Surfactant before the first inflation at birth improves spatial distribution of ventilation and reduces lung injury in preterm lambs

David G. Tingay,1,2,3,4 Megan J. Wallace,5,6 Risha Bhatia,1,3,4 Georg M. Schmölzer,1,3,5 Valerie A. Zahra,5 Melinda J. Dolan,5 Stuart B. Hooper,5,6 and Peter G. Davis1,3,7

1Neonatal Research, Murdoch Childrens Research Institute, Parkville, Australia; 2Neonatology, The Royal Children’s Hospital, Parkville, Australia; 3Neonatal Research, The Royal Women’s Hospital, Parkville, Australia; 4Department of Paediatrics, University of Melbourne, Melbourne, Australia; 5The Ritchie Centre, Monash Institute of Medical Research, Monash University, Clayton, Australia; 6Department of Obstetrics and Gynaecology, Monash University, Clayton, Australia; and 7Department of Obstetrics and Gynaecology, University of Melbourne, Melbourne, Australia

Submitted 15 October 2013; accepted in final form 17 December 2013

Tingay DG, Wallace MJ, Bhatia R, Schmölzer GM, Zahra VA, Dolan MJ, Hooper SB, Davis PG. Surfactant before the first inflation at birth improves spatial distribution of ventilation and reduces lung injury in preterm lambs. J Appl Physiol 116: 251–258, 2014. First published December 19, 2013; doi:10.1152/japplphysiol.01142.2013.—The interrelationship between the role of surfactant and a sustained inflation (SI) to aid ex utero transition of the preterm lung is unknown. We compared the effect of surfactant administered before and after an initial SI on gas exchange, lung mechanics, spatial distribution of ventilation, and lung injury in preterm lambs. Gestational-age lambs (127 days; 9 per effect) received 100 mg/kg of a surfactant (Curosurf) either prior (Surf+SI) or 10 min after birth (SI+Surf). At birth, a 20-s, 35 cmH2O SI was applied, followed by 70 min of positive pressure ventilation. Oxygenation, carbon dioxide removal, respiratory system compliance, end-expiratory thoracic volume (via respiratory inductive plethysmography), and distribution of end-expiratory volume and ventilation (via electrical impedance tomography) were measured throughout. Early markers of lung injury were analyzed using quantitative RT-PCR. During the first 15 min, oxygenation, carbon dioxide removal, and compliance were better in the Surf+SI group (all P < 0.05). End-expiratory volume on completion of the sustained inflation was higher in the Surf+SI group than the SI+Surf group; 11 ± 1 ml/kg vs. 7 ± 1 ml/kg (mean ± SE) (P = 0.043; t-test), but was not different at later time points. Although neither achieved homogeneous aeration, spatial ventilation was more uniform in the Surf+SI group throughout; 50.1 ± 10.9% of total ventilation in the left hemithorax at 70 min vs. 42.6 ± 11.1% in the SI+Surf group. Surf+SI resulted in lower mRNA levels of CYR61 and EGR1 compared with SI+Surf (P < 0.001, one-way ANOVA). Surfactant status of the fetal preterm lung at birth influences the mechanical and injury response to a sustained inflation and ventilation by changing surface tension of the air-fluid interface.

OPTIMIZING THE RESPIRATORY support of preterm infants at birth may reduce the long-term complications of immaturity (4, 28). An initial sustained inflation (SI) at birth is often advocated as a method of aiding lung-liquid clearance, establishing a functional residual capacity (FRC), and uniformly aerating the preterm lung (12, 29, 30). The applied pressure of the SI needs to be sufficient to overcome the high resistance and long time constant of the liquid-filled respiratory system at birth (29, 30, 37). Thereafter, ventilation strategies need to account for the low compliance state of the surfactant-deficient lung. Proven adjunctive therapies such as exogenous surfactant replacement therapy also need to be considered (26).

It is plausible to expect that the therapeutic benefits of exogenous surfactant therapy will be maximized by quickly and uniformly aerating the lung beforehand (9, 22, 35). In preterm rabbits, an SI of 20 s and inflating pressure of 35 cmH2O was sufficient to fully aerate the lung at birth (29). Whether these parameters translate to other animal models or to newly born humans remains unclear (19). Excessively large inflations can cause lung injury (5) and negate the benefits of subsequent surfactant therapy (6). Conversely, inadequate aeration at birth lowers FRC, exposes the lung to regional shear forces and atelectasis, and inhibits surfactant function (35).

