EFFECT OF RADIATION *IN UTERO* ON MOUSE CARDIOVASCULAR FUNCTION

EFFECTS OF LOW-DOSE IONIZING RADIATION *IN UTERO* ON POSTNATAL GROWTH AND CARDIOVASCULAR PHYSIOLOGY IN BALB/cJ MICE

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Lay Abstract

Diagnostic radiation is typically avoided during pregnancy, as the effect of radiation exposure on the fetus is uncertain. I wanted to determine if exposure to radiation would cause stress during pregnancy that would cause prolonged effects in offspring following birth. To test this, I used a mouse model to study the effects of various doses of radiation exposure during pregnancy and if it may affect the offspring growth and cardiovascular function. There was no effect of *in utero* radiation exposure on blood pressure, heart rate, endothelial function, and mitochondrial capacity. High doses of radiation reduced body weight for male and female offspring and decreased inflammatory cytokine expression (TNF-α) in male offspring. This study helps to provide knowledge on the possible effects of radiation exposure during pregnancy, as well as broadens the knowledge on the range of stressors capable of affecting offspring during pregnancy.

Abstract

Diagnostic radiation is typically avoided during pregnancy, as the effect of low-dose radiation exposure on the fetus is uncertain. The objective of this study was to determine if ionizing radiation exposure during late fetal development would cause an adverse intrauterine environment, and lead to growth restriction of offspring and a hypertensive phenotype later in life. To study this, pregnant BALB/cJ mice were exposed to ionizing radiation at 5, 10, 50, 100, 300 or 1000 mGy on gestational day 15. Offspring were weighed weekly from the age of weaning until a mature age of 16 weeks. Cardiovascular effects were assessed every other week via heart rate and blood pressure measurements using tail plethysmography. The expression of genetic markers for endothelial dysfunction, inflammation, mitochondrial capacity, and regulation of the oxidative stress response in the aorta and heart for the 1000 mGy was assessed from tissue collected at 17 weeks of age. We observed no effects of low to mid dose (5-300 mGy) radiation on offspring growth and blood pressure. Growth restriction was observed in male and female offspring exposed to high-dose radiation (1000 mGy). In the heart, there was no observed effect on mitochondrial capacity and oxidative stress response genes. In the aorta, we observed decreased TNF-α expression in male offspring, which may be linked to the growth restriction but was not considered a sign of cardiovascular dysfunction. There were no observed effects of exposure to 1000 mGy on cardiovascular function. This study provides knowledge on the possible effects of radiation on *in utero* development, which broadens the knowledge on the range of stressors capable of affecting offspring growth and development.

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List of Abbreviations and Symbols:

ATP: Adenosine triphosphate

BP: Blood pressure

bpm: Beats per minute

CPT1A: Carnitine palmitoyltransferase 1A

DBP: Diastolic blood pressure

HR: Heart rate

ICAM: Intracellular adhesion molecule

MAP: Mean arterial pressure

MCMC: Markov Chain Monte Carlo

mGy: milligray

Mt-Co1: Cytochrome c oxidase subunit 1

Mt-Cytb: Mitochondrially encoded cytochrome c oxidase

NADPH: Nicotinamide adenosine dinucleotide phosphate

NDUFB6: NADH dehydrogenase (ubiquinone) 1 beta subcomplex subunit 6

NO: Nitric oxide

NOS: Nitric oxide synthase

NRF: Nuclear respiratory factors

OXPHOS: Oxidative phosphorylation

PPARGC1A: Peroxisome proliferative activated receptor, gamma, coactivator 1 alpha

ROS: Reactive oxygen species

SBP: Systolic blood pressure

SEM: Standard error of the mean

TNF: Tumour necrosis factor

UCP2: Uncoupling protein 2

VCAM: Vascular adhesion molecule

Declaration of Academic Achievement

This thesis is arranged in a sandwich format approved by McMaster university. This thesis consists of three chapters. Chapter 1 describes background information on fetal programming, ionizing radiation exposure and the cardiovascular system. Chapter 2 determines fetal programming effects of low- to mid- dose ionizing radiation in BALB/cJ mice with a focus on cardiovascular function and growth. Chapter 3 is a general discussion of the effect of ionizing radiation during *in utero* development on cardiovascular function and growth. This also includes a general discussion on the effects of ionizing radiation and fetal programming.

Chapter 1: General Introduction

Chapter 1 was prepared by Jessica Preston and edited by Joanna Wilson.

Chapter 2: Effects of low-dose ionizing radiation *in utero* on postnatal growth, and cardiovascular physiology in BALB/cJ mice

Experimental planning, data collection, data analysis and writing of the chapter was performed by Jessica Preston under the supervision of Joanna Wilson. Doug Boreham and Lisa Stoa were also involved in the experimental planning. Lisa Stoa and Shayen Sreetharan assisted in experimental work including irradiations and collecting blood pressure and growth data. T. C. Tai and Sujeenthar Tharmalingam were involved in some of the experimental planning of the RT-qPCR work; the experimental work was performed with the assistance of Sujeenthar Tharmalingam in T.C. Tai's lab. Ben Bolker assisted in the statistical analysis of the blood pressure and weight datasets. Assistance with the statistical analysis of the RT-qPCR data was provided by Andrea Murillo. Funding for this research was obtained by Drs. TC Tai, Doug Boreham and Joanna Wilson through an NSERC Collaborative Research and Development Grant with Bruce Power as the partner organization.

Chapter 3: General Discussion

Chapter 3 was prepared by Jessica Preston and edited by Joanna Wilson.

Chapter 1: General Introduction

1.1 Impact of Radiation Exposure and Susceptibility of Disease

There is a consensus that exposure to high-dose radiation can lead to negative health effects, however there is uncertainty around the risk of low-dose ionizing radiation (Brenner, 2003). In animal models, low-dose ionizing radiation is defined as an absorbed radiation dose of less than 100 milligray (mGy), where Gy is a measurement of the deposited energy per unit mass (Tang et al., 2017). Exposure can be through human made sources such as nuclear medicine, industry, and research, or unavoidable natural sources such as natural background radiation present in the environment one lives in. Natural sources include cosmic and terrestrial radiation as well as naturally occurring radionuclides in the environment (NCRP 2009; Morgan and Bair, 2013).

There are two main theories that are used to estimate the risk of radiation exposure, which are contradicting for the low-dose range. The first theory is the linear no threshold model, which describes a risk at all doses and no threshold or dose at which the probability of an effect or risk of exposure is zero (Hall and Giaccia, 2012). Whereas radiation hormesis describes how the effects of high and low doses of radiation may be different; high doses have a toxic effect on an individual, whereas low doses may be beneficial and may have a protective effect (Hall and Giaccia, 2012). It is currently unknown how low-dose radiation effects the body and which theory is more accurate. This is in part because majority of studies, including those in humans, are in the high-dose range.

The uncertainty of low-dose radiation exposure effects stems from the need to understand how different biological factors affect radiation risk. Risk is highly dependent on the type of animal, its susceptibility to exposure, age, sex, total dose exposure and the

type of radiation exposure (ie. photon, positron, proton; Tang et al., 2017). Involvement of these factors can lead to similar dose exposures showing variable responses. Epidemiological studies have shown low to mid-dose ionizing radiation exposure (≤500 mGy) may increase an individual's risk of cancer (Brenner et al., 2013), cardiovascular disease (Baselet et al., 2016) and cataracts (Ainsbury et al., 2009; Tang et al., 2017). However, in mouse disease models, research has shown low-dose-rate radiation exposure over a lifetime may have positive effects including tumour suppression (Sakai et al., 2006) and increased lifespan (Ina and Sakai, 2004; Ina and Sakai, 2005; Tang et al., 2017).

The current study is focused on the risk of radiation exposure on cardiovascular disease development. Current radiation research has been conducted through human studies on atomic bomb survivors, occupational and medical exposures, along with animal studies (Baselet et al., 2016). At high radiation exposures, there is a consensus of increased cardiovascular disease risk, however results for exposures under 500 mGy have been inconclusive (Caselet et al., 2016). This is complicated by the fact that the mechanisms leading to cardiovascular disease from radiation exposure are unclear (Bakashi et al., 2016; Baselet et al., 2016). One potential mechanism is that radiation can cause damage to endothelial cells, leading to a subsequent induction of an inflammatory response (Shultz-Hector and Trott, 2007). This inflammatory response can then promote the development of cardiovascular disease such as atherosclerosis (Shultz-Hector and Trott, 2007; Baselet et al., 2016). Several systems may play a role in this, including endothelial dysfunction, inflammation, oxidative stress, alterations in coagulation, DNA damage, senescence and cell death (Baselet et al., 2016).

Endothelial dysfunction is typically implicated in cardiovascular disease progression, with endothelial cells showing activation and dysfunction post ionizing radiation (Baselet et al., 2019). The endothelium is crucial as it maintains vascular homeostasis within the body and disruptions to its function can be detrimental to vascular health (Baselet et al., 2016). In response to ionizing radiation, pro-inflammatory signalling is enhanced and there is dysregulation of adhesion molecules. Radiation has been shown to upregulate the intracellular adhesion molecule (ICAM)-1 and vascular adhesion molecule (VCAM)-1 following irradiation of endothelial cells in a time and dose dependant manner (Sievert et al., 2015; Hallahan et al., 1998; Baselet at al., 2016). Increased expression (i.e. VCAM-1, ICAM-1) has been observed up to 20 weeks post irradiation (Sievert et al., 2015; Baselet at al., 2016). Even exposure to doses as low as 50 mGy have been observed to lead to differences in expression of these markers (VCAM, ICAM; Mathias et al., 2015). In a mouse model of atherosclerosis ($ApoE^{-/-}$ mice), tumour necrosis factor (TNF)-α has been differentially expressed in the plasma for up to 6 months post irradiation (Mathias et al., 2015). This study has shown adverse effects of low-dose radiation (Mathias et al., 2015); however, other studies have observed an effect only at high doses (Sievert et al., 2015)

Due to uncertainty of the effect of low-dose radiation exposure, diagnostic radiation is typically avoided during pregnancy (Lowe, 2004). Currently there is little knowledge on the effect of low-dose radiation exposure to mothers during fetal development and how it could be a potential stressor and lead to adverse effects on offspring later in life. The effect of radiation exposure on *in utero* development depends on the developmental stage during the exposure and the dose of radiation. As reviewed by

Sreetharan et al. (2017), *in utero* radiation exposure has been associated with postnatal behavioural changes, learning and memory impairments as well as physiological effects including low birth weight and weight reduction of offspring. The threshold for behavioural and learning effects were observed at doses greater than 300 mGy; physiological impairments were at a mid- to high- dose of 400 mGy (Sreetharan et al., 2017). Effects were not clearly observed within the low-dose range, i.e. ≤100 mGy (Sreetharan et al., 2017). There have been very few studies which have looked at how *in utero* radiation exposure may cause changes in heart physiology.

1.2 Fetal Programming and the Cardiovascular System

During early development (i.e. preconceptual to early postnatal development) environment plays a crucial role in the health of an individual. An adverse environment during this time can predispose offspring to disease in adulthood (Morton et al., 2016). Barker and his colleagues were the first to correlate undernutrition during fetal development with a low birth weight, later leading to an increased susceptibility of coronary heart disease during adulthood (Barker et al., 1989). This concept was termed fetal programming (Barker, 1998; Godfrey and Barker, 2001).

Metabolic and physiological function during *in utero* development is relatively plastic in relation to later stages of life. This means that an adverse environment during early development may result in permanent changes to metabolic capacity and physiological responses in adulthood (Jackson, 2000; Lau and Rogers, 2004). This is a mechanism which allows the fetus to adapt to intrauterine environmental conditions, however it may not prepare the offspring for survival in later life (Kwon and Kim, 2017).

Environmental exposures (i.e. toxin exposure) and maternal lifestyle (i.e. nutritional deficits, hypoxia, maternal obesity) can all affect adult disease development. Of interest is how these environmental stressors *in utero* can impact the development of cardiovascular disease in later life (Morton et al., 2016).

The effect of fetal programming is typically not observed at the point of insult, but instead leads to changes at the epigenetic level, causing alterations in gene expression leading to disease phenotypes later in life (Meyer and Zhang, 2007). Mechanisms of fetal programming may include alterations in prenatal glucocorticoids, sex steroids, the renin-angiotensin-aldosterone system, the sympathetic nervous system, and oxidative stress (Alexander et al., 2015).

One of the major indicators of fetal programming is a low birth weight. During postnatal development, offspring may compensate and go through a process called catch-up growth where they have an increased growth rate and grow to a normal size (Eriksson et al., 1999). Low birth weight, along with catch-up growth has been associated with long-term deleterious consequences, leading to an increased susceptibility to cardiovascular diseases (Eriksson et al., 1999; Meyer and Zhang, 2007). Development of hypertension post fetal programming is one of the most commonly studied cardiovascular disease outcomes (Morton et al., 2016).

Growth restriction at birth is commonly observed through programming, however the direct mechanism leading to an increased susceptibility of cardiovascular disease is unclear (Meyer and Zhang, 2007). In response to growth restriction, children have shown cardiovascular changes including cardiac morphology, subclinical cardiac dysfunction and

endothelial dysfunction (Miranda et al., 2017). Whereas, adults show hypertension, impaired glucose tolerance, insulin resistance, obesity and coronary heart disease (Miranda et al., 2017). Fetal growth restriction has been shown to induce apoptosis, reduce nephron number, activate the renin-angiotensin system and cause ventricular hypertrophy leading to increased risk of higher arterial blood pressure in both adolescents and adults (Miranda et al., 2017).

1.3 Development of Cardiovascular Disease through Endothelial Dysfunction

The vascular system is one of the main systems involved in the development of cardiovascular diseases including hypertension**.** Early signs of progression include endothelial dysfunction (Rodriguez-Rodriguez et al., 2018; Meister et al., 2018). Endothelial dysfunction is characterized by an imbalance in the production of mediators important to vascular tone, platelet aggregation, coagulation, and fibrinolysis in the endothelium (Dinh et al., 2014). Vascular inflammation is often implicated, through the induction of vasoconstrictor agents, adhesion molecules, and growth factors (Savoia et al., 2011). This commonly includes the involvement of oxidative stress (Dinh et al., 2014; Teixeira et al., 2014). When there is an imbalance of these factors, the systemic vasculature becomes stiffer and less distensible due to alterations in the collagen and elastin balance during disease progression. This can lead to the development of hypertension (Morton et al., 2016).

One of the main mechanisms leading to alterations in the vasculature, is the alterations in the primary vasodilator pathways for nitric oxide (NO) production. NO is a primary

vasodilator responsible for smooth muscle relaxation. NO dysregulation plays a major role in the progression of endothelial dysfunction (Dinh et al., 2014). The main enzyme responsible in the production of NO is nitric oxide synthase (NOS). There are 3 isoforms including the endothelial (eNOS), neuronal (nNOS) and inducible (iNOS) NOS (Shulz, 2008; Morton et al., 2016). Differential eNOS expression and functionality have been observed in cardiovascular disease models of fetal programming. For example, animal models have shown differential eNOS expression in the aorta of rats exposed to nutritionally restricted diets including protein (Franco et al., 2002; Rodford et al., 2008, Torrens et al., 2012) and zinc restricted (Tomat et al., 2012) diets and diets with high salt (Piecha et al., 2012). Additionally, rabbits have shown differential eNOS in response to hypoxia (Chen et al., 2013; Morton et al., 2016).

When a pro-inflammatory state is induced during cardiovascular disease progression through tissue damage, eNOS regulation is affected (Teixerira et al., 2014; Dinh et al., 2014). For example, TNF-α is capable of destabilising eNOS mRNA, resulting in an attenuation of NO production (Yan et al., 2008). Inflammatory markers are involved in NO synthesis and degradation, which can affect NO availability in the vascular tissue (Dinh et al., 2014). Oxidative stress through an imbalance of reactive oxygen species (ROS) is also known to contribute to vascular disease (Yzydorcyk et al., 2017). The production of ROS is important in cardiovascular disease development as NO can interact with ROS, specifically superoxide, to form a highly reactive and toxic species called peroxynitrite, which is capable of uncoupling eNOS. Peroxynitrite formation may then lead to further NO level impairment as it can also inhibit eNOS activity. When eNOS is uncoupled, eNOS will

further produce superoxide instead of NO leading to increased oxidative stress (Vasquez-Vivar et al., 1998; Dinh et al., 2014). Oxidative stress in the vasculature can cause eNOS uncoupling which can affect NO synthesis and bioavailability, leading to impaired vascular relaxation (Dinh et al., 2014).

Alterations to the vasculature leading to cardiovascular disease can involve dysregulation of the vasodilator NO. When there are changes in eNOS functionality and abundance, often related to a pro-inflammatory state along with oxidative stress this can lead to the changes in NO production (Dinh et al., 2014; Yzydorczyk et al., 2017). NO plays a central role in vascular physiology and its regulation is important for vascular function. When eNOS is dysregulated, NO production can be attenuated affecting vasodilation function. This can cause a dysregulation of vascular tone leading to changes in blood pressure. Impaired endothelial function has been noted to result in cardiovascular disease development such as hypertension (Dinh et al., 2014; Liu and Huang, 2008).

1.4 *In Utero* Oxidative Stress, Radiation and Potential for Cardiovascular Risk

Reactive oxygen species (ROS) play an important role during organ formation in the embryo. ROS are involved in placentation and are signalling molecules involved in gene transcription; they are essential to normal embryonic and fetal development. Due to this, it is essential that production and elimination of ROS during development is kept in balance (Rodriguez-Rodriguez, 2018). Embryo development occurs in a low oxygen environment which causes the embryo to have a low antioxidant capacity and prevents the ability of regulating alterations to ROS levels (Thompson and Al-Hasan, 2012; Dennery et

al., 2010). If unregulated levels of ROS result in a pro-oxidative state, this can cause damage to macromolecules and thus cellular injury. A pro-oxidative state during development can compromise fetal development and size (Rodríguez-Rodríguez et al., 2018). With oxidative stress there is the potential for the induction of epigenetic modifications, especially during *in utero* development when the epigenome is more susceptible to dysregulation (Terry-Adkins et al., 2013; Rodríguez-Rodríguez et al., 2018). Changes in gene regulation through oxidative stress may lead to changes in heart function and disease development (Rodriguez-Rodriguez et al., 2018). Therefore, it is critical to study the effect of radiation causing a pro-oxidative state *in utero* and the resulting disease outcomes in adulthood.

Low birth weight and growth restriction are commonly observed from *in utero* radiation exposure at mid to high doses (Otake and Schull, 1998; Kimler and Norton, 1998; Minamisawa et al., 1990; Sreetharan et al., 2017; Sreetharan et al., 2019). However, it is currently unknown if radiation exposure during pregnancy is capable of increasing risk of cardiovascular diseases including hypertension. Nakashima et al. (2007) studied the effect of exposure to radiation on the atomic bomb survivors from both Hiroshima and Nagasaki. They observed a positive dose effect for adolescents exposed within the second trimester of gestation for systolic hypertension. Tatsukawa et al. (2008) also studied populations that were survivors of Hiroshima and Nagaski with interest in subjects exposed to radiation *in utero*. No statistical significance was found between *in utero* exposure and cardiovascular disease, however a suggestion of an increased cardiovascular risk was observed.

