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ACCEPTANCE

This dissertation, THE IMPACT OF VITAMIN D, ENERGY BALANCE AND BODY COMPOSITON ON PERCEIVED MUSCLE SORENESS IN ACTIVE POSTMENOPAUSAL WOMEN by DORIS JOY MORRIS, was prepared under the direction of the candidate's Dissertation Advisory Committee. It is accepted by the committee members in partial fulfillment of the requirements for the degree, Doctor of Philosophy, in the College of Education & Human Development, Georgia State University.

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THE IMPACT OF VITAMIN D, ENERGY BALANCE AND BODY COMPOSITON ON
PERCEIVED MUSCLE SORENESS IN ACTIVE POSTMENOPAUSAL WOMEN

by

DORIS JOY MORRIS

Under the Direction of L. Jerome Brandon, Ph.D.

ABSTRACT

This study addresses the impact of vitamin D, energy balance and body composition on muscle soreness in active postmenopausal women. Purpose: This descriptive study was designed to assess vitamin D (diet and sun exposure) and energy balance in order to determine their effect on muscle soreness 48 hours after eccentric exercise. Methods: 29 healthy postmenopausal women (mean age: 59.1 ± 4.5 years) were recruited for this study. A 4-day diet/activity record was obtained and analyzed to estimate dietary vitamin D and energy balance (EB) in hourly increments. Total hours spent in EB < zero kcal and < - 400 kcal were calculated from the 4-day dietary log. A sun exposure questionnaire assessed the amount of vitamin D acquired as a result of sun exposure and skin exposed. Body composition was assessed with a multi-current Bioelectrical Impedance Analysis. Muscular strength was assessed with a 10 RM of the elbow flexors. Eccentric exercise was used to induce delayed onset muscle soreness of the elbow flexors. Participants recorded their level of perceived soreness pre and post exercise intervention on a visual analog scale. Spearman correlations evaluated associations between vitamin D and EB and soreness. Results: Soreness was not statistically significant with total vitamin D ($p=1.0$), EB < zero kcal ($p=0.11$) and EB < -400 kcal ($p=0.50$). Soreness was statistically significant with weight ($p=0.0$), waist ($p=0.04$), hip ($p=0.01$), Body Mass Index ($p=0.02$) and VO_{2max} ($p=0.05$). Conclusions: These data suggest that adequate levels of vitamin D offer protection against muscle soreness 48 hours after. However, increase weight, waist, hip and BMI were related to increased soreness.

INDEX WORDS: Vitamin D, energy balance, body composition, muscle soreness, post menopause

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A Dissertation

Presented in Partial Fulfillment of Requirements for the

Degree of

Doctor of Philosophy

in

Kinesiology

in

Department of Kinesiology and Health

in

the College of Education & Human Development

Georgia State University

Atlanta, GA
2021

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DEDICATION

I dedicate this dissertation to my beautiful and loving family. To my wonderful husband, Jerryl, you've been by my side from day one; always encouraging and supportive. You are literally the wind beneath my wings! I love you to the moon and back!! To my children, Tim and Melanie, I can't imagine life without you on my side, cheering me on. Thank you for your love and support. To my parents, Isabel and John, I love you both so much! My mom is not here for this, but she would be so proud. A big thank you to my dear friend, Ann. You were there with me from the beginning. Your support never wavered, gave me great advice, a shoulder to cry on and always had my back. And finally, Ace, who never, and I mean never, left my side.

ACKNOWLEDGMENTS

I would like to thank my advisor and chair of my committee, Dr. L. Jerome Brandon. This dissertation would not be possible without your advice, guidance, feedback and the numerous hours this past year on WebEx. It's been a long process and I appreciate your patience through it all.

I'm extremely grateful to my committee members, Dr. Dan Benardot, Dr. Tiffany Esmat, Dr. Walter Thompson and Dr. Yuri Feito. You have all provided me with countless hours of advice, critique, and most importantly support. I will forever be grateful!

I need to thank my colleagues at Kennesaw State University, Garrett, Trisha and Jerry who never turned me away when I popped into their offices asking for help. I truly appreciate your willingness to help and support me.

A huge thank you goes to my research assistant, Esther Steingold, an undergraduate student at Kennesaw State University. Her assistance in collecting data was invaluable. She was a pleasure to work with and will make a great researcher one day.

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ABBREVIATIONS

7-DHC	7-dehydrocholesterol
1,25(OH)D	1,25-dihydroxyvitamin D
25(OH)D	25-hydroxyvitamin D
ANOVA	Analysis of variance
BF%	Body fat percentage
BMI	Body mass index
BP	Blood pressure
BPM	Beat per minute
DBP	Diastolic blood pressure
DOMS	Delayed onset muscle soreness
EB	Energy balance
HR	Heart rate
HR _{max}	Maximum heart rate
IU	International units
MAP	Mean arterial pressure
PAR-Q+	Physical activity readiness questionnaire
RDA	Recommended dietary allowance
RED-S	Relative energy deficiency in sport
RHR	Resting heart rate
RM	Repetition maximum
RPE	Rate of perceived exertion
SBP	Systolic blood pressure

SDD	Standard vitamin D dose
SEQ	Sun exposure questionnaire
SPF	Sun protection factor
UVB	Ultra-violet B rays
VAS	Visual analog scale
VDR	Vitamin D receptor
VO _{2max}	Maximal oxygen consumption
WC	Waist circumference
WHR	Waist to hip ratio

CHAPTER 1

Introduction

Menopause is the time in a woman's life when the ovaries cease to produce estrogen, marking the end of reproductive years (American College of Obstetricians and Gynecologists, 2018). The average age of menopause in the U.S. is 51 years of age. As reported by the Center of Disease Control and Prevention, life expectancy for females in 2019 was 81.4 years of age (Center for Disease Control and Prevention, 2020). As a result, women can potentially spend one-third of their life with low levels of the female hormones estrogen and progesterone (Cabrera & March, 2018; Hansen, 2018).

Low levels of estrogen have a significant negative impact on the maintenance of muscle mass, and is associated with the loss of skeletal muscle tissue (Sirola & Kroger, 2011). These changes may result in loss of muscular strength, aerobic fitness, increased weight and body fat along with a decrease in bone mineral density. According to Maltais et al., (2009) it is suggested that physiological changes progress more rapidly in postmenopausal women due to low levels of estrogen. Muscle loss decreases approximately 3% - 8% per decade after the age of 30 and the rate of decline is higher after the age of 60, making postmenopausal women especially vulnerable (Bettis et al., 2018; Volpi, 2004).

The decline of estrogen is also associated with an increase in adiposity in the abdominal area and can cause postmenopausal women to experience an increased risk of musculoskeletal and cardiovascular injury that is coupled with delayed recovery from muscle soreness (Diniz et al., 2017; Lesser, 2015; Tiidus, 2005). Postmenopausal women tend to have a higher body fat percentage and an increase in visceral and abdominal fat compared with pre-menopausal women

and are more susceptible to muscle damage and slower recovery from exercise-induced muscle damage than pre-menopausal women (Nelson, 2013; Schiebel, 2018).

A small amount of bone loss occurs after the age of 35 years in women, but during the first 4 – 8 years after menopause, women lose bone more rapidly (American College of Obstetricians and Gynecologists, 2018). If too much bone is lost, it will increase the risk of osteoporosis, which will increase the risk of bone fractures.

Poor vitamin D status can result in reduced bone density, hypertension, abdominal obesity and negatively affect muscle mass and strength (Schub, 2018). Severe vitamin D deficiency can result in osteomalacia in adults, autoimmune and bone diseases, muscle weakness, pain, and impaired gait (Schmitt et al., 2018; Schub, 2018; Shea et al., 2011). Vitamin D deficiency is of concern as it is very prevalent among postmenopausal women (Civelek et al., 2014; Touvier et al., 2015).

Aging is typically associated with a decrease in physical activity, thus maintaining an active lifestyle is important for postmenopausal women. Postmenopausal women who are chronic exercisers should maintain proper energy balance, which is defined as the state achieved when the energy intake equals energy expenditure. Body weight changes are associated with an imbalance between energy content of food and energy expended to maintain life and perform work (Hall et al., 2012; Hill et al., 2013). Adequate energy balance will provide macro and micronutrient delivery and optimize physical performance (Behrens et al., 2020). Relative energy deficiency increases the risk of musculoskeletal injuries and decrease endurance performance (Mountjoy et al., 2014; Mountjoy et al., 2018). Low energy availability is related to endocrine alterations, which lead to health and performance impairing conditions, such as poor bone health and an increase risk of injury (Fahrenholtz et al., 2018). Previous research has shown physical

activity during menopause and post menopause, can minimize and/or prevent many functional limitations, including preserving muscular strength and aerobic capacity; therefore, it is vital that an energy balance deficit in active postmenopausal women be avoided (Keysor, 2003; Russell, 2018; Schiebel, 2018).

Physical activity is defined as any bodily movement produced by contraction of skeletal muscle that results in caloric expenditure above resting energy expenditure. Exercise, though a type of physical activity is structured, planned and consists of repetitive bodily movements (American College of Sports Medicine, 2021a). Exercise improves strength, flexibility, physical function, fall prevalence, bone health, and helps to prevent common conditions that typically occur during postmenopausal years, including reduced metabolism, increased caloric intake, and increased cardiovascular disease risk (Asikainen et al., 2004; Earnest et al., 2010; Mishra et al., 2011; Pinzon Rios, 2019). It is also know to improve psychological and social factors, such as self-esteem, muscular tension, insomnia, and reduce stress and anxiety (Bonganha et al., 2012).

Exercise is an important strategy for the prevention of muscle loss in postmenopausal women. Though muscle loss may result from other conditions, one of its principal causes is low levels of estrogen (Mendoza et al., 2016). Despite a potential prevalence of muscle soreness in postmenopausal women, progressive resistance training programs are known to increase muscle mass and function in postmenopausal women (Mendoza et al., 2016; Pinzon Rios, 2019; Vasconcelos et al., 2016). Exercise will improve muscular strength, overall fitness, relieves fatigue and improves overall physical ability. Exercise, therefore, is also considered medicine (Pedersen & Saltin, 2015).

Estrogen

As women enter menopause, estrogen concentration begins to decline, increasing the incidence of muscle injury as well as a delay in recovery from muscular injuries (Chidi-Ogbolu & Baar, 2018; Enns & Tiidus, 2010). In several studies, estrogen has been shown to protect skeletal muscle from inflammation through attenuation of circulating cytokines (Williams et al., 2015). Estrogen has been shown to inhibit inflammation related leucocyte infiltration into skeletal muscle after unaccustomed exercise (Tiidus, 2005). The mechanism by which estrogen reduces muscle damage and cytokine release is believed to be due to estrogen exhibiting antioxidant properties, acting as a membrane stabilizer, and its ability to bind to estrogen receptors and regulate downstream genes including decreased inflammation (Enns & Tiidus, 2010; Williams et al., 2015).

