96 do CNS/MS.

PARECER:

- A FAVOR DA EMENDA SOLICITADA.

- Os pesquisadores deverão citar quando solicitados o parecer de aprovação do CEP-FHEMIG 077/2008.

Belo Horizonte, 24 de Junho de 2010

Vanderson Assis Romualdo
Coordenador
Comitê de Ética em Pesquisa

Vanderson Assis Romualdo
COORDENADOR DO CEP-FHEMIG

Alameda Vereador Álvaro Celso,100 - Santa Efigênia - Belo Horizonte/MG
CEP: 30150-260 - Fone: 0(xx)31 3239-9500 - Fax: 0(xx)31 3239-9579
Site: http: //www.fhemig.mg.gov.br/ E-mail: fhemig@fhemig.mg.gov.br

ANEXO D – Parecer do Comitê de Ética em Pesquisa do Hospital Municipal Odilon Behrens (HOB)

HOSPITAL MUNICIPAL
ODILON BEHRENS

COMITÊ DE ÉTICA EM PESQUISA

Avaliação de Projeto de Pesquisa

FR: 418198

Número do Parecer: 0681.0.000.216-11

Título do Projeto: "Pré-Eclâmpsia: inter-relação dos sistemas hemostático e inflamatório"

Pesquisador Responsável: Melina de Barros Pinheiro

PARECER DO CEP/ HOB: o projeto em apreço foi avaliado pelo CEP-HOB e aprovado.

Data da reunião: 12 de maio de 2011

Atenciosamente,

Ricardo Melo Marinho
Coordenador do CEP/HOB

ANEXO E – Declaração da Gerência da Unidade Básica de Saúde da Família (UBSF) Guanabara / Betim

Prefeitura Municipal de Betim
Secretaria Municipal de Saúde
Hospital Público Regional de Betim - HPRB
Maternidade do HPRB

SUS

Declaração

Declaro, para os devidos fins, que estou de acordo com o desenvolvimento do projeto intitulado "PRÉ-ECLÂMPSIA: INTER-RELAÇÃO DOS SISTEMAS HEMOSTÁTICO E INFLAMATÓRIO" da Faculdade de Farmácia da UFMG, no posto de saúde UBSF Guanabara Betim.

Betim, 31 de agosto de 2010.

Wilson Ribeiro de Meireles
Gerente UBSF Guanabara

ANEXO F – Declaração da Diretoria do Hospital Público Regional de Betim (HPRB)

Prefeitura Municipal de Betim
Secretaria Municipal de Saúde
Hospital Público Regional de Betim - HPRB
Maternidade do HPRB

SUS

Declaração

Declaramos, para os devidos fins, que estamos de acordo com o desenvolvimento do projeto intitulado "PRÉ-ECLÂMPSIA: INTER-RELAÇÃO DOS SISTEMAS HEMOSTÁTICO E INFLAMATÓRIO" da Faculdade de Farmácia da UFMG, na Maternidade do Hospital Público Regional de Betim, desde que seja aprovado por um comitê de ética em pesquisa.

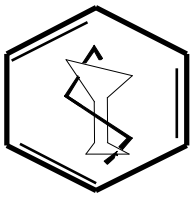
Betim, 09 de novembro de 2010.

Denise da Silva Kattah
Gerente Maternidade

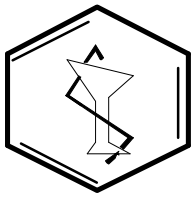
Clésio Gontijo do Amaral
Coordenador SEPPEM
Serviço de Educação Permanente e Pesquisa Multiprofissional

Geraldo Marcio Viana Zanon
Diretor HPRB

ANEXO G – Termos de Consentimento Livre e Esclarecido para os grupos I, II e III



UNIVERSIDADE FEDERAL DE MINAS GERAIS
FACULDADE DE FARMÁCIA
DEPTO. ANÁLISES CLÍNICAS E TOXICOLÓGICAS



TERMO DE CONSENTIMENTO LIVRE E ESCLARECIDO
(Para o grupo de gestantes com pré-eclâmpsia)

PROJETO DE PESQUISA: **"PRÉ-ECLÂMPSIA: INTER-RELAÇÃO DOS SISTEMAS HEMOSTÁTICO E INFLAMATÓRIO"**

Prezada Sra,

Você está sendo convidada para participar de uma pesquisa que tem por objetivo investigar as alterações da coagulação que ocorrem na pré-eclâmpsia e, dessa forma, contribuir para o maior entendimento desta doença.

Para realizar este estudo, gostaríamos de colher 15 mL do seu sangue para realização dos exames e armazenamento em um banco de amostras biológicas para estudos genéticos futuros.