Animal studies have assessed the effect of *in utero* radiation on cardiovascular function. Sreetharan et al. (2019) investigated the effect of low-dose radiation on blood pressure and heart rate in C57Bl/6J mice. This study found no effect of *in utero* radiation up to a dose of 300 mGy. At a high dose of 1000 mGy male mice showed a significant decrease in heart rate along with growth restriction in both sexes. However, it should be noted that there was a transport effect from moving the mice to the irradiation facility, which complicated the interpretation of the results (Sreetharan et al., 2019). Another study by Bakshi et al. (2016) looked at changes in the heart proteome of mice exposed to radiation *in utero*. There were persistent changes to protein expression for mitochondrial respiratory complexes, redox, and heat shock responses (100 mGy). This suggests that *in utero* exposure to radiation may have the potential for cardiovascular programming of disease through oxidative stress and may be an environmental stressor capable of fetal programming.

1.5 Mitochondria and Oxidative Stress in the Cardiovascular System

To ensure proper heart function, the cardiac tissue needs a constant supply of energy. To meet this demand, the heart produces energy using the mitochondria (Siasos et al., 2018). Ionizing radiation can lead to a pro-oxidative state through the production of ROS from the mitochondrial electron transport chain and nicotinamide adenosine dinucleotide phosphate (NADPH) oxidases in the cytoplasm (Tharmalingam et al., 2017). This is of interest within the heart as this tissue has the highest oxygen uptake and density of mitochondria in comparison to other organs, which causes it to be an active source of

ROS and a target of oxidative damage. The mitochondria produce energy in the form of adenosine triphosphate (ATP), through a process known as oxidative phosphorylation (OXPHOS). OXPHOS is the last stage in mitochondrial metabolism, with the mitochondria first undergoing beta oxidation and then the citric acid cycle to produce the necessary biproducts for ATP production. If these processes become dysregulated, this can cause the mitochondria to be an active source of ROS, which makes this organelle an important mechanism of cardiovascular disease (Siasos et al, 2018; Chen and Zweier, 2014).

The first step in mitochondrial metabolism is the break down of its main source of energy, fatty acids, through beta oxidation. Fatty acid oxidation is important in the uptake of long-chain fatty acids in cells for energy homeostasis (Marin-Garcia and Goldenthal, 2002). The mitochondria first transport long-chain fatty acids into the inner mitochondrial membrane using the carnitine palmitoyltransferase 1 (CPT-1), to undergo beta oxidation resulting in Acetyl-CoA. Acetyl-CoA is then used in a process known as the citric acid cycle which produces the coenzymes $FADH_2$ and NADH. These are then used by the electron transport chain through the process of OXPHOS (Marin-Garcia and Goldenthal, 2002).

OXPHOS produces energy through the electron transport train which is made up of four complexes: NADH-dehydrogenase (complex I), succinate dehydrogenase, (complex II), ubiquinone, bc_1 complex (complex III), cytochrome c (Cyt *c*) and cytochrome *c* oxidase (CcO; complex IV) and ATP synthase, as well as two electron carriers located in the inner mitochondrial membrane (Bergman and Ben-Shachar, 2016). Using products created from the citric acid cycle, electrons from both NADH and succinate, are transferred to

ubiquinone once they pass through complex I and II. These then go through complex III and cytochorome c, stopping at complex IV. In complex IV, O2 is reduced to H20 (Bergman and Ben-Shachar, 2016).

Mitochondrial number or biogenesis is regulated in response to environmental stimuli (Sanchis-Gomar et al., 2014). One of the main regulators in the heart is peroxisome proliferative activated receptor, gamma, coactivator 1 alpha (PPARGC1A) (Bhatti et al., 2017). It is a co-transcriptional regulation factor that interacts with various transcription factors and proteins including mitochondrial transcription factor A, uncoupling proteins (UCP2) and nuclear respiratory factors (NRF) 1 and 2. These are all important in the process of mitochondrial biogenesis (Bhatti et al., 2017). UCP2 and NRF2 are not only important for mitochondrial biogenesis but also for the regulation of ROS (Mailloux and Harper, 2011; Ball et al., 2011). As well, NRF2 is involved in the regulation of functions such as autophagy, inflammation and inflammasome signalling, ER stress and the unfolded protein response, and, apoptosis (Ma, 2013). The action of these genes is important in regulating mitochondrial biogenesis and ROS regulation, which is maintains production of ATP while avoiding cellular damage.

Genes involved in beta oxidation, the citric acid cycle, the electron transport chain and mitochondrial biogenesis are important for mitochondrial function. If there is dysregulation of these genes in the cardiac tissue, there is potential for cardiovascular disease. The mitochondria are believed to be a major source of radiation-induced secondary ROS (Baselet et al., 2016). If there is excess electron leakage in the mitochondria leading to ROS production, this can lead to mitochondrial dysfunction and oxidative stress.

Therefore, if mitochondrial function is negatively affected by oxidative stress *in utero* through ionizing radiation, there could be long term effects on heart function.

1.6 Development of the Mouse Cardiovascular System

Proper development of the cardiovascular system is essential to embryonic survival both *in utero* and postnatally. The heart is the first mouse organ to develop and function *in utero* (Savolainen et al., 2009)**.** By embryonic day (E) 7.5 the heart endothelial cells have already differentiated to form the right and left endocardial heart tubes. By E8.0, the endothelial-lined vessels will fuse and form a single beating heart tube, with a regular heartbeat established by E9.0 (Kaufman and Navaratnam, 1981; Savolainen et al., 2009). At E8.5, three regions of the heart are distinguished including the bulbus cordis, primitive left ventricle, and common atrial chamber (Kaufman and Navaratnam, 1981; Savolainen et al., 2009). At this stage primitive circulation is established (Kaufman and Bard, 1999). At E9.5 the atrioventricular and outflow tract including the conus and tuncus (arteriosus), which are necessary to produce the aorta and pulmonary trunk, are distinguished (Savolainen et al., 2009).

Between E11.5-14.5, the main developmental events include the continuous septation of the out-flow tract and continuous septation of the atria and ventricles. By E11.5 the outflow tract consists of aortic and pulmonary channels. Following E12.0 there is the development of systemic veins, including the right and left superior venae cavae, involved in drainage of the embryo head, neck and forelimbs (Savolainen et al., 2009). At E13.5 the aortic arch system has undergone apoptosis and is comparable to the adult mouse. Blood can flow though the systemic circuit by the ascending (thoracic) aorta. The outflow tract

septation is complete by this stage. Normal progression of the outflow tract development is important, as abnormalities can lead to cardiac defects (Savolainen et al., 2009). By E14.5 the atrial septation is complete and after E15.5 the definitive external prenatal configuration of the heart is complete, the main developmental events left are modifications of the atrioventricular valve leaflets and coronary arteries (Savolainen et al., 2009). Between E11.0-E16.0 apoptosis plays an important role in the ventricular morphogenesis (Abdelwahid et al., 1999; Savolainen et al., 2009). Apoptosis is important for normal heart development and plays a major role in the development of the outflow tract and developing the valves for the aorta and pulmonary trunk (Savolainen et al., 2009).

Between E10.0-E12.0 the proliferation level of cardiomyocytes decreases and by postnatal day 5-14 the cardiomyocytes exit their cell cycle completely. Development of the heart during postnatal development is strongly reliant on cardiomyocyte hypertrophy (Takeuchi, 2014). Cell replacement is typically not a mechanism to repair damage during adulthood. Heart cells present at birth may be determinant for heart resilience or vulnerability to disease. If total myocyte number is reduced, the burden of increased afterload results in myocyte enlargement and hypertrophy of the ventricular wall which is used as a compensatory mechanism to normalize wall stress (Barbera et al., 2000; Miranda et al., 2017). If there is a trauma or stress during fetal or postnatal development, which affects the development of the cardiovascular system or the metabolic capability of the heart, there may be negative consequences for cardiac function in later life.

1.7 Research Question

Does *in utero* exposure to ionizing dose radiation during late gestation cause an adverse environment which is capable of fetal programming? Will there be permanent changes to the cardiovascular system and growth rate in offspring in the postnatal period?

1.8 Objective and Hypothesis

The objective of this study is to determine if exposure to ionizing radiation *in utero* during fetal development (E15; past the point of organogenesis), will affect postnatal growth rate and cardiovascular system function. It is hypothesized that maternal exposure to ionizing radiation will cause an adverse intrauterine environment leading to fetal programming of the offspring. Offspring exposed to high doses of radiation will show postnatal growth restriction and cardiovascular disease such as endothelial dysfunction and hypertension in adulthood. Offspring will show signs of oxidative stress and changes to heart mitochondrial capacity. Whether these effects will occur at low doses is unknown.

1.9 References

Abdelwahid E, Pelliniemi LJ, Niinikoski H, Simell O, Tuominen J, Rahkonen O. Apoptosis in the Pattern Formation of the Ventricular Wall During Mouse. 1999;217:208–17.

Ainsbury EA, Bouffler SD, Dörr W, et al. Radiation cataracto- genesis: a review of recent studies. Radiat Res 2009;172:1–9

Alexander BT, Dasinger JH, Intapad S. Fetal Programming and Cardiovascular Pathology. In: Comprehensive Physiology. 2015;5(2);997-1025.

Bakshi M V., Azimzadeh O, Merl-Pham J, Verreet T, Hauck SM, Benotmane MA, et al. In-utero low-dose irradiation leads to persistent alterations in the mouse heart proteome. PLoS One. 2016;11(6):1–14.

Ball WB, Kar S, Mukherjee M, Chande AG, Mukhopadhyaya R, Das PK. Uncoupling Protein 2 Negatively Regulates Mitochondrial Reactive Oxygen Species Generation and Induces Phosphatase-Mediated Anti-Inflammatory Response in Experimental Visceral Leishmaniasis. J Immunol. 2011;187(3):1322–32.

Barbera A, Giraud GD, Reller MD, Maylie J, Morton MJ, Thornburg KL, et al. Right ventricular systolic pressure load alters myocyte maturation in fetal sheep. 2019;3098:1157–64.

Barker DJ, Osmond C, Golding J, Kuh D, Wadsworth ME. Growth *in utero*, blood pressure in childhood and adult life, and mortality from cardiovascular disease. BMJ. 1989;298(6673):564–7.

Baselet B, Rombouts C, Benotmane AM, Baatout S, Aerts A. Cardiovascular diseases related to ionizing radiation: The risk of low-dose exposure (Review). Int J Mol Med. 2016;38(6):1623–41.

Bergman O, Ben-Shachar D. Mitochondrial oxidative phosphorylation system (OXPHOS) deficits in schizophrenia: Possible interactions with cellular processes. Can J Psychiatry. 2016;61(8):457–69.

Bhatti JS, Bhatti GK, Reddy PH. Mitochondrial dysfunction and oxidative stress in metabolic disorders — A step towards mitochondria based therapeutic strategies. Biochim Biophys Acta. 2017;1863(5):1066–77.

Brenner DJ, Doll R, Goodhead DT, Hall EJ, Land CE, Little JB, et al. Cancer risks attributable to low doses of ionizing radiation: Assessing what we really know. Proc Natl Acad Sci. 2003;100(24):13761–6.

Chen Y, Wang Z, Chen Z, Lv G, Ferrari M. intermittent maternal hypoxia has an influence on regional expression of endothelial nitric oxide synthase in fetal arteries of rabbits. Pediatr Res. 2013;73(6):706–12.

Chen Y-R, Zweier JL. Cardiac mitochondria and ros generation. Circ Res. 2014;114(3):524–37.

Dennery PA. Oxidative stress in development: Nature or nurture? Free Radic Biol Med. 2010;49(7):1147–51.

Dinh QN, Drummond GR, Sobey CG, Chrissobolis S. Roles of inflammation, oxidative stress, and vascular dysfunction in hypertension. Biomed Res Int. 2014;2014:406960.

Eriksson JG, Forsen T, Tuomilehto J, Winter PD, Osmond C, Barker DJP. Catch-up growth in childhood and death from coronary heart disease: longitudinal study. BMJ. 1999;318(7181):427–31.

Franco, Carmo PArruda MMP, Paula A, Dantas V, Helena M, Carvalho C, Tostes RCA, et al. Intrauterine undernutrition: expression and activity of the endothelial nitric oxide synthase in male and female adult offspring. Cardiovasc Res. 2002;56:145–53.

Godfrey KM, Barker DJ. Fetal programming and adult health. Public Health Nutr. 2001;4(2b) 611-624.

Goldenthal MJ, Marín-García J. Fatty acid metabolism in cardiac failure: biochemical, genetic and cellular analysis. Cardiovasc Res. 2002;54:516–27.

Hall EJ, Giaccia AJ. Radiobiology for the radiobiologist. 7th ed. Philadelphia: Wolters Kluwer Health/Lippincott Williams & Wilkins; 2012.

Hallahan DE, Virudachalam S, Kuchibhotla J. Nuclear factor κB dominant negative genetic constructs inhibit X-ray induction of cell adhesion molecules in the vascular endothelium. Cancer Res. 1998;58(23):5484–8.

Houten SM, Wanders RJA. A general introduction to the biochemistry of mitochondrial fatty acid β-oxidation. J Inherit Metab Dis. 2010;33(5):469–77.

Ina Y, Sakai K. Further study of prolongation of life span associated with immunological modification by chronic low-dose-rate irradiation in MRL-lpr/lpr mice: effects of whole-life irradiation. Radiat Res 2005;163:418–23

Ina Y, Sakai K. Prolongation of life span associated with immunological modification by chronic low-dose-rate irradi- ation in MRL-lpr/lpr mice. Radiat Res 2004;161:168–73.

Ionizing Radiation Exposure of the Population of the United States (2009). NCRP Report No. 160. Bethesda: National Council on Radiation Protection and Measurements: 2009

Jackson AA. Nutrients, Growth, and the Development of Programmed Metabolic Function. Adv Exp Med Biol. 2000;478:41–55.

Kaufman MH, Bard JBL. The Anatomical Basis of the Mouse Development. Academic Press; San Diego, CA: 1999.

Kaufman MH, Navaratnam V. Early differentiation of the heart in mouse embryos. J Anat. 1981;133(Pt 2):235–46.

Kimler BF, Norton S. Behavioral changes and structural defects in rats irradiated *in utero*. Int J Radiat Oncol Biol Phys. 1988;15(5):1171–7.

Kimler BF, Norton S. Behavioral changes and structural defects in rats irradiated *in utero*. Int J Radiat Oncol Biol Phys. 1988;15(5):1171–7.

Kwon EJ, Kim YJ. What is fetal programming?: a lifetime health is under the control of *in utero* health. Obstet Gynecol Sci. 2017;60(6):506–19.

Lau C, Rogers JM. Embryonic and fetal programming of physiological disorders in adulthood. Birth Defects Res Part C - Embryo Today Rev. 2004;72(4):300–12.

Liu VWT, Huang PL. Cardiovascular roles of nitric oxide: A review of insights from nitric oxide synthase gene disrupted mice †. Cardiovasc Res. 2008;77:19–29.

Lowe SA. Diagnostic radiography in pregnancy: Risks and reality. Aust N Z J Obstet Gynaecol. 2004; 44(3):191–196.

Lu M, Ji J, Jiang Z, You Q. The Keap1 – Nrf2 – ARE Pathway As a Potential Preventive and Therapeutic Target: An Update. Med Res Rev. 2016;36(5):924–63.

Ma Q. Role of Nrf2 in Oxidative Stress and Toxicity. Annu Rev Pharmacol Toxicol. 2013;53:401–26.

Mailloux RJ, Harper ME. Mitochondrial proticity and ROS signaling: Lessons from the uncoupling proteins. Trends Endocrinol Metab. 2012;23(9):451–8.

Marín-García J, Goldenthal MJ. Fatty acid metabolism in cardiac failure: biochemical, genetic and cellular analysis. Cardiovasc Res. 2002;54:516–27.

Mathias D, Mitchel REJ, Barclay M, Wyatt H, Bugden M, Priest ND, et al. Low-dose irradiation affects expression of inflammatory markers in the heart of ApoE -/- Mice. PLoS One. 2015;10(3):1–19.

Matsuno-Yagi A, Hatefi Y. Studies on the Mechanism of Oxidative Phosphorylation. J Biol Chem. 1987;262(29):14158–63.

Meister TA, Rexhaj E, Rimoldi SF, Scherrer U, Sartori C. Fetal programming and vascular dysfunction. Artery Res. 2018;21:69–77.

Meyer K, Zhang, L. Fetal programming of cardiac function and disease. Reprod Sci. 2007;14(3):209–16.

Minamisawa T, Hirokaga K, Sasaki S. Gross morphological changes to the mouse brain exposed prenatally to ionizing radiation. J Radiat Res. 1990;31:214–8.

Miranda JO, Ramalho C, Henriques-Coelho T, Areias JC. Fetal programming as a predictor of adult health or disease: the need to reevaluate fetal heart function. Heart Fail Rev. 2017;22(6):861–77.

Morgan WF, Bair WJ. Issues in Low Dose Radiation Biology: The Controversy Continues. A Perspective. Radiat Res. 2013;179(5):501–10.

Morton JS, Cooke C-L, Davidge ST. *In Utero* Origins of Hypertension: Mechanisms and Targets for Therapy. Physiol Rev. 2016;96(2):549–603.

Nakashima E, Akahoshi M, Neriishi K, Fujiwara S. Systolic blood pressure and systolic hypertension in adolescence of atomic bomb survivors exposed *in utero*. Radiat Res. 2007;168(5):593–9.

Nguyen Dinh Cat A, Montezano AC, Burger D, Touyz RM. Angiotensin II, NADPH Oxidase, and Redox Signaling in the Vasculature. Antioxid Redox Signal. 2013;19(10):1110–20.

Otake M, Schull WJ. Review: Radiation-related brain damage and growth retardation among the prenatally exposed atomic bomb survivors. Int J Radiat Biol. 1998;74(2):159–71.

Piecha G, Koleganova N, Ritz E, Müller A, Fedorova O V, Bagrov AY, et al. High salt intake causes adverse fetal programming — vascular effects beyond blood pressure. 2012;41–50.

Rodford JL, Torrens C, Siow RCM, Mann GE, Hanson MA, Clough GF. Endothelial dysfunction and reduced antioxidant protection in an animal model of the developmental origins of cardiovascular disease. J Physiol. 2008;586(19):4709–20.

Rodríguez-rodríguez P, Ramiro-cortijo D, Reyes-hernández CG, Pablo ALL De, González MC, Arribas SM. Implication of Oxidative Stress in Fetal Programming of Cardiovascular Disease. 2018;9:1–13.

