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The Effects of Diet and Ultra Violet B Exposure on Vitamin D Levels and Muscle Performance In Mice (Mus musculus)

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The Effects of Diet and Ultra Violet B Exposure on Vitamin D Levels and Muscle Performance In Mice (*Mus musculus*)

by

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A THESIS

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Abstract

Vitamin D is a steroid that promotes healthy bone structure and muscle function. It is obtained from the diet and synthesized in the skin during UV-B exposure from sunlight. In muscle, it influences calcium transport and promotes growth, with varied responses depending on muscle type. I hypothesized that reduced vitamin D in the diet and removal of UVB light, as occurs during hibernation or the Polar winter, will impact circulating levels of vitamin D to the extent that muscle contractile performance is altered. I also hypothesize that fast-twitch muscles will show greater responses of muscle contractile capabilities to treatments compared to slow-twitch muscles. Mice were used as a model, and fed diets with normal or low vitamin D, and exposed to normal or no UV-B doses for 3 months. Neither fast nor slow muscle types showed significant differences between treatment groups in force, power, force-velocity measures, or rates of fatigue. Serum vitamin D levels, measured at the midpoint and endpoint of the treatment, varied substantially among individuals, but not, on average, among treatment groups, indicating mechanisms of compensatory homeostasis in the face of reduced vitamin D supply. These results suggest that intermittent interruptions in dietary vitamin D or light exposure in mammals, such as those that hibernate or live above the Arctic Circle, do not impair muscle contractile function.

Preface

This is an original thesis, unpublished, independent work by author, N. Tsao. The experiments reported in the Methods were approved by Animal Ethics Certificate number AC18-0032, issued by the University of Calgary Life and Environmental Sciences Animal Care Committee for the project “Effects of Vitamin D via light exposure and diet on muscle contractile performance” on June 27, 2018.

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Finally, my family and friends who helped me grow as an individual throughout this journey.

Dedication

This thesis is dedicated to Jessica (Minh) Bui

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List of Symbols, Abbreviations and Nomenclature

Symbol	Definition
ANOVA	Analysis of variance
Ca^{2+}	Calcium ions
CSA	Cross-sectional area
EDL	Extensor digitorum longus muscle
Hz	Hertz (cycle/second)
J	Joules
ML/s	Muscle length per second
mN	Millinewton
ms	Millisecond
PTH	Parathyroid hormone
SEM	Standard error of mean
SOL	Soleus muscle
UVB	Ultraviolet B light
UV-	No UVB exposure
UV+	UVB exposure
VD-	Low levels of dietary vitamin D
VD+	Normal levels of dietary vitamin D
W	Watts (J/s)
25OHD	25-hydroxyvitamin D

INTRODUCTION

1.1 Overview:

My thesis explores the impact of reduced ultra-violet B exposure (in sunlight) and vitamin D in the diet on levels of circulating vitamin D and potential impacts on muscle contractile performance in mice. Sunlight is important for animals to create vitamin D (Holick, 1987), a steroid that promotes healthy bone structure and muscle function (Holick, 1987; Hamilton, 2010; Ceglia & Harris, 2013; Stratos et al., 2013). Its synthesis begins in the skin of animals and requires ultraviolet-B rays from sunlight (UVB) (Holick, 1987; Hamilton, 2010; Ceglia & Harris, 2013). This process creates 25-hydroxy vitamin D (25OHD), the main storage form of vitamin D, which circulates in the blood, and is converted into its active form (1,25-dihydroxyvitamin D) in the kidneys (Holick, 1987; Hamilton, 2010; Ceglia & Harris, 2013). The active form of vitamin D is responsible for maintaining important aspects of muscle function and is involved in stimulating muscle growth through protein synthesis (Curry et al., 1974; Birge & Haddad, 1975; Janssen et al., 2002; Hutton et al., 2014). However, without UVB exposure, such as during the Polar winter or during hibernation, vitamin D synthesis in mammals is negligible due to the absence of adequate sunlight (Vestergaard et al., 2011; Andersen et al., 2013). Increased cloud cover even in summer, a result of Polar warming, has the potential to further reduce blood vitamin D in animals living in Polar regions (Wang & Jeffrey, 2003; Yinghui et al., 2008; Abe et al., 2016) and perhaps threaten prolonged vitamin D deficiency. The aim of this study was to investigate the effects of reduced sunlight and dietary vitamin D, as might occur during several months without sunlight and/or hibernation, on circulating levels of vitamin D and subsequently on muscle mass and contractile function. Based on results from previous studies, I hypothesized

that muscle contractile capacity would be influenced by the amount of circulating vitamin D in the blood. More specifically, circulating vitamin D will be reduced in animals exposed to reduced ultraviolet-B light and/or reduced vitamin D in the diet, and muscles in these animals will show signs of impaired contractile capacity. I measured the contractile properties of muscles in slow- and fast-twitch muscles of mice exposed to the different vitamin D and UVB treatment groups, as there is evidence that vitamin D insufficiency has a greater impact on fast muscles than slow (Girgis et al., 2014; Dzik & Kaczor, 2019)**.**

1.2 Background:

1.2.1 Importance of vitamin D

The importance of vitamin D was not investigated until the 18th and 19th century when young children were developing a bone disease, commonly known as rickets, due to vitamin D deficiency associated with living in the air polluted industrial era of Europe and the United States (Holick, 1987; Wacker & Holick, 2013). Rickets is characterized as a bone deforming disease that commonly affects infants and causes retardation in their growth (Rajakumar, 2003; Holick, 2006). Vitamin D deficiency is also known to cause muscle weakness, increase risk of fractures in elderly, osteoporosis in postmenopausal women and depression (Holick, 2007). Vitamin D deficiency is also associated with muscle weakness and atrophy, suggesting that vitamin D stimulates muscle growth through protein synthesis via activation of vitamin D receptors (Curry et al., 1974; Birge & Haddad, 1975; Janssen et al., 2002; Hutton et al., 2014). Birge and Haddad (1975) found that vitamin D supplemented diets caused increased incorporation of the amino acid leucine into the diaphragm muscles of rats. This suggests that vitamin D influences muscle

metabolism and accelerates the incorporation of amino acids into muscle proteins needed for increases in myofibrillar protein synthesis. Furthermore, skeletal muscle fibres express vitamin D receptors and 1-α-hydroxylase, the enzyme required to convert 25(OH)D_3 to the active form of vitamin D (1,25(OH)D_3) (Zanello et al., 1997; Ha et al., 2001; Shanmugasundaram & Selvaraj, 2012; Srikuea et al., 2012; Braga et al., 2017), and thus vitamin D appears to act directly at the level of muscle cells.

What constitutes a healthy vitamin D status has been long debated, and an optimal level has not yet been determined. In general for humans, it is believed that 25OHD levels in serum less than 20 ng ml^{-1} (50 nmol/L) are considered vitamin D deficient, 21-29 ng per millilitre (52-72 nmol/L) is considered insufficient and 30 ng per millilitre (75 nmol/L) or greater is determined to be a sufficient vitamin D status (Heaney et al., 2005; Holick, 2007).Vitamin D deficiency is defined as observable skeletal abnormalities, and vitamin D insufficiency is characterized as having no physically observable skeletal or calcium metabolism abnormalities, however normally mineralized bone at the trabecular and cortical compartments of the hind limbs are abnormal (Holick, 2006; Morris & Anderson, 2010). Normal bone structure and strength is obtained when serum 25OHD levels are sufficient or adequate (Morris & Anderson, 2010). For the purpose of my thesis, deficient levels of serum vitamin D were avoided as to prevent skeletal fractures or breaks from influencing muscle function.

1.2.1.1 Vitamin D in muscle

The biological effects of vitamin D on muscle are still under debate (Beaudart et al., 2014). For example, there are meta-analyses and randomized trial studies using vitamin D

supplementation that indicate no effect of different levels of serum vitamin D on muscle (Stockton et al., 2011; Verschueren et al., 2011). However, there have also been studies indicating that vitamin D supplementation increases muscle repair, muscle protein synthesis and reduces risk of injury from falling in elderly participants (Wong et al., 2004; Pfeifer et al., 2006; Stratos et al., 2013). The mechanism of increasing protein synthesis through actions of vitamin D receptors on muscle cells has been shown to cause hypertrophy in skeletal muscle growth. Hutton et al. (2014) demonstrated that skeletal muscle fibre cross-sectional area increased significantly in fast-twitch pectoralis major muscles after vitamin D treatment, but did not have an effect on slow-twitch muscles of the biceps femoris of broiler chickens (*Gallus gallus domesticus*). The molecular mechanisms by which vitamin D acts to influence muscle properties are not well understood. However, they are thought to be related to calcium-phosphorus homeostasis affecting aspects of muscle contraction (Birge & Haddad, 1975; Matthews et al., 1977; Hutton et al., 2014). During a muscle contraction, calcium ions (Ca^{2+}) stored in membrane bound sarcoplasmic reticulum (SR) within muscle cells are released upon muscle activation. Vitamin D promotes Ca^{2+} storage in the sarcoplasmic reticulum by increasing the number of Ca^{2+} transporter binding sites on the SR or effectiveness of Ca^{2+} uptake into the SR (Birge & Haddad, 1975; Matthews et al., 1977; Hutton et al., 2014). Fast-twitch muscles are adapted for quick responses, requiring fast calcium cycling mechanisms (measured through twitch kinetics), making these muscle types a good indicator of changes due to dietary vitamin D or vitamin D synthesis through UV light exposure on muscle performance (Barclay, 1994). Alternatively, slow-twitch muscles are better adapted for endurance performance (Baldwin et al., 1973) and changes occurring from dietary vitamin D or UV light may be more prominent in the fatigue responses of these muscle types.

Most research focuses on the effects of vitamin D status on the molecular nature of muscle properties. However, nothing is known of the effects of vitamin D status on muscle force production and other measures of muscle performance at the cellular level. Many clinical trials have been conducted to determine if vitamin D has a beneficial impact on muscle strength in humans performing different strength tasks (Stockton et al., 2011; Beaudart et al., 2014). However, there are no studies testing the effects of vitamin D on muscle contractile capacity directly, such as from isolated muscles. Systematic reviews and meta-analyses on humans do not reach the same conclusions and the topic of the effects of vitamin D on muscle strength is still under debate. Thus, we would benefit from additional information about the effects of vitamin D on muscle itself by contributing towards knowledge in muscle rehabilitation research as well as understanding how seasonal changes and hibernation may influence the muscles of animals.

1.2.2 Production of vitamin D in skin and through diet

There are two forms of vitamin D, both involving a pre-cursor reaction with ultraviolet-B radiation (UVB). Plants produce vitamin D_2, commonly known as ergocalciferol, from ergosterol (a plant cell component of fungi and yeast) and animals produce vitamin D_3, also known as cholecalciferol, from lanolin, a sterol ester (Holick, 2007). Both forms follow isomerization paths and are transported to the liver to be further metabolized (Wolpowitz & Gilchrest, 2006; Bouillon & Suda, 2014). Most dietary sources of vitamin D are inadequate to achieve sufficient levels of serum 25OHD, including those fortified with vitamin D. Thus, vitamin D production through sunlight exposure is a major contributing factor to serum 25OHD levels (Holick, 2007; Chen et al., 2009). There are rare but notable exceptions, such as, Inuit populations living above the

Arctic circle mainly consume marine mammals and fatty arctic fishes (Bjerregaard & Young, 1998; Rejnmark et al., 2004; Kuhnlein et al., 2007). These fish contain the highest levels of vitamin D compared to non-arctic fish, hence mammals (including humans) that prey mainly on arctic fish will have higher levels of circulating vitamin D obtained from their diets (Kuhnlein et al., 2005). Vitamin D supplements are most commonly used with adequate calcium intake to ensure sufficient serum 25OHD levels in countries with inadequate dietary vitamin D or sun exposure (Vieth et al., 2001; Rucker et al., 2002; Ovesen et al., 2018).

The epidermis and dermis layers of skin contain 7-dehydrocholesterol in the cytoplasm of the cells, which effectively absorbs ultraviolet radiation between the wavelengths 290-320 nm (Norman, 1998). Ultraviolet light within this range is called ultraviolet-B radiation (UVB), which converts 7-dehydrocholesterol into pre-vitamin D_3 through bond cleavage between carbons 9 and 10 (Holick et. al., 1987; Wacker & Holick, 2013) (Figure 1). Further rearrangement of double bonds occurs due to the thermally unstable nature of pre-vitamin D_3 to form thermodynamically stable vitamin D_3 (cholecalciferol). A majority of pre-vitamin D_3 synthesis occurs in the epidermis due to high-energy photons from UVB being absorbed before reaching the dermis layer. Thermally stable vitamin D_3 is released from the plasma membrane of the cell, into the extracellular space where it diffuses into capillary beds within the dermis and is transported via vitamin D binding protein to the liver (Wacker & Holick, 2013). Vitamin D_2 undergoes a similar hydroxylation process as D_3 and enters the liver through vitamin D binding proteins (Hamilton, 2010). Dietary sources of vitamin D enter the system as cholecalciferol and begins metabolism in the intestines where they are absorbed with fat molecules and transported to the liver using chylomicrons (Figure 1). Chylomicrons are lipoproteins that function as transport particles for lipid-based molecules (such as cholecalciferol), and carry vitamin D in the form of

cholecalciferol to the liver where it undergoes lipolysis, releasing the cholecalciferol in to the hepatocytes to be further metabolized into 25OHD (Haddad et al., 1993) (Figure 1). Vitamin D enters the hepatocytes of the liver and is metabolized into 25-hydroxyvitamin D (25OHD) via enzyme actions of 25-hydroxylase (Holick, 1987; Bikle, 2014). 25OHD is the biologically inactive form and must be transported to the kidneys where it is converted to 1,25-hydroxyvitamin D (1,25OHD) via actions of 25-hydroxyvitamin D-1-hydroxylase (CYP27B1) (Holick et al., 1987). 1,25OHD is the hormonally active form of vitamin D that is transported systematically to vitamin D receptors on a variety of cells to activate transcription of specific genes (Figure 1). These genes influence the mobilization of calcium and phosphorus from the skeleton, as well as cause increased protein synthesis in tissues such as muscles (Holick, 1987; Ceglia & Harris, 2013; Stratos et al., 2013).

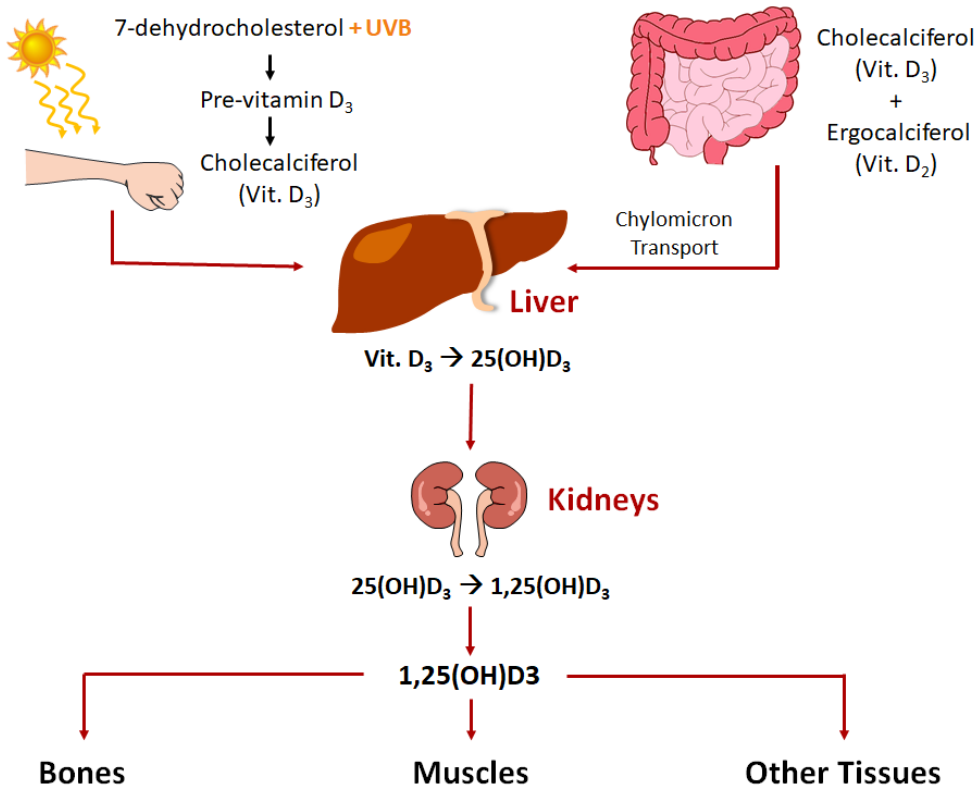


Figure 1: Schematic diagram of vitamin D synthesis and metabolism through dietary sources and UVB light exposure.

Skin pigmentation also plays a large role in the amount of vitamin D synthesis occurring through the skin. People with darker pigmentations contain more melanin within the epidermal cells, reducing the amount of UVB that is able to penetrate into the cell cytoplasm containing 7-dehydrocholesterol (a pre-cursor for vitamin D) (Jablonski & Chaplin, 2000). Dark skinned people have been shown to have a lower level of circulating vitamin D compared to lighter skinned individuals due to melanin in the skin acting as a light filter and blocking UVB from reacting with 7-dehydrocholesterol (Clemens et al., 1982). It is proposed that through evolutionary time, as humans migrated away from the equator towards the North and South, where less UVB rays are able to hit Earth's surface and reducing the amount of vitamin D

produced through the skin, skin pigmentation lightened to allow for more UVB rays to penetrate the epidermis and synthesize vitamin D (Jablonski & Chaplin, 2000).

Over-exposure to sunlight will not cause vitamin D_3 toxicity in animals (including humans) because excess pre-vitamin D_3 in the skin is photodegraded by UVB into 7-dehydrocholesterol, tachysterol, lumisterol or other toxisterols (Dauben & Baumann, 1961; Boomsma et al., 1977; Jacobs et al., 1977). In a similar way, vitamin D_3 is photodegraded into different suprasterols or 5,6-trans-vitamin D_3 (Holick et al., 1987; Wacker & Holick, 2013). All photodegraded products have no calcemic effect, and do not contribute to muscle or bone health (Wacker & Holick, 2013).

Age also seems to be a key factor in the amount of vitamin D synthesized, showing an inverse relationship between concentration of serum 25OHD and age. Lund & Sørensen (1979) found that 25OHD levels were lower in elderly populations compared to younger populations even when both groups were supplemented with vitamin D. It has been suggested that younger people have better hepatic hydroxylation of vitamin D in the intestines compared to older people and there may also be an age-related decline in renal 25-hydroxyvitamin D-1-hydroxylase (CYP27B1) activity (Lund & Sørensen, 1979; Hamstra et al., 2008). Aging rats also appear to exhibit a decrease in calcium absorption, serum 1,25OHD, and 25-hydroxyvitamin D-1-hydroxylase (CYP27B1) enzyme activity (Armbrecht et al., 1978).

25OHD, while not the active form of vitamin D, is the main circulating form of vitamin D and is a good indicator of vitamin D produced in the skin from sun exposure, and obtained from the diet. There are several reasons why 25OHD is the form of vitamin D that most studies use to quantify levels within the body (Holick et al., 1987; Ceglia & Harris, 2013). First, the half-life of 25OHD is 2-3 weeks, as opposed to the half-life of 1,25OHD which is approximately 4-6 hours.

