DIAGNOSTIC EXPOSURE OF IONIZING RADIATION AND ITS LONG-TERM EFFECTS

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TITLE: Low dose radiation and the development of the respiratory system

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LAY ABSTRACT

Radiation is necessary in medicine to observe the internal structures of the body, but it can sometimes cause unwanted biological changes within the body. This risk is heightened when considering exposure to developing baby because of the dynamic changing it is naturally going through and possible lifetime left to experience effects. This thesis aimed to understand what levels of radiation patients receive in hospital, observing one population predicted to receive high levels (Intensive care patients) and one predicted to receive low levels (pregnant patients). Overall, the majority of patients in the two cohorts received less than the recommended yearly public limit of 1 millisievert (mSv). The second aim was to observe the effects on the growth, lungs and hearts of the babies in an animal model when they are exposed during pregnancy. Radiation had no overall effect on the lungs or heart but can reduce body weight at moderate (100 milligrays (mGy) and high (1000 mGy) exposures.

Abstract

Medical radiation is vital in acquiring a patient diagnosis, but some clinicians are concerned with the perceived risks associated with ionizing radiation. This risk is heightened when incorporating *in utero* exposures due to the risk to the developing foetus. Although other organ systems have been studied, there is a paucity of data on the effects to the respiratory system from *in utero* exposures. The aim of this thesis was to understand the long-term effects on the respiratory system from *in utero* exposures, but as a first step, it was important to determine what levels patients receive whilst admitted to hospital. Two polar populations were chosen based on their predicted exposure levels during hospitalisation; one with high levels, intensive care unit (ICU) patients, and one with low levels, pregnant patients. Most patients cumulatively received < 1mSv with median exposures of 0.99 mSv (ICU patients) and 0.02 mSv (pregnant patients). However, both cohorts had patients that surpassed 10 mSv. To assess the effects from *in utero* exposures on the respiratory system, two animal models were conducted both exposed during late gestation, one healthy model and one acute lung injury model. In the health animal model, cardiovascular outcomes were also measured, however, ionizing radiation (50, 300, 1000 mGy) did not appear to influence these two organ systems from the outcomes measured. In the acute lung injury model, lipopolysaccharide (3mg/Kg) stimulated an acute lung inflammatory response, however, there was also no overt effect of radiation from the outcomes measured (10, 100, 1000 mGy). In both models, ionizing radiation did cause growth restriction up to 16

weeks of age, but this was only observed from doses above 100 mGy. Overall, the levels of ionizing radiation patients receive is low and from diagnostic exposures during pregnancy, there does not appear to be any strong effects on the developing foetus.

Declaration

I certify that this thesis does not incorporate without acknowledgment any material previously submitted for a degree or diploma in any university and that to the best of my knowledge and belief, it does not contain any material previously published or written by another person except where due reference is made in the text.

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James McEvoy

Date: 29/8/2019

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To the three simple phrases that got me through the past 3.5 years…

YOU GOT THIS,

WE BELIEVE IN YOU,

WORDS OF ENCOURAGEMENT

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LIST OF ABBREVIATIONS

^{123}I	-	Iodine-123
^{137}Cs	-	Caesium-137
^{18}F	-	Fluoride-18
^{201}Tl	-	Thallium-201
^{99m}Tc	-	Technetium-99m
a.u.	-	Arbitrary units
ALARA	-	As low as reasonably achievable
ANOVA	-	Analysis of variance
APACHE	-	Acute physiology, age, chronic health evaluation
ARPANSA	-	Australian radiation protection and nuclear safety agency
Atm	-	atmospheres
AUS	-	Australian
BAL	-	Bronchoalveolar lavage
Bq	-	Becquerel
C.I.	-	Confidence interval
CAN	-	Canadian
CED	-	Cumulative effective dose
cm	-	Centimetre
CT	-	Computed tomography
CTPA	-	Computed tomography pulmonary angiography
CXR	-	Chest X-ray
DAP	-	Dose area product
DBP	-	Diastolic blood pressure
dH_2O	-	Demineralised water
DLP	-	Dose length product
DNA	-	Deoxyribonucleic acid
EDTA	-	Ethylenediaminetetraacetic acid
ERR	-	Excess relative risk
FEV	-	Forced expiratory volume
FMC	-	Flinders Medical Centre
g	-	Gram

GAM	-	Generalised additive model
GD	-	Gestational day
GLMM	-	Generalised linear mixed model
GPx	-	Glutathione peroxidase
Gy	-	Gray
GYN	-	Gynaecology
h	-	Hour
H_2O_2	-	Hydrogen peroxide
HELLP	-	Haemolysis, elevated liver enzymes, and low platelet count syndrome
HIP	-	Health information portal
I.P.	-	Intraperitoneal
I.T.	-	Intratracheal
I.V.	-	Intravenous
ICCU	-	Intensive and critical care unit
ICRP	-	International commission on radiation protection
ICU	-	Intensive care unit
IgG	-	Immunoglobulin G
IHC	-	Immunohistochemistry
IL	-	Interleukin
INF-γ	-	Interferon-gamma
IQR	-	Interquartile range
J	-	Joules
KC	-	Keratinocyte chemokine
kDa	-	kilodalton
KeV	-	Kiloelectron volts
kVp	-	Peak kilovoltage
L	-	Litre
LAT	-	Lateral image
LD	-	Lethal dose
LNT	-	Linear no-threshold
LOG	-	Logarithm
LOS	-	Length of stay
LPS	-	Lipopolysaccharide

LSS	-	Lifespan study
M	-	Molarity
m	-	Metre
MAP	-	Mean arterial pressure
mAs	-	Milliampere-seconds
mg	-	Milligram
mGy	-	Milligray
min	-	Minute
mL	-	Millilitre
mM	-	Millimolar
mm	-	Millimetre
MRI	-	Magnetic resonance imaging
mSv	-	Millisievert
n	-	Number of experiments
NaCl	-	Sodium Chloride
NCRP	-	National commission on radiation protection
NF-κB	-	Nuclear factor kappa-light-chain-enhancer of activated B cells
ng	-	Nanogram
nm	-	Nanometre
Nrf1/2	-	Nuclear factor erythroid 2-related factor 1 and 2
OACIS	-	Open architecture clinical information system
OBS	-	Obstetrics
PA	-	Posterior-anterior image
PACS	-	Picture archiving and communication system
PBS	-	Phosphate buffered saline
PBST	-	Phosphate buffered saline + Tween
PVDF	-	Polyvinylidene fluoride
RNA	-	Ribonucleic acid
ROS	-	Reactive Oxygen Species
s	-	Second
S.E.M	-	Standard Error of the Mean
SBP	-	Systolic blood pressure
SOD	-	Superoxide dismutase

Sv	-	Sievert
TBE	-	Tris Borate EDTA
TBS	-	Tris Buffered Saline
TGF-β	-	transforming growth factor-beta
TLR4	-	Toll-like receptor 4
TNF-α	-	Tumour necrosis factor-alpha
UK	-	United Kingdom
UNSCEAR	-	United nations scientific committee on the effects of atomic radiation
USA	-	United States of America
V	-	Volt
µg	-	Microgram
µL	-	Microliter
µm	-	Micrometre
µM	-	Micromolar

#	-	Number
%	-	Percentage
~	-	Approximately/no substantial change
<	-	Less than
>	-	Greater than
≤	-	Less than or equal to
≥	-	Greater than or equal to
↑	-	Increased/elevated
↓	-	Decreased/reduced
°	-	Degrees
°C	-	Degrees Celsius
μ	-	Mu
α	-	Alpha
β	-	Beta
γ	-	Gamma
κ	-	Kappa
ρ	-	rho

DECLARATION OF ACADEMIC ACHIEVEMENT

This thesis is arranged in a sandwich format approved by McMaster University and with the permission of the supervisory committee. The thesis contains 7 chapters. Chapter 1 describes the background information of ionizing radiation with an overview of the effects during *in utero* exposure. Chapter 2 and 3 are retrospective audits of patient cumulative exposure to diagnostic radiation. Chapter 4 describes the effects of *in utero* exposures to ionizing on the cardiovascular and respiratory system in a healthy animal model. Chapter 5 examines the effect of *in utero* exposures to ionizing radiation on an acute lung injury model. Chapter 6 summarises and concludes the results found in chapters 2-5 and establishes future directions of research. Lastly, Chapter 7 summarises the Cotutelle experience.

This preface is to certify that a chapter within this thesis contains content that is substantially unchanged from the content of multi-author papers which have either been published or are being prepared for publication, which may lead to some repetition of ideas.

CHAPTER 1 INTRODUCTION

CHAPTER 2 CUMULATIVE RADIATION IN CRITICALLY ILL PATIENTS: A RETROSPECTIVE AUDIT OF IONIZING RADIATION EXPOSURE IN AN INTENSIVE CARE UNIT.

Authors: J.H. McEvoy, S. Bihari, A.M. Hooker, D-L. Dixon

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CHAPTER 3 CUMULATIVE RADIATION IN HOSPITALISED PREGNANT PATIENTS: A RETROSPECTIVE AUDIT OF IONIZING RADIATION EXPOSURE.

Authors: J.H. McEvoy, S. Bihari, A.M. Hooker, D-L. Dixon

Comments: JM, planned and conducted this study under the supervision of SB, AM and DD. JM and SB contributed to collection of data, JM and AH contributed to effective dose calculation, and JM and DD contributed to data analysis. JM, SB, AH and DD contributed to the study design, data interpretation, and the editing of the chapter.

CHAPTER 4 *IN UTERO* EXPOSURE TO LOW DOSE IONIZING RADIATION: LONG TERM CARDIOVASCULAR AND RESPIRATORY OUTCOMES IN C57BL/6 MICE.

Authors: J.H. McEvoy, D. Jones, L. Stoa, D-L. Dixon, T.C Tai, A. Hooker, D. Boreham and J.Y. Wilson

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CHAPTER 5 *IN UTERO* EXPOSURE TO LOW DOSE IONIZING RADIATION AND THE RESPIRATORY RESPONSE TO AN ACUTE LUNG INJURY STIMULUS AT ADOLESCENCE IN BALB/C MICE.

Authors: J.H. McEvoy, S. Sreetharan, L. Stoa, D-L. Dixon, T.C Tai, A. Hooker, D. Boreham and J.Y. Wilson

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CHAPTER 6 DISCUSSION

CHAPTER 7 COTUTELLE PROGRAM EXPERIENCE

CHAPTER 1

INTRODUCTION

1.1 RADIATION

Radiation is the emission of energy through space as subatomic particles or waves including electromagnetic, acoustic or gravitational. These forms can travel with a variety of energy levels. Radio waves, microwaves and visible light are all forms of radiation with low energy termed non-ionizing radiation. When the energy level passes a threshold where it is enough to emit an electron from an atom, thereby ionizing the atom, it is considered ionizing radiation, such as X-rays, gamma rays or beta and alpha particles. Because of this effect, ionizing radiation is more biologically relevant, having the effect to inhibit cell function, mutate cells and even cause cell death. However, understanding how this energy can cause these biological effects is complicated and requires an understanding of source and dose of ionizing radiation.

1.1.1 Sources of ionizing radiation

People are continuously exposed to radiation, from naturally occurring sources to artificial man-made sources. We are exposed to natural radiation from cosmic and terrestrial sources. Stars continuously produce cosmic radiation in the form of electromagnetic waves; however, we are mostly protected by the earth's electromagnetic

field. Terrestrial exposures come from radioactive material or radioactive isotopes found in our air, food, and soil, including uranium, thorium, radon and potassium. Due to instability, these atoms decay and produce ionizing radiation. Interestingly, humans themselves are radioactive due to radioactive isotopes absorbed within the body, for example, potassium-40 and carbon-14. The dose, however, is relatively small compared to those exposed from terrestrial and cosmic sources[1].

Man-made sources of radiation typically harness natural sources of radiation for other intentions, such as war or terrorism, nuclear power plants, and medical radiation[2]. The atomic bombs dropped in Japan during World War II harnessed the natural radioactive decay of uranium and plutonium in an extremely concentrated way. Similarly, nuclear power plants harness decaying radioactive material for electricity production. Ionizing radiation used in the medical industry harnesses both wave (X-ray and Gamma-ray) and particle (beta, alpha) forms for diagnostics or therapeutic uses[1].

1.1.2 Doses of ionizing radiation

To quantify dose, the energy of ionizing radiation deposited per unit mass is measured and reported in grays (Gy). Termed absorbed dose, 1 J of energy deposition per Kg of tissue = 1000 mGy. However, not all types of ionizing radiation elicit the same biological response, this is the concept of radiation biological effectiveness. Photons (gamma rays or X-rays), beta particles, alpha particles etc. deposit different amounts of energy in space and therefore can produce different biological effects. Due to this, a

radiation-specific weighting factor for each radiation type is applied to the absorbed dose to create the equivalent dose, measured in sieverts (Sv)[2] (Table 1-1). Finally, radiation doesn't affect each organ in the body similarly, as some are more radiosensitive than others. Tissues such as lung and bone marrow are more radiosensitive than skin or brain and so have more of a risk of radiation causing stochastic effects. These tissues, therefore, relate to a higher tissue weighting factor. This tissue weighting factor is applied to the equivalent dose to create the effective dose, also measured in sieverts (Table 1-2)[2]. The link between absorbed, equivalent and effective dose can be visualised in Figure 1-1.

Additionally, sources such as naturally occurring radon or medical radiopharmaceuticals are expressed in becquerels (Bq); a measurement of radioactivity[2,3]. Becquerels can, with extensive calculations, be converted into mSv, which generally occurs when considering the effect from multiple forms of radiation to the person, such as yearly background exposures or cumulative patient exposures.

Natural sources of radiation contribute a large amount to the ubiquitous background radiation exposure each year. The average natural background exposure for Australians and Canadians are 1.5 and 1.8 millisieverts (mSv) per year, respectively[4,5]. However, other places in the world receive higher averages such as Ramsar, Iran (6.0 mSv/y), Yangjiang, China (6.4 mSv/y), Kerala, India (6.9 mSv/y) and Poços de Caldas, Brazil (13 mSv/y), with some inhabited rural areas >100 mSv/y[6–9].

Table 1-1: Radiation weighting factors for different forms of radiation used to calculate the equivalent dose, with permission from ICRP[2]. Photons relate to X-ray and gamma-ray radiation.

Radiation type	*Radiation weighting factor*
Photons, Beta particle	1
Protons	2
Alpha particle, heavy ions	20
Neutrons	A continuous function of neutron energy

Table 1-2: Tissue weighting factors for different organs of the body used to calculate effective dose, with permission from ICRP[2]. Organs can differ in their radio-sensitivity, thus each organ within the scan field must be compensated for.

Organs	*Tissue weighting factors*
Red Bone Marrow	0.12
Colon	0.12
Lung	0.12
Stomach	0.12
Breasts	0.12
Gonads	0.08
Bladder	0.04
Liver	0.04
Oesophagus	0.04
Thyroid	0.04
Skin	0.01
Bone surface	0.01
Salivary glands	0.01
Brain	0.01
Remainder of body	0.12
Total	1.00

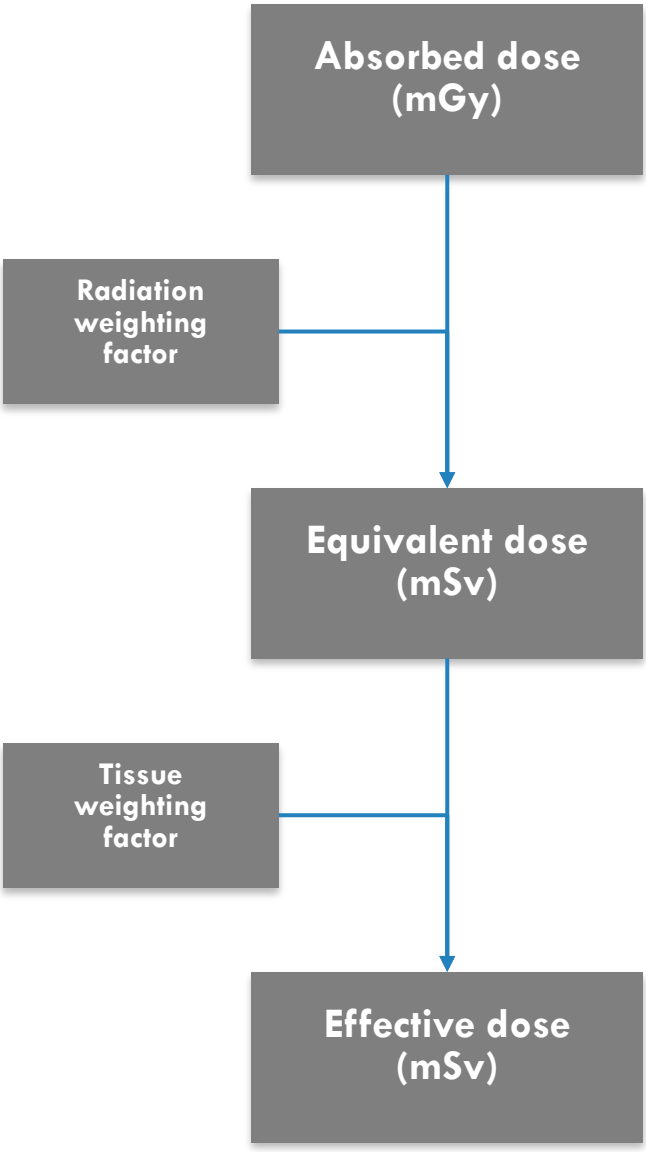

Figure 1-1: Dose quantities and units of ionizing radiation used in radiation research.

Occupational exposures can also be from natural sources but are present only when the person is in proximity to the occupational source. It can, however, have a large contribution to a person's yearly exposure depending on their occupation[4]. Pilots of international flights can receive up to 4 mSv a year, due to extended periods at high altitude resulting in increased exposure to cosmic radiation[10]. On the other hand, some miners working underground are exposed to 3-8 mSv per year due to radioactive uranium, thorium or radon in the rock[11,12]. With the increase in radiation exposure, comes an increase in the perception of risk and thus strict regulations are enforced. The occupational limit for ionizing radiation exposure is 20 mSv per year averaged over 5 years, with a clausal limit of 50 mSv in a single year[13].

Medical radiation comprises the dominant exposure from man-made sources. It accounts for 0.9-1.5 mSv/yr bringing the average yearly exposure to 2.4-3 mSv/yr for Australians and 3.6 mSv/yr for Canadians[4,14]. Radiation is an important tool aiding in the diagnosis and management of disease and as a therapeutic tool to treat disease. While therapeutic exposures use much higher doses of ionizing radiation, diagnostic exposures fall in the low dose radiation range.

1.2 LOW DOSE RADIATION

Diagnostic exposures fall under the category of low dose radiation which the United Nations Scientific Committee on the Effects of Atomic Radiation (UNSCEAR) defines as a single exposure below 100 mSv, in line with the International Council on Radiation Protection (ICRP)[2,15]. Under this threshold, the biological effects of radiation continue to be debated[16–19].

1.2.1 Diagnostic modalities using ionizing radiation

Conventional (plain diagnostic) X-rays, computed tomography (CT), fluoroscopy and nuclear medicine are all forms of ionizing radiation which clinicians use to enable quicker and more accurate patient diagnosis and prognosis.

Conventional radiology, or X-ray radiography, provides a useful non-invasive technique of visualising the internal tissues and structures of the body. Dense tissues, such as bone, absorb X-rays and appear white on photographic films and less dense tissues allow X-rays to pass through resulting in dark areas on the films[20]. X-rays can be taken of any part of the body and can be used for diagnostic assessment and monitoring. Mettler *et al.* collected published data between 1980-2007 from around the world and compiled the adult effective doses for each procedure as given in Table 1-3 and Table 1-4[21]. Although frequent, the amount of radiation received from X-ray procedures is relatively small compared to other procedures. The average chest X-Ray (CXR) accounts for 0.02 mSv, equating 5 days background radiation in Australia (1.5 mSv)[4,21].

Table 1-3**:** Effective doses from various diagnostic radiation modalities including conventional X-rays, computed tomography and fluoroscopy, adapted from Mettler *et al* with permission from RNSA[21].

Examination	*Effective dose (mSv)*
NUCLEAR MEDICINE	
Brain (^{18}F or ^{99m}Tc)	5.7-14.1
Thyroid scan (^{99m}Tc or ^{123}I)	1.9-4.8
Cardiac Stress (^{99m}Tc or ^{201}Tl)	9.4-40.7
Lung ventilation/perfusion (^{99m}Tc)	0.2-2
Renal (^{99m}Tc)	1.8-6.3
Bone (^{99m}Tc)	6.3

^{18}F = fluorine 18, ^{99m}Tc = Technetium 99m, ^{123}I = Iodine 123, ^{201}Tl = Thallium 201.

Table 1-4: Effective doses from various nuclear medicine exams, adapted from Mettler *et al* with permission from RNSA[21].

Examination	*Average effective dose (mSv)*	*Values reported in Literature (mSv)*
CONVENTIONAL RADIOGRAPHY		
Skull	0.1	0.03-0.22
Cervical spine	0.2	0.07-0.3
Thoracic spine	1	0.6-1.4
Lumbar spine	1.5	0.5-1.8
LAT chest	0.1	0.05-0.24
AP chest	0.02	0.007-0.05
Abdomen	0.7	0.04-1.1
Pelvis	0.6	0.2-1.2
Hip	0.7	0.18-2.71
Other extremities	0.001	0.0002-0.1
COMPUTED TOMOGRAPHY		
Head	2	0.9-4.0
Neck	3	…
Head and neck angiography	5	0.8-19.6
Chest	7	4-18
Thoracic angiography	15	13-40
Abdomen	8	3.5-25
Pelvis	7	3.3-10
Abdominal angiography	12	4-48
Spine	6	1.5-10
Coronary angiography	15	7-57
FLUOROSCOPY		
Intravenous urography	3	0.7-3.7
Upper gastrointestinal series	6	1.5-12
Barium Enema	8	2-18

mSv = millisieverts, LAT = lateral position, AP = anteroposterior position

CT is a combination of many X-rays taken from different angles to create cross-sectional images. It was defined as a high dose technique in 1990 due to its slice by slice, axial imaging, and although several improvements have reduced the dose, compared to general radiology, CT procedures are still a high dose technique[20]. These doses can vary significantly based on procedure type and body location of scan, for example, an abdominal CT = 8 mSv, but can vary 5 fold (Table 1-3)[21]. Technological developments and increases in the amount of information received from the exam have led to an increase in the use of CT scans over recent decades. In 1989, the National Radiation Protection Board in the UK estimated CT represent 2% of radiological procedures and contribute 20% to the cumulative effective dose (CED)[22]. Less than a decade later in 1998, the figures were 5% and 40% respectively[23]. In 2014, CT made up 11% of procedures and 82% CED[24]. The significant benefit of CT to healthcare has resulted in continued improvement in scanner technology and clinical application[25]. Currently, a CT scan can compile a 3D reconstruction of the internal organisation of the body within a few seconds, thereby significantly decreasing the dose.

Fluoroscopy uses a continuous X-ray beam. Unlike general radiology, the X-rays are transmitted to a monitor allowing for real-time visualisation of tissue or contrast dye movements throughout the body[20]. Fluoroscopy is used for assessing swallowing or upper and lower gastrointestinal tract movements. Additionally, it is useful for arthrography, angiography and aiding in placement of intravenous catheters and stents[26,27]. Fluoroscopic examinations are high in individual exposures, similar to lower-end CT scans, but are

relatively infrequently prescribed[24]. The dose is highly depended on the time taken thus careful consideration is needed as extended skin exposure to radiation can result in skin burns and ulcerations[27].

Nuclear Medicine is the use of radiopharmaceuticals to visualise internal structures in the place of an X-ray beam. Harnessing gamma (γ) waves, which are very similar to X-rays, radioisotopes are injected, inhaled or swallowed and visualised by recording the emitted radiation[20]. Procedures can include myocardial, lung or renal perfusion tests, and bone density scans and can range from 0.2-40 mSv as seen in Table 1-4[21]. Choice of radioisotope depends on the requirements for the procedure, including the state of isotope, decay rate, or uptake in the target organ, but nuclear medicine procedures are the least frequent modality prescribed[21,24].

1.2.2 Alternative diagnostic procedures

Some diagnostic procedures, such as magnetic resonance imaging and ultrasound, use other types of radiation to visualise the internal structures of the body without harnessing ionizing radiation. Magnetic resonance imaging (MRI) is also a non-invasive technique that harnesses magnetic fields and radio waves. MRI spectroscopically measures the magnetic properties of the nucleus, particularly protons. Beginning in the 1940s, its applications take advantage of its high contrast in soft tissue differentiation, including brain and skeletomuscular scans, contrast perfusion and diffusion imaging and angiography. MRI drawbacks include significant imaging time, image artefact production and cost[20].

Ultrasound is another non-invasive diagnostic technique but rather harnesses soundwaves and the acoustic properties of the body to produce an image. These sounds waves, which exceed the frequencies audible to humans, are pulsed into the tissue and depending on the acoustic properties of different tissues, sound waves are reflected as an echo. This process is repeated, and the collected echoes are transformed into an image. Ultrasound is used during pregnancy to visualise the foetus but can also be used for needle biopsy guidance and cardiovascular disease. Disadvantages of this method include poor image clarity/detail, artefact creation and requirement of significant training to establish and maintain competency[20,28].

1.3 RISKS ASSOCIATED WITH LOW DOSES OF IONIZING RADIATION

The observation that environmental factors can influence the occurrence of mutation, and thus cancer, has been known since the late 18th century[29]. However, the recognition of mutations caused by X-rays came almost 50 years later from Muller[30]. Although failing to cite similar work published earlier that year, Muller was credited with the observation that X-rays cause mutations in Drosophila, which induced transgenerational, phenotypic changes providing a plausible mechanism of evolution. This finding very quickly transformed the radiation field and later gained him the Nobel prize. Indeed, it was from a combination of Muller's pioneering observation, target theory, and some mathematical modelling that the first mechanism based, risk model (linear no-threshold model - LNT) was created[31]. However, as understanding in mutations has

improved, some scientists have begun to rebut the interpretations of his results, as reviewed by Calabrese[32–34].

A cornerstone observation made by Caspari and Stern found that dose rate played an integral role in the effect of radiation[35]. This challenged the LNT model and hypothesised that either a threshold dose does exist or that multiple independent actions are required to initiate a mutation. Over the decades since this observation, numerous studies have not supported the LNT model, which has given rise to other models to calculate risks in the low dose range.

1.3.1 Modelling radiation risk

Of the many current hypothesised models currently debated, Figure 1-2 shows four examples. The low-dose-high-sensitivity hypothesis assumes there are people who are highly sensitive to radiation and thus differ in their DNA damage response to radiation exposure deleteriously[36]. Contrary to this, the hormesis hypothesis assumes that there is a health benefit at low exposures, through stimulation of adaptive responses, compared to non-exposed persons, as reviewed by Vaiserman[37]. However, the top two debated models are the Linear No-Threshold (LNT) model and the threshold model. LNT is a linear extrapolation from high dose radiation and infers no “safe” level of radiation. Although this model is the current international standard enforced by most, if not all, radiation protection agencies, including the International Commission of Radiation Protection (ICRP), International Atomic Energy Agency (IAEA), National Commission of Radiation Protection

(NCRP), and Australasian Radiation Protection and Nuclear Safety Agency (ARPANSA)[2,38,39], the support is foremost as a radiation protection model and not as a cancer risk assessment model due to the difficulty in accurately measuring detrimental effects at low doses. Therefore, it is impractical to produce cancer risk estimates for diagnostic radiation, which is all below 100 mGy. The contradicting evidence to the LNT is substantial, as suggested by Tubiana *et al,* Averbeck *et al* and Calabrese *et al*[34,40–42]. The majority that opposes the LNT hypothesis support the threshold hypothesis, which indicates that under a "threshold" dose there are no radiation-related health detriments[43]. This hypothesis would make it easier to ignore perceived risks associated with very low dose radiation exposure, however as the major governing bodies support the LNT model, the current paradigm follows the 'as low as reasonably achievable' (ALARA) principle which aims at reducing any exposure unless an absolute necessity and thus reducing the perceived risk to the person.

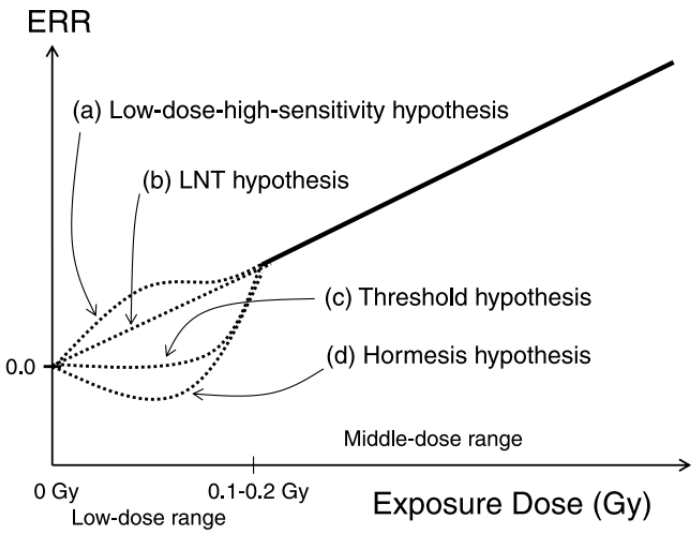

Figure 1-2: Schematic figure for the four dose-response model hypotheses in the low dose range, reprinted with the permission from Wolters Kluwer Health Inc.[164]. (a) the low-dose-high-sensitivity hypothesis, (b) the linear no-threshold (LNT) hypothesis, (c) the threshold hypothesis, and (d) the hormesis hypothesis. *ERR = excess relative risk*

1.3.2 Professional understanding of ionizing radiation

The perception of risk changes with the level of knowledge of radiation. Typically, professionals with more experience in the field of radiation have less fear of low dose radiation whereas those with limited radiation understanding exacerbate risk perception[44]. This, unfortunately, leads to some health care workers being unsure of the doses associated with ionizing radiation procedures or the actual risks associated with those doses[45]. In a survey collected by Lee *et al*, 91% of doctors did not understand the lifetime risk associated with an abdominal CT, or if it contributed to cancer incidence. Therefore, they could not, and did not, impart this information to the patients. Additionally, radiologists (77%), clinicians (74%) and patients (100%) did not know the dose of an abdominal CT (8 mSv) compared to a chest X-ray (0.02 mSv)[21,45]. Another study asked clinicians to compare a fluoroscopic exam to a chest X-ray and demonstrated a similar lack of understanding[46]. Although these studies have small-medium cohorts from single centres, the results imply a limited understanding of radiation dose and the associated risks, which is an essential requirement for examining the risk-to-benefit ratio of diagnostic radiology[3,45–50]. In nearly all cases, the benefit of the diagnostic imaging will outweigh the relatively small risks associated with the exam, however, the lack of understanding, and thus inversely related levels of fear, might lead the clinician away from diagnostic radiation and potential misdiagnoses. In contrast, the scientific evidence does not suggest that diagnostic radiation should be overused as this would increase cumulative exposures of radiation, financial burden and patient time mismanagement but instead prescribe

clinically indicated procedures with a firm understanding of radiation, its effects and risks. It is therefore in the interest of the patient if the clinician has an accurate understanding of all factors relating to the risk to benefit ratio of prescribing any action/intervention including diagnostic radiation.

1.3.3 Decision making with ionizing radiation in clinical practice

In the instance of prescribing diagnostic radiation, the clinical indications and the risks associated with the procedure are important in the decision-making process, but the patient's individual circumstance can also have a huge effect on the outcome of the decision. From this understanding, two polar populations can be chosen based on their predicted levels of ionizing radiation exposures.

Amongst hospitalised patients, those in the Intensive Care Unit (ICU) have a high severity of illness and are most constantly and intensively monitored by equipment. As the patient's conditions are severe with dynamic changes, diagnostic radiation is frequently used, with general radiology the predominant procedure[24]. Up until recent years, it was common for ICUs to administer chest X-rays as daily routine care until it was found to have no benefit in identifying a change in treatment[51–54]. When faced with acute and severe disease, the attention is on addressing the immediate problems and so the stochastic risks of radiation are usually not the focus. Thus, this population can be predicted to receive high levels of ionizing radiation.

Conversely, pregnant patients are commonly admitted with a variety of illness severities, with some requiring acute medical care that may include diagnostic radiation. However, the effects of radiation will not only be to the mother but also to the unborn foetus, thus the clinician's risk assessment must extend to include that of the developing foetus. This shifts the focus to long term outcomes, as there is a lifetime of risk still remaining, meaning that the stochastic effects of radiation are greater. As such, it is predicted that clinicians may reduce exposure to pregnant patients, to reduce the risk to the foetus. There is no dose limit to pregnant patients, instead, the prescription must be clinically justified, however strict care is taken to reduce the foetal dose as much as possible[55].

1.4 Pregnancy

The normal gestation can vary significantly depending on the species, mice have a 19-day gestation which is much small than the 38-40 weeks (280 days) for humans[56,57]. In this time a single-cell zygote progresses through embryogenesis and foetal development until a newborn offspring is birthed. The first stage of development is the preimplantation period, which encompasses the first two weeks of development. In this period the single-celled zygote goes through cleavage, blastocyst formation and implantation, and ends in the development of a bilaminar disk. Next is the embryonic period, which lasts until week 8 and is the beginning of organogenesis. Each of the germ layers proceeds to differentiate into various tissues and by the end of this period, the main organs and major features of

the body are beginning to be established. The period of development from here (week 9) until birth is the foetal period, characterised by the maturation of organs and rapid growth[56].

1.4.1 Prenatal care

Prenatal care is a very important and influential period with small changes in the intrauterine environment having the potential to cause significant and long-lasting changes to the offspring. The premise that prenatal care can have a significant implement to the offspring's phenotype is known as the 'Barker Hypothesis' or foetal programming. It was first discussed in the early 1990s by David Barker, a physician and epidemiologist at the University of Southampton. He originated this term to describe his observations that infants of lower birthweight had more health-related problems later in life[58]. Clinically, these infants had a higher risk of heart disease and diabetes[59]. He suggested that deprivation prenatally, due to poor nutrition, alcohol or drug abuse, or typical all-around poverty, caused changes to the foetus and its organ development, which led to a higher risk of certain diseases later in life. It is now known that there are various stressors, which can induce foetal programming and affect many different organs including the lung.

1.4.2 Normal Development of the respiratory system

In mice, as reviewed by Maeda *et al.* and Pinkerton *et al.* and seen in Figure 1-3[60,61], the embryonic formation of the lung buds begins 9 days post-conception. The development of the major bronchi and division of the tracheal-oesophageal tube commences first. At gestational day 11, lung development reaches the pseudoglandular stage. Branching and budding begin to give the stereotypic structure of the lungs with the development of the bronchial and bronchiolar tubules. Smooth muscle builds around the airway branches and the pulmonary circulation is initiated by neighbouring blood vessel neovascularisation. Day 15 begins the movement into the canalicular phase, where the general structure of the lungs is complete with intertwining airway and vessel branches. The future conducting and respiratory zones are easily distinguishable creating the framework for region-specific differentiation. From the saccular phase until birth, the respiratory epithelium continues cellular differentiation. The peripheral lung spaces become smaller as saccules develop, the lung fluid begins reabsorption and synthesis of surfactant is initiated. Postnatally, the final phase of lung development occurs with alveolarization of saccular structures increasing surface area and pulmonary gas exchange. This concludes the morphogenesis of the lung giving rise to the fully formed and functional pulmonary system.