Sakai K, Nomura T, Ina Y. Enhancement of bio-protective functions by low dose/dose-rate radiation. Dose Response 2006; 4:327–32

Sanchis-Gomar F, Luis Garcia-Gimenez J, Carmen Gomez-Cabrera M, Pallardo, FV. Mitochondrial biogenesis in health and disease. Molecular and therapeutic approaches. Curr Pharm Design. 2014;20(35);5619-5633.

Savoia C, Sada L, Zezza L, Pucci L, Lauri FM, Befani A, et al. Vascular inflammation and endothelial dysfunction in experimental hypertension. Int J Hypertens. 2011;2011:1–8.

Savolainen SM, Foley JF, Elmore SA. Histology Atlas of the Developing Mouse Heart with Emphasis on E11.5 to E18.5. Toxicol Pathol. 2009;37(4):395–414.

Schulz E, Jansen T, Wenzel P, Daiber A, Münzel T. Nitric Oxide, Tetrahydrobiopterin, Oxidative Stress, and Endothelial Dysfunction in Hypertension. Antioxid Redox Signal. 2008;10(6):1115–26.

Siasos G, Tsigkou V, Kosmopoulos M, Theodosiadis D, Simantiris S, Tagkou NM, et al. Mitochondria and cardiovascular diseases—from pathophysiology to treatment. Ann Transl Med. 2018;6(12):256–256.

Sievert W, Trott KR, Azimzadeh, O, Tapio, S, Zitzelsberger H, Multhoff G. Late proliferating and inflammatory effects on murine microvascular heart and lung endothelial cells after irradiation. Radiother Oncol. 2015; 117:376-381.

Sreetharan S, Stoa L, Cybulski ME, Jones DE, Lee AH, Kulesza A V., et al. Cardiovascular and growth outcomes of C57Bl/6J mice offspring exposed to maternal stress and ionizing radiation during pregnancy. Int J Radiat Biol. 2019;1–9. Available from: https://doi.org/10.1080/09553002.2019.1589025

Sreetharan S, Thome C, Tharmalingam S, Jones DE, Kulesza A V., Khaper N, et al. Ionizing Radiation Exposure During Pregnancy: Effects on Postnatal Development and Life. Radiat Res. 2017;187(6):647–58.

Takeuchi T. Regulation of cardiomyocyte proliferation during development and regeneration. Dev Growth Differ. 2014;56:402–9.

Tang FR, Loke WK, Khoo BC. Low-dose or low-dose-rate ionizing radiation-induced bioeffects in animal models. J Radiat Res. 2017;58(2):165–82.

Tarry-Adkins JL, Martin-Gronert MS, Fernandez-Twinn DS, Hargreaves I, Alfaradhi MZ, Land JM, et al. Poor maternal nutrition followed by accelerated postnatal growth leads to alterations in DNA damage and repair, oxidative and nitrosative stress, and oxidative defense capacity in rat heart. FASEB J. 2013;27(1):379–90.

Tatsukawa Y, Nakashima E, Yamada M, Funamoto S, Hida A, Akahoshi M, et al. Cardiovascular disease risk among atomic bomb survivors exposed *in utero*, 1978-2003. Radiat Res. 2008;170(3):269–74.

Teixeira BC, Lopes AL, Macedo RCO, Correa CS, Ramis TR, Ribeiro JL, et al. Inflammatory markers, endothelial function and cardiovascular risk. J Vasc Bras. 2014;13(2):108–15.

Tharmalingam S, Sreetharan S, Kulesza A V., Boreham DR, Tai TC. Low-Dose Ionizing Radiation Exposure, Oxidative Stress and Epigenetic Programing of Health and Disease. Radiat Res. 2017;188(4.2):525–38.

Thompson LP, Al-Hasan Y. Impact of oxidative stress in fetal programming. J Pregnancy. 2012;2012.

Tomat A, Elesgaray R, Zago V, Fasoli H, Fellet A, Balaszczuk AM, et al. Exposure to zinc deficiency in fetal and postnatal life determines nitric oxide system activity and arterial blood pressure levels in adult rats. Br J Nutr. 2010;104(3):382–9.

Torrens C, Brawley L, Anthony FW, Dance CS, Dunn R, Jackson AA, et al. Folate supplementation during pregnancy improves offspring cardiovascular dysfunction induced by protein restriction. Hypertension. 2006;47(5):982–7.

Toyoda M, Shirato H, Nakajima K, Kojima M, Takahashi M, Kubota M, et al. jumonji Downregulates Cardiac Cell Proliferation by Repressing cyclin D1 Expression. 2003;5:85–97.

Vasquez-Vivar J, Kalyanaraman B, Martasek P, Hogg N, Sue B, Masters S, et al. Superoxide generation by endothelial nitric oxide synthase: The influence of cofactors. Proc Natl Acad Sci USA. 1998;95:9220–5.

Yan G, You B, Chen SP, Liao JK, Sun J. Tumor necrosis factor-α downregulates endothelial nitric oxide synthase mRNA stability via translation elongation factor 1-α 1. Circ Res. 2008;103(6):591–7.

Yzydorczyk C, Armengaud JB, Peyter AC, Chehade H, Cachat F, Juvet C, et al. Endothelial dysfunction in individuals born after fetal growth restriction: Cardiovascular and renal consequences and preventive approaches. J Dev Orig Health Dis. 2017;8(4):448–64.

Chapter 2: Effects of low-dose ionizing radiation *in utero* on postnatal growth, and cardiovascular physiology in BALB/cJ mice

2.1 Abstract

Diagnostic radiation is typically avoided during pregnancy, as the effect of low-dose radiation exposure on the fetus is uncertain. The objective of this study was to determine if ionizing radiation exposure during late fetal development would cause an adverse intrauterine environment, and lead to growth restriction of offspring and a hypertensive phenotype later in life. To study this, pregnant BALB/cJ mice were exposed to ionizing radiation at 5, 10, 50, 100, 300 or 1000 mGy on gestational day 15. Offspring were weighed weekly from the age of weaning until a mature age of 16 weeks. Cardiovascular effects were assessed every other week via heart rate and blood pressure measurements using tail plethysmography. The expression of genetic markers for endothelial dysfunction, inflammation, mitochondrial capacity and regulation of the oxidative stress response in the aorta and heart for the 1000 mGy was assessed from tissue collected at 17 weeks of age. We observed no effects of low to mid dose (5-300 mGy) radiation on offspring growth and blood pressure. Growth restriction was observed in male and female offspring exposed to high-dose radiation (1000 mGy). We also observed decreased TNF-α expression in the aorta of male offspring (1000 mGy), which may be linked to the growth restriction but was not considered a sign of cardiovascular dysfunction. This study provides knowledge on the possible effects of radiation on *in utero* development, which broadens the knowledge on the range of stressors capable of affecting offspring growth and development.

2.2 Introduction

During early development (i.e. preconceptual to early postnatal development) environment plays a crucial role in the health of an individual. An adverse environment during this time can predispose offspring to disease in adulthood (Morton et al., 2016). This concept is termed fetal programming (Barker, 1998; Godfrey and Barker, 2001). Metabolic and physiological function during *in utero* development is relatively plastic in relation to later stages of life. This means that an adverse environment during early development may result in permanent changes to metabolic capacity and physiological responses in adulthood (Jackson, 2000; Lau and Rogers, 2004). Fetal programming is a mechanism which allows the fetus to adapt to intrauterine environmental conditions, however it may not prepare the offspring for survival in later life (Kwon and Kim, 2017).

Environmental exposures (i.e. toxin exposure) and maternal lifestyle (i.e. nutritional deficits, hypoxia, maternal obesity) during *in utero* development can all affect adult disease development. Of interest is how these environmental stressors can impact the development of cardiovascular disease in later life (Morton et al., 2016). One of the major indicators of fetal programming is a low birth weight. This has been associated with long-term deleterious consequences, leading to an increased susceptibility to cardiovascular diseases (Eriksson et al., 1999; Meyer and Zhang, 2007). Development of hypertension post fetal programming is one of the most commonly studied outcomes (Morton et al., 2016). In response to growth restriction, children have shown cardiovascular changes including cardiac morphology (i.e. increased heart size, left ventricular mass), subclinical cardiac dysfunction and endothelial dysfunction (Arnott et al., 2015; Miranda et al., 2017).

Whereas, adults show cardiovascular affects such as hypertension and coronary heart disease (Miranda et al., 2017).

Early signs of cardiovascular disease progression include endothelial dysfunction (Rodriguez-Rodriguez et al., 2018; Meister et al., 2018). Endothelial dysfunction is characterized by an imbalance in the production of mediators important to vascular tone, platelet aggregation, coagulation and fibrinolysis in the endothelium (Dinh et al., 2014). Vascular inflammation is often implicated, through the induction of vasoconstrictor agents, adhesion molecules and growth factors (Savoia et al., 2011). This commonly includes the involvement of oxidative stress (Dinh et al., 2014; Teixeira et al., 2014). When there is an imbalance of these factors, the systemic vasculature becomes stiffer and less distensible due to alterations in the collagen and elastin balance during disease progression. This can lead to the development of hypertension (Morton et al., 2016).

Oxidative stress is a common mechanism of fetal programming (Luo et al., 2006). Oxidative stress can be defined as an imbalance in the production and elimination of reactive oxygen species (ROS; Rodriguez-Rodriguez, 2018). ROS are involved in placentation and are signalling molecules involved in regulating gene transcription; they are essential to normal embryonic and fetal development. Due to this, it is essential that production and elimination of ROS during development is kept in balance (Rodriguez-Rodriguez, 2018). Embryo development occurs in a low oxygen environment which causes the embryo to have a low antioxidant capacity and prevents the ability of regulating alterations to ROS levels (Thompson and Al-Hasan, 2012; Dennery et al., 2010). If unregulated levels of ROS result in a pro-oxidative state during development, this can cause

damage to macromolecules and thus cellular injury. This can then compromise fetal development and size (Rodriguez-Rodriguez et al., 2018). Excess ROS production *in utero* may affect offspring growth and predispose offspring to disease later in life as it plays an important role during organ formation in the embryo (Rodriguez-Rodriguez et al., 2018). We are interested in whether radiation exposure during fetal development will cause oxidative stress and lead to programming of the offspring.

Ionizing radiation can lead to a pro-oxidative state through the production of ROS from the mitochondrial electron transport chain and nicotinamide adenosine dinucleotide phosphate (NADPH) oxidases in the cytoplasm (Tharmalingam et al., 2017). The heart has the highest oxygen uptake and density of mitochondria in comparison to other organs, which causes it to be an active source of ROS and a target of oxidative damage. To ensure proper heart function, the cardiac tissue needs a constant supply of energy, and meets this demand by producing energy using the mitochondria (Siasos et al., 2018). Genes involved in mitochondrial biogenesis, beta oxidation, and the electron transport chain are all important to mitochondrial metabolism. The main process which produces energy in the form of adenosine triphosphate (ATP) is oxidative phosphorylation. If this process becomes dysregulated, this can cause the mitochondria to be an active source of ROS. This makes the mitochondria an important mechanism of cardiovascular disease (Siasos et al, 2018; Chen and Zweier, 2014).

There is a consensus that exposure to high-dose radiation can lead to negative health effects, however there is uncertainty around the risk of low-dose ionizing radiation (Brenner, 2003). In animal models, low-dose ionizing radiation has been defined as an

absorbed radiation dose of less than 100 milligray (mGy; Tang et al., 2017). Due to uncertainty of the effect of low-dose radiation exposure, diagnostic radiation is typically avoided during pregnancy (Lowe, 2004). Currently there is little knowledge on the effect of low-dose radiation exposure to mothers during fetal development and how it could be a potential stressor leading to adverse effects on offspring later in life.

This study aims to determine if exposure to low-dose radiation *in utero* affects offspring growth and cardiovascular physiology in a mouse model. We wanted to determine if *in utero* exposure to ionizing radiation during fetal development would cause an adverse environment which is capable of fetal programming. The experiment meant to determine if there were changes to the cardiovascular system and growth rate of offspring. For cardiovascular effects we measured offspring blood pressure, heart rate, and markers for endothelial dysfunction and mitochondrial capacity. We hypothesized that maternal exposure to ionizing radiation would cause an adverse intrauterine environment leading to fetal programming of the offspring. Offspring exposed to high doses of radiation would show postnatal growth restriction and cardiovascular disease such as endothelial dysfunction and hypertension in adulthood, signs of oxidative stress and changes to heart mitochondrial capacity. Whether these effects will occur at low doses was unknown.

2.3 Materials and Methods

2.3.1 Breeding Protocol

BALB/cJ wildtype mice were obtained from Jackson Laboratories, Bar Harbor, ME and brought to McMaster University to be housed in the Central Animal Facility. Females were housed with five per cage until breeding and males were housed alone. Mice were

acclimated without disruptions for one week. Mice were housed with a 12:12 photoperiod and were given food and water *ad-libitum* for the duration of the study.

During breeding, two females were moved to one cage with one male and were left overnight. Females were removed the next morning and housed alone for the duration of the study. Hand palpitation and the presence of vaginal plugs were used to confirm pregnancy. During pregnancy mice did not leave the housing room in the Central Animal Facility except for maternal irradiation (see Irradiation Protocol 2.3.2). The naïve control group did not leave the animal housing room. Mothers were left undisturbed for a minimum of one week following the birth of the litter. At 3-4 weeks of age offspring were weaned from their mothers and female and male offspring were housed with a maximum of four or three per cage, respectively. A maximum of two offspring per mother of each sex were used in the present study. All animal protocols were approved by the Animal Research Ethics Board at McMaster University (AUP#15-11-26).

2.3.2 Irradiation Protocol

Pregnant maternal BALB/cJ mice were exposed to low-dose ionizing radiation during late fetal development at day 15 of gestation. The ionizing radiation source used was a Cesium-137 gamma radiation (662 KeV energy) source located at McMaster University. Since the radiation source was in a separate building from animal holding, pregnant mice were transported using a temperature-controlled vehicle. Prior to the irradiation, mice were left undisturbed to acclimate for 1 hour under the shielded source. After the acclimation, sham mice were removed from under the shield into a control room. Food and water were then restricted for the duration of the planned irradiation dose.

Pregnant mice were exposed to nominal doses of 5, 10, 50, 100, 300 and 1000 mGy. All doses were delivered at a nominal dose rate of 10 mGy min^{-1} with a 8.9 mGy min^{-1} fetal dose rate estimated using thermoluminescent dosimeters (TLDs; Mirion Technologies, Irvine, California, USA), as previously verified by Sreetharan et al. (2019). Both naïve and sham irradiated mice were included as controls; naïve control mice did not leave animal housing whereas the sham irradiated mice were transported to the irradiation facility and followed the same protocol as the mice exposed to radiation but never received a radiation dose. The naïve control was included to compare with the sham irradiated control to determine any potential transportation stress effects.

2.3.3 Blood Pressure, Heart Rate, Weight Collection

After weaning, cardiovascular effects were assessed biweekly and the offspring were weighed weekly until a mature age of 16 weeks. Figure 2.1 shows the experimental timeline. Cardiovascular parameters were assessed using the CODA8 High Throughput Noninvasive Blood Pressure System (Kent Scientific Corporation, Torrington, Connecticut, USA). Mice were restrained in plexiglass tubes and placed on an animal warming platform with the temperature was set to approximately 35-38℃. A tail-cuff with a photosensitive cell was placed at the base of the tail and changes in the systolic blood pressure (SBP), diastolic blood pressure (DBP), mean arterial pressure (MAP) and heart rate (HR) were measured. The CODA software determined the validity of the cycle based on criteria such as a minimum tail volume of 15ml, the length of time between DBP and SBP measurements, a difference between SBP and DBP of at least 5 mmHg and the SBP measurement had to be higher than the DBP measurement.

Measurements of offspring blood pressure (BP) and HR started the week after weaning, at 5 weeks of age. Weeks 5-6 were used to acclimate the mice to the CODA8 system and acclimate them to being restrained in plexiglass tubes. BP measurements were collected every other week from 6 to 16 weeks of age. A total of 30 cycles were run in a given session each day for each mouse, the first 5 cycles were used as acclimation cycles and were removed from analysis. Measurements were obtained three days within a given week where blood pressure measurements were collected for a mouse.

2.3.4 Tissue Collection and mRNA analysis

Mice were euthanized at 17 weeks of age via cervical dislocation. The heart and descending aorta were flash frozen using liquid nitrogen and were stored at -80°C. The expression of marker genes was determined using quantitative RT-qPCR (Reverse Transcriptase-quantitative Polymerase Chain Reaction) for the sham and 1000 mGy treatment groups.

Total RNA was extracted from aorta and heart tissue using TRIzol reagent according to instructions from the manufacturer (Sigma-Aldrich). 1 ml of TRIZol was used for the aorta tissue and 2ml was used for the heart tissue extractions. RNA was quantified using the nanodrop 1000 spectrophotometer. RNA purity (A_{260}/A_{280}) ranged from 1.7 to 2.0. RNA integrity analysis was run for 25% of heart and 22.5% of aorta samples using the TapeStation (Agilent Technologies, 2017). RNA integrity number (RIN^e) ranged from 6.6 to 7.7 in the heart tissue, and 6.3 to 7.3 in the aorta tissue. RNA was treated with a Sigma DNAse 1 treatment according to manufacturers instructions. The final concentration for the DNAse treated RNA was 2 micrograms of RNA per 22 microliter volume. 2 micrograms

of RNA were used to synthesize the cDNA with Promega M-MLV Reverse Transcriptase (RT; FisherScientific), random primers, dNTP mix and 5X Reaction Buffer for Mu-MLV RT according to manufacturers instructions. Gene expression was determined using quantitative RT-qPCR PowerUp SYBR Green Master Mix with gene specific primers using the QuantStudio 5 Real-Time PCR System. Sequences and efficiency for primers used in the heart are listed in Table 2.1, these included genes involved in mitochondrial capacity, a ROS-generating oxidase and a regulator of the oxidative stress response. Sequences and efficiency for primers used for the aorta are listed in Table 2.2, which involves genes related to endothelial function and a regulator of the oxidative stress response. Primer specificity was determined using Primer3 and BLAST. Using melt-curves, a single peak was obtained for each primer. Linear doubling was demonstrated from 0.1 to 25ng cDNA for all primers ($R2>0.93$). Annealing temperature used was 60 degrees Celsius for all quantitative RT-qPCR reactions. Sequences for TNF-α (Yamakawa et al., 2011), VCAM-1 (Lam et al., 2011; Salim et al., 2016; Liu et al., 2017), ICAM-1 (Bros et al., 2007; Salim et al., 2016; Liu et al., 2017) and NRF2 (Liessem-Schmitz et al., 2018; Cong et al., 2018; Zhou et al., 2018; aorta) were obtained from previously published literature.