The circulating levels of 1,25OHD is also much less than 25OHD and most assays would not be capable of measuring such low concentrations (Lips, 2007). Furthermore, as a person becomes vitamin D insufficient and deficient, parathyroid hormone is secreted and it causes an increase in 1,25OHD levels to seemingly normal concentrations (Hamilton, 2010). I will discuss the mechanisms of parathyroid hormone later.

1.2.3 Vitamin D in different animals

The cholesterol synthetic pathway is a ubiquitous and highly conserved pathway throughout evolutionary time that is important for membrane function and the production of steroid hormones, including vitamin D (Summons et al., 2006). Some propose that the use of vitamin D evolved as a strategy to absorb UVB light and thus to prevent DNA, RNA and protein damage due to ultraviolet radiation (Holick, 1987). Vitamin D was thought to absorb energy from UVB rays, and may have acted as an effective protective mechanism against DNA damage for early marine organisms when the ozone layers, which block UVB from reaching the earth surface, were less developed or non-existent (Wacker & Holick, 2013). This implies that vitamin D may have an important function since the beginning of unicellular eukaryote evolution (Bouillon & Suda, 2014). Thus, even simple marine life forms such as phytoplankton and zooplankton contain vitamin D, which is then passed up the food chain. Early in evolution, vitamin D_2 may have acted as a natural sunscreen for plants by dissipating UVB radiation energy through rearranging double bonds (Wacker & Holick, 2013). Less is known about the role of vitamin D in invertebrate evolution. Vitamin D in invertebrates appears to mainly affect the

calcified exoskeleton of these animals, with serum vitamin D obtained from the diet or from ultraviolet rays (He et al., 1992; Bouillon & Suda, 2014; Oonincx et al., 2018).

The degree to which vitamin D is obtained from the diet or is synthesized in the skin varies with species. In the arctic, fish contain the highest levels of vitamin D, hence animals that prey mainly on fish will have high levels of circulating vitamin D obtained from their diets (Kuhnlein et al., 2005). Although fish and other plankton-eating animals obtain vitamin D through their diet, they still obtain some from synthesis in the skin. Rainbow trout (*Oncorhynchus mykiss*) show vitamin D synthesis in the skin when exposed to wavelengths within the blue light spectrum (440-480 nm), which is the deepest penetrating light in water (Bouillon & Suda, 2014b). The vitamin D synthesizing capabilities in the skin of 21 different carnivorous species was investigated, using the omnivorous rat (*Rattus norvegicus*) as a control, and found that most carnivorous species, including some Polar species, such as the Polar bear (*Ursus maritimus*), may not rely on vitamin D synthesis from UVB rays in the skin as initially expected (Corbee et al., 2008). However, for many animals, synthesis in the skin is a significant source (Kuhnlein et al., 2005; Bouillon & Suda, 2014) and thus exposure to adequate levels and durations of sunlight are likely critical to health. Consequently, inadequate exposure to light may have deleterious impacts on several aspects of physiology, and is the focus of my interest in the interactions between light exposure, vitamin D levels, and muscle function, particularly in the context of the Polar environment.

The effects of vitamin D have been shown to have a stronger response in females than in males across many different species (Sharifi et al., 2016; Crescioli & Minisola, 2017). This may be due to gender differences in bone density or vitamin D responses being associated to specific gender-related hormones (Bilezikian, 2002; Spach & Hayes, 2014; Crescioli & Minisola, 2017).

For example, estrogen has been shown to increase the conversion of 25OHD to 1,25OHD in male white leghorn and Sussex-Rhode Island red chicks (*Gallus gallus domesticus*), whereas testosterone had no effect under similar conditions (Pike et al., 1978). As a result, my project used female mice to emphasize potential serum vitamin D and muscle contractile changes that may be occurring due to treatment groups.

1.2.4 Importance of parathyroid hormone

Parathyroid hormone (PTH) is produced in the parathyroid gland and is involved in the regulation of plasma calcium, and affects vitamin D synthesis (Igarashi et al., 2007; Morris & Anderson, 2010). When plasma calcium levels drop, the parathyroid glands release PTH, which upregulates the expression of renal CYP27B1 used to convert 25OHD to 1,25OHD, which in turn helps restore calcium homeostasis. Renal CYP27B1 is an enzyme that functions at very low 25OHD levels. As a result, 1,25OHD continues to be synthesized even at deficient levels of 25OHD, a further reason why this active form (1,25OHD) is inappropriate as a whole-body index of vitamin D stores (Hamilton, 2010; Morris & Anderson, 2010). Thus, even when a patient is clinically diagnosed with vitamin D deficiency, their 1,25OHD levels may be within normal ranges. For this reason, many studies focusing on vitamin D deficiency use 25OHD instead of 1,25OHD as an appropriate index for quantifying vitamin D levels within the blood.

1.2.5 Photoperiods and vitamin D

Most solar UVB radiation from the sun is not able to penetrate the stratospheric ozone layer. Approximately one percent of UVB energy reaches the Earth's surface, and increasing the

path by which UVB photons must travel through the ozone layer will decrease the energy that reaches Earth's surface. This results in people and other animals living above the Arctic Circle (66^{o} latitude) receiving little to no UVB for vitamin D production in the skin (Caldwell & Flint, 1994). Vitamin D deficiency risks are even more extreme in regions above the Arctic Circle where photoperiods are reduced to no sunlight during winter months. To compensate for periods of no sunlight, Polar animal populations receive high levels of vitamin D through their diets, especially from eating foods such as oily fish, and liver from several different animal species (Keiver et al., 1988; Rajakumar, 2003; Rejnmark et al., 2004; Andersen et al., 2013; Kenny et al., 2017).

Altitude also plays a role in the amount of UVB rays that hit the Earth's surface. Mammals living at higher altitudes have greater vitamin D synthesis due to the reduced path UVB needs to travel through the atmosphere (Wacker & Holick, 2013). As demonstrated by Holick et al., (2007), production of pre-vitamin D_3 was almost 5 times greater at the Mt. Everest Base camp (5300 metres above sea level), compared to Agra (city in India 169 metres above sea level).

I increased cloud cover even in summer, predicted as a result of Polar warming, could further reduce blood vitamin D in animals living within Polar regions, perhaps threatening even more prolonged vitamin D deficiency and leading to muscle dysfunction (Birge & Haddad, 1975; Janssen et al., 2002; Holick, 2007; Ceglia & Harris, 2013). Understanding the role of vitamin D in impacting muscle function and the mechanisms involved in its effects on muscle will help us better understand how drastic photoperiods might affect muscle function, which might be even more emphasized in Arctic climates if the effects of climate change impact Polar light exposure.

1.2.6 Mouse model

I chose mice (*Mus musculus*) as a model Polar endotherm because of their panglobal distribution including in cities above the Arctic circle(Michael F. Holick, 1989). More specifically, I used C57BL/6 laboratory mice (*Mus musculus*) which could have several impacts on the scope of this project. For example, polar endotherms have adapted ways to survive in such extreme climates which may not be accurately represented by the use of these model animals. The limitations of using a laboratory mouse as a model polar endotherm will be outlined in section 4.9 (*Limitations of Study*). Furthermore, Female mice were used because females of many different mammalian species appear to synthesize more vitamin D from UVB in the skin, compared to male counterparts (refer to sub-chapter 1.2.3). C57BL/6 strain mice were chosen because they are a widely used laboratory animal in modelling human diseases. They are readily obtainable, husbandry techniques are well established, including health status in the context of vitamin D levels, and diets of controlled composition are available. This strain has been used in several vitamin D related studies and their mechanisms of vitamin D metabolism and synthesis are representative of a typical Polar mammal (Bolton et al., 2013; van der Meijden et al., 2015). They possess muscles (soleus and extensor digitorum longus) of appropriate fibre types (slow vs fast, respectively) and size for experimentation. I considered them a suitable experimental model on which to make initial investigations into whether reduced light availability impacts circulating levels of vitamin D and muscle function in animals that are exposed to such conditions in their natural environment.

1.2.7 Methods to assess muscle contractile capacity

I used several different measures of muscle contractile performance to assess the effects of the treatments on the muscle. I selected them to help identify how the treatments might impact different aspects of muscle cell biology and contractile capacity, including those relevant to animal locomotion. The kinetics of isometric twitches can be used to assess if aspects of sarcolemmal calcium homeostasis have been impacted (Milani-Nejad et al., 2014). Maximum, isometric tetanic force can be used to assess the ability of the muscle to develop force, whether there are changes in the effective cross-sectional area of myofibrils, and if there are changes in the ability of cross bridges to generate force (Milani-Nejad et al., 2014). Force-velocity properties of the muscle are used to evaluate the inherent ability of maximally activated muscle to generate force at different speeds of shortening, how fast the muscle is able to shorten, how much power it can produce and the effect of shortening velocity on power output (Lin, 2008). Measures of muscle fatigue assess the resistance of the muscle to fatigue, and could infer changes in muscle fibre type, particularly differential effects between the two muscle types (Currier, 1969).

I used the work-loop technique to measure muscle contractile performance (work and power output) of fast and slow-twitch muscle types. Measurements of cyclic activation and relaxation of muscle during a twitch contraction, and measures of shortening and lengthening work of muscle as would occur during locomotion were made. This technique was developed by Josephson (1985) and has since been used by many researchers to understand the contractile capacity of muscle when being used as they do in animals during locomotion (Josephson, 1985; James et al., 1996; Layland & Kentish, 2000; Tallis et al., 2014). Before the work-loop technique

was created, most tests could only measure muscle contractions at a constant load (isotonic contractions) or length (isometric contractions). However, *in vivo*, muscles rarely contract with a constant load, and a primary function of many muscles is to shorten and do work rather than just generate force at a constant length. The work-loop technique (Figure 2) allows for measurements of work done by the muscle while it shortens, the work required to re-extend/re-lengthen the muscle after shortening, and the net energy (work) contributed by the muscle during a complete cycle of shortening and lengthening, as would be relevant to a muscle that is moving a limb during locomotion (Josephson, 1985). Further, the work-loop technique allows assessment of dynamic aspects of muscle contraction that help understand its functional capacity, and how they might be impacted by experimental interventions.

Work is a measure of mechanical energy (Joules) produced by the muscle and can be calculated as the product of force produced by the muscle and change in muscle length during one lengthening-shortening cycle (Josephson, 1985; Syme, 2005). Measurements of work can be assessed using a graphical construct referred to as a work loop, which is a plot of the relationship between muscle length (x-axis) versus muscle force production (y-axis) during a complete cycle of shortening during contraction and lengthening during relaxation (Josephson, 1985; Syme, 2005). When so plotted, the area below the lengthening limb of the loop is lengthening work, which is work done on the muscle to stretch/lengthen it from its minimum to maximum length (Figure 2). The area below the shortening limb of the loop is the work produced by the muscle during the shortening phase of a muscle contraction and includes the work produced during a contraction from stimulation (Figure 2) (Josephson, 1985). The difference between work produced during the shortening and lengthening phases gives the area within the loop, and is the net work produced during a complete cycle of lengthening and shortening by the muscle.

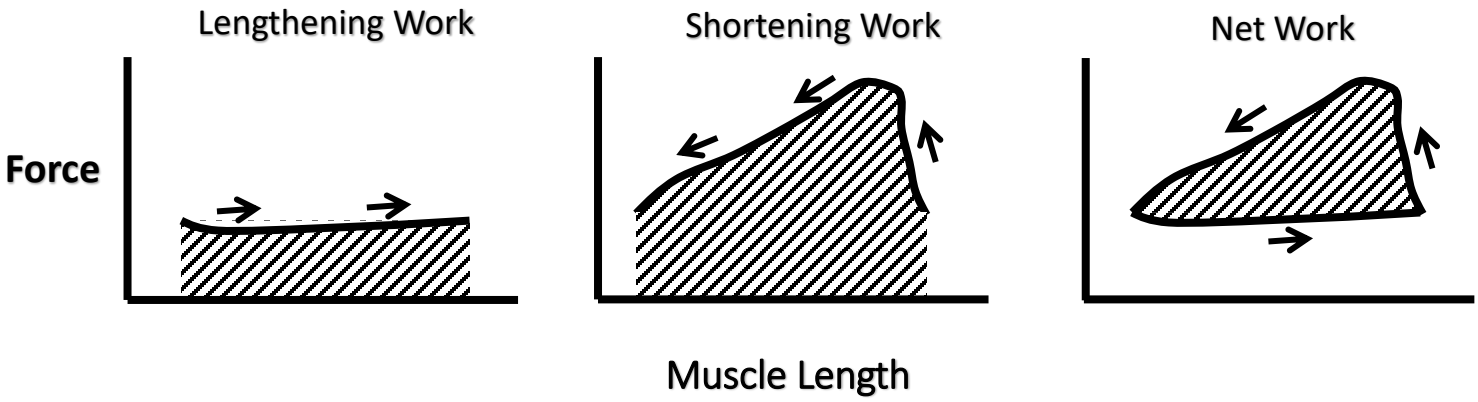


Figure 2: The work-loop technique. Muscle force is plotted against muscle length during a cycle of muscle lengthening (left panel), shortening (middle panel), and the loop formed during a complete cycle (right panel). Dashed lines showing the area under the curves represent the work done. The area within the loop is indicative of net work done during the complete cycle. Adapted from Josephson (1985).

The work-loop technique can provide numerous measurements and insights into muscle contractile performance. For example, muscle power can be calculated from work measurements as the product of work done during a single cycle and the frequency of cycling (analogous to the rate of limb cycling in a running animal). This provides information about the peak power that a muscle can produce when being used during locomotion, and the effects of limb cycling frequency on the ability of muscle to produce power. Power curves are graphs of cycle frequency (x-axis, i.e. the number of shortening-lengthening cycles a muscle performs per second in Hertz) versus the power a muscle produces (y-axis, i.e. the rate at which work is performed in Joules per second, or Watts) (Josephson, 1985; Syme, 2005). In summary, measures of twitch kinetics,

tetanic force, work, power, force-velocity, and rates of fatigue were made to assess different aspects of muscle contractile performance and the effects of light and vitamin D on them, and will be explained in more detail in the "Methods" section.

1.3 Hypotheses:

The purpose of this study was to understand if the absence of UVB light during periods of prolonged darkness or reduced vitamin D in the diet impacts circulating levels of vitamin D and muscle function. My study will contribute to a better understanding of how vitamin D obtained from two different sources (UVB and diet) affects muscle function and if there are differential effects of vitamin D and/or UVB on fast-twitch versus slow-twitch muscle fibres. The experimental design was intended to simulate intermittent, extended interruptions in dietary vitamin D and light exposure in mammals, as would be seen during hibernation or in mammals experiencing a Polar winter. The objective was to measure the effects of these conditions on muscle contractile performance (force production, twitch speed, work, power, fatigue, force-velocity measures), and correlate this to serum vitamin D levels. I hypothesized that if reduced UVB light and dietary vitamin D impaired vitamin D production and thus availability of vitamin D in the animal, then muscle contractile function would subsequently be impaired due to vitamin D's role in promoting protein synthesis and calcium homeostasis. I thus predicted that mice exposed to reduced light and vitamin D in the diet would exhibit the lowest serum vitamin D levels, and show reduced muscle contractile performance as a result. This expectation was based on results obtained from other studies indicating that vitamin D supplementation causes an increase in muscle protein synthesis that subsequently causes hypertrophy of muscle fibres

(Hutton et al., 2014), and reduces the chances of falling or hip fractures in women who were given vitamin D supplementation while hospitalized for strokes (Sato et al., 2005; Ceglia et al., 2013). I also hypothesized that fast-twitch muscles would be more prone to the effects of reduced vitamin D due to their reliance on rapid rates of calcium cycling during contraction and their reliance on high myofibril content to produce high power. Based on previous studies comparing the two muscle types subject to vitamin D treatments, showing an increase in responsiveness by fast-twitch muscles to vitamin D supplementation relative to slow-twitch muscles through an increase in fibre cross-sectional area (Stratos et al., 2013; Hutton et al., 2014), I predicted that fast-twitch muscles (the extensor digitorum longus, EDL) would be more responsive to the vitamin D diet and light treatments compared to slower-twitch muscle (soleus, SOL). Examples of responsiveness include greater force production, improved relaxation periods, faster contractility periods, greater power outputs, higher endurance measures and other related measures.

My study will be an early contribution to the field researching the effects of reduced dietary vitamin D and removal of UV exposure on serum vitamin D and muscle contractile capabilities as this will be a preliminary study and one of the first of its kind. Results of my study will contribute to the knowledge of the effects of climate change on mammalian physiology and help predict ecological changes occurring in the Arctic due to climate change. My research may help other scientists predict behavioural changes that may be occurring from muscular changes following periods of reduced dietary vitamin D or UV light exposure.

METHODS

2.1 Animal care and experimental design

All aspects of animal handling were approved by the University of Calgary animal care committee following CCAC guidelines. C57BL/6 mice were obtained from the breeding colony in the Life and Environmental Sciences Animal Resource Centre at the University of Calgary. During breeding and growth, the mice were housed in rooms containing 32W Sylvania fluorescent lights (Versailles, Kentucky) on a 12:12 h light:dark photoperiod and fed food and water ad libitum until ready for experiments. These fluorescent lights did not emit detectable levels of UVB when measured with a UVB meter (Model 6.2 Sensitive UVB meter, Glenside, Pennsylvania). The normal diet fed to the mice was 9F-5020 mice feed (Canadian Lab Diets), which contains a vitamin D component of 3.4 IU/g of vitamin D_3. This level of vitamin D in the diet ensures sufficient levels of serum vitamin D in mice (80-100 nmol/L) (Seldeen et al., 2017). For the low vitamin D diet used in the experiments, a diet similar in composition to the 9F-5020 mice feed was created by Canadian Lab Diets, but with a vitamin D content of 0.1 IU/g of vitamin D_3. These levels within the diet have been shown to cause insufficient (21-29 ng per millilitre or 52-72 nmol/L) levels of serum vitamin D within mice models (Seldeen et al., 2017). Mice were shaved on the dorsum and lateral sides from the back of the ears to the base of the tail before treatments began to ensure UVB light could reach the skin, and the standard JIRIK ear punch method was used for identification. Subsequent shaving was employed every two weeks, when hairs began growing long enough to obscure the skin surface.

For UVB light treatments, UVB exposure was administered through two adjacent 26W ExoTerra UVB 200 light bulbs (Rolf C. Hagen Inc., Montreal, Quebec) attached to aluminum

reflectors and placed approximately 17.5 cm above the cages. A UVB meter was used to measure UVB power in the spectrum between 250-320 nm. According to the World Health Organization and the International Labour Organization (Prüss-üstün et al., 2006; Vecchia et al., 2017), the minimal erythemal dose (MED) of UVB by which a patch of skin indicates faint signs of sunburn or redness from engorgement of capillaries approximately 8-24 hours after radiation, is approximately 200 J/m^2 in humans. Sleijffers et al. (2002) has indicated that a UVB dose of 1500 J/m^2 is equivalent to one MED in C57BL/6 mice. Furthermore, Rebel et al. (2015) used a UVB dose of 2000 J/m^2 daily administered over 15 minutes (222 $\mu W/cm^2$) on C57BL/6 mice to produce differences in serum vitamin D levels between treatment groups. Rebel et al. (2015) noted that this dose caused discomfort and wounds from scratching to the UV exposed area. Thus, I chose a UVB exposure dose of 2000 J/m^2, using a 15 minute UVB exposure of 222 $\mu W/cm^2$ as the experimental exposure intensity. However, the mice were exposed to this only three days a week, every Monday, Wednesday and Friday for a total of 3 months to reduce symptoms of sunburn that were seen in Rebel et al. (2015). To further reduce the possibility of sunburn, all mice receiving UVB treatment were initially acclimated to the UVB light by being exposed to only 111 $\mu W/cm^2$ for the first two weeks of treatment. Exposure was subsequently increased to 222 $\mu W/cm^2$ for the remainder of the treatment period. Mice were observed closely to ensure symptoms of sunburn were not occurring.