Na coleta de sangue pode ocorrer uma leve dor localizada e formação de um pequeno hematoma. Para minimizar o risco de formação de hematomas, a coleta de sangue será realizada por um profissional experiente. Serão utilizados agulhas e tubos descartáveis.

Seu nome e os resultados dos exames serão mantidos em segredo.

Esclarecemos que caso não queira participar deste estudo, não haverá nenhum comprometimento ao seu atendimento e tratamento.

Para qualquer dúvida sobre esta pesquisa você deverá entrar em contato com as pessoas responsáveis pela mesma, cujos nomes estão abaixo relacionados.

Se você estiver de acordo, por favor, assine esta folha.

Responsáveis:
Luci Maria Sant'AnaDusse – telefone: 3409-6880
Lirlândia Pires de Sousa – telefone: 3409-6883
Melina de Barros Pinheiro – telefone: 3409-6880
Luiza Oliveira Perucci – telefone: 3313-6343

Comitê de Ética em Pesquisa – CEP/HOB: Rua Formiga, 50 - CEP: 31110-430 - Bairro São Cristovão. Telefone: (31) 3277-6198

NOME: _____
Carteira de identidade:_____
Assinatura: _____ DATA: ____/____/____
Agradecemos sua valiosa participação!

UNIVERSIDADE FEDERAL DE MINAS GERAIS
FACULDADE DE FARMÁCIA
DEPTO. ANÁLISES CLÍNICAS E TOXICOLÓGICAS

TERMO DE CONSENTIMENTO LIVRE E ESCLARECIDO

(Para o grupo de gestantes controle)

PROJETO DE PESQUISA: **"PRÉ-ECLÂMPSIA: INTER-RELAÇÃO DOS SISTEMAS HEMOSTÁTICO E INFLAMATÓRIO"**

Prezada Sra,

Você está sendo convidada para participar de uma pesquisa que tem por objetivo investigar as alterações da coagulação que ocorrem na pré-eclâmpsia e, dessa forma, contribuir para o maior entendimento desta doença. Você será incluída no grupo de gestantes controle, ou seja, que não apresentam a doença.

Para realizar este estudo, gostaríamos de colher 15 mL do seu sangue para realização dos exames e armazenamento em um banco de amostras biológicas para estudos genéticos futuros.

Na coleta de sangue pode ocorrer uma leve dor localizada e formação de um pequeno hematoma. Para minimizar o risco de formação de hematomas, a coleta de sangue será realizada por um profissional experiente. Serão utilizados agulhas e tubos descartáveis.

Seu nome e os resultados dos exames serão mantidos em segredo.

Esclarecemos que caso não queira participar deste estudo, não haverá nenhum comprometimento ao seu atendimento e tratamento.

Para qualquer dúvida sobre esta pesquisa você deverá entrar em contato com as pessoas responsáveis pela mesma, cujos nomes estão abaixo relacionados.

Se você estiver de acordo, por favor, assine esta folha.

Responsáveis:
Luci Maria Sant'AnaDusse – telefone: 3409-6880
Lirlândia Pires de Sousa – telefone: 3409-6883
Melina de Barros Pinheiro – telefone: 3409-6880
Luiza Oliveira Perucci – telefone: 3313-6343

Comitê de Ética em Pesquisa – CEP/HOB: Rua Formiga, 50 - CEP: 31110-430 - Bairro São Cristovão. Telefone: (31) 3277-6198

NOME: _____
Carteira de identidade:_____
Assinatura: _____ DATA: ____/____/____
Agradecemos sua valiosa participação!

UNIVERSIDADE FEDERAL DE MINAS GERAIS
FACULDADE DE FARMÁCIA
DEPTO. ANÁLISES CLÍNICAS E TOXICOLÓGICAS

TERMO DE CONSENTIMENTO LIVRE E ESCLARECIDO

(Para o grupo de mulheres não gestantes)

PROJETO DE PESQUISA: **"PRÉ-ECLÂMPSIA: INTER-RELAÇÃO DOS SISTEMAS HEMOSTÁTICO E INFLAMATÓRIO"**

Prezada Sra,

Você está sendo convidada para participar de uma pesquisa que tem por objetivo investigar as alterações da coagulação que ocorrem na pré-eclâmpsia e, dessa forma, contribuir para o maior entendimento desta doença. Você será incluída no grupo-controle, ou seja, de mulheres não gestantes.

Para realizar este estudo, gostaríamos de colher 15 mL do seu sangue para realização dos exames e armazenamento em um banco de amostras biológicas para estudos genéticos futuros.