Estrogen increases during puberty and is about four times higher in women than men during adulthood until menopause when estrogen levels are reduced to negligible levels in most women (Hansen, 2018). The decline in estrogen levels in postmenopausal women affects habitual physical activity, body composition, and bone loss causing a wide range of effects on musculoskeletal quantity and quality, which impacts health and quality of life (Abdel-Razeq & Awidi, 2011; Nedergaard et al., 2013). Due to this known decrease in estrogen both during and following menopause, it is important for postmenopausal women to practice a healthy lifestyle which should include engaging in physical activity.

Estrogen is believed to have a high antioxidant capacity which may have the ability to scavenge free radicals and stimulate the expression of certain antioxidant enzymes, thus limiting oxidative damage. It has been shown to exert significant influence of post-damage leukocyte infiltration into skeletal muscle (Enns & Tiidus, 2010).

Vitamin D

Vitamin D is a fat-soluble vitamin that is synthesized in the skin by sun exposure and to a lesser extent can be obtained in diet. Vitamin D has a role in bone development, and regulation of phosphate and calcium homeostasis (DeLuca, 2004). It is an essential micronutrient that is unique due to its potential endogenous production by the body through the stimulation of UVB rays from the sun. Vitamin D is normally produced in the skin through a photolytic process that acts on a cholesterol precursor (7-dehydrocholesterol) to produce pre-vitamin D₃ (1-hydroxycholecalciferol). Approximately 80% - 90% of vitamin D is synthesized from 7-DHC (Aungst & Rainer, 2014). This inactive form then gets isomerized to 1,25-dihydroxycholecalciferol (Institute of Medicine et al., 2011). Consumption of vitamin D in fortified dairy foods and cereal, fatty fish, mushrooms and supplements provide a modest portion of what is needed daily by the body (10%-20% of the daily requirement of vitamin D). Animal products, including fish oil and eggs, as well as certain supplements, provide a dietary source of vitamin D₃. Plant products such as mushrooms, fortified foods and certain supplements provide a source of vitamin D₂. Both vitamin D₂ and vitamin D₃ are absorbed into the bloodstream and metabolized in the liver as calcidiol (the biomarker used to assess vitamin D status). Calcidiol (25(OH)D) is further hydroxylated in the kidneys to produce 1,25-dihydroxyvitamin D (D₃), the biologically active form of vitamin D and the major circulating form (Agostini et al., 2018).

Vitamin D is essential for calcium homeostasis and bone health, a lack of which may result in low calcium levels and have a negative effect on ossification which can result in a higher risk of bone fractures (Civelek et al., 2014; Qu, 2014). Vitamin D deficiency is also associated with a decrease in muscular strength and a reduction in physical performance as well as inversely associated with muscle pain and risk of falling (Anagnostis et al., 2015; Annweiler,

2009; Beudart et al., 2014; Ceglia & Harris, 2013; Jablonski & Chaplin, 2018; Matyjaszek-Matuszek, 2015; Pike, 2014). Studies show that vitamin D supplementation in older adults improves muscular performance and reduces the risk of falls (Bjelakovic et al., 2014). The vitamin D status of postmenopausal women is therefore of concern, especially for those involved in moderate to vigorous exercise.

Vitamin D has a major role in the regulation and uptake of calcium in muscle cells, promoting protein synthesis and calcium and phosphate transport in muscle, which is important for the strength and contractile properties of muscle (Agostini et al., 2018). When vitamin D is required due to low levels of calcium or phosphate the parathyroid hormone is secreted by the parathyroid glands to stimulate the synthesis of the enzyme 1-alpha-hydroxylase, which results in the increase of 1,25(OH)₂ (Bhattarai et al., 2020; Gil et al., 2018). Athletic women are more susceptible to skeletal injuries than males and low levels of 25(OH)D have been associated with an increase of stress fractures in adult women (Brannstrom et al., 2017). Osteoclasts and osteoblasts are the two major bone cells involved in the bone renewal process. Osteoclasts lead to bone reabsorption, while osteoblasts lead to the formation of new bone tissue (Mori et al., 2020). Estrogen decreases the number and activity of osteoclasts and after menopause there is an accelerated rate of bone loss likely a result of low estrogen levels (Bhattarai et al., 2020; Kim et al., 2020). Bhattarai says that resorption increases by 90% after menopause, while bone formation increases only by 45%.

The mechanism by which vitamin D affects muscle strength and function has not been fully elucidated but it is likely mediated by the VDR and 1-alpha hydroxylase, which are both expressed in muscle tissue (Robinson et al., 2018). As women age, vitamin D receptors (VDR) in the nuclei of muscle cells decrease (Bhattarai et al., 2020; Bischoff-Ferrari et al., 2004). The

underlying mechanism may be due to the decreased levels of vitamin D seen in older adults, which may lead to a decrease expression of VDR (Bhattarai et al., 2020; Bischoff-Ferrari et al., 2004; Girgis et al., 2015).

The enzyme 1-alpha hydroxylase is expressed primarily in the proximal tubules of the kidneys and converts 25(OH)D to the active form 1, 25(OH)D (Robinson, 2018). Vitamin D receptors belong to a subfamily of nuclear receptors that act as transcription factors into the target cells (Gil et al., 2018). The presence of VDR in muscle tissue suggests that vitamin D acts on muscle via a genomic transcriptional effect (Agostini et al., 2018). Bischoff-Ferrari, suggests that when 1,25(OH)D binds to the nuclear VDR in muscle it results in de novo protein synthesis (Bischoff-Ferrari, 2013). A study by (Pojednic et al., 2015) found that human primary myoblasts treated with 1,25(OH)D for 18 hours significantly increased the expression of VDR and its target CYP24A1 gene in a dose-dependent manner and 16 weeks of vitamin D supplementation resulted in a persistent increase in VDR gene expression in older adult muscle tissue.

Vitamin D deficiency is a global health problem, affecting an estimated one billion people world-wide (Cashman, 2019; Sahota, 2014). It is a frequent clinical condition among postmenopausal women due to insufficient time spent outdoors, increasing age and a decrease in the capacity of the skin to synthesize vitamin D (Civelek et al., 2014). By the time a woman reaches the of age 70, vitamin D synthesis has reduced 50% (Grant, 2018; Sakamoto, 2019).

Vitamin D deficiency is a common condition with potential medical ramifications in postmenopausal women and has been associated with reduced sun exposure, low vitamin D dietary intake, and increasing age (Hilger et al., 2014). It is also related to an increased risk of muscle atrophy and impaired cross-bridge formation leading to muscle weakness and fatigue (Zebrowska et al., 2020). In a study by (Nowak et al., 2016), fatigue was linked to low levels of

circulating 25(OH)D, however vitamin D treatment significantly improved fatigue in healthy individuals with vitamin D deficiency. The emerging body of evidence surrounding vitamin D and exercise bolster the support for understanding the effect on active postmenopausal who are at risk of deficiency.

Muscle and Vitamin D

Muscle loss as a result of aging is known as sarcopenia. Sarcopenia is defined as a loss of skeletal muscle mass and strength and is believed to mostly affect older adults (Mohseni, 2017). Studies show the prevalence of sarcopenia in postmenopausal women is estimated to be 10% to 40% (Cabrera & March, 2018; Mendoza et al., 2016). In addition to increased age, the risks for sarcopenia include being female, poor nutrition, reduced levels of estrogen and lack physical activity, thus having a detrimental effect on skeletal muscle mass (Anagnostis et al., 2015). It involves loss of muscle functionality, which leads to mobility restriction, functional impairment, and reduced quality of life (Agostini et al., 2018). Muscle-bone physiological interaction is essential to prevent disease and disability in older adults. Sjoblom, et al., reports that women who suffer from sarcopenia have more than double the risk of fracture and falls compared to those without the disease (Sjoblom, 2013). Postmenopausal women experience an accelerated decline in muscle mass, strength and function as a result of menopause (Hansen, 2018).

There are multiple factors that can influence muscle loss, which include poor nutrition, reduced levels of estrogen, and lack of physical activity. When further examining poor nutrition, vitamin D deficiency has been shown to more than double the risk of sarcopenia (Mitchell et al., 2012).

It has been hypothesized that greater exposure to UVB rays and increased levels of serum 25(OH)D above the normal range could be associated with beneficial adaptations in skeletal

muscle leading to enhanced aerobic performance, force and power production and decreased recovery time (Dahlquist, 2015). Variations in sun exposure and dietary intake have caused vitamin D insufficiency to be quite common in the general population (Holick, 2004; Matyjaszek-Matuszek, 2015; Ogan & Pritchett, 2013). The Institute of Medicine (2011) recommends a minimum of 600 IU per day, or 15 mcg of Vitamin D2 or D3 for the general population of adult females. However, the dietary recommendations of vitamin D are complicated by the vitamin D synthesis from sun exposure. Holick's rule says that exposing $\frac{1}{4}$ of the body to $\frac{1}{4}$ of the minimal erythemal dose (the minimal dose leading to pink coloration of the skin 24 hours after exposure) of sun will produce the vitamin D equivalent of 1000 IU (Dowdy et al., 2010; Grant, 2018). However, vitamin D requirements cannot be based on a recommended level of sun exposure because of existing public health concerns regarding sun exposure and skin cancer and geographical differences in exposure potential (Glass et al., 2009). Instead, the current approach is to describe the relationship between dietary vitamin D intake and serum 25OHD levels under conditions of minimal sun exposure (Institute of Medicine et al., 2011). Holick, (2004), specifies that 5 – 30 minutes of sun exposure between 10am and 3pm at least twice a week to the face, arms, legs, or back without sunscreen provides sufficient UVB exposure to synthesize sufficient vitamin D to satisfy physiological needs, with optimal levels of vitamin D synthesis occurring during noontime exposure to the sun (Holick, 2004; Webb & Engelsens, 2006).

It is common for dermatologists to recommend the use of sun protection factor (SPF \geq 15) to prevent sunburn, which can affect an individual's ability to receive adequate vitamin D from sun exposure. The application of sunscreen is actually effective in blocking the formation of pre-vitamin D₃ (Grant, 2018; Sayre & Dowdy, 2007). Research also suggests that melanin

pigmentation significantly affects the amount of vitamin D synthesis, which results in vitamin D insufficiency and deficiency in individuals with darker skin pigmentation (Ajabshir et al., 2014). High amounts of melanin results in lower production of vitamin D in individuals for the same UVB exposure as those with lower skin melanin. Melanin acts as a barrier in which UVB photons cannot reach the skin's 7-dehydrocholesterol (Sakamoto, 2019). In 1975, the Fitzpatrick skin scale was developed to estimate the skin's response to UVB rays based on the individual's sunburn and tanning experience (Fitzpatrick, 1988). The scale is widely used to determine sun-reactive skin types and to classify skin types (Fasugba et al., 2014).

The association between vitamin D, muscle strength, and performance are not unique to postmenopausal women. Impaired muscle function has been linked to vitamin D deficiency in athletes, young women and elderly men (Geiker et al., 2017; Ogan & Pritchett, 2013). In addition, low levels of vitamin D were associated with poor physical performance, muscle weakness, poor balance and falls in individuals aged 65 years and older (Houston et al., 2007; Vaes, 2019). Research on the effects of vitamin D on muscle in sedentary postmenopausal women is plenty, however data is limited in physically active postmenopausal women.