2.3.5 Data Analysis

2.3.5.1 Experimental Organism Analysis

To determine if litter size affected offspring weight, a linear regression was run for both sexes at 4 and 16 weeks of age. As well, linear regression analysis was run individually for each BP variable (SBP, DBP and MAP) and HR, to determine if there was a relationship to body weight, for both the naïve and sham populations. Regression analysis was run using

the IBM SPSS Statistics 25 (IBM Corporation, Armonk, New York, USA), statistical software with a significance level set at α=0.05.

2.3.5.2 Blood Pressure Data Analysis

For the BP data, the median value of SBP, DBP, MAP and HR measurements for each day was used to calculate the average value for a week for each mouse. If there were less than 5 valid readings according to the CODA software for any BP or HR measurement recorded for a given day, all data from that day was removed from analysis. Statistics were run first between the two control groups, the naïve and sham, to determine any transportation stress effects. Analysis was run separately to compare the sham control to all radiation treatment groups (5, 10, 50, 100, 300, 1000 mGy). Data are presented as parametric coefficient ± standard error. The following statistical analysis was run using R Studio version 1.1.453. All statistical tests had a significance level set at α=0.05.

The BP data was analysed using a linear mixed-effects model with the package *lme4* (Bates et al., 2015). This model analyzed changes in BP between the sham and radiation treatment groups between 8 to 16 weeks of age. To account for the confounding variable of 2 offspring used per mother for each sex, the model used a random effects component to allow for differences among pups within mothers. The model run was: BP ~ 1 + sex * week * dose + (1 | mother:pup). The analysis used the average between the male and female sham control, as a baseline for comparisons to treatment groups (5, 10, 50, 100, 300, 1000 mGy). To determine differences between the fixed effects sex and dose, or an interaction between these, dose groups were compared to the sham control using the Type III Wald F tests with Kenward-Roger degrees of freedom (df).

2.3.5.3 Weight Data Analysis

Statistics were run first between the two control groups, the naïve and sham, to determine any transportation stress effects. Analysis was run separately to compare the sham control to all radiation treatment groups (5, 10, 50, 100, 300, 1000 mGy). Data are presented as parametric coefficient ± standard error. The following statistical analysis was run using R Studio version 1.1.453. All statistical tests had a significance level set at $\alpha=0.05$.

The weight data was analyzed using a generalized additive model using REML method with the *mgcv* package (Wood, 2011). This model analyzed changes in weight based on the fixed effects of dose and sex between 4 and 16 weeks of age. To account for the confounding variable of 2 offspring used per mother for each sex within a treatment group, a random effects/ multilevel component was used to allow for differences among pups within mother random effects. The fit for the growth curves were tested using 28 models based on how sex, dose, and variation across pups within mother random effects interacted to affect the shape and vertical shift (i.e. intercept) of the growth curve and determine the best fit for the data. The were three models considered for future analysis, each with an AIC<10. The model chosen for analysis was: dose*sex+s(week,by=dose)+s(week,by=sex)+s(week,mother_pup). This model was chosen as it was the only one that accounted for an interaction between dose and sex. The analysis used the average between the male and female sham control as a baseline for comparisons to the dose treatment groups (5, 10, 50, 100, 300, 1000 mGy). To determine differences between sex, dose, or an interaction of dose and sex, dose groups were

compared to the sham control based on Wald tests of the estimated covariance matrix of the model.

2.3.5.4 Gene Expression Statistical Analysis

Gene expression data from quantitative RT-qPCR was analyzed using a generalized linear mixed model with the *MCMC.qpcr* package (Matz., 2013). This analysis uses a Bayesian Markov Chain Monte Carlo (MCMC) sampling scheme to determine changes in gene expression caused by fixed factors, while including technical replicates as a random factor (Matz et al., 2013). For this analysis, expression data in the form of cycle threshold (CT) values for candidate genes need to be recalculated into molecule count data, using primer efficiency data and the number of PCR cycles needed to detect one target molecule**.** To fit the data we used the two-way naïve model, which does not use reference genes, and the fixed effects specified were dose and sex. Matz et al., (2013) compared the naïve to the informed model which uses reference genes in the analysis. It was found that despite the inclusion of reference genes the point estimates were nearly identical, with the only benefit being sharper credible intervals (Matz et al., 2013). Log(abundance) was plotted for all genes.

2.4 Results

2.4.1 Experimental Organisms

There were between 5-11 mother mice (4-6 per sex) per treatment group. A maximum of 2 offspring per sex was included for each treatment group, with a total number of 8-10 males and females per group. All offspring used for this analysis were healthy and completed all weeks of data collection. Litter size ranged from 2-11 offspring per mother.

Litter size did not have an affect on offspring weight (Appendix S2.1). There was no significant effect of litter size on weight at 4 weeks of age ($F_{1,64}$=3.333; p=0.073 for female offspring; $F_{1,64}$=2.087; p=0.153 for male offspring) or at 16 weeks of age ($F_{1,64}$=3.953; p=0.051 for female offspring; $F_{1,64}$=2.539; p=0.116 for male offspring).

Naïve and sham offspring showed no significant effect of weight on DBP, MAP (Appendix S2.2) and HR (Appendix S2.3), for either male or female offspring. A weak correlation between male SBP and weight was observed for naïve (R=0.289) and sham offspring (R=0.354). Male weight accounted for 8.6% of the variation in SBP ($F_{1,54}$ = 4.904; p=0.031) for naïve and 12.6% ($F_{1,61}$ = 8.761; p=0.004) for sham offspring (Appendix S2.2). Similar results were observed using allometric regression analysis. Due to there being a low correlation between SBP and body weight in male offspring only, body weight was not included as a factor in models used for analysis of the blood pressure data.

2.4.2 Blood Pressure and Heart Rate

The naïve control had a higher SBP by 8.1mmHg (F=6.31, p=0.01), DBP by 6.5 mmHg (F=4.53, p=0.04) and MAP by 6.73mmHg (F=4.92, p=0.03) compared to the sham control. There was no difference in HR (F=1.68; p=0.20). The sham group showed no difference between males and females for SBP (F=0.66; p=0.24); DBP (F=0.84; p=0.46); MAP (F=0.86; p=0.36) or HR (F=0.59; p=0.45). As well, there was no interaction observed between sex and dose for any BP parameter. Estimates of the intercept and standard error for the fixed effect of dose from the model are shown in Appendix S2.4.

There was no difference for all three BP parameters and HR between the sham and the dose treatment groups (5, 10, 50, 100, 300, 1000 mGy); SBP (F=1.29; p=0.26; Figure

2.2), DBP (F=1.99; p=0.07); MAP (F=1.73; p=0.11); HR (F=1.93; p=0.08). Males and females in the sham group showed no difference in SBP (F=0.64; p=0.423; Figure 2.2); DBP (F=0.67; P=0.41); MAP (F=0.77; p=0.38); HR (F=0.50; p=0.48). As well, there was no interaction observed between sex and dose for any BP parameter (Figure 2.2). Estimates of the intercept and standard error for the fixed effect of dose are shown in Appendix S2.4.

2.4.3 Weight

There was no difference in weight (F=0.214; p=0.644) between the naïve and sham control. However, there was an effect of sex (F=32.32; p=3.05e-08) on offspring weight. Naïve females were on average 5.96g (±0.52) smaller than naïve males throughout the study period. There was no interaction between dose and sex (F=0.276; p=0.60).

There was a significant effect of dose (F=11.22; p=3.29e-12) and sex (F=55.44; p=1.85e-13) on offspring weight for comparisons between sham and the treatment groups (5, 10, 50, 100, 300, 1000 mGy). The 1000 mGy treatment group was 3.59g (±0.55) smaller than the sham group (average between males and females; Figure 2.3). Sham females were on average 5.52g smaller than males throughout the study period (t=-7.45, p=1.85e-13; Figure 2.3). There was no significant effect for the interaction of dose and sex (F=1.23; p=0.287; Figure 2.3).

2.4.4 Gene Expression

In the heart tissue, there was no difference in CPT1A, Mt-Co1, Mt-Cyt, PPARGC1A, NDUFB6, UCP2 or NRF2 expression (p>0.05) between sham and 1000 mGy (Figure 2.5). Levels of NADPH oxidase (NOX1) were below detection levels. In the aorta

tissue, no difference was found in eNOS, ICAM-1, VCAM-1, NRF2 expression between sham and 1000 mGy. However, there was decreased TNF-α expression in the aorta of the 1000 mGy male offspring compared to sham (Figure 2.6).

2.5 Discussion

The aim of this study was to determine if *in utero* exposure to ionizing dose radiation during late gestation would cause an adverse environment which is capable of fetal programming. We wanted to determine if there would be permanent changes to the offspring's cardiovascular system function and postnatal growth rate. We expected that offspring exposed to high doses of radiation would show postnatal growth restriction and cardiovascular disease such as vascular endothelial dysfunction and hypertension in adulthood. We expected offspring to show signs of oxidative stress and changes to heart mitochondrial capacity. Whether these effects would occur at low doses was unknown. We observed no effects of low to mid dose (5-300 mGy) radiation exposure on offspring growth and BP. Only offspring exposed to the highest dose of 1000 mGy showed growth restriction. Due to this, gene expression for the heart and aorta was analyzed at the 1000 mGy dose compared to sham. There was no apparent effect on the expression of gene markers for heart mitochondrial capacity, a regulator of the oxidative stress response, nor were there changes in gene expression in the aorta for endothelial function. A decreased expression of TNFα was observed in the male aorta only.

Tail-cuff plethysmography was used to measure BP and HR in mice. This method is non-invasive and is a great alternative to other methods of BP measurement such as radiotelemetry, which typically involves a surgical implant. Tail cuff plethysmography has

been observed to provide accurate measurements in both mice and rats (Feng et al., 2008). Variability in BP data can be due to time of day, ambient conditions, operator of the CODA 8 machine and handler of the animals (Feng et al., 2008). To try and minimize these effects, tail-cuff measurements were obtained during the day with a randomization in the order mice were measured over the study period. Mice were placed on a warming platform with a warming blanket for consistency in temperature and noise was controlled during measurement sessions. Consistent handlers were used throughout the study period and a total of up to 25 measurements a day were obtained over 3 days within one week for the 5 weeks to be used for analysis. We believed that these methods should increase measurement accuracy and lower variability in overall BP readings.

There has been only one other study to look at the effect of radiation exposure during *in utero* development on offspring BP and HR. Sreetharan et al. (2019) investigated the effect of low-dose radiation on BP and HR in C57Bl/6J mice with the same dose exposures and experimental period as the current study. No effect on BP was observed yet the 1000 mGy male mice showed a significant decrease in HR (Sreetharan et al., 2019). We also found no significant effect of *in utero* radiation exposure on BP but did not find an effect on HR in the BALB/cJ strain. It should be noted that there was a transport effect in the study by Sreetharan et al. (2019), caused by the transport protocol to move and acclimate the mice to the irradiation facility, which complicated the interpretation of the results. Sreetharan et al. (2019) transported pregnant C57Bl/6J mice multiple times to an irradiation facility not connected to the animal holding facilities, to acclimate the animals to the transport, and then used a 20-minute acclimation in room before the irradiation. Sham

controls were found to have a higher SBP, DBP, and MAP than the naïve control (Sreetharan et al., 2019). The current study modified this methodology to transport pregnant mice only once, with a 1-hour acclimation to the irradiation facility. Opposite to Sreetharan et al. (2019), the sham control in the present study had a lower SBP, DBP and MAP then the naïve offspring. This effect was small with less than 10% of a difference in BP parameters between naïve and sham controls. Measurements of MAP in mice can differ by 13% between day and night measurements (Kurtz et al., 2104). As the BP measurements of the sham control were lower than that of the naïve control, and the effect was small, it argues that there was limited or no effect of transportation to the mice in this study.

Cardiovascular measurements of BP and HR showed no difference between sham and treatment groups up to 16 weeks of age in the present study. Other studies on fetal programming through maternal nutritional deficiencies have observed a hypertensive phenotype by this stage. Goyal and Longo (2013) studied maternal protein deprivation in FVB/NJ mice. Elevated BP was observed starting at approximately 10 weeks for female offspring and at 15 weeks for male offspring. Other studies involving a rat model have shown offspring to have an elevated BP from maternal protein deprivation prior to 12 weeks of age (Vehaskari et al., 2001; Cambonie et al., 2007). Using hypoxia stress, programming of hypertension with an elevated MAP was observed in a mouse model at 12 months of age, however, BP measurements were not reported earlier so it is unknown at what time point mice started to show an elevated BP (Walton et al., 2017). The current study used BALB/cJ mice, which are sexually mature at 9 weeks of age (Kempermaann et al., 1997), categorizing these mice as mature adults for majority of the study period. The

current study measured BP and HR during the similar time points as the mentioned studies, with no effect observed on BP and HR up to 16 weeks of age. However, it is important to note that different types of stress may not lead to the same results. It is currently unknown if or when heart disease would be observed after *in utero* radiation exposure in mice. Hypertension is prominent in humans once they are middle aged to older adults (Fryar et al., 2017). The mice in the current study were in the young adult stage (Hagan, 2017). We do not know if changes to cardiovascular health may occur in later stages of adulthood.

Offspring weight was measured from 4 to 16 weeks of age. The 1000 mGy treatment group were significantly smaller then the sham up to 16 weeks of age, showing growth restriction throughout the study period. Birth weight was not obtained, although we would suggest it is likely that birth weight was lower for the 1000 mGy exposure group. This is important to note as low birth weight is an early indicator of fetal programming. Radiation has previously led to growth restriction following *in utero* exposure to high doses of radiation (Otake and Schull, 1998; Kimlet and Norton, 1998; Minamisawa et al., 1990; Sreetharan et al., 2017; Sreetharan et al., 2019).

Weight measurements of mice in the current study were collected before weaning (3-4 weeks) as we did not want to disturb mothers. Sreetharan et al., (2019) reported cannibalism of C57Bl/6J offspring when they were handled prior to weaning, so any contact with offspring was avoided to ensure survival. Due to this, we were unable to determine if there were differences in birth weight between different dose groups. As weight measurements were not collected before 4 weeks of age, it is unknown if treatment groups exposed to low- to mid- dose (≤300 mGy) radiation may have had a low birth

weight. In cases of fetal programming, catch-up growth can be observed in offspring with a low birth weight. Low birth weight offspring will grow at an accelerated rate and grow to normal size (Cianfarani et al., 1999). There is potential that dose groups exposed to low- to mid- dose radiation may have had a low birth weight followed by the catch-up growth during the first 4 weeks. Knowledge of early weights is crucial to further understand if radiation at doses lower than 1000 mGy could also affect offspring growth. Low birth weight and catch-up growth can lead to disturbances in metabolism and may also affect susceptibility of cardiovascular disease (Cianfarani et al., 1999; Meyer and Zhang, 2007).

Bakshi et al. (2016) looked at changes in the heart proteome of mice exposed to radiation *in utero*. There were persistent changes in protein expression for mitochondrial respiratory complexes, redox, and heat shock responses (100 mGy). Protein expression was affected for up to 2 years (Bakshi et al., 2016). Changes to the mitochondrial gene expression are important to note in the heart as the cardiac tissue needs a constant supply of energy and meets this demand by producing energy using the mitochondria. Genes involved in mitochondrial biogenesis, beta oxidation, and the electron transport chain are all important to mitochondrial metabolism and are necessary to meet energy demands of the heart. If there is dysregulation of these genes in the cardiac tissue, there is potential for cardiovascular disease. The mitochondria are believed to be a major source of radiation-induced secondary ROS (Baselet et al., 2016). If there is excess electron leakage in the mitochondria leading to ROS production, this can lead mitochondrial dysfunction and oxidative stress. Thus, we chose to examine the following markers of mitochondrial capacity, a ROS-generating oxidase and a regulator of the oxidative stress response in the

heart tissues: carnitine palmitoyltransferase 1A (CPT1A), NADH dehydrogenase (ubiquinone) 1 beta subcomplex subunit 6 (NDUFB6), mitochondrially encoded cytochrome c oxidase (Mt-Cytb), cytochrome c oxidase subunit 1 (Mt-Co1), peroxisome proliferative activated receptor, gamma, coactivator 1 alpha (PPARGC1A) and uncoupling protein 2 (UCP2), NADPH oxidase (NOX1) and nuclear respiratory factors 2 (NRF2). We found no difference observed in gene expression of these markers in the heart tissue at 17 weeks of age between the sham and 1000 mGy exposure group.

Endothelial dysfunction with differential eNOS expression has been observed in models of *in utero* nutritional restriction. For example, differential eNOS expression has been observed in the aorta of rats exposed to nutritionally restricted diets including protein (Franco et al., 2002; Rodford et al., 2008, Torrens et al., 2012) and zinc restricted (Tomat et al., 2012) diets, and diets with high salt (Piecha et al., 2012). Additionally, rabbits have shown this in response to hypoxia (Chen et al., 2013; Morton et al., 2016). We observed no difference in eNOS expression or in markers of cell adhesion. No signs of endothelial dysfunction were observed by 17 weeks of age in BALB/cJ mice. The aorta of the 1000 mGy male offspring did have a decrease in TNF-α expression which was not expected. Typically, in cases of cardiovascular disease there is an inflammatory response leading to an increase of inflammatory markers such as TNF-α (Dinh et al., 2014). However, low plasma levels of TNF-α have been observed in small for gestational age children in prepubertal stages, in insulin resistant children (Jefferies et al., 2004; Briana and Malamitsi-Puchner, 2009). There is potential that the decrease expression of TNF-α observed in the

current study may be related to the observed growth restriction. Differential TNF-α expression may be related to insulin resistance or other forms of metabolic disease.

In conclusion, we observed no effects of low to mid dose (5-300 mGy) radiation on offspring growth and blood pressure. Growth restriction was observed in male and female offspring exposed to high-dose radiation (1000 mGy). In the heart, there was no observed effect on mitochondrial capacity and regulation of the oxidative stress response. In the aorta, we observed decreased TNF-α expression in male offspring, which may be linked to the growth restriction but was not considered a sign of cardiovascular dysfunction. Future work should determine if there are any effects of *in utero* exposure to radiation on the cardiovascular system during later stages of adulthood (e.g. greater than one year of age). Other signs of common fetal programming diseases such as diabetes and obesity should also be considered. The current study used doses up to 1000 mGy, and a nominal dose rate of 10 mGy/min using a Cesium-137 source. Further studies could consider using different sources or dose rates to further clarify if there is a relationship between radiation exposure *in utero* and fetal programming as there are many variables which may affect result outcomes related to radiation (i.e. age, sex, radiation source). This study helps to provide knowledge on the possible effects of radiation on *in utero* development and suggests that low to mid doses of radiation are unlikely to lead to growth restriction or poor cardiovascular outcomes expected from fetal programming.

