3-26-2021

Exercise and Bone: Older Adults, Type II Diabetes, and Ketogenic Diets

Matthew Casey Scott
Louisiana State University and Agricultural and Mechanical College

EXERCISE AND BONE: OLDER ADULTS, TYPE II DIABETES, AND KETOGENIC DIETS

A Dissertation

Submitted to the Graduate Faculty of the
Louisiana State University and
Agricultural and Mechanical College
in partial fulfillment of the
requirements for the degree of
Doctor of Philosophy

in

The School of Kinesiology

by
Matthew Casey Scott
B.S., Louisiana State University, 2012
M.S., Louisiana State University, 2014
May 2021

ACKNOWLEDGEMENTS

I have been a student at LSU for a while now. I started my undergraduate degree in 2008 with an interest in music composition, and after finishing many of the associated prerequisites, I decided to switch to a science-based degree. Thanks to the friend that helped me discover kinesiology and to the LSU counselor that helped me make the switch; the knowledge gained by this degree has been more than simply academic pursuit. Being here, at LSU, for so long, I am finding that writing this acknowledgment is more difficult than I expected. I have had many iterations, each adding another person who has had some truly meaningful and much appreciated impact on my studies, research, or life in general while pursuing this degree; there is too many. I have deleted the previous 3000-word dedication to many of those who helped me, and I will happily find the time to contact and chat with y'all individually. Luckily, there is an intimate group of people who I can put on a pedestal without needing 8-9 pages or risking the exclusion of others who were similarly integral to my success.

To Jean and Errol, my grandparents, I wish y'all were alive to see me finish this degree. In many ways, my success is tied to the support you two provided. To Jonathan and Jean, my children, you two have done extraordinarily little to help me finish this degree. Dealing with you two has been constant work, and I am certain I would have finished this faster without kids. Thanks for giving me a higher calling; my role as a father will be my greatest achievement in life, and I cannot wait to see what the future holds for both of you. To Kristina Scott, my wife, I love you mama. You are genuinely my favorite person, and without you being there to compensate for all my many faults, I would not have been able to find success in these academic pursuits.

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ABSTRACT

Exercise is a well-appreciated modulator of bone and has other positive implications for overall fitness and health. The purpose of this dissertation was to determine the effects of exercise on bone in conjunction with other known modifiers: old age, type II diabetes mellitus (T2DM), and ketogenic diets. The three studies discussed in this dissertation utilized multiple methods of measuring bone to examine the effect of exercise on bone in individuals with type II diabetes, rodents consuming a ketogenic diet, and older adults participating in a novel resistance training intervention.

The first study examined the effects of a 9-month resistance, aerobic, or combination exercise intervention on bone in individuals with T2DM. Whole-body and whole-body derived regional measures of bone mineral density (BMD) were obtained via dual-energy x-ray absorptiometry (DXA) and used to determine the effects. While the entire cohort, control group included, showed significant increases in BMD after a 9-month intervention, the lack of differences between groups, was surprising. An effect of exercise on bone in individuals with T2DM was not revealed by measures of BMD.

The second study utilized rodents to determine the effects of a 6-week ketogenic dietary intervention interceded by a 3-week exercise intervention on their trabecular and cortical bone morphology, measured via micro-computed tomography. Our results did not identify any detriments in bone morphology in response to a ketogenic diet alone, but positive changes in trabecular morphology and density induced by exercise in mice fed a control diet were negated by the ketogenic diet.

The last study examined the effects of resistance training along with low intensity breaks in sedentary activity on BMD, trabecular bone score (TBS), and serum markers

of bone turnover in older adults. Changes in whole-body BMD, lumbar spine BMD, and TBS were not found in response to the 4-month resistance training intervention. Serum markers for bone turnover did not provide any additional context due to reagent, equipment, and technician error. Future re-analysis may be attempted, but for the purpose of this dissertation, the analysis of blood markers for bone turnover was too poor and not included.

CHAPTER 1. INTRODUCTION

It is not the risk of fall that kills you; it is not the risk of fracture that breaks your bones. While fractures certainly have an economic and social cost, the overall impact of having weaker bone is not clear, in most cases, until catastrophic failure occurs. Still, there is a clear relationship between metrics of bone fragility, such as low bone mineral density (BMD), and incidence of fracture [1]. The relationship between BMD and fracture risk is the case for clinical treatment and a common standard in research for determining the efficacy of potential interventions. However, bone density is not the only metric for understanding bone; measures of bone architecture and biochemical indicators of bone turnover are increasingly commonplace, improving our understanding of the modulation of bone health, strength, and ultimately risk of fracture. Exercise has been shown to be a significant modulator of bone strength and is especially interesting because of the holistic implication for improvements in fitness and health [2]. Further, functional attributes such as balance and strength, gained from exercise, reduce the risk of fracture indirectly by reducing the risk of falling [2]. The purpose of this dissertation is to further examine the relationships between exercise and bone in relation to other known modifiers: type II diabetes mellitus (T2DM), ketogenic diets, and old age.

