THE INFLUENCE OF EARLY LIFE UNDERNUTRITION ON MOUSE MAXIMAL TREADMILL RUNNING CAPACITY IN ADULTHOOD

By

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A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

Kinesiology—Master of Science

2019

ABSTRACT

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Introduction: Undernutrition during early life causes chronic disease with specific impairments to the heart and skeletal muscle. Purpose: To determine the effects of early-life undernutrition on adult exercise capacity as a result of cardiac and skeletal muscle function. **Methods:** Pups were undernourished during gestation (GUN) or lactation (PUN) using a cross-fostering nutritive mouse model. At postnatal day 21 (PN21), all mice were weaned and refed a control diet. At PN67, mice performed a maximal treadmill test. Echocardiography and Doppler velocity analysis was performed at PN72, following which skeletal muscle cross-sectional area (CSA) and fiber type were determined. **Results:** Maximal running capacity was reduced (Diet: P=0.0002) in GUN and PUN mice. Left ventricular mass (Diet: P=0.03) and posterior wall thickness during systole (Diet*Sex: P=0.03) of GUN and PUN mice was reduced, causing PUN mice to have reduced (Diet: P=0.04) stroke volume (SV). Heart Rate (HR) of GUN mice showed a trend (Diet: P=0.07) towards greater resting values than other groups. PUN mice had greater CSA of SOL fibers. PUN had a reduced (Diet: P=0.03) proportion of type-IIX fibers in the EDL and a greater (Diet: P=0.008) percentage of type-IIB fibers in the EDL. **Conclusion:** Gestational and Postnatal undernourishment impairs exercise capacity.

ACKNOWLEDGEMENTS

The authors would like to thank Eric C. Leszczynski, Joseph R. Visker, Ashley N. Triplett, Elizabeth Shimoura, Emily Guy, and David P. Ferguson of the Neonatal Nutrition and Exercise Research Lab at Michigan State University; Karin Pfeiffer and Katharine Currie from the Department of Kinesiology; and the Michigan State University InVivo Animal Core Facility for their help in this project. The project was funded by startup funds from the Department of Kinesiology.

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KEY TO ABBREVIATIONS

AET	Aortic ejection time
A-vO_2 diff	Arteriovenous Oxygen Difference
BPM	Beats Per Minute
BSA	Body Surface Area
CON	Control Group
CSA	Cross-sectional Area
CVD	Cardiovascular Disease
CRF	Cardiorespiratory Fitness
DIA	Diaphragm
DOHaD	Developmental Origins of Health and Disease
EDL	Extensor Digitorum Longus
GUN	Gestational Undernourished Group
HR	Heart Rate
IVCT	Left ventricle isovolumetric contraction time
IVRT	Isovolumetric relaxation time.
LP	Low-Protein
LV	Left Ventricle
LVID; d	Left ventricle internal diameter (diastole).
LVID;s	Left ventricle internal diameter at systole
MV A	Mitral Valve late filling velocity.
MV E	Mitral Valve early filling velocity.
MVDT	Mitral valve deceleration time.
PUN	Postnatal Undernourished Group
Q	Cardiac Output
SOL	Soleus
SGA	Small for Gestational Age
SV	Stroke Volume
VO_2Max	Maximal Oxygen Consumption

INTRODUCTION

More than 30 years ago, Dr. David Barker elucidated a positive correlation between low birth weight and death from ischemic heart disease in adulthood (Barker et al. 1993; Barker et al. 1986; Barker et al. 1989). Following further epidemiological investigation , the "Developmental Origins of Health and Disease" (DOHaD) hypothesis was proposed, stating that brief exposure to a suboptimal nutritive environment in early life can yield physiological programing effects that increase the incidence of chronic disease. While initial observations centered on ischemic heart disease and later type II diabetes, evidence shows that hypertension, sarcopenia, and atherosclerosis risks are elevated among growth restricted individuals (Hennington et al. 2013; Menendez-Castro et al. 2018; Sayer et al. 2008). Some evidence also indicates that growth restriction increases cognitive impairments and cancer risk (Hartkopf et al. 2018; Milne et al. 2009) as well.

High Cardiorespiratory fitness (CRF) has been consistently linked to short- and long-term reductions of cardiovascular disease risk (Gupta et al. 2011). Substrate provisions (oxygen, amino acids, calories, etc.) during pre- and post-natal life is an important regulator of cardiovascular and muscular development, as restriction of vital metabolic components compromises function in adulthood. The function of these contractile tissues is essential to cardiorespiratory fitness (CRF), as they contribute in the delivery and uptake of oxygen to meet demands. In particular, CRF relies on proper maintenance of heart rate (chronotropy), stroke volume (contractility), and muscular function (oxygen uptake and force production). High resting heart rate negatively affects exercise capacity, as maximal heart rate is fixed as a function of age—thus limiting chronotropic increase capacity during exercise (Laukkanen et al. 2009; Nauman et al. 2012); undernutrition during the gestational period elicits high resting heart rate and stress sensitivity in offspring (Augustyniak et al. 2010; Johansson et al. 2007; Ozanne et al.

1999; Petry et al. 2000; Phillips et al. 1997; Tonkiss et al. 1998). Stroke volume is influenced by inotropic strength from left ventricular mass, which is reduced in female mice subjected to postnatal undernutrition (Ferguson et al. 2019). Fiber type and cross-sectional area (CSA) are central factors that are associated with oxygen uptake for a myofiber, with type-I fibers and low CSA associated with increased oxygen uptake capacity (Liu et al. 2012b). Skeletal muscle mass has shown to be reduced with undernourishment during early postnatal life (Fiorotto et al. 2014); however, there is a paucity of research on the effects of early life undernutrition on skeletal muscle fiber type.

Taken together, the combination of cardiovascular and muscular impairments from early life undernutrition could reduce exercise capacity. This reduction in exercise capacity may translate to an additional risk for chronic disease in adulthood. Therefore, understanding the parameters that reduce exercise capacity in the growth restricted population will allow for the development of therapeutic countermeasures to reduce chronic disease risk. These countermeasures may include development of exercise protocols to consider cardiac and muscular limitations in growth-restricted individuals or pharmaceutical interventions to mitigate growth-restricted induced impairments. Prior to deployment of these countermeasures, the crucial first step is to evaluate primary parameters (heart and skeletal muscle) influencing exercise capacity in growth-restricted mice.

We hypothesize that exercise capacity will be reduced in mice that experience growth restriction caused by early life undernutrition. To test our hypothesis we have developed the following aims with specific sub-hypotheses:

Aim 1: to determine the influence of gestational and postnatal undernutrition on exercise capacity. We hypothesize that undernutrition (gestation and postnatal) will cause a reduction in exercise capacity as compared to control mice. Furthermore, postnatally undernourished mice will have a lesser exercise capacity as compared to gestationally undernourished mice.

Aim 2: to determine the influence of gestational and postnatal undernutrition on left ventricular structure and function as measured by echocardiography and Doppler velocity analysis. We hypothesize that postnatally undernourished mice will have a reduction in left ventricle (LV) mass, which will impair diastolic filling and stroke volume as compared to control mice. Mice undernourished during gestation will not have an impairment in LV mass, but will have increased left ventricular stiffness thereby reducing preload and diastolic function leading to reduced stroke volume.

Aim 3: to determine the influence of gestational and postnatal undernutrition on skeletal muscle fiber type and CSA. We hypothesize that undernutrition (gestation and postnatal) will have a smaller cross-sectional area with more Type 1 fibers in the soleus, Extensor Digitorum Longus (EDL), and diaphragm as compared to control mice. The reduced cross-sectional area and resultant muscle mass will reduce skeletal muscle pump function thereby reducing cardiac preload.

Aim 4: to determine if gestational and postnatal undernutrition presents sex-specific differences in treadmill running, cardiac structure/function, or skeletal muscle fiber type and cross sectional area. We hypothesize that female PUN mice will experience decreased maximal treadmill

performance compared to male PUN mice due to reductions in LV mass and posterior wall thickness, as well as a compensatory increase in fiber CSA.

Successful completion of these aims will determine mechanisms by which undernutrition during gestation and postnatal life reduces exercise capacity. As growth restricted individuals have elevated risk for chronic disease, knowledge from this thesis will eventually lead to the development of protocols to promote exercise capacity and reduce mortality from chronic disease.

LITERATURE REVIEW

The following literature review will focus on cardiac and skeletal muscle structure and function as influenced by early life undernutrition in mice. This commentary will describe the role undernutrition has with respect to cardiorespiratory fitness and exercise capacity and thus will lay the foundation for the rationale of this thesis. Fick's equation states that heart rate, stroke volume, and A-vO_2 difference influences exercise capacity (Fick 1870). As undernutrition during early life impairs the heart and skeletal muscle (Ferguson et al. 2019; Fiorotto et al. 2014), the literature review will comment on exercise capacity through the lens of the Fick Equation.

Cardiorespiratory Fitness and Exercise Capacity in Mice

Cardiorespiratory fitness (CRF) is positively associated with short- and long-term reductions in risk of cardiovascular disease for men and women(Jiang et al. 2013; Martins et al. 2011) (Gupta et al. 2011). Typically, CRF is assessed through measurement of maximal oxygen consumption (VO_2max) during a graded exercise test. According to Fick's Equation, VO_2max is a product of cardiac output (Q) and arteriovenous oxygen difference (A-vO_2 diff); this considers both delivery of oxygenated blood and uptake of oxygen (Fick 1870). Cardiac output increases with increasing exercise intensity through increases in heart rate and/or stroke volume in order to circulate oxygen and match the ATP (energy) demands of contracting skeletal muscle (Nystoriak et al. 2018).

In order to utilize appropriate methods to test our hypothesis, it is crucial to understand the strength and limitations in assessing CRF in mice. Assessment of human CRF involves incremental increases in exercise intensity (typically on a treadmill) with continuous

measurement of oxygen consumption to establish VO_2max. In mice, this can be measured using an enclosed metabolic treadmill; however, research published by Knab et. al. showed that measures of VO_2max on sealed metabolic treadmills are not repeatable within the same mouse (Knab et al. 2009). This is due to the discontinuity between the mouse's ventilatory stream and the gas sampling sensor, stress responses to shock administration, inability to use tail tapping for motivation, or due to rodent refusal to participate in the test (Knab et al. 2009).

Through the use of deconvolution equations or direct sampling from the mouse, the moment-to-moment values of VO_2 can be extrapolated accurately (Ferguson et al. 2019; Knab et al. 2009); however, these methods retain the issue of high equipment costs and difficulty of operation (Fernando et al. 1993). Through comparison with these equation-derived metabolic chamber VO_2max measurements, Fernando and colleagues were able to confirm that body weight and running speed could be used to predict VO_2 with a low prediction error (2.4 ± 2.9%)(Fernando et al. 1993). Additionally, Schefer and colleagues found that mouse VO_2 increased linearly with work intensity as measured by the treadmill belt speed(Schefer et al. 1996).

Both body weight and running speed are used in the calculation of work:

*Work (Joules) = 9.8 * maximal speed ($m \cdot min^{-1}$) * grade (radians) * time (min) * weight (Kg)*

Combined with an incremental treadmill test to failure, this calculation can be utilized as a reliable means of estimating VO_2max. This logic has been repeatedly applied in the literature (Barbato et al. 1998; Ferguson et al. 2019; Knab et al. 2009; Lightfoot et al. 2001; Massett et al. 2005; Oydanich et al. 2019; Platt et al. 2015), with repeatable within-mouse work results being

observed by Knab and colleagues (Knab et al. 2009). Knab and colleagues additionally found that direct sampling of VO_2max was significantly correlated with maximal work in mice (Knab et al. 2009). Thus, the use of maximal work equations will allow for determination of mouse CRF and exercise capacity as influenced by early life undernutrition.

Early Life Undernutrition and Heart Rate

Heart rate is affected by a variety of intrinsic and extrinsic factors (Parati et al. 2003); this section will focus on the modulation of heart rate by intrinsic biological variations brought forth through undernutrition. Blood flow demands of the body (i.e., cardiac output) are centrally met through chronotropic regulation with action of the sympathetic nervous system, parasympathetic nervous system, and circulating catecholamines (White et al. 2014). The importance of these factors in relation to exercise capacity was indicated by Lehmann et. al. who showed that sedentary individuals display lower work capacity alongside a heightened catecholamine response (alongside heart rate) to exercise (Lehmann et al. 1981).

Maximization of heart rate is especially important for high-intensity exercise, as exercise-mediated tachycardia shortens diastolic filling time and causes a plateau in stroke volume sooner in the incremental exercise bout for sedentary individuals as compared to individuals with lower catecholamine release (i.e. athletic populations) (Magder 2016; Vella et al. 2005). Similar to sedentary humans—a variety of studies have indicated heightened sensitivity to stressful stimuli among growth-restricted humans and rodents (Augustyniak et al. 2010; Johansson et al. 2007; Ozanne et al. 1999; Petry et al. 2000; Phillips et al. 1997; Tonkiss et al. 1998). In a study by Lister et. al., restraint stimuli induced a significantly greater stress response in rats exposed to gestational undernutrition (Lister et al. 2004). This trait was also

observed among humans in a study published by Johansson et al., where children born small for gestational age or preterm showed higher secretion of catecholamines than control subjects—an effect that was associated with a higher resting heart rate among these children (Johansson et al. 2007). These previous studies indicate that undernourishment during the gestational period induces a higher resting heart rate, a trait which hastens maximization of cardiac output and potentially impairs CRF and exercise capacity.

Early Life Undernutrition and Stroke Volume

Stroke volume is regulated by a multitude of factors including preload (Ilebekk et al. 1979), afterload (Ross 1983), heart rate (Higginbotham et al. 1986; Magder 2016), LV size, and contractility (Solaro 2011). A compromised heart structure is among the most common cardiovascular impairments in the DOHaD literature (Beauchamp et al. 2015; Cheema et al. 2005; Corstius et al. 2005; Ferguson et al. 2019; Kwong et al. 2000; Muaku et al. 1997; Nutter et al. 1979; Thornburg et al. 2011; Visker et al. 2018; Vranas et al. 2017). In a recent example, Ferguson et al. showed reductions in left ventricular mass, wall thickness, and stroke volume with postnatal undernourishment in mice (Ferguson et al. 2019).

Preload describes the end-diastolic pressure in the ventricles and is reflective of venous return via increased skeletal muscle pump action, increased ventricular compliance, increased sympathetic tone (described above), increased blood volume, and respiratory pump action (La Gerche et al. 2010). The skeletal muscle pump relies on rhythmic contraction of skeletal muscle to generate intravenous pressure and venous return (Joyner et al. 2015; Masterson et al. 2006). Considering this, a lifelong reduction in muscle size has been consistently observed with postnatal undernutrition (Desai et al. 1996; Fiorotto et al. 2014; Ontell et al. 1984). The

interaction between low muscle mass and muscle pump function was evaluated by Mondal *et al.* who showed that greater muscle mass as a percentage of body weight positively correlates with muscle pump function (Mondal et al. 2017). Thus, low skeletal muscle mass from postnatal undernutrition may reduce muscle pump function, which may compromise venous return and lead to a reduction of end-diastolic volume and thereby stroke volume. Preload can also be influenced by the reduced elasticity of the ventricle, which can increase filling pressures and cause exercise intolerance *(Pislaru et al. 2014).* Ferguson and colleagues showed that postnatal undernutrition reduced ventricular elasticity and preload in adult female mice (Ferguson et al. 2019). Catecholamine-mediated increases in venous pressure (from sympathetic tone) lead to a higher venous return. Previous literature shows that gestational undernutrition increases catecholamine sensitivity (Aihie Sayer et al. 2001; Chan et al. 2009; Hughson et al. 2003; Johansson et al. 2007); however, any venous return advantage that would accompany this (none have been reported) is likely counteracted by the co-occurance of increased afterload with gestational undernutrition (Aihie Sayer et al. 2001; Bertram et al. 2001; Chan et al. 2009; Hughson et al. 2003; Ozaki et al. 2001). Total blood volume is an additional positive regulator of preload. Some work shows that small for gestational age (SGA) infants have significantly greater blood volume than healthy infants due to high plasma volume (Maertzdorf et al. 1991). Regardless of this, there is no evidence that undernourished rats experience 1) and increase in stroke volume or 2) that this is accompanied by an increase in oxygen carrying capacity which would translate to an increase in VO_2max (Coyle et al. 1990; Kanstrup et al. 1982). From these studies, growth restriction during gestation or lactation is likely to reduce cardiac preload; however, this is likely to occur through impairment of skeletal muscle pump function and ventricular compliance.

