

Lithium from breast-milk inhibits thyroid iodine uptake and hormone production, which are remedied by maternal iodine supplementation

Irfan Ahmed^{1,2} | Victor Ma³ | Yuanchao Liu¹ | Muhammad Shehzad Khan¹ | Zhenhui Liu⁴ | Chi Zhang⁴ | Santosh Kumar Paidi⁴ | Francis A. M. Manno¹ | Noreen Amjad¹ | Sinai H. C. Manno^{1,5}  | Rafay Ahmed¹ | Alan W. L. Law¹ | Ahmed Ali²  | Faizan Raza⁶ | Yanpeng Zhang⁶ | William C. S Cho³ | Ishan Barman^{4,7,8} | Martin Alda⁹  | Veerle Bergink^{10,11} | Condon Lau¹ 

¹Department of Physics, City University of Hong Kong, Hong Kong SAR, China

²Department of Electrical Engineering, Sukkur IBA University, Sukkur, Pakistan

³Department of Clinical Oncology, Queen Elizabeth Hospital, Hong Kong SAR, China

⁴Department of Mechanical Engineering, Johns Hopkins University, Baltimore, MD, USA

⁵Department of Biomedical Sciences, City University of Hong Kong, Hong Kong SAR, China

⁶Key Laboratory for Physical Electronics and Devices of the Ministry of Education & Shaanxi Key Lab of Information Photonic Technique, Xi'an Jiaotong University, Xi'an, China

⁷Department of Oncology, Johns Hopkins University School of Medicine, Baltimore, MD, USA

⁸Department of Radiology & Radiological Science, Johns Hopkins University School of Medicine, Baltimore, MD, USA

⁹Department of Psychiatry, Dalhousie University, Halifax, Canada

¹⁰Department of Psychiatry, Icahn School of Medicine at Mount Sinai, New York, NY, USA

¹¹Department of Obstetrics, Gynecology and Reproductive Science, Icahn School of Medicine at Mount Sinai, New York, NY, USA

Correspondence

Condon Lau, Department of Physics, City University of Hong Kong, Hong Kong SAR, China.

Email: condon.lau@cityu.edu.hk

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Abstract

Background: Lithium is especially taken as a maintenance medication for Bipolar Disorder. In women with bipolar disorder, lithium is often effective during postpartum period, but breast-feeding for medicated mothers is controversial because of harmful effects for her child. At present, the biological mechanisms of lithium are not well-understood, affecting its usage and overall health implications.

Procedure: We developed a rat lithium and breast-feeding model at human therapeutic levels to study the effects of lithium exposure through breast-milk on pups' thyroid function. Novel laser analytical spectroscopy, along with traditional blood and immunohistochemical tests, were applied to further investigate the mechanisms behind the thyroid dysfunction. Maternal iodine supplementation was evaluated as a therapeutic method to address the pups' thyroid dysfunction.

Results: Pups exposed to lithium via breastmilk, even with the dam on a sub-therapeutic level, experienced weight gain, reduced blood thyroxine (T_4), and elevated blood urea nitrogen, indicating effects on thyroid and kidney function. We show that lithium inhibited iodine uptake by thyroid follicles, initiating a mechanism that reduced

iodination of tyrosine, thyroglobulin cleavage, and thyroid hormone production. Importantly, infant thyroid function can be significantly improved by administering supplementary iodine to the medicated dam's diet during breast-feeding.

Conclusion: These results elucidate the mechanisms of lithium in thyroid function, provide valuable information on use postpartum, and suggest a clinically applicable remedy to side-effects. The results are particularly important for patients (and their infants) who respond well to lithium and need, or choose, to breast-feed.

KEYWORDS

analytical chemistry, bipolar disorder, endocrinology, lithium, pediatrics, psychiatry

1 | INTRODUCTION

Bipolar disorder (BD) is a severe psychiatric illness affecting between 2% and 3% of the general population and considered amongst the ten leading causes of reduction in disability-adjusted life years (World Health Organization, WHO).^{1,2} Lithium salts are considered one of the most effective prophylactic treatments of BD³ and one a top choice in most bipolar guidelines.

Importantly, many patients who respond well to lithium do not respond well to other treatments.⁴ However, lithium's benefits come, for some patients, at a cost as long term use of lithium can lead to reduced thyroid function as well as other adverse effects including a risk of kidney damage.⁵⁻⁸ Some of these concerns are particularly relevant in relation to pregnancy and breast feeding. Women with BD are at especially high risk of relapse in the postpartum period (meta-analysis estimated the risk at 38%), especially without medication (66%).⁹ While lithium can be effective, its benefits need to be weighed against the possible risk from exposure of infants to lithium in breast milk. Only few and limited human studies have examined the effects of lithium during the breast-feeding period.¹⁰⁻¹² In absence of strong data, many current practices and guidelines in post-partum often discourage the use of lithium during breast-feeding, due to sleep loss for the mother and transient abnormalities of thyroid-stimulating hormone, blood urea nitrogen, and creatinine in infants.¹² The most recent publications suggest that lithium should be given to nursing mothers "with caution"¹³ and "with monthly monitoring of blood lithium concentration",¹⁴ but other experts advise against breast-feeding altogether.¹⁵ The downside of these recommendations is the loss of the breastfeeding benefits both to the infant and the mother.^{11,16} All of these recommendations are based on case series only. There are several factors contributing to the paucity of data on lithium and breastfeeding. In developed countries, the number of potential research subjects is limited as lithium use has decreased the last decades and many mothers on medication choose infant formula over breast-feeding. In developing regions, breastfeeding with lithium is more common, because lithium is widely used¹⁷⁻¹⁹ and infant formula is expensive and not always available. Breast-feeding in developing regions occurs in 90%²⁰

of mothers, but a limited research infrastructure in these countries hampers the research potential. Last but not least, the low mass lithium atom is difficult to detect at trace levels (mmol/L) in small, solid biological samples (μm^3 – mm^3) using traditional analytical methods such as mass spectroscopy and electrochemistry. Lithium has been detected in blood plasma by capillary ion analysis²¹ and in brain tissue by neutron activation analysis.²² These available techniques are less suited for rapid and in situ analysis of solid biological samples in the μm^3 – mm^3 range.

