The Journal of Maternal-Fetal & Neonatal Medicine

ISSN: 1476-7058 (Print) 1476-4954 (Online) Journal homepage: informahealthcare.com/journals/ijmf20

Secreted phospholipase A_2 is increased in meconium-stained amniotic fluid of term gestations: potential implications for the genesis of meconium aspiration syndrome

Roberto Romero, Bo Hyun Yoon, Piya Chaemsaithong, Josef Cortez, Chan-Wook Park, Rogelio Gonzalez, Ernesto Behnke, Sonia S. Hassan, Francesca Gotsch, Lami Yeo & Tinnakorn Chaiworapongsa

To cite this article: Roberto Romero, Bo Hyun Yoon, Piya Chaemsaithong, Josef Cortez, Chan-Wook Park, Rogelio Gonzalez, Ernesto Behnke, Sonia S. Hassan, Francesca Gotsch, Lami Yeo & Tinnakorn Chaiworapongsa (2014) Secreted phospholipase A_2 is increased in meconium-stained amniotic fluid of term gestations: potential implications for the genesis of meconium aspiration syndrome, The Journal of Maternal-Fetal & Neonatal Medicine, 27:10, 975-983, DOI: 10.3109/14767058.2013.847918

To link to this article: https://doi.org/10.3109/14767058.2013.847918

Published online: 06 Jan 2014.

THE JOURNAL OF MATERNAL-FETAL & NEONATAL MEDICINE

http://informahealthcare.com/jmf
ISSN: 1476-7058 (print), 1476-4954 (electronic)

J Matern Fetal Neonatal Med, 2014; 27(10): 975–983

DOI: 10.3109/14767058.2013.847918

informa healthcare

ORIGINAL ARTICLE

Secreted phospholipase A_2 is increased in meconium-stained amniotic fluid of term gestations: potential implications for the genesis of meconium aspiration syndrome

Roberto Romero[1,2,3], Bo Hyun Yoon[1,4], Piya Chaemsaithong[1,5], Josef Cortez[1,6], Chan-Wook Park[4], Rogelio Gonzalez[7], Ernesto Behnke[8], Sonia S. Hassan[1,5], Francesca Gotsch[1,9], Lami Yeo[1,5], and Tinnakorn Chaiworapongsa[1,5]

[1]Perinatology Research Branch, NICHD/NIH/DHHS, Bethesda, MD and Detroit, MI, USA, [2]Department of Obstetrics and Gynecology, University of Michigan, Ann Arbor, MI, [3]Department of Epidemiology and Biostatistics, Michigan State University, East Lansing, MI, [4]Department of Obstetrics and Gynecology, Seoul National University College of Medicine, Seoul, Republic of Korea, [5]Department of Obstetrics and Gynecology, Wayne State University, Detroit, MI, USA, [6]Department of Pediatrics, Wayne State University, Detroit, MI, USA, [7]Center for Perinatal Diagnosis and Research (CEDIP), Hospital Dr. Sotero del Rio, P. Universidad Catolica de Chile, Puente Alto, Chile, [8]Department of Obstetrics and Gynecology, Center for Perinatal Diagnosis and Research (CEDIP), Sotero del Rio Hospital, Santiago, Chile, and [9]Azienda Ospedaliera Universitaria, Integrata Verona, Ostetricia Ginecologia, Verona, Italy

Abstract

Background: Meconium-stained amniotic fluid (MSAF) represents the passage of fetal colonic content into the amniotic cavity. Meconium aspiration syndrome (MAS) is a complication that occurs in a subset of infants with MSAF. Secreted phospholipase A_2 ($sPLA_2$) is detected in meconium and is implicated in the development of MAS. The purpose of this study was to determine if $sPLA_2$ concentrations are increased in the amniotic fluid of women in spontaneous labor at term with MSAF.

Materials and methods: This was a cross-sectional study of patients in spontaneous term labor who underwent amniocentesis ($n = 101$). The patients were divided into two study groups: (1) MSAF ($n = 61$) and (2) clear fluid ($n = 40$). The presence of bacteria and endotoxin as well as interleukin-6 (IL-6) and $sPLA_2$ concentrations in the amniotic fluid were determined. Statistical analyses were performed to test for normality and bivariate analysis. The Spearman correlation coefficient was used to study the relationship between $sPLA_2$ and IL-6 concentrations in the amniotic fluid.

Results: Patients with MSAF have a higher median $sPLA_2$ concentration (ng/mL) in amniotic fluid than those with clear fluid [1.7 (0.98–2.89) versus 0.3 (0–0.6), $p < 0.001$]. Among patients with MSAF, those with either microbial invasion of the amniotic cavity (MIAC, defined as presence of bacteria in the amniotic cavity), or bacterial endotoxin had a significantly higher median $sPLA_2$ concentration (ng/mL) in amniotic fluid than those without MIAC or endotoxin [2.4 (1.7–6.0) versus 1.7 (1.3–2.5), $p < 0.05$]. There was a positive correlation between $sPLA_2$ and IL-6 concentrations in the amniotic fluid (Spearman Rho = 0.3, $p < 0.05$).

Conclusion: MSAF that contains bacteria or endotoxin has a higher concentration of $sPLA_2$, and this may contribute to induce lung inflammation when meconium is aspirated before birth.

Keywords

Acute phase protein reactant, interleukin-6, intra-amniotic inflammation/infection, prostaglandins, $sPLA_2$

History

Received 30 August 2013
Accepted 19 September 2013
Published online 27 December 2013

Introduction

Meconium-stained amniotic fluid (MSAF) represents the passage of fetal colonic content into the amniotic cavity [1–13]. MSAF is a risk factor for maternal infection-related complications (e.g. chorioamnionitis [8,14–20], puerperal endomyometritis [16,17,20,21]), neonatal sepsis [3,22–25], cerebral palsy [26–29], hypoxic-ischemic encephalopathy [3,30–33], meconium aspiration syndrome (MAS) [3,6,8,12,13,34–54], and fetal death [55–57].

MAS occurs in a subset of infants born to mothers with MSAF [3,6,8,12,13,34–54]. However, why some infants with MSAF develop MAS, and others do not, remains an open question [6,38,41–43,45,51]. Meconium-induced lung injury has been attributed to mechanical obstruction [51,52,58–60], chemical injury [58,61–66], pulmonary cell apoptosis [35,36,60,65,67–70] and an inflammatory response [35,59,67,71–87]. A series of experimental and clinical studies have made a strong case for a role of secreted

Address for correspondence: Roberto Romero, MD, D.Med.Sci., Perinatology Research Branch, NICHD, NIH, DHHS, Wayne State University/Hutzel Women's Hospital, 3990 John R, Box 4, Detroit, MI 48201, USA. Tel: (313) 993-2700. Fax: (313) 993-2694. E-mail: romeror@mail.nih.gov

phospholipase A2 ($sPLA_2$) in MAS [67,88–95]. This enzyme can exert deleterious effects by eliciting inflammation [92,93,96–112] and inactivating lung surfactant [89,90,113–115]. The purpose of this study was to determine if $sPLA_2$ concentration is increased in the amniotic fluid of women in spontaneous labor at term with MSAF.

Materials and methods

Study design and population

A cross-sectional study was conducted which included patients at term with MSAF ($n = 61$) and clear amniotic fluid ($n = 40$, controls). Inclusion and exclusion criteria for the study population were similar to a previous report [116]. All women provided written informed consent before collection of the amniotic fluid samples. The collection and utilization of the samples was approved by the Human Investigation Committee of the participating institutions and the IRB of the *Eunice Kennedy Shriver* National Institute of Child Health and Human Development (NICHD/NIH/DHHS). The clinical definitions, sample collection, microbiological studies, detection of endotoxin, and statistical analysis have been described in a previous report [116]. $sPLA_2$ immunoassay was performed according to the methods defined by Stoner et al. [117,118].

Results

Among women with spontaneous labor at term, 60.4% (61/101) had MSAF and 39.6% (40/101) had clear amniotic fluid. The median maternal age was significantly higher in patients with MSAF than in those with clear fluid ($p = 0.03$). Otherwise, the clinical characteristics of the two study groups were similar ($p > 0.05$).

Microorganisms in the AF were identified in 16.4% (10/61) of patients in the MSAF group and in 5% (2/40) of those with clear fluid ($p < 0.05$). The most common microorganisms were Gram-negative rods ($n = 6$), followed by *Ureaplasma urealyticum* ($n = 2$), Gram-positive rods ($n = 2$) and *Mycoplasma hominis* ($n = 1$). One patient's amniotic fluid had both a Gram-positive rod and *M. hominis.* Two patients with clear amniotic fluid had positive cultures for bacteria (*U. urealyticum*).