Energy Balance

Metabolism involves all the chemical reactions that sustain the life of cells, and thus the organism. The process is comprised of catabolism, which refers to the breakdown of nutrients for energy and anabolism, which refers to the synthesis of compounds needed by the cells. Carbohydrates, protein and fats are essential to the building and repair of body tissue. A major role of carbohydrates is to maintain a constant supply of glucose to the brain. When blood glucose levels get low, mental fatigue sets in followed by muscular fatigue (Benardot, 2012). The energy needs of muscle are determined by how quickly and in what quantity glucose and

glycogen are used during exercise; both of which are essential for vigorous and prolonged strenuous exercise (Karvitz, 2013).

Energy balance is defined as the difference between energy intake and energy expenditure (Geesmann, 2017). The amount of time spent in a catabolic state is an area of concern in exercising postmenopausal women. The Academy of Nutrition and Dietetics recommends moderately active to active women over the age of 51 consume 1,800 – 2,200 kcal per day (Academey of Nutrition and Dietetics, 2021). According to Benardot (2013), individuals who stay at ± 400 kcal avoid peaks and valleys which can negatively affect the body tissues (Benardot, 2013). Energy balance ± 400 kcal is in line with the predicted amount of liver glycogen storage (Behrens et al., 2020). The balance between energy expenditure and energy intake must be maintained in order to guarantee normal physiological function, making it important for postmenopausal women who exercise to avoid excessive energy deficits. Failure to supply adequate levels of carbohydrate to maintain blood glucose and proper glycogen stores, results in an increase in the production of the stress hormone, cortisol. Cortisol is a glucocorticoid hormone secreted from the adrenal cortex in response to physiological and psychological stress and plays an important role during and after exercise (Cordova et al., 2010; Mor et al., 2019). Cortisol catabolizes muscle and converts glucogenic amino acids into glucose (Benardot, 2021). Time spent in severe energy balance deficit results in an increase of cortisol and low blood glucose, which has been associated with low BMD, higher fat mass, a decrease in performance and increased fatigue (Benardot, 2013; Mor et al., 2019; Nassib, 2016). Increased levels of cortisol can also increase a women's risk for bone and muscle breakdown (Hannibal & Bishop, 2014). Carbohydrate intake causes an increase in levels of the anabolic hormone, which then transports glucose to muscle cells for muscle hypertrophy, as well as replenish glycogen

storage and blocks the catabolic effects of cortisol by increasing absorption of amino acids of the muscles (Mor et al., 2019). It protects muscles by initiating protein and glycogen synthesis in the muscles, blocking the effects of cortisol. Prolonged negative EB can lead to health and performance impairments, including an increased risk of injury and delayed muscle recovery (Behrens, 2020; Benardot, 2021; Fahrenholtz et al., 2018; Mountjoy et al., 2014; Mountjoy et al., 2018).

Body Composition

Obesity and metabolic syndrome are three times more prevalent during post menopause than before menopause and linked to hyperlipidemia increasing the risk of cardiovascular disease (Kozakowski et al., 2017; Kwasniewski, 2012). One of the most important concerns of postmenopausal women is the fear of weight gain, which may lead to caloric restriction. Obesity is defined as a body mass index (BMI) $> 30 \text{ kg/m}^2$ and is an established risk factor for the development of cardiovascular disease (American College of Sports Medicine, 2021b). Menopause is associated with reduced lean muscle and an increase in fat mass and when combined with a fat redistribution to central adipose regions, may predispose women to obesity (Schub & Karakashian, 2017; Wooten et al., 2021).

The relationship between the degree of obesity and estrogen also involves other sex hormones. Low sex-hormone binding globulin increases the bioavailability of androgens (Kozakowski et al., 2017; Schub & Karakashian, 2017). Though estrogen declines substantially with the onset of menopause, the ovaries continue to secrete androgens, which promote the accumulation of abdominal fat (Kozakowski et al., 2017; Markopoulos et al., 2015). Thus, the development of obesity with metabolically unfavorable fat redistribution is linked to androgens when estrogen is lacking (Kozakowski et al., 2017).

Wooten et al., (2021), investigated lipid and lipoprotein-cholesterol profiles and total and regional body composition between premenopausal and postmenopausal and concluded that postmenopausal women had a greater prevalence of obesity and a lower prevalence of normal weight than premenopausal women. Overall, being postmenopausal was associated with a greater chance of developing hyperlipidemia compared with premenopausal, increasing the risk of cardiovascular disease (Wooten et al., 2021). The postmenopausal women were borderline hyperlipidemic with higher serum concentrations of total cholesterol, low-density lipoproteins, and non-HDL-C than premenopausal, which is a common features of hyperlipidemia in postmenopausal women (Cui et al., 2016).

Estrogens also have an effect on body composition. 17β -estradiol (E2), the main circulating estrogen, is produced by the ovaries. Estrogen receptors alpha and beta belong to a family of nuclear hormone receptors which comprises 48 different transcription factors and transcriptional regulators (Paterni et al., 2014). Estrogens acting on estrogen receptors alpha and beta are recognized as important regulators of metabolic homeostasis and lipid metabolism. Estrogen deficiency causes metabolic dysfunction, obesity, metabolic syndrome and type 2 diabetes (Paterni et al., 2014). Recent studies highlight the beneficial effect of estradiol on atherosclerosis and metabolic disorders (Gourdy et al., 2018). Experimental data demonstrates the major protective actions of estrogen on arteries, namely that estradiol administration strongly prevents lipid deposition in mouse models of atherosclerosis (Gourdy et al., 2018).

Evidence suggests that estradiol is an important regulator of body composition and bioenergetics (Napoli, 2012). The wide distribution of estrogen receptors and their genomic and nongenomic signaling pathways suggest that the loss of estradiol at menopause has an effect on the body beyond that of reproduction (Cui et al., 2018; Kirshner et al., 2020). Signaling in the

regulation of adiposity is highlighted in a study by Heine, et al., which showed that a whole-body knockout of estrogen receptor alpha resulted in increased fat in both female and male mice when compared with wild-type mice. The parametrial and inguinal fat pads were reported to be 2-fold larger in female estrogen receptor alpha knockout mice (Heine et al., 2000). In another study by Sherk et al., (2019), exercise was shown to not protect ovariectomized mice from weight gain. These suggest that estrogen receptor alpha plays a protective role against fat accumulation.

Metabolic problems, such as weight gain, insulin resistance and glucose and lipid metabolism disturbances emerge during menopause, consequently increasing the risk of type 2 diabetes, osteoporosis, certain cancers and cardiovascular disease (Kozakowski et al., 2017). Barros et al., (2018), investigated the influence of components of body mass on the performance variation of functional physical fitness tests in postmenopausal women. They concluded that higher levels of total adiposity proved to be associated with lower strength and those with higher central adiposity were associated with lower upper body flexibility. Over 81% of the women in this study had intra-abdominal adiposity, the effect of estrogenic depletion, that acts directly on the increase of visceral fat. The older women with higher relative fat mass presented a worse performance in the aerobic fitness test, regardless of the time of menopause.

Menopause is accompanied with weight gain and increased central adiposity causing body fat redistribution and body composition changes that may be due to the hormonal changes and loss of muscle mass (Karvitz, 2013). Exercise reduces the risk of heart disease and diabetes and provides postmenopausal women with an improvement in numerous health outcomes and should be part of every woman's regular regimen.

Muscle Soreness

Exercise-induced muscle damage occurs at the cellular level and is an inherent risk of exercise, such as the loading phase in weight training. It is believed to trigger an inflammatory response generating soreness, tenderness and stiffness (Nelson, 2013). Exercise-induced muscle damage caused by unaccustomed exercise, specifically eccentric exercise, is well documented and is associated with muscle soreness (Delfa de la Morena et al., 2013; Mankovsky-Arnold et al., 2013; Parr et al., 2009). The inflammatory response to exercise-induced muscle damage is associated with the activation of leukocytes, muscle edema, deterioration of muscle function and delayed onset muscle soreness (DOMS) (Jamurtas, 2018). DOMS generally peaks during the first 24 to 72 hours following exercise (Clifford et al., 2020; Corder et al., 2016; Yoon & Kim, 2020).

An important factor in the recovery of muscle soreness is age, as it has been shown that older individuals (≥ 50 years old) have a slower rate of recovery than their younger counterparts (Brisswalter & Nosaka, 2013; Doering et al., 2016). The role of vitamin D in muscle includes, cell maturation, growth, repair and mitochondrial function (Dzik & Kaczor, 2019; Sanha, 2013). Ceglia, et al., investigated the role of vitamin D in muscle physiology, muscle strength and physical performance and found that individuals who received vitamin D supplementation showed beneficial effects on muscle strength and physical performance (Ceglia & Harris, 2013).

Summary

Evidence suggests that muscle training and exercise provide functional benefits for postmenopausal women (Laddu et al., 2017; Mendoza et al., 2016; Mishra et al., 2011). While research exists in the area of vitamin D, energy balance, muscle health and exercise, no studies have explored the impact of these on muscle soreness in postmenopausal women who engage in

moderate or vigorous exercise. In a recent study of 593 women (18 – 50 year old) who played sports at least two times a week it was concluded that the majority of the women did not have sufficient vitamin D (Wrzosek et al., 2019).

With a growing number of postmenopausal women choosing active lifestyles, there is a need to understand how vitamin D, energy balance and body composition impacts muscle soreness. The current research is sparse as it relates to postmenopausal women who exercise. Vitamin D and muscle architecture, the presence of the VDR in muscle, the effect of negative energy balance and age-related changes in muscle, all support the proposition that vitamin D plays in muscle structure and function (Hamilton, 2011). Adequate energy balance and exercise have a major impact on the health of all postmenopausal women but more specifically on those who maintain very active lifestyles.

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CHAPTER 2

Introduction

As women enter menopausal years, they undergo multiple physiological changes that include, but are not limited to, the loss of muscle mass, lower aerobic fitness, and increased proportion of body fat (Greendale et al., 2019; Moreira et al., 2014). The rapid decline in muscle mass is a result of several important factors such as physical inactivity, energy intake, oxidative stress and low estrogen levels (Love, 2019; Maltais et al., 2009). Low levels of estrogen are reported to increase visceral fat, and decrease muscle mass, bone mineral density and strength, which result in a slower recovery from exercise-induced muscle damage (Nelson, 2013; Schiebel, 2018). Maintaining a regular exercise routine, therefore is crucial for the health of bones, muscles and cardiovascular system (Chidi-Ogbolu & Baar, 2018; Rizzoli et al., 2014)

Research has shown that vitamin D is essential for healthy muscle function. Although vitamin D deficiency is common among all age groups, it is especially common among post-menopausal women and may be the result of insufficient sun exposure, poor dietary intake of vitamin D-rich foods or a combination of the two (Bendik et al., 2014; Tayem, 2019). Based on current knowledge of vitamin D and its role in bone and muscle health, immunity, and inflammation, it is possible that low levels of vitamin D increase the risk of over-use injuries in physically active individuals (Angeline et al., 2013; Wyon, 2019). According to a study on vitamin D status and college athletes, the adverse consequences of low levels of vitamin D may negatively affect athletic training and performance (Halliday et al., 2011). Despite many studies conducted in the general population and athletes, less is known about vitamin D and soreness in

physically active postmenopausal women (Angeline et al., 2013; Behrens, 2020; Benardot, 2021; Casey, 2016; Fahrenholtz et al., 2018),

The maintenance of adequate energy balance is also of utmost importance in postmenopausal women who exercise. Sustaining a severe negative energy deficiency can compromise performance and is associated with an increase in fat mass (Behrens et al., 2020; Benardot, 2013). Relative Energy Deficiency in Sport (RED-S) syndrome refers to impairment to physiological function, which includes, but is not limited to, compromised metabolic rate, bone health, immunity, protein synthesis, cardiovascular health and menstrual function (Keay et al., 2018; Mountjoy et al., 2014; Mountjoy et al., 2018). Among the consequences of low energy deficiency are increased injury risk, decreased endurance performance and decreased muscular strength (Mountjoy et al., 2014; Mountjoy et al., 2018). Though RED-S addresses male and female athletes, the issue of sustaining a severe and negative energy balance is also a concern among postmenopausal women who are chronic exercisers (Desbrow et al., 2019).