In our study, we used an animal model of nursing with and without lithium, in combination with a highly sensitive method of measuring low lithium concentrations. Our aim was to test to what extent is lithium detectable in maternal milk and whether it affects the nursed pups' development. We hypothesize that lithium will reach the pups and significantly affect thyroid funding by inhibiting thyroid iodine uptake, similar to our earlier observations in adult rats.^{23,24} Based on the above findings, we also tested whether it was possible to reverse the adverse effects of lithium on pup thyroid function by iodine supplementation of the nursing rats (ie. mothers).

2 | METHODS

2.1 | Overview

In this study, we developed a rat lithium and breast-feeding model adapted from our earlier adult model.²³⁻²⁵ Nursing mother rats received lithium at human therapeutic levels (1000 mg/12 hours/50 kg) and effects on their pups were studied at the organ, tissue, cell, molecular, and ion levels. This model is free of confounding by indication (the underlying maternal disease) and lithium doses are well-controlled. Lithium was primarily analyzed using laser induced breakdown spectroscopy (LIBS), a novel method highly sensitive to lithium in small samples of.²³⁻²⁶ LIBS was performed on thyroid tissues. The LIBS laser ablates an $\sim\mu\text{m}^3$ volume of sample to obtain a spectrum. The spectrum contains emission lines corresponding to elements present in the sample. The amplitude of a line is directly related to the amount of the element in the sample. LIBS is also highly sensitive to other low mass and biologically important elements such as lithium, sodium, magnesium, potassium,

and calcium.^{23–28} Note that rat samples are very small compared with human samples, making LIBS very important for this study. For further details on data acquisition, analysis, and instrumentation of LIBS, see Data S1. Blood and breast-milk from the dams were analyzed for lithium using inductively coupled plasma mass spectrometry (ICP-MS) and LIBS respectively. Blood from pups was analyzed for lithium, along with total thyroxine (T_4), free T_4 , free triiodothyronine (T_3), thyroid stimulating hormones (TSH), and urea nitrogen (BUN), during, shortly after, and long after breast-feeding. T_3 , T_4 , and TSH are markers of thyroid function and BUN is a marker of kidney function. Pup body weight was monitored throughout the study. Pups were behaviorally assessed using a forced-swim test during breast-feeding. The thyroids and brains of pups underwent trace element analysis for lithium, iodine, sodium, potassium, and calcium during and shortly after breast-feeding. Based on the blood test results, thyroid was further analyzed for thyroglobulin expression during breast-feeding. Finally, we tested whether it is possible to reverse the adverse effects of lithium on pups' thyroid function by iodine supplementation of the nursing rats. The study overview is graphically presented in Figure 1.

2.2 | Animals

Pregnant female Sprague Dawley rats (SD) ($N = 26$, weight 350–450 g) entered the study at one week prior to giving birth. The rats were provided by the AAALAC accredited Laboratory Animal Unit of the University of Hong Kong. All the animals were housed individually in the Laboratory Animal Research Unit (LARU) of the City

University of Hong Kong. The unit had 12/12 hour light/dark cycles, constant temperature of 25°C, and humidity of 60% to 70%. Dams had regular access to chow food and drinking water. Each mother's delivery day was noted. On the fourth day postpartum (postnatal day 4 for the pups, P4), all pups (hereon referred to as pups or subjects) from each mother were weighed and the $N = 12$ heaviest were chosen for breast-feeding, with equal numbers of males and females. Fixing the litter size is necessary as rats of this age can deliver 12–18 pups, with the variability affecting subsequent analyses. This study employed three time points: P18 (during nursing), P25 (shortly after weaning), and P60 (long after weaning). Note that the rat nursing period is approximately 21 days. There were a total of $26 \times 12 = 312$ subjects in this study. There were $N = 18$ ($N = 7$ control, $N = 7$ lithium, and $N = 4$ lithium+iodine) dams rearing P18 pups, $N = 6$ ($N = 3$ control and $N = 3$ lithium) rearing P25 pups, and $N = 2$ ($N = 1$ control and $N = 1$ lithium) rearing P60 pups. The pups underwent a range of analyses at the time points, including blood testing, analytical spectroscopy, and immunohistochemistry. This study was approved by the animal research ethics committees of the City University of Hong Kong, the University of Hong Kong, and the Department of Health of the Hong Kong Special Administrative Region.

2.3 | Lithium preparation and administration

Lithium carbonate (Li_2CO_3 , 62470-100G-F) was purchased from Sigma Aldrich (USA). For Li treated dams, we used a dose of 1000 mg per 12 hours of Li_2CO_3 per 50 kilogram of body weight,