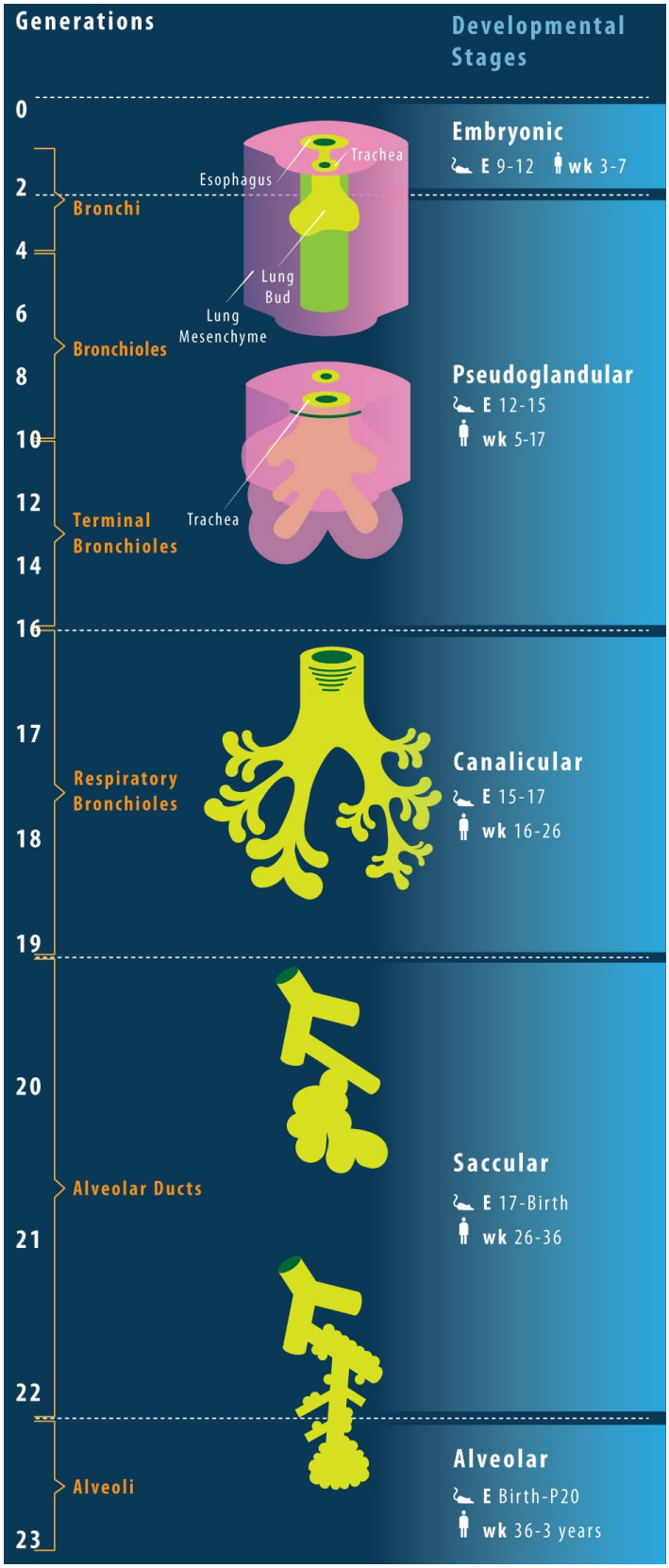

Figure 1-3: Schematic comparing mouse and human progression times through the 5 stages of normal respiratory development[165]. Copyright with permission from LifeMap Discovery.

The generation of lung tissue in humans follows a similar pattern as described for the mouse except with respect to timing. As reviewed by Smith *et al*[62], lung buds begin to develop 3 weeks post-conception during the embryonic period. At roughly 6 weeks, lung development moves into the pseudoglandular stage where branching and budding give rise to the stereotypic structure of the lung. The canalicular phase, with conducting and alveolar epithelial cell differentiation, begins at week 16 and lasts until week 26 where the saccular phase begins. Finally, the alveolar period of lung development begins right before birth and continues postnatally. This has been accepted to be up to 2-3 years postnatally, however current studies suggest the lung could still be developing through childhood and adolescence, although at a much slower rate[63–65].

1.4.3 Foetal programming of the respiratory system

Lung development can be influenced by foetal programming through several stimuli, including smoking and malnutrition. Smoking during pregnancy has been linked to foetal programming disrupting normal growth patterns and causing respiratory restructuring. Maternal smoking may have many inducers of foetal programming, including inhaled chemicals crossing the placental barrier, but it also causes an increase in carboxyhemoglobin and reduced placental blood flow leading to foetal hypoxia[66–68]. In a rat model of maternal smoking, offspring had foetal growth restriction, lung hypoplasia with increased indications of emphysema[69]. Similar outcomes were witnessed in humans with prenatal smoking associated with airway remodelling and the development of asthma[70–74].

Inadequate maternal nutrition during pregnancy can also cause significant disruption to growth patterns and lung development. In a rat model of maternal malnutrition, starved pups had reduced body weight, lung cell differentiation and immature air-blood barrier at birth. Enzymatic activity of alveolar macrophages was also reduced posing implications for post-birth response to infection[75]. Malnutrition can also alter lung lipid differentiation, lung morphology, and lung function that can persist into adulthood[76,77]. However, it may be possible that foetal programming stems from the reduced growth rate and not the stimuli *per se.*

In a multivariant analysis, adjusting for multiple confounding factors, it was low birth weight and not smoking status, maternal height or family history of asthma, that was associated with reduced lung function (forced expiratory volume, FEV, in 0.4 seconds)[78]. Thus, regardless of the stimuli, if it is potent enough to cause intrauterine growth restriction then respiratory development could be affected. This supports Barker *et al* in that low birth weight and low weight at 1 year of age were associated with a worse respiratory function in adulthood, with lower birthweights having lower FEV at 1 second (FEV_1), regardless of smoking status, social status, or early childhood respiratory infection[79]. Turner *et al* provided additional data in that reduced body size was associated with reduced FEV_1, forced vital capacity and expiratory flow rate[80]. Adult asthma, wheezing and bronchial hyperreactivity have also been linked to low birthweight[81,82] and, counterintuitively, asthma severity is associated with an increased head circumference[83]. It could be expected that reduced body size would equate to reduced head circumference

however, the foetus may adapt to preserve cerebral blood and head circumference by redistributing blood flow and nutrients to the brain, at the cost of the rest of the body's development, resulting in asymmetric growth restriction[84]. Twin control cohorts have strengthened these studies by identifying that there is a relationship between reduced birth weight and the development of asthma in both monozygotic and dizygotic twins. These results reinforce the foetal programming hypothesis that reduced foetal growth negatively impacts lung development independent of environment or genetic factors[85].

There are therefore many factors that may cause respiratory foetal programming, with associated intrauterine growth restriction, including environmental, disease, dietary, or stress-related stimuli[66–72,75–77,85–88]. One environmental stimulus that has yet to be explored is ionizing radiation during pregnancy to assess if it causes intrauterine growth restriction and thereby possible respiratory changes. Unlike other methods of foetal programming, like maternal hypotension or dietary restrictions, the effect of radiation may not only occur indirectly through the mother's exposure and foetal programming but also directly on the foetus itself. As X-rays and gamma rays penetrate the body, they will inevitably also penetrate the foetus. Thus, the effects of ionizing radiation during pregnancy may encompass both foetal programming and direct effects.

1.4.4 Foetal radiation exposure

When the foetus is in the field of view of medical procedures it will be directly exposed, however even when outside the field of view, the foetus may still be exposed via

scatter radiation, but this dose would be minimal in comparison. Therefore, procedures of the abdomen and pelvis carry larger doses than those of the chest or head (Table 1-5). In addition, the mother's skin and tissues provide some level of shielding, with increased maternal perimeter and foetal depth in the body associated with decreased foetal dose[89]

Table 1-5 outlines foetal doses following common diagnostic procedures; however, these numbers were calculated in the 1990s using different technology to current modalities. The present technologies allow for better dose reduction and control and so the current exposures would presumably be less[90]. Although these doses from medical radiation typically fall below the threshold of deterministic effects, such as lethality or malformation development, other factors can influence the effect the radiation dose has on the foetus including the type of radiation, as mentioned previously, and timing of exposure[91].

The effect of the radiation on the foetus differ significantly based on the length post-conception and therefore developmental stage of the foetus. In the pre-implantation period, death to the foetus is the dominant effect from large radiation doses, typically from cytogenetic damage. Exposures early in gestation of > 1000 mGy significantly induce embryonic death, whereas exposures later in gestation required higher levels of radiation to attain $LD_{50/30,}$ showing the sensitivity to radiation at the preimplantation stage[92]. Interestingly, the $LD_{50/30}$ varies significantly (1000 – 5500 mGy) and not in a linear fashion[92]. Exposures during organogenesis and foetal development periods tend to produce

malformations or growth retardation but lessen with higher gestational age[93]. Data from survivors of the Hiroshima and Nagasaki bombing demonstrate that there is a significant shift in effect with respect to the timing of exposure and formation of microcephaly, small head circumference. Most microcephaly cases were induced from foetal doses >100 mGy, but the frequency reduces from 1st trimester to 2nd to 3rd, independent of dose exposure, demonstrating the importance of timing in regards exposure[94].

In clinical settings, the physical parameters that change with gestation can also influence exposure. For indirect clinical exposures of diagnostic radiation, through a chest CT, the foetal dose from scattered radiation increases with gestational age[96], presumably due to a larger foetus being closer to the field of view during the procedure. For direct exposures, such as abdominal or pelvic examinations, a larger mass means a decrease in foetal depth and therefore less shielding from the mother's facia, increasing the dose received by the foetus[89].

The importance of gestational timing during exposure to ionizing radiation is clear but what are the actual effects of such exposures during the three main stages of development: pre-implantation, embryogenic and foetal? These effects can be either deterministic, occurring relatively close after exposure, or stochastic, taking many years to come to fruition.

Table 1-5: Foetal doses following common diagnostic procedures taken from UK surveys of diagnostic radiology and adapted from Sharp, Shrimpton and Bury with copywrite permission granted[95].

	Mean (mGy)	*Maximum (mGy)*
CONVENTIONAL X-RAY		
Skull	<0.01	<0.01
Thoracic spine	<0.01	<0.01
Chest	<0.01	<0.01
Lumbar spine	1.7	10
Abdomen	1.4	4.2
Pelvis	1.1	4
FLUOROSCOPY		
Barium meal	1.1	5.8
Barium enema	6.8	24
Intravenous urogram	1.7	10
COMPUTED TOMOGRAPHY		
Head	<0.01	<0.01
Chest	0.06	0.96
Lumbar spine	2.4	8.6
Abdomen	8	49
Pelvis	25	79

mSv = millisieverts

1.4.5 Deterministic effects

The deterministic effects of radiation have been known for 90 years from when Goldstein and Murphy observed a high incidence of neonatal cranial malformations in pregnant women who received radiotherapy, estimated >1000 mGy, for uterine cancer[97]. Many studies identifying the deterministic effects of *in utero* exposure to ionizing radiation typically look at outcomes including lethality, growth restrictions, malformations, and neurological function, but these outcomes can vary in severity depending on the timing of exposure during development.

1.4.5.1 Experimental models – Preimplantation

The preimplantation stage of development can often go unrecognised in clinical settings due to this very early stage of pregnancy being missed during diagnosis and thus there is a scarcity of human epidemiological data with most conclusions coming from animal studies. From 0-8 days post-conception of rodents, the foetus appears to be less radiosensitive to deterministic effects. In this time, the effect of radiation is typically reserved to lethality, a failure of embryo implantation or early abortion, or no long term effect at all: this is known collectively as the all-or-nothing theory[98]. This idea presumes the effects either cause lethality or repair/replacement of damaged cells via a repair mechanism or totipotent stem cell differentiation[98,99]. Doses above 500 mGy result in embryonic fatality and reduced litter size, but the likelihood of malformation during this time is low[92,93,100,101]. This may be because, during preimplantation exposures, only the extraembryonic tissues are exposed, as the primitive streak doesn't develop until the 2nd

week. The structural features of the body are yet to develop and thus malformation effects are unlikely to arise. The contradictory evidence to this statement comes from animal studies where there are species and sex variations, and only occurs for doses higher than 100 mGy, but nonetheless, fatality remains the greatest risk associated during the preimplantation stage of development[102–104].

1.4.5.2 Experimental models – Embryonic

Fatality is still a prominent effect of radiation during the embryonic development stage, however, the incidence of intrauterine growth restriction and malformation, particularly neuropathological, are more likely. One study with swiss albino mice showed exposures that 50 mGy had increased mortality from 10% to 16%, possibly due to high dose rate (900 mGy/min)[105] however, most other studies have a much higher dose threshold (>500 mGy)[100,101,106,107]. Birth weight was reduced for rats and mice exposed to > 450 mGy at both early and late time periods in this developmental stage, suggesting intrauterine growth restriction had occurred[100,108–111], but only doses > 1000 mGy result in a sustained lighter weight later in life[110].

Malformations and physiological changes appear to be variable and inconsistent. A rat study found exposures up to 800 mGy, far above diagnostic exposures, 9 days post-conception resulted in no physiological or preweaning reflex changes but a mouse studies with 500 mGy 9 days post-conception did for similar outcomes[112,113]. Likewise, other studies in rodents > 500 mGy show physical abnormalities of the face or appendages, and

also some motor-neurological abnormalities, such as loss of limb control[92,100,101]. In human, Kinlen and Acheson conducted a case-control study of 605 children with malformations and found no association with prenatal diagnostic radiation[114], which was supported by Ornoy *et al*[115]. Similarly, a prospective study showed no higher incidence of malformation, or mortality, compared to the general population[116]. A recent study identified that maternal exposure to ionizing radiation during conception and early gestation resulted in birth defects in 2/19 categories however lacked the power to remain after more scrutinous statistical analysis[117].

In humans, as with rodents: some studies have found significant increases in cranial malformations, including microcephaly; decreased cognitive function; or a change in behaviours, including anxiety and nervousness, with a threshold dose > 100 mGy[92,94,100,101,111,115,118]. The neurological changes are not just short term and can vary based on the dose received or the timing of the exposure[119–121]. For example, mice irradiated with 100 or 200 mGy during late embryonic development can have behavioural changes that last up to 13 months post-birth, but those exposed to 500 or 1000 mGy only manifest changes after 20 months[120,121]. Therefore, although there are inconsistencies with the report of malformations and neurobiological defects, it appears there is a consensus that these events only occur from doses above 100 mGy, which is far greater than received from diagnostic procedures.

1.4.5.3 Experimental models – Foetal Development

The effects of radiation during the foetal development stage (2nd and 3rd trimesters) are observed less, with a higher dose threshold required to elicit similar responses to the previous stages. Postnatal fatality in rodents does not increase with 500 mGy exposures but does with exposures > 1000 mGy[100,122–124]. Human data available in the early to mid-20th century demonstrated radiation-induced fatality but was not 100% effective. Using a higher dose in the region of 5000 mGy, clinicians would use irradiation as a method to induce abortion[125]. In humans and rodents, intrauterine growth restriction from exposures to radiation is still possible at < 1000 mGy, but they usually recover 1-2 weeks postnatally, – the reduced weight only persists when the foetus is exposed to > 1000 mGy[107,111,122–124].

Similar to the embryonic period, exposures during the foetal development period have variable and inconsistent effects on formation or physiological outcomes. Exposures of 500 mGy in swiss albino mice or 2000 mGy in ICR mice during foetal development showed no malformations[100,101], however, a threshold dose of 0.5 mGy in Swiss albino mice in another study caused cranial and appendage malformations[123]. In Wistar rats, doses above 400 mGy resulted in physiological changes, including cranial malformations, and reduced preweaning reflexes[112,124]. Preweaning and motor reflexes and motor strength in Sprague-Dawley rats were not different from controls when exposed up to 800 mGy[126]. Other neurological outcomes including cognitive ability, learning memory, and other behaviour changes have a threshold effect of 250 mGy, with the persistence of effects only seen > 500 mGy[127–129]. In humans, exposures above 100 mGy early in this period can cause

microcephaly, but later gestational exposures appear not to have any effect regardless of dose[94]. Additionally, Otake *et al* re-evaluated a threshold dose for severe mental retardation in the atomic bomb survivors to 60 – 310 mGy (95% CI) for early foetal development and 250 – 280 mGy for later gestations[130]. Although there are variations in malformations and neurobiological defects, it appears there is a consensus that there is a threshold dose > 100 mGy to which these effects occur. For ease of view, the American College of Radiology tabulated suspected effects from ionizing radiation dictated from ICRP reports[131], Table 1-6.

Amongst all periods of exposure, preimplantation, embryonic and foetal development, it is apparent there is a large variation in outcomes based on species, strain and sex. For instance, Nash *et al.* found a large variation in results when comparing inbred mice and hybrid genotypes indicating varying levels of radioresistance among different strains as well as sex[106]. For a wider comparison, the $LD_{50/30}$ can vary from 1730 – 8000 mGy also based on the differences in sex, species and strain[132–141]. This variation in radiosensitivity is most likely the reason for conflicting results above which it is difficult to draw meaningful and translatable-to-human conclusions. It is therefore important that more research is conducted, with species-specific radiosensitivities in mind, to fill these gaps.

Table 1-6: Summary of suspected *in utero* ionizing radiation-induced deterministic effects, adapted with copywrite permission from ACR[131].

Gestational Age	*<50 mGy*	*50 – 100 mGy*	*>100 mGy*
0 – 2 wks	None	None	None
3 – 4 wks	None	Probably none	Possibly spontaneous abortion
5 – 10 wks	None	Potential effects are scientifically uncertain and probably too subtle to be clinically detectable	Possible malformations increasing in likelihood as dose increases.
11 – 17 wks	None	Potential effects are scientifically uncertain and probably too subtle to be clinically detectable.	Risk of diminished IQ or of mental retardation, increasing in frequency and severity with increasing dose.
18 – 27 wks	None	None	IQ deficits not detectable at diagnostic doses.
>27 wks	None	None	Nonapplicable to diagnostic medicine

mGy = milligray, wks = weeks

1.4.6 Stochastic effects

The long-term effects of ionizing radiation arise from stochastic effects. These originate from damage to the genetic material of a cell in terms of mutations. The level of DNA damage increases with radiation dose and the levels of mutations are predicted to be proportional to the level of DNA damage[142–144]. When these mutations originate in germline cells, there is an association with hereditary effects, and when these mutations originate in somatic cells there is an association to cancer development[144]. As stated by Kamiya[145], it is important to delineate that 'the probability, and not the severity, of stochastic effects increase with radiation dose.'

The Life-Span Study (LSS) is the largest epidemiology study of the atomic bomb survivors allowing for the best understanding of the effects of radiation on the human population. The study examined 94,000 survivors who were <10 km from the hypocentres of either Hiroshima or Nagasaki and 26,000 controls who were out of the cities at the time of exposure. Of the 92% of exposed individuals who had their exposure successfully estimated, the majority received doses <100 mGy (79%), while 55% had less than 5 mGy; only 2.8% received a dose => 1000 mGy[146]. The risk of hereditary effects and cancer from ionizing radiation in humans has largely been estimated from epidemiological studies of the atomic bomb survivors, however, the gestational timing of pregnant women during these exposures is much harder to elucidate as the incidence of effects are small.

The risk of hereditable effects during *in utero* exposures from ionizing radiation is difficult to calculate as there has not been a study to investigate the F2 generation. However, the children (F1) born after parental atomic bomb exposure had no increase in malformations, stillbirths or perinatal death post-birth, and no increased risk of death caused by cancer, death by non-cancer disease, or common adult-onset multifactorial diseases including hypertension, diabetes, heart disease or stroke[147–152]. The mutation rate in children born from exposed survivors is also not different and as reviewed by Neel *et al.* there is no evidence of any genetic effects from radiation occurring in these children[153,154]. For the F1 generation born after parental exposure, the association between these diseases and radiation could only come from the radiation's effect on the germline cells, indicating that the exposures the parents received had no adverse effects on their germline cells. An assumption could then be made that *in utero* exposed foetuses, who carry their germ cells or progenitors at that time of exposure, would also have no increase in stochastic events in their offspring, however, there is no evidence to support this notation currently.

Cancer risk during *in utero* exposure is also difficult to calculate but estimates come from both the LLS and clinical cohorts as well. Data from the 807 *in utero* exposures of the LSS showed only 1 cancer death in the first 15 years of life and 10 more up to 46 years old, most being female[155,156]. These studies from the LSS conclude a slight increase in solid cancer rates, but not leukemia, in those exposed *in utero*. However, caution was recommended in the interpretation of results due to numerous limiting factors including

low cancer death incidence, sex differences, and no apparent dose-response variation[156]. One large clinical study collated data from abdominal and pelvic irradiations during the 1950s and reported an increased rate of childhood cancers and leukemia in their offspring[157]. Recent reviews have stated, with limited certainty, that doses >10 mGy to the foetus may cause a small increase in childhood carcinogenesis[158,159], however, another study found no statistical increase[160]. This variation could be due to the decade's difference between studies in which presumably increased awareness of radiation risk resulted in a reduction in the number of radiological scans received during pregnancy, including in the controls. Thus, the later study would have reduced foetal exposure and consequently saw no significant increase in cancer risk. It is important to recognise that, as reviewed and reported by Boice and Miller[161], the majority of the evidence supporting an association comes from case-controlled studies whereas cohort studies typically find no association. Case-controlled studies observe the outcome (cancer) and then identify which patients received the exposure (radiation), repetitively seen in clinical studies/observations, whereas cohort studies reverse this order; observe the exposure (radiation) and follow to see the outcome (cancer), such as the LSS. Cohort studies are powerful but require large patient numbers to observe rare events. In contrast, case-control studies are good at assessing rare events with small patient numbers but can typically have a reporting bias in patients recalling if they had received the exposure decades ago. This bias is not as evident in cohort studies which may play some role in the difference between results from these methods[162].

A final point to note is that these two populations, pregnant patients who received diagnostic radiation and those that don't, resemble two subpopulations that are inherently different. One medically requires radiation for diagnostic purposes, thus there is clinical justification. Totter and MacPherson argue that the selection factor of the medical decision to prescribe diagnostic radiation, and not the radiation itself, might be responsible for the associated risks[163]. Therefore, it is relatively difficult to epidemiologically delineate the effect of radiation and thus interpretation of such data should be met with caution.

Overall, the integrity and the reliability of the low dose data used in epidemiological studies causes uncertainty in the robustness of the risk estimates, but all who conclude some level of association infer that the risk is not zero. Furthermore, across all periods of development, there appears to be a paucity of research conducted on the deterministic and stochastic effect of radiation on respiratory development, thus there is a clear gap in the literature that needs to be investigated.

1.5 THESIS GOALS

The overall objective of this thesis was to understand what level of exposures patients are receiving whilst admitted to hospital and the potential effects these levels of radiation can have, particularly for the respiratory system. To investigate this goal, clinical levels of diagnostic radiation were first recorded then used in animal models to understand more about its effect.

Clinically, it is important that clinicians have a firm understanding of the risks and benefits to any test or intervention they prescribe. General radiology has to potential to be lifesaving as a diagnostic tool, but clinicians are not as firm with the understanding of its risks. However, before considering the risks of diagnostic radiation, it is necessary to understand the doses being prescribed. For the reason that doses are very patient-centric and clinically justified, two populations at polar ends of the patient spectrum were selected to understand how much radiation admitted patients would receive; those who are the sickest and require constant diagnosis and management of disease, ICCU patients, vs those who are typically restricted in radiographic prescription due to the fear of deterministic and stochastic effects, pregnant patients and their unborn foetus.

The convention surrounding irradiations of pregnant patients is to limit the dose to the foetus to as low as reasonably achievable due to the risks from ionizing radiation, however, these risks are interpreted from much higher doses of ionizing radiation than that which comes from diagnostic radiation. Research into low dose radiation during pregnancy

is emerging and showing little effect on the development of the foetus, however, there is a paucity of data on the respiratory system. To understand these effects, healthy and acute lung injury mouse models were used to investigate if *in utero* exposure to diagnostic levels of ionizing radiation altered the long-term respiratory physiology and immunology.

Together, this thesis wants to explore low dose ionizing radiation during development and its long-term effect on the respiratory system, with specific goals to

1. Examine the cumulative levels of ionizing radiation from diagnostic radiation received by patients admitted to the ICCU.
2. Examine the cumulative levels of ionizing radiation by diagnostic radiation received by pregnant patients admitted to hospital and the resulting foetal exposures.
3. Examine the impact of diagnostic levels of ionizing radiation during late gestation pregnancy on the long-term development of the respiratory system in a healthy mouse model.
4. Examine the impact of diagnostic levels of ionizing radiation during late gestation pregnancy on the physiological and immunological response to an acute lung injury stimulus at adolescence in a mouse model.

1.6 CHAPTER SUMMARY

With a focus on medical uses, this thesis will highlight the clinical apprehensiveness between ionizing radiation and pregnancy, with the intent of informing clinicians to improve risk to benefit analysis of prescribing these procedures. Through the use of mouse models, this work significantly contributed to the current understanding of the long-term effects and aligns with the ideology that the risks associated with low doses ionizing radiation, such as diagnostic radiation, are negligible compared to the benefit of performing the procedure and possibly saving the patient's life.

Chapter 1 is a general introduction to the themes covered in this thesis including ionizing radiation and pregnancy. This chapter also summarises the current knowledge of ionizing radiation during pregnancy highlighting the paucity of data specifically relating to the respiratory system.

Chapter 2 investigated the levels of ionizing radiation received by patients admitted to the ICCU. Radiological records from 526 patients were assessed to calculate the cumulative effective dose for each patient. Clinical and demographic characteristics were also recorded to assess association with cumulative effective doses. This study helped comprehend the levels of ionizing radiation received by the sickest patients who frequently required these procedures.

Chapter 3 investigated the levels of ionizing radiation received by pregnant patients admitted to hospital. Radiological records from 557 patients were assessed to calculated cumulative effective doses. The procedure exposures were then used to calculate foetal exposures. Again, clinical and demographic characteristics were investigated for an association to dose. This study identified the levels of exposures received by patients who sparingly received ionizing radiation.

Chapter 4 used a healthy animal model to examine the effect of *in utero* exposure to ionizing radiation during late gestation. The diagnostic levels of ionizing radiation used, replicate clinical settings and late gestation was chosen to avoid mortality and major malformations and focus on foetal programming effects and functional changes. This study highlighted the temporal effects on the cardiovascular system as well as the long-term response to the respiratory system.

Chapter 5 also used a mouse model but induced acute lung injury, through intratracheal instillation of a respiratory stimulus, to examine if *in utero* exposures to ionizing radiation altered physiological and immunological responses during inflammation. Similar diagnostic levels of ionizing radiation were used but the introduction of acute sickness allows for investigation into the immunomodulatory response of ionizing radiation, and if this response is long term.

Chapter 6 summarises the key findings from the experimental chapters (chapter 2-5) and discusses how these data support the negligible effects, and thus risk, diagnostic

levels of radiation during pregnancy have on the respiratory system. It also discusses perspectives on the findings and potential routes for future research.

Chapter 7 summarises the experience received from embarking on a Cotutelle de Thesis PhD, highlighting the struggles and benefits from participating in this cross-institutional arrangement. This chapter also emphases the value this involvement has added, both academically and professionally.

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CHAPTER 2

CUMULATIVE RADIATION IN CRITICALLY ILL PATIENTS: A RETROSPECTIVE AUDIT OF IONIZING RADIATION EXPOSURE IN AN INTENSIVE CARE UNIT.

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SUMMARY

Ionizing radiation is a valuable tool in modern medicine including for patients in an intensive care unit (ICU). However, clinicians are faced with a trade-off between benefit of information received from procedure versus risks associated with radiation. As a first step to understanding the risk and benefits of radiation exposure to ICU patients, we aimed to assess the cumulative levels of ionizing radiation patients receive during their ICU stay. This audit included 526 patients admitted to the ICU, Flinders Medical Centre, South Australia, for longer than 120hrs (long-stay) over a 12-month period from April 2015 to 2016. The 526 patients audited underwent 4331 procedures totalling 5688.45 mSv of ionizing radiation. The most frequent procedure was a chest X-ray (82%) which contributed 1.2% to cumulative effective dose (CED). Although only 3.6% of total procedures, abdominal/pelvic computed tomography (CT) contributed the most to CED (68%). Over

50% of patients received less than 1 mSv CED during their stay in the ICU. However, 6% received >50 mSv and 2% >100 mSv CED. Trauma patients received significantly higher CED compared to other admission diagnoses and CED increased with length of stay. Most ICU patients received low CED during their stay, with the majority less than the recommended public limit (1 mSv). These results could educate clinicians regarding radiation exposures in ICU settings highlighting the relatively low exposures and thus low risk to the patients.

2.1 INTRODUCTION

Ionizing radiation is continuous and ubiquitous, coming from both natural and artificial sources and can be expressed in millisieverts (mSv), which is the measurement of the biological effect of the absorbed radiation dose. At natural background levels, Australians are exposed to 1.5 millisieverts of radiation annually, while residents in the United States of America (USA) receive approximately 3.1 mSv[1,2]. This level increases with artificial exposures, another 1.7 mSv for Australia and 3.1 mSv for USA, totalling an average yearly exposure of 3.2 mSv and 6.2 mSv respectively[2,3]. Most artificial exposures derive from medical sources. Ionizing radiation is a crucial tool in medicine, aiding both diagnosis and therapy. Among hospitalised patients, diagnostic and interventional radiology is potentially most frequently used for patients admitted to Intensive Care Units (ICU).

Diagnostic radiation is used to aid ICU clinicians in both disease diagnosis and management. Previous studies attempting to calculate patient's cumulative effective dose (CED) over their stay in the ICU have been limited to exclusive populations within the ICU and thus overestimate cumulative exposure[4–12]. The real burden of radiological exposure in ICU patients remains unknown.

Conventional X-rays, computed tomography (CT), fluoroscopy and nuclear medicine are all forms of ionizing radiation that clinicians use to enable quicker and more accurate patient diagnosis. Mettler *et al.* collated average effective doses of each radiological exam worldwide between 1980 and 2008[13]. An anterior-posterior chest x-ray,

the most common radiological procedure prescribed in ICUs, was reported as 0.02 mSv, approximating to 5 days background radiation[1,13]. Generally, higher exposures of radiation are received from CT where median chest and abdominal exposures are reported as 7 mSv and 8 mSv respectively[9,13]. However, when surveyed, it was highlighted that some health care workers have a limited understanding of the doses associated with procedures utilising ionizing radiation and therefore have a misunderstanding of the associated risks[14–19].

Under the current international regulatory framework, it is considered that there is no 'safe' level of radiation and that all radiation can be considered harmful. Currently, the member of public limit has been set at 1 mSv/yr, whilst occupationally exposed workers can receive up to 20 mSv/yr above background[20]. For medical exposures, all treatments must be clinically justified on an individual's needs for successful diagnosis and treatment. The International Commission on Radiation Protection (ICRP) states a cancer risk of 5.5% per Sievert (Sv)[20]. Thus, when extrapolated down to diagnostic levels, a whole-body 10 mSv CT scan would equate to an approximate 0.06% increase (1/1800) in cancer risk. When added to the average risk of cancer by the age of 65, the cancer risk from a CT would increase from 40% to 40.06%[21]. However, it is important to recognise that the ICRP, as well as other governing bodies, state that epidemiological methods do not have the power to isolate cancer risks for exposures below 100 mSv[20,22,23].

As a first step, it is important that we accurately determine the level of radiation exposure of patients in the general ICU cohort, including an assessment of the exposure due to each procedure, to inform clinicians and thereby aid their assessment of the risk-to-benefit ratio when prescribing diagnostic radiation.

The aim of this study was to conduct a retrospective audit of daily and cumulative radiation exposure of all patients admitted for >120 hours to a 32-bed mixed surgical and medical, metropolitan, tertiary level ICU, over a 12-month period.

2.2 METHODS

2.2.1 Study cohort

The study cohort included patients admitted to the ICU, Flinders Medical Centre, Adelaide, Australia, between 1st April 2015 and 1st April 2016 with ICU length of stay >120 hours. Patients with shorter stays were excluded to remove routine post-surgery stays. This audit was reviewed and approved by the South Adelaide Clinical Human Research Ethics Committee (OFR # 131.16) in line with the requirements of the *National Statement on Ethical Conduct in Human Research* and the requirement of informed consent was waived.

2.2.2 Data collection

Hospital records from the Open Architecture Clinical Information System, the Australian Outcomes Research Tool for Intensive Care (AORTIC) database and the radiological database picture archiving and communications system were used to collect

demographic information, clinical data and radiology reports for each patient. All ionizing forms of radiation were recorded including conventional X-rays, CT, fluoroscopy and nuclear medicine.

2.2.3 Calculation of effective dose

Effective doses for each procedure were calculated using values from patient radiology reports and reported conversion factors by Deak *et al* and Hart and Wall[24,25]. In short, values for Dose Area Product (DAP) or Dose Length Product (DLP) produced from the radiology reports were collected and converted to effect dose (mSv) via multiplication by tissue-specific conversion factors (Table 2-1). For conventional X-rays, accepted diagnostic reference levels were used for each individual exam due to lack of information in reports, except in cases where sufficient information enabled calculation of effective doses.

As this study focuses on initial management and intensive care treatment, radiological examinations were only included if they were performed during the time between admission and discharge from the ICU. No radiological procedures were recorded pre or post ICU stay.

Table 2-1: Conversion factors for each modality divided by body region scanned or radiopharmaceutical used adopted from Deak *et al* and Hart and Wall[24,25].

	Conversion factor
CONVENTIONAL X-RAY	0.000028
Head/neck	0.00012
Chest	0.00027
Abdominal and pelvic	0.00001
Extremities	0.0019
COMPUTED TOMOGRAPHY	0.0051
Head	0.0145
Neck	0.0153
Chest	0.0015
Abdominal and pelvic	0.0120
Extremities	0.000028
Spine	0.00003
FLUOROSCOPY	0.00012
Cerebral	0.00012
Oesophageal	0.00026
Pulmonary	0.00018
Cardiovascular	0.00001
Gastrointestinal	0.011
Urinary	0.007
Peripheral	0.0057
NUCLEAR MEDICINE	0.0017
99mTc MAA	0.015
99mTc MAG3	0.000028
99mTc MDP	0.00012
99mTc DISIDA	0.00027
99mTc Tgas	0.00001

CT= computed tomography; abdo/pelv= abdominal and pelvic; 99mTc = Technetium-99m; MMA = macro aggregated albumin; MAG3 mercaptoacetyltriglycine; MDP = methyl diphosphonate; DISIDA = diisopropyliminodiacetic acid; Tgas = Technegas.

2.2.4 Statistics

All outcomes measured in this audit were not normally distributed, therefore data was expressed as median (interquartile range, IQR) and tested using non-parametric tests. The dichotomous variable sex was assessed by Mann-Whitney U test and categorical variables, including patient ethnicity, length of stay, and admission diagnosis, were assessed by Kruskal-Wallis analysis with *post hoc* Mann-Whitney U test. Scale variables, including age and APACHE III score, were assessed using Spearman's rank-order correlations. Statistical significance was dictated by $p < 0.05$

2.3 Results

This cohort included 526 patients that underwent 4331 procedures totalling 5688.45 mSv of ionizing radiation. The median (IQR) age was 65 (51 – 76) years old with a median (IQR) length of ICU stay of 7 (6 – 13) days and overall mortality of 10.3% (Table 2-2). Patients were admitted with either non-operative (medical) or post-operative (surgical) diagnoses with respiratory or cardiovascular complications the main reason for admission.