I conducted experiments on 40 mice, 10 in each of 4 treatment groups. Mice began treatments at 6-weeks of age until 18-weeks of age (12 weeks of treatment). Mice in the same treatment group were housed in groups of five (two cages of 5 mice) in plastic makrolon cages measuring 29.5 cm by 18.7 cm. Housing cages were supplemented with enrichments such as paper houses and shredded paper. Treatment group 1 was comprised of mice being fed a regular

diet with normal levels of vitamin D (VD+) and were not exposed to UVB light (UV-). This group is most similar to a control group, as they are the least modified group to what a regular laboratory mouse would experience. Treatment group 2 was fed a regular vitamin D diet and was exposed to UVB light (VD+/UV+). Treatment group 3 was fed the low vitamin D diet and was not exposed to UVB lights (VD-/UV-), and treatment group 4 was fed the low vitamin D diet and was exposed to UVB lights (VD-/UV+). A group of baseline mice (n = 6) were also obtained at 6 weeks of age, and sacrificed at 6 weeks of age to obtain blood for measures of vitamin D (25OHD) and parathyroid hormone (PTH), and to obtain measures of muscle contractile performance, prior to experimental treatments. This group of younger mice can comparable as a younger control-like group to treatment 1 (VD+/UV-), as they undergo the same treatments, but are sacrificed at a younger age. Through comparisons between the younger baseline mice and its older counterpart group (VD+/UV-), I am able to determine if the changes seen are due to development changes.

To ensure unbiased experimental design, the onset of treatments and subsequent measures of blood parameters and muscle contractile performance were staggered between treatment groups. Half (n = 5) of the mice from treatment group 1 began treatments (i.e. group 1a), about a week later, half of the mice from treatment group 2 began treatments (i.e. group 2a), etc., and then the second half of the mice from treatment group 1 (group 1b) commenced treatments, etc. All mice were 6 weeks of age when treatments commenced, regardless of the group or sub-group they were in. In this way, the treatments and measures of blood parameters and muscle contractile performance were sequentially distributed across the various treatment groups and sub-groups, reducing the possibility of bias in experimentation.

Mice in the UV+ groups were exposed to UVB light as above for 15 minutes every Monday, Wednesday and Friday at approximately 3PM during the treatment period. For the UVB exposure, mice were moved from the room they were housed in to an adjacent room with similar fluorescent lighting. Mice were transferred from the holding cage to a larger clear makrolon cage measuring 47.7cm by 25.6cm. Each treatment cage contained 5 mice and was covered with a coarse wired cage top to allow UVB rays to penetrate the wiring but prevent mice from jumping out of the cage. Mice in the treatment groups that were not subjected to UVB light treatment (UV-) were similarly transferred out of their cages and into the UVB treatment cages for 15 minutes on treatment days, but they were not exposed to UVB light. Mice were weighed every Monday prior to treatments and at the end of treatments before sacrifice.

2.2 Blood tests and analysis

I conducted blood tests were conducted to determine the levels of serum 25OHD (vitamin D) and PTH in all mice. Tests of 25OHD were conducted once at a midpoint during the treatments, and then again when the mice were euthanized at the end of the treatments. 50 µL of blood was required for triplet replications of vitamin D at the midpoint, acquired via a lateral saphenous bleed on the left leg, and assessed using vitamin D ELISA kits (Cayman Chemical, Item number: 501050, Ann Arbor, Michigan). Each blood sample was diluted by eight times and ten times then replicated three times for each dilution.

Mice within each sub-group were sacrificed after 12 weeks of treatment (i.e. sacrificed during the 13^{th} week). Since anesthetics affect muscle relaxation (Stadler et al., 2006), mice were rendered unconscious with carbon dioxide, a cardiac bleed was conducted to obtain blood

samples and affect euthanasia, followed by cervical dislocation to ensure euthanasia prior to dissection of muscles from hind limbs for measures of contractile capacity. This blood was placed in a 1mL centrifuge tube and on ice prior to centrifugation. Blood was centrifuged at 12,100 x g for 15 minutes using a Mini Spin Eppendorf (AG 22331 Hamburg, Brinkmann Instruments Inc., Westbury, New York) to separate serum from blood cells and other particulate fractions. Serum was micropipetted into a cryovial and stored in a -80^{0} C freezer until ready to be transported to the Calgary Laboratory Services (CLS) for analysis for vitamin D (25OHD) and PTH levels.

2.3 Muscle dissection

After euthanasia, I removed the soleus (SOL) and extensor digitorum longus (EDL) muscles of the right hind limb. I submerged the muscles in physiological saline (in mM: 144 NaCl, 10 glucose, 6 KCl, 1 NaH_2PO_4, 1 $MgSO_4$, 10 HEPES, 2 $CaCl_2$; pH 7.4) (Daut & Elzinga, 1989) and dissected under a dissecting microscope on a temperature-controlled plate at 18 $^{\circ}$C. Tendons on both ends of the muscles were left intact. A small piece of bone was left attached to the proximal tendons to ensure suture knots did not slip off. Size 6-0 silk suture was secured to the tendons on each end of the muscle (Figure 3). Both SOL and EDL muscles were tested on each mouse immediately after dissection, consecutively, and the order in which each muscle (SOL and EDL) was tested alternated between mice.

2.4 Muscle tests

The muscle preparation was placed into the muscle chamber and submerged in saline. One tendon was tied onto the arm of a servomotor (Cambridge Technology Inc., Model 350, Series 300 dual mode servo) that controlled muscle length, and the opposite tendon was tied onto a force transducer (Aurora Scientific Inc., Aurora, ON, Model No. 402A). Two platinum plate electrodes placed in close proximity to both sides of the muscle (~2 mm) stimulated the muscle through electrical currents. Electrodes were connected to a stimulator (Isostim A320, WPI, FL, USA) that allowed for voltage and stimulus frequency as well as pulse duration control. To provide sufficient current for the electrode plates, the stimulator gated a custom-made transistor circuit that in turn gated current from a bank of five 12 V gel batteries connected in series (60 V). The stimulus voltage was set to 50% above what was needed to elicit maximum force production from the muscle, stimulus pulse duration was set to 1 ms, and the frequency of the pulses was set to 100 Hz. See Figure 3 for a set-up of the muscle stimulation apparatus. Muscle contraction experimental parameters and data collection were controlled and recorded using custom-written LabView software (ver 6.1, National Instruments, Austin, TX) and a 12-bit analog/digital converter card (PCI MIO 16E-4, National Instruments, Austin, TX).

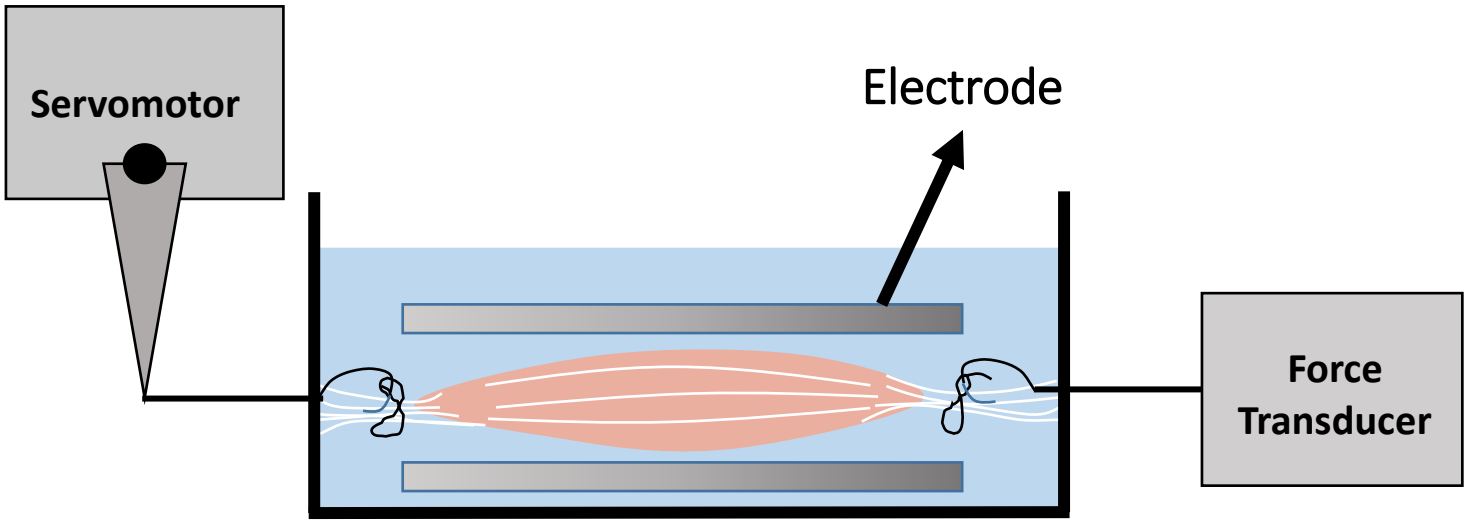


Figure 3: Schematic diagram of apparatus used to stimulate muscle for work-loop and force-velocity tests. Muscle is depicted in orange, submerged in a saline-filled chamber with two platinum electrode bars on either side in close proximity used for stimulation. Muscle tendon is tied at both ends with suture which is attached to servomotor and force transducer pins.

Prior to data collection, the resting length of each muscle was adjusted to give maximum force from an isometric twitch. The resting length was then measured. Maximum isometric tetanic force provides a measure of the ability of fully-activated muscle to produce force, and was measured by stimulating the muscle for 800 ms in the SOL and 300 ms for the EDL, long enough to obtain a plateau in the force response.

Measurements of work were then obtained to assess if the treatments affected the capacity of the muscles to produce power. To assess power output, muscle length was oscillated across a range of frequencies (analogous to the frequency of leg movement during locomotion), work was measured at each frequency, and the associated power output was then calculated (power = work X cycle frequency). The servomotor imposed these length changes (strain) on the muscle in a

sinusoidal pattern, with the amplitude of the length change set at 10% peak-to-peak of the muscle's resting length. Cycle frequencies of 2, 3, 4, 5, 6, 7, 8 and 10 Hz were tested for the EDL muscle, and frequencies of 0.5, 1, 2, 4, 6, and 8 Hz were tested for the SOL, ranges that encompassed the frequencies at which power output was maximal for each muscle. For each cycle frequency, the stimulus phase (i.e. the time point at which the muscle is activated relative to its length-change cycle) and stimulus duration (i.e. the length of time the muscle is stimulated/activated during each cycle) were adjusted through several trials to achieve maximal net work produced by the muscle. When adjusting the stimulus phase, the stimulus duration was simultaneously adjusted so that the stimulus ended at the same point of the strain cycle, to allow independent assessment of the effect of changing the onset and offset of the stimulus on work output. Figure 4 shows a sinusoidal work-loop of a mouse in group VD+/UV-. Work values were obtained through the LabView analysis program.

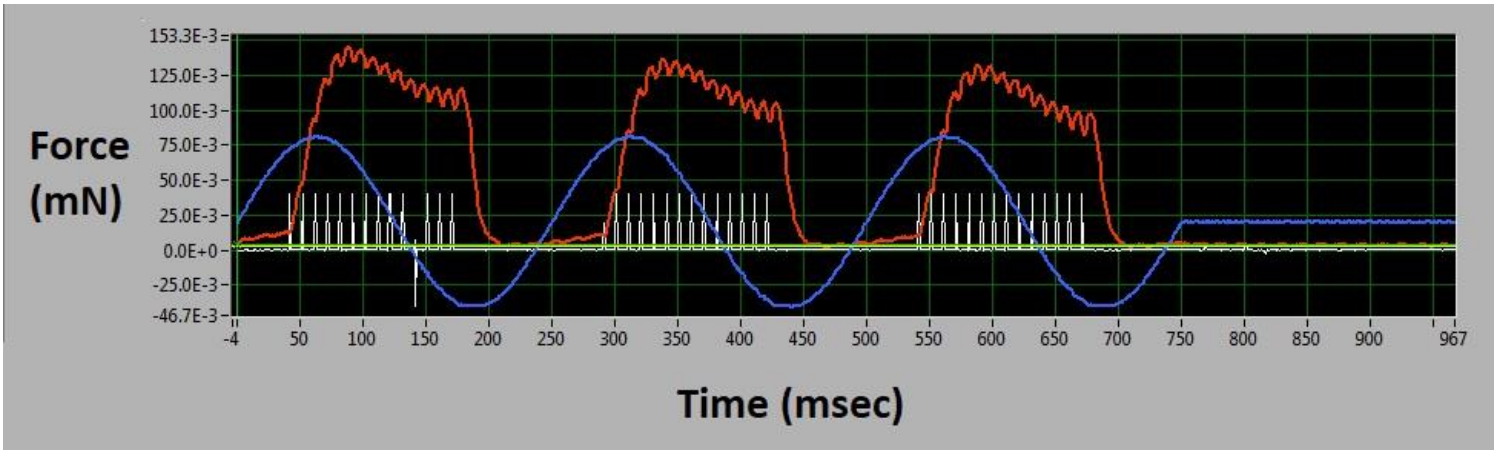


Figure 4: Raw traces obtained from LabView analysis program showing three work-loop cycles of an EDL muscle at a cycle frequency of 4 Hz, a stimulation duration of 132 ms and a stimulus phase of 16%. Red traces represent force, blue traces represent the lengthening and shortening positions of the muscle, and white traces are indications of muscle stimulation (voltage pulses applied to the muscle).

Measures of the relationship between the ability to produce force and the velocity of muscle shortening (i.e. the force-velocity relationship) were then made to provide information about the maximal, steady-state (i.e. not cyclic work) contractile capacity of the muscles. From the force-velocity relationship it was possible to assess power output, the maximum velocity at which the muscle can shorten, and the velocity at which power was maximum. This value of power is different from that calculated from the work-loop measurement because it does not simulate the cyclic activation and relaxation of a muscle during locomotion, but instead it reflects the muscle's maximum and instantaneous abilities.

To obtain force-velocity data, measurements of muscle force were made across a series of different muscle shortening velocities, where shortening velocity was expressed as muscle lengths per second (ML/s). For the EDL muscle, these measurements were made at velocities 0, 1, 2, 3, 4, 5, 6, 7, and 8 ML/s. For the slower SOL muscle, velocities of 0, 0.5, 1, 2, 3, 4, 5 ML/s were used. These ranges of velocities encompass 0 (an isometric contraction) up to almost the fastest the muscle could shorten, and enough intermediate values to reliably fit a curve to the relationships. Because the muscle shortened during these measurements, muscles were initially stretched 10% past the resting length that gave maximal force, to ensure that force-velocity measures would remain along the plateau region of the length-tension curve (Figure 5).

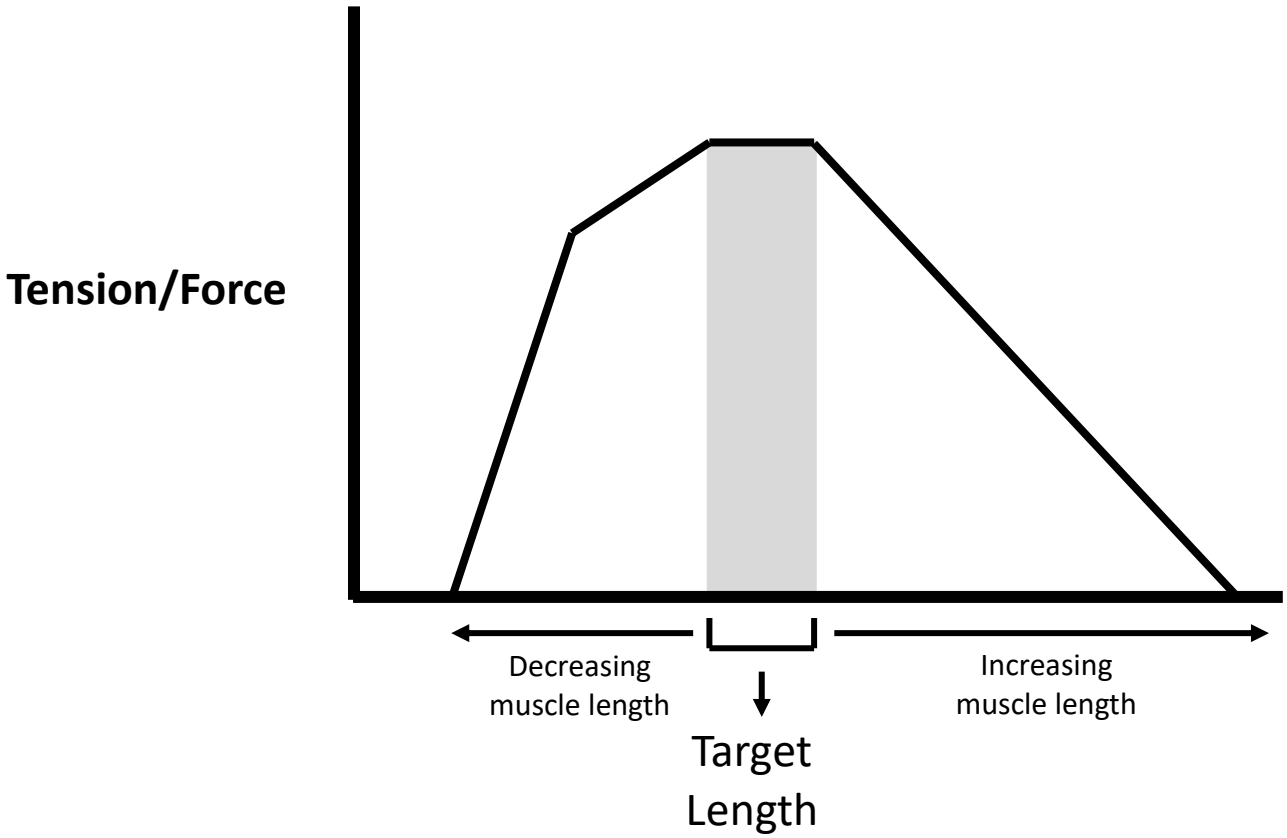


Figure 5: Schematic diagram of length-tension curve with grey area highlighting plateau region in which muscle length produced greatest amount of force. My study aimed at setting muscle length initially just at the right edge of plateau region, so that the muscle shortened through the plateau region during measurements of force-velocity properties. (Adapted from Marieb & Hoehn, 2006)

During an individual measurement, the muscles were first maximally stimulated through a tetanic contraction until they reached maximum force (300 ms stimulus in the EDL muscle and 600 ms in the SOL muscle) to ensure full activation. The peak force during maximal tetanic contraction was measured and denoted as P_o. Muscle length was then suddenly shortened to cause force to drop close to the level it would attain during subsequent shortening. Since force depends on shortening velocity, the size of this shortening step was adjusted for each different velocity. Muscle length was then shortened using the servomotor at a constant velocity, and the

subsequent force produced by the muscle was recorded. The exact same series of muscle length changes were then re-imposed on the muscle, however, it was not stimulated, allowing measurements of the resting force (i.e. passive tension) of the muscle at the same muscle length where active force was measured during the previous contraction. The difference between the recorded active force and the resting force provides a more accurate measurement of the force produced by the muscle (i.e. accounts for elastic components within the preparation) at the associated muscle shortening velocity. This procedure was repeated over the range of shortening velocities mentioned above, to obtain a series of force-velocity pairs, and these data were used to generate the force-velocity curves and to then assess power production of the muscle.