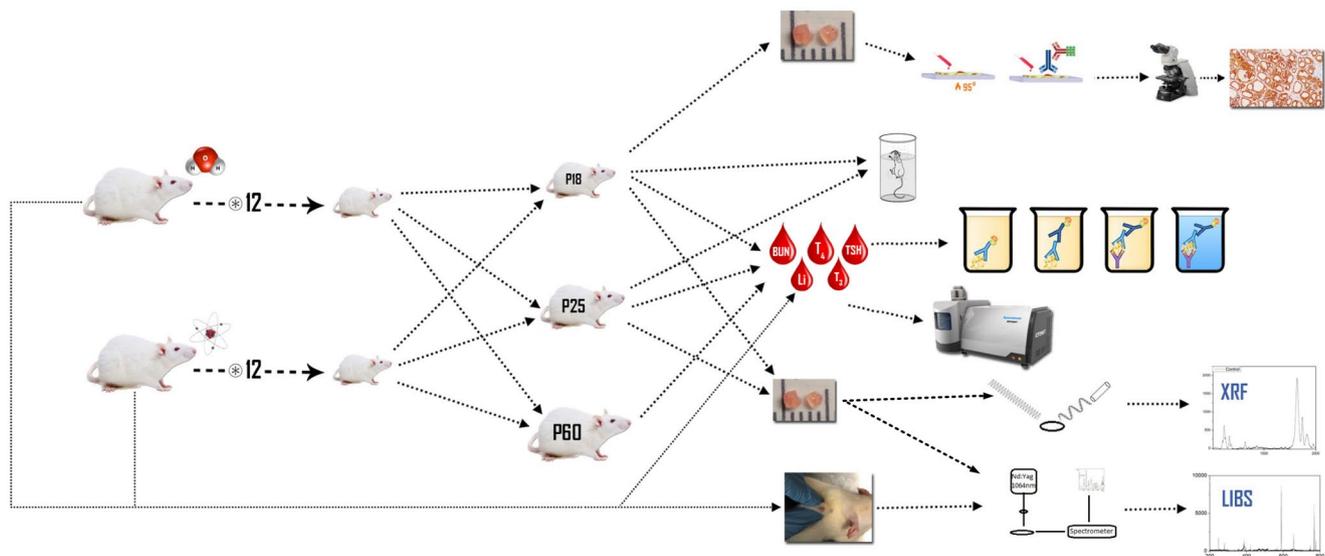


FIGURE 1 Work flow of the study and illustration of methodology. Groups of lithium ($N = 11$), control ($N = 11$) and lithium+iodine ($N = 4$) dams (mother rats) on the left giving birth to pups. Each litter was reduced to 12 pups only. The mother's breast milk is analyzed with LIBS. The pups ($N = 312$) from all groups at time points postnatal day (P) 18, P25 and P60 go through body weight measurements ($N = 312$), immunohistochemistry ($N = 24$), forced swimming ($N = 48$), blood measurements (Li, urea nitrogen, total thyroxine (T_4), free T_4 , free triiodothyronine (T_3), thyroid stimulating hormone (TSH)) ($N = 240$) and multi-elemental spectroscopy methods ($N = 48$)

resulting in plasma Li level of ~ 0.5 mmol/L (see Figure S1, at the lower end of the therapeutic window for humans.²⁹ This measurement was taken at P18 after the pups were removed for analysis. Prior to administering Li_2CO_3 , the animals were weighed daily. Subsequently, subject specific 1 ml solutions of Li_2CO_3 were prepared to the right concentration in individual 10 ml tubes. The Li_2CO_3 salt was dissolved into distilled water with the help of magnetic stirring for 30 minutes at room temperature. Later, the respective 1 ml solutions were fed using gavage. This daily regimen of lithium treatment was continued throughout the nursing period (P4 to approximately P21). Control mothers ($N = 11$) were similarly gavaged with water only.

2.4 | Iodine supplement preparation and administration

Molecular iodine (I_2 , 326143-100G) was purchased from Sigma Aldrich (USA). A 0.05% iodine solution was prepared by adding 0.5 g of crystal molecular iodine to 1000 ml of distilled water. To dissolve the iodine completely, the solutes were magnetically stirred in a light-sealed beaker for 48 hours at room temperature. Each time a fresh 0.05% (500 $\mu\text{g}/\text{ml}$) iodine solution was prepared, it was placed in light-sealed 500 ml drinking bottles. The bottle was placed in the cage of a breast-feeding dam administered lithium supplemented with iodine (lithium+iodine). The water intake of adult rats weighing