Cardiac afterload is the impedance to ventricular emptying by aortic pressure, with high measures typically reflecting the presence of systemic hypertension (Mason 1978). In many cases of early life undernutrition, the presence of high afterload is related to kidney development. Gestational undernourishment causes a reduction in nephron number and subsequent increase in systemic blood pressure (Aihie Sayer et al. 2001; Bertram et al. 2001; Chan et al. 2009; Hughson et al. 2003; Ozaki et al. 2001). Previous literature indicates cardiac impairment associated with other body tissues whereby gestationally undernourished rats and mice show higher sympathoadrenal sensitivity alongside impaired nephron development and increased mean arterial pressure—traits which indirectly reduce stroke volume through either early maximization of heart rate with exercise or by the increased afterload (Aihie Sayer et al. 2001; Chan et al. 2009; Hughson et al. 2003; Johansson et al. 2007). This is supported by data from Zohdi and colleagues which showed offspring from low-protein diet rat dams (gestation and lactation) had higher arterial resistance and lower stroke volumes than offspring from normal-protein diet dams (Zohdi et al. 2014). This occurs because reduction in nephron count or function can decrease clearance of fluid and solutes (Na^+, Cl^-, urea, etc.) from the bloodstream, leading to high blood viscosity and total peripheral resistance (Chmielewski 2003). Moreover, the impaired nephron development in growth-restricted subjects does not recover with refeeding, thus causing an increased workload on the prevailing nephron pool, subsequently greater cell turnover, and eventual decreases in functional capacity with aging (Satomura 2014; Simeoni et al. 2008; Tain et al. 2017). These studies indicate that an increase in afterload arises due to undernourishment during gestation; this may cause a decrease in stroke volume, especially with limitation of Left Ventricular (LV) contractility due to postnatal undernutrition (Mazic et al. 2015). Further, cardiac contractility is an important variable in the maintenance of stroke volume; this is

illustrated with the use of inotropic agents, which increase stroke volume through raising contractility (Tariq et al. 2015). Postnatal undernutrition in mice reduces ventricular mass through a limitation of cardiomyocyte binucleation and size (Ferguson et al. 2019) which serves to reduce contractility, as myocardial thickness is a positive inotropic trait (Nakamura et al. 2018).

Taken together growth restriction caused by either gestational or postnatal undernutrition compromises cardiac output and likely exercise capacity. The other component to exercise capacity is the ability of the skeletal muscle to utilize oxygen for ATP production, which will be discussed below.

Early Life Undernutrition and A-vO_2 Difference

Arteriovenous oxygen difference (A-vO_2 diff; mL/dL) describes the difference in blood oxygen content between the arterial (C_aO_2) and venous blood (C_vO_2), serving to elucidate somatic oxygen utilization. This value can be determined through spectrophotometry and spirometry (Kiel et al. 1983); however, skeletal muscle fiber type distribution is used as a reliable proxy for A-vO_2 Diff (Barstow et al. 1996). Myosin ATPase expression (fiber type) in a particular muscle determines central metabolic tendency. For instance, type-I fibers show greater mitochondrial content and lower fiber cross-sectional area—both of which positively influence the extraction and utilization of oxygen (Kushmerick et al. 1992; Liu et al. 2012b). Thereby, if cardiovascular traits are identical, individuals with greater type-I fiber representation in the musculature will show greater aerobic exercise capacity (Barstow et al. 1996). There is a paucity of work elucidating the role of early life nutritive status on muscle fiber type. Some work showed that gestational undernutrition reduces glycolytic and increase slow-oxidative fiber

distribution in pigs (Bee 2004). Postnatal nutrition can also affect fiber distribution; however, this has only been documented in pigs and with overnutrition prompting an increase in glycolytic fiber distribution (L. Hu et al. 2018). Due to the limited work surrounding fiber type and early life undernutrition, this area warrants further investigation.

Early Life Undernutrition and Skeletal Muscle Mass

Myofiber number and size are positive predictors of muscle mass and strength (Miljkovic et al. 2015; Vaughan et al. 1979). The specific number of myofibers is determined prenatally (humans) or within the first week of life (rodents), indicating that skeletal muscle growth in mice and humans is constrained to hypertrophy rather than hyperplasia in adulthood (Li et al. 1996). Previous work showed that undernourishment during gestation will reduce myofiber number in mice, rats, and sheep (Bedi et al. 1982; Costello et al. 2008; Quigley et al. 2005; Wilson et al. 1988; Woo et al. 2011). Interestingly, this restriction in size from gestational undernourishment is not permanent as compensatory growth occurs with *ad libitum* feeding of a non-restricted diet during postnatal life (Desai et al. 1996; Fiorotto et al. 2014).

Postnatal undernutrition consistently shows lifelong reductions in muscle mass (Desai et al. 1996; Fiorotto et al. 2014; Ontell et al. 1984). Unlike gestational undernourishment however, compensatory growth does not occur in postnatally undernourished subjects (Desai et al. 1996; Fiorotto et al. 2014). According to Fiorotto et. al., compensatory growth is lacking in these mice due to diminished Upstream-Binding Factors which regulate rRNA synthesis and ribosomal amount thereby reducing muscle protein synthesis (Fiorotto et al. 2014; Hannan et al. 2003).

Early life growth restriction can potentially impair the skeletal muscles ability to utilize oxygen during exercise, but there is limited information in the peer reviewed literature. To appropriately address these gaps in the literature an animal model for growth restriction is ideal due to 1) the invasiveness of testing and 2) shortened lifespan compared to humans.

Modeling Early Life Undernutrition

Animal models are consistently utilized in growth restriction research due to practical advantages such as short developmental lifespan, well-established comparative databases, and ability to control environmental stimuli. Among research animals, the mouse has particular advantages for research involving an exercise variable as mice have similar physical activity levels (Letsinger et al. 2019), responses to exercise stimuli (Petrosino et al. 2016), and training adaptations (Garton et al. 2016) to humans. The structure of the mouse heart varies from that of a human in shape (the mouse heart is similar to a "rugby ball"), freedom within the pericardial sac (greater), resting heart rate (greater), and atrial dimensions (smaller), and axis of ejection (Kusunose et al. 2012; Wessels et al. 2003). In particular, the axis is an important factor in the sensitivity of human hearts to LV loading (Demontis et al. 2017; Huttin et al. 2017); this suggests that structural and functional impairments in the LV of the mouse heart may be exacerbated when applied to the human heart. Despite these differences, the use of a mouse model in cardiac research is validated by similarities in cardiac morphogenesis and chamber-to-chamber communication (Krishnan et al. 2014; Wessels et al. 2003). While obvious differences in biomechanics and muscle shape exists, comparisons of muscular transcriptome and metabolic function between human and mice shows similarities and warrants the use of a mouse model (Kho et al. 2006; Schiaffino et al. 2011).

Methods to induce undernutrition in the mouse mode include placental ablation (Krishna et al. 2011), manipulation of liter size (Horton et al. 2016), or dietary restriction (Dickinson et al. 2017; Ferguson et al. 2019; Visker et al. 2018). While all methods are successful in the restriction of organ growth, dietary restriction mimics the natural state of reduced *in-utero* nutrient availability through restricting amino acid intake (Dunlap et al. 2015). Amino acids are critical to the developing fetus, as they contribute not only to protein synthesis, but also growth signaling and hormonal secretion (Dunlap et al. 2015). Further, the restriction of maternal protein intake also limits lactation, with Sampson et al. showing that amino acid-restricted feeds will reduce milk production (Sampson et al. 1986). Hypoxic models of growth restriction (artery ligation) also reduce amino acid delivery to the fetus; however, this model only applies to the intrauterine period and is accompanied by neuroendocrine effects on the fetus that are not observed with undernourishment alone (Vuguin 2007). Litter size manipulation methods also induce global undernutrition, but are limited to only the postnatal period and induce unequal weight distribution among offspring (Yuan et al. 2015). Given that maternal protein restriction affects both the gestational and postnatal nutrient environment for offspring, this method is practical for targeting distinct windows of mouse development.

Use of echocardiograph and Doppler velocity analyses in mice is a well-established cardiac evaluation tool (Gao et al. 2011). Similar to humans, echocardiography in mice can utilize parasternal long- and short- axis views (Scherrer-Crosbie et al. 2008). Additionally, Doppler analysis of mitral flow and strain rate have been validated in mice (Schaefer et al. 2003; Sebag et al. 2005). The heart rates of mice (500-700 bpm necessitates proper adjustment of frame rate for reliable data (Scherrer-Crosbie et al. 2008). Preference for use of M-mode images in mice has emerged, as B-mode images can be difficult to obtain; however, B-mode

measurements of stroke volume and ejection fraction remain essential, as calculations derived from M-mode rely on geometric assumptions (such as heart shape) which can vary (Lindsey et al. 2018; Scherrer-Crosbie et al. 2008). Assessment of stroke volume can be calculated using endocardial systolic and diastolic areas (LV tracing; done during (B-mode) with the following software and equation (VisualSonics, Vevo® 2100 Imaging System)(VisualSonics 2008):

$$\frac{4\pi}{3} \times \frac{End\ Major;\ d}{2} \times \left(\frac{End\ Area;\ d}{\pi\left(\frac{End\ Major;\ d}{2}\right)} \right)^2 - \frac{4\pi}{3} \times \frac{End\ Major;\ s}{2} \times \left(\frac{End\ Area;\ s}{\pi\left(\frac{End\ Major;\ s}{2}\right)} \right)^2$$

The merits of Doppler imaging are most pronounced in evaluation of diastolic function such as E- (early) and A- (late) wave velocity for evaluation of valvular function. Overall, outcomes derived from use of this technique in mice are considered to be externally valid and reliable to humans (Scherrer-Crosbie et al. 2008).

In addition to in-vivo cardiac functional assessment, ex-vivo working heart modeling using the Langendorff perfusion is a reliable method for cardiac function (Eberli et al. 1998; Ferguson et al. 2019; Stampfl et al. 2011); however, the small heart size of growth-restricted mice, and removal of differences in afterload/neuronal regulation during dissection, which is important in analysis of early-life undernutrition, makes in vivo echocardiography the preferred method over the ex-vivo working heart model (Bertram et al. 2001; Johansson et al. 2007; Ozaki et al. 2001; Schechter et al. 2014; Skrzypiec-Spring et al. 2007; Tonkiss et al. 1998).

Sex-Specific Considerations in Mice

Male and female mice show a variety of differences in physiological parameters. Female mice have increased exercise capacity versus males, even when controlling for muscle mass and

body weight (Oydanich et al. 2019). This may result from a more oxidative muscle phenotype among female mice(Oydanich et al. 2019). Additionally, estrogen provides a positive effect on nitric oxide-synthase, resulting in increased blood flow. Resting heart rate of females is greater than that of male counterparts; this exists as a compensation mechanism for smaller heart size among females (Ramaekers et al. 1998). In female mice, postnatal undernutrition reduces reduce heart weight, resting stroke volume, and increases isovolumic contraction time; while there is no effect to of postnatal undernutrition on male mice (Ferguson et al. 2019). Maternal undernutrition shows sex effect on the adrenal gland of adult male offspring (Khorram et al. 2011). Additionally, male rats exposed to gestational undernutrition show greater rates of adipocyte hypertrophy and carcass lipid content—suggesting that males are at increased risk of obesity from maternal undernutrition (Jones et al. 1984). These sex-specific considerations with growth restriction and exercise capacity warrant the use of sex as a covariate in statistical modeling for mouse research.

Conclusion

Much of the research on developmental programming has focused on disease risk and associated physiological mechanisms; however, research considering exercise performance has been largely neglected in the DOHaD realm. Maximal exercise capacity (and thus cardiorespiratory fitness) can be influenced by both cardiac and muscular impairments. Gestational undernourishment causes high resting heart rate, and increased afterload (which reduces stroke volume). Similarly, postnatal undernutrition reduces LVmass, diastolic function, cardiac contractility, stroke volume, and skeletal muscle mass. In principle, these tissues contribute to cardiorespiratory performance; thus, it is warranted to investigate their

developmentally-programmed impairment on maximal exercise performance. This data will inform the application of exercise and pharmaceutical prescriptions to improve exercise capacity and reduce chronic disease risk, respectfully, in growth-restricted populations.

INTRODUCTION *(manuscript)*

The Developmental Origins of Health and Disease (DOHaD) hypothesis, which was first championed by Dr. David Barker (Barker et al. 1986), showed that growth restriction (induced by early life undernutrition) during the early stages of development caused negative health outcomes in adulthood that include an increased risk of cardiovascular disease, type II diabetes, hypertension, and sarcopenia (Barker et al. 1986; Thornburg et al. 2011; Wadhwa et al. 2009; Zardini et al. 1994). Undernutrition during gestation has been documented to reduce skeletal muscle fiber number via a reduction in myogenic cells which participate in initiation of secondary myotube formation (Woo et al. 2011). Moreover, undernourishment during the postnatal period leads to reduced skeletal muscle mass due to a reduction in ribosomal content which decreases protein synthesis rate (Fiorotto et al. 2014). Interestingly—even with refeeding a healthy diet—permanent correction to normal growth pattern is not observed in postnatally undernourished mice; leading to a lifelong reduction in muscle mass. Cardiac function as a result of early life undernutrition is impaired by a reduction cardiomyocyte number and size with a corresponding increase in LV stiffness and reduced cardiac output (Thornburg et al. 2011; Vranas et al. 2017).

From the perspective of the Fick Equation (Fick 1870) [Oxygen Consumption (VO_2) = Heart Rate (HR) x Stroke Volume (SV) x Arterial-Venous Oxygen Difference (A-vO_2 Dif)], we hypothesize maximal oxygen consumption—and subsequently exercise capacity—may be compromised in growth restricted individuals due to the associated cardiac and skeletal muscle impairments. The clinical significance of our hypothesis is that regular participation in exercise reduces the risk of chronic disease (Booth et al. 2012); if exercise capacity is compromised by developmental programming, then understanding of the associated impairments will aid

healthcare practitioners in development of efficient therapeutic countermeasures for growth restricted individuals. Thus, the purpose of this study was to evaluate exercise capacity in mice that were growth restricted during gestation and lactation. Furthermore, we evaluated cardiac function via echocardiography and Doppler velocity analysis and skeletal muscle fiber type and cross-sectional area.

METHODS AND MATERIALS

Animal Model

Animal Model

This study was approved by the Michigan State University Institutional Animal Care and Use Committee (IACUC) in accordance with the Guide for the Care and Use of Laboratory Animals. FVB (Charles River Laboratories, Wilmington, MA, USA) genetically homogeneous mice were used due to tendency of dams to willingly accept pups from separate nests, allowing for use of cross-fostering models (Ferguson et al. 2019; Fiorotto et al. 2014; Leszczynski et al. 2019). Mice were exposed to 12-hour light/dark cycles and allowed access to food and water ad libitum.

Nutritive Model

Two weeks prior to breeding dams were allocated to either a control (CON; 20% protein; Research Diets, New Brunswick, NJ, USA), or an isocaloric low-protein (LP) diet (8% protein; Research Diets, New Brunswick, NJ, USA). The macronutrient breakdown of these diets are shown in Table 1. Male mice were introduced to the female cages for a 24-hour period. Thus, all pups studied were the same age. At birth, (PN1) pups were pooled and distributed to one of three experimental groups (Figure 1; N=3 litters per group). Control (CON) pups were born to dams fed a CON diet and cross-fostered to a different dam fed the CON diet. Gestationally undernourished pups (GUN) were born to dams fed the LP diet throughout gestation, then at PN1 cross-fostered and suckled to dams fed the CON diet. Postnatally undernourished pups (PUN) were born to dams fed the CON diet during gestation, then at PN1 suckled from dams fed the LP diet. This study did not include a group exposed to both GUN and PUN. We have observed from

previous pilot experiments that mouse pups exposed to gestational and postnatal undernourishment show high rates of maternal neglect and low rates of survivability which complicates maintenance of a sample population. Thus, undernourishment only during gestation or early postnatal life was evaluated.

All litters were standardized to equal number of pups and sex ratio (4 males and 4 females per litter). Pups were identified with a unique tattoo and litter size was maintained by donor pups, which were never studied. At PN21 all mice were weaned and fed the control diet, thus isolating undernutrition to gestation or lactation only.

Diet	Grams Protein (% of kcals)	Grams Fat (% of kcals)	Grams Carbohydrate (% of kcals)	Grams Fiber
CON	196.6 (20%)	70 (16%)	609.4 (63%)	50
LP	80.6 (8%)	70 (16%)	728.8 (75%)	60

Table 1: Macronutrient composition of diets. Diets were a casein and cornstarch base with fat content consisting solely of soybean oil. Both Diets contained identical fatty acid content (70g) and composition (10.3g palmitic, 22.8g oleic, 51g linoleic, 6.8g linolenic). CON: Control diet. LP: low-protein diet.