Administration of surfactant into the fetal trachea of the surfactant-depleted lung prior to the first inflation greatly improves the uniformity of aeration (25). This indicates that surface tension plays an important role in defining the spatial pattern of lung aeration, with a lower surface tension facilitating the movement of the air/liquid interface down both daughter airways at each branching point (25). Surfactant therapy is also known to improve the spatial distribution of ventilation in already-aerated preterm animal models (10). In contrast, pre-delivery of surfactant improved oxygenation and lung mechanics but did not mitigate lung injury, compared with surfactant administration at 5 min of life in preterm lambs that were resuscitated without SI (9). Notwithstanding the clinical difficulties of predelivery surfactant, these studies highlight the potential of preexisting surfactant to influence the spatial response of the immature lung to early resuscitation maneuvers at birth.

We hypothesize that the surfactant state of the preterm lung at birth will influence FRC, the distribution of ventilation and injury response to an SI, and early exogenous surfactant therapy. The aims of this study were to determine the effect of administering exogenous surfactant prior to birth compared with administration after resuscitation with an SI and positive pressure ventilation on gas exchange, lung volume, lung mechanics, spatial distribution of ventilation, and early markers of lung injury.

MATERIAL AND METHODS

This study was approved by the Animal Ethics Committee of the Murdoch Children’s Research Institute, Melbourne, Australia, in ac-

http://www.jappl.org 8750-7587/14 Copyright © 2014 the American Physiological Society 251
cordance with National Health and Medical Research Council guidelines.

Experimental instrumentation. Preterm lambs were delivered at 127–128 days of gestation (term is ~145 days) by caesarean delivery on anesthetized date-mated twin-pregnancy ewes. Twin-pregnancy ewes were chosen to minimize maternal and environmental variability, and to optimize the ethical principle of reduction. Prior to delivery, the fetal head was exteriorized, the carotid artery and external jugular vein were cannulated, and the trachea was intubated with a 4.0-mm cuffed ETT. Fetal lung liquid was then passively drained for 15 s prior to clamping the ETT. Exogenous surfactant (100 mg/kg; Curosurf, Chiesi Pharma, Italy) was administered via the ETT into the trachea using a premeasured catheter for those lambs randomized to predelivery exogenous surfactant therapy. The fetal thorax was exteriorized and dried, and 16 custom-built 23G EIT needles were placed subcutaneously equidistant around the fetal chest ~1 cm above the xiphisternum. These were secured using a 5-cm-wide self-adherent bandage (Coban; 3M, St. Paul, MN) (31). After connecting to a Goede-MF EIT system (Carefusion, Hoechberg, Germany), signal quality was confirmed with the proprietary EIT software. Immediately prior to delivery, RIP bands (Respibands; Sensormedics, Yorba Linda, CA) were secured around the chest (parallel to the upper margin of Coban) and immediately above the umbilicus. The lamb was then delivered, weighed, placed supine, and ventilated as per assigned protocol. Anesthesia and sedation of the lamb with iv infusions of ketamine and midazolam at doses sufficient to suppress spontaneous ventilation were maintained throughout the study.

Measurements. SpO2, heart rate, arterial blood pressure, and rectal temperature were displayed continuously from birth (HP48S; Hewlett Packard, Andover, MA). PAO and flow were measured at the airway opening using a Florian respiratory mechanics monitor sampling at 200 Hz (Acutronic Medical Systems, Hirzel, Switzerland). VL RIP was measured with DC-coupled RIP (Respitrace 200; NIMS, North Bay Village, FL) sampling at 200 Hz using a method we have described previously (32, 33). Summated RIP voltage signals from each band were zeroed immediately prior to unclamping the ETT. Relative changes in regional thoracic volumes were measured using EIT sampling at 25 Hz (10, 31, 34).