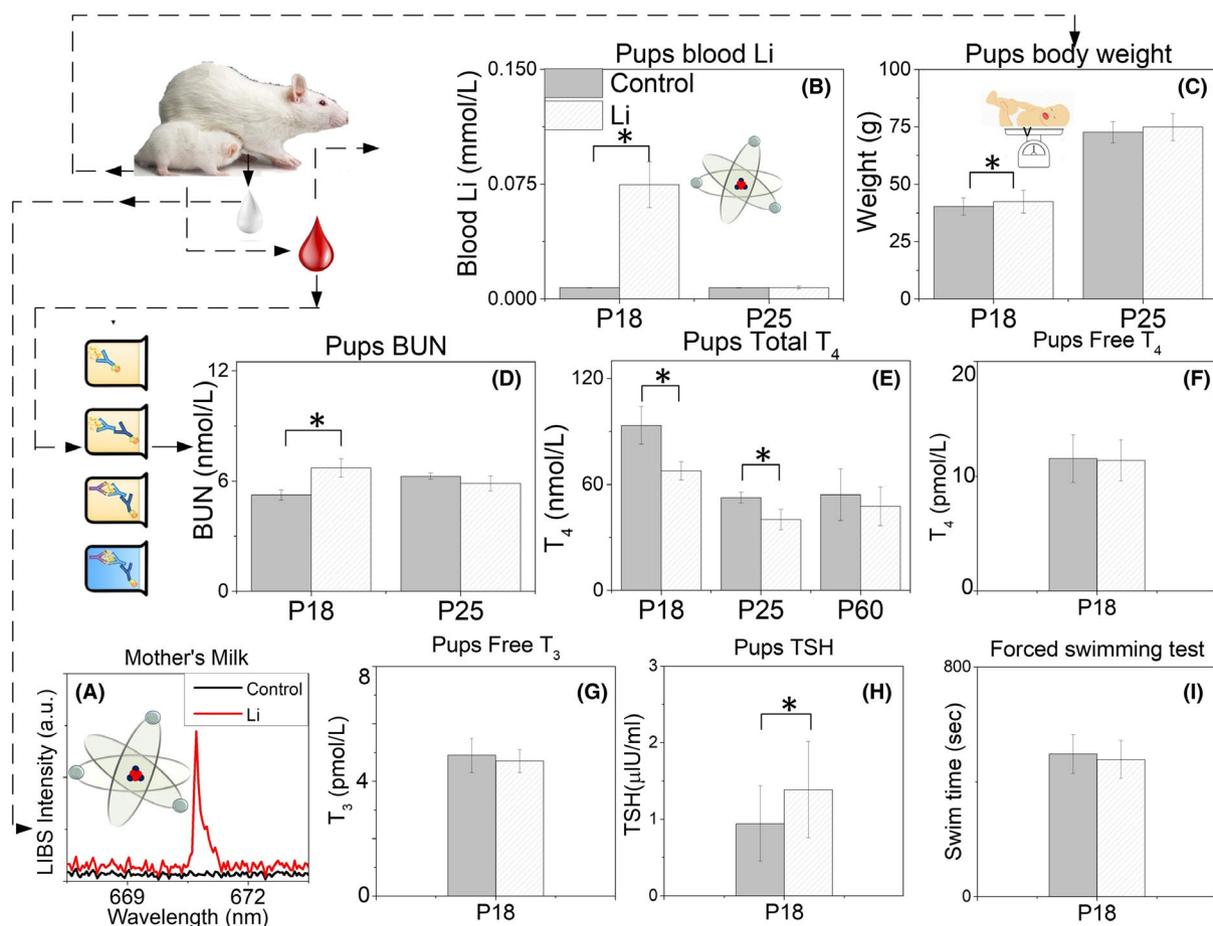


FIGURE 2 Health assessment of pups. (A) Group-averaged LIBS spectra from the breast milk of control lactating dams ($N = 3$) and lithium treated dams ($N = 3$) expanded about 670 nm. A lithium (Li) emission line is observed in the milk of treated subjects only at 670.7 nm. (B) Blood lithium levels in breast-fed pups ($N = 24$) during (P18), shortly after (P25), and long after (P60) breast-feeding. Lithium is detected in the blood of pups of lithium treated dams during breast-feeding only. (C) Body weight of pups at P18 ($N = 268$) and P25 ($N = 48$). Body weights at P60 ($N = 24$) are shown in Figure S2. In general, pups exposed to lithium weigh slightly more and the difference is statistically significant ($p < 0.05$) during breast-feeding. (D) Blood urea nitrogen (BUN) levels in pups ($N = 24$) at P18 and P25. BUN is reduced in lithium pups during breast-feeding, indicating transient kidney dysfunction. (E) Blood total thyroxine (T_4) in pups ($N = 24$) at P18, P25, and P60. In general, total T_4 is lower in lithium pups, indicating prolonged inhibition of thyroid hormone production. Combined with the increased body weight, total T_4 reduction indicates that breast-fed pups of medicated dams suffer symptoms similar to those of hypothyroidism. (F-H) show blood free T_4 and triiodothyronine (T_3), and thyroid stimulating hormones (TSH) in pups ($N = 72$) at P18. Both free T_3 and free T_4 show the same reduction trend with Li as total T_4 , but the effect is less. Therefore, inhibition of thyroid hormone production does not lead to clinically significant effects. This is likely due to the compensatory increase of TSH production with Li. Blood lithium was measured by ICP-MS while BUN, total T_4 , free T_4 , free T_3 , and TSH were measured by ELISA. (i) Swimming times on the forced swim test ($N = 24$) at P18. There are no differences in depressive-like behavior in the two groups. This remains the case at P25 (see Figure S2)

400 to 600 g is around 50 to 70 ml, respectively, per day.³⁰ The supplemented iodine dose in our work ranged from 2 to 3 mg/day for the lithium+iodine group. Note that the breast-fed pups likely received a considerably lower iodine intake. The period of iodine supplementation extended from day P4 to day P18. Note that the rat chow (Labdiet 5053) given to dams contained 0.97 ppm of Iodine, which roughly equates to a normal iodine diet ranging from 2 to 7 $\mu\text{g/day}$.^{31,32} Note that the pups received this indirectly through the breast milk.

2.5 | Statistical analysis

Body weight, blood lithium, total T_4 , free T_4 , free T_3 , TSH, BUN, and/or swimming duration were compared between control and lithium pups at P18 and P25 using one-way analysis of variance (ANOVA) at each time point. Post-hoc testing was performed with Tukey's test. At P60, body weight and total T_4 were compared. Total T_4 , free T_4 , free T_3 , TSH, and body weight were compared between control, lithium, and lithium+iodine pups at P18 using two-way ANOVA with post-hoc test. Trace element contents were compared between control and lithium pups at P18 and P25 using one-way ANOVA with post-hoc test at each time point. For elements measured by both LIBS and the energy dispersive x-ray fluorescence (XRF) spectroscopy (eg. iodine), separate analyses were performed for the two spectroscopy methods. Optical densities (of thyroglobulin for thyroids) from control, lithium, and lithium+iodine pups were compared by one-way ANOVA. A p -value threshold of 0.05 was considered statistically significant.

2.6 | Analytical methods

Breast milk, blood, and tissue samples were analyzed with a range of methods for analytes such as lithium and various thyroid hormones. The analytical methods included laser induced breakdown spectroscopy, x-ray fluorescence spectroscopy (XRF), enzyme-linked immunosorbent assay (ELISA), ICP-MS, immunohistochemical staining, and forced-swim test. Refer to Data S1 for details on these methods, including sample preparation, data acquisition, and data processing.

TABLE 1 Displays the barplots of Figure 2 (panels b–i) in table form. Only P18 and P25 values are shown. Abbreviations: limit of detection (LOD), blood urea nitrogen (BUN), thyroxine (T_4), triiodothyronine (T_3) and thyroid stimulating hormone (TSH)

	P18		P25	
	Control	Lithium	Control	Lithium
Blood Li (mmol/L)	<LOD	0.075 \pm 0.03	<LOD	<LOD
Weight (g)	40.31 \pm 3.75	42.5 \pm 5.51	72.31 \pm 4.6	74.95 \pm 6.2
BUN (nmol/L)	5.25 \pm 0.49	6.71 \pm 0.27	5.8 \pm 0.17	6.26 \pm 0.41
T_4 (nmol/L)	93.41 \pm 10.60	67.2 \pm 5.21	52.61 \pm 3.03	40.06 \pm 5.79
Free T_4 (pmol/L)	11.53 \pm 2.0	11.12 \pm 1.7		
Free T_3 (pmol/L)	4.9 \pm 0.6	4.7 \pm 0.4		
TSH (mIU/ml)	0.94 \pm 0.58	1.38 \pm 0.63		
Swim time (sec)	497.2 \pm 68	447 \pm 65		

3 | RESULTS

Figure 2(A) shows the group averaged LIBS spectra obtained from breast milk samples of control (black) and lithium treated dams (red) at the P18 time point. The control spectrum does not show any lithium emission line while the treated spectrum shows a prominent line at 670.7 nm, which belongs to lithium. This shows the presence of Li in the breast milk of the dams administered with lithium. The unnormalized intensity for lithium in breast milk is 672 \pm 132 arbitrary unit (a.u.) from the treated subject. Breast milk is likely lithium's route of entry from medicated dam to breast-fed pups. It is noteworthy that no comprehensive studies have been reported on the impact of lithium on the lactation process.¹⁰ Figure 2(B) shows the pup blood lithium concentrations in mmol/L at P18 and P25. Note that the barplots in Figure 2 are also shown in table form in Table 1. No lithium (below limit of detection of 0.007 $\mu\text{mol/L}$) was found in controls, whereas 0.075 \pm 0.03 mmol/L was measured in lithium exposed pups at P18 (~15% of the maternal levels). However, no lithium was found in the blood of any pups at later time points (also see Figure S2(A)). As the normal rat weaning period is around P21, this suggests that lithium was cleared from the pups' blood shortly after weaning. Figure 2(C) shows the measured body weights from pups. Lithium treated pups were statistically significantly heavier than controls at P18 (42.5 \pm 5.51 vs. 40.31 \pm 3.75 g, $p < 0.05$). Lithium exposed pups also appear to weigh more than controls at P25 (74.95 \pm 6.2 vs. 72.31 \pm 4.6 g) and P60 (333 \pm 68 vs. 310 \pm 91 g) (see Figure S2(B)), but the differences were not significant. Figure 2(D) shows the measured BUN in nmol/L. BUN from lithium pups at P18 was significantly higher than that of controls (6.71 \pm 0.27 vs. 5.25 \pm 0.49 nmol/L, $p < 0.05$). This indicates reduced kidney function during lithium exposure from breast-feeding. After weaning, this difference disappeared. The BUN in controls at P25 was 6.26 \pm 0.41 nmol/L and in the lithium group it was 5.8 \pm 0.17 nmol/L (not significantly different). At P60, BUN in controls was 6.06 \pm 0.38 nmol/L and in lithium pups it was 5.9 \pm 0.23 nmol/L (see Figure S2(C)). Figure 2(E) shows the blood total T_4 levels in nmol/L from pups. Total T_4 was significantly reduced ($p < 0.05$) in lithium pups, compared with controls, at P18 (67.2 \pm 5.21 vs. 93.41 \pm 10.60 nmol/L, $p < 0.05$) and P25 (40.06 \pm 5.79 vs. 52.61 \pm 3.03 nmol/L, $p < 0.05$). Total

