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The effect of diet-induced serum hypercholesterolemia on the surfactant system and the development of lung injury

Scott Milos [a,1], Joshua Qua Hiansen [a,1], Brandon Banaschewski [a], Yi Y. Zuo [b], Li-Juan Yao [a], Lynda A. McCaig [a], James Lewis [a,c], Cory M. Yamashita [a,c], Ruud A.W. Veldhuizen [a,c,*]

[a] Lawson Health Research Institute, Department of Physiology and Pharmacology, Western University, London, ON, Canada
[b] Department of Mechanical Engineering, University of Hawaii at Manoa, Honolulu, HI, USA
[c] Department of Medicine, Western University, London, ON, Canada

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ABSTRACT

Acute respiratory distress syndrome (ARDS) is a pulmonary disorder associated with alterations to the pulmonary surfactant system. Recent studies showed that supra-physiological levels of cholesterol in surfactant contribute to impaired function. Since cholesterol is incorporated into surfactant within the alveolar type II cells which derives its cholesterol from serum, it was hypothesized that serum hypercholesterolemia would predispose the host to the development of lung injury due to alterations of cholesterol content in the surfactant system.

Wistar rats were randomized to a standard lab diet or a high cholesterol diet for 17–20 days. Animals were then exposed to one of three models of lung injury: i) acid aspiration ii) ventilation induced lung injury, and iii) surfactant depletion. Following physiological monitoring, lungs were lavaged to obtain and analyze the surfactant system.

The physiological results showed there was no effect of the high cholesterol diet on the severity of lung injury in any of the three models of injury. There was also no effect of the diet on surfactant cholesterol composition. Rats fed a high cholesterol diet had a significant impairment in surface tension reducing capabilities of isolated surfactant compared to those fed a standard diet exposed to the surfactant depletion injury. In addition, only rats that were exposed to ventilation induced lung injury had elevated levels of surfactant associated cholesterol compared to non-injured rats.

It is concluded that serum hypercholesterolemia does not predispose rats to altered surfactant cholesterol composition or to lung injury. Elevated cholesterol within surfactant may be a marker for ventilation induced lung damage.

1. Introduction

The Acute Respiratory Distress Syndrome (ARDS) is a pulmonary disorder caused by one or multiple insults to the lung and is characterized by severe hypoxemia (PaO_2:$FiO_2 < 200$ mmHg) with radiographic bilateral lung infiltrates [1]. ARDS carries a mortality rate of approximately 40% and in lieu of effective pharmacological therapies, the mainstay of treatment remains supportive through the use of low tidal volume mechanical ventilation to maintain adequate arterial oxygenation [2,3]. The pathophysiology of ARDS is complex and involves multiple contributing factors including lung edema formation, maladaptive inflammation, and alterations to the pulmonary surfactant system [4,5]. Even prior to the development of ARDS, it has been suggested that certain individuals may be at an increased risk for the susceptibility to disease, and the development of poorer outcomes. An example of such susceptibility is chronic alcohol intake, which may be related to an increased vulnerability to some of these pathogenic mechanisms described above [6].

Among the various pathophysiological processes occurring in ARDS, alterations and dysfunction of the pulmonary surfactant system has been consistently observed across all causes. Dysfunctional surfactant not only contributes directly to altered lung physiology but also impacts local inflammatory responses [5,7,8]. Pulmonary surfactant is a lipoprotein complex synthesized and secreted by alveolar type II cells into the alveolar space where it reduces the work of breathing by stabilizing the lung through reducing the surface tension, especially during low lung volumes [9,10]. Obtained from bronchoalveolar lavage material, two extracellular surfactant subtypes can be isolated; the surface active large aggregates (LA) and the inactive small aggregates (SA) [11].

* Correspondence to: Lawson Health Research Institute, Western University, Room F4-110, 268 Grosvenor Street, London, ON, Canada N6A 4V2.
E-mail address: rveldhui@uwo.ca (R.A.W. Veldhuizen).
[1] SM and JQH have made equal contribution to this manuscript.

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The functional LA are composed of phospholipids (80–90%), neutral lipids (3–10%) – the majority of which is cholesterol, and surfactant associated proteins (5–10%). The precise balance of surfactant's constituent components is paramount to the material's biophysical properties and changes to the composition can impair surfactant's ability to function [10,12].

In the setting of ARDS, numerous studies have demonstrated profound alterations to the surfactant phospholipid species composition, surfactant proteins levels, and decreased levels of the LA surfactant subtype [5,7,8]. These changes, together with biophysical inhibition by serum proteins that have leaked into the lung, have traditionally thought to be the primary contributors to surfactant dysfunction [12–14]. Interestingly, recent in vitro biophysical studies and experiments with animal models of lung injury have demonstrated that elevated cholesterol within surfactant represents a distinct mechanism of surfactant dysfunction [15–17]. For example our group has previously shown that in a rat model of ventilator-induced lung injury (VILI) there was increased surfactant associated cholesterol in LA, contributing directly to surfactant's biophysical dysfunction. Furthermore, a reduction of cholesterol to baseline physiological levels could restore isolated surfactants innate biophysical function [17]. The mechanisms by which cholesterol content within surfactant increases in the setting of ARDS are unknown. It has been shown that under normal physiological conditions the majority of cholesterol found within surfactant is derived by uptake from the circulation [18,19], with subsequent intracellular regulation of this cholesterol to allow for the incorporation into surfactant [20,21]. It was therefore hypothesized that serum hypercholesterolemia would predispose the host to the development of severe lung injury due to changes to the cholesterol content within pulmonary surfactant. In order to test this hypothesis rats fed a standard and high cholesterol diet were subjected to three independent experimental models of lung injury.

