2.4. Discussion

Acute lung injury (ALI) or its severe form, acute respiratory distress syndrome (ARDS) is an important cause of mortality in the human population [Rubenfeld *et al.,* 2005]. The endotoxin, LPS is a well known inducer compound for ALI/ARDS, it induces ALI by mainly dysfunctioning the pulmonary surfactants. For the past 3 decades, intensive research in pulmonary biology has led to a better understanding of the role of lipids during ARDS. However, the mortality associated with ARDS ($\sim 40\%$) remains unchanged [Ware and Matthay, 2000]. The major target organ for ALI/ARDS is respiratory organ, lung and the normal physiology of the respiratory system is maintained by lipid-protein complex called "pulmonary surfactant". ARDS is associated with reduced surfactant phospholipids in the bronchoalveolar lavage particularly phosphatidylcholine (PC) [Oulton *et al.,* 1991; Finley and Ladman, 1972]. LPS is expected to alter the membrane composition, since LPS is known to enter into the lipid layer of biological membranes, local amendments in bilayer organization are modified [Rothfield and Romeo, 1971] and so are the enzymes involved in phospholipid metabolism. Previous reports have shown more about PC alterations, especially DPPC but the regulation of phospholipid during endotoxemia is yet to be elucidated. Hence, the aim of this study was to assess the influence of LPS on alteration of surfactant phospholipid metabolism in terms of surfactant degradation and synthesis of new phospholipids by PLA$_2$ and lyso-phospholipid acyltransferases. Moreover, impact of LPS on surfactant PL fatty acid composition and remodeling enzymes expression are vital and were determined to understand pulmonary dysfunction. The pulmonary surfactant metabolism was studied by *in vitro* as well as *in vivo* model of ALI/ARDS.

2.4.1*. In vitro* metabolic labelling of whole lung and AEC2 phospholipids with LPS shows alteration in major surfactant PL

In the present study, altered surfactant phospholipid metabolism was fully corroborated with experimental evidences using rat as a model system. In order to understand the impact of LPS on phospholipid metabolism, the whole lung tissue and

AEC2 were exposed over a wide range of LPS concentration along with [^{32}P]orthophosphate. We observed reduction in phospholipids, particularly PC and PG when compared to control. All other PLs such as PE, PA, PS, PI and LPC did not show much alteration (Fig. 1A,B).

To further understand the reduction in phospholipid if it is due to defective phospholipid synthesis/increased degradation, we did [^{32}P]orthophosphate labelling studies with LPS lung PL. Profound reduction was observed in PC ($\sim$ 90%) and PG ($\sim$ 75%) and during pre-incubation/post incubation with LPS (Fig. 2B). However, the reduction in PC was not attributed by accumulated level of LPC. In AEC2 cells labelling also a similar trend was observed (Fig. 4A-C) as that of the whole lung. From the above labelling results it was clearly shown that LPS induces significant alterations in major surfactant phospholipids, PC and PG. The decreased content of PC and PG significantly/potentially impairs the normal functioning of the pulmonary surfactant. Major surface tension reducing component in surfactant, is composed of ~85% PC and ~15% PG [Goerke, 1998]. Decreased PC and PG content were observed in BALF from patients with respiratory infection [LeVine *et al.,* 1996; Griese *et al.,* 1997]. Surfactant deficiency is the major factor underlying the pathogenesis of respiratory distress syndrome and alterations of surfactant composition and structure may contribute to the etiology of lung diseases [Kitsiouli *et al.,* 2009; Griese, 1999]. PC, a major class of glycerophospholipids present in all mammalian and some prokaryotic cells, plays critical role in membrane structure and cellular signalling and is involved in several metabolic pathways [Cui and Houweling, 2002; DeLong *et al.* 1999]. An extensive network of phospholipases, acyltransferases and other metabolizing enzymes make PC and it is the source for signaling mediators namely PA, DAG, LPC, and arachidonic acid [Exton, 1994; Billah and Anthes, 1990].