Postmenopausal women are reported to have a high prevalence of muscular soreness due to exercise (Mendoza et al., 2016). Severe energy deficit as well a vitamin D deficiency have been previously implicated in muscular soreness (Keay et al., 2018; Margolis et al., 2014).

Energy and vitamin D deficiency are both important aspects of an active postmenopausal woman. Adequate levels of vitamin D can be protective from muscular soreness and decrease their risk of injury (Barker, Henriksen, et al., 2013). However, no recent research has investigated the impact of vitamin D and energy balance in active postmenopausal women. While vitamin D is obtained through dietary sources, the majority of vitamin D is provided by endogenous UVB dependent biosynthesis, through the conversion of 7-DHC in the skin (Ajabshir et al., 2014). However, high amounts of melanin in the skin reduces the dose

equivalent cutaneous vitamin D production as it competes with 7-DHC for ultraviolet B photons (Jablonski & Chaplin, 2018; Thompson et al., 2018). In 1975, the Fitzpatrick Skin Scale was developed as a tool to determine the skin's ability to burn and tan when exposed to UVB rays (Gupta & Sharma, 2019). The scale ranges from type I (pale white) to type VI (dark brown to black).

This descriptive study aims to examine the impact of vitamin D (dietary and sun exposure), energy balance, and body composition on the perceived level of muscle soreness in active postmenopausal women 48 hours after completing an eccentric exercise. There is evidence in the literature that vitamin D and energy balance have an impact on skeletal muscle but there is no evidence of how they impact muscular soreness in active. My hypothesis is that vitamin D levels, energy balance and body composition can mitigate muscular soreness 48 hours after unaccustomed exercise in active postmenopausal women. This hypothesis will be tested using two specific aims:

Specific aim 1: Dietary vitamin D and sun exposure will be evaluated to determine its impact on muscle soreness.

Specific aim 2: Within-day energy balance will be assessed to determine its impact on muscle soreness.

Methods and Materials

2.1 Participants

Thirty-four, postmenopausal and physically active participants were recruited for this descriptive experimental investigation. The Georgia State University and Kennesaw State University Institutional Review Board approved the procedures for the study (GSU #H20215, KSU # 20-176PR). A priori power analysis was conducted to determine effect size, power, and required sample size to attain a power of 0.8 at an alpha level of 0.05 with the use of G* Power 3.1.9.7 and it was determined that 26 participants were needed. All participants were made aware of all procedures, including risks and benefits. Eligibility requirements included postmenopausal status for a minimum of 12 months, exercise 30 minutes or more, three or more days per week for a minimum of six months, not on hormone replacement therapy, and no diagnosed presence of cardiovascular, pulmonary or metabolic diseases. They agreed to complete a university-approved, informed consent form, a health history questionnaire and the 2018 Physical Activity Readiness Questionnaire (PAR-Q+) (Appendix A). The health history questionnaire included a Fitzpatrick Skin Scale (FSS) (Appendix B) and a sun exposure questionnaire (SEQ) (Appendix C).

All participants visited the exercise physiology laboratory at Kennesaw State University on two occasions. The visits occurred two weeks apart. Prior to the first visit, participants were instructed to drink water ad libitum 24 hours prior to the visit, not to drink caffeine or eat two hours prior to testing; to wear light-athletic clothing and not to exercise 48 hours prior to their laboratory visit. At the first visit anthropometric measures, 10 repetition max (10RM) and aerobic capacity were assessed. Prior to the aerobic capacity assessment, the 2018 PAR-Q+ was completed to evaluate cardiovascular disease risk with exercise. They were given instructions on

how to complete the four day dietary recall, which was to be completed a week before the second visit. Prior to the second visit, they received an email with a digital visual analog scale (VAS) to complete and send back (Appendix D). The VAS ranged from 0 (no soreness) to 10 (extreme soreness) was meant to assess any level of soreness of the elbow flexors. The second visit was used to induce delayed onset muscle soreness (DOMS) in the elbow flexors with an eccentric exercise protocol. Forty-eight hours after the second visit they repeated the VAS, which assessed any perceived muscular soreness of the elbow flexors.

2.2 Anthropometric Measures

Anthropometric data, including height (cm), mass (kg), waist circumference (cm), and hip circumference (cm) were collected by standard methods (American College of Sports Medicine, 2021b). Participants wore light, athletic clothing without shoes during measurements. Standing height was recorded to the nearest 0.1 cm using a stadiometer (Tanita Corporation of America, Arlington Heights, IL). Body mass was measured to the nearest 0.1kg as part of the body composition analysis using a bioelectrical impedance analysis scale (InBody, model 770, Cerritos, CA). Body mass index was calculated as a function of weight and height (kg/m^2). Waist circumference (WC) and hip circumference (HC) were measured in duplicate at the narrowest part of the torso and at the maximum circumference of the hips to the nearest 0.1 cm, respectively, using a Gulick fiberglass tape measure (Country Technology, Inc. Gays Mill, WI), and following established protocols (American College of Sports Medicine, 2021b). Blood pressure (mmHg) and heart rate (beats per minute; bpm) were measured prior to and during aerobic capacity test.

2.3 Body Composition

Body composition was assessed with the direct segmental multifrequency bioelectrical impedance analysis, InBody, model 770, (Cerritos, CA). The 4-compartment impedance is used to determine fat-free mass, body fat, bone and water. It is equipped with eight tactile electrodes utilizing multiple frequencies (1, 5, 50, 250, 500, and 1000 kHz) and alternating current (100 and 500) to analyze impedance in each arm, each leg, and the trunk. (InBody, 2020). The participants stood upright and barefoot on the device, grasped the handles of the analyzer, and extended their arms out from the sides of their body, per manufacturer instructions. The eight tactile electrodes make contact with the palm and thumb of each hand and the anterior and posterior aspects of the sole of each foot.

2.4 Fitzpatrick Skin Scale

The potential of vitamin D₃ production from UVB radiation exposure to the skin is determined by the amount of melanin in the skin. The higher the melanin in the skin, the lower the rate of vitamin D production, as melanin competes with 7-dehydrocholesterol for UVB rays (Thompson et al., 2018). The lower the melanin content, the higher the rate of vitamin D₃ production in the skin (Jablonski & Chaplin, 2018). Since the amount of melanin in the skin influences vitamin D levels, the FSS was utilized to estimate the skin's response to UVB rays.. The scale provided the participants with a visual skin color scale and descriptions of how the skin responds to sun exposure. The scale ranged from type I (light, pale white) to type II (very dark brown, black). Participants were instructed to select the skin color and description that most closely matched the color of their inner forearm. Study participants classified as skin type I (pale white), II (fair white) and III (white to olive) on the FSS.

2.5 Vitamin D and Energy Balance

During the first visit to the laboratory, specific verbal and written instructions and procedures on the dietary recall were given to all participants. Dietary and energy intake was assessed by a four day diet and activity log. Participants recorded hour by hour energy intake and physical activity above resting levels (Appendix E). The information was used to determine each participant's vitamin D intake and their energy balance (EB) with the NutriTiming® data acquisition software. Individuals who spend time in less than 0 kcal are considered to be a catabolic state, whereas time spent in positive kcal is considered to be an anabolic state (fig.1). Maintaining an EB at ± 400 kcal (1,674 kJ), which is in line with the predicted amount of liver glycogen storage and based on previous studies, will prevent extreme highs and lows in hormonal response (Deutz et al., 2000; Fahrenholtz et al., 2018). Time spent in energy balance deficit exceeding -400 kcal results in the body breaking down lean muscle mass (Benardot, 2013). Prolonged negative EB can lead to health and performance impairments, including an increased risk of injury (Behrens, 2020; Benardot, 2021; Fahrenholtz et al., 2018; Mountjoy et al., 2018). Within-day EB assessments included the average daily dietary vitamin D intake, time spent in EB of < -400 kcal and in $< \text{zero}$ kcal but higher than -400 kcal, and the amount of vitamin D obtained during each. Hours in catabolic state, are considered to be the total number of hours each day the participant spent breaking down macronutrients, resulting in the release of energy. Within-day EB accounts for real-time shifts in EB that lead to endocrine responses involving hormones that impact the body, such as cortisol, insulin leptin and ghrelin. Reduced energy intake results in low blood sugar causing the body to go into a state of gluconeogenesis (Benardot, 2013).

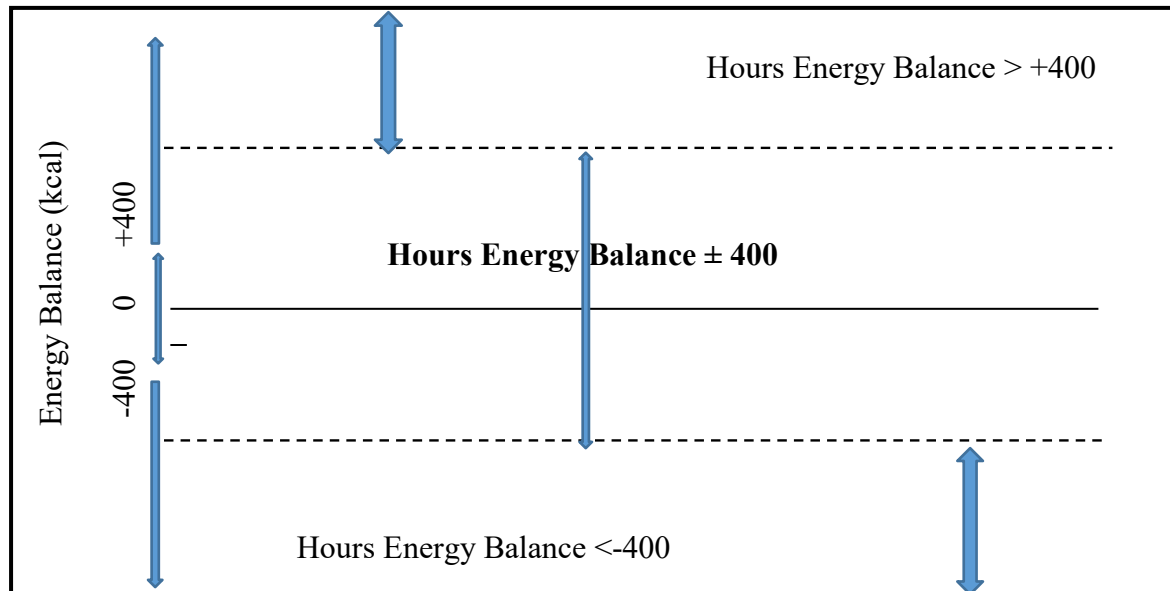


Figure 1: Within-Day energy balance

2.6 Sun Exposure Vitamin D

Participants indicated their average daily sun exposure using a validated sun exposure score (SEQ) that captured information regarding the time spent in sun and amount of skin exposed to the sun (Hanwell et al., 2010; Hildebrand, 2016). The strength of using a SEQ to assess vitamin D status is the cost. It is self-administered and less expensive than serum 25(OH)D testing. Participants were offered three choices for time spent outdoors (0 = ≤ 5 min, 1 = 5-30 min, and 2 = >30 min) and four options for skin exposure while outdoors (1 = face and hands only, 2 = face, hands and arms, 3 = face, hands, arms and legs and 4 = bathing suit). A daily sun exposure score for each day was calculated by multiplying the time spent outdoors score times the skin exposed while outdoors score which. The scale for each day ranged from 0 to 8. The sum of all seven days was used to create the arbitrary Sun Exposure Score (SES) which ranged from 0 (min) to 56 (max). The weekly sun exposure and weekly skin exposure scores were calculated by adding the seven daily scores respectively (min=0, max=14 and min=7, max=28).