Na coleta de sangue pode ocorrer uma leve dor localizada e formação de um pequeno hematoma. Para minimizar o risco de formação de hematomas, a coleta de sangue será realizada por um profissional experiente. Serão utilizados agulhas e tubos descartáveis.

Seu nome e os resultados dos exames serão mantidos em segredo.

Esclarecemos que caso não queira participar deste estudo, não haverá nenhum problema.

Para qualquer dúvida sobre esta pesquisa você deverá entrar em contato com as pessoas responsáveis pela mesma, cujos nomes estão abaixo relacionados.

Se você estiver de acordo, por favor, assine esta folha.

Responsáveis:
Luci Maria Sant'AnaDusse – telefone: 3409-6880
Lirlândia Pires de Sousa – telefone: 3409-6883
Melina de Barros Pinheiro – telefone: 3409-6880
Luiza Oliveira Perucci – telefone: 3313-6343

Comitê de Ética em Pesquisa – CEP/HOB: Rua Formiga, 50 - CEP: 31110-430 - Bairro São Cristovão. Telefone: (31) 3277-6198

NOME: _____
Carteira de identidade:_____
Assinatura: _____ DATA: ____/____/____
Agradecemos sua valiosa participação!

ANEXO H – Fichas clínicas dos grupos I, II e III

FICHA CLÍNICA			
Projeto: "**PRÉ-ECLÂMPSIA: INTER-RELAÇÃO DOS SISTEMAS HEMOSTÁTICO E INFLAMATÓRIO"**			
Data:			
Grupo 1: Gestantes com pré-eclâmpsia	**Paciente nº:**		
Diagnóstico de pré-eclâmpsia dado em: _____/_____/_____			
Médico responsável:			
1. Identificação			
Nome:			
Prontuário número:			
Nacionalidade:	Naturalidade:		
Data de nascimento:	Idade:		
Estado civil:	Escolaridade:		
Endereço:			
Rua/Avenida:			
Número:		Complemento:	
Bairro:		Cidade:	
CEP:		Estado:	
Telefone: ( )			
2. Anamnese			
Presença de doenças intercorrentes? (distúrbios da coagulação, doenças cardiovasculares, doenças renais, doenças autoimunes, doenças hepáticas, diabetes, câncer, sangramento, pré-eclâmpsia na família, complicações em gravidez anterior)			
Fumante?☐ SIM ☐NÃO			
Consumo de álcool?☐ SIM ☐ NÃO	Quantidade:		
Pratica exercício físico?☐ SIM ☐ NÃO			
Freqüência:	Modalidade:		
3. Informações sobre a(s) gestação(ões)			
Idade gestacional**:** _____ semanas			
Pré-natal?☐SIM ☐ NÃO			
Gravidez múltipla? ☐ SIM ☐ NÃO			
GPA (Gravidez Parto Aborto): _____**/**_____**/**_____			
Mesmo pai?			
Partos vaginal(PN) ou cirúrgico (PC)?			
Intervalo interpartal (meses):			
Parto prematuro?			
Filhos vivos:			
Principais queixas:			
☐Cefaléia☐Epigastralgia☐Escotoma☐ Reflexo patelar			
☐ Outros			
4. Uso de medicamentos			
☐Nifedipina☐ Metildopa ☐ Sulfato de magnésio			
☐ Outros			
5. Informações clínicas e laboratoriais			
Altura: _____ cm			
Peso: _____ Kg			

Ganho de peso na gravidez: _____Kg			
Exames laboratoriais: Data da realização:			
Hm: Hb: Ht: Global: N.b N.S E B L M Plaquetas:		TGO: TGP: Bilirrubina total: Bilirrubina direta: Bilirrubina indireta: Ac. Úrico: LDH: Outros:	
Pressão arterial:			
Acompanhamento:			
Data e horário	Pressão arterial	Proteinúria	Edema