PG is second most abundant phospholipid ($\sim$ 10-15%) in lung surfactant, it aids in the spreading of dipalmitoyl-PC, which is presumed to be the main functional component of lung surfactant [Postle *et al.* 2001]. It is well established that the concentration of PG increases during foetal development. PG is a precursor for

cardiolipin (CL), an important component of the inner mitochondrial membrane, where it constitutes about 20% of the total lipid composition. In mammalian cells, cardiolipin (CL) is found almost exclusively in the inner mitochondrial membrane where it is essential for the optimal function of numerous enzymes that are involved in mitochondrial energy metabolism [Schlame *et al.,* 2000]. Moreover, reduction of surfactant phospholipids affects other functions like immunosuppression, inhibition of superoxide generation by granulocytes [Chao *et al.,* 1995], cytokine production by alveolar macrophages [Allen *et al.,* 1995], and proliferation of peripheral blood mononuclear cells [Kremlev *et al.,* 1994].

2.4.2. Effect of LPS on Lung fatty acid composition

Labelling studies showed a marked impact on PC and PG of lung surfactant. To check whether the alteration of PLs is attributed along with alteration in FA molecular species of membrane PLs, Fatty acids were analyzed by GC/MS. Alterations were monitored with two sets of reactions (i) tissues treated along with LPS, (ii) tissues post incubated with LPS in order to find out the changes happens in which fatty acids (newly synthesizing PL/synthesized PL).

Table 1A, B summarizes the fatty acid composition of PC and PG. In lung, FA composition of PC, PG showed significant alteration in post LPS treatment. The UFA/SFA of PC was increased from 0.253 to 0.325, in PG it was 0.348 to 1.249. Fascinatingly AA content was drastically increased in PC ($\sim$ 4.78-fold) and PG ($\sim$ 42-fold). In this present study FA analysis of lung phospholipid clearly showed that LPS has strong impact on synthesized PL. This *in vitro* result has been further validated by *in vivo* experiments. Rats selected from colonies were randomized into 4 groups, comprising of six rats each and are overnight fasted. Group I, Control animals (24 h saline treated); Group II, III and IV were given LPS (10 mg/kg body wt.) intraperitoneally and sacrificed after 6, 12 and 24 h.

2.4.3. LPS administration leads to lung neutrophil sequestration

Myeloperoxidase (MPO), a well known marker enzyme for lung neutrophil sequestration, increased ~ 7-fold in LPS-treated rats (group IV) when compared to control rats (group I). BALF cell analysis also revealed that total cell count increased significantly in LPS-treated rats when compared with control rats (data not shown). During differential count, the neutrophil content in group IV rats are significantly ($p<0.001$) elevated when compared to control rats, which also supported the neutrophil infiltration.

The excessive activation and migration of circulating neutrophils from blood to the alveolar airspace is one of the key events in the early development of ALI [Matusiewicz *et al.,* 1993]. It has been reported that LPS results in activation of the p38 mitogen activated protein kinase (MAPK), and inhibition of pulmonary MAPK activity abrogates LPS-induced TNF production, bronchoconstriction, neutrophil recruitment into the lungs and broncho-alveolar space [Togbe *et al.,* 2007; Schnyder-Candrian *et al.,* 2005].

2.4.4. Protein concentration of BALF confirms vascular leakage

Protein concentration of BALF was increased significantly in LPS-treated rats (group II - 122.32 ± 31.54 mg/dl, group III - 221.57 ± 26.97 mg/dl and group IV -280.47 ± 35.46 mg/dl), when compared with normal rats (Group I - 29.68 ± 14.97 mg/dl). This clearly showed the presence of pulmonary vascular leakage. Clinical studies have reported that IL-1β is one of the most biologically active cytokines in the airway of patients with acute lung inflammation, and IL-1β has been shown to inhibit fluid transport across the distal lung epithelium [Roux *et al.,* 2005] to cause surfactant abnormalities [Hybertson *et al.,* 2000] and to increase protein permeability across the alveolar–capillary barrier [Lee *et al.,* 2000].

LPS-induced oxidative stress in lung is well established and it was characterized by increased MDA, pulmonary MPO, BALF neutrophil content and BALF protein concentration [Mitsopoulos *et al.,* 2008; Rabinovici *et al.,* 1993; Simons *et al.,* 1991].

Hence to start with, it was necessary to determine the basic parameters to confirm oxidative stress and our results were in agreement with the afore-said previous reports. Previous *in vivo* studies using animal models that principally involved oxidative damage to the lung have usually shown severe stages of injury with marked changes in lung dysfunction [Zenri *et al.,* 2004; Dombrowsky *et al.,* 2006]. However, in most studies, level of antioxidants along with serum marker enzymes for organ dysfunction were not measured simultaneously, but our study correlated both.