T_4 was numerically lower in lithium pups at P60 (47.63 ± 11.0 vs. 54.2 ± 14.59 nmol/L), albeit not significantly. Figure 2(F–H) shows free T_4 , free T_3 and TSH in pups at P18. Free T_3 and T_4 show the same trend of Li reducing thyroid hormone output as total T_4 , but the effect is less and not statistically significant. TSH is increased in Li pups, likely as a compensatory mechanism to the Li-induced reduction in thyroid hormone output. The impact of Li on blood T_4 likely appears larger in the total T_4 measurement as total T_4 is >99% of all T_4 in blood. The increased body weight, reduced total T_4 and increased TSH of lithium pups suggests that Li exposure, even indirectly through breast-milk, can inhibit thyroid hormone output, leading to symptoms similar to those of hypothyroidism.³³ The inhibition persists after weaning and after lithium has cleared from the blood. Figure 2(I) shows swimming times on the forced-swim test. P18 pups, control, and lithium treated, perform similarly (see Figure S2 (D) for P25).

Figure 3(A) shows the location of the thyroid in the rat. The extracted thyroid from each subject was analyzed with LIBS and XRF to obtain the trace element information. A range of structurally and physiologically important elements are observed with LIBS (see Figure S3). Figure 3(B–D) shows the LIBS emission lines and intensities for lithium (Li) and iodine (I). Lithium emission is observed at 670.7 nm in the thyroids of lithium treated pups at P18 and P25, but not in control pups. Note that lithium was cleared from the blood by P25 (see Figure 2(B)). In contrast, iodine emission at 746.9 nm is significantly reduced in lithium pups at P18 and P25 ($p < 0.05$). Other elements with levels significantly affected by lithium exposure include calcium and potassium (also see Figure S4). Similar to LIBS, XRF analysis of the same thyroids also observed that lithium exposure significantly reduces thyroid iodine fluorescence at 427 eV (Figure 3(E and F), $p < 0.05$) at P18 and P25. Other elements with levels significantly affected by lithium include chlorine and potassium