Finally, I conducted a fatigue test on the muscles. The muscle performed repeated cycles of work, using the same parameters that resulted in maximal power output from the work-loop measurements (i.e. the same frequency of length change and stimulation parameters). For the EDL muscle, cycle frequencies of 2 Hz were used for fatigue tests and for the SOL muscle tests, a cycle frequency of 1Hz was used. The SOL and EDL muscles were made to work for 25 consecutive cycles. The cycle number at which work became 50% of what it was initially during the first cycle was identified and used as an index of the rate of fatigue. To assess recovery from fatigue, prior to fatigue tests a work-loop test from cycle frequencies of 2 Hz for the EDL and 1 Hz for the SOL was conducted and maximal net work was recorded from the muscle and used as a baseline for comparison. The muscles were allowed to rest for 10 minutes after the end of the fatigue test before another work-loop test was conducted, followed by another 10 minute rest and work-loop test. Recovery work-loop tests following the 10 minute breaks were then compared to initial max net work produced prior to fatigue.

Following muscle tests, the tendons on each end were removed from the muscle under a dissection microscope. Surface moisture was removed by tapping the muscle on a Kim wipe and the muscle was transferred to an aluminum dish before being weighed on a microbalance (Mettler-Toledo AG, Laboratory and Weighing Technologies, CH-9606 Greifensee, Switzerland) immediately to determine muscle wet mass. Muscle wet mass was used to determine if changes from treatments influenced the total muscle mass (excluding tendons) and this value was used to standardize power output values in Watts per kilogram.

2.5 Data analysis

Comparisons among treatment groups for isometric tetanic and twitch force production are shown for absolute force produced by the whole muscle (N) and when force has been standardized by the cross-sectional area of the muscle (N/cm^2). Measurements of peak force produced during an isometric twitch and during a tetanic contraction (Figure 6). Measurements of twitch kinetics included the time (ms) for force to rise from 10% to 90% of maximal force immediately after a stimulus, the time for force to fall from 90% to 10% of maximal force during subsequent relaxation, and the time that force was maintained above 10% during the entire twitch (Figure 7). ANOVA was used to make comparisons among treatment groups and baseline mice. Further significance was determined using non-parametric statistical tests when assumptions of normality were violated. All blood comparisons among treatment groups and baseline mice were done using ANOVA statistical tests and further significance was determined through a Dunn's test.

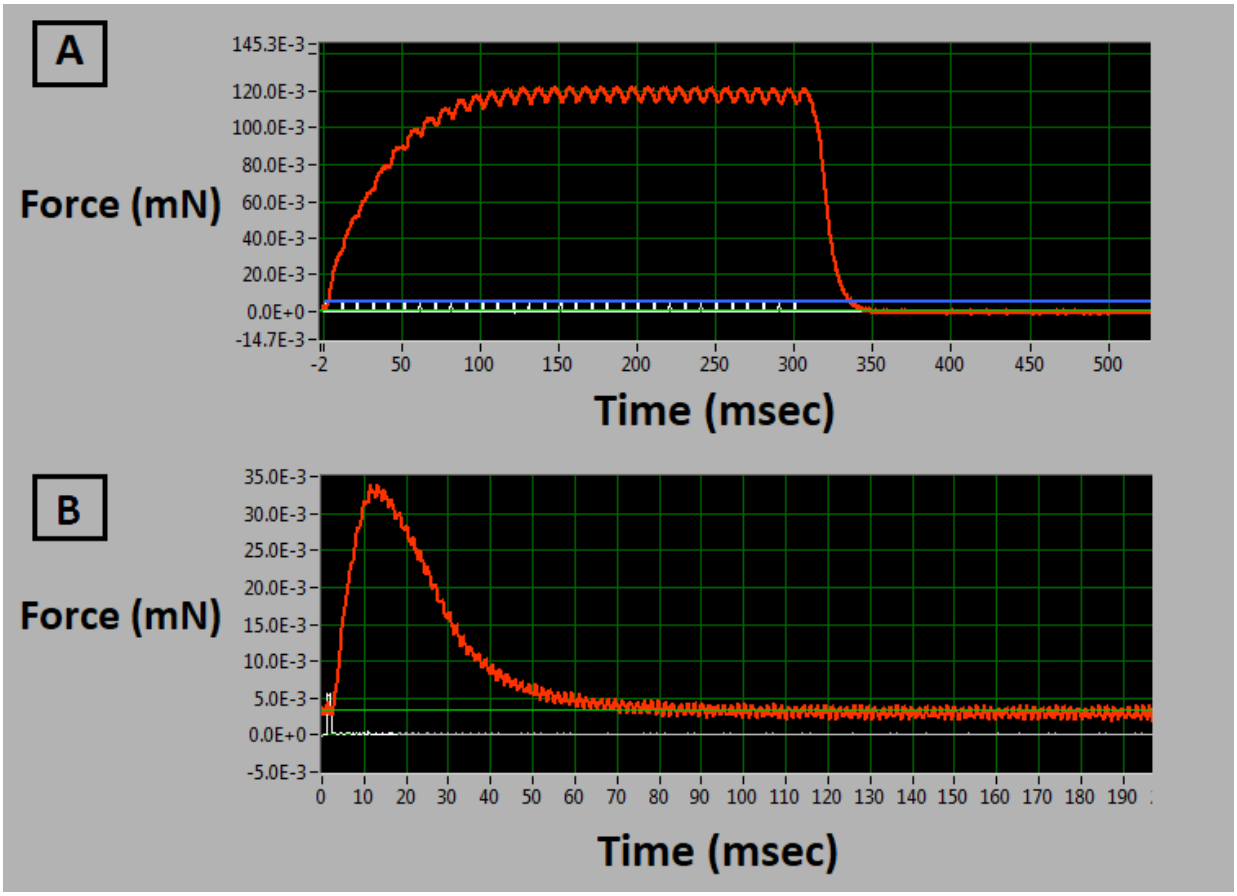


Figure 6: Raw traces obtained from LabView analysis program showing a tetanic contraction (A) and twitch contraction (B) of mouse 4 in group VD+/UV-. Red traces represent force. White traces are indications of stimulation period.

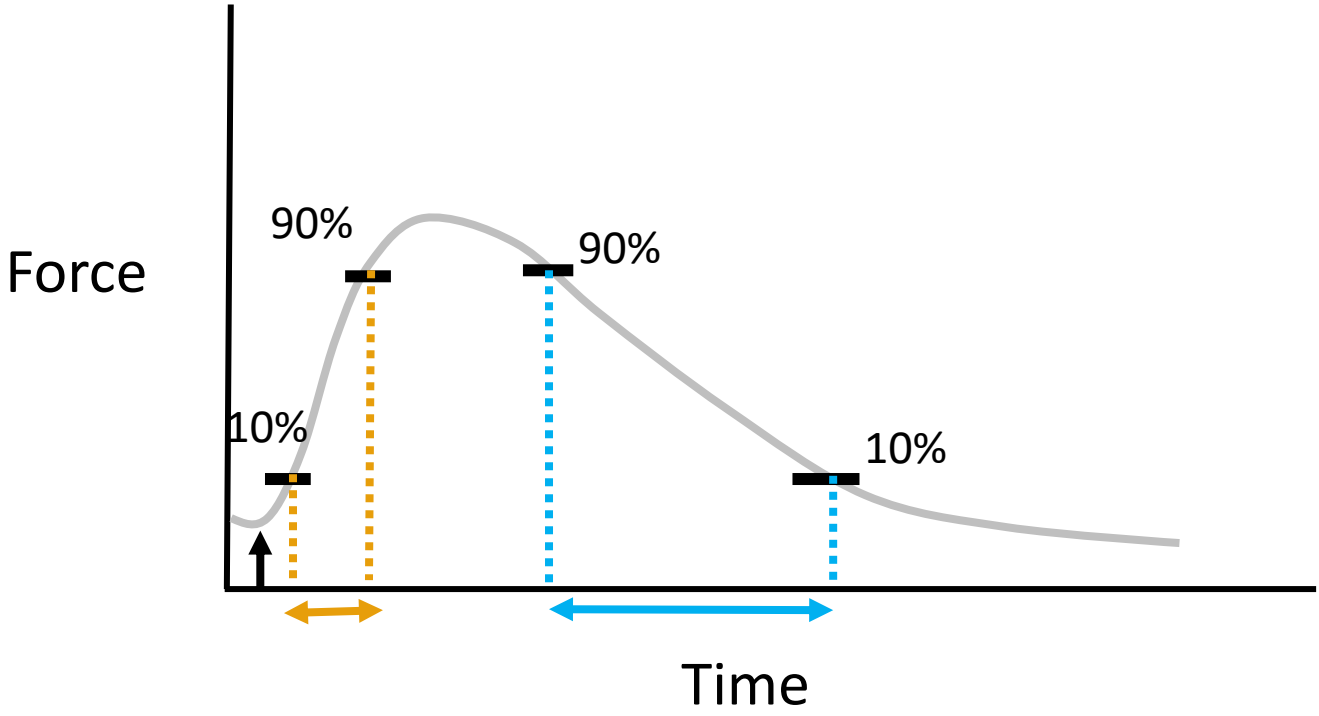


Figure 7: Force versus time graph of a muscle twitch. Black arrow represents stimulation time point. Time labelled by orange arrow is the contraction period and time labelled by blue arrow is relaxation period. Measurements of twitch kinetics from 10%-90% in the contraction period, 90%-10% in the relaxation period.

Net-work from the work-loop recordings was calculated as the integral of muscle force with respect to length change during the complete cycle of lengthening/shortening which is equivalent to net work, and was calculated as the area within the loop formed when muscle force was plotted against length (i.e. difference between lengthening and shortening work), which then allowed for calculations of power (i.e. the product of cycle frequency and net work). In addition, measurements of lengthening work (area under lengthening portion of work loop) were recorded to determine the contribution of series elastic elements within a muscle unit and whether or not vitamin D treatments influenced these properties (Josephson, 1985; Syme, 2005; Roberts, 2016).

Lengthening work is recorded as a negative value measure because the muscle is lengthening and absorbing energy from the system (Josephson, 1985; Syme, 2005). Comparisons of lengthening work were conducted at the closest cycle frequency to which peak power was obtained. Measurements of work and power were standardized to muscle mass. A 3rd order polynomial regression was fit to the power versus cycle frequency data for each muscle using OriginPro 9 (OriginLab Corporation, Northhampton, MA) and used to determine peak power and the cycle frequency at which peak power was achieved by taking the first derivate of the equation, setting it equal to zero, and solving for cycle frequency at which power was maximal and then for maximal power. Changes in this value would indicate a shift in the power and cycle frequency (i.e. limb cycling speed) at which a muscle could reach maximum power output. Calculations of power are presented in both absolute power from the entire muscle (Watts) and standardized to muscle mass (Watts per kilogram of muscle). Cycle frequency is presented in units of Hertz (cycles/second). ANOVA, student t-tests, post-hoc tests were used to make comparisons between treatment groups and baseline mice. Further significance was determined using non-parametric statistical tests (Dunn-sidak and Holm-sidak) when assumptions of normality were violated.

The force-velocity data were fitted to the Hill force-velocity equation (Equation 1; Hill, 1938), where V is the speed at which the muscle shortens, P is the associated force produced by the muscle, P_0 is the maximum isometric force produced by the muscle, and a, and b are all constants. Rearranging the Hill equation can be done to solve for power (i.e. the product of force and velocity), and taking the first derivative and solving for the value at zero yields the force or velocity at maximal power, which can then be used to calculate maximal power from the muscle and the associated force and velocity of shortening. Figure 8 depicts the force-velocity relationship where force declines as the muscle shortening velocity increases. This relationship is

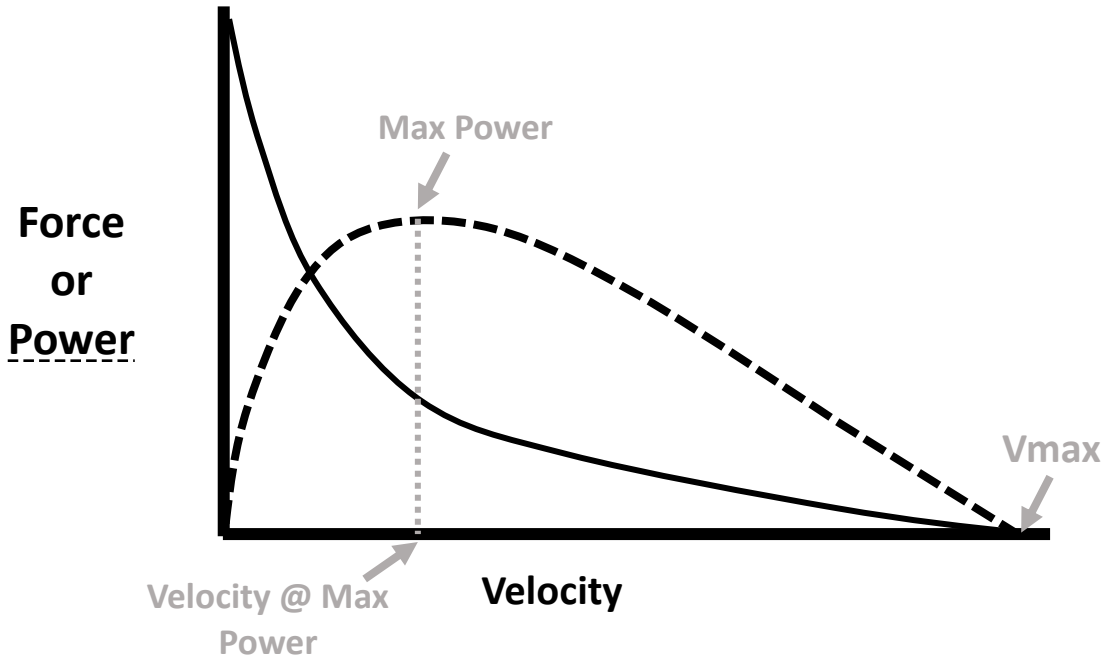


Figure 8: Force-velocity curve (solid black line) and power curve (black dashed line) produced from Hill equation. Maximum power, Vmax and Velocity at which maximum power is achieved is highlighted in grey.

defined by the hill equation and can used to determine the power-velocity relationship as well. The Hill equation was also used to estimate Vmax (i.e. the maximum shortening velocity obtained when force is equal to zero) (Figure 8), and the curvature of the force-velocity relationship (i.e. the ratio of a/Po in the Hill equation), which provides an index of the power of the muscle. Slow-twitch muscles tend to have a larger or deeper curve in the force-velocity relationship (smaller a/Po ratio), while fast-twitch muscle fibres tend to have a straighter curve in the force-velocity relationship (larger a/Po ratio). Vmax and velocity of muscle shortening where power was maximal were calculated in units of metres per second (m/s) and muscle lengths per second (ML/s).

Equation 1 $$(V + b)(P + a) = b(P_0 + a)$$

The ability of a muscle to resist fatigue during a series of work cycles was measured as the number of work cycles completed before work dropped below 50% of the initial value. Furthermore, values of work at cycles 1, 10, 20 and 25, relative to cycle 1, were compared between treatment groups for both the EDL and SOL muscle to compare fatigue rates. The regression values (slope) of relative work at cycle number 1, 10, 20, and 25 were also compared between treatment groups and an ANOVA was used to compare slopes between treatment groups. Further significance testing was done using a non-parametric Dunn's statistical test, as the sample sizes were not equivalent between compared treatment groups. Net work measured during the recovery period (10 and 20 minute intervals following the end of the fatigue trial) was compared to the value before fatigue to provide information on differences in recovery between muscles in different treatment groups.

All statistical measurements were compared between treatment and baseline groups for EDL and SOL muscles using Excel, Origin9 and Sigmaplot. A P-value less than 0.05 was considered significant and ANOVA followed by specific post-hoc tests were used as statistical analyses between treatment group results.

RESULTS

3.1 Serum vitamin D and PTH

Table 1 and Figure 9 show serum vitamin D concentrations comparing the baseline and treatment groups at different time points. Serum vitamin D levels of baseline (i.e. pre-treatment) mice were significantly lower than the midpoint samples of treatment group VD+/UV+, but not different from all other midpoint and endpoint treatment groups. Midpoint treatment group VD-/UV- was significantly lower than all other midpoint treatment groups, but none of the other treatment group comparisons differed (Table 6). When comparing between sub-groups A and B within individual treatments groups, midpoint serum vitamin D levels in sub-group A for treatment group VD-/UV+ was significantly higher than sub-group B. However, subgroups A and B for midpoint treatment groups VD-/UV- and VD+/UV+ did not differ significantly. Midpoint blood analysis for VD+/UV- sub-group A was not successful and so no data are available for this treatment subgroup at the midpoint. Serum vitamin D levels were not significantly different between any treatment groups or baseline mice at the end point of the treatment period (Tables 1 & 6), and serum vitamin D comparisons between subgroups A and B at the endpoint of treatments showed no significant differences (Table 1). There were no significant changes in serum vitamin D levels between midpoint and endpoint concentrations in any treatment groups (Table 1).

Table 1: Blood vitamin D analyses showing results for subgroups (A and B) at the midpoint and endpoint of the treatments. Values are mean (SEM). ANOVA tests were used to compare between treatment groups and further analyses were done using Dunn-Sidak statistical tests.