Ventilation strategies. Figure 1 summarizes the experimental protocol. Lambs were randomized to either predelivery exogenous surfactant therapy (Surf+SI) or surfactant at 10 min of life (SI+Surf) using the same dose as the Surf+SI group. For the Surf+SI groups, surfactant was administered via an in-line system (NeoLINK universal side-port adapter; Viasys MedSystems, Wheeling, IL). In both groups a 20-s SI at an inflating pressure of 35 cmH2O (NeoCuff T-piece Infant Resuscitator; Fisher and Paykel Healthcare, Auckland, New Zealand) was applied immediately upon unclamping the ETT, followed by 5 s at a PEEP of 6 cmH2O. The ETT was then clamped to prevent lung volume loss and PPV commenced in a VT mode (SLE 5000; SLE, South Croydon, UK). A PEEP of 6 cmH2O and inspiratory time of 0.4 s were used throughout the study. Initial VT was set at 7 ml/kg (maximum positive inflating pressure 50 cmH2O) and FIO2 was set at 0.21. FIO2 was adjusted to maintain SpO2 at 88–94%, and VTV was adjusted to maintain Paco2 between 45–60 mmHg on arterial blood gas analysis at 8, 15, 25, 40, 55 and 70 min of life.

At 70 min of life the animals were ventilated in 1.0 FIO2 for 3 min, and the ETT was clamped for 2 min to facilitate lung collapse by oxygen reabsorption. Then lambs were euthanized with 100 mg/kg of pentobarbitone and, after disconnection to atmosphere, a static supersyringe pressure-volume curve was performed to 40 cmH2O.

Data acquisition and analysis. PAO, flow, VL RIP, SpO2, heart rate, temperature, and arterial blood pressure were recorded at 1,000 Hz and digitalized (Powerlab and LabChart V7.2.5; AD Instruments, Sydney, Australia). Along with EIT, these were continuously acquired during the first 15 min of life and then for 2 min immediately prior to subsequent arterial blood gas analysis. EIT and VL RIP data were also recorded during the static pressure-volume curve to allow calibration and normalization. The last 30 s of continuous stable data were analyzed 1 min after completing the SI, at 5 min of life, and then to coincide with each arterial blood gas analysis. Applied inflation pressure, PEEP, and ΔP were determined from the PAO waveform offline. Vr and Cdyn were calculated from the flow and PAO data. Change in EEvRIP from birth was determined from the trough of the VL RIP signal (33) and calibrated to the known volumes from the static pressure-volume curve (LabChart Multipoint calibration module). AaDO2, as a measure of oxygenation, was determined from the arterial blood gas parameters.

To describe the mechanical status of the respiratory system and determine the adequacy of the SI duration, the time constant of the respiratory system during each sustained inflation was calculated using a one-phase association exponential model applied to the VL RIP time-course recording (17, 23) as follows: y = yo + (Plateau – yo)⋅[1 – e(–k⋅t)] where yo is the y value at time (x) zero, Plateau is the y value at infinite times, and k is the rate constant. The time constant (τ) was computed as the reciprocal of k, and was calculated only if the goodness of fit of the model was R2 > 0.7. An SI of at least three τ being required to achieve ≥95% of maximum (stable) VL RIP (17, 23).

EIT data were analyzed using our previously described methodology (2, 11, 31, 34). ΔZ signals were low-pass filtered to the respiratory domain (10 breaths per minute above the second harmonic of the respiratory rate) (1, 7) and referenced to the values immediately prior to the SI to determine relative change from fetal lung state. EEV was defined as the trough of the ΔZ signal. The ΔZ signal for the nondependent (ventral) and dependent (dorsal) hemithorax was normalized to the regional impedance values at 0 cmH2O (0%) and 40 cmH2O (100%) during the static pressure-volume curve to determine ΔZ%VCROI (11, 31).

Functional EIT images were constructed for each time point and the spatial distribution of ventilation was determined from these using the method described by Freerichs and coworkers (10). This allows the relative Vr within 32 nondependent to dependent equal slices of the right and left hemithoraces to be calculated and expressed as a fraction

![Figure 1](https://jap.physiology.org/DownloadedFrom/10.1152/japplphysiol.01142.2013)
of total VT (%) within the image for each of the 64 slices. Histograms of the fraction of ventilation were then generated to demonstrate the gravity-dependent distribution of ventilation within each hemithorax. From these data, the geometric center of gravity-dependent ventilation and the proportion of total VT occurring in each hemithorax were calculated (10).