2.7 Muscular Strength

Muscular strength was estimated with the use of multiple repetition maximum test procedures (American College of Sports Medicine, 2021b). All participants were familiar with the biceps curl exercise and were able to demonstrate proper technique. A short warmup consisting of five submaximal repetitions of biceps curl was conducted prior to the test. A submaximal muscle endurance test with a 10RM load was used to estimate 1-repetition maximum. (National Strength and Conditioning Association, 2008). After completing the warm-up set, participants were provided with a two minute rest period. The sequential load changes for the 10 RM test were about one-half that of a 1RM test. The testing process continued until the load allowed only 10 repetitions. The 10RM was measured within one to three testing sets with a two minute rest period in between each set.

2.8 Aerobic capacity

The health history questionnaire included questions regarding participants exercise intensity, how often they exercised, how much time they exercised and how long they had maintained this exercise regimen. To measure the participants level of fitness, a submaximal aerobic capacity test was conducted. Eighty-five percent of their age-predicted heart rate max (HR_{max}) was used to estimate their maximal oxygen consumption (VO_{2max}). The incremental Bruce protocol was employed to assess aerobic capacity using a motor driven Woodway treadmill (Model Pro 27, Woodway USA, Waukesha, WI). Expired air was measured breath-by-breath by indirect calorimetry using the portable, metabolic measurement system, COSMED K5 (COSMED USA INC, Chicago, IL). Prior to beginning the test, the gas analyzer and flow meter were calibrated according to manufacturer recommendations. Heart rate (HR) was measured prior to and continuously throughout the test using a Polar Heart Rate monitor (Polar, Lake

Success, NY). An appropriately sized blood pressure cuff was secured on the participants' upper arm to measure blood pressure. In accordance with the American College of Sports Medicine, (2021a), submaximal testing on a treadmill was used to assess cardiorespiratory fitness.

Participants warmed up for two – three minutes to acquaint themselves with the treadmill. The initial velocity and inclination were set at 1.7 mph and 10%, respectively, for three minutes. The velocity and inclination were increased to 2.5 mph and 12% in stage 2, 3.4 mph and 14% in stage 3. No participant was able to reach stage 4 (4.2 mph/16% grade). Blood pressure (BP) was measured at rest, every three minutes during exercise, immediately at exercise termination, and at one and five minutes during the post-exercise recovery period. At the end of each three minutes, during the exercise assessment, they were instructed to provide a rate of perceived exertion (RPE) using the Borg scale (American College of Sports Medicine, 2021a), which consists of values ranging from a low of 6 to a high of 20 (Appendix F). Participants verbally informed the investigator the value that corresponded with their perceived level of exertion. The test was terminated when the participants reached 85% of age predicted HR_{max} , or if they requested to stop. Once complete, participants remained on the treadmill and completed an active cool-down until HR and BP stabilized.

2.9 Pre-Exercise Soreness Assessment

Forty-eight hours before the second visit, participants received an email with a link to a previously validated VAS for soreness (Delgado et al., 2018; Yoon & Kim, 2020). The VAS is a classic instrument used to measure muscle soreness. It consisted of a 10cm line horizontal line that represents a continuum between the two ends of the scale, “no soreness” (0) on the left and “extreme soreness” (10) on the right. They recorded the level of perceived muscle soreness in the elbow flexors prior to the eccentric exercise protocol.

3.0 Eccentric Exercise

In this study, the biceps curl exercise was selected because it is commonly performed in resistance training and based on the eligibility requirements, the participants were familiar with it. This exercise protocol was designed to induce soreness in the elbow flexors and has been shown to induce significant changes in muscle damage indicators for adults (Chang et al., 2019; Lavender & Nosaka, 2008; Ra et al., 2013; Tanabe et al., 2019; Tsuchiya et al., 2019; Yoon & Kim, 2020). The protocol consisted of more sets of fewer repetitions which would minimize unexpected injury. Before starting the exercise, participants were instructed on proper exercise form. A dumbbell was used to induce DOMS of the non-dominant arm. The weight of the dumbbell was based on 75% of the previously measured 10 RM (Chang et al., 2019; Janecki et al., 2011; Richens, 2014). Participants positioned their dominant arm in front of their body, resting on an inclined padded bench, with their shoulder at a flexion angle of 45° (Lavender & Nosaka, 2006, 2008; Ra et al., 2013). The upper arm was maintained in a supinated position throughout the exercise. The forearm was flexed at a 45° angle. Participants slowly lowered the dumbbell to a fully extended position over 5 seconds and performed 6 sets of 5 repetitions with 2-min rest interval between sets (Conceicao et al., 2012; Ra et al., 2013). Throughout all exercise testing, verbal encouragement was given to elicit maximal effort. Participants maintained a constant speed of movement with the help of a metronome. After each repetition, the investigator returned the dumbbell to the starting position so that the participant performed no concentric action. Participants rested for 3 seconds between repetitions and 2 minute between sets.

3.1 Perceived Muscle Soreness

Two days after the eccentric exercise protocol, participants received another VAS. The VAS consisted of a perpendicular slide for participants to indicate their level of perceived soreness. Participants slid the perpendicular slide along the 10 cm line to the point that most accurately expressed their perceived degree of soreness.

3.2 Statistical Analysis

Statistical analyses were performed using the Statistical Program for Social Science (SPSS) version 27 software. Descriptive characteristics are presented as mean and standard deviation ($M \pm SD$). Two-tailed Spearman rho correlations were performed to examine associations between: soreness and amount of vitamin D from diet and energy balance, sun exposure, anthropometric and metabolic measures. Mann-Whitney U analysis was used to assess total vitamin D differences at the extremes with soreness. A one-way ANOVA was performed to compare the three different skin types. A p-value of <0.05 was considered statistically significant.

Results

Twenty-nine of the thirty-four participants recruited from the community completed the study. Participants' physical and anthropometric characteristics are presented as mean and standard deviation values (Table 1).

Table 1. Descriptive characteristics (Mean \pm SD)

Variable	All Participants (N=29)
Age (yrs.)	59.1 \pm 4.5
Height (cm)	166.0 \pm 1.0
Weight (kg)	65.0 \pm 15.0
FSS	2.0 \pm 1.0
Years Postmenopausal	10.0 \pm 5.1

FSS = Fitzpatrick Skin Scale

Figure 1. Likert scale of self-reported level of exercise intensity (1=light, 2=light-moderate, 3=moderate, 4=moderate to vigorous and 5=vigorous)

The 29 participants identified their skin type as type I – type III. Eight women selected a FSS of type I and were classified as pale white, 14 selected a type II and were classified as fair white and seven selected type III and were classified as white to olive skin tone (Figure 2). The mean FSS was 2.0 \pm 1.0. A one-way ANOVA did not show significant differences among the three skin type groups and perceived soreness. A Spearman correlation analysis was performed to determine if the number of years the women were postmenopausal correlated with soreness and it was not statistically significant ($\rho = .21$, $p = .27$).

Sun Exposure

Participants reported their daily sun exposure and amount of skin exposed for a typical summer day on a previously validated sun exposure questionnaire (Figure 3). The daily sun exposure score for each day was calculated by multiplying the time spent outdoors score times the amount of skin exposed while outdoors score. The score for each day ranged from 0 to 8. The weekly sun exposure was calculated by adding the daily scores (min=0, max=56).

Day	<u>Time Exposed to Sun</u>			<u>Skin Exposed Sun</u>			
	<5 min	5 -30 min	>30 min	Face and hands	Face, hands and arms	Face, hands, arms and legs	Bathing suit
Mon	0	1	2	1	2	3	4
Tues	0	1	2	1	2	3	4
Wed	0	1	2	1	2	3	4
Thurs	0	1	2	1	2	3	4
Fri	0	1	2	1	2	3	4
Sat	0	1	2	1	2	3	4
Sun	0	1	2	1	2	3	4

Figure 2. Sun Exposure Questionnaire (SEQ)

The scores for time spent outdoors and amount of skin exposed were then converted to a standard vitamin D dose (SDD) which is equivalent to an oral dose of approximately 1000 IU (Fioletov, 2010). The international units were further converted to micrograms. The mean weekly score for time spent outdoors in mid-day sun was 11.0 ± 3.0 and based on the SEQ, the mean score for amount of skin exposed to sun was 19.0 ± 5.0 . The mean weekly Sun Exposure Score calculated was 30.0 ± 12.0 . Based on the SEQ, the mean was 54% above the minimum. The majority of the women (62%) spent more than 30 minutes in mid-day sun, while 35% of them spent 5 – 30 minutes. Only 3% spent less than 5 minutes in the sun on an average summer week. Two women (14%) reported skin exposure in the lowest category (face and hands) and 7% in the highest category (bathing suit). The remaining 79% is a combination of exposed face, hands and arms and face, hands, arms and legs. The mean vitamin D acquired via sun exposure was $45.0 \text{ mcg} \pm 21.1$ (Table 2). There was no statistically significant correlation between the scores of time spent outdoors, amount of skin exposure, weekly sun exposure score and soreness, however it revealed a negative correlation (Table 3).