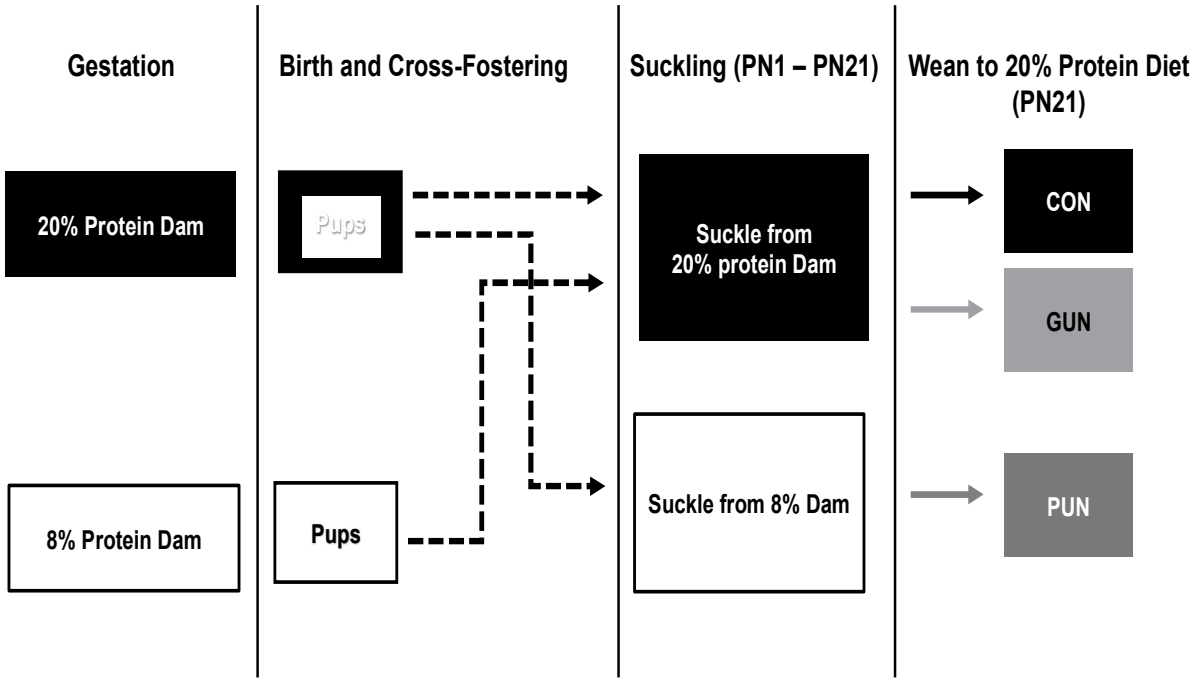

Figure 1: Cross-Fostering Nutritive Model. 20% Protein Dam: Dam fed 20% protein feed. 8% Protein Dam: Dam fed 8% protein feed. PN: postnatal day. CON: control group. GUN: gestational undernourished group. PUN: postnatal undernourished group.

Maximum Exercise Capacity

At PN61, mice began a 5-day treadmill acclimation protocol to allow for familiarization with the procedure. This was not adequate to produce a training effect (Baldwin et al. 1977). Following one day of rest, the maximal treadmill test was performed at PN67. Mice were weighed and placed on a treadmill with a warmup speed of 10 $m \cdot min^{-1}$ at a 10% grade with a 0.75mA shock for 10 minutes. Following warmup, the treadmill speed was increased by 5 $m \cdot min^{-1}$ every two minutes until the mouse either remained on the shock grid for more than 20 seconds or touched the shock grid 5 times in a 30 second period, at which point the test was terminated. Maximal treadmill speed did not exceed 30 $m \cdot min^{-1}$ throughout data collection. Treadmill testing time (start of warm up to test termination) (minutes) and time spent running at maximal speed (minutes) were recorded for each mouse.

Work performed at maximal speed was calculated with the following equation:

Joules = 9.8 * maximal speed ($m \cdot min^{-1}$) * grade (radians) * time at maximal speed (min) * weight (Kg)

Total work performed for the whole treadmill test was calculated with the following equation:

Joules = [9.8 * 20 ($m \cdot min^{-1}$) * 0.1 (radians) * time spent at speed (min) * weight (Kg)] + [9.8 * 25 ($m \cdot min^{-1}$) * 0.1 (radians) * time spent at speed (min) * weight (Kg)] + [9.8 * 30 ($m \cdot min^{-1}$) * 0.1 (radians) * time spent at speed (min) * weight (Kg)].

Echocardiography and Doppler Velocity Analysis

At PN72, echocardiography (Vevo 2100 high frequency ultrasound unit; Toronto, Canada) and Doppler velocity analysis (Visualsonics MS550D; 40MHz) were performed. Mice were briefly anesthetized with 2% isoflurane in oxygen. Hair was removed from the trunk area via clippers followed by Nair hair removal gel. Isoflurane concentration was then reduced and mice were maintained at 1% isoflurane throughout the measurement. Body temperature was maintained at 37□ using a supplemental heat lamp. Two-dimensional echocardiographic images (parasternal long axis and short axis B-mode images at 233 $frames \cdot second^{-1}$, and M-mode short axis views at mid-papillary level) were collected along with Pulsed-wave Doppler mitral valve waveforms. Anesthesia time was less than 10 minutes (including induction and hair removal). All image measurements were performed at a separate workstation by a single technician blinded to the treatment groups. Cardiac mass variables were standardized to body weight while cardiac function measures were standardized to body surface area using Meeh's Formula (Gouma et al. 2012):

Body Surface Area = 9.662 x $(body\ weight)^{0.667}$

Mice were allowed a 24-hour recovery period, after which they were euthanized by cervical dislocation while under 1.5% isoflurane anesthesia. Tibia lengths of each hind leg were measured to estimate body composition (Lang et al. 2005).

Measurement of Skeletal Muscle Fiber Type and Cross-Sectional Area

Following euthanasia, the soleus (SOL) was collected to represent the predominantly-oxidative locomotor musculature, extensor digitorum longus (EDL) to represent the predominantly-glycolytic locomotor musculature, and diaphragm to represent the respiratory musculature (Bedi et al. 1982; Pette et al. 2000). Weight of each muscle was collected and reported as absolute and relative to body weight.

Fiber typing was conducted as previously described with methods utilized to limit cross binding of antibodies (Leszczynski et al. 2019). In concordance with previous research, no antibodies were administered for type-IIX Myosin Heavy Chain due to low binding reliability; this fiber was accounted for through counts of non-immunofluorescent cells (Bamman et al. 1998; Leszczynski et al. 2019; Wu et al. 2017).

Samples were imaged using an Olympus Fluoview 1000 inverted confocal laser microscope. A PlanFluor UPLFN 20X NA.50 objective was used to collect images using a 488 nm Argon Laser or 543/643 HeNe laser for excitation of fluorescence. Single XY confocal images were taken using sequential excitation, and no changes were made to the images post acquisition. Cross-sectional area and type were assessed using ImageJ analyzer software. Samples were only analyzed if they contained 300 or more cells per section per animal. All values of cross-sectional area were scaled to body weight as is the standard when conducting

growth restriction studies (Leszczynski et al. 2019; Nakada et al. 2016; Nonaka et al. 2018; Wang et al. 2018; You et al. 2015).

Statistics

All analysis was performed in JMP Pro 13 software (SAS, Cary, NC). Growth and maximal treadmill running capacity, and skeletal muscle fiber data were analyzed using a two-way ANOVA (3x2 factorial design) with the main comparison effects of diet group (CON, GUN, PUN) and sex (male or female) with alpha level set at 0.05. Echocardiograph measures were analyzed using a two-way ANCOVA with sex and diet group being used as the independent variable and heart rate and body surface area used to standardize functional parameters, while body weight was used to standardize measures related to LV structure (Inoue et al. 2017; Popović et al. 2005). A Tukey's HSD post hoc test was performed on analyses for which significance was observed. All values are presented as mean±standard deviation (SD).

RESULTS

Growth and Tibia Length

The growth rate during PN1-PN21 showed the PUN mice grew at a slower rate (Diet: $P<0.0001$) as compared to the CON and GUN mice (Fig. 2A). During PN22-70, there was a sex effect where growth rate was significantly greater in (Sex: $P<0.0001$) male versus female groups, but there was no diet effect (Fig. 2B).

At PN1, a trend towards significant difference (Diet: $P=0.15$) was observed with GUN mice being smaller than CON and PUN mice (Fig. 2C). Following birth the GUN mice experienced catch up growth to match the weight of CON mice. At weaning (PN21), PUN mice were significantly (Diet: $P<0.0001$) smaller than CON and GUN mice (Fig. 2C).
While there was a trend (Diet*Sex: $P=0.103$) for diet group x sex interaction, body weight measured at PN70 only showed a diet effect (Diet: $P=0.0002$) where PUN mice weighed significantly less than CON and GUN mice (Fig. 2D). Assessment of mouse tibia length did not indicate significant differences (Diet: $P=0.23$) between groups (Fig. 2E).

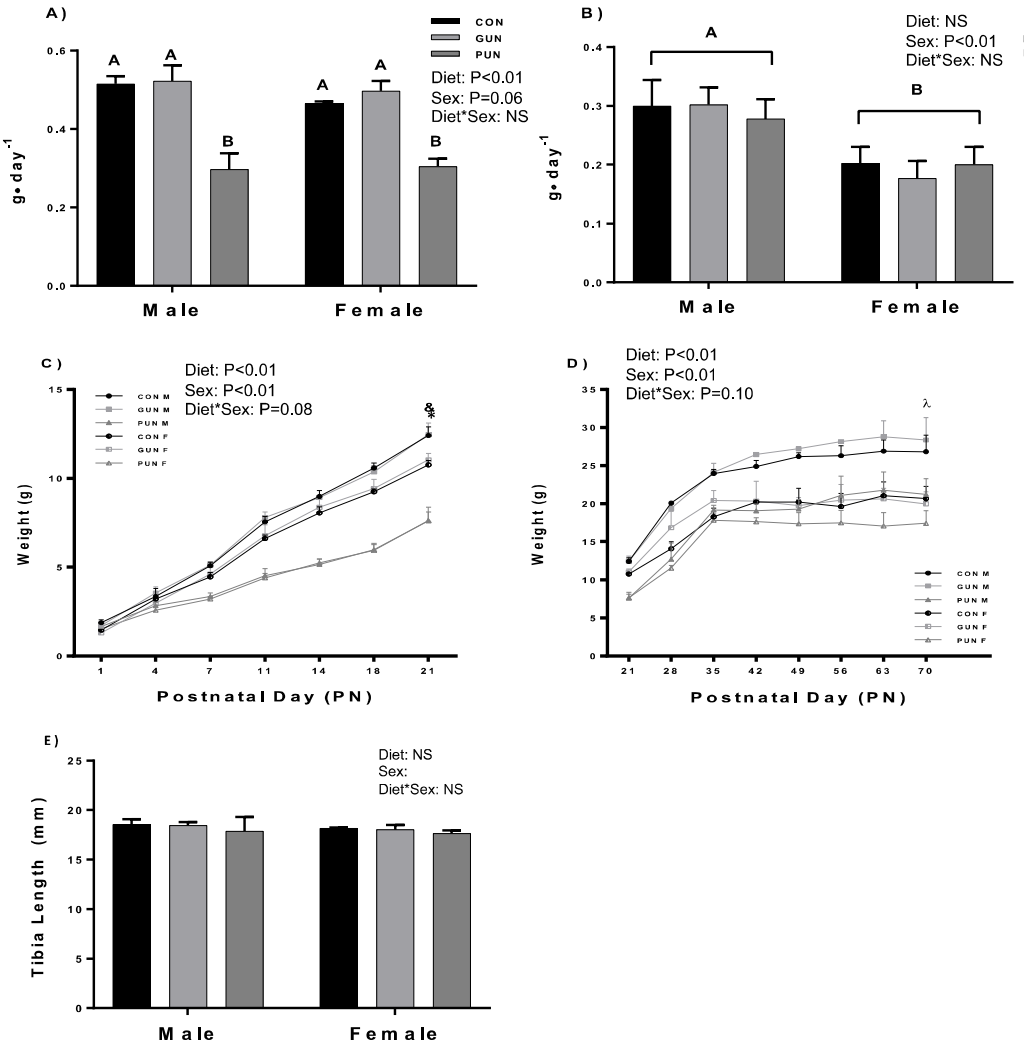

Figure 2: Body weight (g) and Growth. Values are expressed as mean ± SD. A) Growth rate (PN1-PN21) for PUN (male, female) was significantly less (Diet: P<0.0001) than CON (male, female) and GUN (male, female). B) During PN22-PN70, there was a sex effect where growth rate was significantly greater (Diet: P<0.0001) in male versus female groups. C) Bodyweight growth from PN1-PN21. Upon birth at PN1, GUN mice showed a trend towards significantly smaller (Diet: P=0.15) body weight than PUN and CON mice. At weaning (PN21), PUN (male, female) mice were significantly (Diet: *P<0.0001) smaller than CON (male, female) and GUN (male, female) mice. D) Body weight growth from PN21-PN70. At final weight (PN70), PUN mice (male, female) weighed significantly less (Diet: λP=0.0002) than CON (male, female) and GUN (male, female) mice. E) Mouse tibia length. Values are expressed as mean ± SD. No significant difference (Diet: P=0.23) in tibia length was observed among diet groups.

Treadmill Running

Running time to exhaustion on the treadmill showed a non-significant trend (Diet*Sex: P=0.09) where GUN male mice reached exhaustion sooner as compared to GUN female mice; no other differences were observed between groups (Fig. 3A). CON female mice sustained maximal speed for a significantly (Diet*Sex: P=0.012) longer time than PUN male, PUN female, GUN male, GUN female, and CON male mice (Fig. 3B).

A diet effect was observed where work performed at maximal speed was significantly (Diet: P=0.0002) greater in CON mice as compared to GUN and PUN mice (Fig. 3C). Results of total work performed throughout the test were not significantly different (Diet: P=0.32) between groups (Fig. 3D).

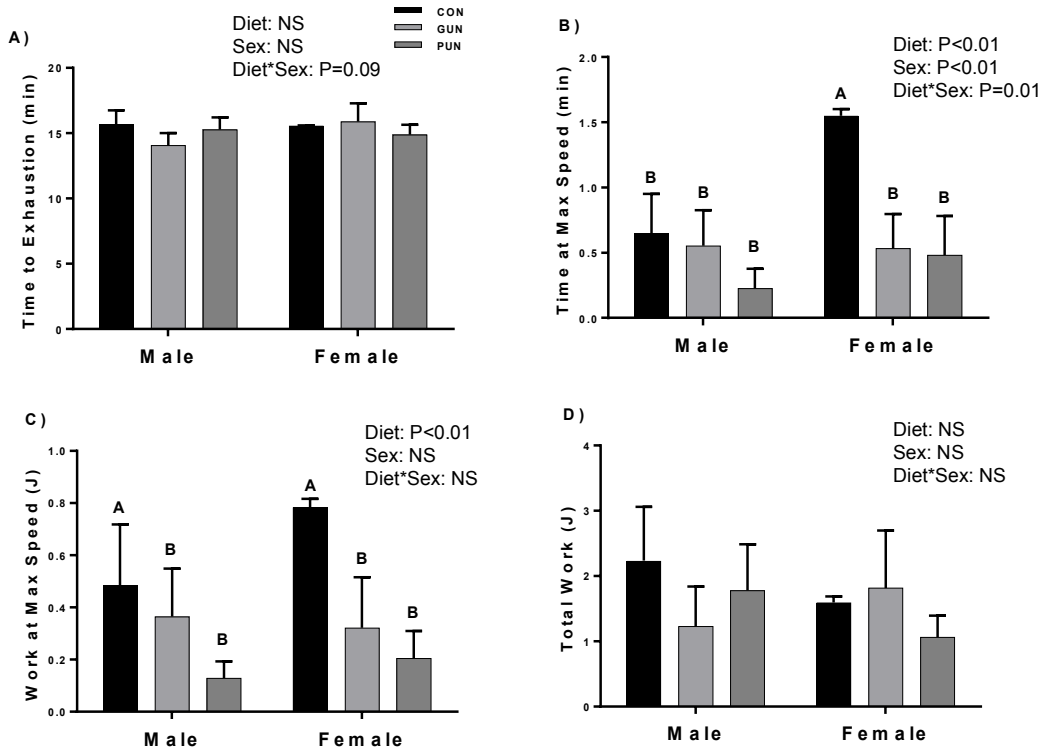

Figure 3: Treadmill running parameters. Values are expressed as mean ± SD. A) Time (in minutes) to exhaustion including the 10-minute warm up. Time to exhaustion during treadmill running showed a trend (Diet*Sex: P=0.09) for significantly quicker time to exhaustion among GUN male mice relative to GUN female mice. B) Time (in minutes) at maximal running speed. CON female mice sustained maximal speed for a significantly (Diet*Sex: P=0.012) longer time than all other groups. C) Work (in Joules) load at maximal speed on the treadmill. Work performed when at maximal speed was significantly (Diet: P=0.0002) greater in CON mice as compared to PUN and GUN mice. D) Total work (in Joules) completed throughout the treadmill test. There was no difference (Diet: P=0.32) between the groups or sexes.