The Limulus amebocyte lysate (LAL) assay for bacterial endotoxin in the amniotic fluid was positive in 32.8% (20/61) of patients with MSAF, but in only 2.5% (1/40) of those with clear amniotic fluid ($p < 0.001$). After heat treatment to eliminate the effect of trypsin [119], the frequency of a positive LAL assay was still significantly higher in the MSAF group compared to those with clear amniotic fluid, even after heat treatment [19.7% (12/61) versus 2.5% (1/40); $p < 0.05$].

Patients with MSAF had a significantly higher median amniotic fluid $sPLA_2$ concentration (ng/mL) than those with clear amniotic fluid [1.7 (0.98–2.89) versus 0.3 (0–0.6); $p < 0.001$] (Figure 1). Moreover, in the MSAF group, those with endotoxin or microorganisms (defined by LAL or amniotic fluid gram stain or positive amniotic fluid culture) had a significantly higher median amniotic fluid $sPLA_2$ concentration (ng/mL) than those with the absence of endotoxin or microorganisms [2.4 (1.7–6.9) versus 1.7 (1.3–2.5); $p = 0.049$] (Figure 2). Amniotic fluid $sPLA_2$ concentration had a significant positive correlation with amniotic fluid IL-6 concentration (Spearman Rho = 0.3, $p = 0.045$).

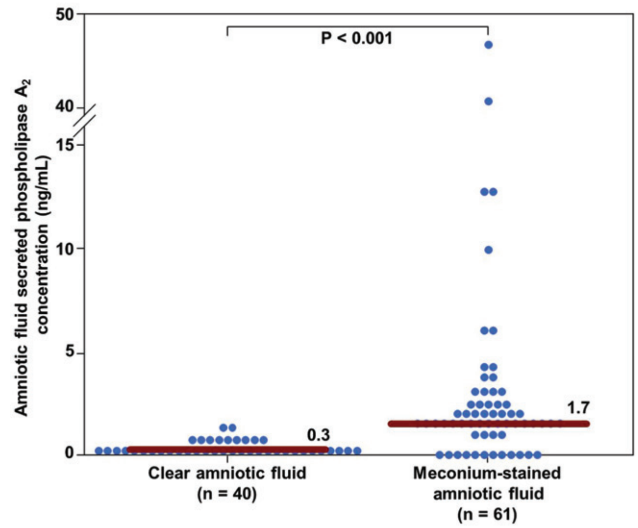

Figure 1. Amniotic fluid secreted phospholipase A_2 concentrations ($sPLA_2$) in women at term with clear amniotic fluid and MSAF. Patients with MSAF had a significantly higher median amniotic fluid secreted phospholipase A_2 concentration (ng/mL) than those with clear amniotic fluid [1.7 (1–2.9) versus 0.3 (0–0.6); $p < 0.001$].

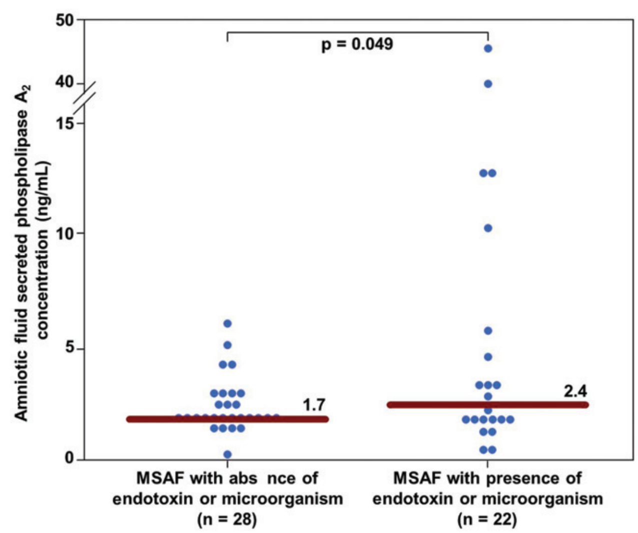

Figure 2. Amniotic fluid secreted phospholipase A_2 concentration ($sPLA_2$) among women with MSAF at term with presence and absence of endotoxin or microorganisms. Patients with MSAF and intra-amniotic inflammation/infection at term had a significantly higher median secreted phospholipase A_2 concentration (ng/mL) than those without intra-amniotic inflammation/infection [2.4 (1.7–6.99) versus 1.7 (1.3–2.5); $p = 0.049$].

Discussion

Principal findings of the study

(1) Patients with MSAF in spontaneous labor at term had a higher median $sPLA_2$ concentration in amniotic fluid than those with clear amniotic fluid; (2) among patients with MSAF, women with either microbial invasion of the amniotic cavity (MIAC; defined as a positive amniotic fluid culture for

microorganisms) or the presence of endotoxin in the amniotic cavity had a higher median $sPLA_2$ concentration in the amniotic fluid than those without MIAC or bacterial endotoxin; and (3) there was a positive correlation between amniotic fluid $sPLA_2$ and amniotic fluid IL-6 concentration. Since $sPLA_2$ is an acute-phase reactant protein induced by IL-6, this observation suggests that an inflammatory response is associated with an increase in $sPLA_2$.

What are phospholipases A_2?

Phospholipase A2 (PLA_2) is a family of enzymes that hydrolyze the ester bond at the *sn*-2 position of phospholipids to generate arachidonic acid and lysophospholipids, which are precursors of eicosanoids and other lipid mediators (leukotrienes and prostaglandins) [110,112,120–135]. These enzymes are broadly classified into two groups: (1) intracellular or cytosolic PLA_2 ($cPLA_2$) and (2) extracellular or secreted PLA_2 ($sPLA_2$) [112,128]. PLA_2 participates in the production of prostaglandins, which are major mediators of the onset of spontaneous labor at term [136–164], as well as preterm labor [151,159,161,163,165–168]. $cPLA_2$ is an intracellular enzyme, while $sPLA_2$ (in particular, group IIA isoform) is an acute phase reactant protein released in response to tissue damage and infection [169–171]. IL-6 can induce the expression of group II $sPLA_2$ from hepatic cells in culture [172]. The properties and functions of $cPLA_2$ and $sPLA_2$ have been reviewed [112,124,128,130,132–134]. Recently, $sPLA_2$ has been implicated in the pathophysiology of meconium-induced lung injury (see below).

More than 10 isoforms of $sPLA_2$ have been described (e.g. groups I, II, III, V, etc.) [110,112,130,132–134]. Individual $sPLA_2$ enzymes act on both cellular membrane phospholipids and non-cellular phospholipids (e.g. surfactant and lipoproteins) including foreign phospholipids (e.g. bacterial membranes and dietary phospholipids) [133]. The functions of $sPLA_2$ depend on: (1) specific $sPLA_2$ isoform; (2) specific target phospholipid or membrane; (3) lipid mediators produced by enzymatic activity; (4) the mechanisms responsible for the activation of $sPLA_2$; and (5) the specific circumstances and site at which a particular $sPLA_2$ isoform is present [133]. For example, the group I $sPLA_2$ isoform is produced in the pancreas, and its primary function is the catalytic cleavage of dietary lipids [173]. The group II $sPLA_2$ isoform is largely expressed and stored in inflammatory cells including neutrophils [174], eosinophils [175,176], T-lymphocytes [177,178], monocytes [179,180], macrophages [181], mast cells [182] and platelets [183]. This particular isoform (group II $sPLA_2$) is detected in high concentrations in biological fluids in the context of inflammation (e.g. synovial fluid in rheumatoid arthritis [105,184–188], bronchoalveolar lavage (BAL) in patients with acute respiratory distress syndrome (ARDS) [100,114], and serum/plasma of patients with septic shock [189], Crohn's disease [190], ulcerative colitis [191], acute pancreatitis [192–194], and rheumatoid arthritis [195].