Table 2-2: Summary of patient demographics and clinical characteristics.

Demographic and clinical characteristics	*Values (%)*
TOTAL NUMBER OF PATIENTS	*526*
AGE *(years), median (IQR)*	*65 (51–76)*
SEX	
Male	*306 (58.2%)*
ETHNICITY	
Aboriginal	*31 (5.9%)*
Caucasian	*477 (90.7%)*
Asian	*5 (1%)*
Other	*3 (0.6%)*
Unknown	*10 (1.9%)*
APACHE III SCORE*, median (IQR)*	*72 (57–86)*
ICU STAY *(days), median (IQR)*	*7 (6–13)*
ICU *mortality*	*54 (10.3%)*
ADMISSION DIAGNOSIS	
Medical	
Cardiovascular	*91 (17.3%)*
Respiratory	*94 (17.9%)*
Gastrointestinal	*45 (8.6%)*
Neurological	*46 (8.7%)*
Sepsis	*52 (9.9%)*
Trauma	*24 (4.6%)*
Metabolic	*25 (4.8%)*
Haematological	*6 (1.1%)*
Renal/genitourinary	*14 (2.7%)*
Other disorders	*1 (0.2%)*
Musculoskeletal	*5 (1.0%)*
Surgical	
Cardiovascular	*50 (9.5%)*
Respiratory	*7 (1.3%)*
Gastrointestinal	*34 (6.5%)*
Neurological	*13 (2.5%)*
Sepsis	*8 (1.5%)*
Renal/genitourinary	*2 (0.4%)*
Gynaecological	*1 (0.2%)*
Musculoskeletal	*8 (1.5%)*
Haematological	*0 (0.0%)*
Metabolic	*0 (0.0%)*

APACHE = Acute Physiology, Age, Chronic Health Evaluation;
ICU = intensive care unit; IQR = interquartile range

All patients who received some diagnostic radiography (98.5%) received at least one conventional X-ray. Of those, 248 patients (48%) received only conventional X-rays. A CT scan was received by 255 patients (49.3%), fluoroscopic exam by 74 (14.3%) and only 6 patients (1.2%) underwent nuclear medicine. The median (IQR) CED of the cohort was 0.91 mSv (0.08 – 11.37mSv). There was no difference in median CED between sexes (p = 0.131), or ethnicities (p = 0.964).

2.3.1 Distribution of procedure dose

As dictated in Table 2-3, the most common modality performed in this ICU was a conventional X-rays, accounting for 85.2% of the 4331 procedures of which the chest X-ray was the most frequent procedure accounting for 82% of all procedures performed. However, because conventional X-rays produce a relatively low dose of radiation, they only accounted for 2.3% contribution to CED. Conversely, CTs contributed the most to CED (93.1%) with the highest contribution coming from abdominal/pelvic, representing 68.8% of the overall 5688.45 mSv. Both the fluoroscopic exams and nuclear medicine accounted for minimal percentages of both number of procedures and total CED.

The highest median effective dose was produced from abdominal/pelvic CTs (21.88 mSv), which also gave the highest individual exposure of any procedure (78.82 mSv) (Table 2-4). There was large variation between doses for the same procedure sometimes up to 1000-fold, for example with gastrointestinal fluoroscopy.

Table 2-3: Distribution of procedures which use ionizing radiation and the frequency at which they contribute number of procedures and total dose.

	Contribution to frequency (n = 4311 procedures)	*Contribution to total dose (n = 5688.45 mSv)*
Conventional X-ray	*85.2%*	*2.3%*
Computed tomography	*12.5%*	*93.1%*
Fluoroscopy	*2.2%*	*4.3%*
Nuclear medicine	*0.1%*	*0.3%*

mSv = millisieverts

Table 2-4: Exposures from each modality divided by body region scanned.

	Median effective dose (mSv) Median (IQR)	*Range of effective dose (mSv)*
CONVENTIONAL X-RAY		
Head/neck	0.01 (0.01–0.03)	0.01–0.03
Chest	0.02 (0.02–0.02)	0.02–0.06
Abdominal and pelvic	0.75 (0.75–1.50)	0.14–3.75
Extremities	0.00 (0.00–0.00)	0.00–0.02
COMPUTED TOMOGRAPHY		
Head	1.64 (1.59–1.79)	1.06–5.20
Neck	4.14 (3.29–5.76)	1.61–12.83
Chest	7.53 (5.07–11.64)	0.98–36.30
Abdominal and pelvic	21.88 (14.05–31.72)	1.55–78.82
Extremities	0.97 (0.31–1.79)	0.25–2.14
Spine	9.66 (5.76–21.70)	5.76–21.70
FLUOROSCOPY		
Cerebral	2.67 (2.05–3.25)	0.11–13.95
Oesophageal	0.02 (0.01–0.26)	0.01–1.14
Pulmonary	5.30 (0.70–11.16)	0.23–12.89
Cardiovascular	0.02 (0.00–0.23)	0.00–28.70
Gastrointestinal	3.86 (0.94–7.36)	0.24–27.78
Urinary	0.02 (0.02–0.02)	0.02–0.02
Peripheral	0.00 (0.00–0.02)	0.00–0.06
NUCLEAR MEDICINE		
Gastrointestinal	2.34 (2.32–2.52)	2.32–2.52
Urinary	5.12 (5.06–5.19)	5.06–5.19

mSv = millisieverts, IQR = Interquartile range

2.3.2 Patient cumulative radiation exposure

In this cohort, 50.5% of patients received under 1 mSv CED during their ICU stay, with 9 patients receiving no ionizing radiation (Figure 2-1). Conversely, 33 patients (6.3%) received more than 50 mSv, of which 7 (1.3%) received more than 100 mSv. The highest CED was 199.89 mSv.

Admission diagnosis also contributed to CED. A patient admitted with trauma received significantly more radiation compared to medical and surgical admissions (Figure 2-2A). Finally, patients who had longer length of stay in ICU had greater CED (Figure 2-2B). There was no correlation between age in years ($\rho = -0.059$, $p = 0.175$) or APACHE III score ($\rho = 0.008$, $p = 0.859$) to CED.

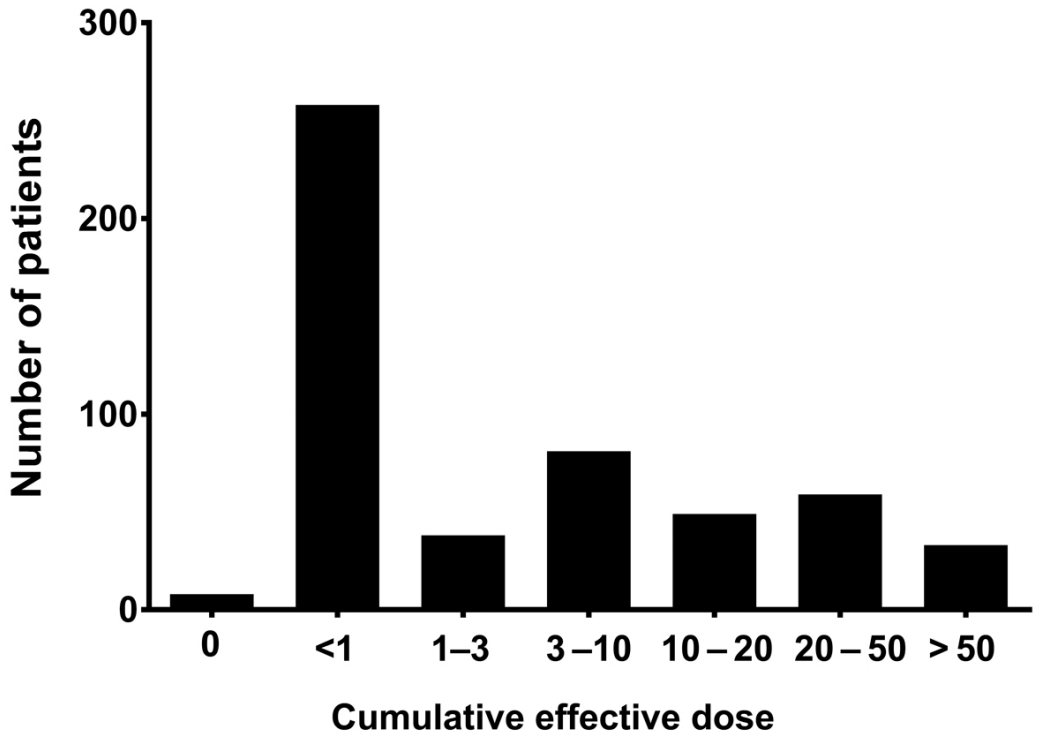

Figure 2-1: Distribution of patient exposure grouped by cumulative effective dose. Majority of patients received less than 1 mSv exposure to ionizing radiation. Bars represent absolute count of total cohort.

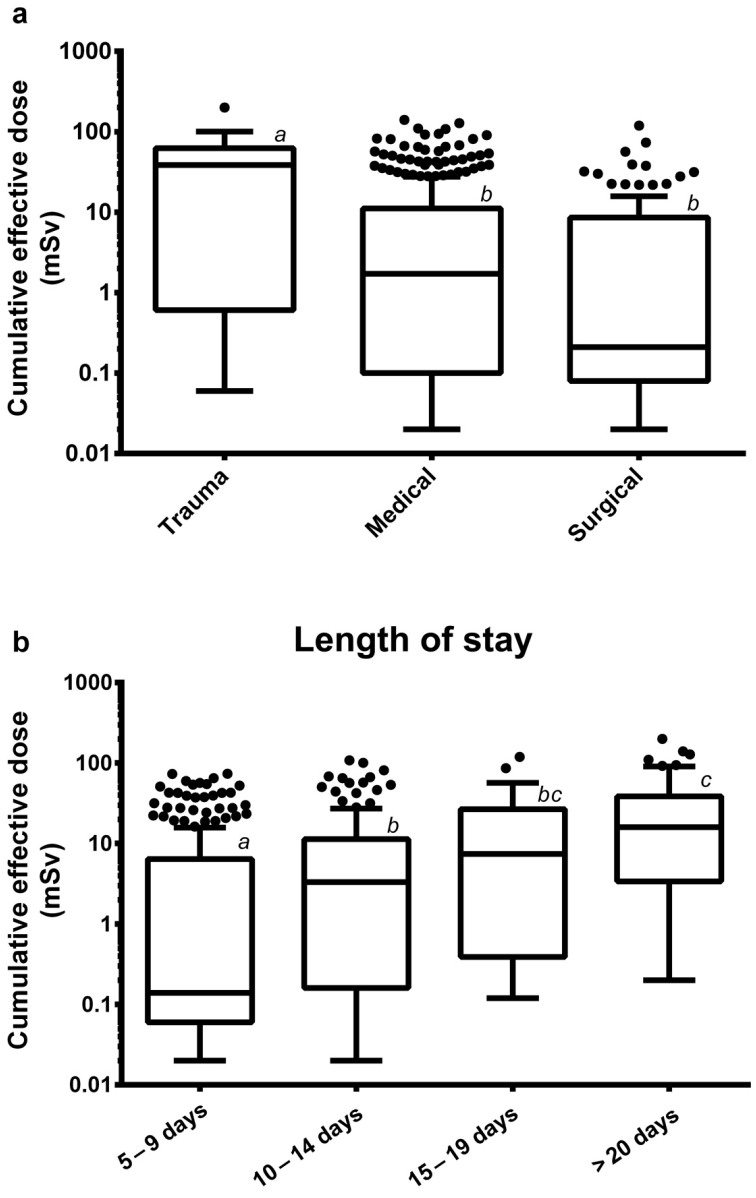

Figure 2-2: Patient factors that contribute to cumulative effective dose. Tukey box and whisker plots of (**a**) admission diagnosis and (**b**) length of stay against cumulative effective dose, with italicised superscripts denoting statistically significant differences ($p < 0.05$) by Kruskal–Wallis analysis with post hoc Mann–Whitney U test. Patients with an admission diagnosis of trauma had significantly higher cumulative effective dose (CED) compared with medical or surgical diagnoses. CED was higher for longer intensive care unit stays. Box plots represent median and interquartile range (IQR), and whisker plots represent 1.5 × IQR below and above the lower and upper quartiles respectively. Single data points represent outliers.

2.4 DISCUSSION

This study is the first to assess cumulative radiation exposures for a large, critically ill cohort without exclusions, based on significantly extended ICU stays, specific diagnosis categorisation or requirements of at least 1 CT or fluoroscopy procedure[4–11]. Although this study excluded stays shorter than five days to remove routine post-surgical stays and thereby focus on those patients intentionally in the ICU, this study did include all radiological exams which used ionizing radiation, not just high dose procedures, and included all admission diagnoses, not just trauma or emergency patients. Therefore, this study is more representative of a typical ICU population and its ionizing radiation exposure than some previous reports. Among previous studies, the median CED was largely overestimated due to these inclusion criteria and ranged from 1.5-104 mSv. However, most patients from this study received less than 1 mSv CED during their stay in the ICU. These exposures are less than both the internationally-recommended exposure limit for members of public (1 mSv) and the man-made portion of the average Australian's yearly radiation exposure (1.7 mSv)[20,26]. However, these patients would have received this exposure over a shorter time period (median stay, 7 days) compared to the year-long period of the public limit exposure and the average Australian's exposure.

Only 6% of patients received more than 50 mSv and 2% above 100 mSv, the upper limit of the low dose radiation spectrum. The health-related risks associated with exposures at these levels are negligible with the large majority of studies showing no harmful effects

below 100 mSv. The most accepted risk assessment model uses the Linear No-Threshold model and predicts that for every 10mSv of radiation, the excess relative risk of cancer incidence increases 0.06%[20]. This is on top of the baseline cancer incidence of 40-42% Australia and USA and 50% for UK[21,27,28]. Thus, these patients, receiving above 100 mSv cumulatively, have a postulated increased risk of cancer from 40-42% to 40.56-42.56%. However, these radiation protection guidelines overcompensate this risk by using modelling based on the atomic bomb survivors and misleading epidemiological studies, which inaccurately extrapolate the risk of low dose radiation from high doses[29,30]. Therefore, although some guidelines calculate increased cancer and other health-related risks, through harnessing the radiation protection modelling, it is the position of many governing bodies to refrain from making such bold statements when dealing with exposures below 100 mSv[20,22,23], to which most diagnostic radiography exposures are found[13]. Long-term, population-based follow up of patients will be required to further decipher this perceived risk.

From this cohort, both trauma categorisation and length of ICU stay were associated with a larger cumulative effective dose (CED). An admission categorisation of trauma typically encompasses patients with multiple sites of major injury, including fractured bones and internal bleeding, leading to scans of most regions of the body. Due to the severity of the injury, secondary follow up scans are also likely leading to increased CED. The length of ICU stay is predominately dictated by the health status of the patient; with sicker or more critical patients associated with longer ICU stays. Continuous

monitoring and re-diagnosis occur in these patients, typically using diagnostic radiation, which additively contributes to higher CED. These, along with a variety of other factors, have also been previously reported in the literature as having an associations to larger CED in ICUs including, active malignancies, readmission to ICU, number of radiology exams, CT scans, and fluoroscopy minutes[6–9].

Consistent with ICUs and hospitals around the world, chest X-rays were the most frequent individual procedure but contributed very little to CED[6,9,11,31–33]. The universal high frequency is likely due to clinical practice and ease of access, with most ICUs having portable X-ray machines. While daily routine chest X-rays were common practice, it was reported than only 2.3% result in a change of management, most often adjustment of antibiotic treatment, implanted devices or central lines[34]. Therefore, practice is moving to clinically indicative administration, saving hospital and patient costs, radiation exposure, and time[35,36].

The median dose of each modality calculated in this cohort is largely consistent with previously published literature, except for the abdominal CT[13]. Typically, abdominal scans result in the highest effective dose of this modality, due to the large number of radiosensitive organs within this region[6,9–11,13,20,31–33]. However, the average abdominal CT in this audit was almost 3x higher than reported in Mettler *et al*[13]. This variation could be due to the defining regions of the scans. It was clinical practice in this ICU to scan both

abdominal and pelvic regions together rather than separately, thereby increasing scan time and radiation exposure to those organs.

This retrospective audit is of a single, medium-sized tertiary centred study and thus these results are limited only to this centre. No conclusions can be made regarding ionizing radiation exposures to ICU patients in other centres without completion of a multicentred approach. It is therefore difficult to discuss these results as being universal across centres in Australia or elsewhere, however, in this centre, majority of patients received negligible doses of ionizing radiation.

Another limitation was that a CT scan was counted as per prescribed regardless of number of actual scans during the procedure. Some CT procedures, such as CT angiograms, require multiple phases to observe how the contrast is circulated around the body. At different time periods different organs are better visualised based on where the contrast is during circulation. Therefore, these procedures can contain multiple scans — for example, a pre-contrast scan, an arterial scan, a venous scan, a delayed scan etc — that are completed at various time intervals after contrast injection, causing the patient to receive a higher dose. Given that some dose reports additively counted the scans as one procedure, separation of these multiphase scans was not possible and, thus, all multiphase CT procedures were reported as one, which explains the large variation in CT dose

2.5 CONCLUSION

Medical radiography is a necessary component of medicine, perhaps particularly in ICU settings. This audit demonstrates that the exposure these patients receive from radiography in a western, tertiary hospital, mixed ICU is relatively small, posing a negligible risk against the potential life-saving benefits. However, further study should be undertaken to identify if these results can be replicated in other tertiary care centres, around Australia and internationally, using similar study criteria.

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CHAPTER 3

CUMULATIVE RADIATION IN HOSPITALISED PREGNANT PATIENTS: A RETROSPECTIVE AUDIT OF IONIZING RADIATION EXPOSURE

SUMMARY

Ionizing radiation is a valuable tool in modern medicine, however, in the case of pregnancy, the potential effects from ionizing radiation are not only to the mother but also to the foetus. As a first step to understanding the risks and benefits of radiation exposure to pregnant patients, we aimed to assess the cumulative levels of ionizing radiation received by pregnant patients during a single admission to a tertiary hospital. From January 2013 till December 2017 inclusive, 28,275 pregnant patients were admitted to Flinders Medical Centre, Adelaide, South Australia. There were 3742 patients who received a radiological procedure, including ultrasound and MRI, but only 547 patients received ionizing radiation. These 547 patients underwent 841 procedures totalling 601.2 mSv of ionizing radiation. The median cumulative effective dose (CED) was 0.02 mSv and only 5 patients received more than 10 mSv, with 19.07 mSv the highest dose received. Stays longer than 10 days had significantly higher CED as did those with cardiovascular or cerebral related admission. The median foetal CED was 0.01 mSv with only 3 foetuses receiving more than 10 mSv. Overall, only 1.9% of patients admitted over the 5 years received ionizing radiation, with

more than 50% of these patients receiving less than a chest X-ray (0.02 mSv). These results suggest that pregnant patients are exposed to relatively low doses of ionizing radiation, in both the individual procedure and cumulative doses, and thus the relative risk to the foetus is negligible. If clinicians are avoiding foetal radiation exposure due to the radiation risk, they should recognise that this risk is negligible from diagnostic procedures.

3.1 INTRODUCTION

We are continuously exposed to ionizing radiation from both natural and artificial sources. The exposure varies around the world, with the average natural background level for Australian's being 1.5 mSv annually whilst the United States of America (USA) residents receive approximately 3.1 mSv[1,2]. In addition to this, levels can increase with artificial exposures, another 1.7 mSv for Australia and 3.1 mSv for the USA, totalling an average yearly exposure of 3.2 mSv and 6.2 mSv respectively[2,3]. Of these artificial exposures, most derive from medical sources with ionizing radiation playing a crucial role in medicine; aiding in both diagnosis and therapy.

Diagnostic radiation typically utilises X-irradiation, for conventional radiography, computed tomography (CT) and fluoroscopy, or gamma-emitting radioisotopes, such as Technetium-99 used in nuclear medicine. Average patient doses can range between 0.001 – 16 mSv, with a chest X-ray about 0.02 mSv and an abdominal CT 8 mSv[4]. When a female of childbearing age requires diagnostic radiation, confirmation of pregnancy is required and can alter the course of diagnosis[5]. During pregnancy, exposure levels can vary significantly between mother and foetus due to the procedure's field of view, the delivery method of nuclear medicine or the shielding used. When the foetus is not in the direct field of the X-rays, such as head and neck radiography, it will only receive a fraction of the dose, limited to scatter radiation[6]. When the radionuclide is administered via a route which is unlikely to travel near the foetus, for example, inhaled gas, then the dose will be low, compared to

methods which come in close contact, such as I.V. injection, which may transfer into placental circulation[7,8]. When pregnant patients receive ionizing radiation, they generally have lead shielding applied to areas not intended for the scan. The lead will absorb radiation and reduce to the dose to the tissues underneath. Lead shielding is applied over the uterus to reduce the dose to the foetus during diagnostic radiation.

Among hospitalised patients, diagnostic and interventional radiology is potentially used least for pregnant patients due to the additional fear of ionizing radiation exposure to the unborn foetus. This fear stems from the reported stochastic effects of cancer risk[9,10], although the reality of this risk, particularly from diagnostic levels of radiation, is continually challenged[11–13]. Similarly, for deterministic effects, there is uncertainty as to the effect of low doses of radiation but the current understanding is that adverse outcomes increase with higher doses and earlier exposures[14–16]. Although controversial within the radiation protection profession, there is no universally recognised threshold for the effects of radiation. The consensus is that there is technically no 'safe level' of ionizing radiation and that any dose carries a potential risk to the developing foetus. Regardless, the possible risk of ionizing radiation to the foetus should always be weighed against the clinical benefit from the diagnostic procedure and against the risk of not receiving that procedure.

As a first step, it is important that we accurately understand the current levels of radiation these patients, and more specifically their unborn foetuses, are receiving. This study will inform clinicians regarding their prescribing practices and the resultant exposures thereby

aiding their future assessment of the risk-to-benefit analysis. The aim of this study was to conduct a retrospective audit of cumulative ionizing radiation exposure of all pregnant patients admitted to Flinders Medical Centre over a 5-year period.

3.2 METHOD

3.2.1 Study cohort

The study cohort included patients admitted to Flinders Medical Centre, Adelaide, Australia, between 1st January 2013 and 31st December 2017 with a pregnancy-related admission code. In some circumstances, patients were coded with pregnancy status, but the primary diagnosis code was unrelated to pregnancy; these patients were still included. In other circumstances, patients were coded as pregnant due to suspicion of pregnancy. Cases of suspected pregnancy which were later confirmed not to be pregnant were removed. This audit was reviewed and approved by the South Adelaide Clinical Human Research Ethics Committee (OFR # 172.18) in line with the requirements of the *National Statement on Ethical Conduct in Human Research* (2007) and *the Australian Code for the Responsible Conduct of Research* (2007) and the requirement of informed consent was waived.

3.2.2 Data collection

Hospital records from Open Architecture Clinical Information System (OACIS), and Health Information Portal (HIP) and the radiological records from Picture Archiving and

Communication System (PACS) were used to collect demographic information, clinical data and radiology reports for each patient.

All ionizing forms of radiation were recorded including conventional X-rays, Computed Tomography (CT), fluoroscopy and nuclear medicine, as well as non- ionizing procedures, such as ultrasound and magnetic resonance imagining.

3.2.3 Calculation of effective dose

Effective doses for each procedure were calculated using values from patient radiology reports and reported conversion factors by Deak *et al* and Hart and Wall[17,18]. In short, values for Dose Area Product (DAP) or Dose Length Product (DLP) produced from the radiology reports were collected and converted to effective dose (mSv) via multiplication by tissue-specific conversion factors (Table 3-1). For conventional X-rays, accepted diagnostic reference levels were used for each individual exam due to lack of information in reports, except in cases where enough information enabled calculation of effective doses.

Effective doses for foetal exposures were derived using values from Sharp et al, Russel et al and Hauer et al[6,7,19]. In brief, average procedure doses were used for common procedures, where available (Table 3-1). Maternal doses from procedures in which the field of view encompassed the foetus were equated to equivalent foetal doses, such in abdominal and pelvic X-ray or CT.

Table 3-1: Conversion factors for each modality, divided by body region scanned or radiopharmaceutical used adopted from Deak *et al* and Hart and Wall[17,18], and foetal doses following common diagnostic procedures, adapted from Sharp *et al*, Russel *et al* and Dauer *et al* [6,7,19].

	Conversion factors	*Foetal doses (mSv)*
CONVENTIONAL X-RAY		
Head/neck	0.000028	0.01[a]
Chest	0.00012	0.01[a]
Abdominal and pelvic	0.00027	MD
Extremities	0.00001	0.01[a]
COMPUTED TOMOGRAPHY		
Head	0.0019	0.01[a]
Neck	0.0051	0.01[a]
Chest	0.0145	0.06[a]
Abdominal and pelvic	0.0153	MD
FLUOROSCOPY		
Cardiovascular	0.00012	0.3[c]
Gastrointestinal	0.00026	MD
Urinary	0.00018	MD
Peripheral	0.00001	0.01[c]
NUCLEAR MEDICINE		
^{99m}Tc MAA	0.011	0.75[b]
^{99m}Tc Tgas	0.015	0.3[b]

mSv = millisieverts, CT = computed tomography,
99mTc = Technetium-99m; MMA = macro aggregated albumin; Tgas = Technegas
Dose from [a] = Sharp et al, [b] = Russel et al, [c] = Hauer
MD = maternal dose

As this study focuses on radiation exposures in pregnancy, only patients who had live pregnancies at the time of the procedure were included. No radiological information was collected for scans during pre-conception, non-viable pregnancies or postpartum.

3.2.4 Statistics

All outcomes measured in this audit were non-normally distributed, therefore data was expressed as median (interquartile range (IQR)) and assessed using non-parametric tests. Categorical variables, including patient indigenous status, gestational age of the foetus, admission unit, admission diagnosis and length of stay, were assessed by Kruskal-Wallis analysis with *post hoc* Mann-Whitney U test. The scale variable of age was assessed using Spearman rank-order correlations. Statistical significance was accepted as $p < 0.05$.

3.3 RESULTS

3.3.1 Overall patient cohort

There were 28,275 patients admitted to Flinders Medical Centre with a pregnancy-related admission over the 5-year period of this study, Figure 3-1. A total of 3742 (13.24%) patients received 5310 diagnostic radiological procedures. Of these patients, 3099 patients (82.82%) received an ultrasound, 96 patients (2.57%) received an MRI and 547 patients (14.62%) received a procedure using ionizing radiation: X-ray, CT, fluoroscopy or nuclear medicine (Table 3-2). Conventional X-ray was the most frequent procedure then CT scans, 11.45% and 2.47% respectively. As ultrasound and MRI do not produce ionizing radiation, the final exposed cohort was reduced to 547 patients, Figure 3-1.

3.3.2 Exposed patient cohort

Within the exposed cohort of 547 patients, the median age was 33 years old (IQR, 28-38) with a median length of stay of 3 days (1-7),Table 3-3. These patients were admitted with either pregnancy or non-pregnancy related primary diagnosis, with delivery the main reason for pregnancy-related admissions and trauma the main reason for non-pregnancy related admissions. The median cumulative effective dose (CED) of the cohort was 0.02 mSv (IQR, 0.02-0.75) and did not significantly change based on indigenous status (p = 0.291).

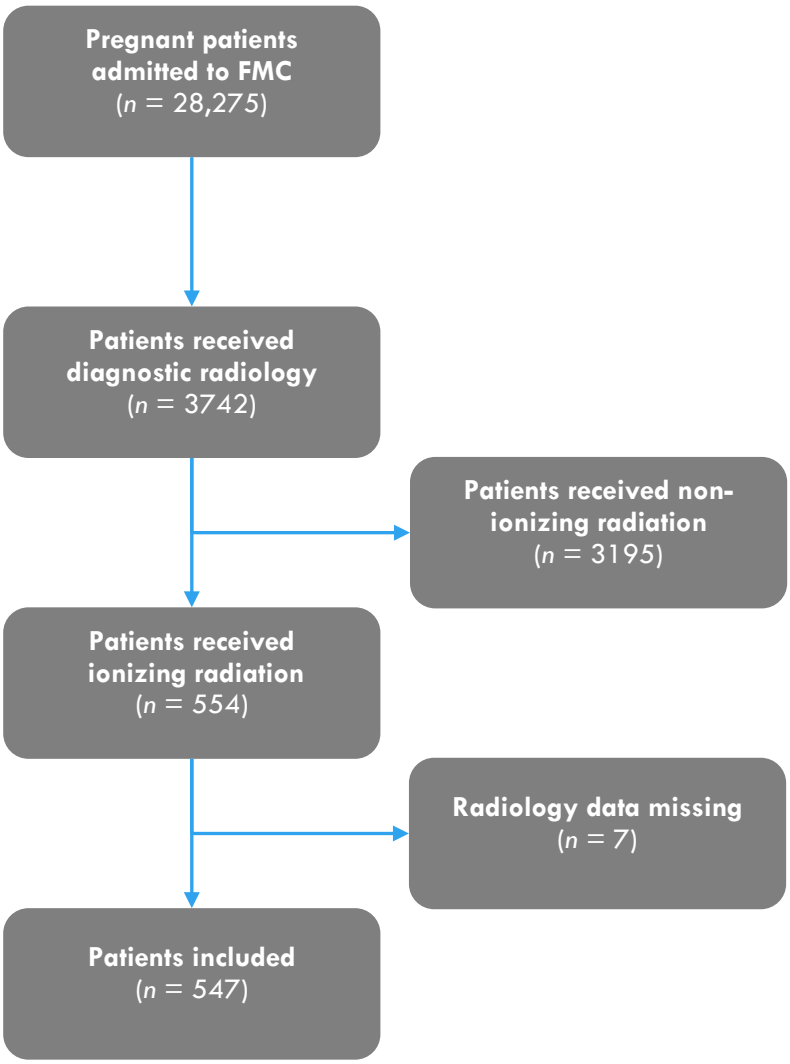

Figure 3-1: Flow chart of patient eligibility criteria for inclusion in retrospective audit.
FMC = Flinders Medical Centre

Table 3-2: Distribution of patient and procedure frequency of pregnant patients who received any diagnostic radiation during the length of stay.

	Contribution to frequency of patients (n = 3742 patients)		*Contribution to frequency of procedures (n = 5310 procedures)*	
NON-IONIZING RADIATION	84.18%		84.15%	
Magnetic resonance imaging		*2.57%*		*1.85%*
Ultrasound		*82.82%*		*82.3%*
IONIZING RADIATION	14.62%		15.85%	
Conventional X-ray		*12.08%*		*11.45%*
Computed tomography		*3.13%*		*2.47%*
Fluoroscopy		*1.39%*		*1.13%*
Nuclear medicine		*1.12%*		*0.79%*

Table 3-3: Summary of patient demographic and clinical characteristics.

Demographic and clinical characteristics	*Values (%)*
TOTAL NUMBER OF PATIENTS	547
AGE (years), *median (IQR)*	33 (28-38)
INDIGENOUS STATUS	
Aboriginal	*54 (9.9%)*
Non-indigenous	*474 (86.7%)*
Data not available	*19 (3.5%)*
LENGTH OF STAY (days), *median (IQR)*	3 (1-7)
GESTATIONAL AGE	
<5 – 13 wks	69 (12.3%)
14 – 19 wks	61 (10.8%)
20 – 25 wks	80 (14.2%)
26 – 34 wks	194 (34.5%)
35 - >37 wks	159 (28.2%)
ADMISSION DIAGNOSIS	
Non-pregnancy related	195 (35.6%)
Cardiovascular	*30 (5.5%)*
Cerebral	*17 (3.1%)*
Gastrointestinal	*27 (4.9%)*
Respiratory	*46 (8.4%)*
Trauma	*48 (8.8%)*
Other	*27 (4.9%)*
Pregnancy related	352 (64.4%)
Cardiovascular	*43 (7.9%)*
Cerebral	*18 (3.3%)*
Delivery	*155 (28.3%)*
Gastrointestinal	*18 (3.3%)*
Respiratory	*75 (13.7%)*
Other	*43 (7.9%)*

IQR = interquartile range; wks = weeks

Non-pregnancy related Other (*n*) = Haematological (1), Cancer (1), Genitourinary (3), Medication related (3), Mental disorder (2), Musculoskeletal (2), Dermatological (4), Infection (5), Observational (7).

Pregnancy-related Delivery (*n*) = False Labour (7), Pre-eclampsia (8), Delivery complications (24) Abortion (26).

Pregnancy-related Other (*n*) = Cancer (1), gestational diabetes (2), Pelvic abnormality (2), Foetal abnormality (2), Dermatological (2), Haematological (4), Musculoskeletal (5), Severe morning sickness (7), Infection (8), Genitourinary (10).

3.3.3 Distribution of procedure frequency and dose

As seen in Table 3-4, 452 patients (82.63%) received conventional X-rays and 117 patients (21.39%) received CT scans. Fluoroscopy was received by 52 patients (9.51%) while 42 patients (7.68%) received nuclear medicine. Conventional X-ray was the most frequent procedure, accounting for 72.29% of 841 procedures but only contributed 4.28% to the 601.2 mSv received by the cohort. The most frequent individual procedure was a chest X-ray accounting for 57.36% of all procedures (Table 3-4). CT scans contributed the most to total dose (74.25%), with chest CT accounting for 46.21% of total dose, but only accounted for 15.58% of all procedures. Although chest CT contributed the most to the total dose, the abdominal and pelvic CT produced the highest median and individual dose of any procedure, 12.00 mSv and 16.31 mSv respectively (Table 3-5). There were 4 abdominal and pelvic CT scans and they produced 4/5 highest dose-producing procedures (Table 3-6). Fluoroscopy was performed more frequently than nuclear medicine but contributed slightly less to total cohort dose, 7.13% vs 4.99% of procedures compared to 10.52 vs 10.95% in dose contribution. Doses for each individual procedure could range significantly, sometimes up to 500-fold (e.g. with gastrointestinal fluoroscopy).

3.3.4 Pregnant patient cumulative radiation exposure

In this cohort, the median CED was 0.02 mSv. The large majority (68.7%) of patients received less than 1 mSv CED during their stay and only 22 patients (4%) received more than 5 mSv (Figure 3-2). Of these, only 5 patients (1%) received more than 10 mSv, with the highest CED recorded as 19.07 mSv.

Table 3-4: Distribution of procedures which use ionizing radiation and the frequency at which they contribute to the number of patients, number of procedures and total dose.