Treatment Group	Subgroup A or B	# weeks into treatment	Average [25OHD] (nmol/L)	Significance comparing between subgroups	Subgroups Combined Average [25OHD] (nmol/L)	Significance (P) comparing Combined Averages	Significance (P) between midpoint and endpoint measurements
Baseline		0	82.350 (±4.634)	N/A	82.350	*See Table 6	
Midpoint Blood Analyses							
VD+/UV-	A	N/A	N/A	N/A	109.079 (±2.426)		
	B	7	109.079 (±2.426)				
VD+/UV+	A	10	134.615 (±11.435)	0.065	120.168 (±7.675)	**0.000258**	
	B	6	105.721 (±5.474)				
VD-/UV-	A	9	70.137 (±5.274)	0.518	66.129 (±5.659)		VD+/UV-: 0.343 VD+/UV+: 0.249 VD-/UV-: 0.0789 VD-/UV+: 0.614
	B	6	62.121 (±10.404)				
VD-/UV+	A	9	140.561 (±6.427)	**3.849×10^{-5}**	105.608 (±12.236)		
	B	6	70.656 (±4.645)				

Endpoint Blood Analyses

VD+/UV-	A	12	105.280 (±4.411)	0.331	152.220 (±43.015)	0.697
	B	12	199.160 (±84.883)			
VD+/UV+	A	12	115.140 (±11.854)	0.326	108.240 (±6.416)	
	B	12	101.340 (±4.573)			
VD-/UV-	A	12	163.780 (±60.024)	0.498	137.510 (±35.804)	
	B	12	111.240 (±42.670)			
VD-/UV+	A	12	166.100 (±73.711)	0.354	126.610 (±38.589)	
	B	12	87.120 (±22.093)			

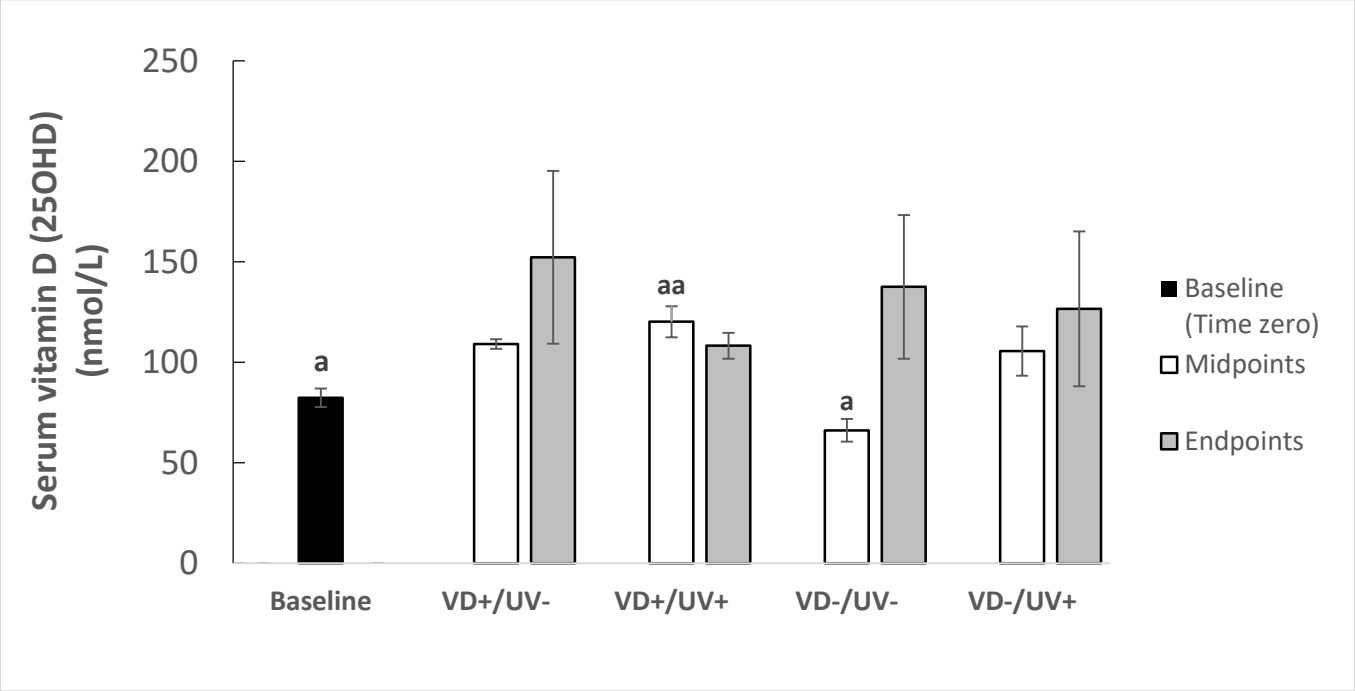


Figure 9: Average (+/-SEM) serum vitamin D levels at three different sampling time points (baseline, midpoint, and endpoint) of the four treatment groups. Baseline mice were significantly lower than midpoint measures of serum vitamin D in group VD+/UV+ (**Table** 6). Midpoint treatment group VD-/UV- had significantly lower serum vitamin D levels compared to all other midpoint treatment groups (**Table** 6). Midpoint and endpoint treatment groups did not differ significantly.

For nearly half of the mice, not enough blood could be collected to allow PTH analyses. Thus, values for serum PTH are not shown and statistical analysis of this parameter was not conducted. Serum PTH levels were not abnormally high in any of the groups, where high levels would be indicative of vitamin D deficiency.

When comparing serum vitamin D levels to physical measures of the mice (mouse mass, muscle mass, muscle length, muscle cross-sectional area), baseline group mice were significantly

lighter in body mass and muscle mass when compared to treatment group mice in the EDL muscle type (Tables 5 & 6). Also, younger baseline control group mice had significantly lower muscle mass and CSA when compared to all other treatment groups in the SOL muscle type (Tables 5 & 6). The muscle length in the SOL muscle type measurements were significantly shorter in treatment group VD+/UV- when compared to groups VD+/UV+ and VD-/UV- (Tables 2 and 6). Group VD-/UV+ showed no significant differences in muscle length compared to any other treatment group. An example of a physical measure (mouse mass) plotted against serum vitamin D concentration is shown in Figure 10. All physical parameters plotted against serum vitamin D concentrations followed a similar pattern to Figure 10, with no significant effect of vitamin D concentration on any of the parameters measured (Table 2). There was no effect of serum vitamin D levels regardless of which treatment groups the mice were in (Table 3).

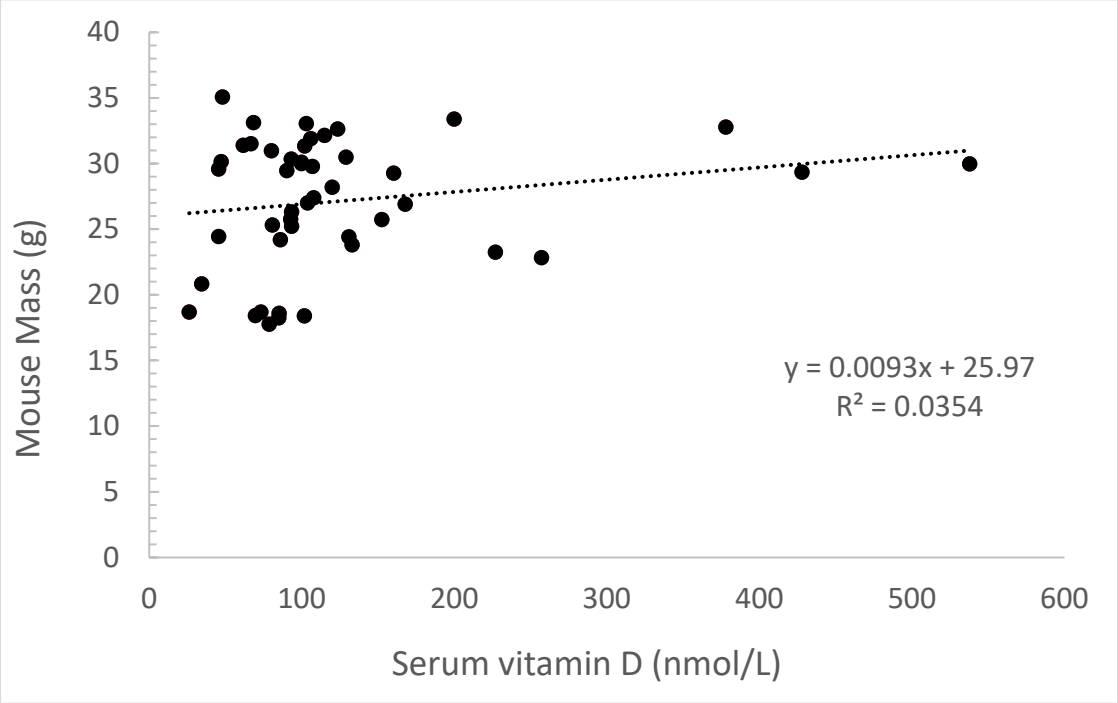


Figure 10: Mouse mass versus serum vitamin D concentrations (nmol/L). See Table 2 for statistical results.

Table 2: Relationship between plasma vitamin D concentration and physical measures of mouse anatomy and muscle contractile performance. Data from all treatment groups are combined. Slope of the relationship, R^2, and statistical significance comparing treatment groups are shown.

Parameter Comparison	Slope of Relationship	R^2	Significance
Mouse mass (g)	9.30×10^{-3}	3.54×10^{-2}	0.211
Muscle mass (g) - EDL	-1.00×10^{-6}	8.70×10^{-3}	0.537
Muscle length (cm) - EDL	-4.00×10^{-4}	5.57×10^{-2}	0.114
Muscle CSA (cm^2) - EDL	6.00×10^{-6}	6.93×10^{-2}	0.077
Muscle mass (g) - SOL	2.85×10^{-6}	7.85×10^{-3}	0.252
Muscle length (cm) - SOL	-5.61×10^{-4}	-2.07×10^{-2}	0.745
Muscle CSA (cm^2) - SOL	3.89×10^{-6}	5.37×10^{-3}	0.272
EDL Muscle			
Max Twitch Force (N)	-1.31×10^{-3}	-2.26×10^{-2}	0.940
Max Twitch Force (N/cm^2)	-2.97×10^{-3}	3.06×10^{-3}	0.292
Max Tetanic Force (N)	3.95×10^{-2}	-1.77×10^{-2}	0.643
Max Tetanic Force (N/cm^2)	-8.59×10^{-3}	-1.31×10^{-2}	0.521
Work-loop: Absolute Max Power (W)	-1.91×10^{-8}	-2.25×10^{-2}	0.924
Work-loop: Max Power (W/kg)	-7.91×10^{-3}	-2.08×10^{-2}	0.772
Work-loop: Cycle Freq. (Hz) @ Max Power	2.62×10^{-3}	7.72×10^{-2}	0.034
Lengthening Work @ 6 Hz (J/kg)	5.76×10^{-4}	-1.26×10^{-2}	0.511
Twitch Kinetics: 10%-10% (ms)	-7.81×10^{-3}	-2.10×10^{-2}	0.787
Twitch Kinetics: 10%-90% (ms)	-2.44×10^{-3}	2.81×10^{-2}	0.137
Twitch Kinetics: 90%-10% (ms)	-4.83×10^{-3}	-2.19×10^{-2}	0.852
Force-velocity: Absolute Max Power (W)	5.52×10^{-4}	2.17×10^{-2}	0.164
Force-velocity: Max Power (W/kg)	5.85×10^{1}	6.40×10^{-3}	0.262
Force-velocity: Velocity (m/s) @ Max Power	1.10×10^{-5}	1.69×10^{-2}	0.190
Force-velocity: Velocity (ML/s) @ Max Power	2.46×10^{-3}	4.80×10^{-2}	0.077
Vmax (m/s)	7.00×10^{-6}	-2.20×10^{-2}	0.859
Vmax (ML/s)	6.76×10^{-3}	3.98×10^{-3}	0.283
SOL Muscle			
Max Twitch Force (N)	-1.79×10^{-3}	-2.23×10^{-2}	0.839
Max Twitch Force (N/cm^2)	-1.26×10^{-3}	6.35×10^{-4}	0.316
Max Tetanic Force (N)	4.95×10^{-2}	-1.10×10^{-2}	0.474
Max Tetanic Force (N/cm^2)	-3.56×10^{-3}	-1.97×10^{-2}	0.699
Work-loop: Absolute Max Power (W)	-1.38×10^{-8}	-2.20×10^{-2}	0.821
Work-loop: Max Power (W/kg)	-6.97×10^{-3}	-4.87×10^{-3}	0.380
Work-loop: Cycle Freq. (Hz) @ Max Power	-3.06×10^{-4}	-1.22×10^{-2}	0.497
Lengthening Work @ 2 Hz (J/kg)	-4.47×10^{-3}	3.18×10^{-2}	0.0319

Twitch Kinetics: 10%-10% (ms)	-5.89×10^{-2}	2.59×10^{-3}	0.297
Twitch Kinetics: 10%-90% (ms)	-1.18×10^{-3}	-1.77×10^{-2}	0.632
Twitch Kinetics: 90%-10% (ms)	-3.37×10^{-2}	-1.23×10^{-2}	0.498
Force-velocity: Absolute Max Power (W)	2.41×10^{-4}	-1.96×10^{-3}	0.344
Force-velocity: Max Power (W/kg)	2.42×10^{-2}	-2.26×10^{-2}	0.865
Force-velocity: Velocity (m/s) @ Max Power	2.63×10^{-6}	-1.77×10^{-2}	0.630
Force-velocity: Velocity (ML/s) @ Max Power	3.45×10^{-4}	-1.86×10^{-2}	0.661
Vmax (m/s)	2.15×10^{-5}	-9.16×10^{-3}	0.443
Vmax (ML/s)	2.47×10^{-3}	-1.30×10^{-2}	0.512

Table 3: Effects of treatment group on measures of mouse and muscle anatomy. Significance shows P value comparing the different groups. Values are mean (SEM). * indicates $P < 0.05$ comparing between treatment groups.

Parameters	Baseline	VD+/UV-	VD+/UV+	VD-/UV-	VD-/UV+	Significance
Mouse Mass (g)	*18.35 (±0.14)	30.37 (±0.65)	27.70 (±0.85)	29.33 (±1.32)	26.41 (±1.52)	3.581×10^{-7}
Muscle Mass (g)-EDL	*5.65×10^{-3} ($\pm 2.87 \times 10^{-4}$)	7.91×10^{-3} ($\pm 1.73 \times 10^{-4}$)	8.21×10^{-3} ($\pm 3.84 \times 10^{-4}$)	7.88×10^{-3} ($\pm 2.52 \times 10^{-4}$)	7.17×10^{-3} ($\pm 2.44 \times 10^{-4}$)	6.722×10^{-6}
Muscle CSA (cm^2)-EDL	7.35×10^{-3} ($\pm 7.11 \times 10^{-4}$)	1.04×10^{-2} ($\pm 7.90 \times 10^{-4}$)	9.48×10^{-3} ($\pm 5.85 \times 10^{-4}$)	9.88×10^{-3} ($\pm 7.28 \times 10^{-4}$)	9.58×10^{-3} ($\pm 7.91 \times 10^{-4}$)	0.13
Muscle Length (cm)-EDL	0.76 (±0.05)	0.76 (±0.05)	0.84 (±0.05)	0.79 (±0.04)	0.75 (±0.05)	0.61
Muscle Mass (g)-SOL	*5.65×10^{-3} ($\pm 2.87 \times 10^{-4}$)	7.91×10^{-3} ($\pm 1.73 \times 10^{-4}$)	8.21×10^{-3} ($\pm 3.84 \times 10^{-4}$)	7.88×10^{-3} ($\pm 2.52 \times 10^{-4}$)	7.17×10^{-3} ($\pm 2.44 \times 10^{-4}$)	1.865×10^{-9}
Muscle CSA (cm^2)-SOL	*5.473×10^{-3} ($\pm 4.64 \times 10^{-4}$)	1.052×10^{-3} ($\pm 6.16 \times 10^{-4}$)	9.418×10^{-3} ($\pm 6.01 \times 10^{-4}$)	9.109×10^{-3} ($\pm 3.26 \times 10^{-4}$)	9.801×10^{-3} ($\pm 7.54 \times 10^{-4}$)	8.197×10^{-5}
Muscle Length (cm)-SOL	0.9 (±0.05)	*0.81 (±0.03)	0.94 (±0.03)	0.96 (±0.01)	0.84 (±0.04)	0.0062

3.2 Effects of vitamin D treatments on isometric contractions

There was no effect of reduced vitamin D in diet and UV light on measures of isometric force (Tables 4 & 5) with the exception of maximum twitch force produced by the SOL muscle in younger control baseline mice being significantly higher than the older control group VD+/UV-, VD+/UV+ and VD-/UV+ (Tables 5 & 6). Analysis of the effects of vitamin D concentration in serum on isometric tetanic and twitch force production showed no trend between the variables (slope was not significantly different from zero) (Table 2).

There were effects of vitamin D in diet or UV light on twitch kinetics when comparing treatment groups to baseline mice, where the baseline group was significantly slower than treatment groups VD+/UV- and VD+/UV+ in the EDL muscle (Tables 4 & 6), and the baseline group was significantly slower than treatment groups VD+/UV+ in the SOL muscle (Figure 11, Tables 5 & 6). These differences occurred for twitch kinetic parameters measuring the period of time when muscle force remained above 10% and during the relaxation period from 90%-10% of the force trace (Figure 7). Vitamin D in diet or UV light did not have an effect on the twitch kinetics amongst groups for either muscle type (Tables 4 & 5).

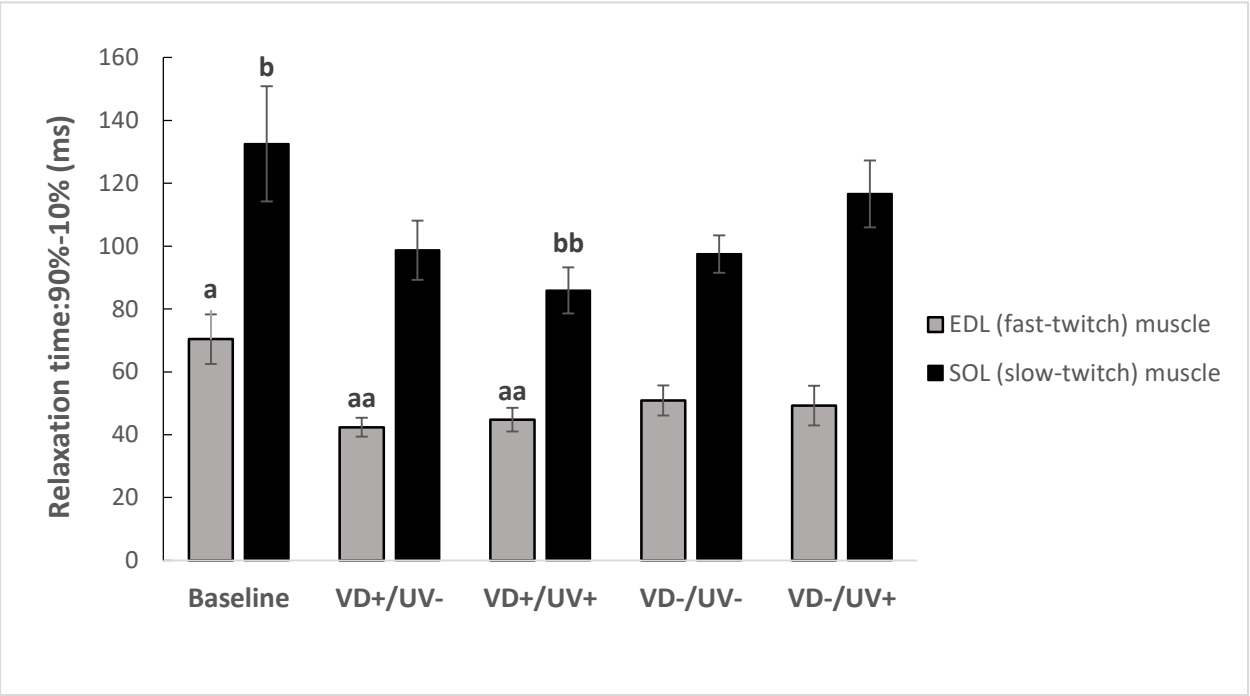


Figure 11: Time for twitch force to relax from 90%-10% of maximal for EDL and SOL muscle. Baseline mice were significantly slower than treatment groups VD+/UV- and VD+/UV+ for the EDL muscle, and SOL muscles of the baseline mice were significantly slower than treatment group VD+/UV+ (see Table 6 for statistical results). Comparisons between SOL and EDL muscle types were not made.