The group II $sPLA_2$ isoform has potent antimicrobial activity [112,171,196–207]. Elsbach et al. purified $sPLA_2$ from polymorphonuclear leukocytes of rabbits, and reported that $sPLA_2$ was bactericidal against *Escherichia coli* and *Salmonella typhimurium*, acting in concert with a ''bactericidal/permeability increasing protein'' [196]. Subsequently, Weinrauch et al. extracted group II $sPLA_2$ from sterile peritoneal fluid of rabbits, and demonstrated that it had potent antimicrobial activity against *Staphylococcus aureus* [198,208]. Similarly, group II $sPLA_2$ isolated from the plasma of baboons after a challenge with *E. coli* has potent bactericidal properties against *S. aureus* and *Streptococcus pyogenes* [198,203]. Such activity can be blocked by a monoclonal antibody against the enzyme [198]. Other investigators have shown antimicrobial activity against *Listeria monocytogenes* [197,209] and *Bacillus anthracis* [203,204]. $sPLA_2$ may also participate in host defense against viruses [210–212] and parasites [213]. The presence of high $sPLA_2$ concentrations in biological fluids (e.g. tears [214,215], semen [216], intestinal lumen [197,217,218], inflammatory exudates [105,184–188], bronchoalveolar lavage [100,114], and serum [189,219]) of both animals and humans with bacterial infections has been interpreted as indicating that $sPLA_2$ is part of the host defense against microbial invasion [112,171].

Group II $sPLA_2$ can induce activation of human neutrophils [101,104], exocytosis in human lung macrophages [102], neutrophils [104], eosinophils [176], and degranulation of mast cells [99]. Triggiani et al. reported that sPLA2 [group I $sPLA_2$ from cobra venom and group II (recombinant synovial fluid) $sPLA_2$] can increase the expression of IL-6 mRNA and the rate of secretion of IL-6 from human lung macrophages, as well as the release of β-glucuronidase (a cytosolic enzyme used as a surrogate marker for cellular exocytosis) [102]. Groups I and II $sPLA_2$ generate an intracellular response that activates both exocytosis and cytokine gene expression in macrophages [92,102,111]. Other investigators have reported that different isoforms of $sPLA_2$ (group IA, IB, IIA, V and X) induce the production of cytokines (e.g. IL-6, TNFα and IL-10) and chemokines [e.g. monocyte chemotactic protein-1(MCP-1)/chemokine (C-C motif) ligand 2 (CCL2), macrophage inflammatory protein-1 (MIP-1α)/CCL3 and MIP1-β/CCL4] from inflammatory cells such as monocytes [105], neutrophils [108] and eosinophils [176]. These observations collectively suggest that $sPLA_2$ has an important role in inflammation. The catalytic action of $sPLA_2$, cleaving membrane phospholipids to generate eicosanoid precursors (arachidonic acid, leukotrienes and prostaglandins), has been implicated in the generation of an inflammatory state [112,114,170,220].

PLA_2 have been localized in lysosomes of chorioamnion [221], decidua [222,223] and amniotic fluid [224,225]. Moreover, its activity in fetal membranes was increased before the onset of labor [221]. Group II $sPLA_2$ mRNA expression and immunoreactivity has been demonstrated in amnion, choriodecidua and placenta [226–228]. Rice et al. concluded that this isoenzyme is a major contributor of the net tissue $sPLA_2$ activity in the human placenta and may contribute to the production of prostaglandins during labor [227]. The expression of this enzyme is increased in placentas of women in labor [228].

Phospholipase A_2 in meconium and meconium-induced lung injury

$sPLA_2$ has been reported in meconium [67,88,90,93]. The administration of meconium into the trachea of neonatal pigs results in severe histologic lung inflammation, increased apoptosis and increased lung $sPLA_2$ activity (measured by the concentration of arachidonic acid following incubation of lung homogenates with 1,2-dipalmitoylphosphatidylcholine (DPPC), a substrate that is specific to $sPLA_2$) [67].

$sPLA_2$ activity has been detected in meconium (determined by measuring DPPC metabolites in suspensions of this material before and after mixing with the substrate) [90]. Enzymatic activity is attributed to $sPLA_2$ (rather than other phospholipases), and has been demonstrated by the formation of lysophosphatidylcholine after samples had been heat-treated ($sPLA_2$ is heat-stable – other lipolytic enzymes are heat-sensitive). $sPLA_2$ extracted from meconium inhibits surfactant activity *in vitro* [90].

$sPLA_2$ activity in lung tissues can be induced by meconium and bile acids [115]. $sPLA_2$ is locally produced in lung tissue and contributes to the total PLA_2 activity during MAS [53,93]. Collectively, the evidence suggests that: (1) meconium contains $sPLA_2$ activity; (2) the lungs of neonates affected with MAS contain higher amounts of $sPLA_2$; (3) $cPLA_2$ was not detected in meconium or alveolar fluid; and (4) there is a correlation between $sPLA_2$ activity and TNF-α concentrations in bronchoalveolar lavage [53,67,90,92,93,115].

Phospholipase A_2 in amniotic fluid with MSAF and microbial invasion of the amniotic cavity

The findings reported herein suggest that the concentration of $sPLA_2$ is higher in MSAF than in clear amniotic fluid among patients in labor at term. After exclusion of samples with MSAF with either bacteria or endotoxin, the difference between clear amniotic fluid and MSAF disappeared. Moreover, $sPLA_2$ concentrations in amniotic fluid correlated positively with IL-6 concentrations. These observations suggest that the elevation in $sPLA_2$ can be attributed to the consequences of MIAC or the resulting inflammatory process.

Our findings and interpretation are consistent with those reported by Koyama et al., indicating that $sPLA_2$ activity (measured by high-performance liquid chromatography) and group II $sPLA_2$ concentration in amniotic fluid were higher in patients with preterm labor (with or without chorioamnionitis) than in preterm controls (i.e. pregnant women without labor who underwent amniocentesis for chromosomal studies between 17–30 weeks of gestation) [229].

We recently reported that the frequency of MIAC and bacterial endotoxin in amniotic fluid is higher among women in spontaneous labor at term with MSAF than in those with clear amniotic fluid [116]. We proposed that microorganisms or microbial products, such as endotoxin, present in amniotic fluid can be swallowed by the fetus, resulting in increased fetal peristalsis and intrauterine passage of meconium. Aspiration of meconium with microorganisms and inflammatory mediators during fetal life could predispose to MAS. Since $sPLA_2$ has been proposed to be a major mediator of lung injury in MAS, our findings suggest that the meconium of patients with MIAC or endotoxin contains higher concentrations of $sPLA_2$. Although exposure to $sPLA_2$ may begin during fetal life, aspirated meconium and microbial products contained in such meconium, as well as inflammatory mediators, may induce further production of $sPLA_2$ and other inflammatory mediators by the lung that may eventually lead to lung injury and respiratory insufficiency observed in MAS.

Conclusion

Term meconium-stained amniotic fluid that contains bacteria or endotoxin has a higher concentration of secreted phospholipase A_2, and this may contribute to induce lung inflammation when meconium is aspirated before birth.

Declaration of interest

The authors report no conflicts of interest.

This research was supported, in part, by the Perinatology Research Branch, Division of Intramural Research, *Eunice Kennedy Shriver* National Institute of Child Health and Human Development, National Institutes of Health, Department of Health and Human Services (NICHD/NIH); and, in part, with Federal funds from NICHD, NIH under Contract No. HHSN275201300006C. The research was also supported by a grant from the Walter Scott Foundation for Medical Research.

References

1. Woods JR, Glantz JC. Significance of amniotic fluid meconium. In: Creasy RK, Resnik R, eds. Maternal fetal medicine principles and practices. 3rd ed. Philadelphia: W.B. Saunders Company; 1994:413–22.
2. Ross MG. Meconium aspiration syndrome – more than intrapartum meconium. N Engl J Med 2005;353:946–8.
3. Ahanya SN, Lakshmanan J, Morgan BL, et al. Meconium passage in utero: mechanisms, consequences, and management. Obstet Gynecol Surv 2005;60:45–56; quiz 73–44.
4. Lakshmanan J, Ross MG. Mechanism(s) of in utero meconium passage. J Perinatol 2008;28:S8–13.
5. Caughey AB, Musci TJ. Complications of term pregnancies beyond 37 weeks of gestation. Obstet Gynecol 2004;103:57–62.
6. Bhat R, Vidyasagar D. Delivery room management of meconium-stained infant. Clin Perinatol 2012;39:817–31.
7. Kariniemi V, Harrela M. Significance of meconium staining of the amniotic fluid. J Perinat Med 1990;18:345–9.
8. Maymon E, Chaim W, Furman B, et al. Meconium stained amniotic fluid in very low risk pregnancies at term gestation. Eur J Obstet Gynecol Reprod Biol 1998;80:169–73.
9. Abramovici H, Brandes JM, Fuchs K, et al. Meconium during delivery: a sign of compensated fetal distress. Am J Obstet Gynecol 1974;118:251–5.
10. Fujikura T, Klionsky B. The significance of meconium staining. Am J Obstet Gynecol 1975;121:45–50.
11. Lee KA, Mi Lee S, Jin Yang H, et al. The frequency of meconium-stained amniotic fluid increases as a function of the duration of labor. J Matern Fetal Neonatal Med 2011;24:880–5.
12. Sheiner E, Hadar A, Shoham-Vardi I, et al. The effect of meconium on perinatal outcome: a prospective analysis. J Matern Fetal Neonatal Med 2002;11:54–9.
13. Oyelese Y, Culin A, Ananth CV, et al. Meconium-stained amniotic fluid across gestation and neonatal acid-base status. Obstet Gynecol 2006;108:345–9.