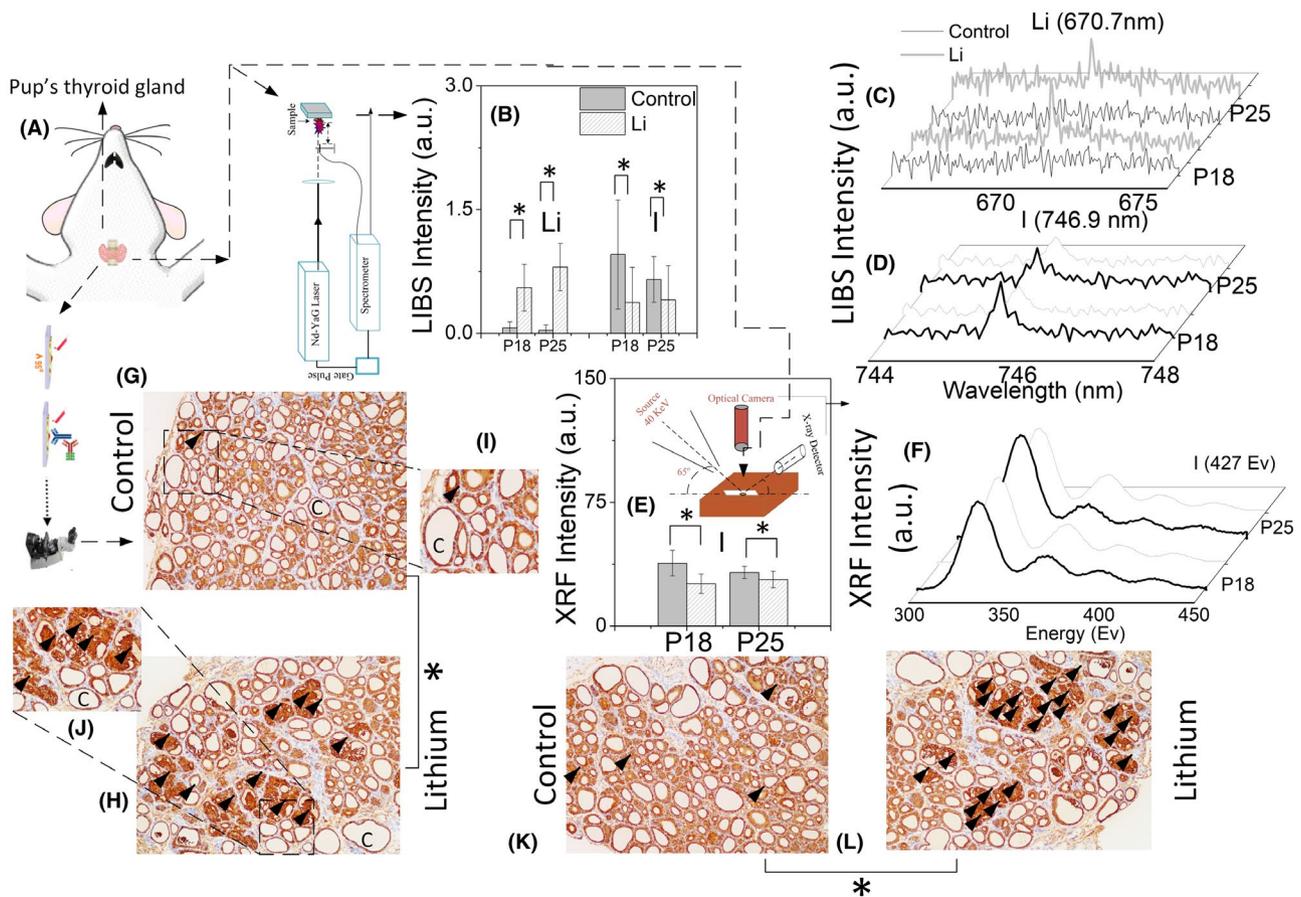


FIGURE 3 Thyroid analysis at P18 and P25. (A) Sketch showing location of thyroid encircling the trachea. (B) Bar plots showing the mean and standard deviation of LIBS emission intensities for lithium (Li) and iodine (I) from the thyroid glands of control ($N = 12$) and lithium groups ($N = 12$). (C) and (D) show the corresponding group averaged LIBS spectra expanded about Li and I respectively. (E) Bar plots showing the mean and standard deviation of XRF emission intensities for I from the thyroid glands of control ($N = 12$) and lithium groups ($N = 12$). (F) shows the corresponding group averaged XRF spectra expanded about I. (G) and (H) show representative immunohistochemical images (10X) of thyroids stained with anti-thyroglobulin from control and lithium groups respectively. (I) and (J) are expanded views (20X) of the windows marked on (G) and (H), respectively, showing enhanced thyroglobulin protein expression in the lithium group. Enhanced thyroglobulin expression marked by arrow heads is obvious in the colloid (C). (K) and (L) shows the same as (G) and (H), respectively. Statistical analysis between groups was performed with two-way ANOVA for P18 and P25, while one-way ANOVA was performed at P18 for thyroglobulin expression. A post hoc analysis using two-tail t -test of equal variance was performed with p -value threshold of 0.05 considered statistically significant. *indicates $p < 0.05$

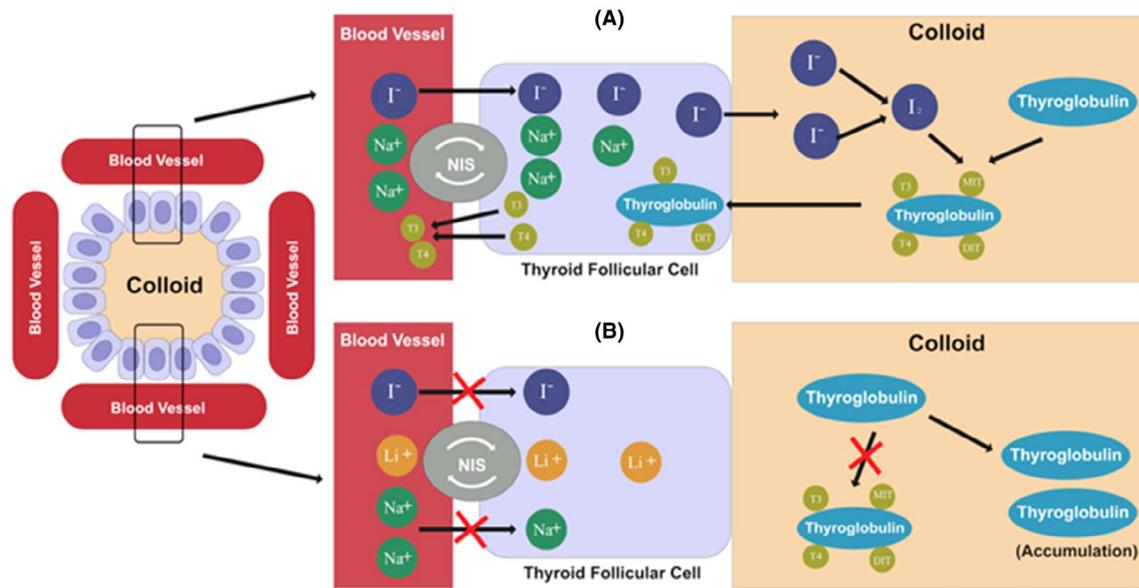


FIGURE 4 In a normal thyroid follicle (ie. control), one I^- ion enters the follicular cell through the sodium-iodide symporter (NIS), along with two sodium (Na^+) ions. The I^- enter the colloid where they bond with tyrosine on thyroglobulin proteins. The iodinated thyroglobulin re-enter the follicular cell and split into T_3 and T_4 hormone molecules, which enter the blood stream. Lithium in the blood stream, such as through breast-milk, significantly alters this process. Li is chemically similar to Na and may enter the follicular cell through the NIS. However, the NIS cannot also bring in I^- while bringing in Li^+ . This leads to Li accumulation and I deficiency in the thyroid. With insufficient I, thyroglobulin molecules are left non-iodinated, resulting in thyroglobulin accumulation and T_3/T_4 deficiency. This leads to clinical symptoms similar to those of hypothyroidism

(see Figure S5 and S6). Lithium exposure through breast-milk reduces iodine uptake by the thyroid. This persists to at least P25, after lithium has cleared from the blood. Note that iodine uptake is required for the thyroid to produce the important T_3 and T_4 hormones.³³ Therefore, inhibition of iodine uptake is a key step in the mechanism behind lithium inhibition of thyroid hormone production. This likely occurs as iodine enters thyroid follicular cells through the sodium-iodide symporter.^{33–35} Lithium and sodium are chemically very similar. Therefore, lithium likely blocks the symporter, preventing iodine from entering the thyroid.

Figure 3(G–L) show thyroglobulin stained sections of thyroid tissue from P18 control and lithium pups. In controls, thyroglobulin is primarily found in follicular cells, which can be located by the blue nuclear counterstain. Follicular cells surround an unstained center that is the colloid. In lithium pups, a number of colloids show high thyroglobulin expression, indicating accumulation of thyroglobulin in some follicles. The overall thyroglobulin optical densities of the sections are significantly higher ($p < 0.05$) in lithium pups versus controls. In the process of producing hormones, iodine bonds with tyrosine molecules on thyroglobulin proteins.³⁶ T_3 and T_4 molecules are then cleaved from the thyroglobulin molecule and exit the thyroid to enter the blood and supply the body. Due to lithium impairing thyroid iodine uptake, iodine does not bond with tyrosine and thyroglobulin does not split into T_3 and T_4 . Therefore, thyroglobulin accumulates, as observed in Figure 3 (G–L), and the pups suffer T_3 and T_4 deficiency. For reference, enhanced expression of thyroglobulin has been reported in adults receiving lithium treatment for mental disorders.³⁷ This mechanism of lithium inhibition of thyroid hormone

production is illustrated in Figure 4. For reference, in our earlier work on adult rat lithium subjects,³⁸ lithium also elevates thyroid tyrosine levels, likely because thyroglobulin is not split into T_3 and T_4 .