2. Materials and methods

2.1. Animal procedures

Eighty-six 6-week old male Wistar rats (Charles River, St. Constant, Quebec, Canada) were used for these experiments. All procedures were approved by the Animal Care Committee at Western University and are in agreement with the guidelines of the Canadian Council of Animal Care. Animals were acclimatized for three days and had *ad libitum* access to water and a standard laboratory diet. Following acclimatization, animals were randomized to either a standard or a high-cholesterol diet (0.5% cholic acid and 1.25% cholesterol by mass; Harlan Teklad, Madison, WI, USA) and allowed food *ad libitum*. Rats were fed their respective diet for 17–20 days. Animals were subsequently exposed to one of three different models of experimental lung injury: 1) acid aspiration, 2) high-tidal volume mechanical ventilation, or 3) whole-lung lavage. In order to obtain non-injured controls, 5–6 animals from each diet group were euthanized by intraperitoneal sodium pentobarbital overdose (110 mg/kg) for determination of baseline serum cholesterol levels and lung lavage analysis as described below. High density lipoprotein (HDL) and triglycerides levels in serum were determined via enzymatic colorimetric assays by London Laboratory Services Group (London On).

At the outset of each experimental model of lung injury, surgical procedures were utilized for anesthesia and instrumentation as previously reported by Maruscak et. al [22]. Briefly, animals were anesthetized via intraperitoneal injection of 75 mg/mL ketamine and 5 mg/mL xylazine in sterile saline. After appropriate sedation, animals were given a subcutaneous injection of 0.1 mg/kg buprenorphine and a 0.2 mL subcutaneous injection of a 0.5% local anesthetic sensorcaine at the ventral neck area. After exposure of the ventral neck area, the left and right jugular veins and the right carotid artery were exposed and catheterized with PE-50 tubing. The left jugular catheter was used to deliver anesthetic/analgesic (0.5–2.5 mg/100 g/h propofol) and the right jugular catheter was used to continuously deliver fluid (sterile saline with 100 IU heparin/L at 0.5–1.0 mg/100 g/h). The carotid artery catheter was used to measure blood pressure, heart rate, collect blood for blood gas measurements (ABL 500 Radiometer, Copenhagen, DK), and continuously deliver fluids (sterile saline with 100 IU heparin/L at 0.5–1.0 mg/100 g/h). All fluids and anesthetics were delivered via infusion pumps (Harvard Instruments, St. Laurent, QC, Canada). After exposure of the trachea, a 14-gauge endotracheal tube was inserted and firmly secured.

Following these surgical preparations, animals were given a 0.1 mL IV bolus of 2 mg/mL neuromuscular inhibitor pancuronium bromide and then immediately connected to a volume-cycled rodent ventilator (Harvard Instruments, St. Laurent, Quebec, Canada) via endotracheal tube. The ventilator was set to initially deliver: 8 mL/kg tidal volume (Vt), respiratory rate (RR) of 54–58 breaths per minute (bpm), 5 cmH_2O positive end-expiratory pressure (PEEP), and a fraction of inspired oxygen (FiO_2) of 1.0. Animals were ventilated for fifteen minutes prior to initial baseline blood-gas measurements. To ensure animals did not have pre-existing lung injury, the baseline (BL) inclusion criteria following this procedure was $PaO_2/FiO_2 \geq 400$ mmHg.

2.2. Experimental models of lung injury

2.2.1. Acid aspiration

A model of acid-aspiration injury was utilized as described previously [23]. Following the initial surgical procedure, rats meeting the BL inclusion criteria were randomized to receive an intra-tracheal

Table 1
Baseline characteristics of non-injured rats fed a standard or high cholesterol diet for 17–20 days.

Baseline characteristics		**Standard diet (n=6)**	**High cholesterol diet (n=5)**
	Weight (g)	368.58 ± 4.97	362.63 ± 4.98
Serum analysis	Cholesterol (mmol/L)	1.50 ± 0.14	7.97 ± 1.57*
	High density lipoprotein (mmol/L)	1.12 ± 0.08	1.94 ± 0.20*
	Triglycerides (mmol/L)	0.735 ± 0.13	2.05 ± 0.20*
Lavage analysis	Total surfactant (mg/kg BW)	2.92 ± 0.30	2.28 ± 0.32
	Large aggregates (LA) (mg/kg BW)	1.79 ± 0.20	1.26 ± 0.14
	Small aggregates (SA) (mg/kg BW)	1.13 ± 0.20	1.02 ± 0.18
	% LA (% of total surfactant)	61.42 ± 4.93%	56.48 ± 2.67%
	Cholesterol (% of LA)	7.31 ± 0.40%	9.34 ± 1.51%
	Protein content (mg/kg weight)	12.17 ± 1.62	10.83 ± 1.39

* $P < 0.05$ Standard versus high cholesterol diet.