	Contribution to patients (n = 547 patients)		*Contribution to procedures (n = 841 procedures)*		*Contribution to total dose (n = 601.2 mSv)*	
CONVENTIONAL X-RAY	82.63%		72.29%		4.28%	
Head/neck		*7.13%*		*5.34%*		*0.04%*
Chest		*70.02%*		*57.36%*		*1.61%*
Abdominal and pelvic		*7.13%*		*7.01%*		*0.02%*
Extremities		*3.66%*		*2.49%*		*2.62%*
COMPUTED TOMOGRAPHY	21.39%		15.58%		74.25%	
Head		*7.50%*		*5.70%*		*17.69%*
Neck		*1.28%*		*0.83%*		*2.00%*
Chest		*12.80%*		*8.55%*		*46.21%*
Abdominal and pelvic		*0.73%*		*0.48%*		*8.35%*
FLUOROSCOPY	9.51%		7.13%		10.52%	
Cardiovascular		*1.65%*		*1.31%*		*0.02%*
Gastrointestinal		*6.95%*		*4.75%*		*10.40%*
Urinary		*0.91%*		*0.59%*		*0.10%*
Peripheral		*0.73%*		*0.48%*		*0.00%*
NUCLEAR MEDICINE	7.68%		4.99%		10.95%	
Pulmonary		*7.68%*		*4.99%*		*10.95%*

mSv = millisieverts

Table 3-5: Exposures from each modality divided by body region scanned.

	Median effective dose (mSv) Median (IQR)	*Range of effective dose (mSv)*
CONVENTIONAL X-RAY		
Head/neck	0.006 (0.006-0.006)	0.006-0.006
Chest	0.020 (0.020-0.020)	0.020-0.020
Abdominal and pelvic	0.750 (0.750-0.750)	0.750-0.750
Extremities	0.002 (0.002-0.002)	0.002-0.002
COMPUTED TOMOGRAPHY		
Head	1.83 (1.52-3.06)	0.47-3.60
Neck	1.63 (1.02-2.53)	0.75-2.97
Chest	3.64 (2.90-4.79)	0.82-12.46
Abdominal and pelvic	12.00 (10.42-14.66)	9.87-16.31
FLUOROSCOPY		
Cardiovascular	0.002 (0.000-0.010)	0.000-0.081
Gastrointestinal	1.102 (0.435-2.613)	0.014-6.278
Urinary	0.132 (0.123-0.144)	0.039-0.164
Peripheral	0.003 (0.000-0.003)	0.000-0.004
NUCLEAR MEDICINE		
Pulmonary	1.53 (1.44-1.64)	1.30-2.30

mSv = millisieverts, IQR = Interquartile range

Table 3-6: Top 5 highest dose procedures with patient characteristics and diagnoses.

Patient	*Age*	*gestation*	*Procedure*	*Dose (mSv)*	*Admission unit*	*Reason for scan*	*Primary diagnosis*	*Confounding factors*
1	28	26-34wks	Abdominal and pelvic CT plain	16.31	ICCU	HELLP syndrome	eclampsia	Kidney disease, cerebral haemorrhage, caesarean delivery of stillborn, asthma, smoker, mental disorder
2	22	05-13wks	Abdominal and pelvic CT w contrast	13.01	ICCU	motor vehicle accident	Trauma	Fracture of femur, thoracic spine, intracranial injuries, pneumonia
3	29	34->37wks	CT pulmonary angiography	12.46	OBS	Suspected plural embolism	Caesarean delivery	Gestational diabetes, excess foetal growth, polyhydramnios, tachycardia, smoker
4	33	05-13wks	Abdominal and pelvic CT plain	10.99	RENAL	Reflux nephropathy post-transplant	Medical abortion w complication	Severe kidney disease, corpus luteum cyst, anaemia
5	40	14-19wks	Abdominal and pelvic CT w contrast	9.87	ICCU	Intraabdominal sepsis	Medical abortion	Sepsis, ARDS, intestinal perforation, intestinal endometriosis, pelvic infection, anaemia

mSv = millisieverts, CT = computed tomography, w = with
ICCU = intensive and critical care unit, OBS = obstetrics, RENAL = renal ward
ARDS = acute respiratory distress syndrome

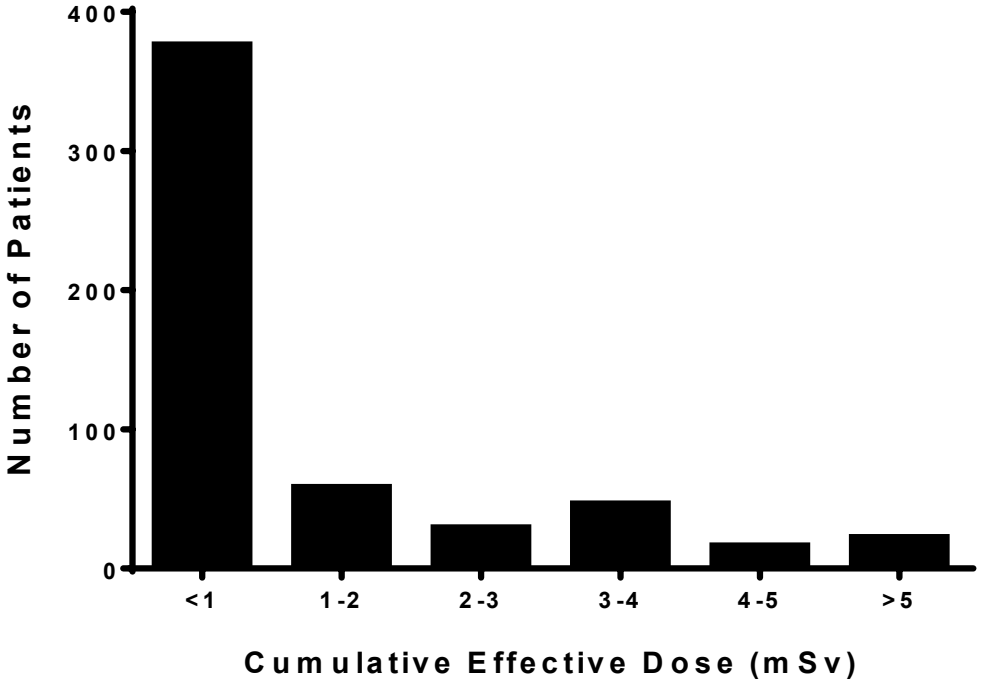

Figure 3-2: Distribution of patient cumulative exposure grouped by cumulative effective dose. Most patients received less than 1 mSv exposure to ionizing radiation. Bars represent absolute count.

There was no significant difference in CED among gestational age groups or admission unit (Figure 3-3, $p = 0.085$ and $p = 0.409$, respectively) or significant correlation between CED and age ($\rho = -0.04$, $p = 0.349$). However, as in Figure 3-3, those patients who stayed more than 10 days had significantly higher CED than any other group ($p < 0.01$). Additionally, admission categories appeared to separate into three distinct groups, regardless of whether the patient was primarily admitted due to pregnancy-associated reasons ($p < 0.001$). Patients with cardiovascular or cerebral admission received a higher CED than the rest and those with gastrointestinal or trauma-related admissions received a lower CED. Finally, there was a significant difference in CED among year of admission ($p < 0.001$), with a slight increase in median CED in 2015 and a peak in 2016 before decreasing again in 2017. A breakdown of modality and dose by year is available in Table 3-7.

3.3.5 Foetal cumulative radiation exposure

The median foetal CED was 0.01 mSv, with only 26 foetuses (4.5%) receiving >1 mSv and 3 (0.4%) receiving >10 mSv. The highest exposure to a foetus was 16.33 mSv. Similar to maternal exposures, there was no significant difference among admission unit ($p = 0.589$) or significant correlation to maternal age ($\rho = 0.021$, $p = 0.621$), but there were slight variations in other factors (Figure 3-4). Mothers who stayed in hospital >10 days had higher foetal exposures than any other group ($p < 0.001$) and foetuses exposed in 2016, but not 2015, had a higher CED compared to other years ($p < 0.001$). Maternal admission diagnosis also resulted in differences in foetal exposures ($p < 0.001$). Foetuses from mothers with cardiovascular admissions, but not cerebral, again received the highest median CED (0.07 mSv) which was significantly higher than the rest of the groups.

Although all remaining groups had a median CED of 0.01 mSv, foetuses from the mothers admitted for delivery had significantly different CED compared to all other groups, due to the increased range of exposures. Of these mothers, 24/26 of foetal exposures were above 1 mSv. Unlike maternal exposures, gestational age was significantly associated with a higher CED (p = 0.013).

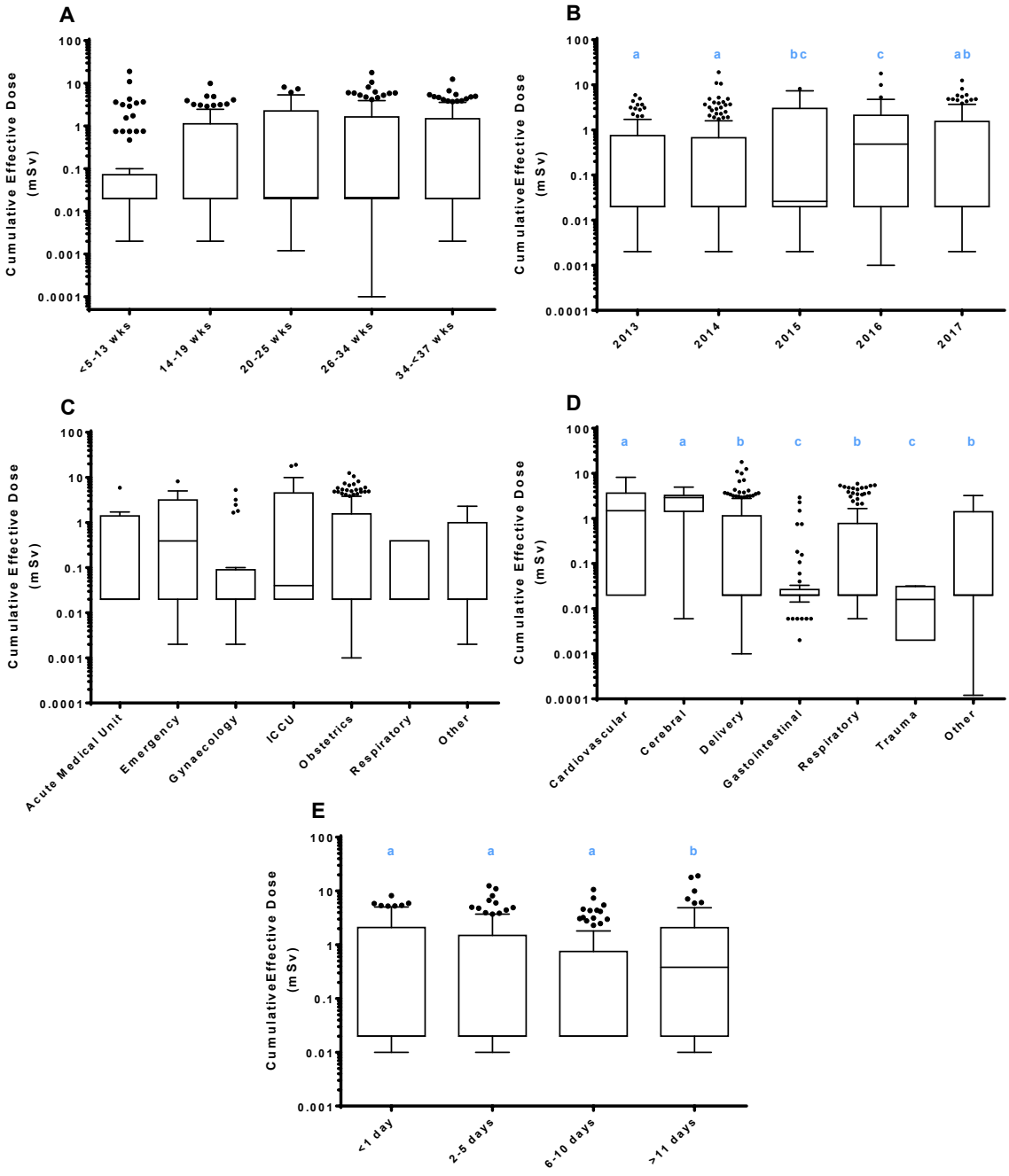
A
Cumulative Effective Dose (mSv)
100
10
1
0.1
0.01
0.001
0.0001
<5-13 wks
14-19 wks
20-25 wks
26-34 wks
34-<37 wks
B
Cumulative Effective Dose (mSv)
a
a
bc
c
ab
2013
2014
2015
2016
2017
C
Cumulative Effective Dose (mSv)
Acute Medical Unit
Emergency
Gynaecology
ICCU
Obstetrics
Respiratory
Other
D
Cumulative Effective Dose (mSv)
a
a
b
c
b
c
b
Cardiovascular
Cerebral
Delivery
Gastointestinal
Respiratory
Trauma
Other
E
Cumulative Effective Dose (mSv)
a
a
a
b
<1 day
2-5 days
6-10 days
>11 days

Figure 3-3: Factors that affect maternal cumulative doses **A)** Gestational age at exposure, **B)** Year of admission, **C)** Admission unit, **D)** Admission diagnosis and **E)** Length of stay**. A)** There was no difference in CED among groups of gestational ages ($p = 0.085$, $n = 69–194$). **B)** There was a significant difference in CED among admission years ($p < 0.001$, $n = 85–121$). Median CEDs increased in 2015 and peaked at 2016 before reducing back down in 2017, 0.026 0.487 and 0.020 respectively. **C)** There was no difference in CED depending on admission unit ($p = 0.409$, $n = 17–354$). **D)** There was a significant difference in the CED based on patient admission diagnoses ($p < 0.001$, $n = 35–155$), separating into three statistically different levels of CED. Cardiovascular and cerebral related admission received the highest CEDs, 1.49 and 2.91 mSv respectively, whereas patients with gastrointestinal or trauma-related admission received the lowest CEDs, 0.02 and 0.016 mSv. Finally, **E),** There was a significant difference among length of stay groups ($p = 0.04$), with patients who stayed more than 10 days having a significantly higher CED, median = 0.16mSv, compared to all other groups, median = 0.02 mSv for all individual groups, $n = 77–204$. Box plots represent the median and interquartile range (IQR), and whisker plots represent 1.5 × IQR below and above the lower and upper quartiles respectively. Single data points represent outliers. Non-visible medians are due to equal and aligned lower quartile and median lines. Factors were statistically assessed by Kruskal Wallis with *post hoc* Mann-Whitney U analysis. Superscripts denote a statically significant difference of $p < 0.05$.

Table 3-7: Breakdown of frequency and dose information for each modality across each admission year.

	CONVENTIONAL X-RAY				CT			
	n (%)	*percent per modality*	*Sum of Dose mSv*	*Median dose (IQR) mSv*	*n (%)*	*percent per modality*	*Sum of Dose mSv*	*Median dose (IQR) mSv*
2013	112 (18.4%)	79.4%	4.9	0.02 (0.02-0.02)	13 (9.9%)	9.2%	33.0	2.70 (1.67-2.98)
2014	158 (26%)	79.4%	4.0	0.02 (0.02-0.02)	29 (22.1%)	14.6%/	101.2	2.49 (1.73-4.16)
2015	116 (19.1%)	69.5%	6.4	0.02 (0.02-0.02)	32 (24.4%)	19.2%	111.3	3.33 (2.50-4.27)
2016	118 (19.4%)	65.6%	6.5	0.02 (0.02-0.02)	31 (23.7%)	17.2%	105.0	3.05 (1.57-3.45)
2017	104 (17.1%)	67.5%	4.1	0.02 (0.02-0.02)	26 (19.8%	16.9%	95.9	3.39 (1.78-4.57)
	FLUOROSCOPY				NUCLEAR MEDICINE			
	n (%)	*percent per modality*	*Sum of Dose mSv*	*Median dose (IQR) mSv*	*n (%)*	*percent per modality*	*Sum of Dose mSv*	*Median dose (IQR) mSv*
2013	9 (15%)	6.4%	3.2	0.14 (0.01-0.37)	7 (16.7%)	5.0%	11.4	1.5 (1.43-1.7)
2014	11 (18.3%)	5.5%	9.4	0.08 (0.00-1.13)	1 (2.4%)	0.5%	1.4	1.45 (1.45-1.45)
2015	11 (18.3%)	6.6%	18.3	0.80 (0.12-3.69)	8 (19%)	4.8%	13.2	1.63 (1.62-1.68)
2016	17 (28.3%)	9.4%	18.7	0.65 (0.03-1.65)	14 (33.3%)	7.8%	21.6	1.45 (1.36-1.64)
2017	12 (20%)	7.8%	13.6	0.87 (0.11-1.83)	12 (28.6%)	7.8%	18.1	1.51 (1.47-1.56)

IQR = Interquartile range, mSv = millisieverts

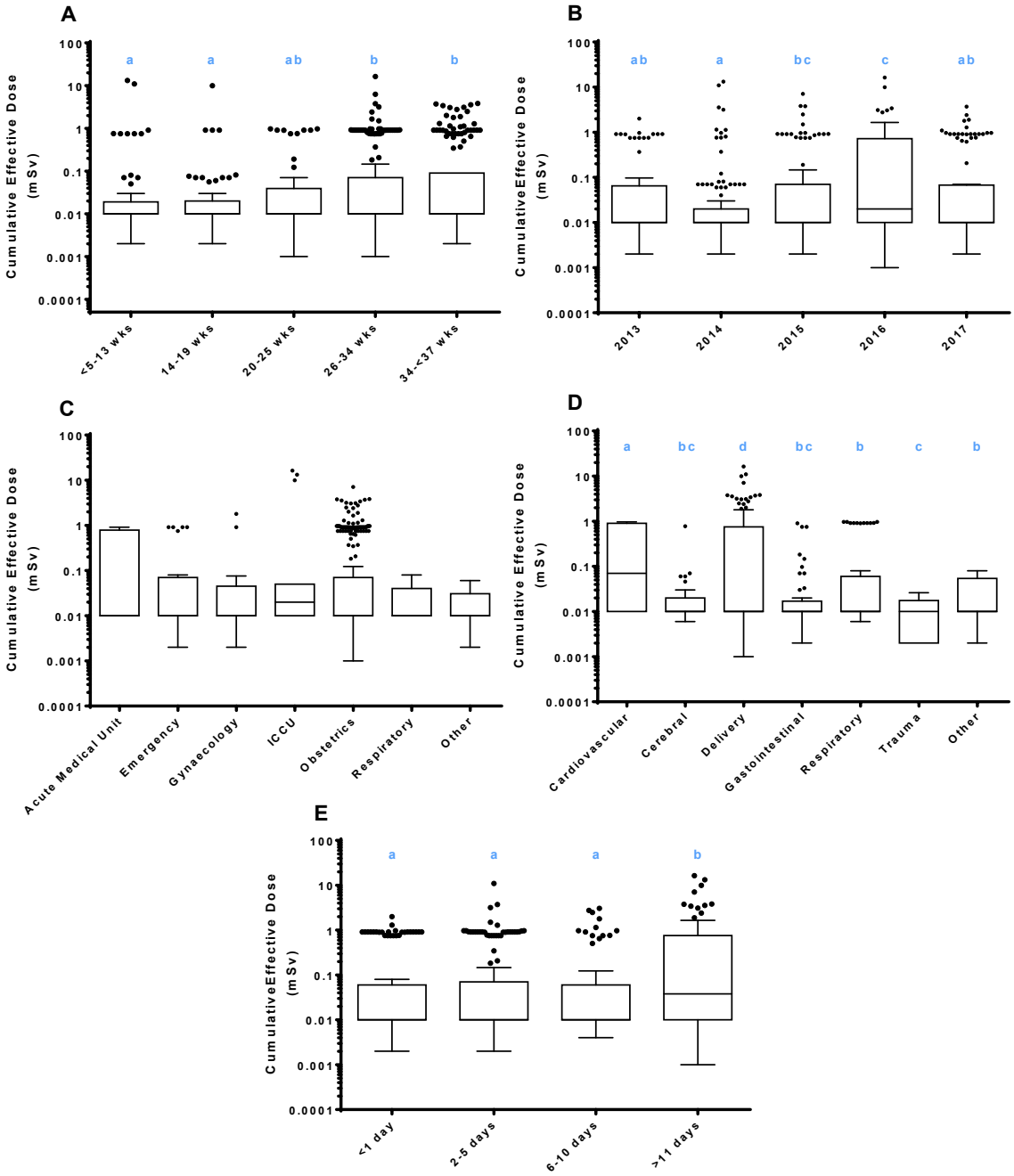
A
Cumulative Effective Dose (mSv)
100
10
1
0.1
0.01
0.001
0.0001
a
a
ab
b
b
<5-13 wks
14-19 wks
20-25 wks
26-34 wks
34-<37 wks
B
Cumulative Effective Dose (mSv)
ab
a
bc
c
ab
2013
2014
2015
2016
2017
C
Cumulative Effective Dose (mSv)
Acute Medical Unit
Emergency
Gynaecology
ICCU
Obstetrics
Respiratory
Other
D
Cumulative Effective Dose (mSv)
a
bc
d
bc
b
c
b
Cardiovascular
Cerebral
Delivery
Gastointestinal
Respiratory
Trauma
Other
E
Cumulative Effective Dose (mSv)
a
a
a
b
<1 day
2-5 days
6-10 days
>11 days

Figure 3-4: Factors that affect foetal cumulative effective doses **A)** Gestational age at exposure, **B)** Year of admission, **C)** Admission unit, **D)** Admission diagnosis and **E)** Length of stay**. A)** There was a significant difference in CED among groups of gestational ages (p = 0.013, n = 69–194). There was a trend that increasing gestational age-related to increased CED however, the median CED was constant among groups, 0.01 mSv. **B)** There was a significant difference in CED among admission years (p < 0.001, n = 85–121). Admissions in 2016 had significantly higher CED, median = 0.02 mSv, compared to the other groups, median = 0.01 mSv **C)** There was no difference in CED depending on admission unit (p = 0.409, n = 17–354). **D)** There was a significant difference in foetal CED based on maternal admission diagnoses (p < 0.001, n = 35–155). Cardiovascular admissions had the highest CED compared to the other admission groups, 0.07 vs 0.01 mSv respectively. Although delivery admission also had a median CED of 0.01 mSv, they were significantly different from the rest of the groups due to the larger and higher distribution of exposures. Finally **E),** There was a significant difference among length of stay groups (p = 0.04), with mothers who stayed more than 10 days having a significantly higher foetal CED, median = 0.036mSv, compared to all other groups, median = 0.01 mSv for all individual groups, n = 77–204. Box plots represent the median and interquartile range (IQR), and whisker plots represent 1.5 × IQR below and above the lower and upper quartiles respectively. Single data points represent outliers. Non-visible medians are due to equal and aligned lower quartile and median lines. Factors were statistically assessed by Kruskal Wallis with *post hoc* Mann-Whitney U analysis. Superscripts denote a statically significant difference of p < 0.05.

3.4 Discussion

In this 5-year, retrospective audit, only 1.9% (546/28275) of patients received ionizing radiation, which was in the form of conventional X-ray, CT, fluoroscopy or nuclear medicine. Of these exposures, most patients accumulated very small CED, median = 0.02 mSv, similar to 5 days of background radiation in Australia[1].

To the author's knowledge, this is the first audit of pregnant patients regardless of procedure-specific inclusion criteria, for example: must have received a CT scan, to include all forms of ionizing radiation in Australia. In 2006, a 10-year retrospective audit at a tertiary care centre in the USA had much higher median doses for each modality with calculated foetal doses for conventional radiography = 0.43 mGy, CT = 4.3 mGy, fluoroscopy = 2.91 mGy, nuclear medicine = and 0.41 mGy[20]. However, during this study, there were several advancements in technology to reduce dose, especially with regards to exposure to venerable patients like foetuses and children, which have continued to now[21]. Additionally, in the scans received by our cohort, the dose reports consistently reported dose reductions of up to 40%, which would significantly reduce the median procedure, as well as cumulative effective, doses.

Other studies focused on foetal doses with inclusion criteria based on the prescription of specific procedures. Some studies focused on fluoroscopic exams and showed that foetal doses were negligible except those from barium enemas which can vary in dose, 1.14-16.27 mGy[22,23]. The majority of studies focused on CT scans during pregnancy and therefore reported much higher calculated mean foetal doses 4.3 – 24.8 mGy[20,24–29]. Whilst some were based on patient cohorts[20,24–26], others were on anthropomorphic

models[27–29], and although there are large variations in mean dose, based on scan location, scan parameters, type of scanner used and method of calculation, the conclusion that abdominal and pelvic CT scans result in the largest dose, remained consistent.

In this cohort, abdominal and pelvic CT scans similarly produced the highest median and individual procedure dose and although there were only 4 abdominal CT scans received, they were all in the top 5 highest dose procedures. During an abdominal/pelvic CT, the foetus is directly exposed and therefore receives a high dose as well. In following the clinical guideline of ALARA, as low as reasonably achievable, clinicians are likely to avoid using this procedure to reduce the dose, and thereby perceived risk, to the foetus. This may explain why the frequency of abdominal and pelvic CT scans are low compared to other patient populations within the hospital[4,30–33].

In the 4 patients that received abdominal and pelvic CT scans, the risk of not performing this procedure had to be weighed against the risk of ionizing radiation to the foetus. These scans were performed on patients who had serious primary diagnoses and confounding diagnoses/factors that specifically required ionizing radiation to confirm and manage diagnosis; these were not healthy patients (Table 3-6). Thus, the risk to mother, and thereby foetus, by not performing the procedure was deemed greater than the radiation risk to the foetus.

Choice of procedure during diagnosis may also change due to confirmation of pregnancy status, as in the diagnosis of pulmonary embolism which can be with CT or nuclear medicine[34]. Chest CT results in higher exposures than respiratory nuclear medicine, with 97.2% (70/72) of chest CT being CT pulmonary angiography (CTPA) and 100% (42/42) of

respiratory nuclear medicine being ventilation and perfusion scans (Table 3-5), which is consistent with the literature[4,35]. It is important to note, that CTPA will result in a higher dose to the breast tissue, which, depending on stage of gestation, could be undergoing proliferation in preparation for lactation. This breast tissue is therefore at increased risk of mutation and, if repeatedly exposed, possible cancer induction[36]. As reviewed by Pahade *et al,* it is estimated that CTPA scans result in breast absorbed doses, not effective doses, of 10 – 70 mGy, compared to 0.22 – 0.28 mGy for ventilation-perfusion scans, however, these numbers would be reduced in pregnancy by a reduction in kVp and mAs[35]. Unfortunately, ventilation and perfusion scans produce a higher dose to the foetus compared to CTPA, as the injected radiopharmaceuticals come in closer proximity to the foetus, 0.8 vs 0.06 mSv respectively[35,37,38]. All these factors need to be considered when prescribing these procedures.

Several factors affected maternal and foetal doses. Pregnant patients who were admitted under cardiovascular and cerebral diagnosis received significantly higher CED than other admissions (Figure 3-3), presumably due to these scans encompassing organs outside the field of view of the foetus and would, therefore, pose little risk to the foetus. Clinicians would, therefore, have an easier risk-to-benefit analysis with prescribing these scans, and thus a higher volume of these higher-dose procedures was performed, increasing maternal dose. However, foetal doses were only higher in mothers with cardiovascular and not cerebral admission. Both diagnoses had CT scans and general radiology, but only cardiovascular patients had ventilation-perfusion scans. The technetium labelled albumin aggregate (^{99m}Tc-MAA) perfused intravenously in these scans can come into close proximity to the foetus via circulation leading to a higher foetal doses[7,8].

A length of stay more than 10 days significantly increased maternal and foetal CED, however, this finding is not unexpected, as patients don't usually stay in the hospital unless clinically required or for patient welfare. Due to disease severity or observational requirements, these patients require continual monitoring for disease progression, cumulatively increasing their CED. As with length of stay, the median CED also changed based on year of admission, but not in a discernible trend. Although 2015 was significantly higher than 2013, 2014 and 2017, the median only increased by 0.006 mSv for maternal exposure, less than 1/3rd the dose of a chest X-ray[4], and this small change was not seen in foetal doses. Admissions in 2016 saw a larger maternal median increase to 0.487 mSv and foetal median to 0.02 mSv. It also had a higher amount of CT, fluoroscopy, and nuclear medicine procedures producing higher total exposures per year (Table 3-7). As admissions per year were comparable among years, a higher total CED would result in a higher median CED.

Gestational age of the foetus did not significantly alter the maternal CED in this study, however, it did slightly increase foetal CED, which has been observed in other studies[27,37]. Increases in gestational age typically align with increases in size. Thus for indirect exposures, such as in chest CT, the foetus becomes closer to the field of view, receiving more scatter radiation and therefore a higher dose[37]. For direct exposures, a larger mass means that the foetus is not as deep in the uterus, and therefore not as shielded by the mother's abdominal fascia. This decrease in foetal depth is also associated with an increased foetal dose[39]. Although these factors increased the foetal exposures, the vast majority still received very low exposures, which are associated with negligible risks.

The use of the term 'risk' or 'safe' needs to be interpreted in the context of the benefits versus the risks. This cohort included three foetal exposures above 10 mSv. When modelling high dose data and extrapolating down to low doses of radiation, this 10 mGy exposure has been linked to a small increase in cancer risk, approximately 0.06%[10,40,41]. However, these models were intended for radiation protection standards which may not accurately calculate cancer risk[42]. Additionally, the authors suggest caution in interpretation of this value due to uncertainties in the risk estimate[10]. Furthermore, there have been several reviews on the deterministic effect of ionizing radiation on the developing foetus and they all conform to the ideology that these doses are too low to pose a comprehendible risk[14,16,43,44]. In fact, the American College of Radiologists, the Royal College of Radiologists, the Royal Australian and New Zealand College of Radiologists, and the American College of Obstetrics and Gynaecology state that risk of carcinogenesis, miscarriage, or malformations from foetal doses of diagnostic radiation <50-100 mGy is negligible, and should not be considered causation to terminate the pregnancy[45–49]. However, understanding of dose and risk by some clinicians and radiologists does not align with this statement[50–54].

To further minimize the risk, radiation protection methods, including lead shielding, can be applied to the patient in areas not intending to be scanned. The intention is that the denser material absorbs the radiation and therefore reduce the dose to the tissues underneath. However, being outside the direct exposure field, these tissues would only receive small amounts of internal scatter radiation, which lead shielding cannot protect against. As reviewed by Marsh and Silosky[55], there is little evidence to suggest a benefit in lead aprons to reduce foetal dose, with the dose reduction to the uterus during a chest

X-ray (0.02mSv) only 4 – 19%[56,57]. Inadvertently, the use of lead apron may potentially increase individual dose or increase repeat scan rates thereby increasing cumulative dose[55]. Modern imaging can have built-in automatic exposure controls to optimize the dose during the scan which, when recognising the attenuation from lead shielding, compensates by increasing dose. This is generally only seen during fluoroscopy-based procedures, as X-ray and CT scan regions are predefined before scan commences. Misplacement of lead shielding can obscure relevant anatomy and therefore require additional scanning[58]. For these reasons, the American Association of Physicists in Medicine recommend the discontinuation of routine lead shielding, inferring that it is only psychologically beneficial for fearful or anxious patient[59] Other methods, such as refining scan area or improving collimation, provide just as much dose reduction as lead aprons without the possibility of interfering with automatic exposure controls or interfering with anatomical definition[60].

This study and its results stimulate two grand questions which require further exploration. Firstly, are clinicians choosing to avoid using ionizing radiation to avoid the perceived radiation risks: this being that if a patient was not pregnant, would they receive the same procedures. The level of in-depth radiation training clinicians receive is minimal, which leads clinicians to believe that radiation is dangerous and should be used sparingly. Radiation itself can be dangerous, but it all comes down to dose. The average LD_{50} for humans is 4.5 Gy, so in this sense it is dangerous, but the doses received during diagnostic radiation, up to 100,000 × less, are not[4,61,62]. Likewise, it is possible to overdose on paracetamol/acetaminophen but the appropriate dosage can be taken safely, even during pregnancy[63]. But to answer this first grand question would require a clinical study

assessing procedures used in patients who are pregnant vs non-pregnant. Disregarding the ethical concerns, a large obstacle for this study is that these two patient populations are inherently different. Most pregnant patients who receive radiation do so because of pregnancy-related problems and therefore would not be present in non-pregnant patients. This makes comparability of these populations very difficult. Another option, such as removing the pregnancy status from a patient cohort and assessing how the clinicians proceed to obtain diagnosis could answer this question but has large ethical or moral impracticalities.

In situations where a disease may present in both pregnant and non-pregnant patients, such as kidney stones, is it warranted to use non-ionizing techniques first, which, if negative, are then followed by ionizing radiation procedures anyway. In pregnant patients, McCollough *et al.* discuss the diagnosis of suspected kidney stones via ultrasound and CT scan[44]. Specifically, they denote that to reduce the exposure to ionizing radiation, ultrasound should be used first and repeated 24 hours later then if still negative a CT scan should then be used. This method not only increases the amount of time the patient is in pain but also increases the patient length of stay and hospital cost. The radiation dose received by patients in the CT assigned group was larger on day of admission, but the specificity of CT scans means that patients receive less follow up radiological procedures, 5% compared to 40% for ultrasonography, which actually trended lower in total cumulative dose to the patient over a 1 month and 6 month period[64]. So, the intention to reduce dose is not necessarily true. Lastly, although there is no difference in misdiagnosis, serious adverse effects or ED readmission, in the case where a patient is negative for kidney stones, CT scans are advantageous in determining

other causes of symptoms than ultrasonography, making it more practical[64]. Thus, regardless if a patient is pregnant or not, should clinicians remove sometimes unnecessary steps to gain the answer quicker at the risk of increasing dose to the patient, which is not necessarily true?

A limitation of this study is the calculation of foetal dose. The most accurate estimation of foetal dose would be dependent on procedure parameters and clinical characteristics including scanner type, kVp, mAs, pitch, x-ray beam collimation, procedure length, procedure time, procedure position, radiopharmaceutical used, amount of radiopharmaceutical, patient size, foetal depth and shielding. This study did not calculate foetal doses based on procedure or clinical factors but instead were based on average foetal doses in the literature applied to each modality and scan position. This limits the individual variability between similar procedures and therefore both over and underestimates individual foetal exposures. The intention was to gain a preliminary overview of foetal exposures and to highlight the procedures which result in large doses due to the proximity to scan field of view or delivery method of radiopharmaceutical. Only by incorporating details of the listed factors above, can realistic and precise foetal doses be calculated, which would improve the accuracy of these results, however, based on this audit's results, the improved foetal doses would still be presumably low.

Another limitation is that this is a single-centred study. No conclusions can be made regarding ionizing radiation levels to admitted pregnant patients at other tertiary care facilities without a multicentred approach. It is thereby difficult to extrapolate these results as common exposures or Australian standards. The finding that foetal doses were

low in this hospital is not surprising given that there is a consistent mindset around the world to keep exposures as low as reasonably achievable[14,46].

3.5 Conclusion

Diagnosis of disease can be difficult for clinicians without the use of diagnostic radiation. There is a stigma surrounding the use of ionizing radiation during pregnancy which is observable in the very low level of radiation received by the cohort in this audit. Was this because clinicians chose not to give ionizing radiation, or because it was not needed. Regardless, attempts to reduce dose to the foetus will decrease the overall perceived risk to the foetus, but if clinically indicated, the procedure should still be prescribed because the perceived risk is negligible. Further investigation into more accurate measurements of foetal dose should occur as well as an expansion of this analysis of pregnant and foetal exposures to other hospitals around Australia and internationally.

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CHAPTER 4

IN UTERO EXPOSURE TO LOW DOSE IONIZING RADIATION: LONG TERM CARDIOVASCULAR AND RESPIRATORY OUTCOMES IN C57BL/6J MICE.