Figure 4 illustrates the proposed mechanism of lithium inhibiting thyroid hormone production observed in this study. In control subjects, iodide (I^-) ions moves from blood vessels to thyroid follicular cells through the sodium-iodide symporter (NIS), along with two sodium (Na^+) ions. The I^- enter the colloid where they bond with tyrosine on thyroglobulin. The iodine helps thyroglobulin to re-enter the follicular cell and split into T_3 and T_4 hormone molecules, which are secreted to the blood stream. I^- also iodates the tyrosine to form monoiodotyrosine (MIT) and diiodotyrosine (DIT). When lithium is found in the blood stream (ie. lithium subjects), the iodination process is significantly altered. Li^+ ions enter the follicular cell through the NIS, resulting in Li accumulation and intrathyroid iodine deficiency. With insufficient I^- because of Li^+ , thyroglobulin molecules cannot split to secrete T_3 and T_4 hormones to the blood stream. This in turn increases thyroglobulin in the colloid and reduces $T_{3,4}$ hormones.

The relationship between thyroidal iodine content and lithium intake is in general, complicated. For example, the thyroid to serum $[T/S]^{131}I^-$ ratio was significantly increased through 38 and 95 days animals administered with low iodine diet +lithium in contrast with 53 days for animal receiving lithium with normal iodine diet.³⁹ The iodine uptake was also increased. Besides, the rate of secretion of $^{131}I^-$ from the thyroid was significantly decreased up to 25 days. Lithium decreases thyroid iodine release, reduces iodide organification, and increases thyroid iodide content. On the other hand,

lithium can cause hypothyroidism^{40,41} by inhibiting thyroid iodine uptake. Hypothyroidism can also be caused by low iodine. Lithium has also been used, with reported success, to treat refractory hyperthyroidism, which can be caused by high iodine intake.⁴² Also, excess iodine produces low thyroglobulin.⁴³ Relating to our study, low thyroid iodine enhances thyroglobulin expression (see Figure 3), which is similar to the reference.⁴⁴ Overall, the relationship between lithium intake and thyroid iodine content is likely affected by a number of factors, including the lithium dose, dose duration, and timing of the iodine measurement relative to the lithium dosing.

Since thyroid uptake of iodine in lithium pups, compared with controls, at P18 is significantly reduced, supplementing iodine may improve iodine uptake and increase hormone production. To test this hypothesis, we supplemented the dams' diet with iodine during breast-feeding. This is recommended in many breast-feeding guidelines,^{45,46} although the recommendations are not related to lithium use. Figure 5(A) shows the body weights from control, lithium, and iodine supplemented lithium pups (lithium+iodine). Note that the barplots in Figure 5 are displayed in table form in Table 2. Figure 5(B–E) shows blood total T_4 , free T_4 , free T_3 and TSH levels, respectively, from control, lithium, and lithium+iodine pups. Iodine supplementation to the dam while on lithium increases pup total T_4 significantly compared with lithium only pups (85.6 ± 8.32 vs. 67.2 ± 5.21 nmol/L, $p < 0.05$). Importantly, total T_4 in lithium+iodine pups is not far from those in controls (85.6 ± 8.32 vs. 93.41 ± 10.60 nmol/L). Similarly, iodine supplementation to the dam while on lithium also balances

TSH (1.38 ± 0.63 vs. 1.15 ± 0.55 μ IU/ml) when compared with controls, whereas there is significant difference ($p < 0.05$) between lithium (0.94 ± 0.58 μ IU/ml) and control groups (1.38 ± 0.63 μ IU/ml). Interestingly the effects in free T_4 and T_3 are similar, but smaller than those observed in total T_4 . Body weight is not reduced after iodine supplementation, but this is likely because children of mothers with higher iodine levels weigh more,⁴⁷ independent of lithium. Thus, the pups are likely in better health with maternal iodine supplementation.⁴⁷ All in all, supplementing the dam's diet with iodine reduces lithium inhibition of pup thyroid hormone production. This suggests a clinically relevant remedy for lithium-induced thyroid disorders. However, the authors note that it is important to carefully consider the background iodine intake of specific patients prior to administering iodine supplementation.⁴⁸ Also the iodine dosage, dosing duration, and dosing method (via an oral supplement to the mother, or directly by gavage to the infant), should be further studied.

Figure 5(F–H) shows an expanded view (20X) of anti-thyroglobulin stained sections of thyroid tissue corresponding to the three pup groups from Figure 5(A–E). From Figure 3, in controls, thyroglobulin is primarily found in follicular cells. In lithium pups, a number of colloids show high thyroglobulin expression. Whereas in lithium+iodine pups, thyroglobulin is also found in colloids (Figure 5(H)), but at lower optical density than in lithium pups. This is likely related to the partial recovery of total T_4 (Figure 5(B)) with maternal iodine supplementation. The overall thyroglobulin optical densities of the sections are significantly higher ($p < 0.05$) in lithium pups vs. controls and