14. Romero R, Hanaoka S, Mazor M, et al. Meconium-stained amniotic fluid: a risk factor for microbial invasion of the amniotic cavity. Am J Obstet Gynecol 1991;164:859–62.
15. Mazor M, Furman B, Wiznitzer A, et al. Maternal and perinatal outcome of patients with preterm labor and meconium-stained amniotic fluid. Obstet Gynecol 1995;86:830–3.
16. Markovitch O, Mazor M, Shoham-Vardi I, et al. Meconium stained amniotic fluid is associated with maternal infectious morbidity in pre term delivery. Acta Obstet Gynecol Scand 1993;72:538–42.
17. Piper JM, Newton ER, Berkus MD, et al. Meconium: a marker for peripartum infection. Obstet Gynecol 1998;91:741–5.
18. Chapman S, Duff P. Incidence of chorioamnionitis in patients with meconium-stained amniotic fluid. Infect Dis Obstet Gynecol 1995; 2:210–12.
19. Usta IM, Sibai BM, Mercer BM, et al. Use of maternal plasma level of zinc-coproporphyrin in the prediction of intrauterine passage of meconium: a pilot study. J Matern Fetal Med 2000;9:201–3.
20. Tran SH, Caughey AB, Musci TJ. Meconium-stained amniotic fluid is associated with puerperal infections. Am J Obstet Gynecol 2003; 189:746–50.
21. Josephson A. An epidemiologic study of postcesarean infection. Am J Infect Control 1984;12:19–25.
22. Berkus MD, Langer O, Samueloff A, et al. Meconium-stained amniotic fluid: increased risk for adverse neonatal outcome. Obstet Gynecol 1994;84:115–20.
23. Escobar GJ, Li DK, Armstrong MA, et al. Neonatal sepsis workups in infants >/=2000 grams at birth: A population-based study. Pediatrics 2000;106:256–63.
24. Kayange N, Kamugisha E, Mwizamholya DL, et al. Predictors of positive blood culture and deaths among neonates with suspected neonatal sepsis in a tertiary hospital, Mwanza-Tanzania. BMC Pediatr 2010;10:39.
25. Schrag SJ, Cutland CL, Zell ER, et al. Risk factors for neonatal sepsis and perinatal death among infants enrolled in the prevention of perinatal sepsis trial, Soweto, South Africa. Pediatr Infect Dis J 2012;31:821–26.
26. Altshuler G, Arizawa M, Molnar-Nadasdy G. Meconium-induced umbilical cord vascular necrosis and ulceration: a potential link between the placenta and poor pregnancy outcome. Obstet Gynecol 1992;79:760–6.
27. Spinillo A, Fazzi E, Capuzzo E, et al. Meconium-stained amniotic fluid and risk for cerebral palsy in preterm infants. Obstet Gynecol 1997;90:519–23.
28. Redline RW. Severe fetal placental vascular lesions in term infants with neurologic impairment. Am J Obstet Gynecol 2005;192: 452–7.
29. McIntyre S, Taitz D, Keogh J, et al. A systematic review of risk factors for cerebral palsy in children born at term in developed countries. Dev Med Child Neurol 2013;55:499–508.
30. Andres RL, Saade G, Gilstrap LC, et al. Association between umbilical blood gas parameters and neonatal morbidity and death in neonates with pathologic fetal acidemia. Am J Obstet Gynecol 1999;181:867–71.
31. Ellis M, Manandhar N, Manandhar DS, et al. Risk factors for neonatal encephalopathy in Kathmandu, Nepal, a developing country: unmatched case-control study. BMJ 2000;320:1229–36.
32. Hayes BC, Cooley S, Donnelly J, et al. The placenta in infants >36 weeks gestation with neonatal encephalopathy: a case control study. Arch Dis Child Fetal Neonatal Ed 2013;98:F233–9.
33. Hayes BC, McGarvey C, Mulvany S, et al. A case-control study of hypoxic-ischemic encephalopathy in newborn infants at >36 weeks gestation. Am J Obstet Gynecol 2013;209:29.e1–29.e19.
34. Vidyasagar D, Harris V, Pildes RS. Assisted ventilation in infants with meconium aspiration syndrome. Pediatrics 1975;56:208–13.
35. Vidyasagar D, Lukkarinen H, Kaapa P, et al. Inflammatory response and apoptosis in newborn lungs after meconium aspiration. Biotechnol Prog 2005;21:192–7.
36. Vidyasagar D, Zagariya A. Studies of meconium-induced lung injury: inflammatory cytokine expression and apoptosis. J Perinatol 2008;28:S102–7.
37. Katz VL, Bowes Jr WA. Meconium aspiration syndrome: reflections on a murky subject. Am J Obstet Gynecol 1992;166:171–83.
38. Cleary GM, Wiswell TE. Meconium-stained amniotic fluid and the meconium aspiration syndrome. An update. Pediatr Clin North Am 1998;45:511–29.
39. Blackwell SC, Carreno CA, Hassan SS, et al. Meconium staining and meconium aspiration syndrome. Is there seasonal variation? Fetal Diagn Ther 2001;16:208–10.
40. Ziadeh SM, Sunna E. Obstetric and perinatal outcome of pregnancies with term labour and meconium-stained amniotic fluid. Arch Gynecol Obstet 2000;264:84–7.
41. Blackwell SC, Moldenhauer J, Hassan SS, et al. Meconium aspiration syndrome in term neonates with normal acid-base status at delivery: is it different? Am J Obstet Gynecol 2001;184: 1422–5; discussion 1425–6.
42. Manganaro R, Mami C, Palmara A, et al. Incidence of meconium aspiration syndrome in term meconium-stained babies managed at birth with selective tracheal intubation. J Perinat Med 2001;29: 465–8.
43. Sedaghatian MR, Othman L, Hossain MM, et al. Risk of meconium-stained amniotic fluid in different ethnic groups. J Perinatol 2000;20:257–61.
44. Srinivasan HB, Vidyasagar D. Meconium aspiration syndrome: current concepts and management. Compr Ther 1999;25:82–9.
45. Dargaville PA, Copnell B. The epidemiology of meconium aspiration syndrome: incidence, risk factors, therapies, and outcome. Pediatrics 2006;117:1712–21.
46. Vain NE, Szyld EG, Prudent LM, et al. Oropharyngeal and nasopharyngeal suctioning of meconium-stained neonates before delivery of their shoulders: multicentre, randomised controlled trial. Lancet 2004;364:597–602.
47. Fraser WD, Hofmeyr J, Lede R, et al. Amnioinfusion for the prevention of the meconium aspiration syndrome. N Engl J Med 2005;353:909–17.
48. Becker S, Solomayer E, Dogan C, et al. Meconium-stained amniotic fluid – perinatal outcome and obstetrical management in a low-risk suburban population. Eur J Obstet Gynecol Reprod Biol 2007;132:46–50.
49. Fanaroff AA. Meconium aspiration syndrome: historical aspects. J Perinatol 2008;28:S3–7.
50. de Beaufort AJ. Early human development at the perinatal interface: meconium stained amniotic fluid (MSAF) and meconium aspiration syndrome (MAS). Early Hum Dev 2009;85:605.
51. van Ierland Y, de Beaufort AJ. Why does meconium cause meconium aspiration syndrome? Current concepts of MAS pathophysiology. Early Hum Dev 2009;85:617–20.
52. Martin GI, Vidyasagar D. Introduction: proceedings of the first international conference for meconium aspiration syndrome and meconium-induced lung injury. J Perinatol 2008;28:S1–2.
53. De Luca D, Minucci A, Tripodi D, et al. Role of distinct phospholipases A2 and their modulators in meconium aspiration syndrome in human neonates. Intensive Care Med 2011;37: 1158–65.
54. Uhing MR, Bhat R, Philobos M, et al. Value of amnioinfusion in reducing meconium aspiration syndrome. Am J Perinatol 1993;10: 43–5.
55. Mandelbaum B. Gestational meconium in the high-risk pregnancy. Obstet Gynecol 1973;42:87–92.
56. Ohana O, Holcberg G, Sergienko R, et al. Risk factors for intrauterine fetal death (1988–2009). J Matern Fetal Neonatal Med 2011;24:1079–83.
57. Brailovschi Y, Sheiner E, Wiznitzer A, et al. Risk factors for intrapartum fetal death and trends over the years. Arch Gynecol Obstet 2012;285:323–9.
58. Tyler DC, Murphy J, Cheney FW. Mechanical and chemical damage to lung tissue caused by meconium aspiration. Pediatrics 1978;62:454–9.
59. Kisala JM, Ayala A, Stephan RN, et al. A model of pulmonary atelectasis in rats: activation of alveolar macrophage and cytokine release. Am J Physiol 1993;264:R610–14.
60. Zagariya A, Bhat R, Uhal B, et al. Cell death and lung cell histology in meconium aspirated newborn rabbit lung. Eur J Pediatr 2000;159:819–26.
61. Clark DA, Nieman GF, Thompson JE, et al. Surfactant displacement by meconium free fatty acids: an alternative explanation for atelectasis in meconium aspiration syndrome. J Pediatr 1987;110: 765–70.
62. Moses D, Holm BA, Spitale P, et al. Inhibition of pulmonary surfactant function by meconium. Am J Obstet Gynecol 1991;164: 477–81.