SUMMARY

Diagnostic radiation is essential to modern medicine. Advancements in technologies have led to improved diagnosis and patient outcomes, however, the effect of ionizing radiation on the patient is still debated. In the case of pregnancy, the potential effects are not only to the mother but also to the foetus. The aim of this study was to observe if exposure from ionizing radiation during pregnancy alters the development of the cardiovascular and respiratory system of the offspring. Pregnant C57Bl/6 mice were irradiated at gestational day 15 with a ^{137}Cs gamma radiation emitting source at 0 mGy (sham), 50 mGy, 300 mGy, or 1000 mGy. After weaning, male and female pup weights and blood pressure measurements were taken weekly until euthanasia at 16-17 weeks postnatal age. Immediately following, the trachea was cannulated, and the lungs and heart excised. The lung was then examined to assess respiratory physiological outcomes. *In utero* exposures to only 1000 mGy caused significant growth reduction, which remained persistent for both

male and female pups. There was no significant change in cardiovascular or respiratory outcomes. Overall, intrauterine exposures to ionizing radiation do not appear to significantly alter the development of the cardiovascular and respiratory system in C57Bl/6 pups up to 17 weeks postnatal age.

4.1 Introduction

Diagnostic radiation is essential to modern medicine, with its use having increased rapidly over the past few decades. Advancements in technologies have led to improved diagnosis and patient outcomes, however, the effect of this radiation on the patient is still debated. In the case of pregnancy, the potential effect is not only to the mother but also to the foetus. Perinatal care is an important and influential period for the development of the foetus, where sudden changes to the intrauterine environment can have long-lasting effects on health trajectory of the foetus[1]. This paradigm, known as foetal programming, can affect many organ systems of the foetus including cardiovascular and respiratory systems[2–4]. Long term changes and dysfunction can lead to disease adding to the already high health care burden cardiovascular and respiratory diseases have in Australia[5,6]. Further to this, ionizing radiation during pregnancy may also penetrate and directly affect the foetus itself.

Most work that encapsulates irradiation *in utero* has focused on mortality, intrauterine growth restriction, malformations or neurobehavioral outcomes[7]. The larger

the exposure, the higher the risk or severity of these outcomes, but typically, irradiations earlier in gestation lead to mortality whereas later leads more towards malformations or behavioural changes[7–9]. Overall, the dose required to elicit these responses is above 300 – 500 mGy, but can change significantly due to gestational timing of exposure[10,11].

At gestational day 15 in the mouse, the foetus has progressed through implantation and embryogenesis and is now in the foetal development stage[12]. The main cardiac structures have been defined, with the refinement of atrioventricular and semilunar valve occurring, and the respiratory system is at the pseudoglandular phase, where the general gland-like structure of the lung is prominent, with a differentiated conducting region[13,14]. However, most work that investigates *in utero* irradiation at gestational day 15 does not examine the cardiovascular or respiratory systems but instead focuses on intrauterine growth restriction or neurobiological defects[15–17]. These studies found a dose threshold of 1000 mGy for persistent growth restriction and 500 – 1000 mGy for neurobiological defects.

Previous attempts to relate human *in* utero irradiation exposure to cardiovascular function later in life used chronic or single high dose exposures and typically do not focus on the early foetal development period, and for respiratory function, there have been no studies found thus far[18–23]. One C57BL/6 study assessed growth and cardiovascular outcomes and saw no significant effect of irradiation on either outcome, except 1000 mGy that caused persistent and reduced weight of the animals[24]. Despite a significant transport-

induced, stress-related response to their irradiation acclimation procedure, which may have interfered with the cardiovascular data, the growth restriction from 1000 mGy was still apparent. This leaves a paucity of data on the relationship between ionizing radiation exposure during the early foetal developmental stage, particularly of acute medical exposures which are low dose, and the development of the cardiovascular and respiratory system. This study aims to address this relationship to identify if ionizing radiation during late gestation/the early foetal development period, influences cardiovascular function or respiratory physiology in adolescence.

4.2 METHODS

4.2.1 Experimental animals and irradiations

Male and female C57Bl/6J mice (Jackson Laboratories, USA) were housed in a 12:12 hour light:dark cycle and allowed food and water *ab libitum.* The mothers were maintained on standard lab mouse chow (9% fat, 44.9% carbohydrate, 19% protein, Teklad Diets Envigo, USA) for the duration of pregnancy and pups received a similar chow post-weaning (6.2% fat, 44.2% carbohydrate, 18% protein, Teklad Diets Envigo, USA) for the duration of the study.

Female mice were paired 2:1 with male mice over a single night to obtain an accurate gestational day 0. The following morning, the females were checked for vaginal plugs, indicative of sexual activity, and then housed singly. Confirmation of pregnancy was

obtained by abdominal palpitations later in gestation. At gestational day 15, pregnant C57Bl/6J female mice were transported across campus to McMaster University's Taylor Radiobiology Source, acclimated *in situ* for 20 minutes and irradiated (sham (irradiation control), 50 mGy, 300 mGy, 1000 mGy) using a ^{137}Cs Gamma radiation source (620 Ci, 662 keV energy, 10 mGy/min). To reduce the transport effect seen in Sreetharan *et al*[24], animals were only transported once to the source for irradiation and then returned back to the animal facility. All animals were restricted from food and water during irradiations.

Pups were weaned at 3-4 weeks of age, and up to 3 male and 4 female pups were housed together, respectively. To maximise group sizes and control maternal effect, up to 2 male and 2 female pups from any one mother were used to create n = 8 of each sex in each radiation group. Postweaning weight and cardiovascular measurements were taken weekly until euthanasia at 16-17 weeks old.

All described animal procedures were reviewed and approved by the Animal Research Ethics Board at McMaster University (AUP #15-11-26) in line with the requirements of the *Canadian Council on Animal Care Guidelines*.

4.2.2 Cardiovascular measurements

Blood pressure was non-invasively measured in non-anesthetised pups via tail-cuff plethysmography using the CODA8 high throughput non-invasive blood pressure system (Kent Scientific Corporation, USA). This method has been previously described and results in minimal discomfort or stress for the animal[25]. Systolic blood pressure (SBP), diastolic

blood pressure (DBP), mean arterial blood pressure (MAP), and heart rate (HR) were collected 3 times weekly/animal and averaged, between 7-16 weeks of age.

4.2.3 Respiratory measurements

At 16-18 weeks of age, animals were anaesthetised with 5% Isoflurane, and a tracheotomy then thoracotomy was performed. Maximal blood volume was collected via a cardiac puncture, following which the heart and lungs were excised. The left lung lobe was resected and freeze-dried for lung lobe wet:dry weight analysis. The remaining lobes were degassed (0.5 atm for 60 sec) and lavaged with 3 × 16 mL/Kg body weight aliquots of cold 0.9% saline. Lavage supernatant was assessed for total protein concentration using the Pierce BCA protein assay kit (Thermo Fisher Scientific, USA). Cells were stained using trypan blue and counted on a haemocytometer to calculate cellular infiltrate. Remaining cells were fixed in 4% paraformaldehyde, smeared then stained with Hematoxylin and Eosin and photographed under a BX50 brightfield microscope (Olympus, Japan) to assess differences in cell populations.

4.2.4 Statistical Analysis

Longitudinal data (weight and cardiovascular outcomes) are presented as mean weekly measurements and were analysed using a generalised additive model (GAM) fitted to a generalised linear mixed model (GLMM) accounting for nesting of pups within mothers. Cross-sectional data (respiratory outcomes) are presented as mean and 95% confidence interval and were analysed using generalised linear mixed models with a normal

distribution, accounting for nesting of pups within mothers. A p-value < 0.05 was considered statistically significant. Statistical analysis was completed using IBM SPSS v.24 statistical software (IBM Corporation, USA).

4.3 RESULTS

4.3.1 Growth rate

Litter size and sex ratio did not differ between doses of radiation (data not shown). There was no difference in weight at first measurement (age wk4) between any radiation group and sham control. However, there was a significant difference in growth over the longitudinal 12 weeks (Figure 4-1). An *in utero* exposure of 1000 mGy significantly altered the growth trajectory resulting in a 1.42 g reduction in weight overall (p = 0.004). Other doses of radiation were not different from sham over the 12 weeks. Sexes also differed in growth curve (p < 0.001), but growth restriction due to 1000 mGy was similar, 8-10%.

4.3.2 Cardiovascular system

In utero exposure to radiation did not significantly alter the cardiovascular outcomes of SBP, DBP, MAP or HR, p = 0.091, p = 0.889, p = 0.227 and p = 0.060, respectively (Figure 4-2). Therefore, *in utero* irradiation at these doses did not cause hypertension or hypotension over 16 weeks post-weaning. Sexes differed on SBP, DBP and MAP (p < 0.05) but not HR.

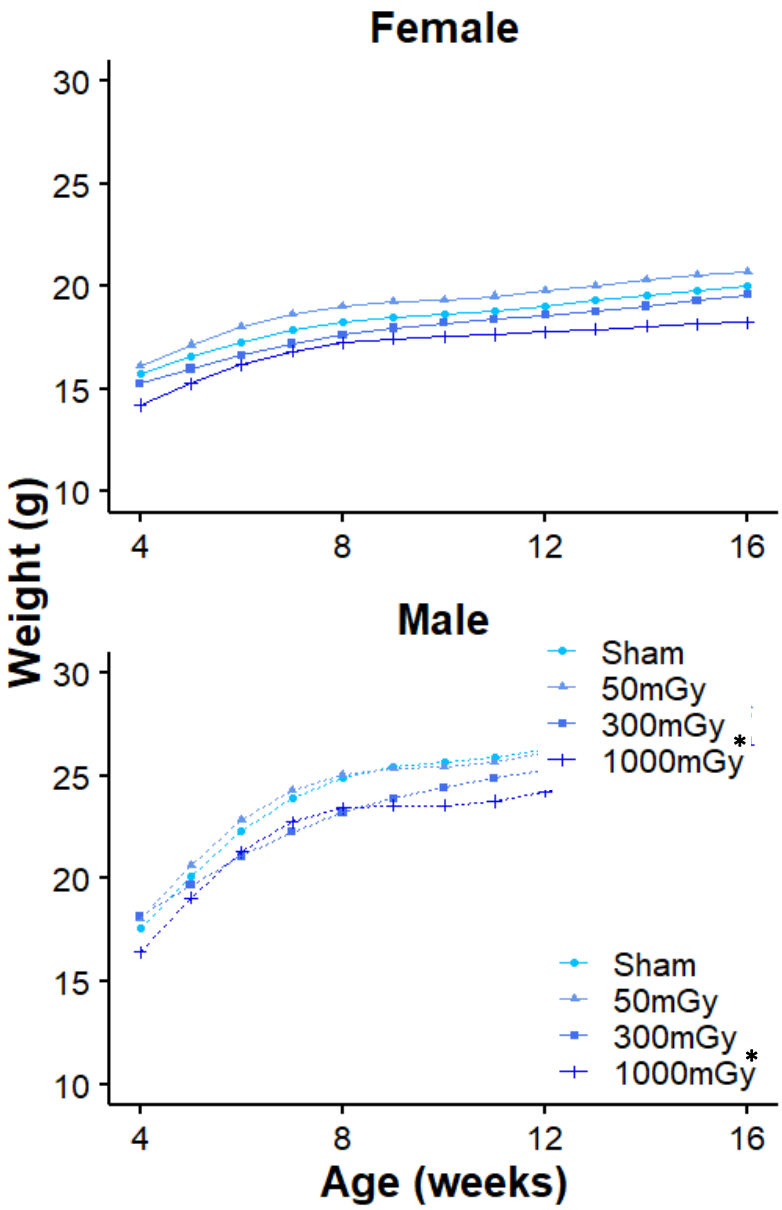

Figure 4-1: longitudinal weight measurements. *In utero* exposure to 1000 mGy significantly alters the trajectory of growth compared to sham control as tested using GAM fitted to GLMM (p = 0.004, n = 7-9) resulting in a 1.42g reduction in weight overall. Mice exposed to other doses of radiation were not different to sham. Sexes differed in growth curves (p<0.001). Graphs represent mean weekly measurements. * indicates statistical significance of $p < 0.05$

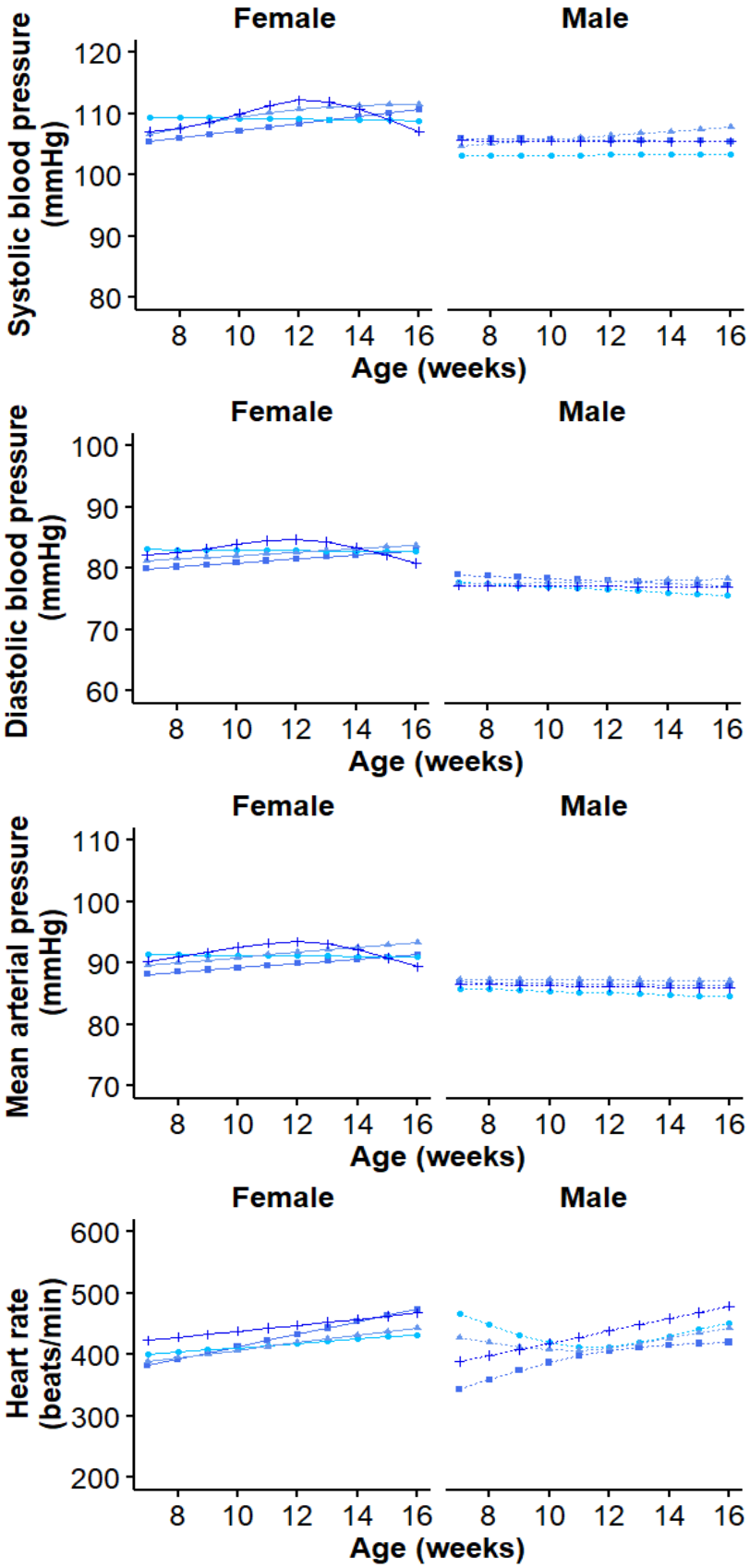
Female
Male
Systolic blood pressure (mmHg)
120
110
100
90
80
8
10
12
14
16
Age (weeks)
Female
Male
Diastolic blood pressure (mmHg)
100
90
80
70
60
8
10
12
14
16
Age (weeks)
Female
Male
Mean arterial pressure (mmHg)
110
100
90
80
70
8
10
12
14
16
Age (weeks)
Female
Male
Heart rate (beats/min)
600
500
400
300
200
8
10
12
14
16
Age (weeks)

Figure 4-2: Longitudinal cardiovascular outcomes of Systolic blood pressure (SBP), diastolic blood pressure (DBP), mean arterial blood pressure (MAP), and heart rate (HR). *In utero* exposure to any dose of radiation did not significantly alter the curve compared to sham for either sex, $p = 0.091$, $p = 0.889$, $p = 0.227$ and $p = 0.060$, respectively, tested with GAM fitted to a GLMM (n = 7-9). Sexes differ for SBP, DBP, MAP but not HR ($p < 0.05$). Graphs represent mean weekly measurements, n = 7-9.

4.3.3 Respiratory system

There was no oedema or change in alveolar-capillary barrier integrity, as indicated by lung lobe wet:dry weight ratio (p = 0.618) and total BAL protein concentration (p = 0.450), by *in utero* exposure to ionizing radiation (Figure 4-3). Similarly, cellular infiltrate did not increase due to *in utero* exposure to ionizing radiation, p = 0.753, nor did the populations of cells within the lung differ, p = 0.413 (Figure 4-4). There was no statistical difference between sex for any outcome except BAL cell count. Female animals had 33% more cells at baseline compared to males, p = 0.022.

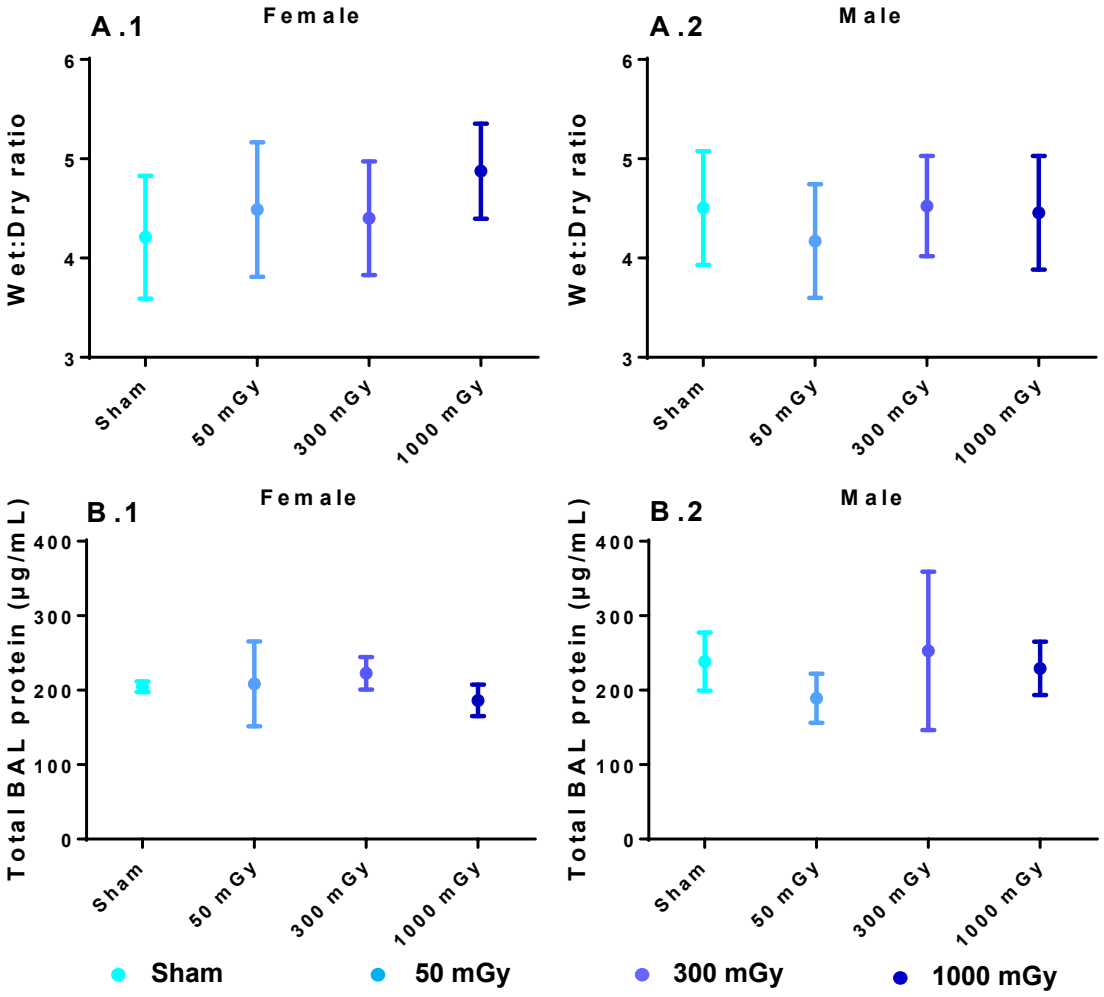

Figure 4-3: Respiratory physiological outcomes of **A)** wet:dry lung weight ratio and **B)** total protein concentration in BAL. *In utero* exposures to any dose of ionizing radiation did not significantly change the wet:dry ratio or BAL total protein concentrations compared to sham control for either sex, $p = 0.450$ and $p = 0.618$, respectively. There was also no difference between sexes, $p = 0.152$ and $p = 0.696$, respectively. Data represented as mean and 95% confidence intervals. Statistical significance was tested by GLMM, $n = 6$-7.

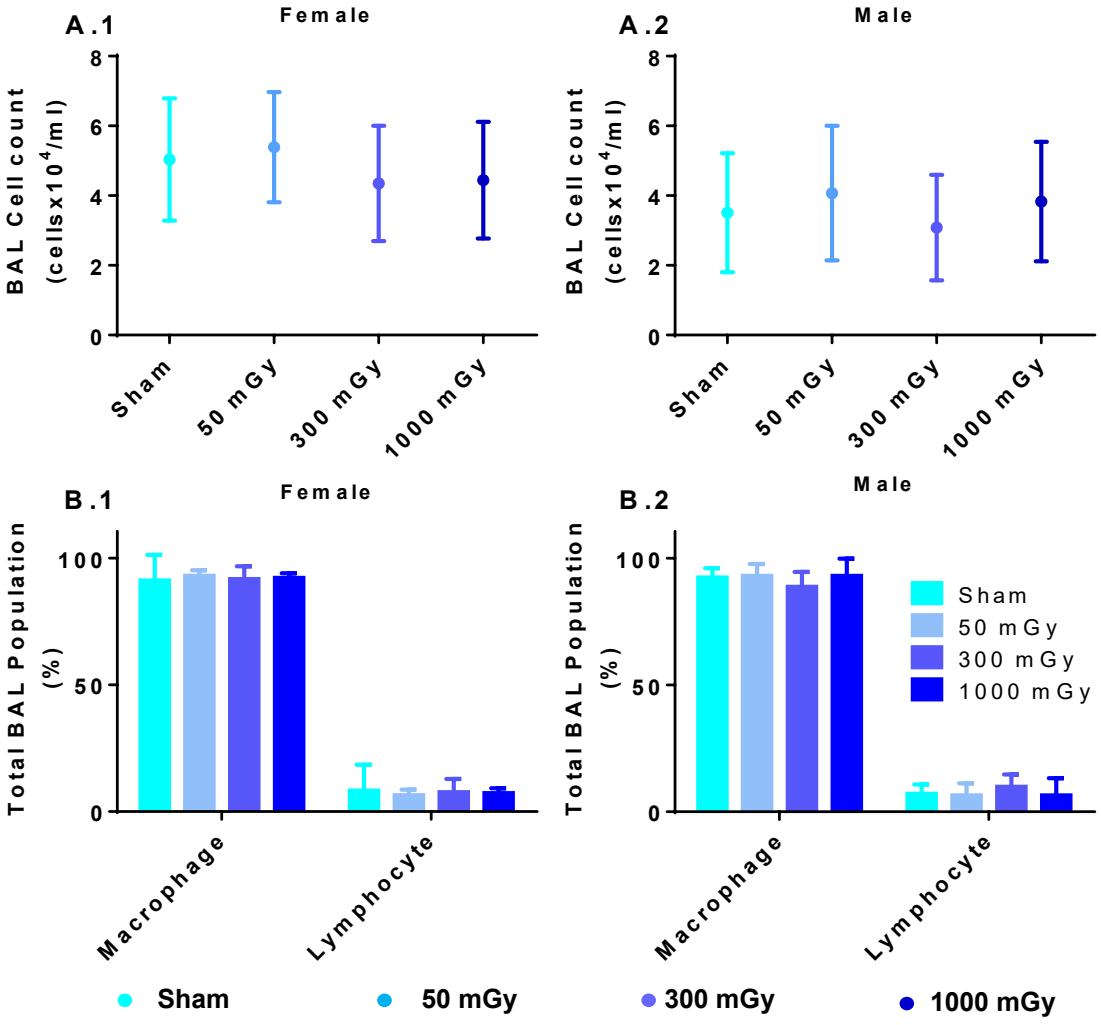

Figure 4-4: Respiratory immunological outcomes of **A)** BAL cell count and **B)** Cell populations within BAL. *In utero* exposures to any dose of ionizing radiation did not significantly change the total cell count or the cell populations within the BAL compared to sham control for either sex, $p = 0.753$ and $p = 0.413$, respectively. There was also no difference between sexes for percentages of populations, $p = 0.839$, but there was for overall cell count, $p = 0.022$. Data represented as mean and 95% confidence intervals. Statistical significance was tested by GLMM, $n = 6$-7.

4.4 DISCUSSION

The current guidelines worldwide have no limit for ionizing radiation exposure during pregnancy but instead suggest that the exposure be clinically justified on an individual basis and kept as low as reasonably achievable[10,26–28]. Therefore, exposure of pregnant patients can vary significantly. In this study, the low dose of 50 mGy is representative of a high-end diagnostic procedure, such as a multi-scan abdominopelvic CT[29,30]. The middle range of 300 mGy represents the amount of radiation someone might accumulate for a severe and extended stay in hospital accruing multiple diagnostic procedures over admission. The highest dose of 1000 mGy is well above diagnostic radiation levels but is still below therapeutic levels. It is important to recognise that the radiosensitivity between humans and mice vary significantly. The human LD50 in 30 days for whole-body exposures is between 2000 – 8000 mGy, 4500 mGy for a healthy adult, whereas for C57Bl/6 mice is 8500 – 9000 mGy[31–33]. Thus, this strain of mice is approximately twice as resistant to ionizing radiation than humans, so it could be postulated that *in utero* exposure to 1000 mGy in mice might equate to 500 mGy in humans in terms of its biological effects.

In this study, only the highest dose of *in utero* radiation caused intrauterine growth restriction, which was about 8-10% reduction in body weight consistently over the lifetime of the mouse. The finding that rodent irradiations during pregnancy, and specifically around gestational day 15, induces intrauterine growth restriction has been reported

before for doses less than 1000 mGy, but matching this study's results, persistent growth restriction is only seen from doses ≥1000 mGy[17,34–37]. Unfortunately, no conclusion can be made regarding the immediate postnatal growth of these animals, as birthweight and postnatal-preweaning weight were not collected in order to reduce maternal stress and cannibalism. It is therefore uncertain if growth restriction occurred from exposures <1000 mGy and the 'catch up' effect resulted in these animals matching the sham control weight post weaning[38]. Additionally, no comment can be made about the long-term growth of these animals to the natural endpoint of their life.

Intrauterine growth restriction, as a hallmark of foetal programming, has been linked individually to cardiovascular and respiratory dysfunction[3,4,39,40]. Low birthweight reduces childhood lung function and increases the risk of hospitalisation in adolescence because of respiratory distress[40,41]. Similarly, the risk of hypertension appears to be set in foetal development from intrauterine growth restriction[39]. This study showed no development of hypertension or hypotension due to intrauterine radiation exposure. However, the risk of cardiovascular disease increases with age and often doesn't arise symptomatically until late adulthood so possibly this study's 3-month follow up was not long enough to observe the full effects of the intrauterine radiation[42]. Additionally, it is important to note that there was large intra-group variation for all cardiovascular outcomes which may contribute to the non-statistically significant inter-group variation.

The longer-term effect of radiation on cardiovascular disease incidence or mortality may have implications to public health given the increase in ionizing radiation use in the medical field over the past few decades[43–45]. Several studies on non-pregnant subjects have recorded that similar-to-medical levels of low dose radiation, but from environmental and occupational exposures, have an association with cardiovascular disease incidence and mortality[18,19,46]. These studies observe chronic exposures instead of acute and have multiple limitations, including cohort age, adjustments for confounding factors, length of exposure, and accurate dosimetry. Studies of medical exposures found a relationship but only at cumulative doses above 150 mGy or from fractionated radiotherapy doses, whereas others, including a large multi-country investigation, found no relationship at all[20–23,47–50]. Studies with pregnant patients are scarce. One study from the Hiroshima and Nagasaki atomic bomb survivors found an association of radiation dose to cardiovascular disease later in life but only at exposure >500 mGy, much higher than diagnostic exposures[51]. Therefore, radiation may have some effect on the foetal cardiovascular system trajectory, but apparently not at doses received from diagnostic radiation sources.

There was also no change in the respiratory outcomes measured in this study. As reviewed by Iles *et al*[52], non-cardiogenic pulmonary oedema can be caused by damage to the alveolar epithelial layer, directly via reactive oxygen species (ROS) and indirectly via infiltrating leukocytes, or by dysregulation of the fluid clearing process. Less than a second after radiation exposure, ROS are formed, primarily through water radiolysis which can induce perpetual cellular oxidative stress to continue cell damage[53]. However, this study

found no measurable oedema, alveolar epithelial layer damage, or cellular infiltrate at 16-17 weeks postpartum suggesting the ROS production from water radiolysis was quenched and damage, if created, was repaired before these measurements were taken. Observations of these outcomes at a much earlier time postnatally would be required to assess the immediate effects of *in utero* exposures however, this study suggests that irradiations ≤1000 mGy do not permanently alter the development of the respiratory system or cause long-term physiological changes indicative of lung injury.

One limitation of this study is that it looked at exposures at a single time point in gestation, as it is well known that similar exposures at different gestational stages result in different outcomes. Although no detrimental cardiovascular or respiratory outcomes were observed in this study, additional work is required at various stages of gestation to fully assess the effect of diagnostic levels of radiation during pregnancy. Equally, additional timings of outcome measurements would strengthen this study, especially to observe the cardiovascular system much later in life or the respiratory system at a younger age.

Another limitation to highlight is that these mice received whole-body irradiations, and thus the foetal dose received is very similar to the maternal dose, whereas clinical application of radiation typically limits exposures to a field of view of interest. For example, a chest CT will result in a much smaller foetal exposure compared to a pelvic CT because the foetus is not in the field of view[30]. This, therefore, will remove or reduce the direct effect of irradiation on the foetus, although the indirect effects from foetal programming

may still exist. Thus, as this experimental model used whole-body exposure, it is not possible to delineate the direct and indirect effects of radiation.

4.5 Conclusion

This study aimed to assess the relationship between *in utero* ionizing radiation exposure at GD15 and the development of the cardiovascular and respiratory system. It appears that the radiation exposure did not significantly induce hypertension or hypotension in these mice, nor did it physiologically change the respiratory system, but exposures of 1000 mGy caused intrauterine growth restriction that remained until adolescence. Overall, from this healthy animal model, exposures to diagnostic levels of ionizing radiation during late gestation caused no significant effects on the growth or cardiovascular and respiratory systems, which support the statement that the effects from low dose radiation at this gestational stage are negligible.

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CHAPTER 5

IN UTERO EXPOSURE TO LOW DOSE IONIZING RADIATION AND THE RESPIRATORY RESPONSE TO AN ACUTE LUNG INJURY STIMULUS AT ADOLESCENCE IN BALB/C MICE.

PREFACE

The animal model used in this chapter, BALB/c, is a different strain of mouse compared to chapter 4, C57Bl/6J. The decision to change mouse strain during this project was due to the high cannibalism rate observed from C57bl/6 mice, which increased the number of pregnancies required to obtain the group sizes; increasing cost, experimental time, and possibly impacting animal welfare. The high possibility of cannibalism caused researchers to be very particular in the way they handled the pregnant C57Bl/6J mice. In comparison, BALB/c mice have a much lower cannibalism rate and were, therefore, easier to handle during pregnancy[1]. This change in mouse strain was adopted by everyone in our group for all future experiments in this program of work. A comparison of the effect this species change had on the results, will be undertaken in the overall thesis discussion, chapter 6.

SUMMARY

Diagnostic radiation during pregnancy is a controversial topic with the potential effects not only to the mother but also to the developing foetus. Interruptions to the intrauterine environment, possibly from maternal exposures to radiation, could alter the development of the foetus and long-term outcomes. Not only this, but the immunomodulatory effects from ionizing radiation exposures could alter the response to inflammation later in life. The aim of this study was to observe if *in utero* exposure to ionizing radiation altered the respiratory immune response during adolescence. Pregnant BALB/c mice were irradiated at gestational day 15 with a ^{137}Cs gamma radiation (662 keV energy) emitting source at 0 mGy (sham), 10 mGy, 100 mGy, or 1000 mGy. At 16-17 weeks postnatal age, 3 mg/Kg of lipopolysaccharide (LPS) was instilled intratracheally. Mice were then euthanized 24h later, the trachea was cannulated and the lungs excised. The left lung lobe was resected for wet:dry weight analysis. A bronchoalveolar lavage (BAL) was performed for total cell count, cell differential and protein analysis. Remaining lung tissue was examined for antioxidant levels. Exposures to 100 and 1000 mGy significantly reduced the growth of the pups for both males and females compared to sham animals ($p<0.05$). Administration of LPS significantly increased lung lobe wet:dry weight ratios, BAL total protein, BAL cell count and transforming growth factor (TGF)-β levels and changed antioxidant levels compared to saline control ($p<0.001$) indicating establishment of acute lung injury, however, there was no overall effect from ionizing radiation. Overall, *in utero* exposures to 100 and 1000 mGy

late in gestation significantly reduced the growth of the animals but had no effect on the response to a respiratory bacterial stimulus.

5.1 INTRODUCTION

Medical uses of ionizing radiation, in which single procedures typically fall below 100 mGy, play an important role in the diagnosis and management of disease. However, there is a bias surrounding its use during pregnancy due to the fear of its effect on the developing foetus. Currently, there has been little investigation into the effect of foetal radiation exposure on the respiratory system with most work instead focusing on mortality, intrauterine growth restriction, malformation and neurobehavioral outcomes[2]. These effects are dependent on dose, with larger doses (>500 mGy) giving higher risk or severity of effect, and timing during gestation, where early exposures (1st trimester) lead to increased mortality and later exposures lead to growth restriction, malformation or neurobehavioral effects[2–4]. For both animals and humans, the current overall threshold for effects to the foetus are >100 mGy but individual thresholds can be higher dependent on outcome and foetal development period[2].

At gestational day 15, the lung is in the pseudoglandular phase, which is a critical time point for the development of the bronchus and bronchioles and the connection of the pulmonary blood supply via peripheral capiliaries[5,6]. Previous work in our laboratory[7], and chapter 4, have shown that irradiation at gestational day 15 in a mouse model causes a reduced growth rate at the highest dose (1000 mGy) but had little effect on the respiratory system. These results came from a healthy animal model; however, radiation also has

immunomodulatory properties, which may affect lung responses during inflammatory injury, resolution and repair.