Echocardiography and Doppler Velocity Analysis

Heart rate showed a trend (Diet: P=0.07) of greater BPM among GUN mice versus CON and PUN (Table 2). Left ventricular (LV) mass of CON mice was larger (Diet: P=0.03) than GUN and PUN mice; this was no longer present when corrected values were obtained from the echocardiography software ($LVmass_{corr}$) (Table 2). The posterior wall of the LV during systole (LVPW;s) was 18.6% thinner in PUN female and 8.5% thinner in GUN female relative to CON female mice (Diet*Sex: P=0.03; Table 2). For the males, LVPW;s was 12.3% thinner in PUN male and 10.6% thinner in GUN male in relation to CON male mice (Diet*Sex: P=0.03; Table

2). Similarly, measures of LV anterior wall thickness during diastole (LVAW;d) showed a trend towards significance (Diet: P=0.09; Table 2) with an 8.8% thinner wall in PUN females and GUN females relative to CON females, while LVAW;d was 13.6% thinner in PUN males and only 1.1% thinner in GUN male mice relative to CON male. There was no other significant differences in left ventricular structure of the diet groups (Diet: P=0.09; Table 2).

There was no difference in systolic function as evaluated by echocardiography (Table 2). However, diastolic function had significant differences in Aortic Ejection Time (AET) and the ratio of early (MV E) to late (MV A) aortic filling velocity (Table 2). CON female mice had a significantly longer (Diet*Sex: P=0.047) AET as compared to GUN male mice (Table 2). No other differences were observed between groups with respect to AET (Table 2). The MV E/A ratio was significantly greater (Diet*Sex: P=0.045) among GUN female in comparison to PUN male mice, with no other differences being observed between groups (Table 2).

Global cardiac function showed stroke volume (SV) was significantly less (Diet: P=0.04) among PUN mice in comparison to CON mice; GUN mice were not significantly different than CON and PUN (Table 2). Myocardial Performance Index (MPI) trended towards significance (Diet*Sex: P=0.16) with CON male, CON female, and GUN female being less than GUN male, PUN male, and PUN female mice (Table 2).

Parameter	Male			Female			*P*		
	CON	GUN	PUN	CON	GUN	PUN	*Diet*	*Sex*	*Diet*Sex*
Heart Rate (BPM)	404±50	426±50	365±50	391±60	416±50	363±50	0.07	NS	NS
Diameter;s (mm)	2.72±0.34	2.50±0.36	2.43±0.36	2.39±0.35	2.39±0.36	2.45±0.36	NS	NS	NS
Diameter;d (mm)	4.07±0.26	3.89±0.28	3.76±0.26	3.76±0.26	3.65±0.26	3.71±0.27	NS	NS	NS
Area;s (mm^2)	12.65±2.38	12.35±2.68	10.56±1.78	8.26±2.22	10.55±1.85	8.65±2.77	NS	NS	NS
Area;d (mm^2)	19.37±2.38	18.96±2.70	18.33±1.78	16.04±2.23	17.46±1.85	16.66±2.78	NS	NS	NS
LV Mass (mg)	$118.9±4.20^{A}$	$107.3±7.74^{B}$	$103.9±5.72^{B}$	$114.6±5.02^{A}$	$99.9±3.72^{B}$	$102.5±12.75^{B}$	0.03	NS	NS
LV Mass Corrected ($mg·g^{-1}$)	95.12±9.2	85.81±9.3	83.15±9.2	91.66±6.5	79.99±11.3	82.02±10.3	NS	NS	NS
LV Anterior Wall;d (mm)	0.88±0.14	0.87±0.14	0.76±0.14	0.91±0.13	0.83±0.14	0.83±0.13	0.09	NS	NS
LV Anterior Wall;s (mm)	1.35±0.24	1.27±0.24	1.26±0.24	1.38±0.22	1.22±0.24	1.27±0.25	NS	NS	NS
LV Posterior Wall;d (mm)	0.81±0.08	0.77±0.08	0.8±0.08	0.82±0.07	0.79±0.08	0.77±0.09	NS	NS	NS
LV Posterior Wall;s (mm)	$1.22±0.12^{A}$	$1.09±0.08^{B}$	$1.07±0.1^{B}$	$1.18±0.07^{A}$	$1.08±0.1^{B}$	$0.96±0.09^{C}$	NS	NS	0.03
Mitral Valve Area (MVA)	6.54±13.08	6.84±13.68	6.76±13.52	7.53±13.04	6.68±13.36	6.14±13.73	NS	NS	NS
Ejection Fraction (%)	62.12±8.4	65.6±8.78	64.93±8.68	66.6±8.37	64.28±8.56	63.68±8.81	NS	NS	NS
Fractional Shortening (%)	33.13±6.3	35.81±6.6	35.27±4.48	37.3±6.25	34.4±6.4	34.24±6.6	NS	NS	NS
LVID;s (mm)	2.68±0.34	2.52±0.36	2.44±0.34	2.38±0.35	2.43±0.34	2.45±0.36	NS	NS	NS
Volume;s (µL)	27.95±7.64	22.83±7.98	21.61±7.88	22.03±7.60	20.14±7.72	22.29±8.0	NS	NS	NS
AET (ms)	$52.35±4.86^{AB}$	$46.57±5.08^{B}$	$50.31±5.02^{AB}$	$58.52±4.83^{A}$	$49.65±4.96^{AB}$	$52.7±5.1^{AB}$	NS	NS	0.047
MV E ($mm·s^2$)	630.18±103	627.42±108	551.94±106	705.5±102	726.22±105	669.34±108	NS	NS	NS
MV A ($mm·s^2$)	336.28±81	368.79±84	353.2±83	464.63±80	310.79±82	346.84±84	NS	NS	0.1
MV E/A	$1.9±0.44^{AB}$	$1.73±0.46^{AB}$	$1.51±0.46^{B}$	$1.51±0.45^{AB}$	$2.61±0.46^{A}$	$1.95±0.47^{AB}$	NS	NS	0.045
MVDT ($mm·s^2$)	-2613±1417	-2872±1479	-2794±1460	-3452±1409	-3928±1443	-3627±1484	NS	NS	NS
IVCT (ms)	10.7±2.56	10.71±2.68	13.24±2.64	8.77±2.55	11.13±2.6	11.43±2.68	NS	NS	NS
IVRT (ms)	16.70±2.18	15.94±2.94	15.84±2.26	16.53±2.18	15.16±2.22	19.6±2.28	NS	NS	NS
LVID (d) (mm)	4.04±0.28	3.88±0.3	3.82±0.3	3.79±0.29	3.65±0.3	3.75±0.29	NS	NS	NS
Volume;d (µL)	73.43±10.36	65.83±10.82	60.94±10.68	61.18±10.31	56.63±10.56	59.56±10.84	NS	NS	NS
Stroke Volume (µL)	$45.47±6.04^{A}$	$43±6.3^{AB}$	$35.3±6.22^{B}$	$39.15±6.01^{A}$	$36.48±6.14^{AB}$	$32.26±6.33^{B}$	0.04	NS	NS
Cardiac Output ($mL·min^{-1}$)	19.96±2.32	17.09±2.42	14.32±2.38	15.8±2.3	14.39±0.36	13.74±2.41	NS	NS	NS
Cardiac Output-LV Tracing in B-mode ($mL·min^{-1}$)	17.96±2.32	17.09±2.42	15.32±2.38	15.38±2.3	14.39±2.36	14.74±2.41	NS	NS	NS
Myocardial Performance Index	0.52±0.08	0.57±0.09	0.58±0.09	0.43±0.08	0.53±0.09	0.59±0.09	NS	NS	0.16

Table 2: Echocardiography and Doppler Velocity Results. Difference in superscripted letters across a row indicates statistical significance. LV: left ventricle. s: systole. d: diastole. LVID;s: LV internal diameter (systole). IVCT: LV isovolumetric contraction time. AET: ejection time from opening to closing of the aortic valve. MV E: Mitral Valve E (early) wave velocity. MV A: Mitral Valve A- (late) wave velocity. MVDT: Mitral valve deceleration time. IVRT: Isovolumetric relaxation time. LVID d: LV internal diameter (diastole).

Skeletal Muscle Weight, Muscle Fiber Type Distribution, and Cross-Sectional Area (CSA)

A representative image of skeletal muscle fiber type and cross-sectional area is shown in Figure 4.

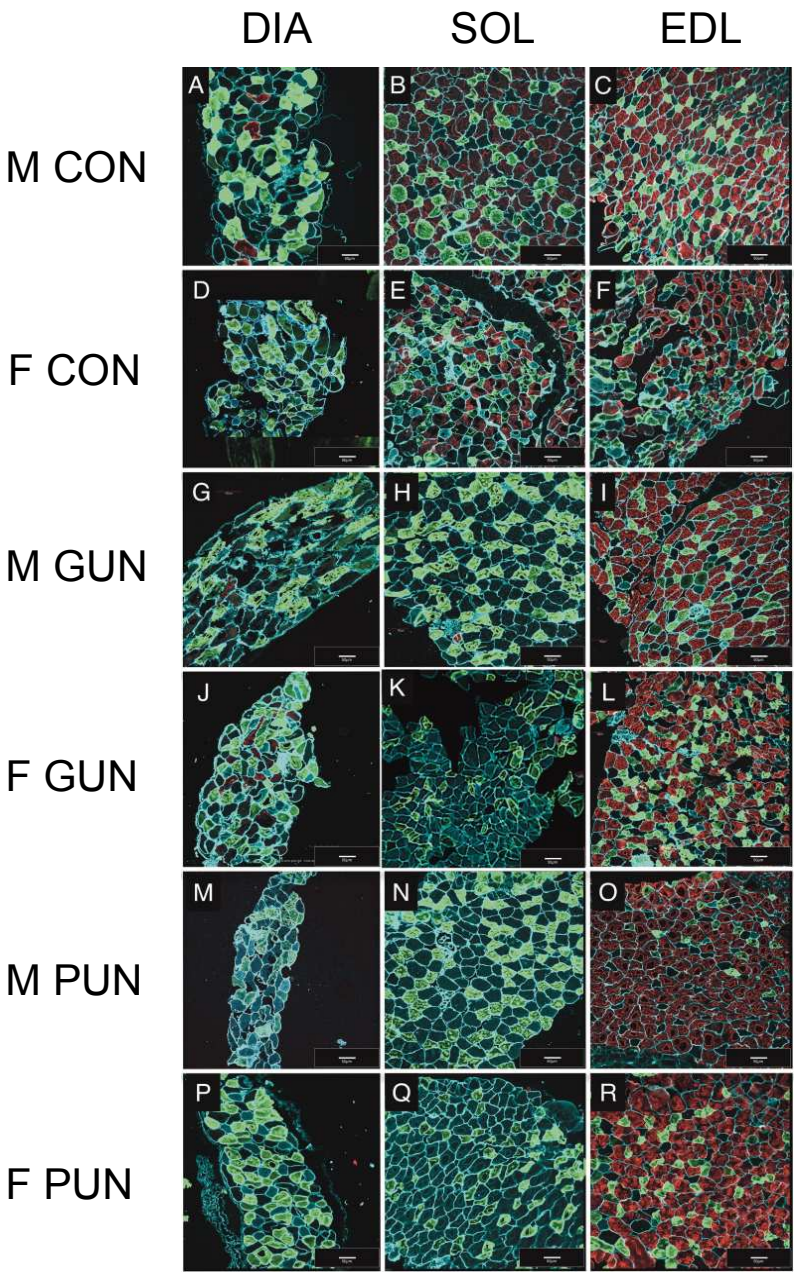

Figure 4: Representative images of skeletal muscle histology. A). CON male diaphragm cross-section. B) CON male SOL cross-section. C) CON male EDL cross section. D) CON female diaphragm cross-section. E) CON female SOL cross-section. F) CON female EDL cross-section. G) GUN male diaphragm cross-section. H) GUN male SOL cross-section. I) GUN male EDL cross-section. J) GUN female diaphragm cross-section. K) GUN female SOL cross-section. L) GUN female EDL cross-section. M) PUN male diaphragm cross-section. N) PUN male SOL cross-section. O) PUN male EDL cross-section. P) PUN female diaphragm cross-section. Q) PUN female SOL cross-section. R) PUN female EDL cross-section.

Diaphragm

Absolute and relative weight of the diaphragm was not significantly different between diet groups (Table 3; Appendix). Type-I fiber distribution was not significantly different (Diet*Sex: P=0.88) between groups (Fig. 5A). PUN female mice had a significantly greater (Diet*Sex: P=0.009) percent of type-IIA fibers in the diaphragm as compared to CON male, CON female, GUN female, and PUN male; GUN males were not significantly different from any group (Fig. 5B). No significant difference (Diet*Sex: P=0.21; Diet*Sex: P=0.70) between groups was found in diaphragm type-IIX (Fig. 5C) or type-IIB fibers (Fig. 5D).

CSA of type-I fibers (Diet*Sex: P=0.0004; Fig. 5E), type-IIA fibers (Diet*Sex: P=0.0007; Fig. 5F) in the diaphragm was significantly larger among CON female mice compared to the other groups. Diaphragm type-IIX fiber CSA showed a sex effect (Sex: P=0.03) where the females had a larger CSA as compared to the males (Fig. 5G). Diaphragm Type-IIB fiber CSA was not significantly different (Diet*Sex: P=0.3) among groups (Fig. 5H).

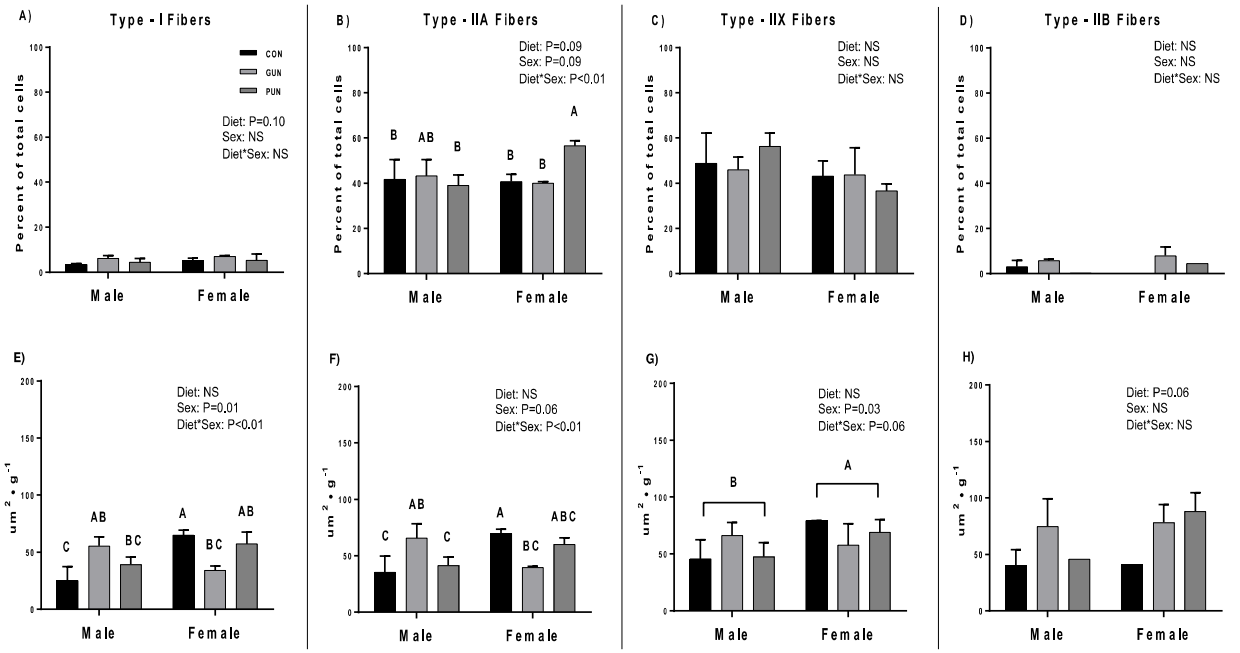

Figure 5: Fiber type distribution of the DIA of mice exposed to gestational and postnatal undernutrition. Values are expressed as mean ± SD. Differing letters within each panel represent statistical difference. A) Percentage of type-I fibers in the DIA. Distribution of type-I fibers was not significantly different (Diet*Sex: P=0.88) between groups. B) Percentage of type-IIA fibers in the DIA. PUN female mice showed a significantly greater percent (Diet*Sex: P=0.009) of type-IIA fibers in the DIA than CON male, CON female, GUN female, and PUN male mice; GUN males were not significantly different from either group. C) Percent of type-IIX fibers in the DIA. Type-IIX fiber distribution was not significantly different (Diet*Sex: P=0.21). D) Percent of type-IIB fibers in the DIA. Percent of type-IIB fibers in the DIA was not significantly different (Diet*Sex: P=0.70). E) CSA of type-I fibers in the DIA. CSA was significantly larger (Diet*Sex: P=0.0004) among CON female mice than PUN male, GUN female, and CON male. PUN female and GUN male CSA was only significantly larger than CON male. F) CSA of type-IIA fibers in the DIA. CSA was significantly larger (Diet*Sex: P=0.0007) in CON female than in GUN female, PUN male, and CON male. GUN male CSA was only larger than CON male and PUN male. G) CSA of type-IIX fibers in the DIA. DIA type-IIX fiber CSA showed a sex affect (Sex: P=0.03) where the females (CON,GUN, PUN) had a larger CSA as compared to the males (CON, GUN, PUN). H) CSA of type-IIB fibers in the DIA. No significant difference (Diet*Sex: P=0.3) was found in CSA of type-IIB fibers in the DIA.