63. Sun B, Curstedt T, Robertson B. Surfactant inhibition in experimental meconium aspiration. Acta Paediatr 1993;82:182–9.
64. Sun B, Herting E, Curstedt T, et al. Exogenous surfactant improves lung compliance and oxygenation in adult rats with meconium aspiration. J Appl Physiol 1994;77:1961–71.
65. Zagariya A, Bhat R, Navale S, et al. Inhibition of meconium-induced cytokine expression and cell apoptosis by pretreatment with captopril. Pediatrics 2006;117:1722–7.
66. El Shahed AI, Dargaville P, Ohlsson A, et al. Surfactant for meconium aspiration syndrome in full term/near term infants. Cochrane Database Syst Rev 2007;CD002054.
67. Holopainen R, Aho H, Laine J, et al. Human meconium has high phospholipase A2 activity and induces cellular injury and apoptosis in piglet lungs. Pediatr Res 1999;46:626–32.
68. Lukkarinen H, Laine J, Lehtonen J, et al. Angiotensin II receptor blockade inhibits pneumocyte apoptosis in experimental meconium aspiration. Pediatr Res 2004;55:326–33.
69. Rosenfeld CR, Zagariya AM, Liu XT, et al. Meconium increases type 1 angiotensin II receptor expression and alveolar cell death. Pediatr Res 2008;63:251–6.
70. Zagariya A, Sierzputovska M, Navale S, et al. Role of meconium and hypoxia in meconium aspiration-induced lung injury in neonatal rabbits. Mediators Inflamm 2010;2010:204831.
71. Lopez A, Bildfell R. Pulmonary inflammation associated with aspirated meconium and epithelial cells in calves. Vet Pathol 1992; 29:104–11.
72. Clark P, Duff P. Inhibition of neutrophil oxidative burst and phagocytosis by meconium. Am J Obstet Gynecol 1995;173: 1301–5.
73. de Beaufort AJ, Pelikan DM, Elferink JG, et al. Effect of interleukin 8 in meconium on in-vitro neutrophil chemotaxis. Lancet 1998;352: 102–5.
74. Kytola J, Uotila P, Kaapa P. Meconium stimulates cyclooxygenase-2 expression in rat lungs. Prostaglandins Leukot Essent Fatty Acids 1999;60:107–10.
75. Tamura DY, Moore EE, Partrick DA, et al. Acute hypoxemia in humans enhances the neutrophil inflammatory response. Shock 2002;17:269–73.
76. de Beaufort AJ, Bakker AC, van Tol MJ, et al. Meconium is a source of pro-inflammatory substances and can induce cytokine production in cultured A549 epithelial cells. Pediatr Res 2003;54: 491–5.
77. Kytola J, Kaapa P, Uotila P. Meconium aspiration stimulates cyclooxygenase-2 and nitric oxide synthase-2 expression in rat lungs. Pediatr Res 2003;53:731–6.
78. Lindenskov PH, Castellheim A, Aamodt G, et al. Complement activation reflects severity of meconium aspiration syndrome in newborn pigs. Pediatr Res 2004;56:810–17.
79. Castellheim A, Lindenskov PH, Pharo A, et al. Meconium is a potent activator of complement in human serum and in piglets. Pediatr Res 2004;55:310–18.
80. Zagariya A, Bhat R, Chari G, et al. Apoptosis of airway epithelial cells in response to meconium. Life Sci 2005;76: 1849–58.
81. Castellheim A, Lindenskov PH, Pharo A, et al. Meconium aspiration syndrome induces complement-associated systemic inflammatory response in newborn piglets. Scand J Immunol 2005;61:217–25.
82. Mollnes TE, Castellheim A, Lindenskov PH, et al. The role of complement in meconium aspiration syndrome. J Perinatol 2008; 28:S116–19.
83. Castellheim A, Pharo A, Fung M, et al. Complement C5a is a key mediator of meconium-induced neutrophil activation. Pediatr Res 2005;57:242–7.
84. Okazaki K, Kondo M, Kato M, et al. Serum cytokine and chemokine profiles in neonates with meconium aspiration syndrome. Pediatrics 2008;121:e748–53.
85. Salvesen B, Fung M, Saugstad OD, et al. Role of complement and CD14 in meconium-induced cytokine formation. Pediatrics 2008; 121:e496–505.
86. Cayabyab RG, Kwong K, Jones C, et al. Lung inflammation and pulmonary function in infants with meconium aspiration syndrome. Pediatr Pulmonol 2007;42:898–905.
87. Salvesen B, Nielsen EW, Harboe M, et al. Mechanisms of complement activation and effects of C1-inhibitor on the meconium-induced inflammatory reaction in human cord blood. Mol Immunol 2009;46:688–94.
88. Pulkkinen MO, Eskola J, Kleimola V, et al. Pancreatic and catalytic phospholipase A2 in relation to pregnancy, labor and fetal outcome. Gynecol Obstet Invest 1990;29:104–7.
89. Hite RD, Seeds MC, Jacinto RB, et al. Hydrolysis of surfactant-associated phosphatidylcholine by mammalian secretory phospholipases A2. Am J Physiol 1998;275:L740–7.
90. Schrama AJ, de Beaufort AJ, Sukul YR, et al. Phospholipase A2 is present in meconium and inhibits the activity of pulmonary surfactant: an in vitro study. Acta Paediatr 2001;90:412–16.
91. Kaapa P. Meconium aspiration syndrome: a role for phospholipase A2 in the pathogenesis? Acta Paediatr 2001;90:365–7.
92. Granata F, Petraroli A, Boilard E, et al. Activation of cytokine production by secreted phospholipase A2 in human lung macrophages expressing the M-type receptor. J Immunol 2005;174: 464–74.
93. Sippola T, Aho H, Peuravuori H, et al. Pancreatic phospholipase A2 contributes to lung injury in experimental meconium aspiration. Pediatr Res 2006;59:641–5.
94. Kaapa P, Soukka H. Phospholipase A2 in meconium-induced lung injury. J Perinatol 2008;28:S120–2.
95. De Luca D, Capoluongo E, Rigo V. Secretory phospholipase A2 pathway in various types of lung injury in neonates and infants: a multicentre translational study. BMC Pediatr 2011;11:101.
96. Pruzanski W, Vadas P, Fornasier V. Inflammatory effect of intradermal administration of soluble phospholipase A2 in rabbits. J Invest Dermatol 1986;86:380–3.
97. Niewoehner DE, Rice K, Duane P, et al. Induction of alveolar epithelial injury by phospholipase A2. J Appl Physiol 1989;66: 261–7.
98. Tocker JE, Durham SK, Welton AF, et al. Phospholipase A2-induced pulmonary and hemodynamic responses in the guinea pig. Effects of enzyme inhibitors and mediators antagonists. Am Rev Respir Dis 1990;142:1193–9.
99. Murakami M, Hara N, Kudo I, et al. Triggering of degranulation in mast cells by exogenous type II phospholipase A2. J Immunol 1993;151:5675–84.
100. Kim DK, Fukuda T, Thompson BT, et al. Bronchoalveolar lavage fluid phospholipase A2 activities are increased in human adult respiratory distress syndrome. Am J Physiol 1995;269:L109–18.
101. Takasaki J, Kawauchi Y, Yasunaga T, et al. Human type II phospholipase A2-induced Mac-1 expression on human neutrophils. J Leukoc Biol 1996;60:174–80.
102. Triggiani M, Granata F, Oriente A, et al. Secretory phospholipases A2 induce beta-glucuronidase release and IL-6 production from human lung macrophages. J Immunol 2000;164:4908–15.
103. Fonteh AN, Marion CR, Barham BJ, et al. Enhancement of mast cell survival: a novel function of some secretory phospholipase A(2) isotypes. J Immunol 2001;167:4161–71.
104. Silliman CC, Moore EE, Zallen G, et al. Presence of the M-type sPLA(2) receptor on neutrophils and its role in elastase release and adhesion. Am J Physiol Cell Physiol 2002;283:C1102–13.
105. Triggiani M, Granata F, Oriente A, et al. Secretory phospholipases A2 induce cytokine release from blood and synovial fluid monocytes. Eur J Immunol 2002;32:67–76.
106. Fuentes L, Hernandez M, Fernandez-Aviles FJ, et al. Cooperation between secretory phospholipase A2 and TNF-receptor superfamily signaling: implications for the inflammatory response in atherogenesis. Circ Res 2002;91:681–8.
107. Beck G, Yard BA, Schulte J, et al. Secreted phospholipases A2 induce the expression of chemokines in microvascular endothelium. Biochem Biophys Res Commun 2003;300:731–7.
108. Jo EJ, Lee HY, Lee YN, et al. Group IB secretory phospholipase A2 stimulates CXC chemokine ligand 8 production via ERK and NF-kappa B in human neutrophils. J Immunol 2004;173:6433–9.
109. Ramoner R, Putz T, Gander H, et al. Dendritic-cell activation by secretory phospholipase A2. Blood 2005;105:3583–7.
110. Triggiani M, Granata F, Frattini A, et al. Activation of human inflammatory cells by secreted phospholipases A2. Biochim Biophys Acta 2006;1761:1289–300.
111. Granata F, Frattini A, Loffredo S, et al. Signaling events involved in cytokine and chemokine production induced by secretory phospholipase A2 in human lung macrophages. Eur J Immunol 2006;36:1938–50.