Table 4: Effects of treatment group on different measures of muscle contractile performance in EDL muscle type. Values are mean (SEM). Significance shows the P value comparing treatment groups (including baseline). * indicates $P < 0.05$ comparing among treatment groups.

Parameters	Baseline	VD+/UV-	VD+/UV+	VD-/UV-	VD-/UV+	Significance
Max Twitch Force (N)	0.029 (±0.004)	0.032 (±0.004)	0.039 (±0.003)	0.040 (±0.004)	0.037 (±0.003)	0.263
Max Twitch Force (N/cm^2)	4.501 (±0.920)	3.397 (±0.587)	4.239 (±0.415)	4.315 (±0.616)	4.222 (±0.530)	0.762
Max Tetanic Force (N)	0.155 (±0.010)	0.177 (±0.027)	0.197 (±0.016)	0.204 (±0.012)	0.165 (±0.016)	0.324
Max Tetanic Force (N/cm^2)	23.170 (±4.586)	19.070 (±3.900)	21.666 (±2.137)	21.895 (±2.254)	18.590 (±2.270)	0.805
Work-loop: Absolute Max Power ($W \times 10^{-4}$)	2.359 (±0.315)	3.137 (±0.511)	3.477 (±0.447)	3.637 (±0.410)	2.695 (±0.308)	0.255
Work-loop: Max Power (W/kg)	43.831 (±8.917)	40.269 (±9.848)	42.654 (±5.013)	46.920 (±5.586)	38.408 (±4.840)	0.870
Work-loop: Cycle Freq. (Hz) @ Max Power	5.755 (±0.167)	6.177 (±0.357)	5.492 (±0.292)	5.567 (±0.305)	5.796 (±0.240)	0.405
Lengthening work @ 6 Hz (J/kg)	-1.0972 (±0.332)	-0.816 (±0.111)	-0.795 (±0.149)	-1.0655 (±0.258)	-0.639 (±0.113)	0.424
Twitch Kinetics: 10%-10% (ms)	*86.867 (±8.528)	59.480 (±4.060)	60.080 (±4.373)	66.120 (±5.333)	63.980 (±7.210)	0.039
Twitch Kinetics: 10%-90% (ms)	6.533 (±0.281)	6.800 (±0.531)	6.740 (±0.426)	6.180 (±0.180)	6.760 (±0.200)	0.711
Twitch Kinetics: 90%-10% (ms)	*70.400 (±7.909)	42.360 (±3.005)	44.820 (±3.764)	50.860 (±4.789)	49.240 (±6.296)	0.013
Force-velocity: Absolute Max Power ($W \times 10^{-3}$)	5.159 ($\pm 1.373 \times 10^{-3}$)	4.652 ($\pm 6.865 \times 10^{-4}$)	8.247 ($\pm 2.193 \times 10^{-3}$)	6.993 ($\pm 1.526 \times 10^{-3}$)	5.803 ($\pm 1.014 \times 10^{-3}$)	0.077

Table 4: cont'd

Force-velocity: Max Power (W/kg)	1018.3 (±444.6)	596.4 (±93.3)	945.2 (±192.3)	911.4 (±209.2)	831.2 (±165.3)	0.494
Force-velocity: Velocity (m/s) @ Max Power	1.270×10^{-2} ($\pm 1.989 \times 10^{-3}$)	*1.898×10^{-2} ($\pm 1.359 \times 10^{-3}$)	1.263×10^{-2} ($\pm 1.444 \times 10^{-3}$)	1.397×10^{-2} ($\pm 1.868 \times 10^{-3}$)	1.164×10^{-2} ($\pm 1.729 \times 10^{-3}$)	0.022
Force-velocity: Velocity (ML/s) @ Max Power	1.776 (±0.319)	2.655 (±0.286)	1.593 (±0.229)	1.885 (±0.291)	1.703 (±0.319)	0.077
Vmax (m/s)	0.077 (±0.006)	0.089 (±0.004)	0.101 (±0.014)	0.086 (±0.004)	0.080 (±0.007)	0.297
Vmax (ML/s)	10.195 (±0.622)	12.207 (±1.004)	12.600 (±2.330)	11.195 (±0.848)	10.868 (±0.983)	0.776
a/Po ratio	0.130 (±0.014)	0.152 (±0.021)	0.117 (±0.019)	0.126 (±0.014)	0.143 (±0.022)	0.682
% work recovery 10 min after fatigue	28.229 (±1.885)	*35.504 (±8.331)	19.237 (±3.404)	20.696 (±2.705)	19.553 (±1.874)	0.041
% work recovery 20 min after fatigue	34.465 (±2.558)	33.530 (±1.209)	25.695 (±3.558)	25.411 (±1.786)	25.691 (±2.851)	0.058

Table 5: Effects of treatment group on different measures of muscle contractile performance in SOL muscle type. Values are mean (SEM). Significance shows the P value comparing treatment groups (including baseline). * indicates $P < 0.05$ comparing between treatment groups.

Parameters	Baseline	VD+/UV-	VD+/UV+	VD-/UV-	VD-/UV+	Significance
Max Twitch Force (N)	1.501×10^{-2} ($\pm 1.097 \times 10^{-3}$)	1.350×10^{-2} ($\pm 2.027 \times 10^{-3}$)	1.425×10^{-2} ($\pm 2.256 \times 10^{-3}$)	1.712×10^{-2} ($\pm 1.418 \times 10^{-3}$)	1.356×10^{-2} ($\pm 1.920 \times 10^{-3}$)	0.661
Max Twitch Force (N/cm^2)	*2.784 (±0.197)	1.368 (±0.236)	1.619 (±0.277)	1.901 (±0.168)	1.473 (±0.250)	0.005
Max Tetanic Force (N)	0.109 (±0.008)	0.133 (±0.013)	0.137 (±0.018)	0.167 (±0.010)	0.124 (±0.015)	0.121
Max Tetanic Force (N/cm^2)	20.385 (±1.817)	13.138 (±1.546)	15.606 (±2.543)	16.566 (±2.066)	13.237 (±1.752)	0.164
Work-loop: Absolute Max Power (W)	8.641×10^{-5} ($\pm 7.568 \times 10^{-6}$)	9.047×10^{-5} ($\pm 1.136 \times 10^{-5}$)	1.004×10^{-4} ($\pm 1.577 \times 10^{-5}$)	1.163×10^{-4} ($\pm 1.305 \times 10^{-5}$)	7.937×10^{-5} ($\pm 1.227 \times 10^{-5}$)	0.321
Work-loop: Max Power (W/kg)	17.071 (±0.982)	10.503 (±1.608)	11.442 (±2.110)	12.613 (±1.190)	9.686 (±1.542)	0.058
Work-loop: Cycle Freq. (Hz) @ Max Power	*2.956 (±0.087)	2.772 (±0.055)	2.513 (±0.080)	2.549 (±0.098)	2.601 (±0.106)	0.013
Lengthening work @ 2 Hz (J/kg)	-1.992 (±0.280)	-2.656 (±0.657)	-1.933 (±0.539)	-1.877 (±0.322)	-1.320 (±0.115)	0.331
Twitch Kinetics: 10%-10% (ms)	*173.767 (±16.797)	142.000 (±14.135)	118.500 (±8.028)	136.244 (±6.797)	150.440 (±11.451)	0.047
Twitch Kinetics: 10%-90% (ms)	14.400 (±0.242)	14.260 (±0.499)	13.800 (±0.793)	14.289 (±0.430)	13.780 (±0.426)	0.894
Twitch Kinetics: 90%-10% (ms)	*132.567 (±18.294)	98.700 (±9.395)	85.880 (±7.338)	97.511 (±5.964)	116.640 (±10.642)	0.032
Force-velocity: Absolute Max Power ($W \times 10^{-3}$)	1.483 ($\pm 1.066 \times 10^{-4}$)	2.043 ($\pm 1.691 \times 10^{-4}$)	1.762 ($\pm 3.445 \times 10^{-4}$)	2.272 ($\pm 2.691 \times 10^{-4}$)	1.800 ($\pm 2.547 \times 10^{-4}$)	0.519

Table 5: cont'd

Force-velocity: Max Power (W/kg)	292.880 (±20.787)	234.373 (±21.203)	198.972 (±40.834)	244.990 (±28.025)	217.968 (±29.686)	0.084
Force-velocity: Velocity (m/s) @ Max Power	1.519×10^{-2} ($\pm 4.043 \times 10^{-4}$)	1.639×10^{-2} ($\pm 1.119 \times 10^{-3}$)	1.263×10^{-2} ($\pm 1.438 \times 10^{-3}$)	1.384×10^{-2} ($\pm 1.191 \times 10^{-3}$)	1.383×10^{-2} ($\pm 1.110 \times 10^{-3}$)	0.170
Force-velocity: Velocity (ML/s) @ Max Power	1.719 (±0.133)	*2.050 (±0.168)	1.379 (±0.174)	1.443 (±0.115)	1.683 (±0.148)	0.024
Vmax (m/s)	6.651×10^{-2} ($\pm 3.681 \times 10^{-3}$)	7.710×10^{-2} ($\pm 6.274 \times 10^{-3}$)	6.459×10^{-2} ($\pm 7.798 \times 10^{-3}$)	7.256×10^{-2} ($\pm 5.743 \times 10^{-3}$)	6.643×10^{-2} ($\pm 4.534 \times 10^{-3}$)	0.551
Vmax (ML/s)	7.516 (±0.645)	9.617 (±0.861)	7.020 (±0.982)	7.594 (±0.630)	8.045 (±0.596)	0.168
a/Po ratio	0.101 (±0.011)	0.082 (±0.004)	0.069 (±0.013)	0.065 (±0.006)	0.078 (±0.007)	0.108
% work recovery 10 min after fatigue	86.299 (±1.664)	86.215 (±2.661)	89.333 (±2.598)	79.271 (±2.566)	85.483 (±3.553)	0.161
% work recovery 20 min after fatigue	89.493 (±1.471)	88.987 (±2.284)	*52.755 (±8.314)	91.052 (±2.599)	85.699 (±5.174)	1.407×10^{-6}

3.3 Effects of reduced vitamin D in diet and UV light on cyclic work and power production

The cycle frequency at which maximum power was achieved indicated that treatment groups VD+/UV+ and VD-/UV- in the SOL muscle type attained maximum power at significantly slower cycle frequencies when compared to that of young baseline mice (Tables 5 & 6). Vitamin D treatment groups and circulating vitamin D levels did not impact measurements of power production or the frequency at which power was maximal, for either the EDL (fast-twitch) or SOL (slow-twitch) muscles. Most comparisons of power measured through the work-loop technique were not statistically different among treatment groups (Tables 5 & 6), and none of these parameters showed a trend when plotted against concentrations of circulating vitamin D (Table 2).

Analysis of lengthening work, measured at the cycle frequency where power was maximal, indicated no significant differences among treatment groups for either muscle type (Tables 5 & 6). However, there was a significant negative correlation when comparing lengthening work and serum vitamin D concentrations ($P=0.0319$) in the SOL muscle (Tables 2 & 6). Noting that lengthening work is negative in value, this data suggests that lengthening work increases as serum vitamin D increases, indicating that series elastic components may be influenced by serum vitamin D.

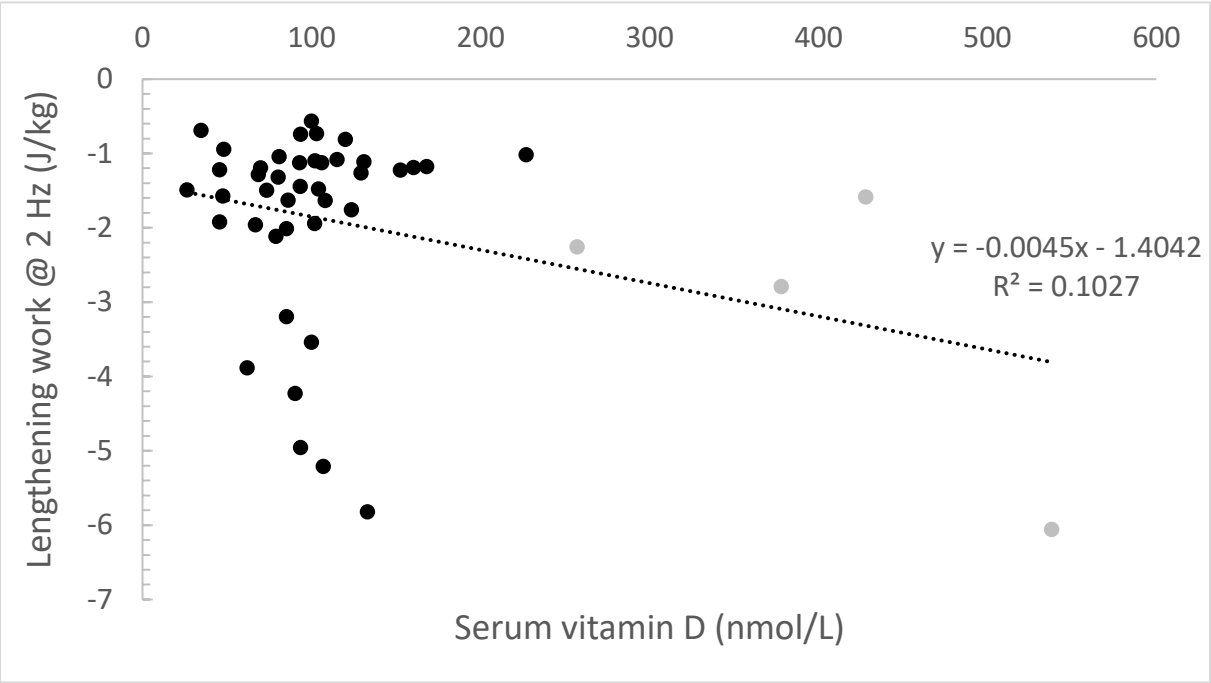


Figure 12: Lengthening work at 2 Hz (J/kg) versus serum vitamin D concentrations (nmol/L) from SOL muscle. Higher than normal serum vitamin D concentrations are considered vitamin D levels > 250 nmol/L and are filled in grey. P=0.0370 with high range individuals (grey dots) and P=0.917 without grey dots. See Table 2 for statistical results.

3.4 Effects of vitamin D treatments on force-velocity measures of power

The velocity of muscle shortening at which peak power was achieved was significantly different among treatment groups (Figure 13, Tables 4, 5 & 6). EDL muscles from treatment group VD+/UV- produced maximum power at a significantly faster shortening velocity than group VD-/UV+, when measured in m/s (Tables 4 & 6). Similarly, the velocity of shortening at which maximal power occurred in the SOL muscles was significantly faster in treatment group VD+/UV- compared to VD+/UV+, but only when compared as ML/s, not m/s (Figure 13, Tables

5 & 6). In addition, the velocity at which a muscle shortens to reach maximum power did not differ between baseline mice and treatment group mice for either EDL or SOL muscles. None of the force-velocity measures of maximal power and Vmax, for both the EDL and SOL muscle types, were affected by vitamin D in the diet or UV light treatment, and likewise the a/Po ratio, which is indicative of the curvature of the force-velocity relationship, did not show significant differences between treatment groups (Tables 4 & 5).

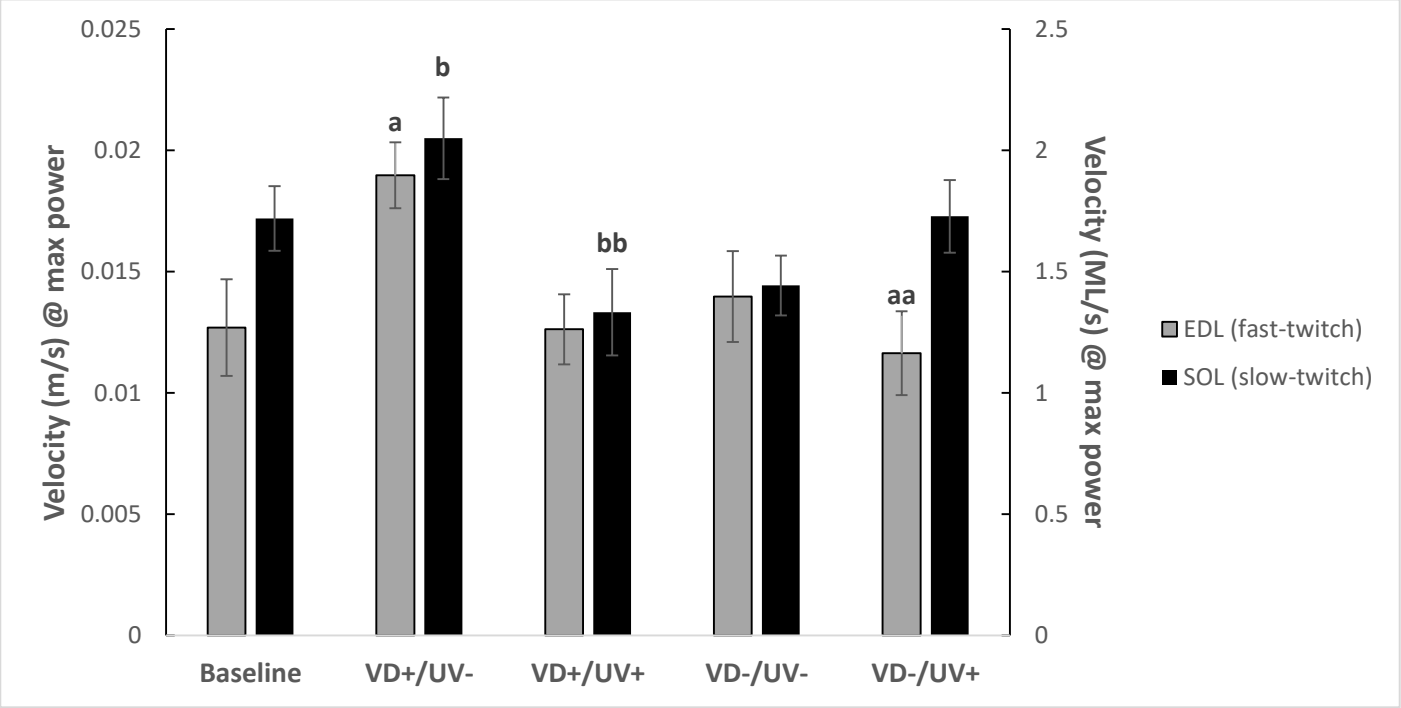
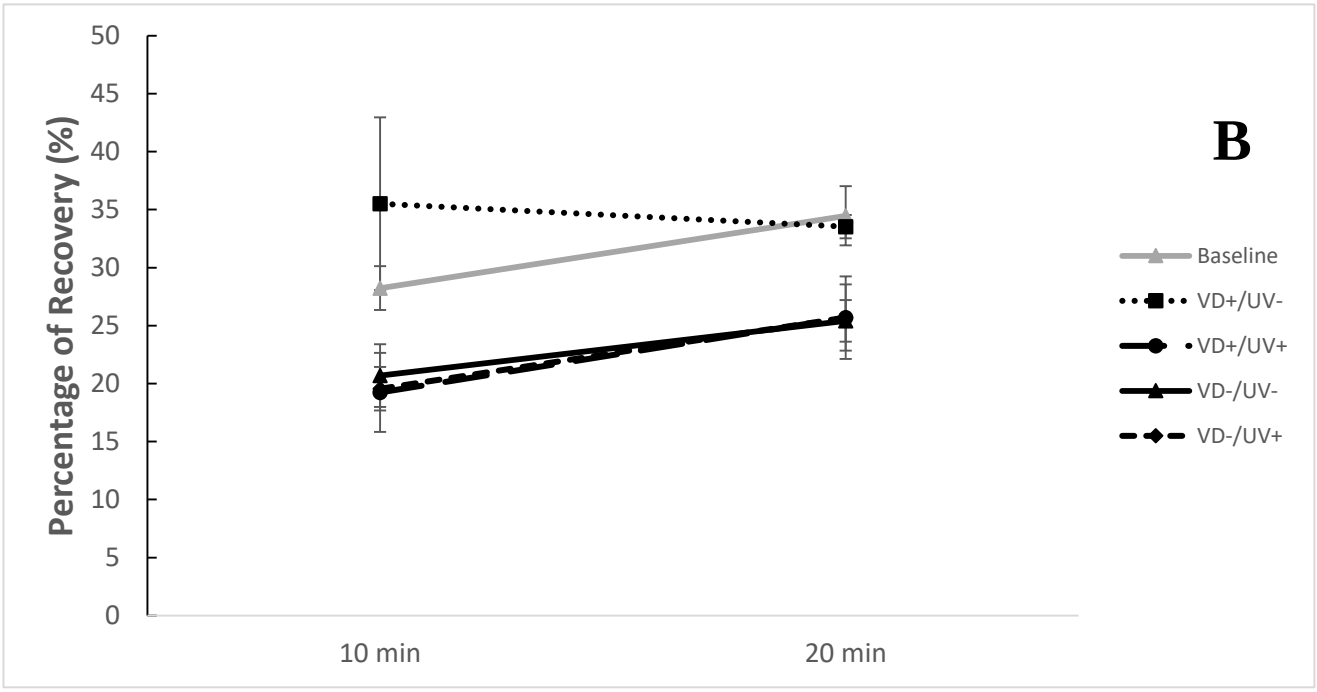


Figure 13: Muscle shortening velocity needed to attain maximum power, in m/s for the EDL muscle and ML/s for the SOL muscle. Treatment group VD+/UV- of the EDL muscle was significantly faster than treatment group VD-/UV+, and treatment group VD+/UV- of the SOL muscle was significantly faster than VD+/UV+ (See **Table** 6 for statistical results). Comparisons between SOL and EDL muscle types were not made.