Table 2. Weekly sun exposure and vitamin D mcg (Mean \pm SD)

Variable	All Participants ($N=29$)
Time Spent Outdoors Score	11.0 \pm 3.0
Skin Exposure Score	19.0 \pm 5.0
Weekly Sun Exposure Score	30.0 \pm 12.0
Avg. Sun Vitamin D mcg	45.0 \pm 21.1

Time spent outdoors score: 0 = 0 to 5 min; 1 = 5 to 30 min; 2 = >30 min

Skin exposure score: 1 = hands & face, 2 = hands, face and arms, 3 = hand, face, arms and legs, 4 = bathing suit

Weekly sun exposure score: minimum = 0, maximum = 56

Table 3. Relationship (Spearman rho) between weekly sun exposure and perceived soreness

Variable	<i>rho</i> All Participants ($N=29$)	(<i>p</i>)
Time Spent Outdoors Score	-.23	0.23
Skin Exposure Score	-.13	1.0
Weekly Sun Exposure Score	-.30	0.12
Avg. Sun Vitamin D mcg	-.01	1.0

Time spent outdoors score: 0 to 5 min =0; 5 to 30 min = 1; >30 min = 2

Skin exposure score: 1 = hands & face, 2 = hands, face and arms, 3 = hand, face, arms and legs, 4 = bathing suit

Weekly sun exposure score: minimum = 0, maximum = 56

Dietary Intake and Energy Balance

All participants completed a four day dietary analysis which was assessed with the use of the NutriTiming software. The analysis provided the mean amount of dietary vitamin D intake, mean time spent in EB less than zero kcal, and mean time spent in EB less than -400 kcal. The dietary analysis revealed 26 of the 29 women (90%) did not consume the recommended amount of vitamin D and only 10% met the RDA of 15 mcg. All participants

spent a mean 18 hours in EB less than zero kcal and a mean 13 hours in EB < -400 kcal (Table 4).

Table 4. Dietary intake and energy balance (Mean \pm SD)

Variable	All Participants (N=29)
Avg daily dietary Vitamin D (mcg)	6.0 \pm 8.0
EB Hours < Zero kcal	18.0 \pm 8.0
EB Hours < -400 kcal	13.0 \pm 8.2

EB = Energy Balance

The Spearman rho correlation analysis showed no significant relationship between perceived soreness and dietary vitamin D and time in catabolic state. (Table 5).

Table 5. Relationship (Spearman rho) between perceived soreness and dietary vitamin D and time in catabolic state

Variable	All Participants (N=29)	(<i>p</i>)
Avg. Dietary Vitamin D (mcg)	-.14	0.50
EB Hours < Zero kcal	.23	0.24
EB Hours < -400 kcal	.22	0.30

p = probability (two-tailed)

* Correlation is significant at the 0.05 level (2-tailed)

The mean amount of dietary vitamin D intake during the hours in EB < zero kcal or hours in EB < -400 kcal was analyzed in all 29 participants and revealed no statistically significant association with soreness (Table 6).

Table 6. Amount of vitamin D (mcg) consumed during catabolic state (Mean \pm SD)

Variable	All Participants($N=29$)
EB Hours < Zero kcal	.94 \pm 2.3
EB Hours < -400 kcal	1.3 \pm 2.0

EB = Energy Balance

p = probability (two-tailed)

* Correlation is significant at the 0.05 level (2-tailed)

Table 7. Relationship (Spearman rho) between perceived soreness and amount of dietary vitamin D consumed during catabolic state

Variable	All Participants ($N=29$)	(p)
EB Hours < Zero kcal	.30	0.11
EB Hours < -400 kcal	.14	0.50

EB = Energy Balance

p = probability (two-tailed)

* Correlation is significant at the 0.05 level (2-tailed)

Spearman's rho was computed to assess the relationship between the average daily dietary vitamin D, average daily sun exposure vitamin D, the average daily total vitamin D and perceived level of soreness (Table 8). Spearman rho revealed no significant relationship between perceived soreness and the average daily dietary vitamin D, average daily sun exposure vitamin D nor a combination of both.

Table 8. Relationship (Spearman rho) between average dietary vitamin D, average sun exposure vitamin D, and perceived soreness

Variable	All Participants (<i>N</i>- 29)	(<i>p</i>)
Avg. daily dietary vitamin D (mcg)	-0.14	0.50
Avg. daily sun exposure vitamin D (mcg)	-0.01	1.0
Avg. total vitamin D (mcg)	-0.10	1.0

p = probability (two-tailed)

Though the mean vitamin D acquired through diet was negligible (6.0 mcg), when added to the mean vitamin D acquired as a result of sun exposure (45.0 mcg), the total daily mean of vitamin D equaled 51.0 mcg.

Since the participants were a fairly homogenous group, the total amount of vitamin D acquired in diet and sun exposure was converted to standardized z scores and divided into 3 groups; below and above 1.0, .50 and .25 SD above the mean to determine if there was a statistically significant with soreness (Table 9). The Mann-Whitney U analysis revealed no statistically significant association between groups categorized by z scores and soreness.

Table 9. Mann-Whitney U analysis of population distribution by z scores*

Total Vitamin D z Scores	Sample Size	Group Mean	U - Statistic	(p)
< -.25	<i>n</i> =1	4.0		
> +.25	<i>n</i> =7	5.0	3.0	1.0
< -.50	<i>n</i> =9	7.2		
> +.50	<i>n</i> =6	9.2	20.0	0.50
< -1.0	<i>n</i> =28	15.2		
> +1.0	<i>N</i> =1	9.0	8.0	0.62

p = probability (two-tailed)

*No statistical significance was found between groups categorized by z scores and soreness

Anthropometric Characteristics

Anthropometric characteristics and body composition are presented in Table 9.

Table 10. Anthropometric characteristics and body composition (Mean ± SD)

Variable	All Participants (<i>N</i>=29)
Weight (kg)	65.0 ± 15.0
Height (cm)	166.0 ± 1.0
Waist (cm)	75.2 ± 11.0
Hip (cm)	98.3 ± 10.1
WHR (ratio)	.76 ± .05
BMI (wt.-kg/ht-m²)	23.5 ± 4.1
BF%	29.0 ± 8.2

BF = Body fat

BMI = Body mass index

WHR = Waist to hip ratio

Spearman correlations revealed a statistically significant association between weight, waist, hip, and BMI and soreness (Table 10). The mean BF% of 29.0% is categorized as fair by the American College of Sports Medicine, (2021b).

Table 11. Spearman ρ correlations between perceived soreness and anthropometric and body composition characteristics

Variable	All Participants ($N=29$)	(p)
Weight (kg)	.54	0.00**
Height (cm)	.11	0.60
Waist (cm)	.40	0.04*
Hip (cm)	.50	0.01*
WHR (ratio)	-.05	0.80
BMI	.43	0.02*
BF%	.32	0.10

WHR = Waist-to-hip ratio

BMI = Body Mass Index

BF% = Body fat percentage

p = probability (two-tailed)

** Correlation is significant at the 0.01 level

* Correlation is significant at the 0.05 level

Metabolic Characteristics

The metabolic characteristics are present in Table 10. None of the blood pressure values suggested risk for cardiovascular disease. Spearman correlations found a negative association with VO_{2max} and soreness ($\rho = -.40, p = 0.05$).

Table 12. Metabolic characteristics (Mean \pm SD)

Variable	All Participants (N=29)
RHR (bpm)	68.0 \pm 13.0
VO_{2max} ml/kg/min	30.0 \pm 6.1
SBP (mmHg)	118.2 \pm 9.0
DBP (mmHg)	74.0 \pm 7.0
MAP (mmHg)	88.4 \pm 7.0

RHR=Resting Heart Rate

VO_{2max}=Maximal Oxygen Uptake

SBP=Systolic Blood Pressure

DBP=Diastolic Blood Pressure

MAP=Mean Arterial Pressure

Perceived Soreness

Muscular soreness was assessed with a VAS. Participants reported their perceived level of soreness 48 hours before and after the eccentric exercise protocol. A score of zero was classified as no soreness and a score of 10, extreme soreness. None of the participants reported any muscular soreness 48 hours prior to the eccentric exercise protocol. Following the exercise intervention, the mean level of soreness reported was 2.24 ± 2.0 . A higher percentage (97%) of participants reported low soreness level of zero to five and only one participant reported a high soreness level of seven. No one reported soreness above seven (Figure 5).

Discussion

The purpose of this study was to investigate the impact of vitamin D in diet (including supplements) and sun exposure, energy balance and body composition on perceived muscle soreness in postmenopausal women who are physically active. The main findings of this study were that: 1 – The combination of dietary vitamin D and sun exposure vitamin D was not statistically associated with perceived muscle soreness. 2 – Energy balance was not statistically

significant with soreness, however the analysis revealed extended time in negative energy balance; 3 - All of the women in the study cohort received more than the recommended amount of vitamin D (diet, supplements, sun).

The literature is essentially void of studies on vitamin D levels and delayed onset muscle soreness in active postmenopausal women. An analysis of the participants' diet with the NutriTiming® software, revealed that the amount of vitamin D consumed was negligible (6.0 mcg). However, the analysis of sun exposure vitamin D was acquired based on sun exposure was quite substantial (45.0 mcg). Added together, the analysis showed that the women acquired three times the RDA amount of vitamin D (51.0).

The finding that sufficient dietary vitamin D and sun exposure vitamin D combined, did not correlate with muscle soreness is consistent with the results observed in previous studies (Barker, Henriksen, et al., 2013; Pilch et al., 2020; Smith-Ryan et al., 2020). Barker, et al., investigated the relationship between serum 25(OH)₂ and inflammatory cytokines in healthy, recreationally active adults. Inflammatory cytokines are small, regulatory proteins that are key modulators of inflammation and vitamin D is known to be a pleiotropic micronutrient (Lai, 2013; Taherkhani et al., 2020). The results revealed interleukin-2, interferon-gamma, interleukin-1 beta, and tumor necrosis factor alpha to be significantly elevated in vitamin D deficient compared to vitamin D sufficient adults (Barker, Martins, et al., 2013). All of these studies report that sufficient vitamin D helps in the recovery of muscle damage.

The majority of women in this study reported involvement in outdoor activities, such as cycling, marathon and ultra-endurance running. They reported whether or not they wore sunscreen during outdoor activities and if so, the specific SPF number. According to Sayre and Dowdy (2007), the use of sunscreen SPF > 15 limits the body's ability to adequately receive

vitamin D. The majority of the participants reported they do not wear sunscreen and those who did, did so inconsistently. The results of the SEQ reveals that the majority of the women spent the recommended 5 – 15 minutes of mid-day sun exposure to acquire the RDA (15 mcg) of vitamin D. This resulted in vitamin D that was triple the recommended amount. The Spearman correlation analysis found no significant relationship between sun exposure vitamin D and soreness. The average level of muscle soreness reported was 3.4 on a scale of 1 to 10 and the high level of sun exposure vitamin D may have been responsible for attenuation of muscular soreness during the 48 hours post exercise.

The women who volunteered for this study were pale white, fair white and white to olive in skin tone as classified by the FSS. This may explain the reason there was no statistical significance between vitamin D and perceived levels of muscle soreness. The amount of melanin in the skin determines the amount of vitamin D the body can synthesize; therefore, the results may have been different with skins types IV, V and VI (Jablonski & Chaplin, 2018; Thompson et al., 2018)

A reduction in energy consumption and subsequent inadequate energy balance can contribute to vitamin D levels that are less than the RDA, since it increases the likelihood of inadequate consumption of vitamin D-rich foods. This can impact muscle viability and function, which may result in muscle soreness following resistance training. This is one interpretation of the results observed in the study and it's consistent with the findings of other work published in the literature (Cooper et al., 2011; Smith-Ryan et al., 2020).