112. Lambeau G, Gelb MH. Biochemistry and physiology of mammalian secreted phospholipases A2. Annu Rev Biochem 2008;77:495–520.
113. Holm BA, Keicher L, Liu MY, et al. Inhibition of pulmonary surfactant function by phospholipases. J Appl Physiol 1991;71:317–21.
114. Arbibe L, Koumanov K, Vial D, et al. Generation of lysophospholipids from surfactant in acute lung injury is mediated by type-II phospholipase A2 and inhibited by a direct surfactant protein A-phospholipase A2 protein interaction. J Clin Invest 1998;102:1152–60.
115. De Luca D, Minucci A, Zecca E, et al. Bile acids cause secretory phospholipase A2 activity enhancement, revertible by exogenous surfactant administration. Intensive Care Med 2009;35:321–6.
116. Romero R, Yoon BH, Chaemsaithong P, et al. Bacteria and endotoxin in meconium-stained amniotic fluid at term: could intra-amniotic infection cause meconium passage? J Matern Fetal Neonatal Med. 2013; Sep 12 [Epub ahead of print].
117. Stoner CR, Reik LM, Donohue M, et al. Human group II phospholipase A2. Characterization of monoclonal antibodies and immunochemical quantitation of the protein in synovial fluid. J Immunol Methods 1991;145:127–36.
118. Romero R, Brandt F, Sepulveda W, et al. Extracellular phospholipase A2 in term and preterm parturition. Abstract 278. 39th Annual Meeting of the Society for Gynecologic Investigation: March 18–21, 1992: scientific program and abstracts: San Antonio Marriott Rivercenter, San Antonio, Texas.
119. Lisowska-Myjak B, Pachecka J. Trypsin and antitrypsin activities and protein concentration in serial meconium and feces of healthy newborns. J Matern Fetal Neonatal Med 2006;19:477–82.
120. Verheij HM, Slotboom AJ, de Haas GH. Structure and function of phospholipase A2. Rev Physiol Biochem Pharmacol 1981;91:91–203.
121. Dennis EA. The enzymes. New York: Academic Press; 1983.
122. Vadas P, Pruzanski W. Role of secretory phospholipases A2 in the pathobiology of disease. Lab Invest 1986;55:391–404.
123. Waite M. The phospholipases. New York: Plenum Press; 1987.
124. Dennis EA. Diversity of group types, regulation, and function of phospholipase A2. J Biol Chem 1994;269:13057–60.
125. Six DA, Dennis EA. The expanding superfamily of phospholipase A(2) enzymes: classification and characterization. Biochim Biophys Acta 2000;1488:1–19.
126. Valentin E, Lambeau G. Increasing molecular diversity of secreted phospholipases A(2) and their receptors and binding proteins. Biochim Biophys Acta 2000;1488:59–70.
127. Kudo I, Murakami M. Phospholipase A2 enzymes. Prostaglandins Other Lipid Mediat 2002;68–69:3–58.
128. Murakami M, Kudo I. Phospholipase A2. J Biochem 2002;131:285–92.
129. Schaloske RH, Dennis EA. The phospholipase A2 superfamily and its group numbering system. Biochim Biophys Acta 2006;1761:1246–59.
130. Burke JE, Dennis EA. Phospholipase A2 structure/function, mechanism, and signaling. J Lipid Res 2009;50:S237–42.
131. Murakami M, Taketomi Y, Girard C, et al. Emerging roles of secreted phospholipase A2 enzymes: lessons from transgenic and knockout mice. Biochimie 2010;92:561–82.
132. Boyanovsky BB, Webb NR. Biology of secretory phospholipase A2. Cardiovasc Drugs Ther 2009;23:61–72.
133. Murakami M, Taketomi Y, Sato H, et al. Secreted phospholipase A2 revisited. J Biochem 2011;150:233–55.
134. Burke JE, Dennis EA. Phospholipase A2 biochemistry. Cardiovasc Drugs Ther 2009;23:49–59.
135. Murakami M, Taketomi Y, Miki Y, et al. Recent progress in phospholipase A(2) research: from cells to animals to humans. Prog Lipid Res 2011;50:152–92.
136. Wiqvist N, Bygdeman M, Green K, et al. Endogenous prostaglandins and the initiation of labor. Acta Obstet Gynecol Scand Suppl 1974;37:7–16.
137. Schwarz BE, Schultz FM, Macdonald PC, et al. Initiation of human parturition. III. Fetal membrane content of prostaglandin E2 and F2alpha precursor. Obstet Gynecol 1975;46:564–8.
138. Csapo AI. Prostaglandins and the initiation of labor. Prostaglandins 1976;12:149–64.
139. Kinoshita K, Satoh K, Sakamoto S. Prostaglandin F2alpha and E1 in plasma and amniotic fluid during human pregnancy and labor. Endocrinol Jpn 1977;24:155–62.
140. Liggins GC, Forster CS, Grieves SA, et al. Control of parturition in man. Biol Reprod 1977;16:39–56.
141. Mitchell MD, Flint AP. Progesterone withdrawal: effects on prostaglandins and parturition. Prostaglandins 1977;14:611–14.
142. Mitchell MD, Bibby JG, Hicks BR, et al. Possible role for prostacyclin in human parturition. Prostaglandins 1978;16:931–7.
143. Liggins GC. Initiation of parturition. Br Med Bull 1979;35:145–50.
144. Okita JR, MacDonald PC, Johnston JM. Initiation of human parturition. Am J Obstet Gynecol 1982;142:432–5.
145. Mitchell MD. Mechanisms of human parturition: role of prostaglandins and related compounds. Adv Prostaglandin Thromboxane Leukot Res 1985;15:613–15.
146. Okazaki T, Sagawa N, Ban C, et al. Regulation of prostaglandin formation during human parturition. Adv Prostaglandin Thromboxane Leukot Res 1985;15:617–18.
147. Jenkins DM. Prostaglandins and parturition. Lancet 1985;2:163.
148. Romero R, Mazor M, Wu YK, et al. Bacterial endotoxin and tumor necrosis factor stimulate prostaglandin production by human decidua. Prostaglandins Leukot Essent Fatty Acids 1989;37:183–6.
149. Romero R, Wu YK, Mazor M, et al. Amniotic fluid concentration of 5-hydroxyeicosatetraenoic acid is increased in human parturition at term. Prostaglandins Leukot Essent Fatty Acids 1989;35:81–3.
150. Romero R, Wu YK, Sirtori M, et al. Amniotic fluid concentrations of prostaglandin F2 alpha, 13,14-dihydro-15-keto-prostaglandin F2 alpha (PGFM) and 11-deoxy-13,14-dihydro-15-keto-11,16-cyclo-prostaglandin E2 (PGEM-LL) in preterm labor. Prostaglandins 1989;37:149–61.
151. Walsh SW. 5-Hydroxyeicosatetraenoic acid, leukotriene C4, and prostaglandin F2 alpha in amniotic fluid before and during term and preterm labor. Am J Obstet Gynecol 1989;161:1352–60.
152. Lundin-Schiller S, Mitchell MD. The role of prostaglandins in human parturition. Prostaglandins Leukot Essent Fatty Acids 1990;39:1–10.
153. Dowling DD, Romero RJ, Mitchell MD, et al. Isolation of multiple substances in amniotic fluid that regulate amnion prostaglandin E2 production: the effects of gestational age and labor. Prostaglandins Leukot Essent Fatty Acids 1991;44:253–5.
154. Romero R, Baumann P, Gonzalez R, et al. Amniotic fluid prostanoid concentrations increase early during the course of spontaneous labor at term. Am J Obstet Gynecol 1994;171:1613–20.
155. Romero R, Gonzalez R, Baumann P, et al. Topographic differences in amniotic fluid concentrations of prostanoids in women in spontaneous labor at term. Prostaglandins Leukot Essent Fatty Acids 1994;50:97–104.
156. Neulen J, Breckwoldt M. Placental progesterone, prostaglandins and mechanisms leading to initiation of parturition in the human. Exp Clin Endocrinol 1994;102:195–202.
157. Mitchell MD, Romero RJ, Edwin SS, et al. Prostaglandins and parturition. Reprod Fertil Dev 1995;7:623–32.
158. Romero R, Munoz H, Gomez R, et al. Increase in prostaglandin bioavailability precedes the onset of human parturition. Prostaglandins Leukot Essent Fatty Acids 1996;54:187–91.
159. Gomez R, Romero R, Edwin SS, et al. Pathogenesis of preterm labor and preterm premature rupture of membranes associated with intraamniotic infection. Infect Dis Clin North Am 1997;11:135–76.
160. Romero R, Gotsch F, Pineles B, et al. Inflammation in pregnancy: its roles in reproductive physiology, obstetrical complications, and fetal injury. Nutr Rev 2007;65:S194–202.
161. Mitchell MD, Chang MC, Chaiworapongsa T, et al. Identification of 9alpha,11beta-prostaglandin F2 in human amniotic fluid and characterization of its production by human gestational tissues. J Clin Endocrinol Metab 2005;90:4244–8.
162. Lee SE, Romero R, Park IS, et al. Amniotic fluid prostaglandin concentrations increase before the onset of spontaneous labor at term. J Matern Fetal Neonatal Med 2008;21:89–94.