2.6 References

Bakshi M V., Azimzadeh O, Merl-Pham J, Verreet T, Hauck SM, Benotmane MA, et al. In-utero low-dose irradiation leads to persistent alterations in the mouse heart proteome. PLoS One. 2016;11(6):1–14

Barker DJ, Osmond C, Golding J, Kuh D, Wadsworth ME. Growth *in utero*, blood pressure in childhood and adult life, and mortality from cardiovascular disease. BMJ. 1989;298(6673):564–7.

Bates D, Mächler M, Bolker B, Walker S. Fitting Linear Mixed-Effects Models Using lme4. J Stat Softw. 2015;67(1).

Brenner DJ, Doll R, Goodhead DT, Hall EJ, Land CE, Little JB, et al. Cancer risks attributable to low doses of ionizing radiation: Assessing what we really know. Proc Natl Acad Sci. 2003;100(24):13761–6.

Briana DD, Malamitsi-Puchner A. Intrauterine growth restriction and adult disease: The role of adipocytokines. Eur J Endocrinol. 2009;160(3):337–47.

Cambonie G, Comte B, Yzydorczyk C, Ntimbane T, Germain N, Lê NLO, et al. Antenatal antioxidant prevents adult hypertension, vascular dysfunction, and microvascular rarefaction associated with *in utero* exposure to a low-protein diet. Am J Physiol Integr Comp Physiol. 2007;292(3):R1236–45.

Chen Y, Wang Z, Chen Z, Lv G, Ferrari M. intermittent maternal hypoxia has an influence on regional expression of endothelial nitric oxide synthase in fetal arteries of rabbits. Pediatr Res. 2013;73(6):706–12.

Chen Y-R, Zweier JL. Cardiac mitochondria and ros generation. Circ Res. 2014;114(3):524–37

Cianfarani S, Germani D, Branca F, Dis A, Fetal C, Ed N. Low birthweight and adult insulin resistance : the " catch-up growth " hypothesis. Arch Dis Child Fetal Neonatal Ed. 1999;81:F71-73.

Dennery PA. Oxidative stress in development: Nature or nurture? Free Radic Biol Med. 2010;49(7):1147–51.

Dinh QN, Drummond GR, Sobey CG, Chrissobolis S. Roles of inflammation, oxidative stress, and vascular dysfunction in hypertension. Biomed Res Int. 2014;2014:406960.

Eriksson JG, Forsen T, Tuomilehto J, Winter PD, Osmond C, Barker DJP. Catch-up growth in childhood and death from coronary heart disease: longitudinal study. BMJ. 1999;318(7181):427–31.

Feng M, Whitesall S, Zhang Y, Beibel M, D'Alecy L, DiPetrillo K. Validation of volume-pressure recording tail-cuff blood pressure measurements. Am J Hypertens. 2008;21(12):1288–91. Godfrey KM, Barker DJ. Fetal programming and adult health. Public Health Nutr. 2001;4(2b) 611-624.

Goyal, R., & Longo, L. D. (2012). Maternal protein deprivation: Sexually dimorphic programming of hypertension in the mouse. Hypertension Research,36(1), 29-35. doi:10.1038/hr.2012.129

Hagan, C. When are mice considered old?. The Jacksons Laboratory. 2017. Available from: https://www.jax.org/news-and-insights/jax-blog/2017/november/when-are-mice-considered-old

Ionizing Radiation Exposure of the Population of the United States (2009). NCRP Report No. 160. Bethesda: National Council on Radiation Protection and Measurements: 2009.

Jackson AA. Nutrients, Growth, and the Development of Programmed Metabolic Function. Adv Exp Med Biol. 2000;478:41–55

Jefferies C, Hofman P, Keelan J, Robinson E, Cutfield W. Insulin resistance is not due to persistently elevated serum tumor necrosis-alpha levels in small for gestational age, premature, or twin children. Pediatr Diabetes. 2004;5(1):20–5.

Kempermann G, Kuhn HG, Gage FH. Genetic influence on neurogenesis in the dentate gyrus of adult mice. Proc Natl Acad Sci U S A. 1997;94:10409–14.

Kwon EJ, Kim YJ. What is fetal programming?: a lifetime health is under the control of *in utero* health. Obstet Gynecol Sci. 2017;60(6):506–19.

Kurtz TW, Lujan HL, DiCarlo SE. The 24 h pattern of arterial pressure in mice is determined mainly by heart rate-driven variation in cardiac output. Physiol Rep. 2014;2(11):1–10.

Lau C, Rogers JM. Embryonic and fetal programming of physiological disorders in adulthood. Birth Defects Res Part C - Embryo Today Rev. 2004;72(4):300–12.

Lowe SA. Diagnostic radiography in pregnancy: Risks and reality. Aust N Z J Obstet Gynaecol. 2004; 44(3):191–196.

Luo ZC, Fraser WD, Julien P, Deal CL, Audibert F, Smith GN, et al. Tracing the origins of "fetal origins" of adult diseases: Programming by oxidative stress? Med Hypotheses. 2006;66(1):38–44.

Meister TA, Rexhaj E, Rimoldi SF, Scherrer U, Sartori C. Fetal programming and vascular dysfunction. Artery Res. 2018;21:69–77.

Meyer K, Lubo Z. Fetal programming of cardiac function and disease. Reprod Sci. 2007;14(3):209–16.

Minamisawa T, Hirokaga K, Sasaki S. Gross morphological changes to the mouse brain exposed prenatally to ionizing radiation. J Radiat Res. 1990;31:214–8.

Miranda JO, Ramalho C, Henriques-Coelho T, Areias JC. Fetal programming as a predictor of adult health or disease: the need to reevaluate fetal heart function. Heart Fail Rev. 2017;22(6):861–77.

Morgan WF, Bair WJ. Issues in Low Dose Radiation Biology: The Controversy Continues. A Perspective. Radiat Res. 2013;179(5):501–10.

Morton JS, Cooke C-L, Davidge ST. *In Utero* Origins of Hypertension: Mechanisms and Targets for Therapy. Physiol Rev. 2016;96(2):549–603.

Rodríguez-rodríguez P, Ramiro-cortijo D, Reyes-hernández CG, Pablo ALL De, González MC, Arribas SM. Implication of Oxidative Stress in Fetal Programming of Cardiovascular Disease. 2018;9:1–13.

Savoia C, Sada L, Zezza L, Pucci L, Lauri FM, Befani A, et al. Vascular inflammation and endothelial dysfunction in experimental hypertension. Int J Hypertens. 2011;2011:1–8.

Siasos G, Tsigkou V, Kosmopoulos M, Theodosiadis D, Simantiris S, Tagkou NM, et al. Mitochondria and cardiovascular diseases—from pathophysiology to treatment. Ann Transl Med. 2018;6(12):256–256.

Sreetharan S, Thome C, Tharmalingam S, Jones DE, Kulesza A V., Khaper N, et al. Ionizing Radiation Exposure During Pregnancy: Effects on Postnatal Development and Life. Radiat Res. 2017;187(6):647–58.

Sreetharan S, Stoa L, Cybulski ME, Jones DE, Lee AH, Kulesza A V., et al. Cardiovascular and growth outcomes of C57Bl/6J mice offspring exposed to maternal stress and ionizing radiation during pregnancy. Int J Radiat Biol. 2019;1–9. Available from: https://doi.org/10.1080/09553002.2019.1589025

Tang FR, Loke WK, Khoo BC. Low-dose or low-dose-rate ionizing radiation-induced bioeffects in animal models. J Radiat Res. 2017;58(2):165–82.

Teixeira BC, Lopes AL, Macedo RCO, Correa CS, Ramis TR, Ribeiro JL, et al. Inflammatory markers, endothelial function and cardiovascular risk. J Vasc Bras. 2014;13(2):108–15.

Tharmalingam S, Sreetharan S, Kulesza A V., Boreham DR, Tai TC. Low-Dose Ionizing Radiation Exposure, Oxidative Stress and Epigenetic Programing of Health and Disease. Radiat Res. 2017;188(4.2):525–38.

Thompson LP, Al-Hasan Y. Impact of oxidative stress in fetal programming. J Pregnancy. 2012;2012.

Vehaskari VM. Prenatal Programming of Hypertension: Lessons from Experimental Models. J Am Soc Nephrol. 2005;16(9):2545–56.

Walton SL, Bielefeldt-Ohmann H, Singh RR, Li J, Paravicini TM, Little MH, et al. Prenatal hypoxia leads to hypertension, renal renin-angiotensin system activation and exacerbates salt-induced pathology in a sex-specific manner. Sci Rep. 2017;7(1):1–13.

Wood, S.N. Fast stable restricted maximum likelihood and marginal likelihood estimation of semiparametric generalized linear models. J R Stat Soc B. 2011;73(1):3-36

2.7 Figures

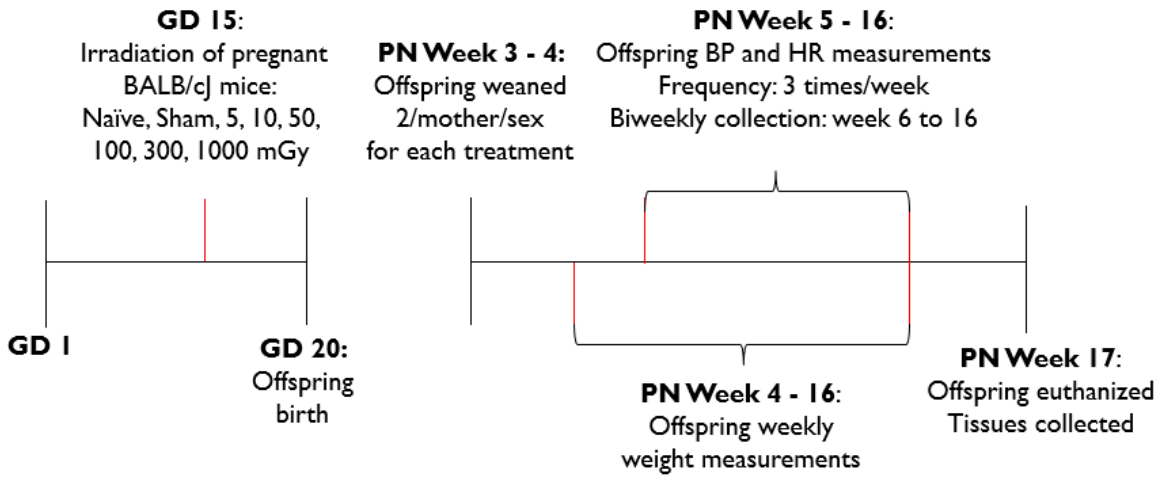


Figure 2. 1. Experimental timeline. Gestational day (GD) 1 represents when males were removed from the females after breeding. On GD15 pregnant BALB/cJ mice were irradiated using Cesium-137. Weight measurements started at 4 weeks of age and blood pressure (BP) and heart rate (HR) measurements started at 5 weeks of age. Both measurements were collected up to 16 weeks. Mice were euthanized and tissues were collected for RT-qPCR analysis at 17 weeks of age. PN = postnatal day.

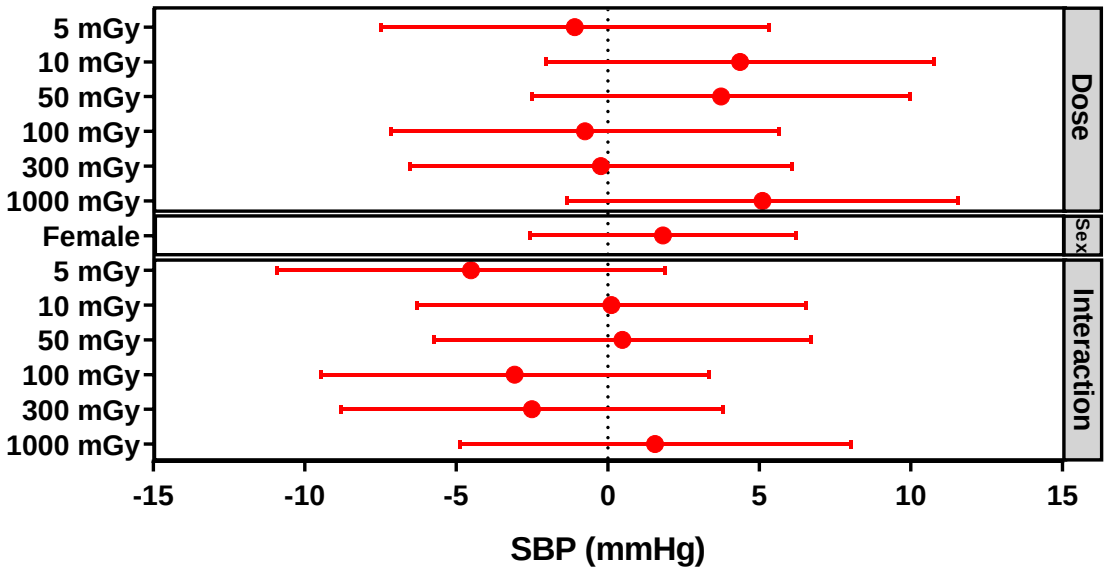


Figure 2. 2.Systolic blood pressure (SBP) comparison between dose treatment groups, sex, and interaction between dose and sex. The dotted line at zero represents the average SBP between males and females for the sham control group. The red lines are depicting 95% confidence intervals of the mean for the model intercept. The top panel (dose) shows the average SBP of males and females for each dose. The middle panel (sex) shows the average female SBP for the sham group. The bottom panel (interaction) shows the interaction between the female sex and dose groups. A generalized linear mixed effects model was used for analysis.

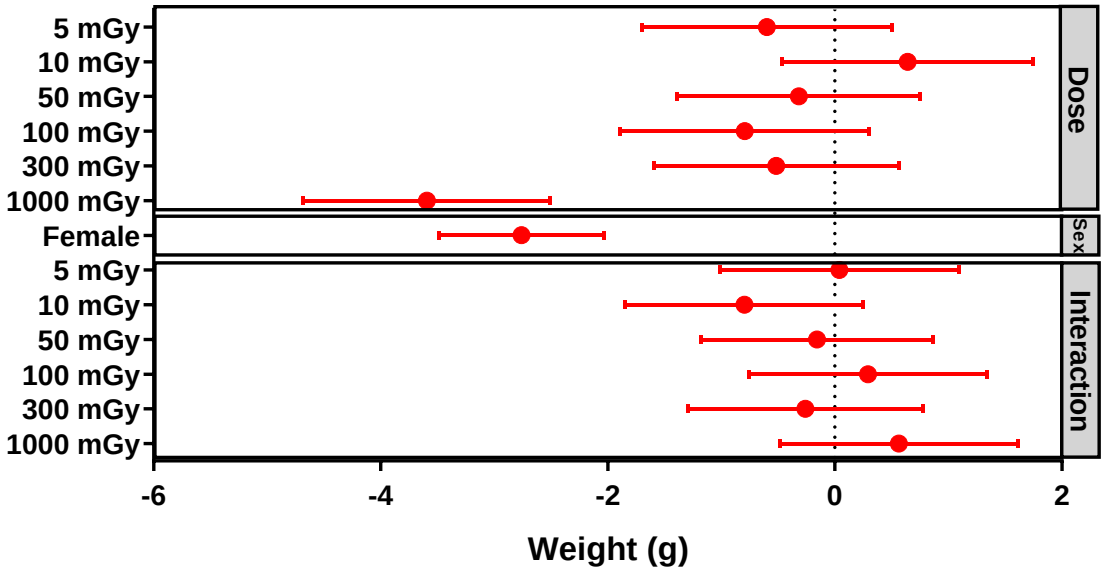


Figure 2. 3. Weight (grams) difference between dose treatment groups, sex, and interaction between dose and sex. The dotted line at zero represents the average weight between males and females for the sham control group. The red lines are depicting 95% confidence intervals for the mean for the model intercept. The top panel (dose) shows the average weight of males and females for each dose. The middle panel (sex) shows the average female weight for the sham group. The bottom panel (interaction) shows the interaction between the female sex and dose groups. A generalized additive model was used to analyze changes in weight over time, which included a random-effects / multilevel component to allow for differences among pups within mother random effects. The 1000 mGy treatment group was significantly smaller than the sham control. Sham females were significantly smaller than sham males.

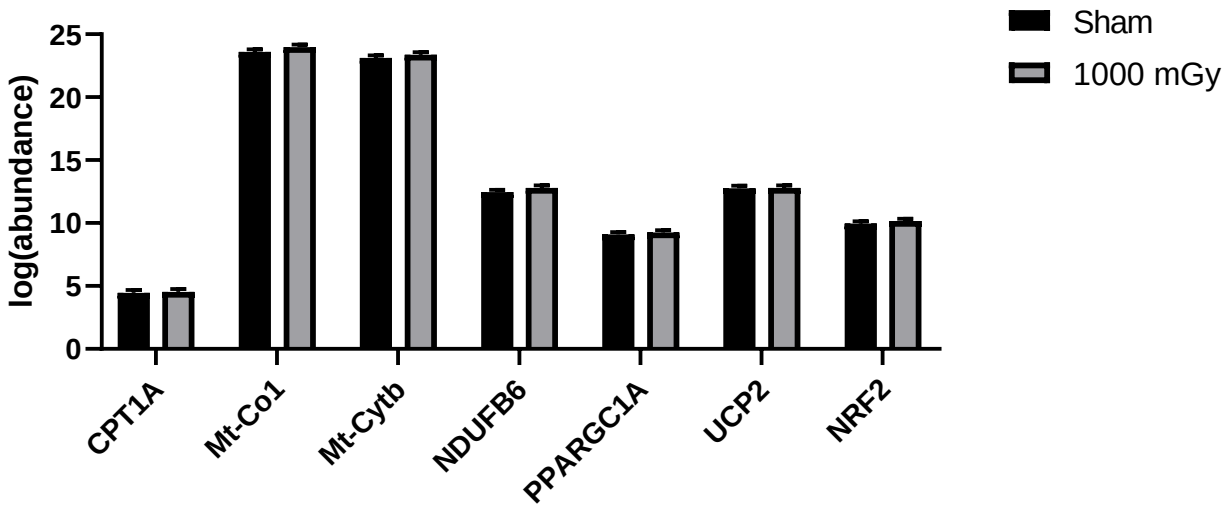


Figure 2. 4. Average mRNA levels (log(abundance)) of male heart tissue for markers of mitochondrial capacity and a regulator of the oxidative stress response. Gene expression comparison between sham and 1000 mGy treatment through Markov Chain Monte Carlo algorithm analysis. Error bars are SD. Abbreviations are defined as: CPT1A, carnitine palmitoyltransferase 1A; Mt-Co1, cytochrome c oxidase subunit 1; Mt-Cytb, mitochondrially encoded cytochrome c oxidase; NDUFB6, NADH dehydrogenase (ubiquinone) 1 beta subcomplex subunit 6; PPARGC1A, peroxisome proliferative activated receptor, gamma, coactivator 1 alpha; UCP2, uncoupling protein 2; NRF2, nuclear respiratory factor 2.