2.4.5. Elevated serum marker enzymes depicted multiple organ failure

We showed dysfunction of lung, liver, heart, kidney and pancreas at a much lower dose of 10 mg/kg of LPS, even after a short exposure of 6 h and LPS-induced ARDS resulted in multiple organ failure that led to inflammation and tissue damage. Dysfunction in renal and hepatic tissues has been observed in animals after 24 h of LPS (7 mg/kg) exposure when induced intratracheally [Turner *et al.,* 1993]. Tissue injuries and functional disorders in lung, stomach, small intestine and liver have been reported after 180 min of post-intravenous LPS (10 mg/100g) administration in rats [Yoshikawa *et al.,* 1994]. In clinical studies with patients, it was shown that ARDS is associated with multiple organ failure [Clermont *et al.,* 2002].

Importantly, our data has showed that elevation of serum marker enzymes LDH, SGOT, CPK, amylase and creatinine, blood urea levels are responsible for multiple organ failure such as lung, liver, heart, pancreas and kidney damage in a time-dependent manner. The LD_{50} value of *E. coli* LPS was reported to be ~50 mg/kg. We showed dysfunction of multiple organs at a much lower dose of 10 mg/kg, even after a short exposure of 6 h.

2.4.6. LPS induced oxidative stress

The status of oxidants in lung tissue was represented by TBARS levels and had increased significantly ($p<0.001$) in group IV rats (0.3583 ± 0.02 μmol/mg protein of

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MDA) when compared to control rats (0.1747 ± 0.018 µmol/mg protein of MDA). The elevated TBARS levels provided evidence for massive oxidative stress.

2.4.7. LPS exposure leads to deficiency in lung antioxidants

The antioxidant enzymes were decreased significantly, such as SOD by 57%, CAT by 46% and GPx by 39% in group IV (24 h LPS-treated) rats, when compared with control rats. LPS administration resulted in a rapid and significant loss of more than 80% of pulmonary EC-SOD in a time and dose- dependent manner [Ueda *et al.,* 2008]. The non-enzymatic antioxidants (ascorbic acid and GSH) also declined in LPS-treated rats when compared to control rats. The decrease in ascorbic acid level was also reported in plasma of patients with sepsis or septic syndrome [Galey *et al.,* 1996]. A significant decrease in plasma levels of glutathione, ascorbate and elevated levels of lipid peroxidation products has been reported in patients with ARDS [Quinlan *et al.,* 1996; Kumar *et al.,* 2000].

An increase in the levels of antioxidant enzyme activities has been reported in serum of ARDS patients [Leff *et al.,* 1993; Leff *et al.,* 1992]. Increased lipid peroxidation and CAT activity, diminished GPx and SOD activity have been reported in lung tissues of animals inhaling 0.5 mg/ml LPS (3,000,000 EU/mg) dissolved in 2 ml of saline using an inhalation chamber daily for 5 days [Valenca *et al.,* 2008]. Improved oxidants and CAT activity after 180 min, declined activity of SOD has been observed in guinea pigs with 0.7 mg/kg of LPS, when given intraperitoneally [Matsuda *et al.,* 1995]. Increased TBARS Level and reduced SOD activity with no alterations in GPx activity is also reported, when LPS is administered to rats (10 mg/100 g) intravenously [Yoshikawa *et al.,* 1994].

Importantly in our present study, after LPS exposure, lungs showed a significant decrease in the levels of SOD, CAT, GPx and non-enzymatic antioxidants like ascorbic acid and GSH in group IV (24 h LPS-treated) rats, when compared with control rats. This pattern contradicted with the previous reports [Yoshikawa *et al.,* 1994; Valenca *et al.,* 2008; Matsuda *et al.,* 1995] and we would expect increased levels of antioxidant enzyme activities to compensate for the increased oxidative stress, so as to defend the animal

against ROS. The average half-life of lung epithelial cells is 17 months [Rawlins and Hogan, 2008]. The differing results observed might be explained by the fact that the life-span of lung cells is much longer than the observation interval or induction of ARDS and the resulting alterations were studied for a short span (0-24 h) and hence there was no sufficient time for adaptation to occur. Furthermore, it might be due to the down regulation of NF-kappaB signaling, which is linked to decreased antioxidant levels, increased oxidative stress, and enhanced cell death [Gilmore, 2006]. Histopathological examination also clearly revealed the effect of LPS on lung tissue damage. The present study demonstrated that intraperitoneal administration of a relatively lower dose of LPS (10 mg/kg body weight) led to ARDS in rats.