Acute pathogenic lung injury occurs when a foreign stimulus, such as lipopolysaccharide (LPS), activates inflammation in the lung. Although there are several methods of lung injury, none accurately model human acute lung injury, however, LPS is an effective stimulus to observe the acute inflammation phase of acute lung injury. LPS, found on gram-negative bacteria, bind to toll-like receptor 4 (TLR4) found on alveolar macrophages and epithelial cells[8–11]. This starts a cascade of events to trigger increased pro-inflammatory mediator release, increased leukocyte infiltration into alveolar spaces, increased epithelial cell damage, increased alveolar-capillary permeability and pulmonary oedema, resulting in decreased lung function[11–13]. Most of these outcomes occur to enable neutralisation and clearance of the pathogen, eventually stimulating resolution and repair of the injury.

Many *in vitro* studies investigating the immunomodulatory response of ionizing radiation focus on monocyte or macrophage responses using both primary and immortalised cell lines in an unstimulated setting. Exposures to >1000 mGy, result in increased release of pro-inflammatory cytokines, for example, IL-1β, which can also be seen *in vivo*[14–17]. Additionally, high doses can cause increase leukocyte adhesion to endothelial cells, increasing leukocyte infiltration and promoting inflammation[18,19]. In contrast, doses below 1000 mGy stimulate an anti-inflammatory environment through decreased pro-inflammatory and increased anti-inflammatory mediator release[14,20–23]. These doses

reduce leukocyte migration through downregulation of adhesion molecules, reducing leukocyte infiltration and slowing the inflammation process[24–28].

Most studies that explore the immunomodulatory response of radiation during inflammation/stimulation do so using *in vitro and ex vivo* models. Exposure of LPS stimulated *in vitro* and *ex vivo* mouse macrophages to 500 mGy showed anti-inflammatory responses, including reduced TNF-α and IL-1β levels and increased TGF-β levels[20,21,23]. Contrarily, IL-1β levels were increased from IFN-γ stimulation in similar conditions with a similar cell type[29]. However, whether monocultured or co-cultured with endothelial cells, 100 – 500 mGy exposure with LPS, IL-1β or TNF-α stimulated mononuclear cells show decreased adhesion and migration, which can be replicated *in vivo*[23–25,30]. Thus, although there can be stimulus-dependent outcomes for cytokine profiles, the overall immunological effect of low dose ionizing radiation on migration, infiltration and inflammation appears similar, irrespective of stimuli.

While these models can loosely relate to respiratory infection, as macrophages and infiltrating leukocytes have major roles in stimulating and propagating inflammation within the lung, there is limited data specifically looking at the effects of ionizing radiation on an acute lung injury model and furthermore, the long-term effects from *in utero* exposures in a whole animal system. This study aims to address this missing research by exploring if *in utero* exposure to ionizing radiation influences the response to an acute respiratory immune stimulus in adolescent BALB/C mice.

5.2 METHODS

5.2.1 Experimental animals and irradiations

Male and female BALB/cJ mice (Jackson Laboratories, USA) were housed in a 12:12 hour light:dark cycle and allowed food and water *ab libitum.* The mothers were maintained on standard mouse chow (9% fat, 44.9% carbohydrate, 19% protein, Teklad Diets Envigo, USA) for the duration of pregnancy and pups received a similar chow post-weaning (6.2% fat, 44.2% carbohydrate, 18% protein, Teklad Diets Envigo, USA) for the duration of the study.

Female mice were paired 2:1 with male mice once overnight to obtain an accurate gestational day 0. The following morning, the females were checked for vaginal plugs, an indication for sexual activity, and then housed singly. Confirmation of pregnancy was obtained by abdominal palpitations later in gestation. At gestational day 15, pregnant BALB/c mice were transported across campus to McMaster University's Taylor Radiobiology Source, acclimated for *in situ* for 20 mins and irradiated (sham (irradiation control), 10 mGy, 100 mGy, 1000 mGy) using a ^{137}Cs Gamma radiation source (620 Ci, 662 keV energy, 10 mGy/min). All animals were restricted from food and water during irradiations. A transport control group (naïve) was added to determine the possible transport-related stress effect.

Pups were weaned at 3-4 weeks of age and up to 3 male and 4 female pups were housed together. To maximise group sizes and control maternal effect, up to 2 male and 2 female pups from any one mother were used in each challenge group (saline or LPS) for each

radiation group. Postweaning weight measurements were taken weekly until euthanasia at 16-17 weeks old.

All described animal procedures were reviewed and approved by the Animal Research Ethics Board at McMaster University (AUP #15-11-26) in line with the requirements of the *Canadian Council on Animal Care Guidelines*.

5.2.2 LPS Model

At 16-17 weeks postnatal age, mice were anaesthetised with 5% isoflurane and 3mg/Kg of lipopolysaccharide (LPS, *Escherichia coli* O55:B5, Sigma-Aldrich, USA) in 50 µL of 0.9% saline was instilled intratracheally followed by an air bolus (0.15 mL). Mice were allowed to recover and monitored for 24 hours until euthanasia.

5.2.3 Surgery and tissue collection

Animals were anaesthetised by 5% isoflurane and a tracheotomy then thoracotomy was performed. Maximal blood volume was collected via a cardiac puncture into lithium heparin blood collection tubes, following which the heart and lungs were excised. The blood was spun at 500g and the plasma was collected and stored at -80°C. The left lung lobe was resected and freeze-dried for lung lobe wet:dry weight analysis. The remaining lobes were degassed (0.5 atm for 60s) and lavaged with 3x 16mLs/Kg volume aliquots of cold 0.9% saline. Lobes were then resected and snap-frozen in liquid nitrogen. Bronchoalveolar lavage was spun at 500g to pellet cells and the supernatant was collected for protein analysis. Cells in the lavage were counted and stained to assess cellular infiltrate

and different cell populations with BAL. Cells were stained using trypan blue and counted on a haemocytometer to calculate cellular infiltrate. Remaining cells were fixed in 4% paraformaldehyde, smeared then stained with Hematoxylin and Eosin and photographed under a BX brightfield microscope (Olympus, Japan) to assess differences in cell populations.

5.2.4 Immunoassays

Bronchoalveolar lavage supernatant was assessed for total protein concentration using the Micro BCA protein assay kit (Thermo Fisher Scientific, USA). Transforming growth factor-beta (TGF-β) was assessed in lavage supernatant and plasma by enzyme-linked immunosorbent assay (Rat TGF-β Duoset ELISA, RnD system, USA) as per manufactures instructions. The ELISA had a lower detection limit of 32pg/mL.

5.2.5 Tissue homogenisation and western blot

The snap-frozen, lower-right, lung lobe was homogenised in Pathscan buffer (25mM Tris, 150mM NaCl, 1mM EDTA and 1% Triton-X) containing sodium fluoride (20mM), sodium orthovanadate (2mM), and protease inhibitor cocktail (Sigma Aldrich, USA) using a Bead Ruptor 4 (Omni International, USA). Samples were then centrifuged at 90,000g for 25 minutes (Optima-Max TL Ultracentrifuge with TLA-55 rotor, Beckman Coulter, USA) and supernatant was collected and stored at -80°C. Protein content was quantified by EZQ protein assay as per the manufacturer's instructions (Thermo Fisher Scientific, USA). Next, 50µg of total protein from each sample was boiled and then 4X loading buffer

(+Bromophenol Blue) with 4% fresh dithiothreitol (DTT, Sigma-Aldridge, USA) was added. The samples were separated on 4-20% Criterion TGX, stain-free, precast, polyacrylamide gel (Bio-Rad, USA) in a Criterion cell (Bio-Rad, USA) using an electrophoresis power supply (EPS 1001, Amersham Pharmacia Biotech, UK) set to 300 volts for 20 minutes. The gel was transferred to Immuno-Blot low fluorescence PVDF (Bio-Rad, USA) using a Trans-Blot Turbo transfers system (Bio-Rad, USA) and imaged on a Gel Doc EZ imager (Bio-Rad, USA).

The membranes were blocked with 5% skim milk in PBS-T (Phosphate buffered saline + 0.1% tween 20) for 1 hour at room temperature, and then incubated with appropriate primary antibody overnight at 4°C. Following washing in PBS-T, the membranes were incubated with the appropriate horseradish peroxidase (HRP)-conjugated secondary antibody for 1 hour at room temperature. After washing again, the protein levels were visualised with enhanced chemiluminescence reagent and imaged on a ChemiDoc Touch imaging system (Bio-Rad, USA). Protein bands were analysed using Bio-Rad Image Lab software (Bio-Rad, USA) and were normalised to total protein concentration. Primary antibodies included: rabbit anti-glutathione peroxidase 1 (GPx 1/2000 dilution, [ab22604]), rabbit anti-superoxide dismutase 2 (SOD2 1/2000 dilution, [ab68155]), and rabbit anti-catalase (1/2000 dilution, [ab209211]) all purchased from Abcam, UK. The secondary antibody was a Donkey anti-rabbit IgG conjugated to HRP ([715-035-152], Jackson Laboratories, USA). All antibody dilutions were made in 2.5% skim milk PBS-T.

5.2.6 Statistical Analysis

Sample size for the primary outcome of bronchoalveolar lavage total protein concentration was calculated to be $n = 7$ per group, using previous data ($\alpha = 0.05$, $1-\beta = 0.8$, effect size = 0.641).

Longitudinal data (weight) is presented as a mean weekly measurement and was analysed using a generalised additive model fitted (GAM) to a generalised linear mixed model (GLMM) accounting for nesting of pups within mothers. Cross-sectional data (respiratory outcomes) are presented as mean and 95% confidence interval (95% C.I.), and were analysed using GLMM, accounting for nesting of pups within mothers. A p-value < 0.05 was considered statistically significant.

5.3 Results

5.3.1 Transport effect

There was no difference between naïve transport controls and sham irradiation controls for the number of living males ($p = 0.711$), living females ($p = 0.357$) or total living pups ($p = 0.685$) indicating no transport effect on litter size (Figure 5-1). Similarly, there was no difference in weight at 4 wks or growth trajectory over the 12 weeks measured between naïve mice and sham irradiated mice indicating no transport effect on growth rate (Figure 5-2). There was also no difference in respiratory physiological outcomes of lung lobe wet:dry weight ratio, $p = 0.773$, or BAL total protein concentration, $p = 0.709$ (Figure

5-3); and respiratory immunological outcomes of BAL cell count, p = 0.828, or cell populations within BAL (Figure 5-4). From these findings, it appears there was no significant transport effect and so naïve animals were not included in the subsequent statistical assessments between groups.

5.3.2 Litter size and growth rate

There was no difference in number of living males, living females or total living pups among sham and irradiated groups, p = 0.484, p = 0.128, and p = 0.078, respectively. Mice exposed to 1000 mGy *in utero* weighed significantly less than sham control at 4 wks of age, males = 2.3 g difference (95% C.I. = 0.5 – 3.9 g.) and females = 1.7 g difference (95% C.I. = 0.1 – 3.4 g). This lighter weight continued throughout the 12 weeks of measurements with the overall growth trajectory of mice exposed to both 100 mGy and 1000 mGy significantly lower compared to the sham control, p = 0.002 and p < 0.001, respectively (Figure 5-2). Specifically, for the 1000 mGy exposed mice, there was a 15% reduction in weight for the males by 16 weeks of age and 25% reduction for the females.

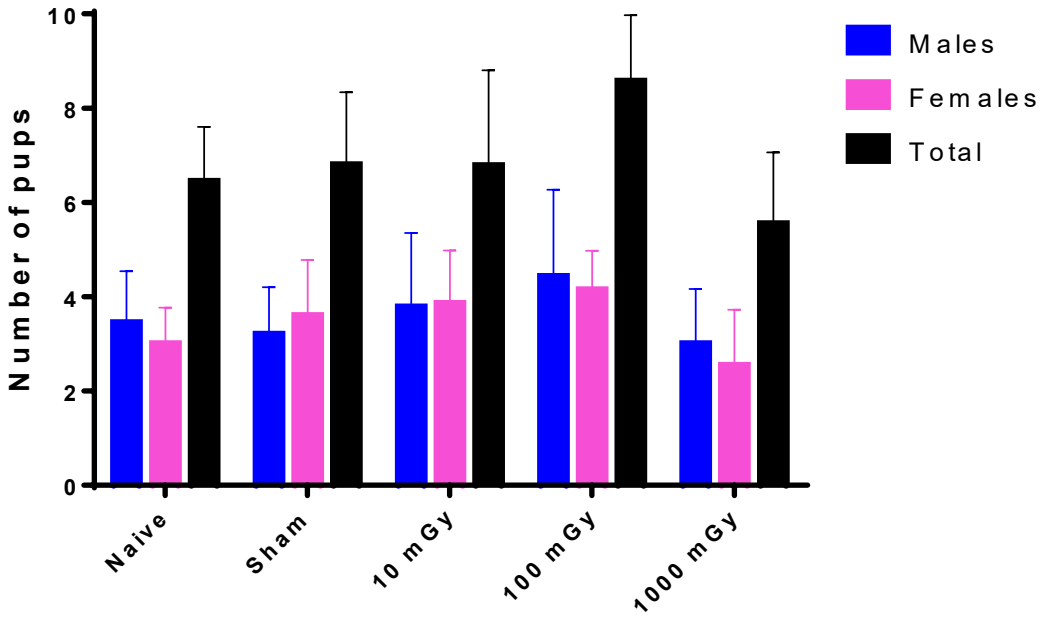


Figure 5-1: Litter size of irradiated mothers**.** There was no significant difference in number of living male pups, living female pups, or total living pups between Naïve transport control and Sham irradiation control groups (p = 0.711, p = 0.357, p = 0.685 respectively), as tested by independent t tests, n = 9-10. There was also no difference among control and irradiated groups (p = 0.484, p = 0.128, and p = 0.078 respectively), as tested by one-way ANOVA n = 7-11. Bars and error bars represent mean and 95% confidence interval of the mean.

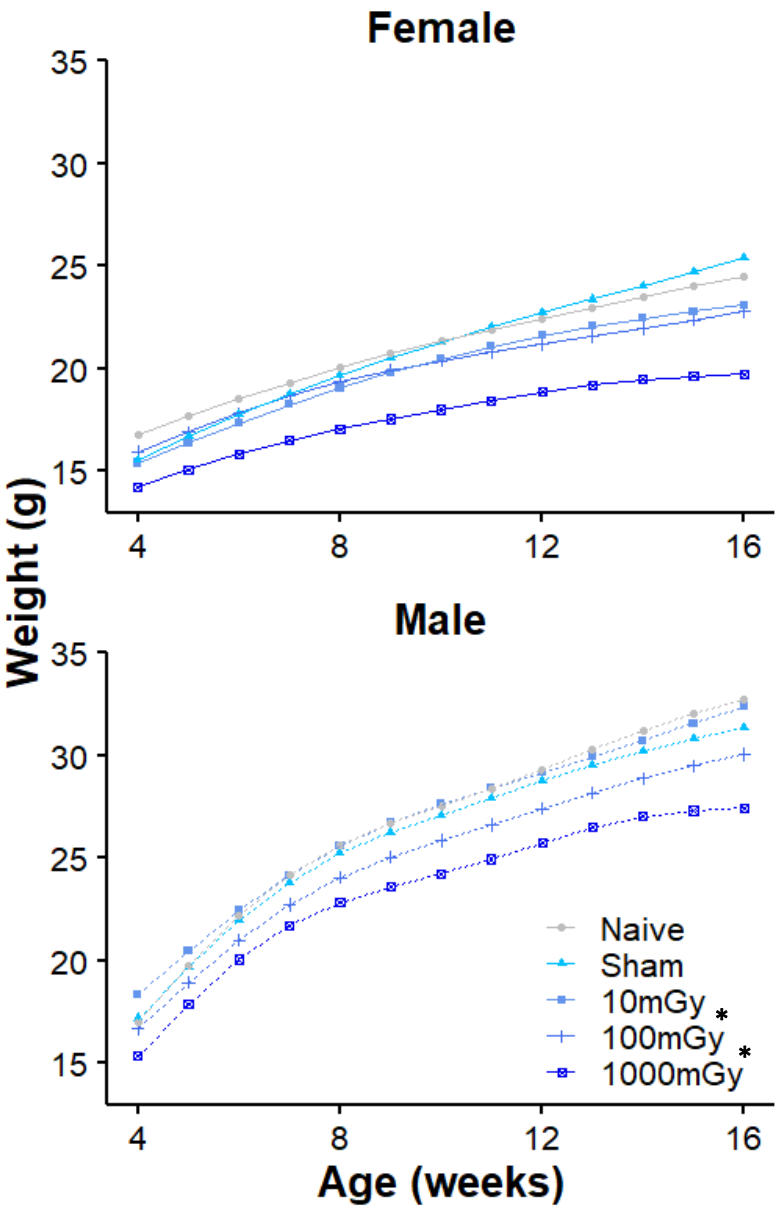


Figure 5-2: longitudinal weight measurements. *In utero* exposure to 100 mGy and 1000 mGy significantly alters the trajectory of growth compared to sham control ($p = 0.0024$ and $p < 0.001$). Exposure to 10 mGy of radiation was not different to sham. Sexes differed in growth curves ($p<0.001$). Graphs represent mean weekly measurements, n = 7-9. * indicates statistical significance of $p < 0.05$.

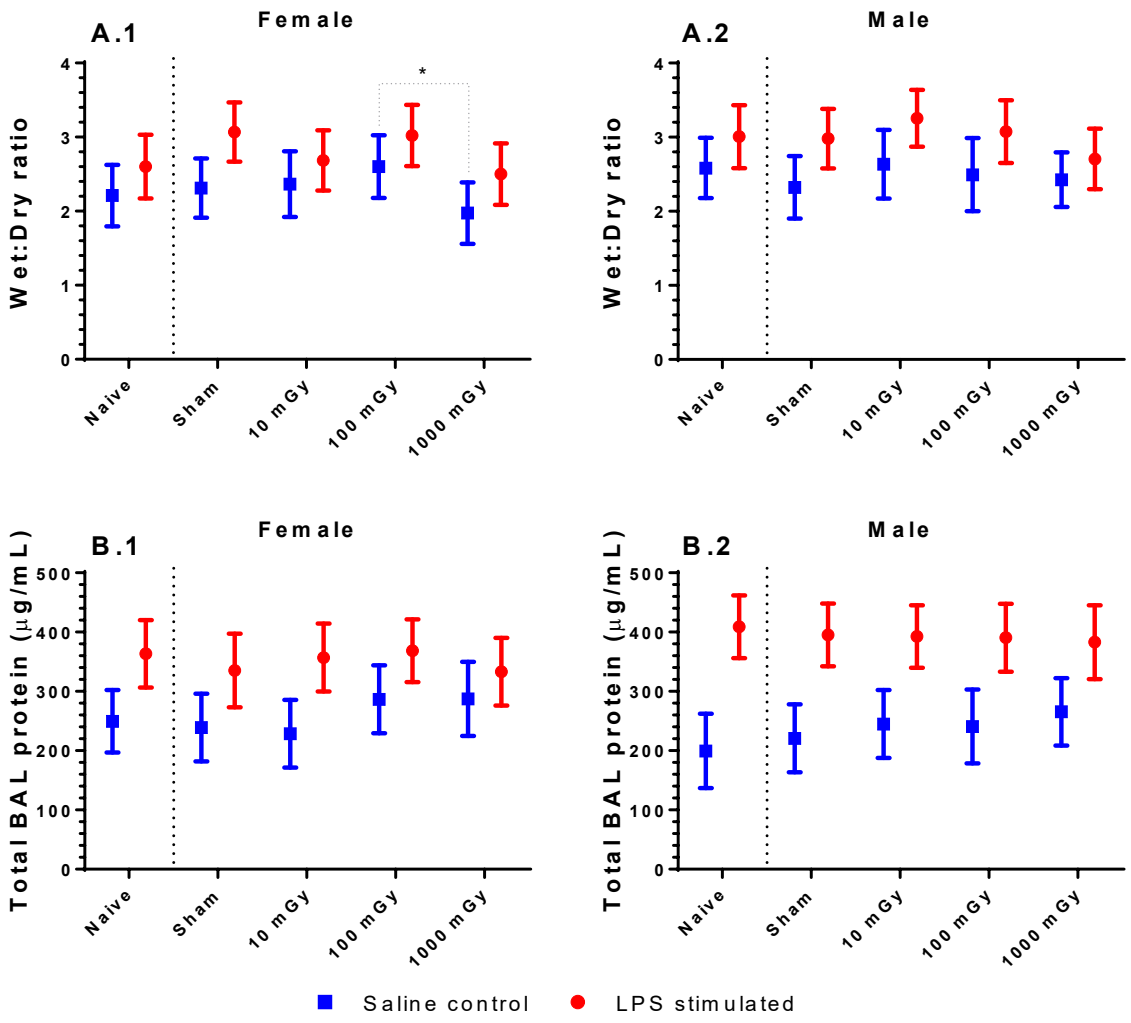


Figure 5-3: Physiological outcomes of the respiratory system, **A)** Lung lobe wet:dry weight ratio and **B)** Total BAL protein concentration. Naïve transport control animals were not significantly different to sham irradiation control animals indicating no effect of transport on these physiological outcomes **A)** LPS significantly increased the wet:dry weight ratio of the lung lobe, from 2.29 – 2.78 for females and 2.49 – 3.00 for males ($p < 0.001$, $n = 7 – 12$). *In utero* exposure to ionizing radiation did not significantly change the wet:dry ratio for saline or LPS stimulated animals back to the sham control for either sex, however, there was a significant difference for Female saline animals between 100 mGy and 1000 mGy, 2.60 – 1.97 ($p = 0.038$). **B)** LPS significantly increased the total BAL protein concentration, from 258.2 – 341.4 µg/mL for females and 234.3 – 394.1 µg/mL for males ($p < 0.001$, $n = 5 – 7$). *In utero* exposure to ionizing radiation did not significantly change the concentration of total BAL protein for saline or LPS stimulated animals back to sham control for either sex. Data represented as mean and 95% confidence intervals. Statistical significance was tested by GLMM with *post hoc* least significant difference (LSD). * denotes statistical significance between groups $p < 0.05$.

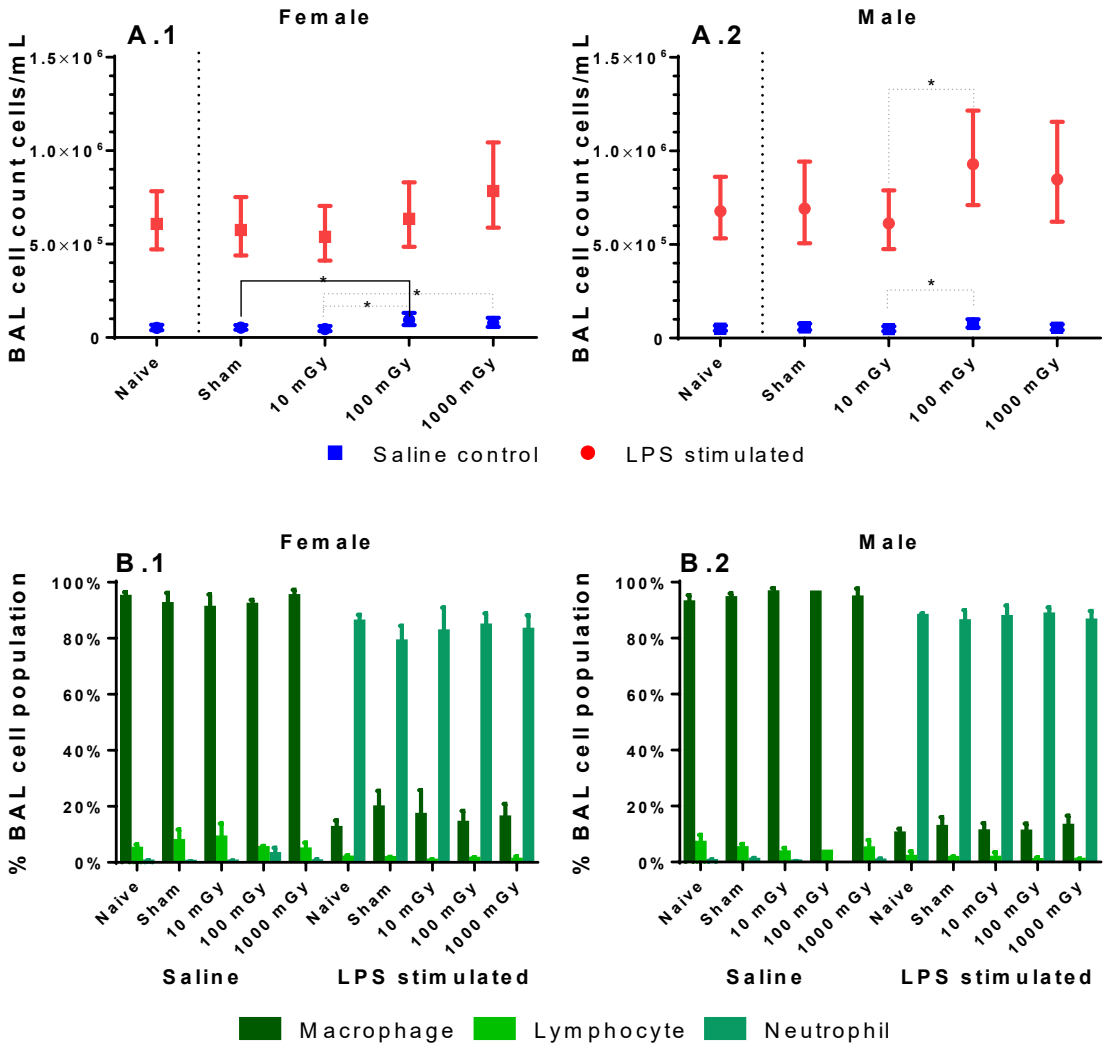


Figure 5-4: Immunological cell outcomes of the respiratory system, **A)** BAL cell count and **B)** Cell populations within BAL. Naïve transport control animals were not significantly different to sham irradiation control animals indicating no effect of transport on these immunological outcomes **A)** LPS significantly increased the BAL cell count, from $6.2x10^4$ – $6.2x10^5$ cells/mL for females and $5.7x10^4$ – $7.4x10^5$ cells/mL for males ($p < 0.001$, $n = 6 – 11$). *In utero* exposure to ionizing radiation did not significantly change the cell counts for LPS stimulated female animals back to the sham control, but for saline animals 100 mGy was significantly higher compared to sham and 10 mGy groups, $p = 0.007$ and 0.002 respectively. Animals exposed to 10mGy were also significantly higher to 1000 mGy animals ($p = 0.017$). In males, *in utero* exposure to ionizing radiation did not significantly alter any group back to sham control, but, 10 mGy was significantly less than 100 mGy for both saline ($p = 0.025$) and LPS stimulated ($p = 0.027$) mice. **B)** LPS significantly increased the percent of neutrophils (orange) seen in BAL cells, from 0.82% – 83.0% for females and 0.37% – 87.2% for Males ($p < 0.001$, $n = 3 – 7$). *In utero* exposure to ionizing radiation did not significantly change the any percentage of cells between any radiation group in saline or LPS stimulated animals of either sex. Data represented as mean and 95% confidence intervals. Statistical significance was tested by GLMM with *post hoc* least significant difference (LSD). * denotes statistical significance between groups $p < 0.05$.

5.3.3 Respiratory physiology

Instillation of LPS caused oedema within the lung and disrupted the alveolar-capillary barrier, as indicated by significantly increased lung lobe wet:dry weight ratio and BAL total protein compared to saline control, $p < 0.001$ (Figure 5-3). For the lung lobe wet:dry weight ratio, both females and males increased 21% and for BAL protein concentration, females increased 36% and males 68%. For both saline and LPS stimulated mice, *in utero* exposure to ionizing radiation did not significantly alter the lung lobe wet:dry weight ratio or the BAL total protein concentration compared to sham irradiated animals for either sex. There was only one inter dose difference noted, which was between saline-treated females exposed to 100 mGy and 1000 mGy, $p = 0.038$ (Figure 5-3A.1)

5.3.4 Respiratory immunology

Instillation of LPS significantly increased BAL cell count ($p < 0.001$, Figure 5-4) and altered the cell populations collected in BAL. Total cell count after LPS instillation increased 1000% for females and 1300% for males. For females, *in utero* exposure to ionizing radiation did not alter cell count in LPS stimulated mice but did for saline mice, where there was a significant difference between sham and 100 mGy animals, but not for 10 or 1000 mGy. There were also some significant differences noted among doses of radiation, as seen in Figure 5-4A.1, but not in a discernible trend. Irradiated male mice did not differ in cell counts compared to sham irradiated control, for either saline or LPS stimulated mice, but 10 mGy was significantly different to 100 mGy for both instillation groups, $p = 0.025$ and $p = 0.027$ respectively (Figure 5-4A.2). For both females and males, LPS instillation caused a

significant increase in the percent of neutrophils within BAL cell count, 10121% and 23567%, respectively. *In utero* exposed female and male mice did not differ in the population of macrophages, lymphocytes or neutrophils within the BAL for either instillation groups.

Instillation of LPS significantly increased the amount of active TGF-β in BAL by 30%, $p = 0.03$ (Figure 5-5A). Irrespective of sex or stimulus, animals exposed to 100 mGy *in utero* had 45%-65% higher TGF-β concentrations than the other groups, $p = 0.018$. When considering sex and stimulus, the difference is not retained for females and only occurs in LPS males, but the difference is now 141%-202%, $p < 0.05$. Instillation of LPS significantly decreased the amount of Latent TGF-β levels in plasma by 45%, $p = 0.002$. There were no differences in latent plasma TGF-β between LPS males, saline males or LPS females, but there was for saline females (Figure 5-5). Both 10 mGy and 1000 mGy exposed animals were statistically higher than sham and 100 mGy irradiated animals, $p < 0.05$.

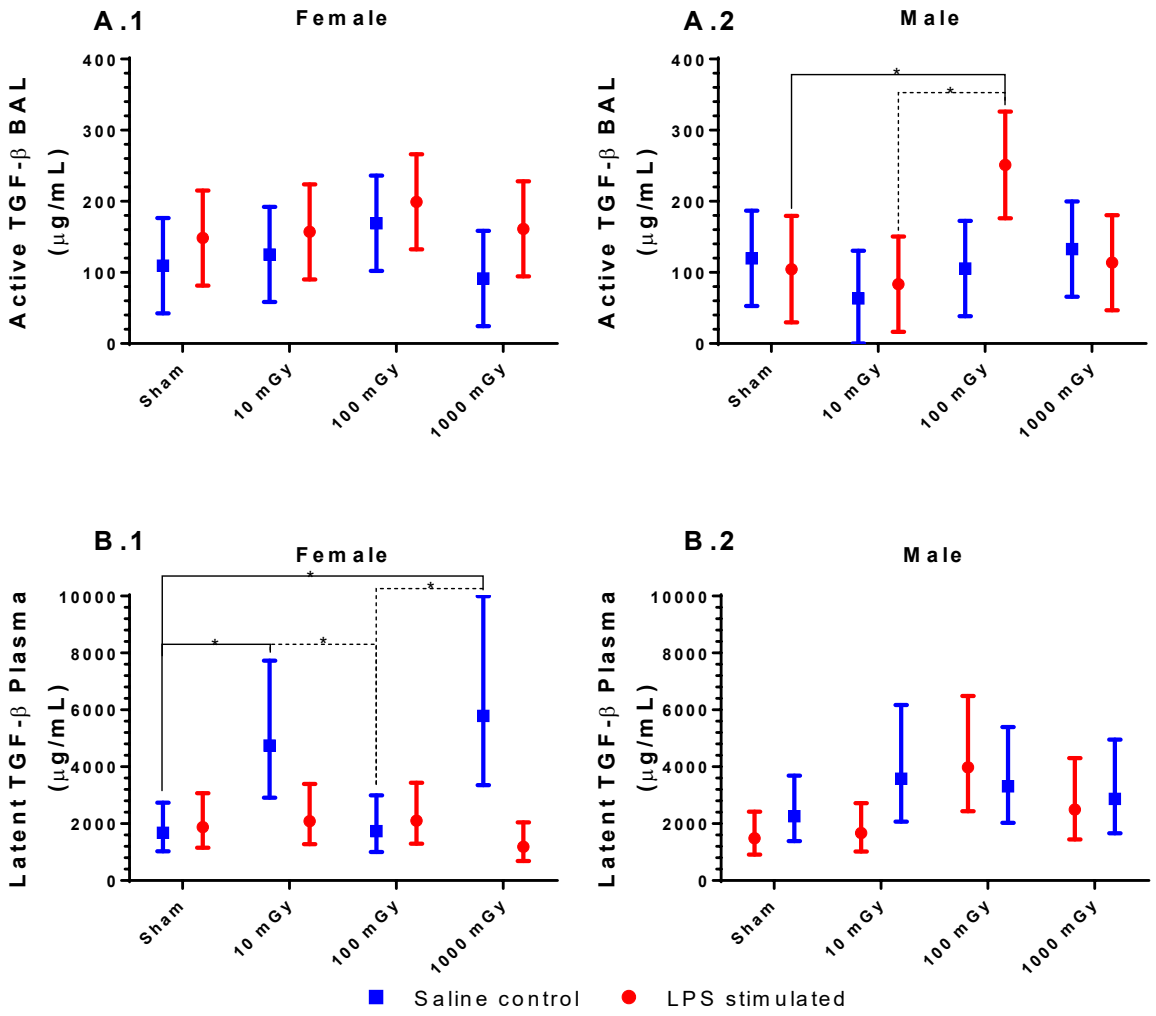


Figure 5-5: Soluble TGF-β concentrations by ELISA in BAL and Plasma as **A)** Active TGF-β in BAL supernatant and **B)** latent TGF-β in plasma samples. Active TGF-β concentrations was assessed in the BAL supernatant and latent TGF-β concentrations was assessed in plasma samples by acid activation of sample. **A)** LPS significantly increased TGF-β concentrations in BAL supernatants of both sexes, $p = 0.03$, $n = 4$-5. Irrespective of sex or stimulus, *in utero* exposure to 100 mGy resulted in a higher TGF-β concentrations than the other groups, $p = 0.018$. However, with *post hoc* analysis, female irradiated groups, for both saline and LPS stimulated animals, were not statistically different: only LPS males exposed to 100 mGy were different to the rest, $p < 0.05$. **B)** Both females and males exposed to LPS had significantly less TGF-β concentrations than saline animals, $p = 0.002$, $n = 4$-5. There was a significant interaction between dose and sex leading to opposite trends with increasing dose, $p = 0.035$. In *post hoc* analysis, 10 mGy and 1000 mGy saline animals had statistically higher TGF-β concentrations in the plasma compared to sham and 100 mGy irradiated animals, $p < 0.05$. Data represented as mean and 95% confidence intervals. Statistical significance was tested by GLMM with *post hoc* least significant difference (LSD). BAL samples were analysed using untransformed data and plasma samples were analysed using log transformed data and then antilog transformed for graphical representation. * denotes statistical significance between groups $p < 0.05$.