Chapter 2 expands on the relationships mentioned above, starting with a review of fracture risk, bone biology, and methods for measuring bone. The latter half of chapter 2 addresses the specific purpose of this dissertation with a discussion of the systematic reviews that organize the literature on older adults, bone and exercise, followed by a specific review of the literature associated with exercise and bone in relation to T2DM and ketogenic diets. Both the literature on T2DM and ketogenic diets,

in relation to bone and exercise, is sparse; there are only a handful of studies that examine the relationships.

The first study, chapter 3, in this dissertation is an ancillary analysis of a large exercise intervention trial in individuals with type II diabetes mellitus (T2DM) [3]. Original research on bone health in individuals with T2DM is budding but seems to suggest BMD is not indicative of fracture risk in this population, as individuals with T2DM have higher than normal BMD, but also have higher than normal incidence of fracture compared to age matched controls [4]. Further, there are only a couple studies that examine the potential effects of exercise on bone in individuals with T2DM and only one true intervention study to date using human subjects [5]. The ancillary analysis in chapter 3 examines the effects of a 9-month exercise intervention with multiple modalities of exercise being included. Although this analysis is mainly based on whole-body scans via dual-energy x-ray absorptiometry (DXA), it is currently the second of only two studies to examine the effects of an exercise intervention on bone and T2DM, although there is a large intervention trial currently underway in Italy, with a 2025 anticipated completion date.

The next study in this dissertation, chapter 4, utilizes rodents to examine the interactions between ketogenic diets and exercise in relation to trabecular and cortical bone morphology. The current literature examining the effects of a ketogenic diet on bone are sparse, but there does seem to be an indication that a high fat, very-low carbohydrate diets negatively impact bone in rodents and in humans. With the rising popularity of ketogenic diets in relation to weight loss and health, the addition of exercise in the context of ketogenic diets and bone has implications for individuals who

may adopt modifications to both their diet and exercise habits. While there are currently two studies that examine this relationship [6], [7], the research outlined in chapter 4 adds to these studies by examining bone morphology, metrics that predict bone strength based on architecture, rather than just BMD.

The third study, chapter 5, adds to the literature associated with older adults, bone, and exercise. Older adults participated in a 4-month resistance training intervention with a novel modification of sedentary activity and had BMD, trabecular bone score (TBS), and serum markers of bone turnover measured before and after the intervention. The relationship between exercise and bone in older adults has been thoroughly researched, but mainly with respect to bone density. The addition of TBS in this study allows us to explore the potential nuance associated with a novel methodology for estimating bone quality, while the utilization of BMD and blood markers of bone turnover was intended to give additional context for explaining any potential changes.

Chapter 6 utilizes the findings in chapters 3-5 to briefly re-examine the literature on T2DM, ketogenic diets, and older adults as it was presented in chapter 2. The research in this dissertation does not drastically adjust our current understanding of bone in relation to exercise but can be used to develop future research directions. Potential research directions for bone and exercise will first be discussed in relation to T2DM, ketogenic diets, and older adults; general implications for future research, in relation to bone and exercise, conclude this dissertation.

1.1. Notes

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CHAPTER 2. LITERATURE REVIEW

2.1 Introduction: Bone Health and Why it Matters

Why study bone? Rationales for better understanding bone have a fair amount of variability between researchers, each with varying degrees of efficacy, but osteoporosis would most likely be number one with the effects of microgravity being a distant contender. It's no surprise that osteoporosis is so commonplace in the introductory paragraph for much of the research examining bone health as its social and economic costs are substantial. Worldwide, there are nearly 9 million fractures per year [1], and costs associated with a fracture in the United States can range from $4,000 to $30,000, with hip fractures being costliest [1] [2]. This is in addition to the cost of treating those with non-fracture osteoporosis and those with osteopenia, although it appears that treating these individuals is economically sensible in the long run [2] [2]. The problem of osteoporosis and fracture incidence in the worldwide population becomes more pronounced with age, suggesting increased overall costs for the treatment and prevention of osteoporotic fractures as the population of older adults grows. Persons aged 65 and older account for nine percent of the global population in 2019 and are expected to account for sixteen percent of the population, 1.5 billion older adults, by 2050 [3]. For the United States, projections suggest larger than average costs as the percentage of older adults is expected to grow from 16 percent in 2016 to 22 percent in 2050 [4].