2.4.8. LPS impairs surfactant phospholipid metabolism

Pison and co-workers (1986) characterized the lung phospholipid alterations seen in septic patients and suggested that damage to the surfactant system is of primary importance in ARDS. LPS-induced acute pulmonary inflammation causes rapid changes in the composition of the surfactant pool and the resident cell population of the lung [Van Helden *et al.,* 1997]. In this present study, altered surfactant phospholipid metabolism was fully corroborated with experimental evidences using rat as a model system. We observed reduction of phospholipids, particularly PC, PG and was not accompanied with increased generation of lysophospholipids, but significant elevated level of SM (4.3-fold), PI (2.7-fold) were observed during 6 h LPS treatment. The decreased content of PC and PG potentially spoils the normal function of pulmonary surfactant.

The fraction of anionic phospholipids PG and PI in surfactant has been proposed to impart stability to the interfacial films during dynamic compression–expansion cycling [Ingenito *et al.,* 2000] through the interaction with the non-lipidic component, particularly hydrophobic proteins SP-B and SP-C [Perez-Gil *et al.,* 1995; Takamoto *et al.,* 2001].

A moderate change in the PL pattern in acute inflammatory lung diseases such as ARDS, pneumonia [Gregory *et al.,* 1991; Gunther *et al.,* 1996; Hallman *et al.,* 1982], idiopathic pulmonary fibrosis (IPF) patients had been observed [Honda *et al.,* 1988; Hughes and Haslam, 1989; Robinson *et al.,* 1988], comprising a reduction in the relative amount of PC and PG and a concomitant increase in PI and sphingomyelin (SPH/SM). This profile of altered distribution of PL classes in ARDS was prominently corroborated in our present study. The increase of membrane fluidity is determined by two other major factors, the relative content of SPH and the degree of unsaturation of the phospholipid acyl chains [Stubbs and Smith, 1984; Kawato *et al.,* 1978].

2.4.9. Impact of LPS on fatty acid composition of lung phospholipids

Alteration of surfactant composition and enhanced degradation may significantly influence the fatty acid composition of surfactant phospholipids. In our present study, we also observed significant changes in fatty acids with LPS. Lung exposed to LPS showed significant differences in the fatty acid composition of individual phospholipids, the most striking observations were the loss of palmitic acid (~14 %) in PC. This reduction was paralleled by a pronounced increase of unsaturated fatty acids in PC, mainly AA (C20:4), (C20:1), and (C20:3). This is in accordance with ARDS and severe pneumonia conditions where about 15-20% reduction in palmitic acid content was observed [Schmidt *et al.,* 2001]. Furthermore, Hallman and colleagues [Hallman *et al.,* 1982] reported a reduction of palmitic acid in PC (BALF) by ~47% in patients with ARDS. Both findings support our data of a marked reduction of palmitic acid in the PC fraction with LPS treatment. In addition to PC, we have also observed significant changes in PG, PE, PI and SPH fatty acid profiles.

In the current investigation, PI presented a FA profile nearly equal to that of PG, which is not surprising in view of the common synthesis pathway of these two PLs, whereby both originate from the same precursor, cytidine diphosphodiacylglycerol (CDP-DAG) [Batenburg, 1992]. This observation further supports the assumption that the acidic PI may fully replace PG during the neonatal development period [Beppu *et al.,*

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1983], as also suggested by *in vitro* studies addressing the biophysical activity of PG-enriched versus PI-enriched PL mixtures [Liau *et al.,* 1985; Hallman *et al.,* 1985].