Soleus

Absolute SOL weight was significantly greater (Diet*Sex: P=0.003) among CON male as compared to CON female mice, no other differences were observed (Table 3; Appendix). Relative SOL weight was significantly greater (Diet*Sex: P=0.02) among CON male and PUN

female versus CON female mice (Table 3; Appendix); no other differences were observed between groups. Type-I fiber (Diet*Sex: P=0.62; Fig. 6A), Type-IIA fiber (Diet*Sex: P=0.89; Fig. 6B), Type-IIX fiber (Diet*Sex: P=0.30; Fig. 6C), and Type-IIB fiber (Diet*Sex: P=0.55, Fig. 6D) distribution in the SOL was not significantly different between groups.

In the SOL, CSA of type-I (Diet: P=0.02; Fig. 6E), type-IIA (Diet: P=0.01, Fig. 6F), and type-IIX (Diet: P<0.0001; Fig. 6G) fibers was larger among PUN mice compared to GUN and CON mice. CSA of SOL type-IIB fibers was significantly larger (Diet*Sex: P=0.05) among PUN males and PUN females than CON females, CON males, and GUN males; GUN females were not significantly different from any group (Fig. 6H).

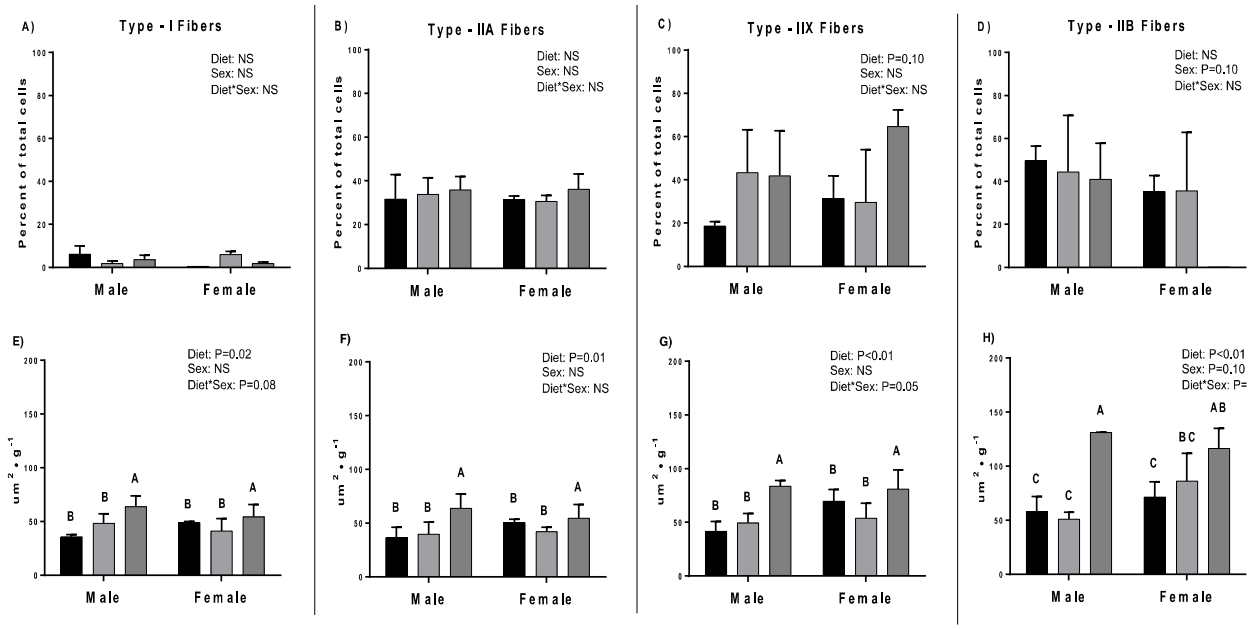

Figure 6: Fiber type distribution from the SOL of mice exposed to gestational and postnatal undernutrition. Values are expressed as mean ± SD. A) Percentage of type-I fibers in the SOL. Type-I fiber percentage was not significantly different (Diet*Sex: P=0.62). B) Percent of type-IIA fibers in the SOL. Fiber distribution was not significantly different (Diet*Sex: P=0.89) between groups. C) Percent of type-IIX fibers in the SOL was not significantly different (Diet*Sex: P=0.30) between groups. D) Percent of type-IIB fibers in the SOL. Distribution of type-IIB fibers was not significantly different (Diet*Sex: P=0.55). E) CSA of type-I fibers in the SOL. There was a significant diet effect (Diet: P=0.02) with CSA of PUN mice being significantly larger than GUN and CON. F) CSA of type-IIA fibers in the SOL. There was a significant diet effect (Diet: P=0.01) where CSA in PUN mice was larger than GUN and CON. G) CSA of type-IIX fibers in the SOL. There was a significant effect of diet (Diet: P<0.0001) on CSA as PUN was significantly larger than GUN and CON. H) CSA of type-IIB fibers in the SOL. CSA of SOL type-IIB fibers was significantly different (Diet*Sex: P=0.05), with the larger fibers in PUN male mice and PUN females versus CON females, CON males, and GUN males. GUN female CSA was not different from any group.

Extensor Digitorum Longus

Absolute EDL weight was significantly greater (Diet*Sex: P=0.02) in CON male and female mice versus GUN males (Table 3; Appendix). Relative EDL weight was significantly greater (Diet*Sex: P=0.002) among PUN male and CON female mice versus GUN males (Table 3; Appendix). Type-I and type-IIA fiber distribution in the EDL was not significantly different (Diet*Sex: P=0.14; Diet*Sex: P=0.46) between groups (Fig. 7A-B). Distribution of type-IIX

fibers in the EDL showed a diet effect with significantly greater (Diet: P=0.03) percent of Type-IIX fibers among CON versus PUN; the GUN group was not different from either group (Fig. 7C). Type-IIB fiber distribution in the EDL showed a diet effect with significantly greater (Diet: P=0.008) percent of Type IIB fibers in PUN versus CON; the GUN mice were not significantly different from either group (Fig. 7D).

Type-I (Sex: P=0.01, Fig. 7E) and Type-IIA (Sex: P=0.0002, Fig. 7F) fibers in the EDL had a sex effect with significantly larger CSA in females than in males. Cross-sectional area of EDL type-IIX (Diet*Sex: P=0.67; Fig. 7G) and type-IIB (Diet*Sex: P=0.83; Fig. 7H) fiber CSA was not significantly different among groups.

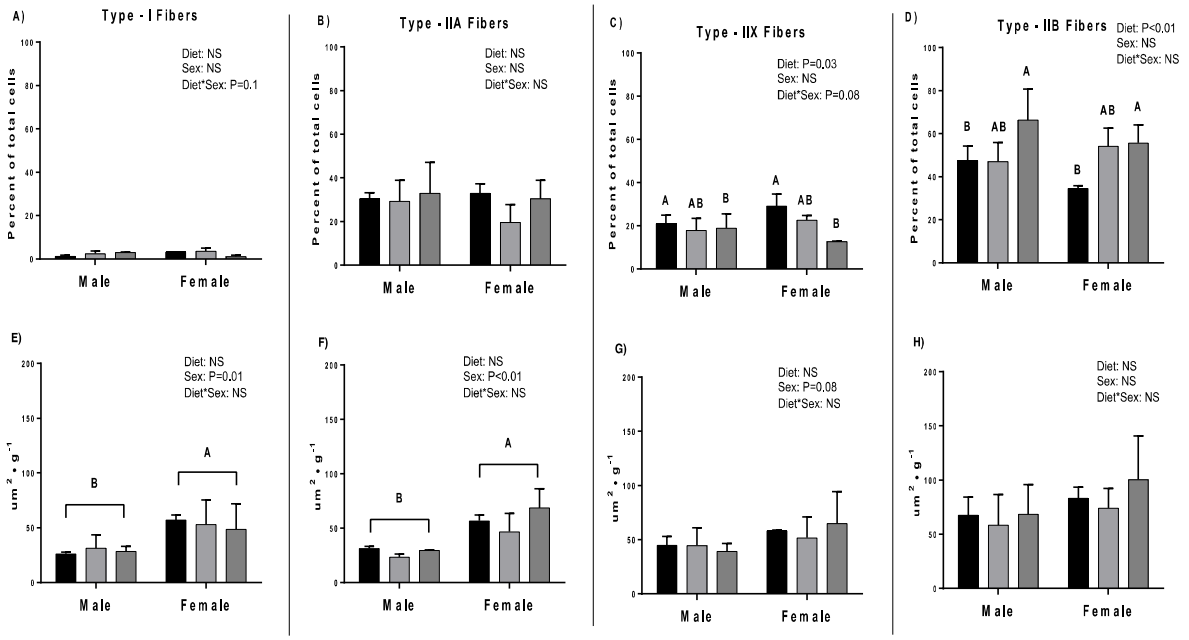

Figure 7: Fiber type distribution from the EDL of mice exposed to gestational and postnatal undernutrition. Values are expressed as mean ± SD. A) Percentage of type-I fibers in the EDL. Type-I fiber distribution in the EDL was not significantly different (Diet*Sex: P=0.14) between groups. B) Percent of type-IIA fibers in the EDL. Percentage of type-IIA fibers in the EDL was not significantly different (Diet*Sex: P=0.46) between groups. C) Percent of type-IIX fibers in the EDL. Type-IIX fiber distribution in the EDL showed a diet effect with significantly greater (Diet: P=0.03) percent among CON versus PUN; GUN was not different from either group. D) Percent of type-IIB fibers in the EDL. Distribution of type-IIB fibers showed a diet effect as percent in PUN mice was significantly greater (Diet: P=0.008) than CON; GUN was not different from either group. E) CSA of type-I fibers in the EDL. A sex effect was observed as female mouse CSA was significantly larger (Sex: P=0.01) than male. F) CSA of type-IIA fibers in the EDL. A sex effect was observed as female mouse CSA was significantly greater (Sex: P=0.0002) than male. G) CSA of type-IIX fibers in the EDL. No significant difference (Diet*Sex: P=0.67) was found in CSA of type-IIX fibers in the EDL. H) CSA of type-IIB fibers in the EDL. No significant difference (Diet*Sex: P=0.83) was found in CSA of type-I fibers in the EDL.

DISCUSSION

The DOHaD hypothesis asserts that low birth weight and growth restriction during postnatal life increases the risk of chronic disease in adulthood (Barker et al. 1986). A therapeutic countermeasure to mitigate the incidence of chronic disease is regular participation in exercise (Booth et al. 2012). However, growth restriction influences a variety of physiological processes that can affect exercise capacity; therefore it is necessary to document exercise capacity in a growth restricted model through the lens of Fick's equation to determine if and how exercise capacity is compromised. Surprisingly, there is a gap in literature regarding the influence of growth restriction on exercise capacity.

We showed that our nutritive model trended to reduce the weight of the GUN group at birth; yet once refeeding occurred, GUN mice experienced catch-up growth. This ability of GUN mice to regain weight to match CON has been observed previously (Fiorotto et al. 2014; Leszczynski et al. 2019). The PUN group however, did not experience catch-up growth following refeeding (Fig. 2).

In order to evaluate exercise capacity we performed a maximal treadmill running test (surrogate for oxygen consumption), assessed cardiac function via echocardiography & Doppler velocity analysis (i.e. oxygen delivery to working muscle), and determined skeletal muscle fiber type & cross-sectional area via immunohistochemistry (ability of muscle to utilize oxygen for work) in adulthood.

Measurement of oxygen consumption during exercise in mice using metabolic treadmills does not yield repeatable measures of maximal VO_2, whereas calculated work obtained during maximal graded treadmill elicits repeatable measures of exercise capacity (Brock et al. 2010; Knab et al. 2009). With enclosed treadmills, oxygen consumption readings reach a peak early in

the test and decline over time. This occurs because the mouse moves towards the back of the treadmill as the test proceeds, reducing the continuity between the mouse's ventilatory stream and the gas sampling airstream on the enclosed treadmill (Knab et al. 2009). A maximal work test is highly correlated with aerobic capacity (VO_2) and thus exists as an appropriate measure of exercise capacity in mice (Barbato et al. 1998; Fernando et al. 1993).

Our results showed that both GUN and PUN mice were unable to withstand high running intensities to the degree of CON mice (work at max speed; Fig. 3C), partly due to an inability to sustain running at max speed (Fig. 3B). Maximal exercise capacity is cardiac-limited (Andersen et al. 1985; Levine 2008). As such, we evaluated echocardiography and Doppler velocity.

LV chamber size was not significantly different among groups for this study (Table 2). However, the PUN and GUN groups had a reduced LV mass, LV posterior wall thickness, and LV anterior wall thickness in relation to CON (Table 2). Myocardial thickness in the LV is a positive regulator of ventricular inotropy (Nakamura et al. 2018). Our data agree with previous work in humans which shows a reduced wall thickness with exposure to growth restriction (Bjarnegard et al. 2013; Lewandowski et al. 2013). Thus, it appears that postnatal undernutrition reduces LV myocardium inotropy and contractility through myocardial size deficit leading to reduced SV, whereas gestationally undernourished mice experienced a restored LV wall thickness with refeeding such that SV was not impaired. Research has shown that increases in posterior wall thickness are associated with an increase in VO_2 max (Adamopoulos et al. 2003), likely as a function of greater SV from inotropic strength. Thus, we conclude that the thinner LV wall in PUN mice acted as a negative inotropic trait, leading to a reduced SV and exercise capacity.

The GUN group in this study showed a reduction in LV wall thickness and mass without accompanying reduction in SV. In GUN mice, LV posterior wall at systole (LVPW;s) was not affected as greatly (female: 8.5% thinner; male: 10.6% thinner) as in PUN mice (female: 18.6% thinner; male: 12.3% thinner) with respect to CON values. Furthermore, the GUN mice displayed a higher Mitral Valve E-to-A filling velocity ratio (MV E/A) and faster aortic ejection time as compared to PUN. Ratio of early-to-late filling velocity reflects both the compliance and the relaxation capacity of the ventricle during diastole; thus, a greater MV E/A would indicate a diastolic advantage for GUN mice relative to PUN. A shorter aortic ejection time indicates a more effective ventricular systole (Cakmak et al. 2007); this also may explain the preserved stroke volume in GUN mice. As such, we conclude that the timing of refeeding in the GUN mice restored LV functional capacity to a degree that stroke volume impairment was not present.

Despite the preservation of stroke volume, GUN mice showed impairment in maximal running capacity. This observation is important, as it indicates a phenomenon where GUN mice are similar to CON mice at rest (body weight and stroke volume), yet impairment becomes evident when a stressor is presented. It was observed that GUN mice had higher resting heart rates relative to other groups. Previous growth restriction literature showed that maternal protein restriction induces stress hyperresponsiveness that manifests as increased heart rate and circulating catecholamines in offspring (Augustyniak et al. 2010; Johansson et al. 2007; Tonkiss et al. 1998). As higher resting heart rate compromises maximal cardiac output and reduces maximal exercise capacity (Laukkanen et al. 2009; Nauman et al. 2012), we conclude that the greater proximity to maximal heart rate reduced cardiac output during maximal exercise, thus limiting maximal exercise capacity for GUN mice.