Many women reduce caloric intake in an effort to avoid weight gain, which may explain why the women in this study spent a mean 18.0 hours in EB < zero kcal and 13.0 hours in EB < -400 kcal. This is concerning since restricting macronutrients or skipping meals can deplete the

body's fuel sources resulting in fatigue and increased risk of injury to muscle and/or bone (Schonberg, 2020). The deleterious impact of a negative EB has been previously established (Behrens, 2020; Louis, 2020; Melin et al., 2015; Viner, 2015) and is a situation that should be avoided by exercising postmenopausal women. Viner's study investigated the dietary patterns and energy balance of competitive male and female cyclists and found they were restricting CHO before and during exercise to increase fat oxidation and reduce BF%. She also found that low energy intake and specifically low CHO intake appeared to be the cause of chronic energy deficit (Viner, 2015). Most of the women in this study are endurance athletes and need sufficient energy to sustain their level of activity.

The literature has multiple studies which indicate that aging is accompanied with a loss of muscle mass and strength (Liebman, 2020; Maden-Wilkinson et al., 2015). Current research suggests there may be an age effect on recovery kinetics of sedentary older adults following resistance exercise (Bouzid et al., 2014; Munoz-Canoves, 2019). However, less is known about the recovery kinetic in master athletes, including muscle soreness in postmenopausal women who are chronic exercisers. Borges, et al. (2016) report that a reduced recovery capacity for active older adults could have a negative effect on training and performance and increase the risk of musculoskeletal soreness (Borges et al., 2018). Our results were different than Borges et al., (2016) and may have been due to the fact that the training intensities of the two studies were different.

The anthropometric and metabolic Spearman correlation results show high weight, BMI, waist and hip and low VO_{2max} were associated with a high level of perceived soreness in all 29 women. These results may partially be a result of the loss of estrogen, since previous research has identified decreased estrogen levels during menopause negatively impacts body composition

and results in a redistribution of fat to the abdomen (Casey, 2016; Chidi-Ogbolu & Baar, 2018; Hansen, 2018; Love, 2019; Marlatt et al., 2020). Adipose tissues are a source of pro-inflammatory cytokines, which lead to low-grade chronic inflammation and induce oxidative stress (Margaritelis et al., 2019). The oxidative stress results in membrane disruption (Kawamura et al., 2018), which after eccentric exercise, results in increased delayed onset muscle soreness (Yoon & Kim, 2020).

Another possible explanation for the positive relationship between weight, waist, hip and BMI is the catabolic stress hormone, cortisol. Unlike other hormones that decrease with aging, cortisol does not decline with age. Estrogen and cortisol exert opposing effects on bodily systems, which in postmenopausal women means the benefits of estrogen's mitigating properties are reduced (Herrera, 2015). Cortisol is elevated during energy deficit and can increase a women's risk of osteoporosis, muscle soreness and weight gain during menopause (Hannibal & Bishop, 2014; Schiebel, 2018; Schorr et al., 2015). Physical exercise will elevate cortisol as does excessive time spent in negative EB (Archer et al., 2011; Schorr et al., 2015). The women in this study spent an average of 18 hours in less than zero kcal, which may potentially increase cortisol. Severe negative EB initiates a higher cortisol production, which has a profound impact on muscle loss and result in an increase of muscular injury (Benardot, 2021). This study emphasizes the importance of active postmenopausal women to match their energy intake to their energy expenditure and in doing avoid the negative effects of energy deficiency.

Summary

The aim of this study was to identify the impact of vitamin D, energy balance and body composition on perceived muscle soreness in active postmenopausal women. Little research exists on postmenopausal women who are chronic exercisers. The result of this study suggests

that there is a relationship between vitamin D and reduced muscle soreness. This indicates that vitamin D may help mitigate muscular soreness, which is consistent with what others have found. The literature suggests that sufficient vitamin D helps in the recovery of muscle damage and may reduce muscle soreness 48 hours later. This study also highlights the importance of maintaining EB. It brings to light the issue of excessive energy deficit which may be the reason weight, waist, hip and BMI correlated with soreness.

Limitations of the study include the accuracy of recall associated with the diet and sun exposure score and the duration of more than 30 minutes outdoors may not be adequately quantified. Future research is needed to better understand the impact of vitamin D, energy balance and body composition on soreness, in active postmenopausal women. They should include women with darker skin tones in order to be able to differentiate between women who absorb more UVB rays and those who don't.

Based on this study, excessive time in negative energy balance should be avoided by active postmenopausal women. They should focus on adequate energy intake to support muscle and bone health and optimize their performance.

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APPENDICES

Appendix A

2018 PAR-Q+

The Physical Activity Readiness Questionnaire for Everyone

The health benefits of regular physical activity are clear; more people should engage in physical activity every day of the week. Participating in physical activity is very safe for MOST people. This questionnaire will tell you whether it is necessary for you to seek further advice from your doctor OR a qualified exercise professional before becoming more physically active.

GENERAL HEALTH QUESTIONS

Please read the 7 questions below carefully and answer each one honestly: check YES or No	YES	NO
1) Has your doctor ever said that you have a heart condition OR high blood pressure ?	<input type="checkbox"/>	<input type="checkbox"/>
2) Do you feel pain in your chest at rest, during your daily activities of living, OR when you do physical activity	<input type="checkbox"/>	<input type="checkbox"/>
3) Do you lose balance because of dizziness OR have you lost consciousness in the last 12 months? (Please answer NO if your dizziness was associated with over-breathing (including during vigorous exercise).)	<input type="checkbox"/>	<input type="checkbox"/>
4) Have you ever been diagnosed with another chronic medical condition (other than heart disease or high blood pressure)? PLEASE LIST CONDITION(S) HERE: _____	<input type="checkbox"/>	<input type="checkbox"/>
5) Are you currently taking prescribed medications for a chronic medical condition. PLEASE LIST CONDITION(S) AND MEDICATIONS HERE: _____	<input type="checkbox"/>	<input type="checkbox"/>
6) Do you currently have (or have had within the past 12 months) a bone, joint, or soft tissue (muscle, ligament, or tendon) problem that could be made worse by becoming more physically active? (Please answer NO if you had a problem in the past, but it does not limit your current ability to be physically active). PLEASE LIST CONDITION(S) HERE: _____	<input type="checkbox"/>	<input type="checkbox"/>
7) Has your doctor ever said that you should only do medically supervised physical activity?	<input type="checkbox"/>	<input type="checkbox"/>

If you answered **NO** to all of the questions above, you are cleared for physical activity. Go to page 4 to sign the **PARTICIPANT DECLARATION**. **You do not need to complete Pages 2 and 3.**

- Start becoming much more physically active – start slowly and build up gradually.
- Follow International Physical Activity Guidelines for your age (www.who.int/dietphysicalactivity/en/).
- You may take part in a health and fitness appraisal.
- If you are over the age of 45 and **NOT** accustomed to regular vigorous to maximal effort exercise, consult a qualified exercise professional before engaging in this intensity of exercise.
- If you have any further questions, contact a qualified exercise professional.

If you answered **YES** to one or more of the questions above, **COMPLETE PAGES 2 AND 3.**

⚠ Delay becoming more active if:

- ✓ You have a temporary illness such as a cold or fever, it is best to wait until you feel better.
- ✓ You are pregnant – talk to your health care practitioner, your physician, a qualified exercise professional, and/or complete the ePARmed-X+ at www.eparmedx.com before becoming more physically active.
- ✓ Your health changes – answer the questions of Pages 2 and 3 of this document and/or talk to your doctor or a qualified exercise professional before continuing with any physical activity program.

2018 PAR-Q+

FOLLOW-UP QUESTIONS ABOUT YOUR MEDICAL CONDITION(S)

- 1. Do you have Arthritis, Osteoporosis, or Back Problems?**
If the above condition(s) is/are present answer questions 1a-1c If **NO** go to question 2
- 1a Do you have difficulty controlling your condition with medications or other physician prescribed therapies? YES NO
(Answer **NO** if you are not currently taking medications or other treatments)
-
- 1b Do you have joint problems causing pain, a recent fracture or fracture caused by osteoporosis or cancer, displaced vertebra (e.g., spondylolisthesis), and/or spondylolysis/pars defect (a crack in the bony ring on the back of the spinal column)? YES NO
-
- 1c Have you had steroid injections or taken steroid tablets regularly for more than 3 months? YES NO
-
- 2. Do you have Cancer of any kind?**
If the above condition(s) is/are present, answer questions 2a-2b If **NO** go to question 3
- 2a Does your cancer diagnosis include any of the following types; lung/bronchogenic, multiple myeloma (cancer of plasma cells), head, and neck? YES NO
-
- 2b Are you currently receiving cancer therapy (such as chemotherapy or radiotherapy)? YES NO
-
- 3. Do you have a Heart or Cardiovascular Condition? This includes Coronary Artery Disease, Heart Failure, Diagnosed Abnormality of Heart Rhythm**
If the above condition(s) is/are present, answer questions 3a-3d If **NO** go to question 4
- 3a Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? (Answer **NO** if you are not currently taking medications or other treatments) YES NO
-
- 3b Do you have an irregular heart beat that required medical management? (e.g., atrial fibrillation, premature ventricular contraction) YES NO
-
- 3c Do you have chronic heart failure? YES NO
-
- 3d Do you have diagnosed coronary artery (cardiovascular) disease and have not participated in regular physical activity in the last 2 months? YES NO
-
- 4. Do you have High Blood Pressure?**
If the above condition(s) is/are present, answer questions 4a-4b If **NO** go to question 5
- 4a Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? (Answer **NO** if you are not currently taking medications or other treatments) YES NO
-
- 4b Do you have a resting blood pressure equal to or greater than 160/90 mmHg with or without medication? (Answer **YES** if you do not know your resting blood pressure) YES NO
-
5. **Do you have any Metabolic Conditions? This includes Type 1 Diabetes, Type 2 Diabetes, Pre-Diabetes**
If the above condition(s) is/are present, answer questions 5a-5e If **NO** go to question 6
- 5a Do you often have difficulty controlling your blood sugar levels with foods, medications, or other physician-prescribed therapies? YES NO
-
- 5b Do you often suffer from signs and symptoms of low blood sugar (hypoglycemia) following exercise and/or during activities of daily living? Signs of hypoglycemia may include shakiness, nervousness, unusual irritability, abnormal sweating, dizziness or light-headedness, mental confusion, difficulty speaking, weakness, or sleepiness. YES NO
-
- 5c Do you have any signs or symptoms of diabetes complications such as heart or vascular disease and/or complications affecting your eyes, kidneys, **OR** the sensation in your toes and feet? YES NO
-
- 5d. Do you have other metabolic conditions (such as current pregnancy-related diabetes, chronic kidney disease or liver problems)? YES NO
-
- 5e Are you planning to engage in what for you is unusually high (or vigorous) intensity exercise in the near future? YES NO