Lung injury analysis. Lung samples were taken from the right lower lobe and immediately frozen in liquid nitrogen for qRT-PCR analysis of early markers of lung injury: CTGF; Cyr61; Egr1; and Il-1β, Il-6, and Il-8 mRNA. Wallace and coworkers have previously published a detailed description of our methodology (36). Results of qRT-PCR were analyzed using the 2−ΔΔCT method (36). Messenger RNA levels in each group were compared with an unventilated control group consisting of four 127-day gestation fetal lambs euthanized at delivery while on placental support and prior to lung aeration. One control fetus was excluded due to significant intrauterine growth restriction.

Statistical analysis. Data were tested for normality and analyzed with t-tests, the Mann-Whitney test, one-way ANOVA, or two-way ANOVA with posttests as appropriate. PRISM 4.02 (GraphPad Software, San Diego, CA) was used for analysis, and P < 0.05 was considered significant. Unless otherwise specified, reported P values refer to two-way ANOVA with a Bonferroni posttest.

RESULTS

Nine lambs were studied in each group. There was no difference in birth weight, cord pH, PaO2, or gender distribution; the pooled weight of animals was 3.0 ± 0.4 kg (mean ± SD), and pH 7.32 ± 0.07. In the Surf+SI group, 33% (3 lambs) were first-born, compared with 89% (8 lambs) in the SI+Surf group.

The exponential model could describe the change in VLRIP during the sustained inflation in both groups ($R^2 = 0.702–0.966$). The median $\tau$ was 1.7 s (range, 0.7–22.2 s) and 2.4 s (range, 0.5–10.3 s) for SI+Surf and Surf+SI, respectively ($P = 0.74$; Mann-Whitney test).

Thoracic volume. Predelivery surfactant administration resulted in a higher EEV RIP on completion of the 20-s SI compared with SI+Surf: 11 ± 1 ml/kg vs. 7 ± 1 ml/kg (mean ± SE) ($P = 0.043$, t-test). One minute after commencing PPV there was no statistical significant difference in EEV RIP: 11 ± 2 ml/kg in the Surf+SI group and 6 ± 2 ml/kg in the SI+Surf (means ± SE) ($P = 0.08$, t-test). Although EEV appeared greater in the Surf+SI group in the first 15 min of life, there was no statistical difference in EEV RIP between the two groups (Fig. 2A). By 70 min of life both strategies resulted in an EEV RIP of ~27 ml/kg using a PEEP of 6 cmH2O. There was no statistical difference in the regional end-expiratory volumes (Fig. 2, B and C), although Surf+SI lambs tended to a higher $\Delta Z_{\text{SI, VCrit}}$ in the nondependent lung. Neither strategy was able to achieve regional end-expiratory volumes above 75% of vital capacity in any lung region.

Gas exchange. AaDO2 over time is shown in Fig. 3A. Overall, predelivery surfactant resulted in better oxygenation ($P < 0.0001$; two-way ANOVA), especially in the first 15 min of life ($P < 0.05$). By 25 min of life there was no significant difference in oxygenation between the two groups.

Paco2 was outside of target range in both groups at 8 min of life (Fig. 3B): 40.1 ± 3.3 mmHg and 62.1 ± 6.7 mmHg for Surf+SI and SI+Surf, respectively (means ± SE) ($P < 0.01$). Paco2 remained below target range, and significantly lower than SI+Surf group, until 25 min of life in the Surf+SI group; thereafter, there was no difference between the groups.
ysis this was significant only at 70 min; mean (95% CI) difference 10.3 cmH2O (0.7–19.9) (95% CI). Overall, \( C_{\text{dyn}} \) was higher in the Surf+SI group (Fig. 3E), with greatest differences at 1 and 8 min (\( P < 0.05 \)). All data are means ± SE.

Spatial distribution of ventilation. Figure 4 shows the spatial distribution at key time points in the study for both groups. Overall, Surf+SI resulted in more uniform ventilation between the right and left lungs from birth that persisted throughout the study (Fig. 5). In the SI+Surf group, the right lung received disproportionately more ventilation than the left from birth, especially in the dependent hemithorax. Surfactant administration did not alter the nonuniform ventilation; in fact, right to left lung discrepancy was greatest at 70 min (\( P < 0.05 \), Fig. 6). There was no difference in the gravity-dependent geometric center of ventilation between the two groups, although Surf+SI trended to more uniform distribution.