The changed fatty acid profile severely affects the surfactant function. The lowering of surface tension especially at end-expiration is a result of a complex interaction of several surfactant compounds and is still not completely understood. The physicochemical considerations imply that DPPC may only be substituted by a limited number of other fatty acid in lowering surface tension. DPPC is predominant surfactant phospholipid in a gel-crystalline state at physiologic body temperatures, with rigid saturated acyl groups allowing dense packing of the molecule upon lateral compression of the film and thus lowering surface tension values [Von Wichert and Kohl, 1977; Pison *et al.,* 1996; Wright *et al.,* 2000; Perez-Gil and Weaver, 2010]. The altered fatty acid composition results in decreased alveolar stability, thereby contributing to the impairment of gas exchange as observed with ARDS and also during pulmonary dysfunction [Hohlfeld *et al.,* 1997; Gunther *et al.,* 2001; Schmidt *et al.,* 2001; Whitsett *et al.,* 2010]. In addition, a number of additional pathogenic events appear to contribute to the deterioration of surfactant function in acute respiratory inflammation, i.e., protein leakage into the alveolar space and changes in the surfactant protein content, especially SP-B and SP-C [Weaver, 1998; Brasch *et al.,* 2004; Whitsett *et al.,* 2010; Nogee, 2002], these findings suggest that the changes in the DPPC composition contributes significantly to the prominent loss of surface activity.

Our results reveal that LPS significantly diminishes the surfactant phospholipids by enhancing deacylation process accompanied with drastic changes in the fatty acid composition of major surfactant phospholipids, PC and PG. The acyl-chain composition of phospholipids is mainly determined by a deacylation-reacylation cycle named Lands' cycle [Lands, 1958]. Our fatty acid analysis reveals decreased palmitic acid content was compensated by increased AA content in PC and PG of LPS exposed lung. Acyltransferases are important in the regulation of membrane phospholipid fatty acyl composition along with PLA_2 enzymes and regulate AA incorporation and remodeling within phospholipids. Lysophospholipid acyltransferases are key control points for

cellular responses to a variety of stimuli including inflammation. Recently lung specific acyltransferase has been identified and characterised in mice. LPCAT is believed to play a specific role in the generation of the major constituents of pulmonary surfactant, DPPC [Chen *et al.,* 2006; Nakanishi *et al.,* 2006] and dioleoyl phosphatidylglycerol (DOPG) by LPGAT [Yang *et al.,* 2004; Funkhouser *et al.,* 1981; Sanford and Frosolono, 1983]. Regulation or manipulation of lysophospholipid acyltransferases may thus provide important mechanisms for novel anti-inflammatory therapies for ARDS. Hence, we examined the reacylation pathways by characterizing the remodeling enzyme mRNA expression in the presence of LPS.

2.4.10. Impact of LPS on the gene expression of PL remodeling enzymes.

Our results suggest that LPS significantly decreases the major surfactant phospholipids PC, and PG. These results have led us to investigate the influence of LPS on expression of PLA$_2$s. We have analysed four specific phospholipases (PLD$_1$, PLD$_2$, sPLA$_2$-IIA, and LPLA$_2$) and two specific acyltransferases (LPCAT, LPGAT), based on their importance in surfactant degradation/reacylation. Our results showed significant reduction in the mRNA expression of PLD$_1$, PLD$_2$, LPCAT and LPLA$_2$.

PLD, a phospholipid phosphohydrolase, catalyzes the hydrolysis of PC to PA and choline [Billah and Anthes, 1990]. Mammalian PLD isoforms, PLD1 and PLD2, share 55% sequence homology. Several studies have implicated a PLD-derived signaling pathway in the generation of prostaglandin in many cell types [He *et al.,* 2008; Kim *et al.,* 2004; Grab *et al.,* 2004; Zhang *et al.,* 2001; He *et al.,* 2006]. PLD activity and its product PA play an important role in a wide range of physiological processes and diseases, including inflammation, diabetes [Hammond et al. 1995], phagocytosis [Kusner et al. 1996], and the oxidative respiratory burst in neutrophils [Waite et al. 1997].

Apart from sPLA$_2$-IIA other PLA$_2$s like cytosolic-PLA$_2\alpha$, LPLA$_2$ also have an important role during lung inflammatory response, surfactant degradation [Ohtsuki *et al.,* 2006; Hite *et al.,* 1998; Hite *et al.,* 2005; Chabot *et al.,* 2003; Arbibe *et al.,* 1998; Abe

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et al., 2006; Balsinde *et al.,* 2006]. LPLA$_2$ is highly expressed in the lung and are participates in surfactant catabolism [Hiraoka *et al.,* 2006; Abe *et al.,* 2004]. LPLA$_2$ displays a substrate preference for PE and PC which is consistent with its role in surfactant degradation [Abe *et al.,* 2006 and 2004; Hiraoka *et al.,* 2006].