163. Kamel RM. The onset of human parturition. Arch Gynecol Obstet 2010;281:975–82.
164. Menon R, Fortunato SJ, Milne GL, et al. Amniotic fluid eicosanoids in preterm and term births: effects of risk factors for spontaneous preterm labor. Obstet Gynecol 2011;118:121–34.
165. Romero R, Emamian M, Wan M, et al. Prostaglandin concentrations in amniotic fluid of women with intra-amniotic infection and preterm labor. Am J Obstet Gynecol 1987;157:1461–7.
166. Romero R, Wu YK, Mazor M, et al. Amniotic fluid prostaglandin E2 in preterm labor. Prostaglandins Leukot Essent Fatty Acids 1988;34:141–5.
167. Romero R, Wu YK, Mazor M, et al. Amniotic fluid arachidonate lipoxygenase metabolites in preterm labor. Prostaglandins Leukot Essent Fatty Acids 1989;36:69–75.
168. Hanna N, Bonifacio L, Weinberger B, et al. Evidence for interleukin-10-mediated inhibition of cyclo- oxygenase-2 expression and prostaglandin production in preterm human placenta. Am J Reprod Immunol 2006;55:19–27.
169. Hack CE, Wolbink GJ, Schalkwijk C, et al. A role for secretory phospholipase A2 and C-reactive protein in the removal of injured cells. Immunol Today 1997;18:111–15.
170. Uozumi N, Kume K, Nagase T, et al. Role of cytosolic phospholipase A2 in allergic response and parturition. Nature 1997;390:618–22.
171. Buckland AG, Wilton DC. The antibacterial properties of secreted phospholipases A(2). Biochim Biophys Acta 2000;1488:71–82.
172. Crowl RM, Stoller TJ, Conroy RR, et al. Induction of phospholipase A2 gene expression in human hepatoma cells by mediators of the acute phase response. J Biol Chem 1991;266:2647–51.
173. Slotboom AJ, van Dam-Mieras MC, Jansen EH, et al. Relationship between structure and activity of pancreatic phospholipase A2. Adv Exp Med Biol 1978;101:137–52.
174. Rosenthal MD, Gordon MN, Buescher ES, et al. Human neutrophils store type II 14-kDa phospholipase A2 in granules and secrete active enzyme in response to soluble stimuli. Biochem Biophys Res Commun 1995;208:650–6.
175. Blom M, Tool AT, Wever PC, et al. Human eosinophils express, relative to other circulating leukocytes, large amounts of secretory 14-kD phospholipase A2. Blood 1998;91:3037–43.
176. Triggiani M, Granata F, Balestrieri B, et al. Secretory phospholipases A2 activate selective functions in human eosinophils. J Immunol 2003;170:3279–88.
177. Asaoka Y, Yoshida K, Sasaki Y, et al. Possible role of mammalian secretory group II phospholipase A2 in T-lymphocyte activation: implication in propagation of inflammatory reaction. Proc Natl Acad Sci U S A 1993;90:716–19.
178. Ho IC, Arm JP, Bingham 3rd CO, et al. A novel group of phospholipase A2s preferentially expressed in type 2 helper T cells. J Biol Chem 2001;276:18321–6.
179. Inada M, Tojo H, Kawata S, et al. Preferential distribution of group-II-like phospholipase A2 in mononuclear phagocytic cells in rat spleen and liver. Eur J Biochem 1991;197:323–9.
180. Sipka S, Farkas T, Gergely P, et al. Secretion of phospholipase A2 induced by interactions of human platelets with monocytes. Ann Hematol 1994;69:307–10.
181. Hidi R, Vargaftig BB, Touqui L. Increased synthesis and secretion of a 14-kDa phospholipase A2 by guinea pig alveolar macrophages. Dissociation from arachidonic acid liberation and modulation by dexamethasone. J Immunol 1993;151:5613–23.
182. Fonteh AN, Bass DA, Marshall LA, et al. Evidence that secretory phospholipase A2 plays a role in arachidonic acid release and eicosanoid biosynthesis by mast cells. J Immunol 1994;152:5438–46.
183. Kramer RM, Hession C, Johansen B, et al. Structure and properties of a human non-pancreatic phospholipase A2. J Biol Chem 1989;264:5768–75.
184. Hara S, Kudo I, Chang HW, et al. Purification and characterization of extracellular phospholipase A2 from human synovial fluid in rheumatoid arthritis. J Biochem 1989;105:395–9.
185. Seilhamer JJ, Pruzanski W, Vadas P, et al. Cloning and recombinant expression of phospholipase A2 present in rheumatoid arthritic synovial fluid. J Biol Chem 1989;264:5335–8.
186. Fierlbeck G, Rassner G, Muller C. Psoriasis induced at the injection site of recombinant interferon gamma. Results of immunohistologic investigations. Arch Dermatol 1990;126:351–5.
187. Kortekangas P, Aro HT, Nevalainen TJ. Group II phospholipase A2 in synovial fluid and serum in acute arthritis. Scand J Rheumatol 1994;23:68–72.
188. Bidgood MJ, Jamal OS, Cunningham AM, et al. Type IIA secretory phospholipase A2 up-regulates cyclooxygenase-2 and amplifies cytokine-mediated prostaglandin production in human rheumatoid synoviocytes. J Immunol 2000;165:2790–7.
189. Vadas P. Elevated plasma phospholipase A2 levels: correlation with the hemodynamic and pulmonary changes in gram-negative septic shock. J Lab Clin Med 1984;104:873–81.
190. Haapamaki MM, Gronroos JM, Nurmi H, et al. Elevated group II phospholipase A2 mass concentration in serum and colonic mucosa in Crohn's disease. Clin Chem Lab Med 1998;36:751–5.
191. Haapamaki MM, Gronroos JM, Nurmi H, et al. Phospholipase A2 in serum and colonic mucosa in ulcerative colitis. Scand J Clin Lab Invest 1999;59:279–87.
192. Nevalainen TJ, Gronroos JM, Kortesuo PT. Pancreatic and synovial type phospholipases A2 in serum samples from patients with severe acute pancreatitis. Gut 1993;34:1133–6.
193. Kemppainen E, Hietaranta A, Puolakkainen P, et al. Bactericidal/permeability-increasing protein and group I and II phospholipase A2 during the induction phase of human acute pancreatitis. Pancreas 1999;18:21–7.
194. Miura M, Endo S, Kaku LL, et al. Plasma type II phospholipase A2 levels in patients with acute pancreatitis. Res Commun Mol Pathol Pharmacol 2001;109:159–64.
195. Lin MK, Farewell V, Vadas P, et al. Secretory phospholipase A2 as an index of disease activity in rheumatoid arthritis. Prospective double blind study of 212 patients. J Rheumatol 1996;23:1162–6.
196. Elsbach P, Weiss J, Franson RC, et al. Separation and purification of a potent bactericidal/permeability-increasing protein and a closely associated phospholipase A2 from rabbit polymorphonuclear leukocytes. Observations on their relationship. J Biol Chem 1979;254:11000–9.
197. Harwig SS, Tan L, Qu XD, et al. Bactericidal properties of murine intestinal phospholipase A2. J Clin Invest 1995;95:603–10.
198. Weinrauch Y, Abad C, Liang NS, et al. Mobilization of potent plasma bactericidal activity during systemic bacterial challenge. Role of group IIA phospholipase A2. J Clin Invest 1998;102:633–8.
199. Laine VJ, Grass DS, Nevalainen TJ. Resistance of transgenic mice expressing human group II phospholipase A2 to Escherichia coli infection. Infect Immun 2000;68:87–92.
200. Beers SA, Buckland AG, Koduri RS, et al. The antibacterial properties of secreted phospholipases A2: a major physiological role for the group IIA enzyme that depends on the very high pI of the enzyme to allow penetration of the bacterial cell wall. J Biol Chem 2002;277:1788–93.
201. Koprivnjak T, Peschel A, Gelb MH, et al. Role of charge properties of bacterial envelope in bactericidal action of human group IIA phospholipase A2 against *Staphylococcus aureus*. J Biol Chem 2002;277:47636–44.
202. Koduri RS, Gronroos JO, Laine VJ, et al. Bactericidal properties of human and murine groups I, II, V, X, and XII secreted phospholipases A(2). J Biol Chem 2002;277:5849–57.
203. Gimenez AP, Wu YZ, Paya M, et al. High bactericidal efficiency of type iia phospholipase A2 against Bacillus anthracis and inhibition of its secretion by the lethal toxin. J Immunol 2004;173:521–30.
204. Piris-Gimenez A, Paya M, Lambeau G, et al. In vivo protective role of human group IIa phospholipase A2 against experimental anthrax. J Immunol 2005;175:6786–91.
205. Femling JK, Nauseef WM, Weiss JP. Synergy between extracellular group IIA phospholipase A2 and phagocyte NADPH oxidase in digestion of phospholipids of *Staphylococcus aureus* ingested by human neutrophils. J Immunol 2005;175:4653–61.
206. Huhtinen HT, Gronroos JO, Gronroos JM, et al. Antibacterial effects of human group IIA and group XIIA phospholipase A2 against *Helicobacter pylori* in vitro. APMIS 2006;114:127–30.
207. Sitkiewicz I, Stockbauer KE, Musser JM. Secreted bacterial phospholipase A2 enzymes: better living through phospholipolysis. Trends Microbiol 2007;15:63–9.