FICHA CLÍNICA			
Projeto: "**PRÉ-ECLÂMPSIA: INTER-RELAÇÃO DOS SISTEMAS HEMOSTÁTICO E INFLAMATÓRIO"**			
Data:			
Grupo 2: Gestantes normotensas	**Paciente nº:**		
1. Identificação			
Nome:			
Prontuário número:			
Nacionalidade:	Naturalidade:		
Data de nascimento:	Idade:		
Estado civil:	Escolaridade:		
Endereço:			
Rua/Avenida:			
Número:		Complemento:	
Bairro:		Cidade:	
CEP:		Estado:	
Telefone: ( )			
2. Anamnese			
Presença de doenças intercorrentes? (distúrbios da coagulação, doenças cardiovasculares, doenças renais, doenças autoimunes, doenças hepáticas, diabetes, câncer, sangramento, pré-eclâmpsia na família, complicações em gravidez anterior)			
Fumante?☐ SIM ☐ NÃO			
Consumo de álcool?☐ SIM ☐ NÃO	Quantidade:		
Pratica exercício físico?☐ SIM ☐ NÃO			
Freqüência:	Modalidade:		
3. Informações sobre a(s) gestação(ões)			
Idade gestacional**:** _____ semanas			
Pré-natal?☐ SIM ☐ NÃO			
Gravidez múltipla? ☐ SIM ☐ NÃO			
GPA (Gravidez Parto Aborto): _____**/**_____**/**_____			
Mesmo pai?			
Partos vaginal(PN) ou cirúrgico (PC)?			
Intervalo interpartal (meses):			
Parto prematuro?			
Filhos vivos:			
4. Uso de medicamentos? ☐ SIM ☐ NÃO			
SE SIM. Quais medicamentos?			
5. Informações clínicas			
Altura: _____ cm			
Peso: _____ Kg			
Ganho de peso na gravidez:			
Pressão arterial: _____/_____ mmHg			

FICHA CLÍNICA			
Projeto: "**PRÉ-ECLÂMPSIA: INTER-RELAÇÃO DOS SISTEMAS HEMOSTÁTICO E INFLAMATÓRIO"**			
Data:			
Grupo: 3 - Mulheres não gestantes	**Paciente nº:**		
1. Identificação			
Nome:			
Nacionalidade:	Naturalidade:		
Data de nascimento:	Idade:		
Estado civil:	Escolaridade:		
Endereço:			
Rua/Avenida:			
Número:		Complemento:	
Bairro:		Cidade:	
CEP:		Estado:	
Telefone: ( )			
2. Anamnese			
Presença de doenças intercorrentes? (distúrbios da coagulação, doenças cardiovasculares, doenças renais, doenças autoimunes, doenças hepáticas, diabetes, câncer, sangramento, história familiar)			
Fumante?☐ SIM ☐ NÃO			
Consumo de álcool?☐ SIM ☐ NÃO	Quantidade:		
Pratica exercício físico?☐ SIM ☐ NÃO			
Freqüência:	Modalidade:		
Uso de medicamentos?☐ SIM ☐ NÃO			
SE SIM. Quais medicamentos?			
Gestações?☐ SIM ☐ NÃO			
Se SIM. Quantas?			
Intercorrências durante a gestação? (hipertensão, pré-eclâmpsia, aborto, parto prematuro)			
3. Exame físico			
Altura: _____ cm			
Peso: _____ Kg			
IMC:			
Pressão arterial: _____/_____ mmHg			

ANEXO I - Produção científica e técnica durante o doutorado

- **Artigos publicados**

PERUCCI, L. O.; VIEIRA, E. L. M.; TEIXEIRA, A. L.; GOMES, K. B.; DUSSE, L. M.; SOUSA, L. P. Decreased plasma concentrations of brain derived neurotrophic factor in preeclampsia. **Clinica Chimica Acta**, v. 464, p. 142-147, nov. 2016.

PERUCCI, L. O.; SANTOS, P. C.; RIBEIRO, L. S.; SOUZA, D. G.; GOMES, K. B.; DUSSE, L. M.; SOUSA, L.P. Lipoxin A4 Is Increased in the Plasma of Preeclamptic Women. **Am J Hypertens**, v. 10, n. 29, p. 1179-85, maio 2016.

RIOS, D. R.; ALPOIM, P. N.; GODOI, L. C.; **PERUCCI, L. O.;** SOUSA, L. P.; GOMES, K. B.; DUSSE, L .M. Increased Levels of sENG and sVCAM-1 and Decreased Levels of VEGF in Severe Preeclampsia. **Am J Hypertens**, out. 2015. (Artigo publicado eletronicamente antes da versão impressa)

PERUCCI, L. O.; CARNEIRO, F. S.; FERREIRA, C .N.; SUGIMOTO, M. A.; SORIANI, F. M.; MARTINS, G. G.;LIMA, K. M.; GUIMARÃES, F. L.; TEIXEIRA, A. T.; DUSSE, L. M.; GOMES, K. B.; SOUSA, L. P. Annexin A1 Is Increased in the Plasma of Preeclamptic Women**. PLoS ONE***,* v. 10, n.9, p 1-12, set. 2015.