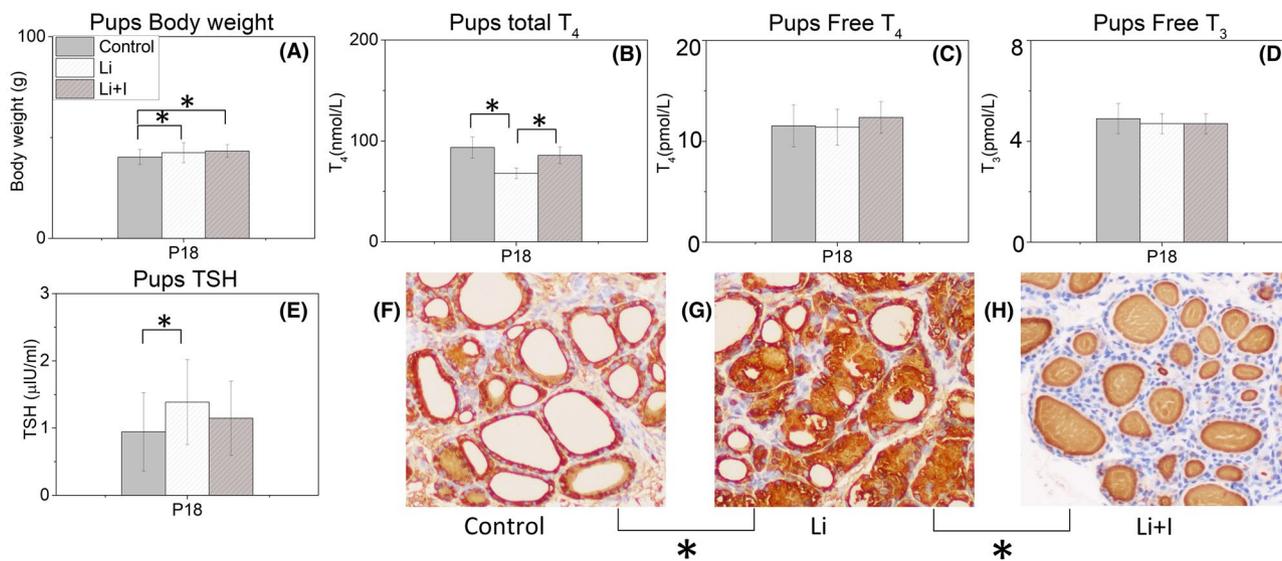


FIGURE 5 Iodine supplementation of maternal diet. (A) shows the body weight of pups at P18 from control, lithium, and lithium on supplemented iodine (dam) groups (lithium+iodine). The control and lithium group measurements are copied from Figure 2. (B)–(E) shows the blood total T_4 , free T_4 , free T_3 and TSH, respectively, in pups ($N = 36$ each) at P18 from control, lithium, and lithium+iodine groups. Lithium decreases T_3 and T_4 (significant for total T_4) and increases TSH. Iodine supplementation of the mother's diet restores total T_4 and TSH closer to control levels, indicating an "improvement". (F–H) are expanded views (20X) of anti-thyroglobulin stained sections of thyroid tissue at P18 from control, lithium, and lithium+iodine groups respectively. (F and G) are copied from Figure 2. Lithium increases thyroglobulin in the thyroid, especially in colloids. Iodine supplementation reduces thyroglobulin expression, but unlike in controls, thyroglobulin is now concentrated in colloids instead of follicular cells. Statistical analysis between groups was performed with one-way ANOVA at P18. A post hoc analysis using two-tail t -test of equal variance was performed with p -value threshold of 0.05 considered statistically significant. *indicates $p < 0.05$

TABLE 2 Displays the barplots of Figure 5 (panels a–e) in table form

	P18		
	Control	Lithium	Lithium+Iodine
Weight (g)	40.31 ± 3.75	42.5 ± 5.51	42.9 ± 4.6
T ₄ (nmol/L)	93.41 ± 10.60	67.2 ± 5.21	85.6 ± 8.32
Free T ₄ (pmol/L)	11.5 ± 2.0	11.12 ± 1.7	12.38 ± 1.5
Free T ₃ (pmol/L)	4.9 ± 0.6	4.7 ± 0.4	4.7 ± 0.4
TSH (mIU/ml)	0.94 ± 0.58	1.38 ± 0.63	1.15 ± 0.55

lithium+iodine group. Altogether, this thyroglobulin immunostaining supports the blood measurements that iodine supplementation reduces lithium inhibition of the thyroid.

Lastly, trace element analysis of the frontal cortex of the brain by LIBS observes lithium emission in lithium subjects at P18 and P25 (see Figure S7). No lithium is observed in controls (below limit of detection). All elements observed by LIBS are presented in Figure S4. Thus, lithium persists in the brain after weaning and after lithium clears from the blood, similar to in the thyroid.

4 | DISCUSSION

We investigated lithium transmission from lithium-treated rats to their nursed pups, and the impact of lithium in maternal milk on health of the pups. Lithium is transmitted through breast-milk, and exposed pups have increased body weight, reduced blood thyroxine (T₄), and elevated blood urea nitrogen, indicating thyroid and kidney impairment, while mothers had low therapeutic blood lithium levels (in human scales). A transient increase in BUN is observed, suggesting reduced kidney function that resolved shortly after weaning (and lithium clearance). In contrast, lithium is not rapidly cleared from the brain after weaning, although no behavioral abnormalities are observed. In the thyroid, exposed pups have higher TSH and reduced blood T₄. These abnormalities seem related to hypothyroidism and persist after weaning, and after lithium has been cleared from the blood. Thyroid iodine uptake is similarly reduced during breast-feeding and shortly after. Likely due to lithium's chemical similarity with sodium, lithium ions may interfere with the sodium-iodide symporter, preventing iodine from entering the thyroid. This initiates a mechanism where iodine cannot bind with tyrosine molecules on thyroglobulin, resulting in accumulation of thyroglobulin and under production of the key T₃ and T₄ hormones. This results in the pups suffering from hypothyroidism symptoms. Importantly, supplementing the dam's diet with iodine resolves much of the pup's thyroid abnormalities. Despite recommendations for adequate iodine intake for pregnant and nursing mothers, iodine deficiency is widespread in developing countries⁴⁹ and a rapidly growing problem in developed countries.⁵⁰ The results of this study provide valuable information for doctors and patients regarding lithium use during breast-feeding and the need for iodine supplementation. The study also provides considerable new insight on lithium mechanisms in the thyroid, kidney and the

brain. In the future, iodine supplementation may be tested in patients using lithium in scenarios where existing thyroid treatments are less suited.

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CONFLICT OF INTEREST

The authors declare no competing financial interests.

AUTHOR CONTRIBUTION

IA, VM, YL, FM, and SHM performed the experiments. ZL, CZ, SKP, IB, and CL helped in analyzing the data. NA, MSK, RA, AWLL, AI, FR, YZ, MA, VB, and CL contributed in writing the manuscript. IA and CL supervised the whole work. All authors have given approval to the final version of the manuscript.

DATA AVAILABILITY STATEMENT

The data sets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

ORCID

Sinai H. C. Manno  <https://orcid.org/0000-0001-6410-6873>

Ahmed Ali  <https://orcid.org/0000-0002-2645-7258>

Martin Alda  <https://orcid.org/0000-0001-9544-3944>

Condon Lau  <https://orcid.org/0000-0002-6569-5814>

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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