Lung injury. The qRT-PCR mRNA data are shown in Table 1. Surf+SI resulted in lower mRNA levels of \( CYR61 \) and \( EGR1 \) compared with SI+Surf (\( P < 0.001 \); one-way ANOVA with Tukey’s posttest). Compared with the unventilated control group, the SI+Surf group had significantly greater mRNA levels of all markers, except \( IL-6 \) (all \( P < 0.05 \); Tukey’s posttest). Surf+SI was significantly higher than the unventilated control group only for \( CTGF \), \( IL-1\beta \), and \( IL-8 \) (all \( P < 0.05 \); Tukey’s posttest).

DISCUSSION

Despite the acceptance of early surfactant therapy (26) and optimizing aeration at birth (12), the interaction between the two has been poorly examined using current approaches to newborn ventilation. This is the first study to explore the influence of surfactant status at birth on the ability of an SI to facilitate lung aeration, establishment of FRC and uniform ventilation, and subsequent protection of the lung from severe injury. In our preterm animal model, we demonstrated improved FRC during an SI and persistent uniform distribution of ventilation if exogenous surfactant was delivered to the lung prior to an SI. These factors resulted in better lung mechanics and gas exchange and reduced activation of early biomarkers of lung injury. More importantly, this highlights that the surfactant status of the lung will influence the response to an initial SI at birth.

Fig. 3. Changes in \( \text{AaDO}_2 \) (A), \( P_{\text{ACO}_2} \) (B), \( V_T \) (C), \( \Delta P \) (D), and \( C_{\text{dyn}} \) (E) from birth after a sustained inflation with either surfactant administration predelivery (Surf+SI; open diamonds) or at 10 min of life (SI+Surf; closed diamonds). †Significant difference between two strategies at the denoted time point (\( P < 0.05 \)). All data are means ± SE.
Rapidly establishing FRC and lung aeration is critical to successful ex utero transition (25, 30). This process requires the clearance of fetal lung liquid via the distal movement of the air-liquid interface through the respiratory tree (12). Surfactant reduces surface tension and improves lung mechanics, and early postdelivery administration reduces lung injury (26). The association between the pattern of ventilation and early improvements in lung mechanics and $P_{aCO_2}$ observed in the group treated with surfactant prior to birth support the hypothesis that surface tension is also important.

Fig. 4. Fractional distribution of ventilation within 32 nondependent to dependent slices of the left (open bars) and right (gray bars) hemithorax at key time points after surfactant administration at 10 min of life (SI+Surf; top) and before birth (Surf+SI; bottom). Data are mean and SD of functional EIT values.

Fig. 5. Difference between the gravity-dependent distribution of $V_T$ in the right and left thorax at 1, 15, and 70 min of life in the SI+Surf and Surf+SI groups. A value of 0% indicates uniform $V_T$ between each hemithorax within that thoracic region. Data are mean and SD.
in determining the movement of the air-liquid interface through the respiratory tree during lung aeration, and subsequent uniformity of aeration (25).

Sustained inflations have been proposed as a method of providing sufficient duration and magnitude of transpulmonary pressure to facilitate uniform aeration at birth (29, 31). Nonuniform aeration results in heterogeneous regional lung volumes and mechanics, and increased risk of lung injury from atelectasis (21, 27, 38), shear forces (4, 8), and regional volutrauma (14, 18). The finding that post-SI ΔEEV_RIP was almost double in the Surf + SI group and Surf + Surf (closed diamonds) groups. A value <50% of the chest diameter indicates that ventilation was distributed toward the nondependent regions of the thorax. Data are mean and SD.

Table 1. Markers of lung injury determined by qRT-PCR

<table>
<thead>
<tr>
<th>Strategy</th>
<th>CTGF</th>
<th>CTGF_6H</th>
<th>EGR1</th>
<th>IL-1β</th>
<th>IL-6</th>
<th>IL-8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Surf + Surf</td>
<td>6.3 ± 1.9*</td>
<td>16.8 ± 15.7†</td>
<td>18.5 ± 24.0†</td>
<td>26.4 ± 16.9*</td>
<td>19.3 ± 9.4</td>
<td>37.5 ± 16.4*</td>
</tr>
<tr>
<td>Surf + SI</td>
<td>6.2 ± 5.8*</td>
<td>2.2 ± 2.0</td>
<td>0.2 ± 0.1</td>
<td>21.2 ± 16.7*</td>
<td>11.3 ± 12.5</td>
<td>52.6 ± 45.0*</td>
</tr>
<tr>
<td>UVC</td>
<td>1.0 ± 0.4</td>
<td>1.0 ± 0.7</td>
<td>1.0 ± 0.3</td>
<td>1.0 ± 0.3</td>
<td>1.0 ± 0.1</td>
<td>1.0 ± 0.7</td>
</tr>
</tbody>
</table>