Fiber type expression and fiber CSA serve as independent regulators of O_2 uptake at the fiber level, with type-I fibers showing the highest oxygen utilization capacity due to greater capillarization, myoglobin content, mitochondrial abundance, and oxygen transit (Liu et al. 2012a). Additionally, high fiber CSA leads to a greater O_2 transit time, decreasing the oxidative capacity of a given fiber (Liu et al. 2012a). Models of postnatal undernourishment are typically associated with a decrease in muscle mass due to reduced translational capacity from lower ribosome content (Fiorotto et al. 2014). Absolute muscle wet weight differences were only observed in CON male and females for the soleus and EDL (data not shown). When standardizing muscle wet weights to body weight the soleus of PUN female and CON males were larger than CON females, while the EDL of CON female and PUN males were larger than GUN males. There were no differences in relative or absolute wet weights of the diaphragm. According to standard practices (Leszczynski et al. 2019; Nakada et al. 2016; Nonaka et al. 2018; Wang et al. 2018; You et al. 2015), to more closely evaluate muscle mass differences our experiment normalized muscle CSA to body weight and showed type-I, type-IIA, and type-IIX fibers of the SOL were significantly larger in PUN mice than CON and GUN. We suspect that the loading frequency of the SOL—due to primary locomotive reliance (X. Hu et al. 2017)—led to a preservation of the muscle in PUN mice despite their restriction of body weight. Previous growth restriction research has indicated that postnatal undernourishment affects the SOL muscle to a lesser degree (Ward et al. 1993), which supports our observation.

The EDL of mice from the PUN group showed a significantly greater percent of type-IIB fibers compared to CON—who in turn showed a greater percent of type-IIX fibers than PUN. Type-IIB fibers fatigue more quickly at maximal intensity than type-IIX, due mostly to lower mitochondrial content (Schiaffino et al. 2011). We conclude that the shift towards the more

highly-fatiguing type-IIB fibers in the EDL of PUN mice contributed to earlier fatigue and subsequent exercise impairment at maximal speed. This finding is supported by findings in previous models, which indicate increased glycolytic activity at the expense of oxidative capacity in rats undernourished from birth (Raju 1974).

PUN female mice showed an increased percent of type-IIA fibers in the diaphragm. This increased percent appears to arise at the expense of type-IIB fiber distribution. Given the contractile demands of the diaphragm, this would suggest a positive adaptation in PUN female mice allowing for a greater ability to delay diaphragmatic fatigue (Pette et al. 2000). In isolation, this may be true; however, as VO_2 increases with exercise, the relative contribution of the diaphragm to ventilation decreases and accessory inspiratory musculature involvement rises (Laroche et al. 1988). As such, diaphragm weakness only subtly affects exercise capacity (Johnson et al. 1993). While diaphragm differences minimally affect exercise, these fiber differences may be of interest for future research on respiratory dynamics in PUN mice. Skeletal muscle of GUN mice did not show marked variation from that of CON with respect to fiber distribution or CSA in the diaphragm, SOL, or EDL (Fig.5-7). These data confirm previous literature on gestational undernourishment with refeeding at birth, where skeletal muscle phenotype "catches up" to that of control groups (Fiorotto et al. 2014; Leszczynski et al. 2019). Thus, impairment in maximal running performance among GUN mice is isolated to chronotropic dysregulation—while PUN mice show both cardiac and skeletal muscle impairment.

Muscle fiber distribution of the DIA and EDL muscles in this experiment were similar to those observed in previous studies (Baán et al. 2013; Greising et al. 2015; Guido et al. 2010). However, the type-I fiber distribution in the SOL was atypical. There are limited data available on the fiber distribution of this muscle in the FVB strain; however, some researchers have

observed a reduction in type-I fibers in the FVB strain (Ferguson et al. 2019; Leszczynski et al. 2019). Furthermore, these investigators confirmed low type-I fiber expression using both SDS Page and confocal imaging, thus given this support we conclude are results are accurate.

Limitations

There is a paucity of literature related to growth restriction and exercise capacity, which is alarming, as exercise could attenuate the incidence of chronic disease in this population. We demonstrate maximal exercise capacity is cardiac-limited in gestational undernourished mice, while undernutrition during lactation impairs treadmill running via cardiac and skeletal muscle impairment. It should be noted that our echocardiography and Doppler velocity measurements were performed with the animal at rest. While we were able to elucidate differences, future studies could couple cardiac imaging with pharmacological stimulants to evaluate cardiac function under similar loads of maximal treadmill running. This experiment did not utilize stress echocardiography, as previous literature shows that resting measures of cardiac structure are predictive of maximal functional capacity (Arbab-Zadeh et al. 2014; Dini 2011; Mihl et al. 2008; Pluim et al. 2000). We showed skeletal muscle differences using histology, the next step in future studies could implement functional measures such as mitochondrial function via high-resolution respirometry or force fatigue measures of isolated skeletal muscles. Our objective in this investigation was to determine the developmental programming of exercise capacity through cardiac and skeletal muscle function as they have been consistently validated as the primary variables associated with maximal exercise capacity (Fick 1870). However, future studies should evaluate the role of other tissues (lungs and vasculature) on exercise capacity. Furthermore, we showed exercise capacity differences that were not due to a pathological state (strength of this

study); however, as mice age cardiovascular disease could contribute to exercise capacity impairments and the cause of them occur should be studied. We did not observe any statistical differences in myocardial performance index (indicator of global cardiac health), but with greater aging this variable could become significant, so the logical next step in this investigation is to evaluate mice at advanced ages rather than adulthood alone.

APPENDIX

A reviewer for this document has requested data regarding the individual weights of muscles for the mice in this study. This table was not included in the original manuscript.

	Male			Female			*P*		
Muscle	**CON**	**GUN**	**PUN**	**CON**	**GUN**	**PUN**	***Diet***	***Sex***	***Diet*Sex***
DIA wt. (mg)	**21.2±6.41**	**20.87±13.42**	**21.97±12.48**	**16.16±4.45**	**22.87±17.06**	**17.33±9.85**	**NS**	**NS**	**NS**
SOL wt. (mg)	**33.4±12.88^{A}**	**26.27±18.68^{AB}**	**14.3±2.98^{AB}**	**9.95±5.42^{B}**	**24.27±18.29^{AB}**	**23.73±27.02^{AB}**	**NS**	**NS**	**0.0034**
EDL wt. (mg)	**31.37±11.62^{A}**	**15±7.23^{B}**	**29.05±20.58^{AB}**	**31±9.12^{A}**	**23.07±15.83^{AB}**	**16.25±9.17^{AB}**	**0.004**	**NS**	**0.02**
Rel. DIA wt. (mg · g^{-1} BW)	**0.76±0.22**	**0.76±0.49**	**1.07±0.6**	**0.79±0.27**	**1.14±0.81**	**0.94±0.5**	**NS**	**NS**	**NS**
Rel. SOL wt. (mg · g^{-1} BW)	**1.2±0.47^{A}**	**0.94±0.67^{AB}**	**0.75±0.11^{AB}**	**0.5±0.3^{B}**	**1.16±0.82^{AB}**	**1.35±1.48^{A}**	**NS**	**NS**	**0.02**
Rel. EDL wt. (mg · g^{-1} BW)	**1.13±0.43^{AB}**	**0.54±0.26^{B}**	**1.49±1.02^{A}**	**1.48±0.34^{A}**	**1.13±0.74^{AB}**	**0.9±0.52^{AB}**	**0.02**	**NS**	**0.002**

Table 3: Absolute and Relative Muscle Weights. Difference in superscripted letters across a row indicates statistical significance

BIBLIOGRAPHY

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Adamopoulos, S., Parissis, J. T., Paraskevaidis, I., Karatzas, D., Livanis, E., Georgiadis, M., et al. 2003. Effects of growth hormone on circulating cytokine network, and left ventricular contractile performance and geometry in patients with idiopathic dilated cardiomyopathy. *Eur Heart J, 24*(24): 2186-2196.

Aihie Sayer, A., Dunn, R., Langley-Evans, S., and Cooper, C. 2001. Prenatal exposure to a maternal low protein diet shortens life span in rats. *Gerontology, 47*(1): 9-14. doi:10.1159/000052764

Andersen, P., and Saltin, B. 1985. Maximal perfusion of skeletal muscle in man. *J Physiol, 366*: 233-249.

Arbab-Zadeh, A., Perhonen, M., Howden, E., Peshock, R. M., Zhang, R., Adams-Huet, B., et al. 2014. Cardiac remodeling in response to 1 year of intensive endurance training. *Circulation, 130*(24): 2152-2161. doi:10.1161/CIRCULATIONAHA.114.010775

Augustyniak, R. A., Singh, K., Zeldes, D., Singh, M., and Rossi, N. F. 2010. Maternal protein restriction leads to hyperresponsiveness to stress and salt-sensitive hypertension in male offspring. *Am J Physiol Regul Integr Comp Physiol, 298*(5): R1375-1382. doi:10.1152/ajpregu.00848.2009

Baán, J. A., Kocsis, T., Keller-Pintér, A., Müller, G., Zádor, E., Dux, L., et al. 2013. The compact mutation of myostatin causes a glycolytic shift in the phenotype of fast skeletal muscles. *The journal of histochemistry and cytochemistry : official journal of the Histochemistry Society, 61*(12): 889-900. doi:10.1369/0022155413503661

Baldwin, K. M., Cooke, D. A., and Cheadle, W. G. 1977. Time course adaptations in cardiac and skeletal muscle to different running programs. *J Appl Physiol Respir Environ Exerc Physiol, 42*(2): 267-272. doi:10.1152/jappl.1977.42.2.267

Bamman, M. M., Clarke, M. S. F., Feeback, D. L., Talmadge, R. J., Stevens, B. R., Lieberman, S. A., et al. 1998. Impact of resistance exercise during bed rest on skeletal muscle sarcopenia and myosin isoform distribution. *Journal of Applied Physiology, 84*(1): 157-163. doi:10.1152/jappl.1998.84.1.157

Barbato, J. C., Koch, L. G., Darvish, A., Cicila, G. T., Metting, P. J., and Britton, S. L. 1998. Spectrum of aerobic endurance running performance in eleven inbred strains of rats. *J Appl Physiol (1985), 85*(2): 530-536. doi:10.1152/jappl.1998.85.2.530

Barker, D. J., Gluckman, P. D., Godfrey, K. M., Harding, J. E., Owens, J. A., and Robinson, J. S. 1993. Fetal nutrition and cardiovascular disease in adult life. *Lancet, 341*(8850): 938-941.

Barker, D. J., and Osmond, C. 1986. Infant mortality, childhood nutrition, and ischaemic heart disease in England and Wales. *Lancet, 1*(8489): 1077-1081.

Barker, D. J., Winter, P. D., Osmond, C., Margetts, B., and Simmonds, S. J. 1989. Weight in infancy and death from ischaemic heart disease. *Lancet, 2*(8663): 577-580.

Barstow, T. J., Jones, A. M., Nguyen, P. H., and Casaburi, R. 1996. Influence of muscle fiber type and pedal frequency on oxygen uptake kinetics of heavy exercise. *J Appl Physiol (1985), 81*(4): 1642-1650. doi:10.1152/jappl.1996.81.4.1642

Beauchamp, B., Thrush, A. B., Quizi, J., Antoun, G., McIntosh, N., Al-Dirbashi, O. Y., et al. 2015. Undernutrition during pregnancy in mice leads to dysfunctional cardiac muscle respiration in adult offspring. *Biosci Rep, 35*(3). doi:10.1042/bsr20150007

Bedi, K. S., Birzgalis, A. R., Mahon, M., Smart, J. L., and Wareham, A. C. 1982. Early life undernutrition in rats. 1. Quantitative histology of skeletal muscles from underfed young and refed adult animals. *Br J Nutr, 47*(3): 417-431.

Bee, G. 2004. Effect of early gestation feeding, birth weight, and gender of progeny on muscle fiber characteristics of pigs at slaughter. *J Anim Sci, 82*(3): 826-836. doi:10.2527/2004.823826x

Bertram, C. E., and Hanson, M. A. 2001. Animal models and programming of the metabolic syndrome. *Br Med Bull, 60*: 103-121.

Bjarnegard, N., Morsing, E., Cinthio, M., Lanne, T., and Brodszki, J. 2013. Cardiovascular function in adulthood following intrauterine growth restriction with abnormal fetal blood flow. *Ultrasound Obstet Gynecol, 41*(2): 177-184. doi:10.1002/uog.12314

Booth, F. W., Roberts, C. K., and Laye, M. J. 2012. Lack of exercise is a major cause of chronic diseases. *Compr Physiol, 2*(2): 1143-1211. doi:10.1002/cphy.c110025

Brock, D. W., Chandler-Laney, P. C., Alvarez, J. A., Gower, B. A., Gaesser, G. A., and Hunter, G. R. 2010. Perception of exercise difficulty predicts weight regain in formerly overweight women. *Obesity (Silver Spring), 18*(5): 982-986. doi:10.1038/oby.2009.318

Cakmak, N., Cakmak, M., Akyol, A., Oguz, E., Sayar, N., Eksik, A., et al. 2007. Effect of radiofrequency catheter ablation on Doppler echocardiographic parameters in patients with Wolff-Parkinson-White syndrome. *Int Heart J, 48*(2): 165-175.

Chan, L. L. Y., Sébert, S. P., Hyatt, M. A., Stephenson, T., Budge, H., Symonds, M. E., et al. 2009. Effect of maternal nutrient restriction from early to midgestation on cardiac function and metabolism after adolescent-onset obesity. *Am J Physiol Regul Integr Comp Physiol, 296*(5): R1455-R1463. doi:10.1152/ajpregu.91019.2008

Cheema, K. K., Dent, M. R., Saini, H. K., Aroutiounova, N., and Tappia, P. S. 2005. Prenatal exposure to maternal undernutrition induces adult cardiac dysfunction. *British Journal of Nutrition, 93*(4): 471-477. doi:10.1079/BJN20041392

Chmielewski, C. 2003. Renal anatomy and overview of nephron function. *Nephrol Nurs J, 30*(2): 185-190; quiz 191-182.

Corstius, H. B., Zimanyi, M. A., Maka, N., Herath, T., Thomas, W., van der Laarse, A., et al. 2005. Effect of Intrauterine Growth Restriction on the Number of Cardiomyocytes in Rat Hearts. *Pediatric Research, 57*: 796. doi:10.1203/01.PDR.0000157726.65492.CD

Costello, P. M., Rowlerson, A., Astaman, N. A., Anthony, F. E. W., Sayer, A. A., Cooper, C., et al. 2008. Peri-implantation and late gestation maternal undernutrition differentially affect fetal sheep skeletal muscle development. *The Journal of physiology, 586*(9): 2371-2379. doi:10.1113/jphysiol.2008.150987

Coyle, E. F., Hopper, M. K., and Coggan, A. R. 1990. Maximal oxygen uptake relative to plasma volume expansion. *Int J Sports Med, 11*(2): 116-119. doi:10.1055/s-2007-1024774

Demontis, G. C., Germani, M. M., Caiani, E. G., Barravecchia, I., Passino, C., and Angeloni, D. 2017. Human Pathophysiological Adaptations to the Space Environment. *Frontiers in physiology, 8*: 547-547. doi:10.3389/fphys.2017.00547

Desai, M., Crowther, N. J., Lucas, A., and Hales, C. N. 1996. Organ-selective growth in the offspring of protein-restricted mothers. *Br J Nutr, 76*(4): 591-603.

Dickinson, H., Ellery, S., Davies-Tuck, M., Tolcos, M., Nitsos, I., Walker, D. W., et al. 2017. Description of a method for inducing fetal growth restriction in the spiny mouse. *J Dev Orig Health Dis, 8*(5): 550-555. doi:10.1017/s2040174417000393

Dini, F. L. 2011. Assessment of cardiac dynamics during stress echocardiography by the peak power output-to-left ventricular mass ratio. *Future Cardiol, 7*(3): 347-356. doi:10.2217/fca.11.12

Dunlap, K. A., Brown, J. D., Keith, A. B., and Satterfield, M. C. 2015. Factors controlling nutrient availability to the developing fetus in ruminants. *Journal of animal science and biotechnology, 6*(1): 16-16. doi:10.1186/s40104-015-0012-5

Eberli, F. R., Sam, F., Ngoy, S., Apstein, C. S., and Colucci, W. S. 1998. Left-ventricular structural and functional remodeling in the mouse after myocardial infarction: assessment with the isovolumetrically-contracting Langendorff heart. *J Mol Cell Cardiol, 30*(7): 1443-1447. doi:10.1006/jmcc.1998.0702

Ferguson, D. P., Monroe, T. O., Heredia, C. P., Fleischman, R. E., Rodney, G. G., Taffet, G. E., et al. 2019. Postnatal undernutrition alters adult female mouse cardiac structure and function leading to limited exercise capacity. *The Journal of physiology, 0*(ja). doi:10.1113/JP277637

Fernando, P., Bonen, A., and Hoffman-Goetz, L. 1993. Predicting submaximal oxygen consumption during treadmill running in mice. *Can J Physiol Pharmacol, 71*(10-11): 854-857.