208. Weinrauch Y, Elsbach P, Madsen LM, et al. The potent anti-Staphylococcus aureus activity of a sterile rabbit inflammatory fluid is due to a 14-kD phospholipase A2. J Clin Invest 1996;97: 250–7.
209. Gronroos JO, Laine VJ, Nevalainen TJ. Bactericidal group IIA phospholipase A2 in serum of patients with bacterial infections. J Infect Dis 2002;185:1767–72.
210. Fenard D, Lambeau G, Maurin T, et al. A peptide derived from bee venom-secreted phospholipase A2 inhibits replication of T-cell tropic HIV-1 strains via interaction with the CXCR4 chemokine receptor. Mol Pharmacol 2001;60:341–7.
211. Mitsuishi M, Masuda S, Kudo I, et al. Group V and X secretory phospholipase A2 prevents adenoviral infection in mammalian cells. Biochem J 2006;393:97–106.
212. Kim JO, Chakrabarti BK, Guha-Niyogi A, et al. Lysis of human immunodeficiency virus type 1 by a specific secreted human phospholipase A2. J Virol 2007;81:1444–50.
213. Deregnaucourt C, Schrevel J. Bee venom phospholipase A2 induces stage-specific growth arrest of the intraerythrocytic *Plasmodium falciparum* via modifications of human serum components. J Biol Chem 2000;275:39973–80.
214. Aho HJ, Saari KM, Kallajoki M, et al. Synthesis of group II phospholipase A2 and lysozyme in lacrimal glands. Invest Ophthalmol Vis Sci 1996;37:1826–32.
215. Saari KM, Aho V, Paavilainen V, et al. Group II PLA(2) content of tears in normal subjects. Invest Ophthalmol Vis Sci 2001;42: 318–20.
216. Nevalainen TJ, Meri KM, Niemi M. Synovial-type (group II) phospholipase A2 human seminal plasma. Andrologia 1993;25: 355–8.
217. Nevalainen TJ, Haapanen TJ. Distribution of pancreatic (group I) and synovial-type (group II) phospholipases A2 in human tissues. Inflammation 1993;17:453–64.
218. Qu XD, Lloyd KC, Walsh JH, et al. Secretion of type II phospholipase A2 and cryptdin by rat small intestinal Paneth cells. Infect Immun 1996;64:5161–5.
219. Nevalainen TJ, Eerola LI, Rintala E, et al. Time-resolved fluoroimmunoassays of the complete set of secreted phospholipases A2 in human serum. Biochim Biophys Acta 2005;1733:210–23.
220. Henderson Jr WR, Chi EY, Bollinger JG, et al. Importance of group X-secreted phospholipase A2 in allergen-induced airway inflammation and remodeling in a mouse asthma model. J Exp Med 2007;204:865–77.
221. Schwarz BE, Schultz FM, MacDonald PC, et al. Initiation of human parturition. IV. Demonstration of phospholipase A2 in the lysosomes of human fetal membranes. Am J Obstet Gynecol 1976; 125:1089–92.
222. Gustavii B. The distribution within the placenta, myometrium, and decidua of 24Na-labelled hypertonic solution following intra-amniotic or extra-amniotic injection. Br J Obstet Gynaecol. 1975;82:734–9.
223. Akesson B, Gustavii B. Occurrence of phospholipase A1 and A2 in human decidua. Prostaglandins 1975;9:667–73.
224. Gebhardt DD, Beintema A, Reman FC, et al. Phospholipase-A2 in amniotic fluid. Lancet 1978;2:1159.
225. Gebhardt DO, Beintema A, Reman FC, et al. The lipoprotein composition of amniotic fluid. Clin Chim Acta 1979;94:93–100.
226. Farrugia W, Aitken MA, van Dunne F, et al. Type II phospholipase A2 in human gestational tissues: subcellular distribution of placental immuno- and catalytic activity. Biochim Biophys Acta 1993;1166:77–83.
227. Aitken MA, Farrugia W, Wong MH, et al. Type II phospholipase A2 in human gestational tissues: extractable immuno- and enzymatic activity in fetal membranes. Biochim Biophys Acta 1993;1170:314–20.
228. Farrugia W, Rice GE, Wong MH, et al. Release of Type II phospholipase A2 immunoreactivity and phospholipase A2 enzymatic activity from human placenta. J Endocrinol 1997;153:151–7.
229. Koyama M, Ito S, Nakajima A, et al. Elevations of group II phospholipase A2 concentrations in serum and amniotic fluid in association with preterm labor. Am J Obstet Gynecol 2000;183: 1537–43.