Messenger RNA levels are shown as mean ± SD expressed relative to a UVC group (n = 3). *Indicates a significant difference with UVC. †Indicates significant difference with Surf + SI (one-way ANOVA with Tukey’s posttest).
ratory mechanical monitoring (22) and expired CO₂ (13) have been proposed as solutions, and RIP and EIT also offer promise (31, 38).

Neither group was able to achieve uniformity of EEV throughout the thorax. In particular, relative aeration was poor within the dependent hemithorax in both groups. This is in contrast to the study by Siew and coworkers that demonstrated more uniform aeration during the first 7 min of life in preterm rabbit pups receiving surfactant prior to birth and PPV with PEEP (25). There are a number of plausible explanations. Phase-contrast radiography was used to image the lung in the study by Siew and coworkers. This technique provides significantly greater spatial resolution than EIT, although it has no clinical application. We studied a preterm lamb model, which is likely to have significantly different chest wall mechanics than a preterm rabbit pup. Unlike the study by Siew and coworkers, we applied an SI to the lung to facilitate aeration. Our choice of SI duration and applied pressure was based on existing data (29). At birth, the time constant of the lung, and hence duration and magnitude of SI to augment lung liquid clearance, is unknown. The modeled time constants of the volume response during the SI suggested that full aeration might not have been achieved in all lambs.

The finding that lung mechanics and injury were different when surfactant was administered after the SI is important in the context of translating animal model research. Administration of surfactant prior to birth is common practice in animal model studies of preterm ventilation (20), but clearly impractical in clinical environments. Our study shows that clinical interpretation of the outcomes of SI strategies in preterm animal models needs to be considered within the context of the animal model experimental design.

Limitations. This study has some additional limitations. Our lambs were not exposed to antenatal corticosteroids. Whether the same differences would have been observed requires investigation. Spontaneous respiratory effort was intentionally suppressed in our study, and ventilation was applied using auffed ETT, unlike preterm infants. Our findings, in an established animal model of prematurity that requires higher pressures to ventilate than in humans, should be considered in this context. The strategy of mechanical ventilation employed after the initial resuscitation maneuver is also important, and warrants further investigation, particularly the role of different modalities such as volume-targeting and high-frequency ventilation (18, 22, 27, 33, 35). The endogenous surfactant state was not known at birth and may have influenced outcomes. Clinicians are unaware of this variable at birth too, although recently simple bedside tests to indicate surfactant state have been described (3). The method of draining lung liquid before birth was standardized, but there may still have been differences in the effective residual volume that may have influenced the response to SI. The limitations of EIT and RIP to measure relative changes in volume have been well described previously (16, 33) and are pertinent to this study. RIP in particular cannot differentiate between intrathoracic air and liquid changes, both of which are significant during ex utero transition.

Conclusions. In preterm lambs, we showed that the presence of exogenous surfactant in the fetal lung liquid at birth improved the mechanical response of lungs to an SI. The benefits in $C_{\text{dyn}}$, gas exchange, and early biomarkers of lung injury were only partially mitigated by surfactant therapy shortly after birth. Our study illustrates the influence of surfactant status within the lungs at birth with short- and longer-term responses to an SI and subsequent PPV. The interaction between adjunctive therapies and mechanical ventilation in early preterm life need to be considered in the development of resuscitation strategies for preterm infants.

ACKNOWLEDGMENTS

We thank Dr. M. Sourial and S. Osterfield for their assistance in preparing the ewe and lamb model.

GRANTS

This study is supported by National Health and Medical Research Council (NHMRC) Project Grant 1009287 and by the Victorian Government Operational Infrastructure Support Program. D. Tingay is supported by NHMRC Clinical Research Fellowship Grant 1009287. P. Davis is supported by NHMRC Practitioner Fellowship Grant 556600 and Program Grant 556600. M. Wallace is supported by NHMRC Project Grant 1044775.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS


REFERENCES