Fick, A. 1870. Uber die Messung des Blutquantums in den Herzventrikeln,. *Seitung der Physikalisches und Medicinisches Gesellschaft zu Würzburg, 2*: 290-291.

Fiorotto, M. L., Davis, T. A., Sosa, H. A., Villegas-Montoya, C., Estrada, I., and Fleischmann, R. 2014. Ribosome abundance regulates the recovery of skeletal muscle protein mass upon recuperation from postnatal undernutrition in mice. *J Physiol, 592*(23): 5269-5286. doi:10.1113/jphysiol.2014.279067

Gao, S., Ho, D., Vatner, D. E., and Vatner, S. F. 2011. Echocardiography in Mice. *Curr Protoc Mouse Biol, 1*: 71-83. doi:10.1002/9780470942390.mo100130

Garton, F. C., North, K. N., Koch, L. G., Britton, S. L., Nogales-Gadea, G., and Lucia, A. 2016. Rodent models for resolving extremes of exercise and health. *Physiol Genomics, 48*(2): 82-92. doi:10.1152/physiolgenomics.00077.2015

Gouma, E., Simos, Y., Verginadis, I., Lykoudis, E., Evangelou, A., and Karkabounas, S. 2012. A simple procedure for estimation of total body surface area and determination of a new value of Meeh's constant in rats. *Lab Anim, 46*(1): 40-45. doi:10.1258/la.2011.011021

Greising, S. M., Medina-Martínez, J. S., Vasdev, A. K., Sieck, G. C., and Mantilla, C. B. 2015. Analysis of muscle fiber clustering in the diaphragm muscle of sarcopenic mice. *Muscle & nerve, 52*(1): 76-82. doi:10.1002/mus.24641

Guido, A. N., Campos, G. E. R., Neto, H. S., Marques, M. J., and Minatel, E. 2010. Fiber Type Composition of the Sternomastoid and Diaphragm Muscles of Dystrophin-Deficient mdx Mice. *The Anatomical Record, 293*(10): 1722-1728. doi:10.1002/ar.21224

Gupta, S., Rohatgi, A., Ayers, C. R., Willis, B. L., Haskell, W. L., Khera, A., et al. 2011. Cardiorespiratory fitness and classification of risk of cardiovascular disease mortality. *Circulation, 123*(13): 1377-1383. doi:10.1161/circulationaha.110.003236

Hannan, K. M., Brandenburger, Y., Jenkins, A., Sharkey, K., Cavanaugh, A., Rothblum, L., et al. 2003. mTOR-dependent regulation of ribosomal gene transcription requires S6K1 and is mediated by phosphorylation of the carboxy-terminal activation domain of the nucleolar transcription factor UBF. *Molecular and cellular biology, 23*(23): 8862-8877. doi:10.1128/MCB.23.23.8862-8877.2003

Hartkopf, J., Schleger, F., Keune, J., Wiechers, C., Pauluschke-Froehlich, J., Weiss, M., et al. 2018. Impact of Intrauterine Growth Restriction on Cognitive and Motor Development at 2 Years of Age. *Frontiers in physiology, 9*: 1278-1278. doi:10.3389/fphys.2018.01278

Hennington, B. S., and Alexander, B. T. 2013. Linking intrauterine growth restriction and blood pressure: insight into the human origins of cardiovascular disease. *Circulation, 128*(20): 2179-2180. doi:10.1161/CIRCULATIONAHA.113.006323

Higginbotham, M. B., Morris, K. G., Williams, R. S., McHale, P. A., Coleman, R. E., and Cobb, F. R. 1986. Regulation of stroke volume during submaximal and maximal upright exercise in normal man. *Circ Res, 58*(2): 281-291.

Horton, D. M., Saint, D. A., Owens, J. A., Kind, K. L., and Gatford, K. L. 2016. Spontaneous intrauterine growth restriction due to increased litter size in the guinea pig programmes postnatal growth, appetite and adult body composition. *J Dev Orig Health Dis, 7*(5): 548-562. doi:10.1017/s2040174416000295

Hu, L., Han, F., Chen, L., Peng, X., Chen, D., Wu, D., et al. 2018. High nutrient intake during the early postnatal period accelerates skeletal muscle fiber growth and maturity in intrauterine growth-restricted pigs. *Genes & nutrition, 13*: 23-23. doi:10.1186/s12263-018-0612-8

Hu, X., Charles, J. P., Akay, T., Hutchinson, J. R., and Blemker, S. S. 2017. Are mice good models for human neuromuscular disease? Comparing muscle excursions in walking between mice and humans. *Skeletal Muscle, 7*(1): 26. doi:10.1186/s13395-017-0143-9

Hughson, M., Farris, A. B., 3rd, Douglas-Denton, R., Hoy, W. E., and Bertram, J. F. 2003. Glomerular number and size in autopsy kidneys: the relationship to birth weight. *Kidney Int, 63*(6): 2113-2122. doi:10.1046/j.1523-1755.2003.00018.x

Huttin, O., Mandry, D., Eschalier, R., Zhang, L., Micard, E., Odille, F., et al. 2017. Cardiac remodeling following reperfused acute myocardial infarction is linked to the concomitant evolution of vascular function as assessed by cardiovascular magnetic resonance. *Journal of cardiovascular magnetic resonance : official journal of the Society for Cardiovascular Magnetic Resonance, 19*(1): 2-2. doi:10.1186/s12968-016-0314-6

Ilebekk, A., and Kiil, F. 1979. Role of preload and inotropy in stroke volume regulation at constant heart rate. *Scand J Clin Lab Invest, 39*(1): 71-78.

Inoue, S., Shibata, Y., Kishi, H., Nitobe, J., Iwayama, T., Yashiro, Y., et al. 2017. Decreased left ventricular stroke volume is associated with low-grade exercise tolerance in patients with chronic obstructive pulmonary disease. *BMJ open respiratory research, 4*(1): e000158-e000158. doi:10.1136/bmjresp-2016-000158

Jiang, X., Ma, H., Wang, Y., and Liu, Y. 2013. Early Life Factors and Type 2 Diabetes Mellitus. *Journal of Diabetes Research, 2013*: 11. doi:10.1155/2013/485082

Johansson, S., Norman, M., Legnevall, L., Dalmaz, Y., Lagercrantz, H., and Vanpee, M. 2007. Increased catecholamines and heart rate in children with low birth weight: perinatal contributions to sympathoadrenal overactivity. *J Intern Med, 261*(5): 480-487. doi:10.1111/j.1365-2796.2007.01776.x

Johnson, B. D., Babcock, M. A., Suman, O. E., and Dempsey, J. A. 1993. Exercise-induced diaphragmatic fatigue in healthy humans. *J Physiol, 460*: 385-405.

Jones, A. P., Simson, E. L., and Friedman, M. I. 1984. Gestational undernutrition and the development of obesity in rats. *J Nutr, 114*(8): 1484-1492. doi:10.1093/jn/114.8.1484

Joyner, M. J., and Casey, D. P. 2015. Regulation of increased blood flow (hyperemia) to muscles during exercise: a hierarchy of competing physiological needs. *Physiological reviews, 95*(2): 549-601. doi:10.1152/physrev.00035.2013

Kanstrup, I. L., and Ekblom, B. 1982. Acute hypervolemia, cardiac performance, and aerobic power during exercise. *J Appl Physiol Respir Environ Exerc Physiol, 52*(5): 1186-1191. doi:10.1152/jappl.1982.52.5.1186

Kho, A. T., Kang, P. B., Kohane, I. S., and Kunkel, L. M. 2006. Transcriptome-scale similarities between mouse and human skeletal muscles with normal and myopathic phenotypes. *BMC musculoskeletal disorders, 7*: 23-23. doi:10.1186/1471-2474-7-23

Khorram, N. M., Magee, T. R., Wang, C., Desai, M., Ross, M., and Khorram, O. 2011. Maternal undernutrition programs offspring adrenal expression of steroidogenic enzymes. *Reproductive sciences (Thousand Oaks, Calif.), 18*(10): 931-940. doi:10.1177/1933719111404613

Kiel, J. W., and Shepherd, A. P. 1983. Continuous measurement of arteriovenous oxygen difference and VO2 by microcomputer. *American Journal of Physiology-Heart and Circulatory Physiology, 245*(1): H178-H182. doi:10.1152/ajpheart.1983.245.1.H178

Knab, A. M., Bowen, R. S., Moore-Harrison, T., Hamilton, A. T., Turner, M. J., and Lightfoot, J. T. 2009. Repeatability of exercise behaviors in mice. *Physiol Behav, 98*(4): 433-440. doi:10.1016/j.physbeh.2009.07.006

Krishna, U., and Bhalerao, S. 2011. Placental insufficiency and fetal growth restriction. *Journal of obstetrics and gynaecology of India, 61*(5): 505-511. doi:10.1007/s13224-011-0092-x

Krishnan, A., Samtani, R., Dhanantwari, P., Lee, E., Yamada, S., Shiota, K., et al. 2014. A detailed comparison of mouse and human cardiac development. *Pediatr Res, 76*(6): 500-507. doi:10.1038/pr.2014.128

Kushmerick, M. J., Meyer, R. A., and Brown, T. R. 1992. Regulation of oxygen consumption in fast- and slow-twitch muscle. *American Journal of Physiology-Cell Physiology, 263*(3): C598-C606. doi:10.1152/ajpcell.1992.263.3.C598

Kusunose, K., Penn, M. S., Zhang, Y., Cheng, Y., Thomas, J. D., Marwick, T. H., et al. 2012. How Similar Are the Mice to Men? Between-Species Comparison of Left Ventricular Mechanics Using Strain Imaging. *PLOS ONE, 7*(6): e40061. doi:10.1371/journal.pone.0040061

Kwong, W. Y., Wild, A. E., Roberts, P., Willis, A. C., and Fleming, T. P. 2000. Maternal undernutrition during the preimplantation period of rat development causes blastocyst abnormalities and programming of postnatal hypertension. *Development, 127*(19): 4195-4202.

La Gerche, A., and Gewillig, M. 2010. What Limits Cardiac Performance during Exercise in Normal Subjects and in Healthy Fontan Patients? *International journal of pediatrics, 2010*: 791291. doi:10.1155/2010/791291

Lang, D. H., Sharkey, N. A., Lionikas, A., Mack, H. A., Larsson, L., Vogler, G. P., et al. 2005. Adjusting Data to Body Size: A Comparison of Methods as Applied to Quantitative Trait Loci Analysis of Musculoskeletal Phenotypes. *J Bone Miner Res, 20*(5): 748-757. doi:10.1359/jbmr.041224

Laroche, C. M., Carroll, N., Moxham, J., and Green, M. 1988. Clinical significance of severe isolated diaphragm weakness. *Am Rev Respir Dis, 138*(4): 862-866. doi:10.1164/ajrccm/138.4.862

Laukkanen, J. A., Laaksonen, D., Lakka, T. A., Savonen, K., Rauramaa, R., Makikallio, T., et al. 2009. Determinants of cardiorespiratory fitness in men aged 42 to 60 years with and without cardiovascular disease. *Am J Cardiol, 103*(11): 1598-1604. doi:10.1016/j.amjcard.2009.01.371

Lehmann, M., Keul, J., Huber, G., and Da Prada, M. 1981. Plasma catecholamines in trained and untrained volunteers during graduated exercise. *Int J Sports Med, 2*(3): 143-147. doi:10.1055/s-2008-1034601

Leszczynski, E. C., Visker, J. R., and Ferguson, D. P. 2019. The Effect of Growth Restriction on Voluntary Physical Activity Engagement in Mice. *Med Sci Sports Exerc*. doi:10.1249/mss.0000000000002040

Letsinger, A. C., Granados, J. Z., Little, S. E., and Lightfoot, J. T. 2019. Alleles associated with physical activity levels are estimated to be older than anatomically modern humans. *PloS one, 14*(4): e0216155. doi:10.1371/journal.pone.0216155

Levine, B. D. 2008. V̇(O(2),max): what do we know, and what do we still need to know? *J Physiol, 586*(Pt 1): 25-34. doi:10.1113/jphysiol.2007.147629

Lewandowski, A. J., Bradlow, W. M., Augustine, D., Davis, E. F., Francis, J., Singhal, A., et al. 2013. Right ventricular systolic dysfunction in young adults born preterm. *Circulation, 128*(7): 713-720. doi:10.1161/circulationaha.113.002583

Li, F., Wang, X., Capasso, J. M., and Gerdes, A. M. 1996. Rapid transition of cardiac myocytes from hyperplasia to hypertrophy during postnatal development. *J Mol Cell Cardiol, 28*(8): 1737-1746. doi:10.1006/jmcc.1996.0163

Lightfoot, J. T., Turner, M. J., Debate, K. A., and Kleeberger, S. R. (2001). *Interstrain variation in murine aerobic capacity* (Vol. 33).

Lindsey, M. L., Kassiri, Z., Virag, J. A. I., de Castro Brás, L. E., and Scherrer-Crosbie, M. 2018. Guidelines for measuring cardiac physiology in mice. *American Journal of Physiology-Heart and Circulatory Physiology, 314*(4): H733-H752. doi:10.1152/ajpheart.00339.2017

Lister, J. P., Schwagerl, A. L., Tonkiss, J., McCormick, C. M., and Galler, J. R. 2004. Prenatal Protein Malnutrition in Rats Alters the c-Fos Response of Neurons in the Anterior Cingulate and Medial Prefrontal Region to Behavioral Stress AU - Rosene, Douglas L. *Nutritional Neuroscience, 7*(5-6): 281-289. doi:10.1080/10284150400015573

Liu, G., Mac Gabhann, F., and Popel, A. S. 2012a. Effects of Fiber Type and Size on the Heterogeneity of Oxygen Distribution in Exercising Skeletal Muscle. *PLOS ONE, 7*(9): e44375. doi:10.1371/journal.pone.0044375

Liu, G., Mac Gabhann, F., and Popel, A. S. 2012b. Effects of fiber type and size on the heterogeneity of oxygen distribution in exercising skeletal muscle. *PloS one, 7*(9): e44375-e44375. doi:10.1371/journal.pone.0044375

Maertzdorf, W. J., Aldenhuyzen-Dorland, W., Slaaf, D. W., Tangelder, G. J., and Blanco, C. E. 1991. Circulating blood volume in appropriate and small for gestational age full term and preterm polycythaemic infants. *Acta Paediatr Scand, 80*(6-7): 620-627.

Magder, S. 2016. Volume and its relationship to cardiac output and venous return. *Critical care (London, England), 20*(1): 271-271. doi:10.1186/s13054-016-1438-7

Martins, V. J. B., Toledo Florêncio, T. M. M., Grillo, L. P., do Carmo P Franco, M., Martins, P. A., Clemente, A. P. G., et al. 2011. Long-lasting effects of undernutrition. *International journal of environmental research and public health, 8*(6): 1817-1846. doi:10.3390/ijerph8061817

Mason, D. T. 1978. Afterload reduction and cardiac performance. Physiologic basis of systemic vasodilators as a new approach in treatment of congestive heart failure. *Am J Med, 65*(1): 106-125.

Massett, M. P., and Berk, B. C. 2005. Strain-dependent differences in responses to exercise training in inbred and hybrid mice. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology, 288*(4): R1006-R1013. doi:10.1152/ajpregu.00476.2004

Masterson, M., Morgan, A., Multer, C., and Cipriani, D. (2006). *The Role of Lower Leg Muscle Activity in Blood Pressure Maintenance of Older Adults* (Vol. 60).