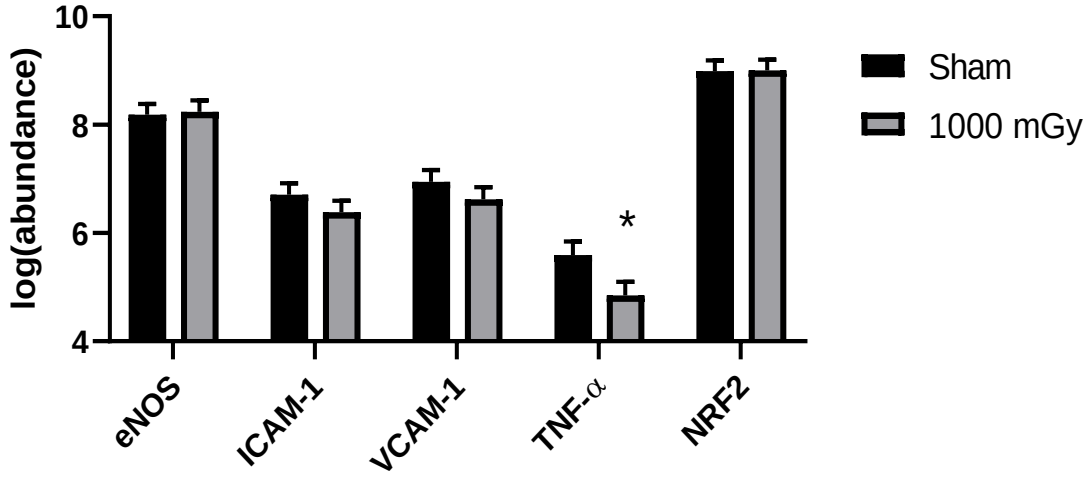


Figure 2. 5. Average mRNA levels (log(abundance)) of male aorta tissue for markers of endothelial dysfunction and a regulator of the oxidative stress response. Gene expression comparison between sham and 1000 mGy treatment through Markov Chain Monte Carlo Analysis. * $p < 0.05$. Error bars are SD. Abbreviations defined as: eNOS, endothelial nitric oxide synthase; ICAM-1, intracellular adhesion molecule; VCAM-1, vascular adhesion molecule- 1; TNF-α, tumour necrosis factor-α; NRF2, nuclear respiratory factor 2.

2.8 Tables

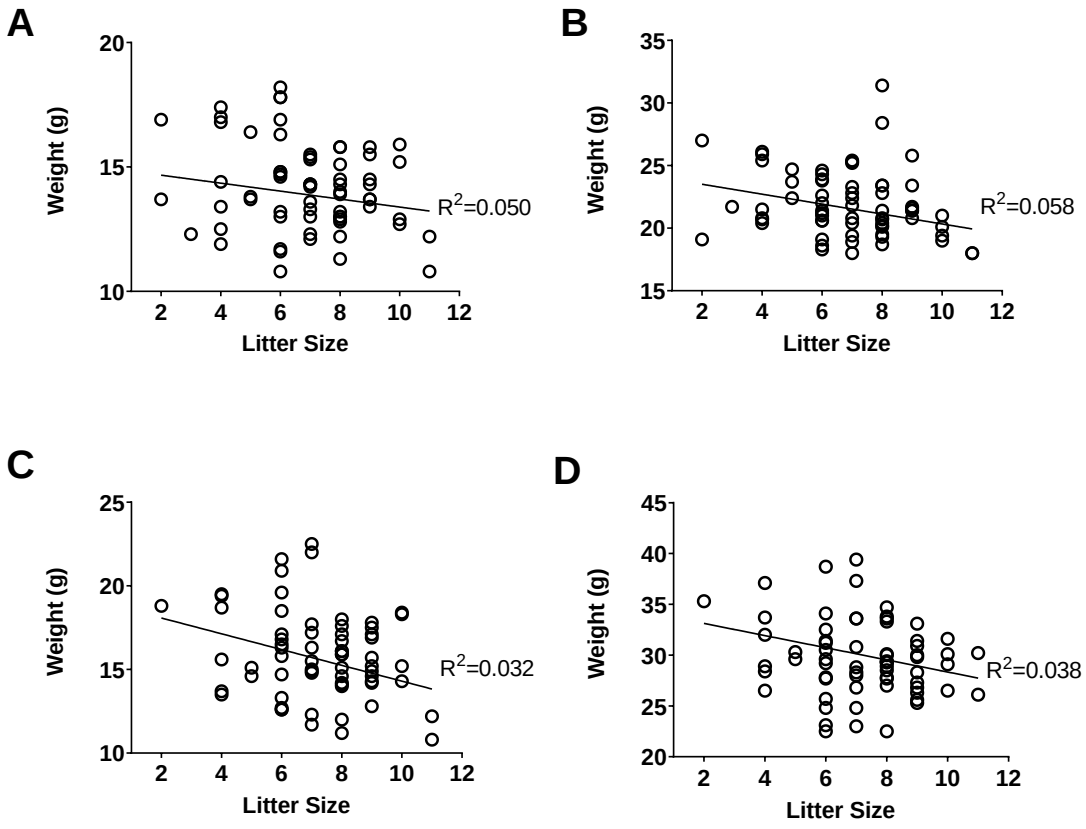
Table 2. 1. Summary of quantitative RT-qPCR oligonucleotide primers used for measurement of mRNA expression in heart. Markers are for mitochondrial capacity, a reactive oxygen species (ROS)-generating oxidase, and a regulator of the oxidative stress response. Abbreviations defined as: F, Forward; R, Reverse; CPT1A, carnitine palmitoyltransferase 1A; NDUFB6, NADH dehydrogenase (ubiquinone) 1 beta subcomplex subunit 6; Mt-Cytb, mitochondrially encoded cytochrome c oxidase; Mt-Co1, cytochrome c oxidase subunit 1; PPARGC1A, peroxisome proliferative activated receptor, gamma, coactivator 1 alpha; UCP2, uncoupling protein 2; NOX1, NADH oxidase 1; NRF2, nuclear respiratory factor 2.

Gene Name	Accession Number	Primer Sequence	Product (bp)	Efficiency
Mitochondrial Capacity				
CPT1A	NM_013495.2	F: TCGGTGAGCCTGGCCT	**85**	**93.05**
		R: TTGAGTGGTGACCGAGTCTG		
NDUFB6	NM_001033305.3	F: AGAACATGGTCTTTAAGGCGT	**139**	**98.52**
		R: ATCCTGGGCTTCGAGCTAAC		
Mt-Cytb	NC_005089.1	F: ACGCAAACGGAGCCTCAATA	**131**	**105.11**
	14145 - 15288	R: TGTGGCTATGACTGCGAACA		
Mt-Co1	NC_005089.1	F: TCGGAGCCCCAGATATAGCA	**145**	**102.69**
	5328 – 6872	R: TTTCCGGCTAGAGGTGGGTA		
PPARGC1A	NM_008904.2	F: TGAAAAAGCTTGACTGGCGTC	**91**	**92.39**
		R: AGCAGCACACTCTATGTCACTC		
UCP2	NM_011671.5	F: TGCGGTCCGGACACAATAG	**108**	**105.92**
		R: GTTCTTCAAAGCTGCCGGTG		
Oxidative Stress Generation and Regulation				
NOX1	NM_172203	F: GGTTGGGGCTGAACATTTTTC	**167**	**91.34**
		R: TCGACACACAGGAATCAGGAT		
NRF2	NM_010902.4	F: CAGCACATCCAGACAGACACCA	**117**	**96.22**
		R: TGGGAATGTCTCTGCCAAAAGCT		

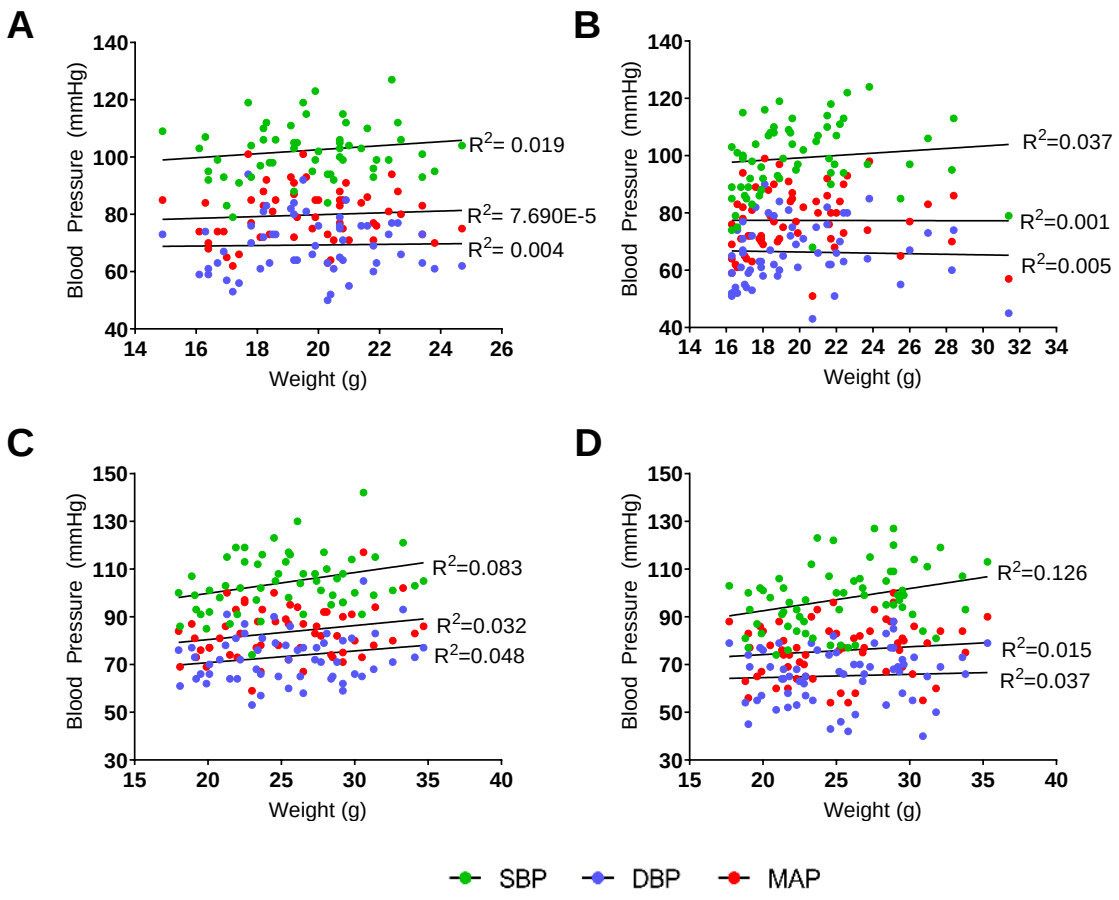
Table 2. 2. Summary of quantitative RT-qPCR oligonucleotide primers used for measurement of mRNA expression in aorta for endothelial dysfunction and a regulator of the oxidative stress response. TNF-α (Yamakawa et al., 2011), VCAM-1 (Lam et al., 2011; Salim et al., 2016; Liu et al., 2017), ICAM-a (Bros et al., 2007; Salim et al., 2016; Liu et al., 2017) and NRF2 (Liessem-Schmitz et al., 2018; Cong et al., 2018; Zhou et al., 2018) primer sequences were obtained from previously published literature. Abbreviations defined as: F, Forward; R, Reverse; eNOS, endothelial nitric oxide synthase; TNF-α, tumour necrosis factor-α; VCAM-1, vascular adhesion molecule- 1; ICAM-1, intracellular adhesion molecule; NRF2, nuclear respiratory factor 2.

Gene Name	Accession Number	Primer Sequence	Product (bp)	Efficiency
Endothelial Dysfunction and Inflammation				
eNOS	NM_008713.4	F: TTTGCTGCCCTTGGCCTGCG	**117**	**91.00**
		R: CTCTGAACTCATGTACCAGCCG		
TNF-α	NM 013693.3	F: GCCTCTTCTCATTCCTGCTTG	**115**	**102.00**
		R: CTGATGAGAGGGAGGCCATT		
VCAM-1	NM_011693.3	F: CCCGTCATTGAGGATATTGG	**186**	**100.80**
		R: GGTCATTGTCACAGCACCAC		
ICAM-1	NM_010493.3	F: TTCACACTGAATGCCAGCTC	**182**	**91.18**
		R: GTCTGCTGAGACCCCTCTTG		
Regulator of the Oxidative Stress Response				
NRF2	NM_010902.4	F: CCCAGCAGGACATGGATTTGA	**106**	**91.10**
		R: AGCTCATAGTCCTTCTGTCGC		

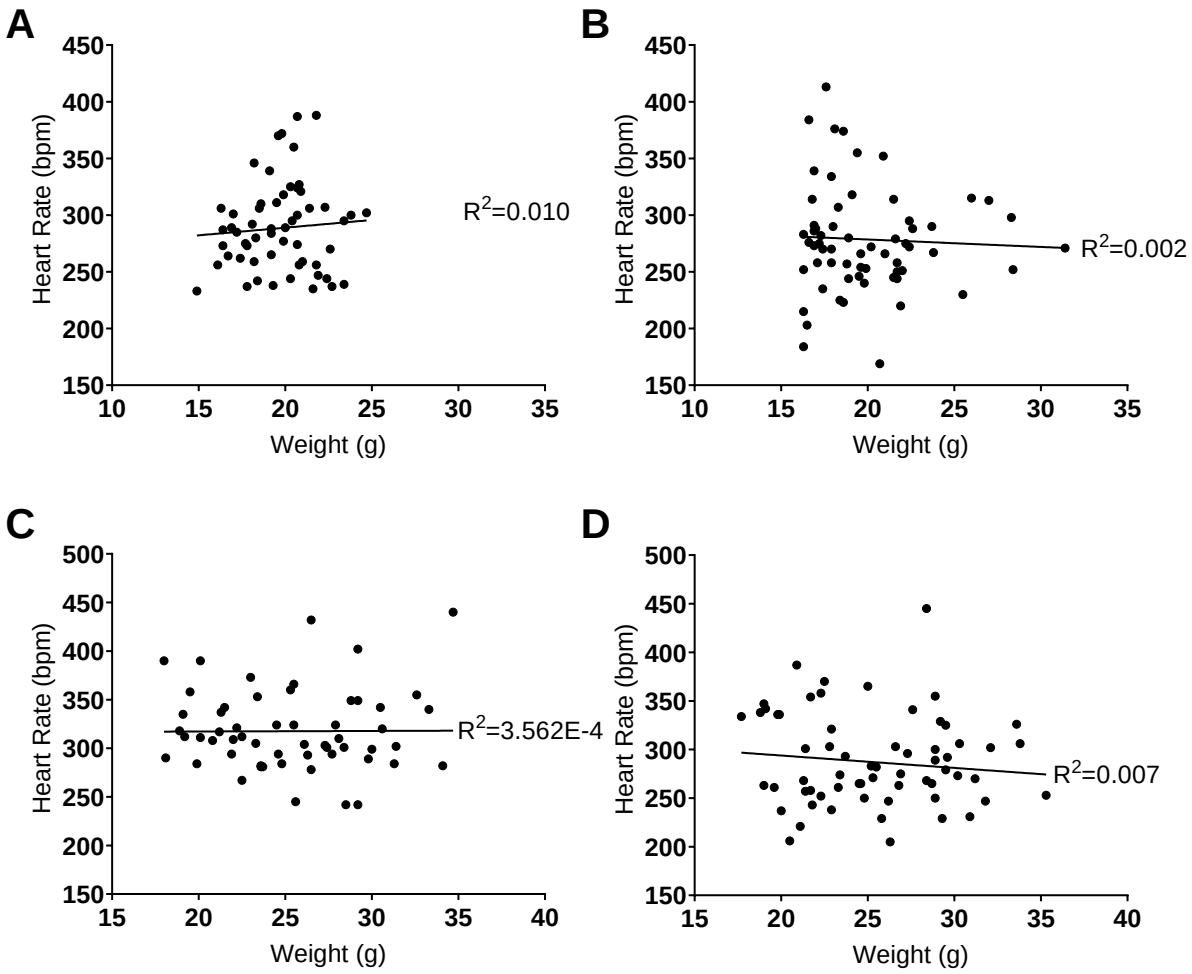
2.9 Supplemental Material



Appendix S2. 1: Weight in grams (g) for female offspring at (A) 4 weeks of age and (B) 16 weeks of age, and male offspring at (C) 4 weeks of age and (D) 16 weeks of age according to litter size. Linear regression showed no significant effect of litter size on offspring weight for either sex.



Appendix S2. 2. Blood pressure (mmHg) measurements for systolic (SBP), diastolic (DBP) and mean arterial pressure (MAP) for female (A) naïve and (B) sham populations and male (C) naïve and (D) sham populations in relation to weight (g). Linear regression showed a significant effect of male body weight on systolic blood pressure, accounting for 8.6% ($F_{1,54}$ = 4.904; p=0.031) of variation in naïve offspring weight and 12.6% ($F_{1,61}$ = 8.761; p=0.004) of variation in the sham offspring weight. Body weight did not show an effect on any other male blood pressure variables and did not account for any variation in female blood pressure.



Appendix S2. 3. Heart Rate (bpm) for female (A) naïve and (B) sham populations and male (C) naïve and (D) sham populations according to weight (g). According to linear regression, there was no significant effect on body weight on the heart rate variable.

Appendix S2. 4. Model estimated intercept (+/-sem) of blood pressure and heart rate measurements for the average between male and female offspring at various irradiation doses between 8 and 16 weeks of age including sample size for each treatment group.

	Naïve	Sham	5 mGy	10 mGy	50 mGy	100 mGy	300 mGy	1000 mGy
N	16	18	16	16	18	16	17	17
Systolic Pressure (mmHg)	**105.71 (3.23)**	97.53 (2.24)	96.42 (3.27)	101.87 (3.27)	101.25 (3.18)	96.76 (3.27)	97.28 (3.22)	102.62 (3.29)
Diastolic Pressure (mmHg)	**73.06 (3.05)**	66.52 (2.27)	65.71 (3.31)	70.83 (3.31)	72.60 (3.22)	63.35 (3.31)	67.09 (3.26)	70.83 (3.33)
Mean Arterial Pressure (mmHg)	**83.72 (3.03)**	76.79 (2.21)	75.59 (3.22)	80.72 (3.22)	81.92 (3.13)	74.19 (3.22)	77.06 (3.17)	81.21 (3.24)
Heart Rate (bpm)	**304.74 (12.154)**	288.90 (8.84)	284.34 (12.81)	311.60 (12.90)	310.83 (12.47)	279.59 (12.90)	290.75 (12.62)	301.99 (12.99)

Chapter 3: Discussions and Conclusions

3.1 Summary of Irradiation Effects

This thesis aims to determine if exposure to low-dose radiation *in utero* during fetal development (E15) affects offspring growth and cardiovascular physiology in the BALB/cJ mouse model. It is currently unknown if *in utero* exposure to ionizing radiation during late gestation would cause an adverse environment which is capable of fetal programming. The experiment meant to determine if there were changes to the cardiovascular system and growth rate of offspring. For cardiovascular effects we measured offspring blood pressure (BP), heart rate (HR), and markers for vascular endothelial dysfunction and mitochondrial capacity. Previous literature has shown that an adverse environment during *in utero* development through environmental exposures (i.e. toxin exposure) and maternal lifestyle (i.e. nutritional deficits, hypoxia, maternal obesity) lead to a low birth weight and predisposed offspring to cardiovascular disease in adulthood (Morton et al., 2016). There have been few studies that have used radiation as a stressor for fetal programming. We hypothesized that maternal exposure to ionizing radiation would cause an adverse intrauterine environment leading to fetal programming of the offspring. Offspring exposed to high doses of radiation would show postnatal growth restriction and cardiovascular disease such as endothelial dysfunction and hypertension in adulthood, with signs of oxidative stress, and changes to heart mitochondrial capacity. Whether these effects would occur at low doses was unknown.