3.5 Effects of vitamin D treatments on muscle fatigue and recovery of work output

The effects of treatment group on muscle fatigue was assessed in two different ways. First, slopes of linear regressions fit to work values from cycles 1, 10, 20, and 25 of the fatigue tests were compared. For this analysis, values of work were standardized as a percentage of work at cycle 1 to account for differences in muscle mass between preparations. No significant differences in the slopes (rates of muscle fatigue) were found between treatment groups or baseline mice for either EDL ($P=0.641$) or SOL ($P=0.631$) muscle types.

As a second measure of fatigue, the rate that work recovered following the end of fatigue was assessed. The work produced by the muscle after a 10 and 20 minute rest following the fatigue test was calculated as a percentage of the work produced from the first contraction immediately prior to the fatigue test. From the results, the EDL recovered faster than the SOL muscle type, however, the SOL muscle type recovered more following the fatigue test. Specifically, for the EDL muscle, work recorded from treatment group VD+/UV- recovered significantly more than treatment groups VD+/UV+ and VD-/UV- 10 minutes following the fatigue test, but there were no significant differences between percentages of recovery at 20 minutes following the fatigue test (Figure 14, Tables 4 & 6). The SOL muscle type showed significant increased muscle percentage work recovery following a 20 minute rest when compared between treatment groups at this time point, with treatment group VD+/UV+ having a significantly lower recovery compared to all other treatment groups (including the baseline mice) (Tables 5 & 6). However, this was not the case for percentage of work recovery 10 minutes following the fatigue test, where there were no differences between any of the treatment groups or baseline mice (Figure 14, Tables 5 & 6).



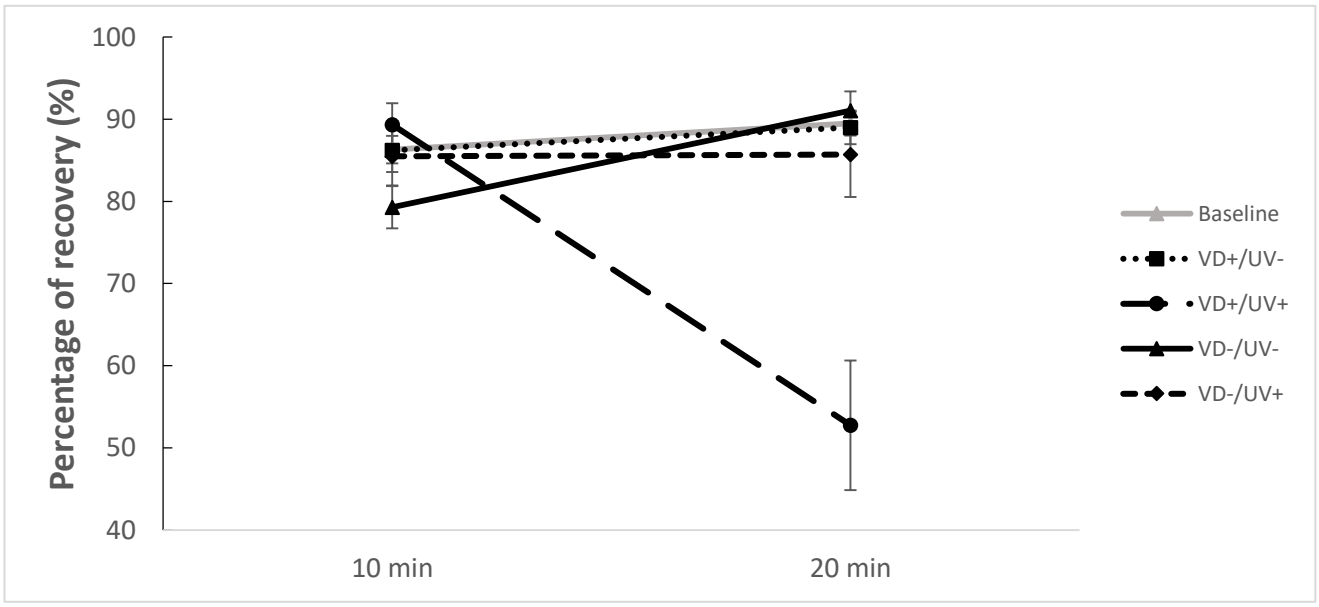


Figure 14: Percentage recovery of work following fatigue at two time points for the EDL (panel A) muscle type and SOL (panel B) muscle type. Treatment group VD+/UV- in the EDL muscle had significantly greater percentages of work recovery compared to VD+/UV+ and VD-/UV- at

10 minutes following fatigue. Treatment group VD+/UV+ in the SOL muscle had the lowest percentage of work recovery when compared with all other groups (including baseline) 20 minutes after fatigue.

Table 6: Post-hoc comparisons for all statistically significant ANOVA tests.

Parameter	Group Comparisons		Test Type	Significance
Serum Vit. D Concentration (nmol/L)	Baseline	VD+/UV+ (midpoint)	Holm-Sidak	5.780×10^{-3}
	VD-/UV- (midpoint)	VD+/UV- (midpoint)	Holm-Sidak	3.350×10^{-3}
		VD+/UV+ (midpoint)		2.432×10^{-5}
		VD-/UV+ (midpoint)		1.130×10^{-3}
	Sub-group A: VD-/UV+ (midpoint)	Sub-group B: VD-/UV+ (midpoint)	Two-Sample T-test	3.849×10^{-5}
Mouse mass (g)	Baseline	VD+/UV-	Dunn-Sidak	2.337×10^{-8}
		VD+/UV+		3.504×10^{-6}
		VD-/UV-		1.635×10^{-7}
		VD-/UV+		3.725×10^{-5}
Muscle mass (g) – EDL	Baseline	VD+/UV-	Holm-Sidak	6.538×10^{-6}
		VD+/UV+		7.196×10^{-7}
		VD-/UV-		7.897×10^{-6}
		VD-/UV+		1.230×10^{-3}
Muscle mass (g)-SOL	Baseline	VD+/UV-	Holm-Sidak	3.488×10^{-9}
		VD+/UV+		4.739×10^{-10}
		VD-/UV-		6.361×10^{-10}
		VD-/UV+		8.256×10^{-8}
Muscle CSA (cm^2)-SOL	Baseline	VD+/UV-	Holm-Sidak	3.492×10^{-6}
		VD+/UV+		1.420×10^{-4}
		VD-/UV-		4.847×10^{-4}
		VD-/UV+		3.999×10^{-5}

Table 6: cont'd

Muscle Length (cm)-SOL	VD+/UV-	VD+/UV+ VD-/UV-	Holm-Sidak	0.00482 0.00214
Twitch Kinetics: 10%-10% (ms) - EDL	Baseline	VD+/UV- VD+/UV+	Holm-Sidak	4.310×10^{-3} 5.150×10^{-3}
Twitch Kinetics: 90%-10% (ms) - EDL	Baseline	VD+/UV- VD+/UV+	Holm-Sidak	9.854×10^{-4} 2.390×10^{-3}
Force-velocity: Velocity (m/s) @ Max Power - EDL	VD+/UV-	VD-/UV+	Holm-Sidak	2.410×10^{-3}
Max Twitch Force (N/cm^2) - SOL	Baseline	VD+/UV- VD+/UV+ VD-/UV+	Holm-Sidak	4.985×10^{-4} 3.350×10^{-3} 1.130×10^{-3}
Work-loop: Cycle Freq. (Hz) @ Max Power - SOL	Baseline	VD+/UV+ VD-/UV-	Holm-Sidak	2.360×10^{-3} 5.660×10^{-3}
Twitch Kinetics: 10%-10% (ms) - SOL	Baseline	VD+/UV+	Holm-Sidak	3.440×10^{-3}
Twitch Kinetics: 90%-10% (ms) - SOL	Baseline	VD+/UV+	Holm-Sidak	4.260×10^{-3}
Force-velocity: Velocity (ML/s) @ Max Power - SOL	VD+/UV-	VD+/UV+	Holm-Sidak	2.600×10^{-3}

Table 6: cont'd

% Recovery 10 minute after fatigue test - EDL	VD+/UV-	VD+/UV+	Dunn-Sidak	$P < 0.05$
		VD-/UV-		$P < 0.05$
% Recovery 20 minute after fatigue test - SOL	VD+/UV+	Baseline		6.048×10^{-5}
		VD+/UV-	Dunn-Sidak	1.400×10^{-5}
		VD-/UV-		5.399×10^{-5}
		VD-/UV+		1.722×10^{-5}

DISCUSSION

4.1 Impacts of ultraviolet (UV) light on muscle physiology

Solar radiation is mainly comprised of UVA and UVB wavelengths (Battie et al., 2014). Although UVB rays contain more energy than UVA, the abundance of UVA radiation reaching Earth's surface is high compared to UVB, and is capable of penetrating deeper into the dermis layers of human skin (Battie et al., 2014). As such, the majority of solar radiation reaching our body is made up of UVA rays. UVA rays are within the wavelengths 320-400 nm and are produced in large quantities by the ExoTerra 26W UVB light bulbs that were used in this study. The Reptile UVB 200 ExoTerra 26W light bulb informational guides indicated that at a distance of 10cm these bulbs emit 225 uW/cm^2 UVB and 1350 uW/cm^2 UVA. Thus, a majority of the UV rays emitted were in the UVA spectrum. Previously, UVA has been found to be associated with the production of nitric oxide in the skin, which is a free radical and its effects on physiology is not well understood (Paunel et al., 2005; Opländer et al., 2009; Suschek et al., 2010). However, nitric oxide influences several biological components related to vasodilation, neurotransmission, apoptosis and cell motility (Juzeniene & Moan, 2012). Thus, UVA rays emitted by the ExoTerra lightbulbs used in this study may have influenced several muscle contractile measurements through neurotransmission, causing older mice in treatment groups to have similar relaxation periods when compared to younger baseline mice. This is not conclusive, as UVA was not measured in this study and metabolites associated with UVA, was also not regulated or measured.

The second type of UV radiation in sunlight are UVB rays which are most physically damaging to biological organisms (Battie et al., 2014). Naturally occurring aerobic contractile

activity in muscles is associated with free radical production, which have been thought to cause exercise-induced muscle damage, but the data is inconclusive. Additional free radical production from UVB exposure may contribute to similar muscle damage (Jackson, 1999). For example, Kazerouni et al., (2016) suggested that reduced muscle locomotor performance in mosquitofish was due to UVB induced reactive oxygen species (ROS) damage to receptors required for calcium cycling (i.e. ryanodine receptors) and myofibrillar proteins involved in muscle contractions.

Both types of UV exposure were present in my study and some changes seen in the results may be explained by pathways not involving vitamin D synthesis. Thus, this section will be referred back to in the rest of the thesis.

4.2 Effects of age on muscle contractile capabilities

Age is a factor that should be considered when interpreting my results, as developmental changes can influence most of the measured parameters of my study. For example, the age of the mice may play a role in serum vitamin D levels, where there were large differences in serum vitamin D levels between two treatment groups at the treatment midpoint, when the mice were only 12-15 weeks of age, compared to the absence of any significant differences between treatment groups in blood vitamin D levels at the endpoint of treatments, when the mice were 18 weeks old. Baseline mice acted as a younger control-like group when compared to the older treatment group VD+/UV- which underwent the same treatment exposures (i.e. normal vitamin D in diet and no UV exposure), but are 3 months older than the baseline mice. By comparing these two groups, I can rule out developmental changes that might be causing the results observed and

will be referring back to these age related changes in the next sections. Age affected several factors of this study and can be considered a limitation of the study.

4.3 Effects of vitamin D treatments on circulating levels of vitamin D and PTH

The effects of dietary vitamin D and UV light treatments on circulating levels of vitamin D (25OHD) were apparent at the midpoint sampling period, when mice were 6-9 weeks into the treatments (Figure 9, Tables 1 & 6). These differences between treatment groups suggest that serum vitamin D levels were influenced by the treatments. As I predicted, the treatment group receiving the least amount of vitamin D through the diet and UVB exposure (VD-/UV-) had the lowest levels of serum vitamin D when compared to all other treatment groups at the midpoint. Furthermore, the treatment group receiving the highest levels of vitamin D (VD+/UV+) had significantly higher serum vitamin D levels than the non-treated baseline mice, but was not different than the other treatment groups. However, at 12 weeks through treatment, endpoint analyses of blood indicated similar levels of circulating vitamin D across all groups (Figure 9). This suggests a homeostatic response may have occurred as the treatments progressed, where factors that may regulate vitamin D levels compensated for the treatment effects, such as PTH levels when vitamin D supplies are low. Differences in vitamin D levels may be due to the age difference between midpoint and endpoint mice.

Serum parathyroid hormone (PTH) levels were only measured in half of all blood samples due to the inability to collect enough blood for both 25OHD and PTH assays to be performed accurately. From PTH results that were available, there was no indication of vitamin D deficiency or toxicity, which was supported by no observable symptoms of vitamin D

deficiency or toxicity (Holick, 2007b; Reinhold Vieth, 2007). Based on the levels of vitamin D (25OHD) and PTH measured in the serum, mice were not deficient in vitamin D (< 50 nmol/L) at any point of the treatment period in any treatment group. The lowest treated diet vitamin D and UV group (VD-/UV-) had insufficient levels of serum vitamin D (52-72 nmol/L) at the midpoint of analyses, but this level increased to sufficient levels by the endpoint analyses. All mice had sufficient levels of serum vitamin D at the endpoint of the treatment period, with four individuals within the range that would be considered toxic levels. However, only chronic exposure to these levels of vitamin D will cause detrimental symptoms of vitamin D toxicity (Reinhold Vieth, 2007), which was not seen in my study. These values were considered in the analysis of all the data, as the mice did not exhibit behaviours or have muscle contractile measures different from those within the adequate/normal range of serum vitamin D.

Dietary vitamin D and UV light treatments and circulating levels of vitamin D were not related to any of the measured physical properties of the mice, such as muscle mass, muscle cross-sectional area, and body mass (Figure 10, Tables 2, 3 & 6). As expected, baseline mice had significantly lower body and muscle mass for both EDL and SOL muscle types when compared to all treatment groups including the older counterpart treatment group VD+/UV- suggesting that age may be a large reason as to the results observed. Further, there was no correlation between concentrations of vitamin D in the blood on any parameters of muscle contractile capacity measured in this study, with the exception of lengthening work (Table 2). However, muscle length measurements of the SOL muscle type indicated that treatment group VD+/UV- was significantly shorter than that of treatment groups VD+/UV+ and VD-/UV- (Table 6). No significant differences were found for muscle length in the EDL muscle type measurements. This study did not produce significantly different serum vitamin D levels between treatment groups,

thus changes in muscle length may be due to chance of selecting mice with longer muscles or other factors not associated with vitamin D may be involved.

It thus appears that there is an inherently wide range of vitamin D levels (Figure 10) in the blood of mice at the endpoint of reduced vitamin D in diet and UV light treatments. Age might affect the ability of mice to regulate vitamin D levels in the blood, but that this variability, at least over the range observed in the present study and through 3 months of treatment, does not have a notable impact on muscle contractile capacity.

4.4 Effects of treatments and circulating vitamin D on force production and twitch kinetics

Maximum twitch force produced by the SOL muscle was significantly greater in baseline mice when compared to treatment groups VD+/UV-, VD+/UV+ and VD-/UV+ (Tables 5 & 6). This difference may be due to younger mice having healthier and stronger muscles. Furthermore, comparisons of force production as a function of serum concentration of vitamin D (i.e. independent of which treatment group mice were assigned) showed that vitamin D did not impact force production (Table 2). Reduced vitamin D in the diet and UV light exposure did not affect maximum force produced from tetanic contractions.

Rates of force rise during isometric contraction and rates of force decline during muscle relaxation are associated with calcium transport mechanisms occurring during muscle contractions (Larsson & Salviatit, 1989). EDL muscles from treatment groups VD+/UV- and VD+/UV+ had significantly shorter relaxation periods (i.e. faster rates of muscle relaxation) when compared to baseline mice (Figure 11, Tables 4 & 6). In addition, SOL muscles from treatment group VD+/UV+ had significantly shorter relaxation periods when compared to

baseline mice (Tables 5 & 6), but treatment group VD-/UV+ was not significantly different from all other treatment groups excluding group VD+/UV+. Thus, this data is inconsistent with an effect of UV exposure on increased speeds of relaxation and thus rates of calcium sequestration in muscle. Rodman & Baker (1978) indicated that rats deficient in vitamin D showed significantly slower hindlimb muscle relaxation periods, and normal relaxation speeds were restored when vitamin D levels were brought back to normal. Furthermore, this effect was independent of dietary calcium intake. In the present study, average serum vitamin D levels were not different between treatment groups and baseline mice at the endpoint of the study. There was also a lack of an association between serum vitamin D and muscle relaxation (Tables 1 & 2), which suggest that mechanisms not associated with vitamin D pathways may be influencing muscle contraction. Perhaps an effect of the UV radiation caused the results seen, refer back to section 4.1 for the effects of UV on muscle function.