5.3.5 Respiratory antioxidant levels

Levels of three antioxidant enzymes, catalase, SOD2, and GPx, were assessed using western blot techniques, (Figure 5-6 and Figure 5-7). A protein curve confirmed that 50μg/well of total protein was sufficient to obtain protein bands without antibody saturation (Figure 5-8). For both sexes, intratracheal instillation of LPS significantly decreased catalase levels by 28% but increased SOD2 levels by 64% and GPx levels by 27%, $p = 0.038$, $p < 0.001$ and $p = 0.047$, respectively. SOD2 levels were 75% higher in female than males, $p < 0.001$, and there was a significant interaction between dose and sex, with increasing foetal radiation dose leading to opposite trends in protein levels, $p = 0.01$. There were no statistical differences between sexes or among irradiation groups for catalase and GPx

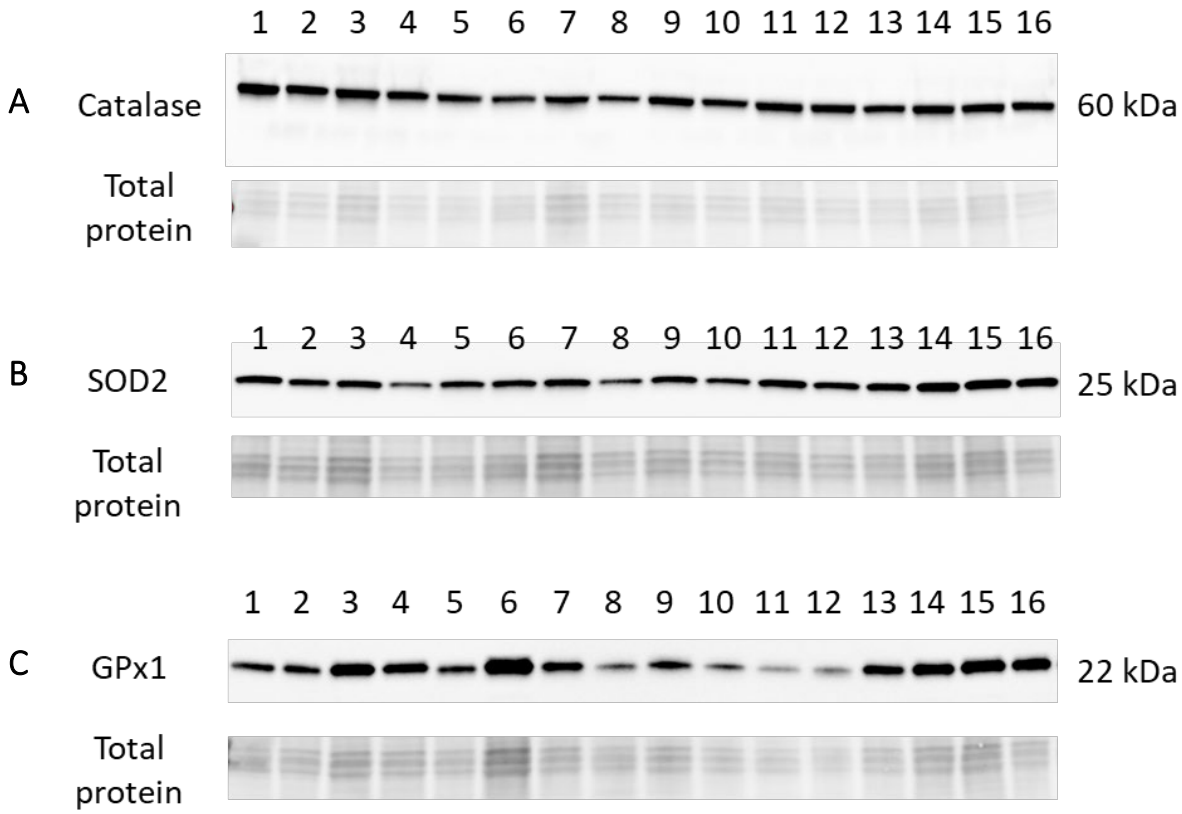


Figure 5-6: Western blot membrane and gel bands for antioxidant proteins catalase, SOD2, and GPx. Band intensities on membrane were normalised back to respective intensities from total protein bands on gel. 1 = Male Saline sham, 2 = Male Saline 10 mGy, 3 = Male Saline 100 mGy, 4 = Male Saline 1000 mGy, 5 = Male LPS sham, 6 = Male LPS 10 mGy, 7 = Male LPS 100 mGy, 8 = Male LPS 1000 mGy9 = Female Saline sham, 10 = Female Saline 10 mGy, 11 = Female Saline 100 mGy, 12 = Female Saline 1000 mGy, 13 = Female LPS sham, 14 = Female LPS 10 mGy, 15 = Female LPS 100 mGy, 16 = Female LPS 1000 mGy.

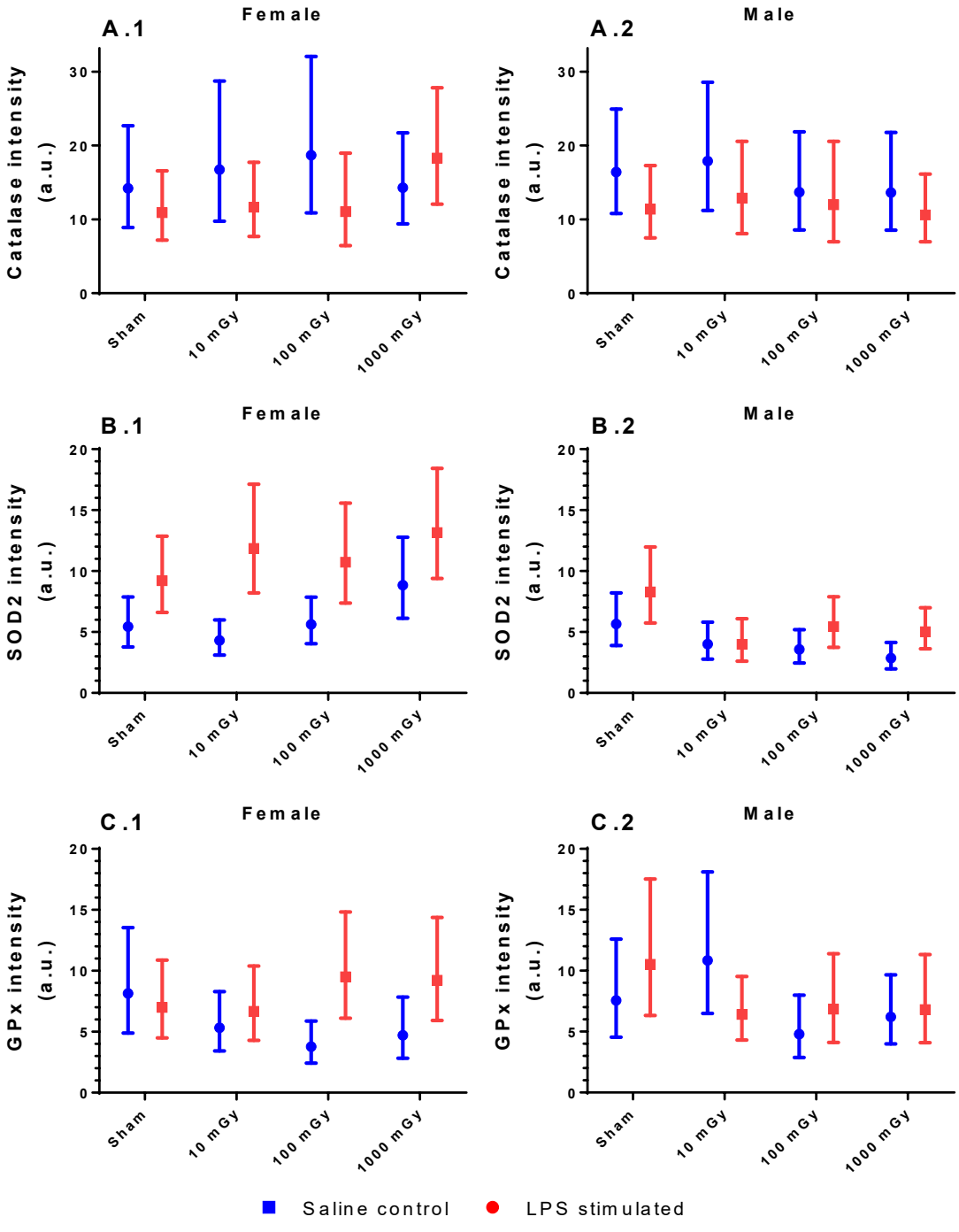
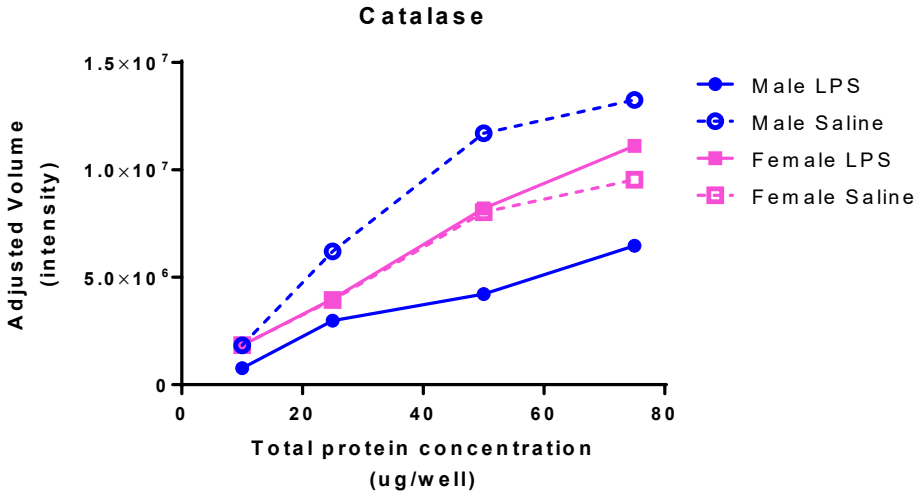
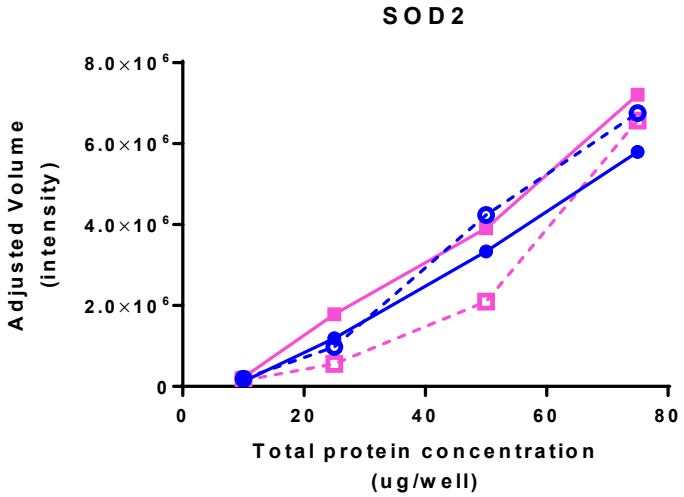

A .1
Female
Catalase intensity (a.u.)
A .2
Male
B .1
Female
SOD2 intensity (a.u.)
B .2
Male
C .1
Female
GPx intensity (a.u.)
C .2
Male
Sham
10 mGy
100 mGy
1000 mGy
Saline control
LPS stimulated

Figure 5-7: Western blot intensities of antioxidant proteins **A)** Catalase, **B)** SOD2, and **C)** GPx. **A)** LPS significantly lowered Catalase protein levels but *in utero* exposure to ionizing radiation had no effect for either sex ($p = 0.038$, $n = 3\text{-}5$). **B)** LPS significantly increased SOD2 protein levels but *in utero* exposure to ionizing radiation had no effect for either sex ($p < 0.001$, $n = 4\text{-}5$). There was a statistical difference between sex ($p < 0.001$) and an interaction between dose and sex leading to opposite trends when increasing dose ($p = 0.002$). **C)** LPS significantly lowered GPx protein levels but *in utero* exposure to ionizing radiation had no effect for either sex ($p = 0.047$, $n = 3\text{-}4$). Data represented as mean and 95% confidence intervals. Data was log transformed for statistical analysis by GLMM and antilog transformed for graphical representation. SOD2 = Superoxide dismutase 2, GPx = Glutathione peroxidase 1.


Catalase
Adjusted Volume (intensity)
1.5×10^7
1.0×10^7
5.0×10^6
0
0
20
40
60
80
Total protein concentration (ug/well)
Male LPS
Male Saline
Female LPS
Female Saline


SOD2
Adjusted Volume (intensity)
8.0×10^6
6.0×10^6
4.0×10^6
2.0×10^6
0
0
20
40
60
80
Total protein concentration (ug/well)

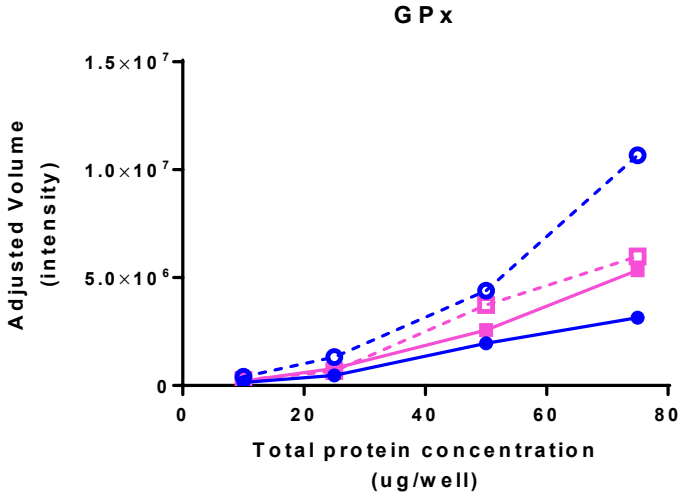

GPx
Adjusted Volume (intensity)
1.5×10^7
1.0×10^7
5.0×10^6
0
0
20
40
60
80
Total protein concentration (ug/well)

Figure 5-8: Saturation curves of antioxidant proteins **A)** Catalase, **B)** SOD2, and **C)** GPx proteins. All concentrations of total protein appear to be increasing and not hitting the asymptote; thus, saturation of antibody has not occurred. There were 4 increasing concentrations of total homogenised protein (*n* = 1): 10, 25, 50, and 75 ug/well. SOD2 = Superoxide dismutase 2, GPx = Glutathione peroxidase 1.

5.4 Discussion

This study is the first to look at the long-term immunomodulatory effects of radiation on these respiratory outcomes in an *in utero* exposed setting. Therefore, there is a paucity of data with which to compare and relate the separate and combined immunomodulatory effects of radiation and LPS. The intention behind this study was to understand if radiation during pregnancy, at a time that had minimal possibility of foetal resorption or severe physical malformations, had any long-term effects on physiology or immunology during a respiratory bacterial infection. To test this aim, three doses were used in this study relating to different plausible clinical exposures. The lowest exposure, 10 mGy, represents the dose of a typical abdominal and pelvic CT scan[31]. Although not routinely received by pregnant patients, as evident in Chapter 3, clinical justification can result in its use e.g. in trauma, such as motor vehicle accidents, or delivery complication, such as placenta accreta. Although almost all diagnostic procedures fall below the middle exposure of 100 mGy, there are limited instances in which the foetus is exposed to a single procedure above this level[32], nonetheless, this dose represents an unlikely, but possible, cumulative exposure to the foetus. The last exposure of 1000 mGy is well above diagnostic levels, and although it falls below therapeutic level of medical radiation, it was selected as the high dose control for this study.

In terms of translation of this animal model to a clinical setting, the gestational development at exposure and radiosensitivities between BALB/c mice and humans are very

similar. The gestational day 15 in mice (GD15) falls around the time of the pseudoglandular phase[5]. This phase in development relates to 5 - 17 weeks gestation in humans, with transcriptomics narrowing GD15 to approximately 12 – 15 weeks in human gestation[33]. GD15, and 15 weeks in humans, are both on the late end of the pseudoglandular, early canicular phase of development, where the stereotypic lung structure is finalising development complete with intertwining airways and vessel branching. In terms of the overall radiosensitivity, BALB/c mice are also very comparable to humans. The human LD50 in 30 days from full body exposure is between 2000 – 8000 mGy, 4500 mGy for a healthy adult, and for BALB/c mice, is about 4500 mGy[34–36]. Therefore, the radiobiological effects seen from irradiation of these mice at GD15 with these doses should be, at least partially, translatable to human exposures during early 2nd trimester of pregnancy.

In this study, there were significant growth restrictions from weeks 4 – 16 in mice exposed to 100 and 1000 mGy *in utero*. Although the result that a high dose of 1000 mGy can cause a persistently reduced growth rate among mice was expected, as previously seen in chapter 4, the degree of growth restriction in this study was much greater[7,37–41]. Exposures of 1000 – 1500 mGy, at similar gestational ages, previously resulted in 11-15% reduction in body weight in mice and rats but this mouse study showed 15% reduction for males and 25% for females[7,37,38]. This larger difference in weight may be due to species and strain variation, with varying radiosensitivities, or sex differences, where due to combining sexes rather than examining individual sexes despite inherently different weights and growth curves[7,35–38]. As reviewed by both Alur and Clifton[42,43], the sex-based differences of the placenta and

foetus allow for differential coping mechanisms to adverse environmental stimuli via protein, micro-RNA and gene expression variations. Their findings indicate that females conserved more energy, thereby reducing growth, as a coping mechanism to possibly prepare against further insult.

A surprising result from the growth outcomes was the reduction in body weight from 100 mGy exposed animals. To the author's knowledge, this is the first report of persistent growth restriction at this dose; an 8% reduction for males and 10% for females. Although it is statistically significant, the biological difference may be less significant. Even though this study was not able to assess weight preweaning, and therefore cannot state this phenomenon existed from birth, when extrapolated to human growth, a 10% reduction in body weight may not result in significant physiological effects, even from birth weight. For example, the average birth weight in Australia is 3.33kg and the average adult weight is 82 kg for males and 72 kg for females. A 10% reduction would not meet the criteria for low birth weight status, <2.5 kg or 25% reduction from the average birth weight, and the adults would actually be healthier in terms of average BMI[44,45]. In comparison, the 25% reduction from the 1000 mGy exposure would result in a low birth weight status, increasing risk of complications and delayed postnatal growth, as well as push adult BMI closer to being underweight[46]. Therefore, although statistically significant, the growth restriction by 100 mGy may not be biologically significant as in the 1000 mGy exposed animals.

LPS is a lipoglycan and endotoxin found on the outer membrane of gram-negative bacteria[10,11]. It is a common, non-infectious, non-replicating, pro-inflammatory stimulus used in acute lung injury models to understand host inflammatory responses during bacterial infection[13]. Intratracheal instillation of LPS in this study increased both respiratory physiological outcomes of total BAL protein concentration and lung lobe wet:dry weight ratio, indicative of a successful acute lung injury model. Total BAL protein is an indicator for decreased alveolar-capillary barrier integrity, and therefore vascular leak, and is an expected finding following LPS instillation[47]. Lung wet:dry weight ratio, an indicator of pulmonary oedema, is also very common with LPS instillation in the lung[48,49]. Even measured by other methods, the concept that LPS induces pulmonary oedema is well known[50]. In both a clinical and animal setting, increased vascular permeability can cause non-cardiogenic pulmonary oedema characterised by the influx of protein-rich fluid into the interstitium and alveolar space leading to reduced pulmonary function and reduced gas exchange[51–54]. Intratracheal instillation of similar and lower doses of LPS (0.05-4 mg/Kg) reduced pulmonary function, including lung compliance, elastance and airway resistance, compared to saline control animals[55,56]. Even via a different delivery method (inhalation), LPS still altered lung function 24 hours post instillation[47]. These outcomes of dysfunction of alveolar-capillary barrier, pulmonary oedema, and changes in lung function, are all aspects present in human acute lung injury, indicating that LPS instillation produces a similar physiological profile observed in clinical patients[12,57].

The mechanisms through which LPS causes physiological outcomes are heavily influenced by its ability to stimulate a pro-inflammatory response. Intratracheal instillation of LPS increased total BAL leukocyte cell count, which was dominated by neutrophilic infiltration. This is again well known, even for LPS concentrations much less than 3mg/Kg[47,48,55,58]. Intratracheal instillation of LPS stimulates alveolar epithelial cells and macrophages to promote a pro-inflammatory milieu that mediates chemotactic, predominately neutrophilic, leukocyte infiltration. The activated and infiltrated neutrophils release additional pro-inflammatory mediators while attempting to neutralise the stimuli, which perpetuate inflammation and inevitably damage healthy host tissues.

TGF-β is produced by macrophages and fibroblasts in a latent protein form and circulates in the blood or is secreted to the extracellular matrix. Activation of TGF-β can occur via integrin-dependent cell-mediated cleavage, or integrin independent means such as acidification, ROS and proteases[59–61]. In response to tissue damage, local TGF-β is activated in an integrin-mediated process, as was indicated by LPS instillation significantly increasing active TGF-β in the lung[62]. Once activated, TGF-β binds to TGF-β receptors on epithelial, endothelial, stromal cells and leukocytes to mediate anti-inflammatory/pro-fibrotic responses[59,63]. Instillation of LPS also significantly reduced the amount of latent TGF-β in the plasma in this study, which may be due to transfer into the lung. Other studies of intratracheal instillation of LPS (0.1 – 0.5 mg/kg) demonstrated increased pro-inflammatory mediators in BAL, including IL-6 and TNF-α, 24h post instillation. However, these levels were lower than earlier time points, and in conjunction with an increase in TGF-β, this

suggests that the inflammatory process could have been shifting from the recruitment phase to the resolution/repair phase[55,56,58].

As reviewed by Matès *et al*[64]*,* reactive oxygen species, for example, hydroxyl radicals, can be created via several pathways, including directly by aerobic respiration or radiolysis of water and indirectly by LPS stimulation[65,66]. SOD2 converts hydroxyl radicals created in the mitochondria to hydrogen peroxide (H_2O_2), which is also considered a reactive oxygen species. When entered into the cytosol, this H_2O_2 is further catalysed to oxygen and water by both GPx and catalase, thereby defusing the reactiveness. In this study, intratracheal instillation of 3 mg/Kg LPS decreased levels of catalase and increased levels of SOD2 and GPx, with a pronounced increase of SOD2 indicating a mechanistic preference for this antioxidant. This could be due to oxidative stress activating intracellular redox-sensitive transcription factors, such as Nrf2, which translocate to the nucleus to upregulate antioxidant enzyme transcription, strongly for SOD2, and weakly for catalase and GPx1[67,68]. In a similar study, mice exposed to a lower LPS concentration (0.5 mg/kg), had comparable outcomes for SOD2 and catalase[55]. SOD2 continually increased up to 24hrs post instillation but catalase was increased at 6hrs then decreased below control levels at 24hrs. The levels of catalase had an inverse relationship with lipid and protein oxidative damage levels, indicating that an increase in H_2O_2 by SOD, but insufficient removal by catalase or GPx, resulted in H_2O_2 mediated lipid and protein oxidation and damage. Therefore, along with the LPS mediated reactive oxygen species damage, SOD2-created H_2O_2 may additively be contributing to damage within the lung.

Similar to chapter 4, this study also found no significant effect of *in utero* exposure to ionizing radiation compared to sham irradiated animals for physiological outcomes in control animals, nor in the LPS stimulated animals. Previously, in healthy, adolescent mice exposed to 2000 mGy and above, lung lobe wet:body weight ratio increased 6 weeks post-irradiation, and returned to baseline before 16 weeks post irradiation[69]. This increase in damage associated oedema was likely due to leaky vasculature or damage to the alveolar-capillary barrier. Therefore, it is plausible that at 6 weeks there would have also been increased BAL total protein concentration in these animals, that would have been reabsorbed before 16 weeks post-irradiation. Thus, it is likely that the timeframe between irradiation and outcome for chapters 4 and 5 may have been too great to observe any overall radiation-related effects on LPS response in these outcomes. In this current study (Figure 5-3), there was one statistical difference in control female animals; a slight decrease in wet:dry weight from 100 to 1000 mGy exposed animals that were also trending in the same direction for LPS simulated animals. However, this difference may not be biologically different as such small changes in ratio have not been previously associated with altered lung function.

Radiation did not have a pronounced effect on the respiratory immunological outcomes examined. *In utero* exposure to ionizing radiation did not significantly alter the amount of cellular infiltrate during inflammation. In saline control females, 100 mGy exposed animals had significantly more BAL cells than all other irradiated groups including the sham irradiated animals. Although statistically higher, the variation in groups resulted in a count

of only 45 cells between the lower confidence interval of the 100 mGy females and the upper interval of the sham irradiation control, a technically unmeasurable amount. From Figure 5-4B, it appears that the increased cell count could consequentially be from neutrophil influx. However, this influx is not comparable to the influx of total cells and neutrophils from LPS instillation, and so the effect of these infiltrated cells in the saline 100 mGy mice would presumably be subtle. This statement should be confirmed by assessment of the level of activation of these cells extra infiltrated cells. Males exposed to 100 mGy were also statistically different to 10 mGy for both saline and LPS stimulated animals. The variation between these two doses is unusual, especially for LPS stimulated animals, as both 10 and 100 mGy falls under the anti-inflammatory range of ionizing radiation's effect[24,25,30]. Overall, the variations in LPS stimulated animals, both statistical and trend-like, should be replicated to confirm a true finding and begin exploration as to why this phenomenon occurs.

There were no obvious trends in the type of cells infiltrating into the lung due to ionizing radiation. As previously reported, it was predominately macrophages for control animals and neutrophils following LPS stimulation, but radiation exposure did not alter this. Changes in cell subset populations might not have been visible due to macrophage or neutrophil cells dominating the population percentages. Additionally, it is also possible that small changes in subset populations were unable to be seen with the limited methodology used in this study. Cell differentials by hematoxylin and eosin staining lacks the ability to correctly identify subpopulations with physiologically similar cell types. Flow cytometry

might have been able to distinguish between subpopulations of cells that ionizing radiation commonly affect, such as T lymphocytes and macrophages. It is possible that the immunomodulatory effects of radiation were not strong enough to cause significant changes in overall populations 16 weeks post-irradiation. Most work that focuses on the immunomodulatory effects of ionizing radiation use macrophages, peripheral mononuclear cells or T lymphocytes, generally investigating short term outcomes[22–25,70,71]. One mouse study found effects the 12 weeks later but in a specialised T lymphocyte subset (T regulatory cells) stimulated by very high dose exposure (15 Gy)[71]. Additionally, there is a scarcity of evidence on the radio-immunomodulatory effects on neutrophil responses, however, attachment and migration of these cells from the vasculature to the site of infection use similar adhesion molecules that are known to have a down-regulated response to low dose radiation[30,72]. Thus, although there were no significant changes in cell number or cell population, likely due to length of time post-irradiation, improving the methodological technology could confirm that there was truly no change in populations.

TGF-β concentration was affected by *in utero* exposure to ionizing radiation but not in a dose-dependent pattern. In BAL supernatant, active TGF-β was unchanged in females and saline males, but BAL from LPS stimulated, 100 mGy irradiated males was significantly greater than the rest. In short term studies, the current understanding is that low doses (<1000 mGy) of ionizing radiation activate TGF-β, through both integrin meditated and non-mediated pathways, and that increased activation of TGF-β results in an anti-inflammatory response, at least in part, though TGF-β- mediated decreased leukocyte

migration[27,28,30,73–75]. However, this is not the case in our results, where there is a trend of increasing cellular infiltrate for 100 mGy LPS stimulated animals. In longer-term experiments, up to 16 weeks post-irradiation, doses much higher than ours are associated with increased active TGF-β, and resulting fibrosis, but there is a lack of evidence associating low doses with similar effects at this timepoint[76,77]. Additionally, if this were to be true and did extrapolate down to lower doses, it would be expected that both 100 and 1000 mGy exposed animals, regardless of stimuli, would be higher, rather than just 100 mGy LPS stimulated animals. Therefore, this result is a surprising anomaly in which further investigation is required to understand this outcome and its relation to LPS induced pathophysiological changes.

In latent TGF-β plasma concentrations, it was saline females that showed variations due to *in utero* irradiation. Animals exposed to 10 mGy and 1000 mGy had significantly higher latent TGF-β levels compared to both sham and 100 mGy irradiated animals. This biphasic shape itself is not unusual for TGF-β, as similar shapes can be seen for active TGF-β at similar exposures, but in a much shorter timeframe[78]. Additionally, as the literature focuses on active TGF-β, there is limited data on the effects of irradiation on latent TGF-β. These results would need to be repeated with a small pilot group of saline instilled females to confirm these results. The difference between irradiated animals in the saline females was not visible in the LPS animals, suggesting that LPS stimulation activated all of the extra latent TGF-β, caused by *in utero* irradiation, to a similar level to sham and 100 mGy animals. However, when incorporating Figure 5-5A.1, the activated LPS did not accumulate in the

lungs. Soluble latent TGF-β has a longer half-life in the plasma (> 100 minutes) than active TGF-β (2 – 3 minutes), which is readily taken up by tissues[63]. This shorter half-life of active TGF-β, the non-significant finding in active TGF-β in the lungs of females, and the large difference in units between latent and active TGF-β levels, could suggest that the TGF-β response had occurred within the lung before the 24 hours measurement. This hypothesis would need to be confirmed by exploring a time-response curve.

Finally, antioxidant levels in the lung appeared unchanged in 16-week-old mice following *in utero* ionizing radiation exposure. There is large variation between groups for all three antioxidants examined in the lung tissue. Increasing sample size may reduce this variation and allow more accurate determination of potential differences. Overall, radiation did not significantly alter the response to LPS for any antioxidant measured. However, when removing grouping by stimulus factor (saline or LPS), the concentration of SOD2 was influenced by radiation based on sex. This sex:radiation-dose interaction led to directionally different outcomes, with SOD2 concentrations increasing for females and decreasing for males with increasing dose. The sham irradiated levels between sexes are very similar, so it appears that there is a sex-associated difference that is triggered by radiation that is causing this variation in direction. There is a lack of evidence supporting sex differences in SOD2 expression following ionizing radiation, so it is difficult to propose any causation. In *in vitro* models, doses < 1000 mGy have shown increased SOD2 expression, via a Nrf1/2 pathway, but only for short periods of up to 24 hours[79]. Doses > 1000 mGy have shown increased concentrations of SOD2 and GPx protein, due to radiation-mediated chronic

mitochondrial stress, but only up to 1-month post irradiation[80]. Although these studies are conducted post-embryonic development and even though this dose:sex interaction is present, the timing in our study post-irradiation may be too long to observe specific effects due to irradiation.

The design of this study presents several limitations. The use of LPS as the model stimulus comes with several advantages and disadvantages. Due to its non-replicating ability, the use of LPS allows examination of specific phases of the inflammation process, for example, recruitment, resolution or repair, based on time periods post instillation. However, in a clinical setting, infection occurs with live pathogens which perpetuates the inflammation processes continually replicating and stimulating the immune system until cleared[13]. Additionally, live bacteria also have other immune triggering stimuli including exotoxins and effector protein infiltrations through type III secretion systems[81,82]. Finally, although LPS triggers an immune response that is similar to intratracheal exposure of LPS in humans, the model does not include all of the complex pathophysiological changes associated with clinical acute lung injury, or its more severe form, acute respiratory distress syndrome (ARDS)[12,13]. Instillation of LPS differs in its ability to cause severe alveolar epithelial, and thus, it is limited in its ability to accurately replicate clinical situations[13,83].

Although longitudinal measurements were taken for the weight of these animals, the respiratory outcomes were only collected at a single time point. This limits the examination of the effects of *in utero* exposure to ionizing radiation on the developing respiratory

system. This time point was chosen to allow for the successful assessment of growth restriction, as a validated outcome to show irradiation affected the pups. In future studies, shortening the time frame between ionizing radiation and respiratory outcomes will prove useful in understanding the temporal immunomodulatory effects of low dose ionizing radiation.

Lastly, expanding the current outcomes to increase specificity will provide more information on the potential effect of radiation on the respiratory system. Incorporating flow cytometry would provide extensive additional information regarding the circulating and tissue infiltrating cell phenotypes. Expanding the cytokine selections to include pro-inflammatory and additional anti-inflammatory mediators will provide greater knowledge into the long-term cytokine profile of irradiated and stimulated respiratory systems. Additionally, examining deeper the relationships between outcomes and understanding the pathways would better help the interpretation of results, such as defining if there is a radiation-induced association between infiltrating cell type and mediators present in BAL. Finally, addition of lung function testing, via blood oxygenation or respiratory mechanics, will confirm if radiation has any effect on the respiratory function directly, or if it attenuates function during acute lung injury.

5.5 Conclusion

Overall, this study has demonstrated that clinically relevant exposures to ionizing radiation during late gestation have little to no effect on the long-term physiology and immunology of the mouse lung during inflammation from a bacterial stimulus. Addition and refinement of some techniques would allow for a greater understanding, but the current results provide a foundation of knowledge. There was some growth restriction occurring at both 100 and 1000 mGy exposures, but no effect was seen at the most clinically relevant dose of 10 mGy. These results support the hypothesis that low dose irradiation has little effect on the foetus during development, however, significant work is required to solidify this ideal.

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CHAPTER 6

OVERALL DISCUSSION

This thesis investigates diagnostic levels of ionizing radiation in hospital patient populations and how these doses may affect long-term respiratory outcomes, with a primary focus on pregnancy-related exposures. Retrospective audits of two separate hospitalised cohorts, ICU patients and pregnant patients, showed that most patients cumulatively received < 1 mSv. Two animal models, assessing the long-term respiratory effects from *in utero* radiation exposure, demonstrated that compared to controls, ionizing radiation had no overall effect on the healthy respiratory system, nor did it alter the overall response to an acute lung injury stimulus for the outcomes measured. It did, however, cause persistent reduced growth from 4 – 16 weeks of age but only at doses >100 mGy.

6.1 CLINICAL AUDITS COMPARISON

Chapters 2 and 3 explored the doses of ionizing radiation received from diagnostic procedures to patients admitted to a tertiary hospital. Two polar populations were chosen based on the predicted exposure they would receive. Admitted ICU patients were predicted to have the highest levels of exposure in the hospital (Chapter 2), and admitted pregnant patients were predicted to have among the lowest exposure (Chapter 3). This predicted difference stems from the risk of prescription, which was discussed in their respective chapters. ICU patients are typically older and severely ill, thus the clinician's

risk to benefit analysis is focused more on the acute timeframe and the greater risk to the patient of not prescribing the scan. The risk of stochastic effects (chance-like relationship), such as cancer, are less than the chances of them surviving long enough to develop that cancer. In contrast, pregnant patients are generally younger, and the clinician's risk assessment extends to include that of the developing foetus. This shifts the focus to long term outcomes, as there is a lifetime of risk remaining for the mother, but more importantly for her foetus. This allows for stochastic effects of radiation to hold more weight in the risk to benefit ratio analysis. In addition, there are several studies that have highlighted that some clinicians do not accurately understand radiation dose and risk, misunderstanding procedure doses such as CT or fluoroscopy, and inferring incorrect levels of risk, such as cancer induction[1–6]. This inevitably ends in risk overestimation, which may make these clinicians reluctant to prescribe lifesaving diagnostic radiation. These two different points of focus, predicted to lead to different perception-based hypotheses when applied to the risk to benefit analysis, were ultimately supported with the median and highest cumulative exposures being 0.91 and 199.89 mSv for ICU patients (Chapter 2) and 0.02 and 19.07 mSv for pregnancy patients (Chapter 3).