We observed no effects of low- to mid- dose (5-300 mGy) radiation exposure on offspring growth, BP and HR. Only offspring exposed to the highest dose of 1000 mGy showed growth restriction. Due to this, gene expression for the heart and aorta was analyzed

at the 1000 mGy dose compared to sham. There were no effects on gene expression for markers of heart mitochondrial capacity, and a regulator of the oxidative stress response. There was also no change in gene expression in the aorta for endothelial function. A decreased expression of tumour necrosis factor (TNF)-α was observed in the male aorta (1000 mGy) only. This study only observed effects of high-dose ionizing radiation (1000 mGy) on offspring growth and inflammatory cytokine (TNF-α) expression. There were no apparent effects in the low- to mid- dose range (5-300 mGy). This data does not support fetal programming of the cardiovascular system by low-dose radiation.

3.2 Growth Restriction of Offspring

One of the major indicators of fetal programming is a low birth weight (Eriksson et al., 1999; Meyer and Zhang, 2007). During postnatal development, offspring may compensate and go through a process called catch-up growth where they have an increased growth rate and grow to a normal size (Eriksson et al., 1999). Both have been associated with long-term deleterious consequences, leading to an increased susceptibility to cardiovascular diseases (Eriksson et al., 1999; Meyer and Zhang, 2007). Due to this, it is important to measure birth weight and growth rate during postnatal development, as these are early indicators of fetal programming. The effect of high doses of radiation *in utero* on growth can be defined by the timing of exposure during different stages of development, however it is uncertain how low doses of radiation may affect offspring weight and growth rate.

There are three stages of *in utero* development, each stage showing a different response to radiation dependant on the total dose exposure (Hall and Giaccia, 2012). These stages include pre implantation, organogenesis and fetal development. Characteristic effects

include lethality, malformations and growth restrictions (Hall and Giaccia, 2012). Mice and rat offspring exposed to high doses (>100 mGy) of radiation during pre implantation (E1-6) tend to show an all or none response. This means that during this stage there is typically no effect observed or lethality (Valentin, 2003; Schlesinger and Brent,1978; Hall and Giaccia, 2012). During organogenesis (E6-13), the stage where body structures are formed, radiation exposures (>50 mGy) have the highest risk of malformations (Russell and Russell; 1954; Hall and Giaccia, 2012). Additionally, low birth weight and growth restriction are observed (Prakash Hande et al, 1990). Growth restriction may be permanent or temporary with offspring recovering postnatally, depending on the point of organogenesis and the total radiation exposure (Prakash Hande et al, 1990; Hall and Giaccia, 2012). During fetal development (E14-20), malformations are less prominent as offspring are past the stage of organ development. In response to high-dose (>1000 mGy) exposures, there is typically a persistence in growth restriction that can be permanent from birth throughout offspring life (Otake and Schull, 1998; Kimler and Norton, 1988; Minamisawa et al., 1990; Sreetharan et al., 2017; Sreetharan et al., 2019; Hall and Giaccia, 2012). Adverse responses to radiation can be observed to high doses of radiation at all stages of development, however responses to low-dose radiation may show no effect or vary from the observed responses to high doses.

In the current study, offspring were exposed to radiation during fetal development on embryonic day (E) 15. Offspring weight was measured post weaning from 4 to 16 weeks of age. The 1000 mGy treatment group were significantly smaller than the sham group up to 16 weeks of age, showing growth restriction throughout the whole study period. We

assumed this growth restriction indicated a permanent growth reduction of offspring as there were no signs of catch up growth throughout the study period. This is commonly observed from high-dose exposures *in utero* during fetal development (Hossain et al., 1999; Sreetharan et al., 2017; Sreetharan 2019).

The current study showed no effect of low- to mid- dose (<300 mGy) radiation on offspring growth. We did not obtain weight measurements before 4 weeks of age. Due to this, it is uncertain if there were differences in weight between the sham and treatment groups at birth. There is the potential for treatment groups in the low- to mid- dose range (<300 mGy) to have shown low birth weight or growth restriction with catch up growth in these early weeks of development. Hossain et al. (1999) observed a low birth weight in Swiss Albino mice following Cobalt-60 exposures greater than 500 mGy on the 17th day of gestation. The 500 mGy group showed catch up growth within one week of birth. Doses of 1000 mGy and above showed persistent growth restriction (Hossain et al., 1999). This is important to note as both low birth weight in combination with catch up growth are strong indicators of fetal programming. This is known to cause deleterious long-term consequences on health and disease (Meyer and Zhang, 2007; Eriksson et al., 1999; Cianfarani et al., 1999). If possible, future studies should measure birth weight and postnatal growth following *in utero* radiation exposure, to further clarify the effects of low-dose radiation.

3.3 Cardiovascular Effects

Development of hypertension is the most commonly studied cardiovascular disease outcome for fetal programming studies (Morton et al., 2016). Fetal programming has been

observed in response to various stressors including maternal nutrition, preeclampsia, maternal diabetes, maternal obesity, as well as maternal alcohol consumption and smoking. As there are various types of stressors, there are different mechanisms which lead to cardiovascular disease. These can include altered prenatal glucocorticoids, sex steroids, renin angiotensin aldosterone system, oxidative stress, endothelin system, inflammatory cytokines, and epigenetic processes (Alexander, 2006). One of the main systems involved in the development of cardiovascular disease is the vascular system through endothelial dysfunction (Rodriguez-Rodriguez et al., 2018; Meister et al., 2018).

It is still unknown if radiation exposure *in utero* is capable of fetal programming. To determine cardiovascular effects, one technique used in this study was tail cuff plethysmography which measures BP and HR in mice. Tail cuff plethysmography has been observed to provide accurate measurements in both mice and rats (Feng et al., 2008). This method is non-invasive and is a great alternative to other methods of BP measurement such as radiotelemetry. Radiotelemetry typically involves the stress of a surgical implant and is expensive, making tail cuff plethysmography a great alternative (Feng et al., 2008). Tail cuff plethysmography allows the user to obtain BP and HR readings from mice with high throughput screening.

Endothelial dysfunction can be an early indicator of cardiovascular disease (Rodriguez-Rodriguez et al., 2018) and changes in RNA expression in the aorta for markers of endothelial dysfunction have been observed in response to fetal programming of the cardiovascular system (Franco et al., 2002; Tomat et al., 2012; Piecha et al., 2012). To explore changes in endothelial function, qPCR was utilized to examine changes in RNA

expression in the aorta. We also used qPCR to investigate potential changes in gene expression for markers of mitochondrial capacity and a regulator of the oxidative stress response in the heart. Markers of mitochondrial capacity were explored as ionizing radiation is known to cause oxidative stress and can deregulate mitochondrial proteins in the heart (Bakshi et al., 2016; Tharmalingam et al., 2017). Deregulation of genes involved in mitochondrial function can cause the mitochondria to be an active source of ROS, which makes this organelle an important mechanism of cardiovascular disease (Siasos et al, 2018; Chen and Zweier, 2014).

3.3.1 Blood Pressure and Heart Rate

The current study analyzed changes in BP and HR between 8 and 16 weeks of age. We used the BALB/cJ mouse strain, which are sexually mature at 9 weeks of age, categorizing these mice as a mature adult for most of the study period. Mice are a mature adult between 3 and 6 months of age, are middle aged between 10-14 months, and are old between 18-24 months; thus, this study focused on mature but not necessarily old individuals (Hagan, 2017). We observed no difference in BP and HR for mature adult BALB/cJ mice up to 16 weeks of age between the sham control and treatment groups (up to 1000 mGy).

Fetal programming through maternal nutritional deficiencies have predisposed offspring to a hypertensive phenotype, with effects typically observed by 16 weeks of age. Goyal and Longo (2013) studied maternal protein deprivation in FVB/NJ mice. Elevated BP was observed starting at approximately 10 weeks for female offspring and at 15 weeks for male offspring. Other studies involving a rat model have shown offspring to have an

elevated BP from maternal protein deprivation prior to 12 weeks of age (Vehaskari et al., 2001; Cambonie et al., 2007). Using hypoxia stress, programming of hypertension with an elevated MAP was observed in a mouse model at 12 months of age. However, BP measurements were not reported earlier so it is unknown at what time point mice started to show an elevated BP (Walton et al., 2017). We observed no changes in BP and HR between sham and treatment groups up to 16 weeks of age in the present study.

It is currently unknown if or when heart disease would be observed after *in utero* radiation exposure in mice. We do not know if changes to cardiovascular health may occur in later stages of adulthood as cardiovascular disease is typically more prominent in older adults. For example, the prevalence of hypertension in humans increases with age. Majority of reported hypertension in humans occurs above the age of 40. 33.2 % of cases occur in those aged 40-49, and 63.1 % occur in those aged 60 and above (Fryar et al., 2017). When related back to mice, a human age of 40-49 is relatable to a middle-aged mouse (10-14 months) and a human age of 60 is related to an old mouse (18-24) months. In mice, an age-related increase in BP can also be observed. There can be increases of 15-20%, when comparing young mice (5-8 weeks) to middle aged mice (1 year of age) (Wirth et al., 2016). The current study followed mice up to 16 weeks of age (~4 months), which is still in the beginning of middle age adult stages and found no effect. It is unknown if there may have been an effect on BP and HR if we had followed mice until they were older, at stages where hypertension is more prevalent.

There have been a few studies investigating the effect of *in utero* radiation on cardiovascular physiology. Nakashima et al. (2007) studied the effect of exposure to

radiation on the atomic bomb survivors from both Hiroshima and Nagasaki. They observed a positive dose effect for adolescents exposed within the second trimester of gestation for systolic hypertension. This effect was not observed for adolescents exposed during the first and third trimester. There was a range of doses between below 5 mGy and above 1000 mGy, with majority of subjects receiving a dose below 500 mGy (Nakashima et al., 2007). Sreetharan et al. (2019) investigated the effect of exposure to low-dose radiation during fetal development, on BP and HR in C57Bl/6J mice. This study found no effect of *in utero* radiation up to a dose of 300 mGy. At a high dose of 1000 mGy male mice showed a significant decrease in HR along with growth restriction in both sexes. However, it should be noted that there was a transport effect from moving the mice to the irradiation facility, which complicated the interpretation of the results (Sreetharan et al., 2019). Another study by Bakshi et al. (2016) looked at changes in the heart proteome of mice exposed to radiation *in utero* on E11. There were persistent changes to protein expression for mitochondrial respiratory complexes, redox, and heat shock responses (100 mGy). However, this study did not measure BP and HR. These studies suggest that *in utero* exposure to radiation may have the potential for cardiovascular programming of disease through oxidative stress and may be an environmental or clinical stressor capable of fetal programming.

The timing of exposure to radiation *in utero*, animal species or strain, and time that cardiovascular physiology is explored may all influence whether changes in are observed. Sreetharan et al. (2019) and Nakashima et al. (2007) found changes to cardiovascular function when exposures *in utero* were during fetal development (mouse E15, human second trimester respectively) with exposure to high radiation doses. Bakshi et al. (2016)

found differences in the heart proteome at much lower doses (100 mGy) in C57Bl/6J mice exposed to radiation during organogenesis (E11). Exposure to radiation *in utero* has a greater effect on the health of a fetus compared to exposures during adulthood (Hall and Giaccia, 2012). Further studies should explore if there would be changes to cardiovascular physiology in response to low-dose radiation (<100 mGy), if exposure was during earlier time points such as organogenesis when the heart was still forming.

3.3.2 Endothelial Function

The vascular system is one of the main systems involved in the development of cardiovascular diseases including hypertension**.** Early signs of progression of cardiovascular disease include endothelial dysfunction (Rodriguez-Rodriguez et al., 2018; Meister et al., 2018). The current study observed no effects on BP and HR on mice by 16 weeks of age for all dose exposures up to 1000 mGy. As we observed growth restriction at 1000 mGy, we wanted to test for earlier signs of cardiovascular disease progression that may be present at a molecular level in these offspring. Endothelial dysfunction is characterized by an imbalance in the production of mediators important to vascular tone, platelet aggregation, coagulation, and fibrinolysis in the endothelium (Dinh et al., 2014). Vascular inflammation is often implicated, through the induction of vasoconstrictor agents (i.e. NO), adhesion molecules (i.e. ICAM-1, VCAM-1), and growth factors (Savoia et al., 2011). This commonly includes the involvement of oxidative stress (Dinh et al., 2014; Teixeira et al., 2014). When there is an imbalance of these factors, the systemic vasculature becomes stiffer and less distensible due to alterations in the collagen and elastin balance

during disease progression. This can lead to the development of hypertension (Morton et al., 2016).

One of the main mechanisms leading to alterations in the vasculature, is the alterations in the primary vasodilator pathways for nitric oxide (NO) production. NO is a primary vasodilator responsible for smooth muscle relaxation. NO dysregulation plays a major role in the progression of endothelial dysfunction (Dinh et al., 2014). The main enzyme responsible in the production of NO is nitric oxide synthase (NOS). Endothelial dysfunction with differential eNOS expression has been observed in models of *in utero* nutritional restriction. For example, differential eNOS expression and activity have been observed in the aorta of rats exposed to nutritionally restricted diets including protein (Franco et al., 2002; Rodford et al., 2008, Torrens et al., 2012) and zinc restricted (Tomat et al., 2012) diets and diets with high salt (Piecha et al., 2012). Additionally, rabbits have shown differential eNOS expression in fetal carotid and femoral arteries in response to hypoxia (Chen et al., 2013; Morton et al., 2016).

After exposure to ionizing radiation, endothelial cells can show activation and dysfunction (Baselet et al., 2019). In response to ionizing radiation, pro-inflammatory signalling is enhanced and there is dysregulation of adhesion molecules. Radiation has been shown to upregulate the ICAM-1 and vascular adhesion molecule VCAM-1 following irradiation of endothelial cells in a time and dose dependant manner (Sievert et al., 2015; Hallahan et al., 1998; Baselet at al., 2016). Increased expression of these adhesion molecules (i.e. ICAM-1 and VCAM-1) have been observed up to 20 weeks post irradiation (Sievert et al., 2015; Baselet at al., 2016). In a mouse model of atherosclerosis ($ApoE^{-/-}$

mice), even exposure to doses as low as 50 mGy have been observed to lead to differences in expression of VCAM (dose rate 150 mGy/min) and ICAM (1 mGy/min) up to 3 months post irradiation in the mouse heart vasculature, dependent on the dose rate (Mathias et al., 2015). TNF-α shows increased expressed in the plasma for up to 6 months post irradiation (>100 mGy; Mathias et al., 2015).

No signs of endothelial dysfunction were observed by 17 weeks of age in BALB/cJ mice. We observed no difference in the aorta tissue for expression of eNOS and markers of cell adhesion. The 1000 mGy male offspring did have a decrease in TNF-α expression in the aorta which indicates an anti-inflammatory effect. Typically, in cases of cardiovascular disease there is an inflammatory response leading to an increase of inflammatory markers such as TNF-α (Dinh et al., 2014). High doses of radiation are also observed to cause an inflammatory response and upregulation in inflammatory cytokines (Hallahan et al., 1989). One potential explanation is that low plasma levels of TNF-α have been observed in small for gestational age children in prepubertal stages, in insulin resistant children (Jefferies et al., 2004; Briana and Malamitsi-Puchner, 2009). There is potential that the decreased expression of TNF-α observed in the current study may be related to the observed growth restriction. Differential TNF-α expression may be related to insulin resistance or other forms of metabolic disease.

3.3.3 Mitochondria and Oxidative Stress in the Cardiovascular System

Genes involved in beta oxidation, the citric acid cycle, the electron transport chain and mitochondrial biogenesis are important for mitochondrial function. If there is dysregulation of these genes in the cardiac tissue, there is potential for cardiovascular disease. The

mitochondria are believed to be a major source of radiation-induced secondary reactive oxygen species (ROS; Baselet et al., 2016). If there is excess electron leakage in the mitochondria leading to ROS production, this can lead to mitochondrial dysfunction and oxidative stress. This can cause the mitochondria to be an active source of ROS, which makes this organelle an important mechanism of cardiovascular disease (Siasos et al, 2018; Chen and Zweier, 2014). In the current study we quantified gene expression in the heart for genes involved in mitochondrial capacity and a regulator of the oxidative stress response. These included carnitine palmitoyltransferase 1A (CPT1A), NADH dehydrogenase (ubiquinone) 1 beta subcomplex subunit 6 (NDUFB6), mitochondrially encoded cytochrome c oxidase (Mt-Cytb), cytochrome c oxidase subunit 1 (Mt-Co1), peroxisome proliferative activated receptor, gamma, coactivator 1 alpha (PPARGC1A), uncoupling protein 2 (UCP2), and nuclear respiratory factors 2 (NRF2). We chose these markers as indicators of mitochondrial capacity, to determine if radiation exposure *in utero* may affect mitochondrial gene expression in the heart which may indicate the potential of cardiovascular disease.

The first step in mitochondrial metabolism is the break down of its main source of energy, fatty acids, through beta oxidation. Fatty acid oxidation is important in the uptake of long-chain fatty acids in cells for energy homeostasis (Marin-Garcia and Goldenthal, 2002). The mitochondria first transport long-chain fatty acids into the inner mitochondrial membrane using the CPT-1, to undergo beta oxidation resulting in Acetyl-CoA. Acetyl-CoA is then used in a process known as the citric acid cycle which produces the coenzymes

$FADH_2$ and NADH. These are then used by the electron transport chain through the process of oxidative phosphorylation (OXPHOS; Marin-Garcia and Goldenthal, 2002).

OXPHOS produces energy through the electron transport train made up of four complexes: NADH-dehydrogenase (complex I), succinate dehydrogenase, (complex II), ubiquinone, bc_1 complex (complex III), cytochrome c (Cyt *c*) and cytochrome *c* oxidase (CcO; complex IV) and ATP synthase, as well as two electron carriers located in the inner mitochondrial membrane (Bergman and Ben-Shachar, 2016). The electron transport chain is the main producer of ATP. The proper function of each of its complexes is essential for mitochondrial bioenergetics and metabolism.