2018 PAR-Q+

- 6. Do you have any Mental Health Problems or Learning Difficulties?** *This includes Alzheimer's Dementia, Depression, Anxiety Disorder, Eating Disorder, Psychotic Disorder, Intellectual Disability, Down Syndrome*
If the above condition(s) is/are present, answer questions 6a-6b If **NO** go to question 7
- 6a Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? (Answer **NO** if you are not currently taking medications or other treatments) YES NO
- 6b Do you ALSO have back problems affecting nerves or muscles? YES NO
-
- 7. Do you have a Respiratory Disease?** *This includes Chronic Obstructive Pulmonary Disease, Asthma, Pulmonary High Blood Pressure*
If the above condition(s) is/are present, answer questions 7a-7d If **NO** go to question 8
- 7a Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? (Answer **NO** if you are not currently taking medications or other treatments) YES NO
- 7b Has your doctor ever said your blood oxygen level is low at rest or during exercise and/or that you require supplemental oxygen therapy? YES NO
- 7c If asthmatic, do you currently have symptoms of chest tightness, wheezing, labored breathing, consistent cough (more than 2 days/week), or have you used your rescue medication more than twice in the last week? YES NO
- 7d Has your doctor ever said you have high blood pressure in the blood vessels of your lungs? YES NO
-
- 8. Do you have a Spinal Cord Injury?** *This includes Tetraplegia and Paraplegia*
If the above condition(s) is/are present, answer questions 8a-8c If **NO** go to question 9
- 8a Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? (Answer **NO** if you are not currently taking medications or other treatments) YES NO
- 8b Do you commonly exhibit low resting blood pressure significant enough to cause dizziness, light-headedness, and/or fainting? YES NO
- 8c Has your physician indicated that you exhibit sudden bouts of high blood pressure (known as Autonomic Dysreflexia) YES NO
-
- 9. Have you had a Stroke?** *This includes Transient Ischemic Attack (TIA) or Cerebrovascular Event*
If the above condition(s) is/are present, answer questions 9a-9c If **NO** go to question 10
- 9a Do you have difficulty controlling you condition with medications or other physician-prescribed therapies? (Answer **NO** if you are not currently taking medications or other treatments) YES NO
- 9b Do you have any impairment in walking or mobility? YES NO
- 9c Have you experienced a stroke or impairment in nerves or muscles in the past 6 months? YES NO
-
- 10. Do you have any other medical condition not listed above or do you have two or more medical conditions?**
If you have other medical conditions, answer questions 10a-10c If **NO** read the Page 4 recommendations
- 10a Have you experienced a blackout, fainted, or lost consciousness as a result of a head injury within the last 12 months **OR** have you had a diagnosed concussion within the last 12 months? YES NO
- 10b Do you have a medical condition that is not listed (such as epilepsy, neurological conditions, kidney problems)? YES NO
- 10c Do you currently live with two or more medical conditions? YES NO
- PLEASE LIST YOUR MEDICAL CONDITIONS(S)** _____
AND ANY RELATED MEDICATIONS HERE: _____

GO to Page 4 for recommendations about your current medical condition(s) and sign the PARTICIPANT DECLARATION.

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☑ If you answered **NO** to all of the follow-up questions about your medical condition, you are ready to become more physically active – sign the **PARTICIPANT DECLARATION** below:

- It is advised that you consult a qualified exercise professional to help you develop a safe and effective physical activity plan to meet your health needs.
- You are encouraged to start slowly and build up gradually – 20 to 60 minutes of low to moderate intensity exercise, 3-5 days per week including aerobic and muscle strengthening exercises.
- As you progress, you should aim to accumulate 150 minutes or more of moderate intensity physical activity per week.
- If you are over the age of 45 yr and **NOT** accustomed to regular vigorous to maximal effort exercise, consult a qualified exercise professional before engaging in this intensity of exercise.

● If you answered **YES** to one or more of the follow-up questions about your medical condition: You should seek further information before becoming more physically active or engaging in a fitness appraisal. You should complete the specially designed online screening and exercise recommendations program – the ePARmed-X+ at www.eparmedx.com and/or visit a qualified exercise professional to work through the ePARmed-X+ and for further information.

⚠ Delay becoming more active if:

- ✓ You have a temporary illness such as a cold or fever; it is best to wait until you feel better.
 - ✓ You are pregnant – talk to your health care practitioner, your physician, and qualified exercise professional, and/or complete the ePARmed-X+ at www.eparmedx.com before becoming more physically active.
 - ✓ Your health changes – talk to your doctor or qualified exercise professional before continuing with any physical activity program.
- You are encouraged to photocopy the PAR-Q+. You must use the entire questionnaire and NO changes are permitted.
 - The authors, the PAR-Q+ Collaboration, partner organizations, and their agents assume no liability for persons who undertake physical activity and/or make use of the PAR-Q+ or ePARmed-X+. If in doubt after completing the questionnaire, consult your doctor prior to physical activity.

NAME _____ DATE _____

Submit

Appendix B

Fitzpatrick Skin Type Classification



Appendix C

Sun Exposure Questionnaire

Day	Time Exposed to Sun			Skin Exposed to Sun			
	<5 min	5-30 min	>30 min	Face and hands uncovered	Arms uncovered	Legs uncovered	Bathing suit
Mon	0	1	2	1	2	3	4
Tues	0	1	2	1	2	3	4
Wed	0	1	2	1	2	3	4
Thurs	0	1	2	1	2	3	4
Fri	0	1	2	1	2	3	4
Sat	0	1	2	1	2	3	4
Sun	0	1	2	1	2	3	4

Total days of time exposed to sun x total days skin exposed = Daily Sun Exposure Score
(min=0, max=8)

All days Sun Exposure Scores added together = Weekly Sun Exposure Score (min=0, max=56)

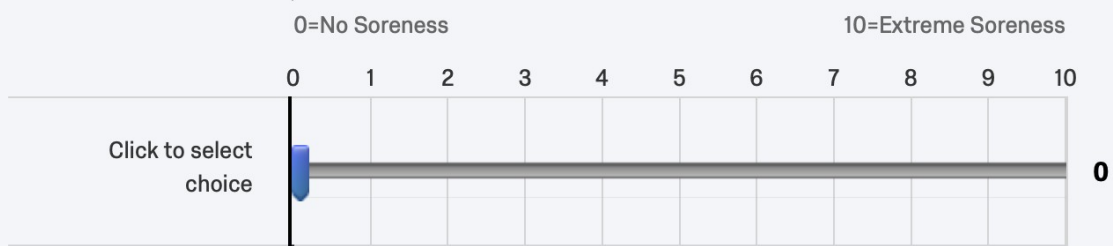
Total weekly time exposed to sun = min 0, max 14

Total weekly skin exposed to sun = min 7, max 28

Appendix D

Visual Analog Scale

Consider the overall severity of soreness in your biceps upon movement. Drag the perpendicular slide along the scale and indicate your level of soreness



Appendix E

NutriTiming™ Data Entry Form

Name: _____ Age: _____ Years Gender: M or F
 Height: _____ Feet _____ Inches Weight: _____ Pounds Date Analyzed ____/____/____
MM DD YYYY

Instructions: Completing this form will help us understand whether the amount of energy (calories) you consume comes close to matching the energy (calories) you expend. This form provides a way of entering your energy expended by using an 'Activity Factor', and your energy consumed by using a description of the foods and drinks you ate. The information is entered by hourly units, so you don't have to remember precisely the time you had an activity or ate some food. Rather, you are asked to enter when you had an activity, its intensity by using the activity factor scale, and how long you did it (example: I had a slow jog between 10 and 11 in the morning that lasted for 30 minutes). Use the NutriTiming Activity Factor Scale Descriptions to help you figure out the best factor to enter when describing an activity. When entering food, describe the food and the way it was prepared fully (example: chicken breast with no skin that was baked; or fried, battered chicken breast, etc), and the amount you consumed (example: 1 apple; 1 ½ cups; 15 red grapes; 1 large banana, etc.). A factor of 1.5 is considered normal daytime activity, and we will assume a factor of 1.5 unless you indicate otherwise. A factor of 1 is equal to sleep, and a factor greater than 1.5 suggests you are doing something more vigorous than normal daytime activity. Please enter a full 24 hours of all your activities and all the foods/drinks you consume. Use the example below to help you understand how to enter the information.

NutriTiming Activity Factor Scale	
Factor	Description
1	Resting, Reclining: Sleeping, reclining, relaxing
1.5	Rest +: Normal, average sitting, standing daytime activity
2.0	Very Light: More movement, mainly with upper body. Equivalent to tying shoes, typing, brushing teeth
2.5	Very Light +: Working harder than 2.0
3.0	Light: Movement with upper and lower body. Equivalent to household chores
3.5	Light +: Working harder than 3.0; Heart rate faster, but can do this all day without difficulty
4.0	Moderate: Walking briskly, etc. Heart rate faster, sweating lightly, etc but comfortable
4.5	Moderate +: Working harder than 4.0. Heart rate noticeably faster, breathing faster
5.0	Vigorous: Breathing clearly faster and deeper, heart rate faster, must take occasional deep breath during sentence to carry on conversation
5.5	Vigorous +: Working harder than 5.0. Breathing noticeably faster and deeper, and must breath deeply more often to carry on conversation
6.0	Heavy: You can still talk, but breathing is so hard and deep you would prefer not to. Sweating profusely. Heart rate very high
6.5	Heavy +: Working harder than 6.0. You can barely talk but would prefer not to. This is about as hard as you can go, but not for long
7.0	Exhaustive: Can't continue this intensity long, as you are on the verge of collapse and are gasping for air. Heart rate is pounding

Begin Hour	End Hour	Activity Factor	Activity Description	Food/Drink Description	Food/Drink Amount
Begin Example					
12am	7am	1.0	Sleep		
7am	8am	1.5	Nothing Special	Whole Wheat Waffles (Frozen-Kellogg)	3
				Maple Syrup	2 Tablespoons
				1 % Milk	1 Cup
				Orange Juice (from concentrate)	1.5 Cups
				Coffee	2 Cups
				1 % Milk for Coffee	2 Tablespoons
10am	11am	5.0	Jog 30 minutes	Gatorade	16 Ounces
12noon	1pm	1.5	Nothing Special	Medium size beef sandwich with white bread, mayonnaise, lettuce, and tomato.	1 Sandwich
				Coffee	2 Cups
				Artificial Coffee Creamer	2 Packets
				Apple Pie	1 Slice (small)
5pm	6pm	4.0	Walk 1 hour	Water	16 ounces
7pm	8pm	1.5	Nothing Special	Lasagna with ground beef and cheese	Large Plate
				Lettuce Salad with Tomatoes and Cucumbers	Medium Size Salad
				Blue Cheese Salad Dressing	1 Tablespoon
				Red Wine	1 Medium Glass
10pm	11pm	1.5	Nothing Special	Popcorn (air popped; no butter)	100 Calorie Pack
End Example					

Appendix F**Borg's Rating of Perceived Exertion**

Rating	Perceived Exertion
6	No exertion
7	Extremely light
8	
9	Very light
10	
11	Light
12	
13	Somewhat hard
14	
15	Hard
16	
17	Very hard
18	
19	Extremely hard
20	Maximum exertion