Mazic, S., Suzic Lazic, J., Dekleva, M., Antic, M., Soldatovic, I., Djelic, M., et al. 2015. The impact of elevated blood pressure on exercise capacity in elite athletes. *Int J Cardiol, 180*: 171-177. doi:10.1016/j.ijcard.2014.11.125

Menendez-Castro, C., Rascher, W., and Hartner, A. 2018. Intrauterine growth restriction - impact on cardiovascular diseases later in life. *Molecular and cellular pediatrics, 5*(1): 4-4. doi:10.1186/s40348-018-0082-5

Mihl, C., Dassen, W. R. M., and Kuipers, H. 2008. Cardiac remodelling: concentric versus eccentric hypertrophy in strength and endurance athletes. *Netherlands heart journal : monthly journal of the Netherlands Society of Cardiology and the Netherlands Heart Foundation, 16*(4): 129-133. doi:10.1007/bf03086131

Miljkovic, N., Lim, J.-Y., Miljkovic, I., and Frontera, W. R. 2015. Aging of skeletal muscle fibers. *Annals of rehabilitation medicine, 39*(2): 155-162. doi:10.5535/arm.2015.39.2.155

Milne, E., Royle, J. A., de Klerk, N. H., Blair, E., Bailey, H., Cole, C., et al. 2009. Fetal Growth and Risk of Childhood Acute Lymphoblastic Leukemia: Results From an Australian Case-Control Study. *American Journal of Epidemiology, 170*(2): 221-228. doi:10.1093/aje/kwp117

Mondal, H., and Mishra, S. P. 2017. Effect of BMI, Body Fat Percentage and Fat Free Mass on Maximal Oxygen Consumption in Healthy Young Adults. *Journal of clinical and diagnostic research : JCDR, 11*(6): CC17-CC20. doi:10.7860/JCDR/2017/25465.10039

Muaku, S. M., Thissen, J.-P., Gerard, G., Ketelslegers, J.-M., and Maiter, D. 1997. Postnatal Catch-Up Growth Induced by Growth Hormone and Insulin-Like Growth Factor-I in Rats

with Intrauterine Growth Retardation Caused by Maternal Protein Malnutrition1. *Pediatric Research, 42*: 370. doi:10.1203/00006450-199709000-00019

Nakada, S., Ogasawara, R., Kawada, S., Maekawa, T., and Ishii, N. 2016. Correlation between Ribosome Biogenesis and the Magnitude of Hypertrophy in Overloaded Skeletal Muscle. *PLOS ONE, 11*(1): e0147284. doi:10.1371/journal.pone.0147284

Nakamura, M., and Sadoshima, J. 2018. Mechanisms of physiological and pathological cardiac hypertrophy. *Nature Reviews Cardiology, 15*(7): 387-407. doi:10.1038/s41569-018-0007-y

Nauman, J., Aspenes, S. T., Nilsen, T. I., Vatten, L. J., and Wisloff, U. 2012. A prospective population study of resting heart rate and peak oxygen uptake (the HUNT Study, Norway). *PLOS ONE, 7*(9): e45021. doi:10.1371/journal.pone.0045021

Nonaka, K., Une, S., Komatsu, M., Yamaji, R., and Akiyama, J. 2018. Heat stress prevents the decrease in succinate dehydrogenase activity in the extensor digitorum longus of streptozotocin-induced diabetic rats. *Physiol Res, 67*(1): 117-126. doi:10.33549/physiolres.933617

Nutter, D. O., Murray, T. G., Heymsfield, S. B., and Fuller, E. O. 1979. The effect of chronic protein-calorie undernutrition in the rat on myocardial function and cardiac function. *Circ Res, 45*(1): 144-152.

Nystoriak, M. A., and Bhatnagar, A. 2018. Cardiovascular Effects and Benefits of Exercise. *Front Cardiovasc Med, 5*: 135. doi:10.3389/fcvm.2018.00135

Ontell, M., and Kozeka, K. 1984. The organogenesis of murine striated muscle: a cytoarchitectural study. *Am J Anat, 171*(2): 133-148. doi:10.1002/aja.1001710202

Oydanich, M., Babici, D., Zhang, J., Rynecki, N., Vatner, D. E., and Vatner, S. F. 2019. Mechanisms of sex differences in exercise capacity. *Am J Physiol Regul Integr Comp Physiol, 316*(6): R832-r838. doi:10.1152/ajpregu.00394.2018

Ozaki, T., Nishina, H., Hanson, M. A., and Poston, L. 2001. Dietary restriction in pregnant rats causes gender-related hypertension and vascular dysfunction in offspring. *J Physiol, 530*(Pt 1): 141-152.

Ozanne, S. E., Wang, C. L., Dorling, M. W., and Petry, C. J. 1999. Dissection of the metabolic actions of insulin in adipocytes from early growth-retarded male rats. *J Endocrinol, 162*(2): 313-319.

Parati, G., and Di Rienzo, M. 2003. Determinants of heart rate and heart rate variability. *Journal of Hypertension, 21*(3).

Petrosino, J. M., Heiss, V. J., Maurya, S. K., Kalyanasundaram, A., Periasamy, M., LaFountain, R. A., et al. 2016. Graded Maximal Exercise Testing to Assess Mouse Cardio-Metabolic Phenotypes. *PloS one, 11*(2): e0148010-e0148010. doi:10.1371/journal.pone.0148010

Petry, C. J., Dorling, M. W., Wang, C. L., Pawlak, D. B., and Ozanne, S. E. 2000. Catecholamine levels and receptor expression in low protein rat offspring. *Diabet Med, 17*(12): 848-853.

Pette, D., and Staron, R. S. 2000. Myosin isoforms, muscle fiber types, and transitions. *Microsc Res Tech, 50*(6): 500-509. doi:10.1002/1097-0029(20000915)50:6<500::Aid-jemt7>3.0.Co;2-7

Phillips, D. I., and Barker, D. J. 1997. Association between low birthweight and high resting pulse in adult life: is the sympathetic nervous system involved in programming the insulin resistance syndrome? *Diabet Med, 14*(8): 673-677. doi:10.1002/(sici)1096-9136(199708)14:8<673::Aid-dia458>3.0.Co;2-9

Pislaru, C., Urban, M. W., Pislaru, S. V., Kinnick, R. R., and Greenleaf, J. F. 2014. Viscoelastic properties of normal and infarcted myocardium measured by a multifrequency shear wave method: comparison with pressure-segment length method. *Ultrasound in medicine & biology, 40*(8): 1785-1795. doi:10.1016/j.ultrasmedbio.2014.03.004

Platt, C., Houstis, N., and Rosenzweig, A. 2015. Using Exercise to Measure and Modify Cardiac Function. *Cell Metabolism, 21*(2): 227-236. doi:https://doi.org/10.1016/j.cmet.2015.01.014

Pluim, B. M., Zwinderman, A. H., van der Laarse, A., and van der Wall, E. E. 2000. The athlete's heart. A meta-analysis of cardiac structure and function. *Circulation, 101*(3): 336-344.

Popović, Z. B., Sun, J. P., Yamada, H., Drinko, J., Mauer, K., Greenberg, N. L., et al. 2005. Differences in left ventricular long-axis function from mice to humans follow allometric scaling to ventricular size. *The Journal of Physiology, 568*(Pt 1): 255-265. doi:10.1113/jphysiol.2005.090779

Quigley, S. P., Kleemann, D. O., Kakar, M. A., Owens, J. A., Nattrass, G. S., Maddocks, S., et al. 2005. Myogenesis in sheep is altered by maternal feed intake during the peri-conception period. *Anim Reprod Sci, 87*(3-4): 241-251. doi:10.1016/j.anireprosci.2004.11.005

Raju, N. V. 1974. Effect of early malnutrition on muscle function and metabolism in rats. *Life Sci, 15*(5): 949-960.

Ramaekers, D., Ector, H., Aubert, A. E., Rubens, A., and Van de Werf, F. 1998. Heart rate variability and heart rate in healthy volunteers. Is the female autonomic nervous system cardioprotective? *Eur Heart J, 19*(9): 1334-1341. doi:10.1053/euhj.1998.1084

Ross, J., Jr. 1983. Mechanisms of cardiac contraction. What roles for preload, afterload and inotropic state in heart failure? *Eur Heart J, 4 Suppl A*: 19-28.

Sampson, D. A., Hunsaker, H. A., and Jansen, G. R. 1986. Dietary protein quality, protein quantity and food intake: effects on lactation and on protein synthesis and tissue composition in mammary tissue and liver in rats. *J Nutr, 116*(3): 365-375. doi:10.1093/jn/116.3.365

Satomura, K. 2014. Developmental Origins of Health and Disease（DOHaD）—The effects of embryonic and infantile environment on kidney disease—. *Nihon Shoni Jinzobyo Gakkai Zasshi, 26*(2): 250-255. doi:10.3165/jjpn.26.250

Sayer, A. A., Syddall, H., Martin, H., Patel, H., Baylis, D., and Cooper, C. 2008. The developmental origins of sarcopenia. *The journal of nutrition, health & aging, 12*(7): 427-432. doi:10.1007/bf02982703

Schaefer, A., Klein, G., Brand, B., Lippolt, P., Drexler, H., and Meyer, G. P. 2003. Evaluation of left ventricular diastolic function by pulsed Doppler tissue imaging in mice. *J Am Soc Echocardiogr, 16*(11): 1144-1149. doi:10.1067/s0894-7317(03)00679-5

Schechter, M. A., Southerland, K. W., Feger, B. J., Linder, D., Jr., Ali, A. A., Njoroge, L., et al. 2014. An isolated working heart system for large animal models. *Journal of visualized experiments : JoVE*(88): 51671. doi:10.3791/51671

Schefer, V., and Talan, M. I. 1996. Oxygen consumption in adult and AGED C57BL/6J mice during acute treadmill exercise of different intensity. *Exp Gerontol, 31*(3): 387-392.

Scherrer-Crosbie, M., and Thibault, H. B. 2008. Echocardiography in translational research: of mice and men. *J Am Soc Echocardiogr, 21*(10): 1083-1092. doi:10.1016/j.echo.2008.07.001

Schiaffino, S., and Reggiani, C. 2011. Fiber types in mammalian skeletal muscles. *Physiol Rev, 91*(4): 1447-1531. doi:10.1152/physrev.00031.2010

Sebag, I. A., Handschumacher, M. D., Ichinose, F., Morgan, J. G., Hataishi, R., Rodrigues, A. C., et al. 2005. Quantitative assessment of regional myocardial function in mice by tissue Doppler imaging: comparison with hemodynamics and sonomicrometry. *Circulation, 111*(20): 2611-2616. doi:10.1161/circulationaha.104.474411

Simeoni, U., Boubred, F., Buffat, C., and Vaiman, D. (2008). *The Developing Kidney and the Fetal Origins of Adult Cardiovascular Disease*.

Skrzypiec-Spring, M., Grotthus, B., Szeląg, A., and Schulz, R. 2007. Isolated heart perfusion according to Langendorff—Still viable in the new millennium. *Journal of Pharmacological and Toxicological Methods, 55*(2): 113-126. doi:https://doi.org/10.1016/j.vascn.2006.05.006

Solaro, R. J. (2011). Integrated Systems Physiology: From Molecule to Function to Disease. In *Regulation of Cardiac Contractility*. San Rafael (CA): Morgan & Claypool Life Sciences

Stampfl, A., Maier, M., Radykewicz, R., Reitmeir, P., Göttlicher, M., and Niessner, R. 2011. Langendorff Heart: A Model System To Study Cardiovascular Effects of Engineered Nanoparticles. *ACS Nano, 5*(7): 5345-5353. doi:10.1021/nn200801c

Tain, Y.-L., and Hsu, C.-N. 2017. Developmental Origins of Chronic Kidney Disease: Should We Focus on Early Life? *International journal of molecular sciences, 18*(2): 381. doi:10.3390/ijms18020381

Tariq, S., and Aronow, W. S. 2015. Use of Inotropic Agents in Treatment of Systolic Heart Failure. *International journal of molecular sciences, 16*(12): 29060-29068. doi:10.3390/ijms161226147

Thornburg, K., Jonker, S., O'Tierney, P., Chattergoon, N., Louey, S., Faber, J., et al. 2011. Regulation of the cardiomyocyte population in the developing heart. *Prog Biophys Mol Biol, 106*(1): 289-299. doi:10.1016/j.pbiomolbio.2010.11.010

Tonkiss, J., Trzcinska, M., Galler, J. R., Ruiz-Opazo, N., and Herrera, V. L. 1998. Prenatal malnutrition-induced changes in blood pressure: dissociation of stress and nonstress responses using radiotelemetry. *Hypertension, 32*(1): 108-114.

Vaughan, H. S., and Goldspink, G. 1979. Fibre number and fibre size in a surgically overloaded muscle. *J Anat, 129*(Pt 2): 293-303.

Vella, C. A., and Robergs, R. A. 2005. A review of the stroke volume response to upright exercise in healthy subjects. *British journal of sports medicine, 39*(4): 190-195. doi:10.1136/bjsm.2004.013037

Visker, J. R., and Ferguson, D. P. 2018. Postnatal undernutrition in mice causes cardiac arrhythmogenesis which is exacerbated when pharmacologically stressed. *J Dev Orig Health Dis, 9*(4): 417-424. doi:10.1017/s2040174418000156

VisualSonics. (2008). *VisualSonics Vevo® 2100 Imaging System—Operator Manual*. In.

Vranas, S., Heinemann, G. K., Liu, H., De Blasio, M. J., Owens, J. A., Gatford, K. L., et al. 2017. Small size at birth predicts decreased cardiomyocyte number in the adult ovine heart. *Journal of Developmental Origins of Health and Disease, 8*(5): 618-625. doi:10.1017/S2040174417000381

Vuguin, P. M. 2007. Animal models for small for gestational age and fetal programming of adult disease. *Horm Res, 68*(3): 113-123. doi:10.1159/000100545

Wadhwa, P. D., Buss, C., Entringer, S., and Swanson, J. M. 2009. Developmental origins of health and disease: brief history of the approach and current focus on epigenetic mechanisms. *Seminars in reproductive medicine, 27*(5): 358-368. doi:10.1055/s-0029-1237424

Wang, Q.-Q., Jing, X.-M., Bi, Y.-Z., Cao, X.-F., Wang, Y.-Z., Li, Y.-X., et al. 2018. Human Umbilical Cord Wharton's Jelly Derived Mesenchymal Stromal Cells May Attenuate Sarcopenia in Aged Mice Induced by Hindlimb Suspension. *Medical science monitor : international medical journal of experimental and clinical research, 24*: 9272-9281. doi:10.12659/MSM.913362

Ward, S. S., and Stickland, N. C. 1993. The effect of undernutrition in the early postnatal period on skeletal muscle tissue. *British Journal of Nutrition, 69*(1): 141-150. doi:10.1079/BJN19930016

Wessels, A., and Sedmera, D. 2003. Developmental anatomy of the heart: a tale of mice and man. *Physiol Genomics, 15*(3): 165-176. doi:10.1152/physiolgenomics.00033.2003

White, D. W., and Raven, P. B. 2014. Autonomic neural control of heart rate during dynamic exercise: revisited. *The Journal of physiology, 592*(12): 2491-2500. doi:10.1113/jphysiol.2014.271858

Wilson, S. J., Ross, J. J., and Harris, A. J. 1988. A critical period for formation of secondary myotubes defined by prenatal undernourishment in rats. *Development, 102*(4): 815-821.

Woo, M., Isganaitis, E., Cerletti, M., Fitzpatrick, C., Wagers, A. J., Jimenez-Chillaron, J., et al. 2011. Early life nutrition modulates muscle stem cell number: implications for muscle mass and repair. *Stem Cells Dev, 20*(10): 1763-1769. doi:10.1089/scd.2010.0349

Wu, P., Zhang, S., Spinner, R. J., Lizardi, M. T., Gu, Y., Yu, C., et al. 2017. A novel triple immunoenzyme staining enables simultaneous identification of all muscle fiber types on a single skeletal muscle cryosection from normal, denervated or reinnervated rats. *Neural regeneration research, 12*(8): 1357-1364. doi:10.4103/1673-5374.213560

You, J. S., Anderson, G. B., Dooley, M. S., and Hornberger, T. A. 2015. The role of mTOR signaling in the regulation of protein synthesis and muscle mass during immobilization in mice. *Dis Model Mech, 8*(9): 1059-1069. doi:10.1242/dmm.019414

Yuan, T.-l., Zhu, Y.-h., Shi, M., Li, T.-t., Li, N., Wu, G.-y., et al. 2015. Within-litter variation in birth weight: impact of nutritional status in the sow. *Journal of Zhejiang University. Science. B, 16*(6): 417-435. doi:10.1631/jzus.B1500010

Zardini, D. M., and Parry, D. J. 1994. Identification, distribution, and myosin subunit composition of type IIX fibers in mouse muscles. *Muscle & nerve, 17*(11): 1308-1316. doi:10.1002/mus.880171110

Zohdi, V., Lim, K., Pearson, J. T., and Black, M. J. 2014. Developmental programming of cardiovascular disease following intrauterine growth restriction: findings utilising a rat