In our present study reduction in PC was not associated with elevated LPC and it was supported by decreased mRNA expression of LPLA$_2$ which is involved in surfactant PC remodeling and degradation, further corroborated by the depleted mRNA expression of LPCAT. Furthermore our results suggest that reduction in PC was not due to PLD also. Hence reduction in PC may be due to the hydrolysis of PC-specific phospholipase C (PC-PLC)/due to defective PC synthesis during endotoxemia.

Exposure of LPS on lung preferentially increases the mRNA expression of sPLA$_2$-IIA and LPGAT. In pathological conditions such as asthma, ARDS, and bronchial pneumonia, elevated amounts of sPLA$_2$ were found in the bronchial/alveolar lavage fluid (BALF) and lung tissue of patients [Arbibe *et al.,* 1998; Balestrieri and Arm, 2006; Chabot *et al.,* 2003; Masuda *et al.,* 2005; Okamoto *et al.,* 2007; Seeds *et al.,* 2003]. Increased expression of sPLA$_2$ colocalizes with areas of intense inflammation [Arbibe *et al.,* 1998].

From these results we confirmed that the reduction in PG was due to sPLA$_2$-IIA and further it was corroborated with increased RT-PCR expression of LPGAT. The activities of certain acyltransferases were significantly increased by lipopolysaccharide exposure [Schmid *et al.,* 2003]. Defective remodeling of PG and cardiolipin are part of the pathophysiology associated with Barth syndrome with mutations of an acyltransferase gene [Vreken *et al.,* 2000; Schlame *et al.,* 2003].
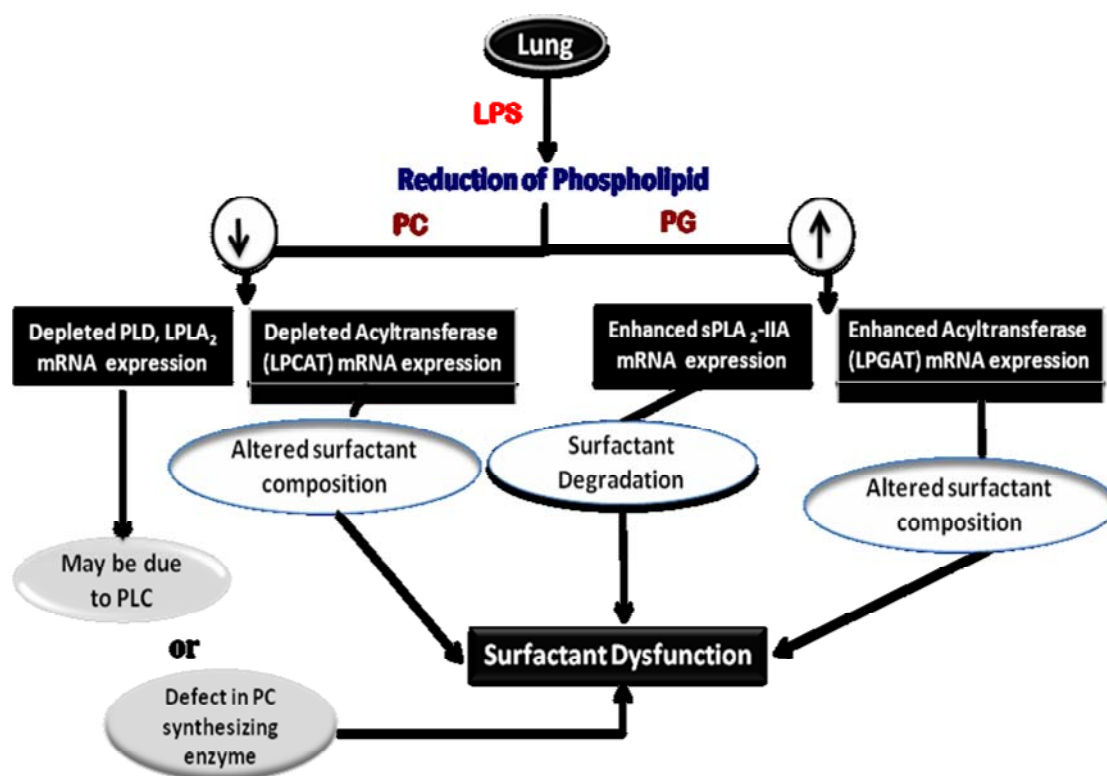
To summarize the present study, *in vitro* metabolic labelling with [32P] in the presence of LPS reveals that significant alteration in major surfactant PC and PG associated with changes in fatty acid composition of synthesized PL. To further understand the PL metabolism we have developed an animal model for ALI/ARDS via intraperitoneal mode administration of LPS (10 mg/kg body wt.) in male Wistar rats. The induction of ARDS was confirmed by neutrophil sequestration, vascular leakage and multiple organ failure (hallmark of ARDS). The antioxidants deficiency also attenuated the inflammation and tissue damage. LPS impairs lung phospholipid metabolism. The FA distribution in individual PL showed an overall increase in UFA content. This may be due to the sensitive response of the organs upon LPS treatment. The changes in the FA may directly or indirectly affect the membrane structure, fluidity. Reduction in PC was not associated with elevated LPC and it was further supported by decreased mRNA expression of LPLA₂ (which is involved in surfactant PC degradation) and LPCAT. Furthermore our results suggest that reduction in PC was not due to PLD. Hence, reduction in PC may be due to the hydrolysis of PC-specific phospholipase C (PC-PLC)/due to defective PC synthesis during endotoxemia. The defect in PG metabolism was confirmed by enhanced mRNA expression of sPLA₂-IIA and it was further validated by elevated LPGAT mRNA expression.