NDUFB6 is a subunit in complex 1 and is required for electron transfer activity (Loublier et al., 2011). It is located on the inner membrane of the mitochondria and transfers electrons from NADH to the respiratory chain (Mimaki et al., 2012). Mt-Cytb is part of complex III and is one of the proteins which make up the catalytic core. It plays a fundamental role in the energy production of the mitochondria. This enzyme catalyses electron transfer from ubiquinol to cytochrome c coupled to translocation of protons across the membrane (Blakely et al., 2005). Mt-Co1 is the main subunit of complex IV and is the subunit which catalyzes the reduction of oxygen to water. When there are impairments of this complex it can result in ROS intermediates promoting oxidative stress (Lloyd and McGeehan, 2013). Dysfunction of these genes has been associated with mitochondrial diseases which may lead to cardiovascular disease (Mimaki et al., 2011; Holvoet et al., 2016; Jarreta et al., 2000).

Mitochondrial number or biogenesis is regulated in response to environmental stimuli (Sanchis-Gomar et al., 2014). One of the main regulators in the heart is PPARGC1A (Bhatti et al., 2017). It is a co-transcriptional regulation factor that interacts with various transcription factors and proteins including mitochondrial transcription factor A, uncoupling proteins (UCP2) and nuclear respiratory factors 1 and 2. These are all important in the process of mitochondrial biogenesis (Bhatti et al., 2017). Of the mentioned transcription factors, UCP2 and NRF2 are not only important for mitochondrial biogenesis but also for the regulation of ROS (Mailloux and Harper, 2011; Ball et al., 2011). UCP proteins are anion carriers located in the inner mitochondrial membrane. They regulate membrane potential, which is important for the oxidation and reduction reactions performed in the electron transport chain (Laskowski and Russell, 2008). UCP2 also minimizes ROS produced from the electron transport chain. Whereas, NRF2 is a major regulator of the oxidative stress response by regulating the antioxidant defense system. It is important in the regulation of functions such as autophagy, inflammation and inflammasome signalling, ER stress and the unfolded protein response, as well as, apoptosis and mitochondrial biogenesis (Ma, 2013). The action of theses genes is important in regulating mitochondrial biogenesis and ROS, which is important in maintaining production of ATP while avoiding cellular damage.

Changes to mitochondrial gene expression in the heart is important to explore as the cardiac tissue needs a constant supply of energy, so proper function of mitochondrial complexes is important. We found no difference observed in gene expression for markers

of mitochondrial capacity, and regulation of the oxidative stress response in the heart at 17 weeks of age of mice in the study.

3.4 Conclusions and Future directions

This thesis aimed to determine if exposure to low-dose radiation *in utero* during fetal development (E15) would affect offspring growth and cardiovascular physiology in the BALB/cJ mouse model. Dose exposures between 5 to 1000 mGy with a nominal dose rate of 10 mGy/min using a Cesium-137 source were explored in the current study. The exposure was during fetal development (E15). We observed no effects of low to mid dose (5-300 mGy) radiation on offspring growth, BP and HR. Growth restriction was observed in male and female offspring exposed to high-dose radiation (1000 mGy). In the heart, there was no observed effect on genes involved in mitochondrial capacity and a regulator of the oxidative stress response. In the aorta, we observed decreased TNF-α expression in male offspring of the 1000 mGy treatment. Further studies could consider using different types of radiation, different dose rates, or an exposure during different points of development to further clarify if radiation is capable of fetal programming. There are many variables which may affect result outcomes related to radiation. Risk is highly dependent on the type of animal, its susceptibility to exposure, age, sex, total dose exposure and the type of radiation exposure (ie. photon, positron, proton; Tang et al., 2017).

Radiation dose, dose rate and type of radiation play a large role in effects observed after exposure. We used a total dose of 1000 mGy. Doses up to 2000 mGy have been observed to not cause lethality in offspring (Russell and Russell, 1954). Higher doses could be tested to determine if the dose used in the current study may not have been high enough

to program cardiovascular disease. In our study, we used a nominal dose rate of 10 mGy/min. Dose rate can have a large effect in observed responses. Mathias et al., found different response in inflammatory and adhesion molecule responses dependent on the dose rate in the mouse heart. High dose rates may lead to a stronger phenotype. When considering different types of radiation there may also be different cellular responses. For example, X-rays may cause distinct physiologic responses to Cesium-137 (Gibson et al., 2015). There is potential that the threshold for physiological responses from X-rays may be lower than Cesium-137 (Sreetharan et al., 2017).

The current study explored radiation exposure *in utero* on fetal development (E15) using a Cesium-137 source. By E15.5 the definitive external prenatal configuration of the heart is complete, and the main developmental events left are modifications of the atrioventricular valve leaflets and coronary arteries. At this point there are only minor modifications occurring in the cardiovascular system (Savolainen et al., 2009). Radiation exposure has been observed to have more impact on offspring when exposure is during earlier stages of development during organogenesis. During this stage, high doses of radiation have been observed to lead to malformations, growth restriction, and changes in postnatal behaviour (Hall and Giaccia et al, 2012). It would be interesting to explore how exposure to low-dose radiation would affect offspring when exposed earlier in gestation such as during organogenesis when the heart is still forming. The cardiovascular system may show a stronger response to low-dose radiation at earlier development. During organogenesis, growth restriction may be temporary, where offspring may be capable of increasing growth rate to grow to normal size (Hall and Giaccia, 2012). Catch-up growth

along with low birth rate can both be mechanisms observed with fetal programming and can lead to cardiovascular disease.

The age of exposure to radiation is important, however it is also important to consider the age which disease is tested for. Future work should determine if there are any effects of *in utero* exposure to radiation on the cardiovascular system during later stages of adulthood (e.g. greater than one year of age). Using hypoxia stress, programming of hypertension with an elevated MAP was observed in a mouse model by 12 months of age (Walton et al., 2017). It is currently unknown if or when heart disease would be observed after *in utero* radiation exposure in mice. We do not know if changes to cardiovascular health may occur in later stages of adulthood as cardiovascular disease is typically more prominent in older adults (Fryar et al., 2017; Wirth et al., 2016). The current study followed mice up to 16 weeks of age (~4 months) which is still in the earlier adult stages and found no effect. It is unknown if there may have been an effect on BP and HR if we had followed mice until they were middle aged or older, at stages where hypertension is normally observed.

This thesis found that there were no observable effects of low-dose radiation *in utero* on growth or cardiovascular physiology. We found a growth reduction at 1000 mGy for both male and female offspring, along with a decreased expression of TNF-α in male aortas. Considering fetal programming outcomes, we found a growth restriction at a high dose which may indicate disease later in life. We did not observe any phenotype up until early adulthood in mature BALB/cJ mice. It would be beneficial to extend the study to include middle aged mice, while also exploring other fetal programming diseases such as diabetes and other signs of metabolic syndrome. This study helps to provide knowledge on the

possible effects of radiation on *in utero* development and suggests that low- to mid- doses of radiation are unlikely to lead to growth restriction or poor cardiovascular outcomes expected from fetal programming.

3.5 References

Alexander BT, Dasinger JH, Intapad S. Fetal Programming and Cardiovascular Pathology. In: Comprehensive Physiology. 2015;5(2);997-1025

Bakshi M V., Azimzadeh O, Merl-Pham J, Verreet T, Hauck SM, Benotmane MA, et al. In-utero low-dose irradiation leads to persistent alterations in the mouse heart proteome. PLoS One. 2016;11(6):1–14

Ball WB, Kar S, Mukherjee M, Chande AG, Mukhopadhyaya R, Das PK. Uncoupling Protein 2 Negatively Regulates Mitochondrial Reactive Oxygen Species Generation and Induces Phosphatase-Mediated Anti-Inflammatory Response in Experimental Visceral Leishmaniasis. J Immunol. 2011;187:1322–32.

Baselet B, Rombouts C, Benotmane AM, Baatout S, Aerts A. Cardiovascular diseases related to ionizing radiation: The risk of low-dose exposure (Review). Int J Mol Med. 2016;38(6):1623–41.

Bergman O, Ben-Shachar D. Mitochondrial oxidative phosphorylation system (OXPHOS) deficits in schizophrenia: Possible interactions with cellular processes. Can J Psychiatry. 2016;61(8):457–69.

Bhatti JS, Bhatti GK, Reddy PH. Mitochondrial dysfunction and oxidative stress in metabolic disorders — A step towards mitochondria based therapeutic strategies. Biochim Biophys Acta. 2017;1863(5):1066–77.

Blakely EL, Mitchell AL, Fisher N, Meunier B, Nijtmans LG, Schaefer AM, et al. A mitochondrial cytochrome b mutation causing severe respiratory chain enzyme deficiency in humans and yeast. FEBS J. 2005;272:3583–92.

Briana DD, Malamitsi-Puchner A. Intrauterine growth restriction and adult disease: The role of adipocytokines. Eur J Endocrinol. 2009;160(3):337–47.

Chen Y, Wang Z, Chen Z, Lv G, Ferrari M. intermittent maternal hypoxia has an influence on regional expression of endothelial nitric oxide synthase in fetal arteries of rabbits. Pediatr Res. 2013;73(6):706–12.

Chen Y-R, Zweier JL. Cardiac mitochondria and ros generation. Circ Res. 2014;114(3):524–37

Cianfarani S, Germani D, Branca F, Dis A, Fetal C, Ed N. Low birthweight and adult insulin resistance : the " catch-up growth " hypothesis. Arch Dis Child Fetal Neonatal Ed. 1999;81:F71-73.

Dinh QN, Drummond GR, Sobey CG, Chrissobolis S. Roles of inflammation, oxidative stress, and vascular dysfunction in hypertension. Biomed Res Int. 2014;2014:406960.

Eriksson JG, Forsen T, Tuomilehto J, Winter PD, Osmond C, Barker DJP. Catch-up growth in childhood and death from coronary heart disease: longitudinal study. BMJ. 1999;318(7181):427–31.

Feng M, Whitesall S, Zhang Y, Beibel M, D'Alecy L, DiPetrillo K. Validation of volume-pressure recording tail-cuff blood pressure measurements. Am J Hypertens. 2008;21(12):1288–91. Godfrey KM, Barker DJ. Fetal programming and adult health. Public Health Nutr. 2001;4(2b) 611-624.

Fryar CD, Ostchega Y, Hales CM, Zhang G, Kruszon-Moran D. Hypertension Prevalence and Control Among Adults: United States, 2015-2016. NCHS Data Brief. 2017;289.

Goyal, R., & Longo, L. D. (2012). Maternal protein deprivation: Sexually dimorphic programming of hypertension in the mouse. Hypertension Research,36(1), 29-35. doi:10.1038/hr.2012.129

Hagan, C. When are mice considered old?. The Jacksons Laboratory. 2017. Available from: https://www.jax.org/news-and-insights/jax-blog/2017/november/when-are-mice-considered-old

Hall EJ, Giaccia AJ. Radiobiology for the radiobiologist. 7th ed. Philadelphia: Wolters Kluwer Health/Lippincott Williams & Wilkins; 2012.

Hallahan DE, Virudachalam S, Kuchibhotla J. Nuclear factor κB dominant negative genetic constructs inhibit X-ray induction of cell adhesion molecules in the vascular endothelium. Cancer Res. 1998;58(23):5484–8.

Holvoet P, Vanhaverbeke M, Bloch K, Baatsen P, Sinnaeve P, Janssens S. Low MT-CO1 in monocytes and microvesicles is associated with outcome in patients with coronary artery disease. J Am Heart Assoc. 2016;5(12):1–13.

Hossain PM, Uma Devi P, Bisht KS. Effect of Prenatal Gamma Irradiation During the Late Fetal Period on the Postnatal Development of the Mouse. Teratology. 1999;59:133–8.

Jarreta D, Orus J, Barrientos A, Miro O, Roig E, Heras M, et al. Mitochondrial function in heart muscle from patients with idiopathic dilated cardiomyopathy. Cardiovasc Res. 2000;45:860–8655.

Jefferies C, Hofman P, Keelan J, Robinson E, Cutfield W. Insulin resistance is not due to persistently elevated serum tumor necrosis-alpha levels in small for gestational age, premature, or twin children. Pediatr Diabetes. 2004;5(1):20–5.

Kimler BF, Norton S. Behavioral changes and structural defects in rats irradiated *in utero*. Int J Radiat Oncol Biol Phys. 1988;15(5):1171–7.

Laskowski KR, Russell RR. Uncoupling proteins in heart failure. Curr Heart Fail Rep. 2008;5(2):75–9.

Lloyd RE, McGeehan JE. Structural Analysis of Mitochondrial Mutations Reveals a Role for Bigenomic Protein Interactions in Human Disease. PLoS One. 2013;8(7).

Loublier S, Bayot A, Rak M, El-khoury R, Bénit P, Rustin P. Biochemical and Biophysical Research Communications The NDUFB6 subunit of the mitochondrial respiratory chain complex I is required for electron transfer activity : A proof of principle study

on stable and controlled RNA interference in human cell lines. Biochem Biophys Res Commun. 2011;414(2):367–72.

Ma Q. Role of Nrf2 in Oxidative Stress and Toxicity. Annu Rev Pharmacol Toxicol. 2013;53:401–26.

Mailloux RJ, Harper ME. Mitochondrial proticity and ROS signaling: Lessons from the uncoupling proteins. Trends Endocrinol Metab. 2012;23(9):451–8.

Marín-García J, Goldenthal MJ. Fatty acid metabolism in cardiac failure: biochemical, genetic and cellular analysis. Cardiovasc Res. 2002;54:516–27.

Mathias D, Mitchel REJ, Barclay M, Wyatt H, Bugden M, Priest ND, et al. Low-dose irradiation affects expression of inflammatory markers in the heart of ApoE -/- Mice. PLoS One. 2015;10(3):1–19.

Meister TA, Rexhaj E, Rimoldi SF, Scherrer U, Sartori C. Fetal programming and vascular dysfunction. Artery Res. 2018;21:69–77.

Meyer K, Zhang, L. Fetal programming of cardiac function and disease. Reprod Sci. 2007;14(3):209–16.

Mimaki M, Wang X, McKenzie M, Thorburn DR, Ryan MT. Understanding mitochondrial complex I assembly in health and disease. Biochim Biophys Acta - Bioenerg. 2012;1817(6):851–62.

Minamisawa T, Hirokaga K, Sasaki S. Gross morphological changes to the mouse brain exposed prenatally to ionizing radiation. J Radiat Res. 1990;31:214–8.

Morton JS, Cooke C-L, Davidge ST. *In Utero* Origins of Hypertension: Mechanisms and Targets for Therapy. Physiol Rev. 2016;96(2):549–603.

Morton JS, Cooke C-L, Davidge ST. *In Utero* Origins of Hypertension: Mechanisms and Targets for Therapy. Physiol Rev. 2016;96(2):549–603.

Nakashima E, Akahoshi M, Neriishi K, Fujiwara S. Systolic blood pressure and systolic hypertension in adolescence of atomic bomb survivors exposed *in utero*. Radiat Res. 2007;168(5):593–9.

Otake M, Schull WJ. Review: Radiation-related brain damage and growth retardation among the prenatally exposed atomic bomb survivors. Int J Radiat Biol. 1998;74(2):159–71.

Prakash Hande M, Uma Devi P, Jagetia GC. Effect of "*In Utero*" Exposure to Low Doses of Low Energy X-ryas on the Postnatal Development of the Mouse. J Radiat Res. 1990;31:354–60.

Rodríguez-rodríguez P, Ramiro-cortijo D, Reyes-hernández CG, Pablo ALL De, González MC, Arribas SM. Implication of Oxidative Stress in Fetal Programming of Cardiovascular Disease. 2018;9:1–13.

Russell LB, Russell WL. An analysis of the changing radiation response of the developing mouse embryo. J Cell Physiol Suppl. 1954;43:103–49.

Sanchis-Gomar F, Luis Garcia-Gimenez J, Carmen Gomez-Cabrera M, Pallardo, FV. Mitochondrial biogenesis in health and disease. Molecular and therapeutic approaches. Curr Pharm Design. 2014;20(35);5619-5633.

Savoia C, Sada L, Zezza L, Pucci L, Lauri FM, Befani A, et al. Vascular inflammation and endothelial dysfunction in experimental hypertension. Int J Hypertens. 2011;2011:1–8.

Schlesinger DM, Brent RL. Effects of X Irradiation during Preimplantation Stages of Gestation on Cell Viability and Embryo Survival in the Mouse. Radiat Res. 1978;75(1):202–16.

Siasos G, Tsigkou V, Kosmopoulos M, Theodosiadis D, Simantiris S, Tagkou NM, et al. Mitochondria and cardiovascular diseases—from pathophysiology to treatment. Ann Transl Med. 2018;6(12):256–256.

Sievert W, Trott KR, Azimzadeh, O, Tapio, S, Zitzelsberger H, Multhoff G. Late proliferating and inflammatory effects on murine microvascular heart and lung endothelial cells after irradiation. Radiother Oncol. 2015; 117:376-381.

Sreetharan S, Stoa L, Cybulski ME, Jones DE, Lee AH, Kulesza A V., et al. Cardiovascular and growth outcomes of C57Bl/6J mice offspring exposed to maternal stress and ionizing radiation during pregnancy. Int J Radiat Biol. 2019;1–9. Available from: https://doi.org/10.1080/09553002.2019.1589025

Sreetharan S, Thome C, Tharmalingam S, Jones DE, Kulesza A V., Khaper N, et al. Ionizing Radiation Exposure During Pregnancy: Effects on Postnatal Development and Life. Radiat Res. 2017;187(6):647–58.

Teixeira BC, Lopes AL, Macedo RCO, Correa CS, Ramis TR, Ribeiro JL, et al. Inflammatory markers, endothelial function and cardiovascular risk. J Vasc Bras. 2014;13(2):108–15.

Thompson LP, Al-Hasan Y. Impact of oxidative stress in fetal programming. J Pregnancy. 2012;2012.

Valentin J, Cox R, Streffer C. Biological effects after prenatal irradiation (embryo and fetus). Elsevier Ltd. 2003.

Vehaskari VM. Prenatal Programming of Hypertension: Lessons from Experimental Models. J Am Soc Nephrol. 2005;16(9):2545–56.

Walton SL, Bielefeldt-Ohmann H, Singh RR, Li J, Paravicini TM, Little MH, et al. Prenatal hypoxia leads to hypertension, renal renin-angiotensin system activation and exacerbates salt-induced pathology in a sex-specific manner. Sci Rep. 2017;7(1):1–13.

Wirth A, Wang S, Takefuji M, Tang C, Althoff TF, Schweda F, et al. Age-dependent blood pressure elevation is due to increased vascular smooth muscle tone mediated by G-protein signalling. Cardiovasc Res. 2016;109:131–40.