These results suggest that younger baseline mice have slower muscle relaxation compared to older treatment group mice for both EDL and SOL muscle. However, Petrella et al. (1989) and Paasuke et al. (2000) note that while age influences the speed of contraction and relaxation of fast-twitch fibres, older individuals have prolonged relaxation periods, possibly due to a decrease in the rate of calcium re-uptake following a muscle contraction. This is opposite to the results found in my study, where younger individuals of the baseline group had slower muscles than older individuals in some of the treatment groups. The lack of an effect of age on rates of relaxation, or faster rates in some treatment groups in the present study, may be related to the relatively small age range (6 weeks compared with 18 weeks) of the mice used.

One possible explanation for increased relaxation rates seen in some treatment groups in this study could be ultra-violet A (UVA) rays causing the changes seen in some older treatment

mice, which would be associated with a pathway independent of UVB and vitamin D metabolism. The effects of UV rays on muscle physiology was discussed in section 4.1 (*The effects of ultraviolet rays on muscle physiology*)

4.5 Effects of treatments and circulating vitamin D on work-loop measurements

The cycle frequency at which maximum power is achieved indicated that groups VD+/UV+ and VD-/UV- respectively, reached maximum power at slower cycle frequencies when compared to baseline mice of the SOL muscle (Tables 5 & 6), but not in the EDL muscle (Table 4). When considering that the two mentioned groups showed the same effect, along with the observation that there were similar average levels of circulating vitamin D in both of these groups, the decreased cycle frequency to attain maximum power is likely the result of a mechanism not involving vitamin D. The significance of reaching maximum power at slower cycle frequencies may depend on the situation or the task an animal is performing. This may not be beneficial for an animal trying to quickly escape from a predator, as faster muscle lengthening and shortening cycles to achieve maximum power may aid in escaping or outrunning a predator. However, animals walking up a steep slope may benefit from attaining maximum power at slower cycle frequencies due to factors involved with energy use or stability (Sawicki & Ferris, 2009). Nevertheless, the benefits or disadvantages of attaining maximum power at slower cycle frequencies to an animal's survival will depend on the situation or task being performed by the animal.

One possible explanation is that age related changes are occurring, causing a decrease in the speed of muscle contractions that only reached statistical significance in two of the four

treatment groups. An age related decline in proportion of fast-twitch fibres could cause a decrease in muscle contraction speed (Kugelberg, 1976; Larsson & Edstrom, 1986; Eddinger et al., 1985). However, results of these studies are inconsistent, with some indicating no changes in proportions of fast-twitch fibres as an animal's muscle aged (Kugelberg, 1976; Eddinger et al., 1985; Larsson & Edstrom, 1986). Alternatively, Larsson & Salviatit (1989) indicate that fast-twitch muscle contraction speeds decreased with age due to an impairment of intrinsic sarcoplasmic reticulum function and a decrease in sarcoplasmic reticulum volume, which are both involved in calcium transport activity. Similar results were seen in slow-twitch muscles. However, this muscle type appeared to be influenced by age-related changes that were associated less with changes in sarcoplasmic reticulum properties, but with fibre type transitions and myosin heavy chain alterations (Larsson & Edstrom, 1986). Myosin heavy chain (MHC) isoforms were found to change with age in a rat model, which may be associated with the decrease in muscle contraction speed (Matoba et al., 1992) and thus a slowing of the cycle frequency for maximal power in muscles from mice in the present study.

Vitamin D in the diet or reduced UV light treatments did not affect work and subsequently power production in either EDL or SOL muscles (Tables 4 & 5). Furthermore, comparisons of power as a function of serum concentrations of vitamin D indicated no effect for both muscle types (Table 2). Thus, circulating levels of vitamin D achieved under conditions of my treatment groups do not influence muscle power production measured using the work-loop technique.

4.6 Effects of treatments and circulating vitamin D on force-velocity measures

EDL muscles from treatment group VD+/UV- produced maximum power at significantly faster muscle shortening velocities (in m/s) compared to treatment group VD-/UV+ (Figure 13, Tables 4 & 6). Similarly, for the SOL muscle type, treatment group VD+/UV- had muscles that attained maximum power at significantly faster shortening velocities (in ML/s) when compared to group VD+/UV+ (Figure 13, Tables 5 & 6). These observations indicate that vitamin D obtained through the diet may influence the speed at which a muscle shortens. However, as serum vitamin D levels between treatment groups did not differ significantly, vitamin D pathways do not appear to play a role in the changes seen. Another explanation could be that a factor other than vitamin D, perhaps in the UVB light, could influence muscle shortening velocity (See section 4.1 and 4.2). Treatment conditions and circulating concentrations did not influence force-velocity measurements of muscle power output and maximum velocity of muscle shortening (Vmax) (Tables 2, 4 & 5).

My results indicate that treatment group VD+/UV- had significantly faster muscle shortening velocities needed to reach maximum power than treatment groups VD-/UV+ (in m/s) in the EDL, and VD+/UV+ (in ML/s) in the SOL. In other words, when referring to the dashed power-velocity curve in Figure 8, the peak of this power curve is shifted towards the right. This might suggest that the presence of UVB may slow the muscle shortening velocity needed to attain peak power output. It is difficult to determine whether or not a faster or slower muscle shortening speed to attain maximum power is beneficial because many factors are involved. The task the muscle is performing is an important factor. For example, if an animal is sprinting then mainly fast-twitch fibres are recruited to obtain burst speed (Heglund & Cavagna, 1985).

However, when an animal is foraging, or walking at a slower pace, slow-twitch fibres are mainly used (Heglund & Cavagna, 1985). There are several factors that should be considered before stating if faster muscle shortening speeds to reach maximum power output are beneficial, unrelated or detrimental to an animal's survival. I believe that the benefits or disadvantages of having faster muscle shortening speeds to attain peak power would depend on the situation or task the animal is performing. A quick strike from a predator may benefit from attaining maximum power at faster muscle shortening velocities, however a large animal bearing its own weight from standing up may benefit more from slower muscle shortening velocities to attain maximum power. From my results, both muscle types showed the rightward shift towards faster muscle shortening speeds to attain maximum power output, thus the benefits of having faster moving muscles does not depend solely on whether an animal uses a majority of fast-twitch muscles for burst performance or slow-twitch muscles for more steady locomotion, but also on the task the animal is performing. Explanations for the results seen are undetermined, but because the results are inconsistent and regression analyses between velocity needed to attain maximum power and serum vitamin D concentrations indicate no strong trends, it suggests that the changes seen are not due to vitamin D pathways.

4.7 Effects of treatments on work recovery following fatigue

Both muscle types showed significant effects of vitamin D in the diet and UV light on fatigue recovery (Figure 14, Table 6), but it is not clear what the cause might be. Normal dietary vitamin D (VD+) in combination with low amounts of UV radiation (UV-) resulted in improved recovery in the EDL, while normal dietary vitamin D in combination with high UV resulted in

reduced recovery in the SOL. This might suggest that increased UV radiation impairs recovery, but this pattern was not seen in the other treatment groups with high UV radiation. In addition, serum vitamin D levels did not differ between treatment groups or baseline mice by the end point of analysis, thus, the differences in fatigue recovery between treatment groups appear unrelated to vitamin D in the blood. Furthermore, regression analyses of fatigue recovery against serum vitamin D concentrations indicate no significant trends, suggesting that vitamin D may not influence percentage of muscle work recovery following fatigue.

Age difference between baseline mice and treatment group mice might also be a factor affecting the ability of muscle to recover from fatigue (see section 4.2). Baseline mice were younger than treatment mice, and studies have indicated that muscles of younger individuals tend to produce greater force and recover from fatigue significantly faster than muscles of older individuals (Klein et al.,1988; Gonazalez & Delbono, 2001). As seen in my study, younger baseline mice recovered faster than older treatment groups VD+/UV- and VD+/UV+ in the EDL fatigue recovery tests and treatment group VD+/UV+ in the SOL fatigue recovery tests. Thus, vitamin D does not appear to influence muscle work recovery during a twitch test for either muscle type, but age may be a factor in the observed results.

A possible explanation for the results from fatigue tests could be similar to that which was as described in earlier and explained in more detail in section 4.7, in that UVA rays could be responsible for the work recovery results seen. As stated in this section, UVA rays are associated with the production of nitric oxide which influences several biological targets related to neurotransmission, cell apoptosis, vasodilation, etc. (Paunel et al., 2005; Opländer et al., 2009; Suschek et al., 2010; Juzeniene & Moan, 2012). As a result, older treatment group mice show

similar percentages of force recovery to younger baseline mice due to potential influences of UVA on neurotransmission, causing muscles to relax faster.

4.8 Differences between fast and slow muscle types in responses to dietary vitamin D and UV light treatments

The results of this study did not show any consistent differences between fast-twitch and slow-twitch muscles in response to the treatments, including differences in force production in response to altered vitamin D that have been suggested in previous studies (Pleasure et al., 1979; Stratos et al., 2013; Wyon et al., 2014). This might suggest that there are no differences between fast and slow muscle types in their responses to vitamin D. Also it may suggest that vitamin D plays a limited role in affecting muscle contractile capacity, or that within the sufficient range does not have a notable impact on muscle. However, it is important to recognize that the purpose of this study was not necessarily to alter the vitamin D concentrations in the serum, but to determine whether relatively low dietary vitamin D and high versus low UVB light exposure, as would be seen in a Polar environment, has sufficient impact on vitamin D to affect muscle. Blood analyses indicated that these treatments did not result in significant differences in average serum vitamin D levels between baseline and any of the treatment groups, thus, it should not be concluded from the present study that vitamin D does not impact muscle. Alternatively, there was substantial variability in vitamin D levels between mice even within treatment groups, and the regression analyses of the effects of vitamin D concentration on the various measures of muscle performance all suggest there was no effect. Thus, it could be concluded that within a relatively wide, but normal, range of serum vitamin D there is little impact on muscle, including

that there is no observable difference in sensitivity to treatments between fast-twitch and slow-twitch muscles.

4.9 Limitations of the study

A limitation of this study is that the mice strain used may not have been an ideal representation of wild animals that experience Polar conditions of low UVB light and reduced vitamin D exposure. C57BL/6 mice were chosen because they are well-established animal models and their mechanisms and rates of vitamin D synthesis have been shown to be comparable to humans and most other animals. This was the first investigation of the effects of altered dietary vitamin D and UVB exposure on muscle contractile capacity, and so laboratory mice are a suitable first step on which to base future studies. The primary issue with using in-bred strains of laboratory mice that have been raised in laboratory conditions under fluorescent lighting (i.e. no UVB) for many generations, may be a loss or reduced ability to synthesize vitamin D through their skin. Although studies have shown that UVB light exposure can increase vitamin D in the blood of mice (Armas et al., 2007; Rebel et al., 2015), the levels measured in the blood may not be representative of wild animals. Furthermore, the levels of vitamin D in commercial mouse chow are reaching concentrations that would be considered more than adequate. According to Seldeen et al. (2017) and Mallya et al. (2016), the standard mouse feed fed to laboratory mice in most animal facilities is considered to be around 1.0 IU/g and the recommended daily intake of vitamin D is 1.0 IU/g. These are levels which would allow laboratory mice to maintain serum vitamin D just below or in the lower range of what is considered the adequate range (80-100 nmol/L). A supplemented diet is considered to be above

2.5 IU/g and can reach 5.0 IU/g vitamin D in many studies, which will maintain serum vitamin D levels within and well above the adequate range (Angeline et al., 2013; Mallya et al., 2016; Seldeen et al., 2017). In my study, a normal diet was considered to be 3.4 IU/g of vitamin D and a low vitamin D diet was considered to have 0.1 IU/g of vitamin D added. Also, tests done on rodent diets from one manufacturer (Lab diets, St. Louis, MO) have shown large variability in vitamin D contents between batches (Mallya et al., 2016). Thus, this variation may influence the endpoint serum vitamin D results examined in my study, and hence the typical levels of vitamin D in the diets of lab mice may not be a good representation of what a daily intake of vitamin D in wild rodents may experience.

Another limitation of this study is the large amount of variance seen in the data which would have concealed any actual significant data that may have been present. Due to the large variance, especially in endpoint blood results, it is difficult to determine whether or not serum vitamin D had an impact on muscle contractile abilities. It is possible that there is naturally more variation in serum vitamin D as mice approach adulthood, however it may also have been the assays performed. The vitamin D assays performed at the midpoint of the treatment period were done using Cayman ELISA tests which is an indirect competitive spectrophotometrical immunoassay, whereas those performed at the endpoint were done using Liaison 25 OH Vitamin D assays which is a direct competitive chemiluminescence immunoassay. Future tests will be done using the same assay techniques to maintain consistency of results. Furthermore, the sample size of 10 mice per group is relatively small with large variation within each group which would contribute to any false positive or negative results. I suggest future work use a larger sample size of approximately 30 animals per group. In addition, many of the significant results seen in my

study were inconsistent, making it difficult to conclude findings. This inconsistency may be due to several reasons, some of which have been mentioned above.

Lastly, hair is known to be a large factor in influencing how much UVB light reaches the skin and thus vitamin D synthesis in the skin. Although the dorsa of the mice were initially shaved prior to treatments, hair growth was not consistent between mice within and between groups. An attempt was made to shave dorsa of mice often to keep hair length as short as possible. This resulted in melanin within the hair binding in the skin follicles after each cut, which caused patchy darkening of the skin pigmentation. Melanocytes in actively growing hair produce the greatest amount of melanin, and as a result, skin pigmentation spread to produce larger and darker patches after each subsequent shaving (Kwon & Sevick-muraca, 2017). Skin pigmentation is also known to change rates of vitamin D synthesis by limiting UVB exposure (Wacker & Holick, 2013). Although all mice began with pink skin, after about a month of dorsa shaving, patches of black pigmentation began occurring in all mice, independent of which treatment group they were in. Thus, hair growth and skin pigmentation were both factors that could have influenced vitamin D levels within the blood between treatment groups.

Future studies may consider measuring 1,25OHD, the hormonal active form of vitamin D to determine if this form is being influenced significantly by the treatment groups and if this would subsequently affect muscle performance. Furthermore, I may consider measuring other vitamin D biomarkers such as vitamin D binding protein or vitamin D receptors on specific tissue cells to determine at what extend does vitamin D influence muscle tissue cells.

4.10 Significance of results

There are studies comparing the effects of vitamin D from the diet or from UVB light exposure on fractures, muscle cellular properties and muscle strength on a whole-animal and molecular level (Pfeifer et al., 2006; Stockton et al., 2011). However, to the best of my knowledge, there is currently no research on the effects of vitamin D resources on the contractile capacity of isolated mammalian muscles, particularly whether the extent of UVB deprivation that animals experience in Polar environments might impact circulating vitamin D and if this might impact muscle. The intent of this thesis was to simulate photoperiods experienced by animals living above the Arctic Circle. The levels of vitamin D in the diet were intended to produce adequate levels of serum vitamin D and insufficient levels without reaching deficient levels and corresponding detrimental effects of being deficient in this major vitamin. The objective was to assess the effects of these vitamin D manipulations through changes in UVB availability and dietary resources on muscle contractile capabilities of fast-twitch and slow-twitch mammalian muscles. This research is beneficial in assessing the effects of reduced UVB exposure, simulating Polar photoperiods of animals living above the Arctic circle, on muscle contractile capabilities, as they receive relatively less sunlight (UVB light exposure) in winter seasons compared to animals living below the Arctic circle. Furthermore, Taylor et al. (2015) has found an increase cloud cover in the Arctic from warming weather, which reduces all sun rays (UVB included) from reaching Earth's surface. As a result, animals living above the Arctic circle will rely much more on dietary vitamin D, which may eventually become an unstable resource due to changing climates influencing ecosystems.

In all, my study has revealed that reduction in UVB exposure and dietary vitamin D, as might be seen in Polar climates or hibernators, will not detrimentally impact the muscle contractile capabilities of mammalian muscles significantly if experienced in periods of 3-4 months. Thus, with evidence showing that muscle contractile capabilities did not change from baseline following treatment, altering levels of vitamin D obtained from the diet and through UVB exposure over a 3 month period does not appear to influence the force, work or power producing capabilities of fast-twitch or slow-twitch muscles. Previous studies (Ceglia et al., 2013; Hutton et al., 2014) indicate that vitamin D influences protein synthesis of muscles through changes in gene expression; however, under the conditions of my study there were no significant differences in muscle mass, cross-sectional area, and very few differences in contractile capacity, when compared to baseline mice. These results do not necessarily refute the notion that vitamin D is an important regulator of protein expression in skeletal muscle, as the treatments did not produce significantly different serum vitamin D levels between treatment groups and baseline mice. But they do suggest that relatively brief periods of deprivation as might be experienced in Polar environments are not detrimental to muscle contractile capacity, and do not appear to affect circulating levels of vitamin D.

CONCLUSION

The results of this study provide little evidence that a 3 month period of reduced levels of vitamin D in the diet and/or manipulation of epidermal vitamin D synthesis through the removal of UVB exposure influenced serum vitamin D levels in mice. Furthermore, there did not seem to be a large impact of the treatment group conditions and circulating levels of vitamin D on muscle contractile capabilities of fast and slow-twitch muscles. The statistically significant differences between treatment groups in muscle contractile capacity that were observed in this study were relatively few and small, and there was a lack of any correlation of these differences to serum vitamin D levels, suggesting that the effects were not related to vitamin D concentrations, regardless of which treatment group the mice were exposed to. Age may have had a large impact on serum vitamin D regulation and muscle contractile capabilities, with results indicating some differences between treatment groups in serum vitamin D levels at the midpoint of the treatment when mice were about 12 weeks of age, but a homeostatic mechanism to regulate circulating vitamin D concentrations within the adequate range in all groups by the end of the treatment period when mice were 18 weeks of age, despite having reduced vitamin D supply in some groups. Thus, other factors related to UVA or UVB exposure may have impacted muscle contractile capacities through pathways unrelated to vitamin D. In addition, muscle fibre type (fast vs slow twitch) did not show significant differences in sensitivity to treatments. Therefore, my study suggests that Polar animals or animals in hibernation receiving intermittent periods of reduced dietary vitamin D or the absence of epidermal vitamin D synthesis from lack of UVB exposure may not be at risk of having impaired muscle contractile performance during winter months (~3 months) or from reduced sunlight exposure due to climate change.

Future directions may involve using wild species of mice, or other animals, inhabiting regions above the Arctic circle. Alternatively, one could sample tissues and serum from animals living in Polar environments at different times of the year, during the summer when UVB light is prevalent and during the winter when it is absent, to assess if there are effects on vitamin D and muscle. Also, it would be useful to account for age differences within the study design. In addition to muscle contractile tests, measures of calcium regulation within muscles could provide information on vitamin D's effects on calcium cycling. Further information about the effects of vitamin D on muscle contractile capacity would also be very beneficial in assessing how and if the Polar environment might be impacting muscle.

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