In comparison to other departments or patient populations within a hospital, the median doses from Chapter 2 and 3 are low. ED, cancer patients, cardiovascular patients, haematological patients, and hospital-wide studies have reported median cumulative exposures of 11.7 – 34.1 mSv[7–11]. These cumulative exposures are large and variable due to the low number of patients and specific disease inclusion criteria for each cohort, such as cancer patient that present as effective doses per year to include follow up visits and therefore a longer period to accumulate dose. In comparison, the dose from Chapter 2

(0.91 mSv) is low, but it is comparable to a recent ICU cohort which had a median cumulative dose of 0.72 mGy[12]. The slight variation between ICU cohorts may be because this study included short-term stays (<5 days) which could have reduced the median dose. Chapter 3 had a median dose of 0.02 mSv, which is very low in comparison to other hospitalized populations but is also low in comparison to a large, USA-based pregnant cohort[13]. Lazarus *et al* reported an average cumulative exposure between 0.82 – 2.1 mGy between 1997 and 2006, which is higher compared to the mean dose from Chapter 3, 0.71 mSv[13]. That study has not clearly defined how they completed dose calculations and therefore it is possible mathematical differences could account for some variability. Additionally, when assessing individual modalities, the proportions of procedures are similar, but they reported much higher average doses for all modalities, possibly indicating that better technology and protocols to reduce dose more effectively are currently used.

However, as stated in Chapter 2 and 3, median cumulative exposures can also vary when eligibility criteria require specific procedures for inclusion, such as CT scans or fluoroscopic examination, which are relatively high dose procedures and therefore increase cumulative exposure. This is evident in an ICU study which only included patients that received a CT and reported a median cumulative exposure of 12.6 mSv[14]. In deciding not to limit based on this criteria, Chapter 2 and 3 were able to include a larger cohort, therefore showing a more representative and accurate understanding of dose delivered to these populations, as well as the frequency of procedures prescribed.

A deeper comparison between the two chapters highlights that the main reason for the variation in median dose is due to the use of high dose techniques, such as abdominal and

pelvic CT. In the ICU cohort (*n* = 526), 156 abdominal and pelvic CT scans were received totalling 3867.8 mSv, whereas in the pregnant cohort (*n* = 547), 4 scans totalled 50.2 mSv. Of the pregnant patients that did receive a CT scan, 3 out of the 4 were performed due to delivery-related admissions and 3 patients were admitted to the ICU, evidently describing how sick these patients were. Most scans in the ICU cohort (50.6%) came from gastrointestinal/sepsis patients, which accounted for 26.5% of total patients. In contrast, gastrointestinal/sepsis-related admissions only accounted for 10.6% of the pregnancy cohort population and none received a CT. This raises the question: are CT scans avoided due to pregnancy? From the sheer difference in the number of scans, it would appear that clinicians do avoid CT in pregnant patients, but this comparison cannot be accurately answered without matching patients that did or did not receive CT based on diagnosis and reason for prescription; for which this investigation was not designed.

This leads to a secondary question: if a diagnostic radiation procedure is prescribed, is the dose the pregnant patient receives reduced? Again, this question could be answered in the aforementioned comparison study design, but from the ICU and pregnancy cohorts in this thesis, the answer suggests yes. The average abdominal and pelvic CT scan in the ICU and pregnancy cohorts was 25.1 and 12.6 mGy, respectively. However, the literature suggests that on average, pregnant patients may receive more. Using foetal dose as a proxy for pregnant patient dose, from studies that solely look at CT scans, and from studies that are not matched by diagnosis or scan intention, the average abdominal and pelvic CT ranges between 13.3 – 21.9 mGy for ICU studies and 17.1 – 28.7 mGy for pregnancy studies[12,13,15–19]. This higher exposure could be explained by pregnant patients only receiving CT scans in severe cases, as seen in Chapter 3, which might also explain the

low number of recruited patients, but high doses observed by these studies. Nonetheless, without further exploration, these overarching questions cannot be answered.

The intention behind these clinical audits was twofold. First, they are an educational tool to understand what dose of radiation our clinicians are prescribing, with the intent to relay this information back to the clinicians and to inform how these doses relate to risk. From these chapters, we observed that;

- Both cohorts had negligible exposures to ionizing radiation from diagnostic procedures, with medians of both cohorts below the recommended public limit of 1 mSv.
- There were factors associated with increased cumulative effective dose, however, these factors combined illustrated patients with a higher severity of illness and longer hospital stays.

Overall, these results should highlight to the clinician the relatively low cumulative exposures, and thus low level of radiation risk, these patients receive. This should lead to a better understanding of dose and risk and an easement surrounding the fear of ionizing radiation. The second intention behind these audits was to provide a translational foundation to choose clinically related exposure levels for the animal studies to investigate the long-term effects of *in utero* exposures.

6.2 Animal studies comparison

Chapter 4 and 5 explored the long-term effect on growth and the respiratory system of *in utero* exposure to ionizing radiation. For both chapters, exposures up to 1000 mGy did

not significantly alter the outcomes measured for the respiratory system nor did the exposures in Chapter 4 affect cardiovascular parameters. A previous similar mouse model with similar doses reported no overall cardiovascular effects arising from a single, late gestation, ionizing radiation exposure[20]. No such study has occurred in humans, but adult chronic environmental and occupational exposures have also not linked ionizing radiation exposure to negative cardiovascular outcomes[21–23]. To the author's knowledge, there have been no long-term investigations into the effect of foetal exposure to ionizing radiation on the respiratory system. Some studies have observed respiratory fibrotic responses but these studies are on adults and required much higher doses, sometimes 20 – 60 Gy[24–26]. Instillation of LPS to cause acute lung injury in Chapter 5 worked as expected, but *in utero* radiation exposure had no effect on the manifestation of acute lung injury or the immune response. It is hypothesised in both studies that the length of time between irradiation and outcome was the reason for lack of immunomodulation observed, suggesting that an examination at a shorter time period post-irradiation would have found radiation-induced immunomodulation of the immune system, however, further studies are required to confirm this hypothesis.

The intentions behind the significant time delay between irradiation and main outcomes were threefold. Firstly, growth rate was observed over a long period of time to ensure that the pups didn't 'catch-up' the weight reduction back to the non-exposed normal level. As can be seen from the growth curves of both chapters, the curves trend separately and do not appear to realign as the pups age. Secondly, this outcome acted as our positive control that irradiation had occurred, as growth reduction from irradiations on gestational day 15 had already been observed[20]. Lastly, to our knowledge, no one had looked at the

respiratory system in response to *in utero* ionizing radiation, thus, to avoid outcomes of death or malformations and focus on the possible 'foetal programming' effects of organ development, late gestation was chosen. Gestational Day 15 is in between two major stages of respiratory development responsible for structural integrity and cellular differentiation, both important in function and physiology later in life[27,28]. Additionally at this time, the liver begins devotion to metabolic activities and so the main site of haematopoiesis moves to the thymus and spleen, before final relocation to the bone marrow[29,30]. Thus, gestational day 15 is also situated in an important phase of the immune system development and disruption at this time may also cause 'programmable' effects.

It would be intriguing to understand if the growth restriction, a hallmark of foetal programming, was caused by maternal effects, direct exposure, or if it required the combination/interaction of both effects[31]. However, due to ionizing radiation affecting the tissue in both an indirect and direct method, it is hard to delineate the cause of the effect. Irradiating a mother and surgically swapping her offspring *in utero* with unirradiated offspring could demonstrate both maternal exposure with control foetuses (indirect) and foetal exposure within control mothers (direct), but technically this would be almost impossible and come with a plethora of maternal and pup stress that would inevitably make the findings redundant. Interestingly, irradiated pups have reduced growth postnatal and non-irradiated pups are unchanged regardless if they are fostered by irradiated or non-irradiated mothers, indicating that it is an intrauterine event that programs long-term reduced weight and not the mother's ability to nurture her pups[32]. Nevertheless, the delineation of cause of growth restriction has yet to be answered.

In respiratory outcomes, a baseline understanding was required (Chapter 4) before further investigation into inflammatory responses during acute lung stimulus (Chapter 5) could commence. It is important to recognise that when comparing the respiratory outcomes from these two animal studies, one used a healthy model (Chapter 4) whilst the other used an acute lung injury model (Chapter 5). Although there were non-stimulated animals in Chapter 5 (Saline control), they were anaesthetised, intubated and instilled with saline, which inevitably causes tracheal damage and slight lung inflammation[33]. Thus, these cohorts are intrinsically different from each other methodologically.

Additionally, as noted in Chapter 5, the two animal studies used different strains of mice; Chapter 4 used C57Bl/6 mice while Chapter 5 used BALB/c mice. The rationale for changing the strain was due to the offspring cannibalism rates of C57BL/6 dams, which was also the reason why pre-weaning weights were not collected. BALB/c mice are generally considered to be less cannibalistic, but as this was the first trial by our group with this strain of mice, caution was taken to observe their motherhood behaviour and cannibalism potential with the least interference possible, which resulted in pre-weaning weight not being collected[34]. It is possible that the growth restriction seen in both chapters could extrapolate to preweaning and even birthweight as BALB/c mice and Wistar rats showed birth weight reductions after *in utero* exposures, even down to 200 mGy[35,36]. Typically, this lower dose recovers, and it is only the high doses (>1000 mGy) that persist long term.

From the outcomes measured, growth was permanently affected by *in utero* ionizing radiation in both sexes and both strains. The reduction in growth following 1000 mGy in

C57BL/6 mice was found with only 100 mGy in BALB/c mice. Others have observed growth restrictions at 1000 – 1500 mGy exposures, but this is the first study in BALB/c to observe persistent growth restriction in both sexes at 100 mGy[20,32,36–39]. The significant difference between strains may be due to the dissimilarity in radiosensitivity levels. C57BL/6 mice are more radioresistant than BALB/c mice, with adult $LD_{50/30}$ of 8.5 and 4.5, respectively[40–43]. Grahn *et al* reported that the $LD_{50/30}$ of ionizing radiation increases with age, so when inferring retrospectively, *in utero* or postnatal exposures should require less dose than adults[44]. However, an earlier study identified a nonlinear age relation to dose in C57Bl/6 mice, in which postnatal day 1 mortality was similar to postnatal day 60[45]. It is unclear if this phenomenon is consistent with other strains, but the actual $LD_{50/30}$ of foetal exposures are still unknown and should be explored in the future.

The rationale behind the difference in adult radiosensitivity is unknown but a few interesting observations regarding genomic instability have been postulated in trying to answer this question. BALB/c mice, or crossbreds with maternal BALB/c background, had less mitochondrial DNA damage and more mitochondrial permeability after 1000 mGy irradiation[46]. The increased permeability triggers mitochondrial-induced apoptosis leading to a higher mortality and therefore a higher radiosensitivity. As mitochondria are passed down via the ovum, maternal inheritance is observed. Additionally after 1000 mGy, cytogenetic aberrations were observed only in BALB/c mice, not C57BL/6 mice[47,48]. BALB/c mice have a detrimental yet normal genetic variation in DNA protein kinases that repair DNA double-strand breaks, whereas C57BL/6 mice have normal functioning kinases[49]. A genomic analysis of crossbreeds identified that numerous positive DNA repair genes are from paternal transfer and not maternal transfer, indicating the importance of

paternal inheritance[50]. Thus, radiosensitivity is an element of both maternal and paternal susceptibility in terms of genomic instability, however, further research is required to fully understand how different radiosensitivities occur.

Due to the strain variation, an interesting question arises: would the respiratory results in Chapter 5 be similar if C57Bl/6 mice were used? Comparison between lung injury models can be difficult due to the LPS dose and delivery technique, BAL technique, and method of outcome quantification between individual studies. Keeping the radiosensitivities aside, there are some slight differences between these strains that need to be highlighted to answer this question.

In normal healthy adulthood, these strains are fairly similar in body weight, so any calculations made during respiratory measurement should be consistant[51]. Respiratory anatomy is very similar in structure and lung volume but 3D visualisation shows bulging of main airways in C57BL/6 mice compared to BALB/c[51]. After intratracheal instillation of LPS, C57BL/6 and BALB/c mice have similar physiological responses[52]. They both have pulmonary oedema, alveolar-capillary barrier breakdown, and lung hyperinflation[52,53]. In terms of immunological responses, they both have cellular infiltrate that is dominated by neutrophils but there are some slight differences in BAL cytokine profile[52,54]. BALB/c mice appear hyperresponsive to LPS 4 h post instillation, with increased BAL concentrations of pro-inflammatory mediators, but at 24 h, C57BL/6 and BALB/c were comparable[54]. Thus, when discounting any effects of radiation, it appears that if C57BL/6 mice were used in Chapter 5, it could be assumed that the physiological and immunological responses 24

hours after intratracheal LPS would be very similar to the BALB/c results, even though this is not necessarily the case for other lung injury models.

In a fibrosis model, intratracheal instillation of bleomycin causes severe fibrosis with reduced lung function in C57Bl/6 mice but BALB/c mice were less susceptible[55]. Matrix metalloproteinases, college deposition, TGF-β, and connective tissue growth factor are all increased in C57Bl/6 compared to BALB/c, with connective tissue growth factor being a key player in strain-variable fibrotic response[56–60]. In an ovalbumin asthma model, methacholine causes less airway constriction, measured via airway resistance and Penh, in C57Bl/6 mice compared to BALB/c[61]. Both had peribronchial and alveolar cellular infiltrate, but C57Bl/6 appeared to have a more eosinophilic, mixed T helper lymphocyte subset 1 ((Th_1) pro-inflammatory type) and subset 2 ((Th_2) allergic type), response whereas BALB/c had a mast cell dominant, Th_2 response, deemed responsible for the airway hyperresponsiveness. Similar airway responsiveness was observed for an LPS/methacholine model[53].

In terms of combining inflammation and radiation into strain differences in immunomodulation, there appear to be no studies assessing this effect. In fact, there are few that investigate immunomodulation *in vivo,* with only 2 from the same author, who used the C57BL/6 mouse strain[62,63]. The majority instead use cell lines or *ex vivo* BALB/c primary cells, and co-cultures typically using human cells[64–68]. As the mouse strains react similarly to LPS, as discussed above, the assumptions of *in vivo* strain differences would be based on the radiosensitivity differences between the animal strains themselves. Therefore, evidence suggests that if C57BL/6 mice were used in Chapter 5, no biologically

significant immunomodulatory effects would be seen, due to the higher radioresistance in C57Bl/6 mice.

The intention behind these animal models was to investigate the effect on the respiratory system from *in utero* exposures to diagnostic levels of ionizing radiation. From *in utero* exposure to ionizing radiation, it was observed that:

- There was no effect on the normal and challenged respiratory systems later in life, nor the cardiovascular system over the 8 weeks of measurement.
- There were significant reductions in body weight of both strains, at doses > 100 mGy.

These results support the idea that a single, whole-body, exposure of ionizing radiation, up to 1000 mGy during pregnancy, in two strains of mice, does not significantly affect the cardiovascular and respiratory system during adolescence, and at doses received from diagnostic radiation, does not affect animal growth. However, the growth outcomes between 10 and 100 mGy should be explored to support this statement.

6.3 TRANSLATING ANIMAL FINDINGS TO HUMANS

Extrapolating animal studies to human exposures is imperfect, and for therapeutic studies can be disheartening when effective treatments appear promising *in vivo* but fail in clinical trials. It is approximated that 30-40% of high citing animal research translates to clinical trials and only about 4% passes to phase 2 testing[69,70]. Several factors contribute to this lack of translation including funding, ethical consideration, technical limitations, statistical

power, and methodological inconsistencies, either between studies or due to complex comorbidities, external stimuli and genetic diversity apparent in human life[71–74]. But some lack of translation also comes from the obvious limitation that animals are not humans. Although there are many similarities, there are significant differences in many mouse systems that ultimately inhibit translation of results to humans. For example, the immune system differs in normal cellular proportions and stimulated transcriptomics, and the respiratory system differs in structure and physiology[75–77]. However, there are several obvious positives to animal research, such as ease of environmental or genetic manipulation, ability to screen new substances or interventions, exploratory insight into the pathophysiology of disease, and the ability to model the whole organism rather than at a cellular level, but care in interpretation and translation should be taken.

The results from BALB/c mice (Chapter 5) most resemble what could be predicted upon human exposures due to their radiosensitivity similarities[44,78]. A main outcome from this chapter was that ionizing radiation during late gestation caused persistently reduced weights of offspring, but only at 100 and 1000 mGy. When recalling data from Chapter 2 or 3, and from the literature, no single diagnostic procedure reached 100 mGy, nor do pregnant patients come close to receiving this dose cumulatively[79]. Therefore, pregnant patients receiving diagnostic radiation are unlikely to incur intrauterine growth restriction or long-term weight reduction of their foetus. However, testing of doses between 10 and 100 mGy would need to occur to confirm this statement, as there are some patients who receive > 10 mGy. Nevertheless, reductions in infant weight due to exposures equalling 100 mGy, a weight reduction of 8-10%, would remain well within normal birthweight variation of the Australian population[80]. Therefore, even though the radiation exposure

has stunted the growth potential of the offspring, the weight reduction would not be observable through the population's variation from environmental and genetic factors. Importantly, this weight reduction does not meet the requirements for low birth weight and therefore would not have increased risks associated with low birth weight. As the patient's exposure rises over 100 mGy and advances closer to 1000 mGy, it could be assumed that the weight reduction would follow the trend of the mouse models, eventually leading to low birth weight infants, however this assumption would need to be confirmed by mapping the dose-response between 100 and 1000 mGy. If this assumption was supported, the clinical impact of why the patient required so many or such detailed procedures in order to receive doses that high would need to be acknowledged[80].

Although no changes were observed in respiratory and cardiovascular outcomes measured, changes in other animal model's organ systems have been observed as dose increases up to the 1000 mGy exposure, suggesting similar changes could occur in humans. When observing studies that used single whole-body exposures at similar gestational ages to Chapters 4 and 5, *in utero* exposures up to 500 mGy can cause slight changes in mouse brain physiology and cognitive behaviour, decreasing anxiety and increasing exploration up to 1-year post irradiation[81–84]. Female rats have growth reduction and males appear to have slight reflex delays and slowed appearance of some physiological markers[35,85]. At exposures up to 1000 mGy, changes in brain physiology and cognitive behaviour are more apparent, with mice less nervous and more adventurous but with some reduced locomotion and learning ability up to 1.5 years post irradiation[81–83,86,87]. The growth of the rat continues to be reduced and there is further reduction in reflex and physiological markers[85,88]. At exposures above 1000 mGy, there is reduced pup

viability and persistent growth reduction in both males and females of mice and rats[20,32,36–39,89]. Female rats are less fertile, and there are severe deficits in mice and rat brain physiology with less neurogenesis[32,38,81,90–92]. Mice have severely reduced learning ability, memory and spatial awareness and have changes to their circadian rhythm[82,83,86,93]. This shows that *in utero* exposures of rodent models can have negative effects on other organ systems in the offspring in which severity increases with dose.

At equivalent gestational exposures to ionizing radiation, humans can have similar outcomes. Much of the evidence of radiation harm to humans comes from the lifespan study (LSS) of the atomic bombing in WWII. Growth reduction, neurobiological defects, behavioural changes and mental retardation have been reported from humans exposed *in utero* to the from bomb, with severity based on gestation at exposure and dose[94–97]. Similar to animal experiments, there appears to be a threshold dose of 300 – 500 mGy for these outcomes. When considering all of this evidence, many governing bodies agree that exposures below 100 mGy pose no significant negative outcomes for humans, nor does it merit foetal termination[98–102]. The animal studies in this thesis support this statement as, at diagnostic levels of ionizing radiation received during late gestation, there were no biologically significant effects in the outcomes assessed.

Additional to the deterministic effects, the universal question that arises from radiation exposure is the presumed stochastic effects – e.g. the risk of cancer. As introduced in Chapter 1, this topic is highly contentious with clinical studies showing both increased risk and no association[103–109]. Many that state a risk of cancer from *in utero* exposure to ionizing radiation use case-control study designs, which do not have the power to

delineate cause and effect, only an association, whereas those that use cohort study designs, which can delineate cause and effect, find no relationship[110]. However, if an assumption is made that there is a significant risk of cancer, this risk from *in utero* exposures is less than from childhood exposures[103]. This finding does not fit with the typical understanding that older individuals are more resistant to radiation-induced cancer than younger individuals[111]. The finding is supporting by foetal exposures, for both mice and humans, having less genetic instability than child or adult exposures[112,113]. Evidence suggests that aberrant cells, induced by radiation, are eliminated during postnatal growth resulting in lower susceptibility to cancer, as inferred from exposed neonatal mice having cytogenetic aberrations persistent only up to 48 hours[113].

When inferring any of these translational results to what is expected from exposures to diagnostic radiations, there are a few limitations that need consideration. Comparison between mouse gestation and human gestation is often difficult due to the difference in organ development between species. Gestational day (GD) 15 in mice can relate to 6 – 12 weeks in humans depending on the organ system under comparison, for example, the cardiovascular and respiratory system equate to approximately 10 – 12 weeks human gestation[27,28,114,115].

For direct human-human comparison, the subjects in the Lifespan study are inherently different from those of patients who receive diagnostic radiation as they have very different covariates. The blast from the atomic bomb resulted in poverty and famine in the surrounding region, adding to the already worn-torn state of the communities. Increased anxiety, post-traumatic stress and other mental illnesses arose not only from

their experiences of loss of family and community but also from the prejudice and discrimination in employment and marriage[116,117]. People who receive diagnostic radiation do so based on a clinical justification highlighting an underlying issue or disease requiring attention and thus healthy people are not exposed to medical radiation. It is important to acknowledge these limitations in human-human comparisons, especially when translating results and inferring outcomes from one to another.

The idea that healthy people are not exposed to medical radiation is the underlying reasoning behind Totter and MacPherson's rebuttal to the question: does CT cause cancer[118]? As highlighted in Chapter 1, they argue that comparison between patients that received CT scans and those that don't, is itself, a limiting factor because they inherently resemble two separate subpopulations. One group has a clinical justification that requires a CT scan whereas the other does not. Thus, is it this selection factor of the clinical justification to prescribe diagnostic radiation, and not the radiation itself, that is responsible for any associated risks? Does the disease cause the CT or does the CT cause the disease? This question is impossible to answer without irradiating healthy people and following them long term. All these limitations make it difficult to interpret and translate animal and epidemiological studies and so it should be conducted with a level of caution.

6.4 Overall conclusions

The main objective of this thesis was to examine the long-term effects on the respiratory system of low doses of ionizing radiation relevant to medical exposures. However, it was first necessary to understand what levels of ionizing radiation hospitalised patients

receive. At the time of beginning this thesis, there was little current understanding of *in utero* radiation exposure and no understanding in terms of the respiratory system. Through the approaches discussed in chapter 2 – 5, this thesis has expanded this knowledge by

1. Demonstrating that most patients admitted to the ICU of a tertiary hospital cumulatively received less than 1 mSv.
2. Demonstrating that most pregnant patients admitted to a tertiary hospital cumulatively received less than 0.2 mSv, with a median cumulative foetal dose of 0.01 mSv.
3. Demonstrating that *in utero* exposure to diagnostic levels of ionizing radiation did not cause any negative long-term outcomes on growth, cardiovascular system or respiratory system in a healthy animal model. However, at 1000 mGy exposures, persistent growth reduction was observed.
4. Demonstrating that *in utero* exposure to diagnostic levels of ionizing radiation does not significantly alter the pulmonary immune response within an acute lung injury model. However, at 100 and 1000 mGy exposures, persistent growth reduction was observed.

The clinical and animal work which contributed to this thesis, and the interpretation of the translation between them, strengthens the hypothesis that current doses received from diagnostic radiation during pregnancy are unlikely to have any long-term detrimental effects on the offspring.

6.5 FUTURE DIRECTIONS

Future studies carrying on from the clinical work should try to answer the following questions: Are the cumulative exposures received at FMC replicable at other hospitals around the world? Are clinicians cautious about using diagnostic radiation? Will improving understanding of dose and risk improve patient outcomes? Using similar eligibility criteria, simple comparisons could be made to gain an Australia-wide and worldwide understanding of patient exposures. Although single patient exposures depend on several factors, gaining a uniform understanding of cumulative exposures for similar populations around the world, could also highlight which countries/hospitals freely prescribe diagnostic radiation and which are more conservative. If there are differences observed, it would be helpful to know if clinicians understanding of radiation could relate to the cumulative levels received at their hospitals. As discussed in Chapter 1, the past 2 decades of assessments have highlighted a lack of professional understanding of ionizing radiation, and even though the scoring slightly improves with time, the level of misunderstanding of dose and risk may inversely relate to the clinician's use of diagnostic radiation[1–6,112–116]. This may also help to answer the second question: are clinicians cautious about using ionizing radiation? This question could be answered simply by qualitative questionnaires, but this questionnaire could also be used to explore two related questions: If clinicians are cautious about using diagnostic radiation, what techniques or methods have they replaced this with to aid in diagnosis? And, would providing additional education change the clinician's view of ionizing radiation and their attitude towards its use?

The use of other techniques, such as ultrasound or magnetic resonance imaging, to replace ionizing radiation procedures during diagnosis has occurred with some success, but only in certain diseases[124–126]. Although they reduce ionizing radiation exposure, some procedures are limited in their ability to give a conclusive diagnosis, without requiring additional scans or methods, when the primary suspicion was negative. It would be important to delineate if clinicians are opting for alternative methods due to the perceived risks associated with ionizing radiation or due to other personal reasons.

Finally, it would be interesting to observe if providing additional education to the clinicians about ionizing radiation would change their opinion and usage rates. If they accurately understood the dose and the risks involved with ionizing radiation, would the clinicians still choose alternative methods of diagnosis or would they use ionizing radiation? Assessing usage rates of procedures that use ionizing radiation before and after education would provide this answer, but more importantly, is there a link between improved education and improved patient outcomes? This could be possible by addressing disease positive findings, length of stay or other patient outcomes associated with improved diagnosis, in the previous study design and may also, inadvertently, answer the question does ionizing radiation avoidance detrimentally affect the patient. Consideration should be applied in that each clinician has likely received different training or experiences that all influence their clinical judgement but educating clinicians on the current understanding of radiation dose and risk could reduce the current 'scared' stigma that may inevitably save a life.

Future studies relating to the animal models should focus on filling in the gaps of this thesis in dose, timeframe and outcomes, to adequately assess at what point growth is affected and whether respiratory and cardiovascular outcomes are altered by *in utero* radiation. BALB/c mice should be continued in the investigation of further questions as they relate better to humans in radiosensitivity.

For the growth outcome, the dose curve between 10 – 1000 mGy should be further explored to assess at what point ionizing radiation is sufficient to cause statistically significant growth reduction, and to assess if this outcome is linear, quadratic, biphasic, or suits another model. Collection of preweaning weight could delineate if any catch-up effect was present but not observed in the mice exposed to 10 mGy. Additionally, extending the study would confirm that the dose reduction observed by 100 and 1000 mGy remain permanent.

The timeframe should also be altered for the respiratory and cardiovascular outcomes of this thesis. For respiratory outcomes, the animal study should be shortened to observe the effects on the postnatal and childhood timeframes. It would be interesting to report the effects of ionizing radiation on the development of respiratory physiology by cataloguing different developmental stages post-irradiation. As the lungs are still developing post-birth, changes to physiology might have been resolved before this thesis examined the lungs[127–129]. Shortening the time between irradiation and outcome may also allow the immunomodulatory effects from ionizing radiation to be observable and therefore relate clinically to infant respiratory infections post *in utero* exposures.

Conversely, the length of the study should be extended for the cardiovascular outcomes as changes, especially hypertension, are more common in late adulthood timeframe[130].

Finally, this thesis unfortunately missed investigating a few key outcomes that may help improve the overall understanding of the effect of ionizing radiation. Assessing body composition could help determine if the reduced growth rate observed in the animal studies was a reduction of body fat, muscle or was proportional for both. Investigating metabolic activity, through tissue analysis, could help understand if the reduced weight is an effect of altered metabolism. Additionally, exploring if low birth weight animals have any other outcomes associated with low birth weight infants. Adding functional changes in the respiratory outcomes, such as incorporation of respiratory mechanics, breath rate or blood oxygenation readings, would provide a basic understanding of radiation effects on the functionality of the respiratory system. Lastly, although no functional changes to the cardiovascular system were observed from the outcomes measured, confirmation could be obtained via more sensitive echocardiographic measurements. Expanding outcomes to include hypertrophy, or fibrotic responses by Masson's trichrome may assist in assessing long-term risk of cardiovascular disease. There are many directions in how these studies can be continued to further contribute to the area of prenatal radiation exposure. This has the ability to further clinical understanding of diagnostic radiation and if used appropriately, has the potential to save a life.

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CHAPTER 7

COTUTELLE PROGRAM EXPERIENCE

A Cotutelle De Theses is a doctoral degree program where a PhD candidate undertakes their studies jointly between two universities. The candidate is jointly supervised from both institutions. The candidate must complete the requirements for both universities, including coursework, yearly milestones and examination processes, and then is conferred a doctorate from both institutions.

7.1 UNIVERSITY INFORMATION

Home (primary) Institution	Host (secondary) Institution
College of Medicine and Public Health (formally Department of Critical Care Medicine, faculty of MNHS)	Department of Biology, Faculty of Science
Flinders University, Sturt Rd, Bedford Park, SA, 5048, Australia	McMaster University 1280 Main St W, Hamilton, ON, L8S 4L8, Canada

Co-Supervisors

Associate Professor Dani-Louise Dixon BSc, PhD.	Professor Joanna Wilson, BSc, MSc, PhD

Supervisory committee

Associate Professor Antony Hooker BBiotech, PhD	Professor Douglas Boreham, BSc, PhD
Associate Professor Shailesh Bihari MD, PhD	Professor Grant McClelland, BSc, PhD

Study Term

Flinders University: Feb 2016 – May 2017

McMaster University: June 2017 – May 2018

Flinders University: June 2018 – July 2019

7.2 COTUTELLE AGREEMENT

To begin this process, a collaboration was established with Prof. Joanna Wilson and Prof. Douglas Boreham, and McMaster University. A Memorandum of Understanding was negotiated between Flinders University and McMaster University. It was approximately 8 months from first contact with Flinders University Cotutelle liaison until the signing of the final Memorandum of Understanding by both parties, thus my experience in the application process was quite lengthy. After the agreement, enrollment into the McMaster Biology Doctoral Program was completed and the Cotutelle De Thesis PhD was established.

Some main points from the Memorandum of Understanding:

- The schedule of research work will be done in alternating periods at Flinders University and McMaster University with a total of one (1) calendar year completed at the host institute (McMaster University).
- The candidate shall be concurrently enrolled at both institutions for the period of their candidature but shall be exempt from tuition fees at the host institute.
- Any intellectual property rights, data, and information resulting from research activities conducted under this agreement on academic cooperation shall be

jointly owned by the two parties and both parties shall be allowed to use such property.

- The candidate shall be required to fulfil all academic requirements of each institution including required coursework, department graduate workshops, comprehensive examinations, yearly milestones, thesis defence and any other requirements. The thesis must include a separate chapter describing the Cotutelle experience and detail the value added to the degree.

7.3 COTUTELLE EXPERIENCE

7.3.1 Visa and travel

After enrolment was confirmed at both institutions, the next process was gaining the student visa and required documentation to study in Canada. This process was rather straightforward with generous help from the international departments of both institutions. Flinders University also generously supports its Cotutelle students with a Cotutelle travel grant which aided in the travel to Canada.

7.3.2 Learning from a different lab

The Critical Care Lab has been a great source of support, development and emotional release throughout this doctoral journey. This relatively small, close-knit group have pushed me in many professional and personal achievements. The first year with this group changed my communication skills immensely. My PhD experience began with experiments and learning the necessary practical skills however my communication skills developed passively. The lack of awareness around my communication skills was quickly highlighted by this group which led to the development and refinement of intentionality,

clarity and expression. In addition, the practical skills learned from this lab enabled me to become the 'expert' of many techniques during my time abroad; which is a powerful but sometimes frustrating position to be in. I gained clinical experience through my audit with patient data and outcomes being the principal results for my 1st and 2nd data chapters. I also learned extensive animal handling and experimental skills through the lab's rodent models which was brought to McMaster University for my 3rd and 4th data chapters. In addition to the physical skills learned, the workup prior to the methods, including the ethics and governance applications, was a smooth process as this lab has dealt with both clinical and animal studies in the past. Without the lab's expertise in both the clinical and animal model studies, I would have had a much harder time and longer process getting these data chapters to completion.

The Wilson lab was very accommodating and inclusive throughout the year in Canada. However, this lab is very different to the Critical Care lab at Flinders University, which allowed for professional and personal growth in many areas. When I entered, the Wilson lab encompassed 21 people from 2nd year undergrads to graduate researchers (Masters and PhD), Research Assistants, Postdoctoral researchers and Joanna herself. This size created a larger scope of academics/researchers than I had been exposed to previously therefore allowing development of communication and group engagement skills. Switching interactions between people from different levels of scientific backgrounds changed: the structure of support, such as receiving support from post docs and giving support to undergrads; and the terminology of communication, including having intellectual debates with experts and giving explanations to lay researchers. In addition, many strong friendships were created with this group that still exist to this date.

Overall, this PhD would not have been completed if not from the valuable lessons learned at both universities, both: practical and passive; and professional and personal.

7.3.3 Implications on scientific learning

As mentioned before, the first year, under Assoc. Prof. Dani-Louise Dixon, taught me everything I needed to know to begin my 1st and 2nd data chapter. The clinical attachment of the lab meant that the research completed in the lab could be seen by clinicians and aid in the translational aspect of the research. Having clinician Assoc. Prof. Shailesh Bihari easily assessable allowed for a clearer understanding of what the clinical data meant and thus a better understanding of the results. Assoc. Prof. Antony (Tony) Hooker provided the radiation expertise which implicated all data chapters in this thesis. Discussions with Tony provided clarity on dosimetry in the clinical work and radiobiological responses in the animal work.

A main reason for collaborating with Assoc. Prof. Joanna Wilson and Prof. Douglas Boreham was the animal model they have maintained. This animal model would have taken a significant amount of time to prepare at Flinders University, thus saving research time and costs. Along with this model, a significant variety of skills were present surrounding animal handling/techniques and this model, which have now been learnt. However, the experimental outcomes measurable by the techniques available in the Wilson Lab lacked some desirable interests. Unfortunately, there was no ability to capture functional changes in the lungs of the animals, including respiratory function and gas exchange analysis, which is something obtainable back in the Critical Care Lab. Overall I am content with the science produced in this PhD and would not have been able to

produce these experiments and advance my scientific knowledge without participating in this exchange.

7.3.4 Co-Supervision

It was valuable to see how different supervisors manage their students. I was fortunate to have two extremely helpful supervisors that have different managerial styles. From the co-supervision, I was able to experience times of continuous support vs times of independence, times when I was communicating to the expert vs communicating as the expert, and times of variation in perspectives on ideas, that particularly helped in interpretation of results. I believe that I received the appropriate level of support and guidance from both supervisors in my PhD at the times where I needed them the most. Dani began this PhD experience as the expert who aided in the development of my project and closely observed my progression. Joanna then gave me the space to become the expert and allowed self-development and growth into an independent researcher. Both aided in the final year of this PhD, defining and improving articulation of the final thesis.

7.3.5 Final statement

The Cotutelle program was an interesting and particularly rewarding experience of professional development and personal refinement. In addition to the obvious pros of living abroad in a new community and the experiences attached to a new routine, this thesis and my professional and personal development is solely a product of the scientific, educational format and supervisory differences created through this exchange. I would urge any PhD student who is looking to forward their growth and a variety of skills sets to investigate the Cotutelle program within their university.

THE END