PIETRANI, N. T.; RODRIGUES, K. F.; BOSCO, A. A.; VIEIRA, C. M. A. F; **PERUCCI, L. O.;** OLIVEIRA, M. C.; TEIXEIRA, A. L.; FERREIRA, A. V.; GOMES, K. B.; SOUSA, L.P. Peripheral activation of inflammatory intracelular signaling pathways and their correlation with IL6, IL10 and TNFα in obesity and type 2 diabetes mellitus. **Inflammation & Cell Signaling***,* v.2, n. e926, p. 1-8, 2015.

VAGO, J. P.; TAVARES, L. P.; GARCIA, C. C.; LIMA, K. M.; **PERUCCI, L. O.;** VIEIRA, E. L.; NOGUEIRA, C. R. C.; SORIANI, F. M.; MARTINS, J. O.; SILVA, P. M. R.; GOMES, K. B.; PINHO, V.; BRUSCOLI, S.; RICCARDI, C.; BEAULIEU, E.; MORAND, E. F.; TEIXEIRA, M. M.; SOUSA, L. P. The Role and Effects of

Glucocorticoid-Induced Leucine Zipper in the Context of Inflammation Resolution. **The Journal of Immunology**, v.194, p.4940-4950, 2015.

JARDIM, L. L.; RIOS, D. R. A.; **PERUCCI, L. O.;** SOUSA, L. P.; GOMES, K. B.; DUSSE, L. M. S.Is the imbalance between pro-angiogenic and anti-angiogenic factors associated with preeclampsia? **Clinica Chimica Acta,** v.447, p.34-38, 2015.

PERUCCI, L. O.; GOMES, K. B.; FREITAS, L. G.; GODOI, L. C.; ALPOIM, P. N.; PINHEIRO, M. B.; MIRANDA, A. S.; TEIXEIRA, A. L.; DUSSE, L. M.; SOUSA, L. P. Soluble Endoglin, Transforming Growth Factor-Beta 1 and Soluble Tumor Necrosis Factor Alpha Receptors in Different Clinical Manifestations of Preeclampsia. **Plos One**, v.9, n. 5, p.e 97632, maio 2014.

- **Manuscritos em fase de revisão**

PERUCCI, L. O.; CORRÊA, M. D.; DUSSE, L. M.; GOMES, K. B.; SOUSA, L. P. Resolution of inflammation pathways in preeclampsia – A narrative review. Submetido na revista **Immunologic Research** em agosto de 2016.

SOUZA-TESTASICCA, M. C.; OLIVEIRA, L. G.; VAGO, J. P.; FIGUEIREDO, A. B.; CANAVACI, A. M. C.; **PERUCCI, L. O.**; FERREIRA, T. P. T.; COELHO, E. A. F.; GONÇALVES, D. U.; ROCHA, M. O. C.; SILVA, P. M. R.; QUEIROZ-JUNIOR, C. M.; SOUSA, L. P.; FERNANDES, A. P. Annexin A1 is involved on the resolution of inflammatory responses during *Leishmania braziliensis* infection. Submetido na revista **The Journal of Immunology** em novembro de 2016.

- **Manuscritos submetidos**

PERUCCI, L. O.; GOMES, K. B.; DUSSE, L. M.; SOUSA, L. P. A endoglina solúvel circulante é útil para o diagnóstico da pré-eclâmpsia? (Is circulating soluble endoglin useful for preeclampsia diagnosis?) Submetido na **Revista Brasileira de Ginecologia e Obstetrícia** em agosto de 2016.

PIETRANI, N. T.; FERREIRA, C. N.; RODRIGUES, K. F.; **PERUCCI, L. O.;** CARNEIRO, F. S.; BOSCO, A. A.; OLIVEIRA, M. C.; PEREIRAS, S.; TEIXEIRA, A. L.; ALVAREZ-LEITE, J. I.; FERREIRA, A. V.; SOUSA, L. P.; GOMES, K. B. Proresolving protein annexin a1: the role in type2 diabetes mellitus and obesity. Submetido na revista **Diabetes/Metabolism Research and Reviews** em junho de 2016.

- **Manuscritos em fase de submissão**

PERUCCI, L. O.; GOMES, K. B.; DUSSE, L. M.; TEIXEIRA, M. M.; SOUSA, L. P. The Role of Annexin A1 and Specialized Pro-Resolving Lipid Mediators in Human Inflammatory Diseases.

- **Informes técnicos publicados**

PERUCCI, L. O.; MAGALHÃES, H. P. B.; BORGES, K. B. G. Interferências pré-analíticas da urinálise. Analisando- Gold Analisa Diagnóstica Ltda. N° 18 - Ano 5 Fev/Abr 2016.