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Synopsis

LPS is a potent stimulator of immune response. The immunological effect of LPS is well documented in spleen and thymus; however impact on lipids particularly phospholipid metabolism is yet to be studied. Hence we are aimed to understand the role of phospholipid and relationship between fatty acids changes and immune impairment during endotoxemia. We are sought to focus on this by means of both *in vitro* and *in vivo* experiments. [^{32}P]orthophosphate metabolic labelling was used to study the spleen and thymus PL metabolism with different concentration of LPS (0-100 µg). For the first time we are reporting here that LPS specifically alters the major structural phospholipid, PC and PG of spleen and PC of thymus tissue. The fatty acid analysis showed marked alteration of UFA and SFA respectively of spleen PC, PG and thymus PC. We believe that these *in vitro* lipid metabolic results could open up new vistas for exploring LPS-induced immune impairment in spleen and thymus, besides underlying the mechanism. To understand the actual in living condition we have developed an animal model for ALI/ARDS and studied the role of phospholipids. In both spleen and thymus LPS impairs phospholipid metabolism. The FA distribution of individual PL showed overall increase in SFA content. This may be due to the adaptive response of the organs upon LPS treatment. The changes in the FA may directly or indirectly affect the membrane fluidity and structure. The mRNA expression of PC, and PG remodeling enzymes showed the reduction in PC was not due to PLD and it may be due to PC-specific phospholipase C or defective PC synthesis. The reduction in PG was due to sPLA$_2$-IIA.

Key words: LPS, spleen, thymus, phospholipids, fatty acids, immune impairment.

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3.1. Introduction

The mammalian immune system, a cooperative venture between the innate and acquired arms, offers an optimal environment for defense against the invasion of pathogens at any site in the body. Immune cells originate in bonemarrow and are found circulating in the blood stream, organized into lymphoid organs such as the thymus, spleen, lymphnodes and gut-associated lymphoid tissue, or dispersed in other locations. Different immune cell types have highly specialised roles (e.g. phagocytosis, antigen presentation, antibody production, destruction of virally infected cells) and acting together they create a coordinated and regulated immune response.

An effective immune response to pathogens is central to host defence and is essential to preventing infectious disease, a breakdown in the mechanisms that act to provide tolerance to self or to benign environmental allergens can lead to inappropriate immunologic activity and damage the host. Research conducted over the last 30 years or so suggests that changing the nature of fatty acid nutrition can be a means by which immune cell behaviour and the immune response can be modified, including its inflammatory component. Changes in membrane phospholipid fatty acid composition might be expected to influence immune cell function. It is generally accepted that biomembrane composition may be altered because of nutritional, environmental or xenobiotic exposure. [Yorio and Frazier, 1990].

Reported evidences mainly focused on immunological effect of LPS and there is paucity or lack of report of their effect in phospholipid metabolism and membrane remodeling in spleen and thymus. Hence understanding the role of phospholipid during endotoxemia may provide a better understanding between the relationship of fatty acids changes and immune impairment.