[1] The average cost of a wrist, vertebral, and hip fracture were $4,000, $8,000 and $30,000, respectively [2].

[2] The average annual cost of osteoporotic fracture prevention was between $300 and $900 [2].

The cost of osteoporosis provides an economic context for concern, but it's also important to outline the social cost of a fracture. Postmenopausal women are most at risk for an osteoporotic fracture with 1 out of 3 women expected to have a fracture within their lifetime [5]. For men the risk is a bit lower, 1 out of 5 [6], and fractures are expected later in life. These statistics may change if there is a significant shift in life expectancy as women typically suffer fractures earlier in life due to hormone deficiencies after menopause, and both men and women continue to lose bone with increased age. For both sexes, a fracture and the resulting immobility and recovery have pronounced effects on risk for loss of independence and death. For women over 60 years, a hip fracture is associated with a 30 percent chance of death within 5 years, and while less likely to suffer a fracture, men are twice as likely to die within 5 years after a hip fracture occurs [7]. In both cases, deaths are more prevalent in the first year after fracture and fracture associated mortality tapers off considerably after 5 years [7]. For survivors, risk of a recurring fracture is at least 25% percent [8] and loss of independence is expected for about a third of individuals [9]; overall, less than 50 percent of individuals regain normal function [10].

Osteoporosis isn't the only bone related disease, but others are not as prevalent. For example, about one million individuals in the United States have Paget's disease and about 50,000 have osteogenesis imperfecta; both are substantially lower than the 10 million individuals in the US diagnosed with osteoporosis, not to mention the additional 34 million individuals with osteopenia [11]. With respect to treatment of the above-mentioned diseases, osteoporosis and the associated fracture risk stands out as being well mitigated by lifestyle modifications which may further explain its prevalence in

the literature, as the benefits of lifestyle modification are clear but implementation into society and optimization of diets or activity are not. In comparison, Osteogenesis Imperfecta is genetic [12] and the cause of Paget's disease is not well-defined, although genetic factors play a role [13].

Prevention and treatment of osteoporosis is a significant driver of scientific interest in bone physiology, and research has yielded a wealth of useful knowledge. General recommendations for prevention and treatment typically include a combination of dietary modulation, supplementation, and weight bearing activity [14], which is in line with living a healthy life overall; however, achieving the highest possible peak bone mass in the decade after puberty is especially effective for prevention. Children and young adults demonstrate substantially larger capacity to increase stored mineral compared to older adults, and a large store of bone combined with proper diet and exercise in later life can potentially attenuate bone loss enough to avoid fractures during the lifespan.

The literature is generally clear concerning the use of exercise as a mitigator of fracture risk. Exercise, especially weight bearing exercise, can improve peak bone mineral density (BMD) and attenuate losses over the lifespan [15]; BMD is highly correlated with bone strength [16]–[19] and the most common metric used in determining osteoporosis status[3] [20]. In addition to improving BMD, weight bearing exercise may also positively impact bone morphology/geometry and while the metrics associated with improved bone morphology do not directly determine osteoporotic status, they are related to bone strength [21]–[23]. Furthermore, exercise also has the

[3] A BMD 1 Standard deviation below the average young adult population is considered Osteopetrosis and 2.5 below is considered Osteoporosis [20].

benefit of improving strength and balance, which are not necessarily related to bone but do play a role in reducing fracture due to fall and add to the rationale for physical activity in older adults [24]. Overall, exercise seems to play a significant role in bone physiology and considering the structural purpose bone serves, it's no surprise. While the effects of exercise on bone in the general population are clear, effects on more specific subsets of the population have less clarity. In order of increasing ambiguity, older adults, individuals with type II diabetes and individuals undergoing a keto genic diet demonstrate a less clear relationship between bone and exercise. The purpose of this literature review is to discuss the basic biology of bone, review the general effects of exercise on bone in older adults, review the specific literature associated with T2DM, bone and exercise, and review the specific literature associated with ketogenic diets, bone and exercise.

2.2 Basic Bone Biology

Microarchitecture

Many subsets of information about bone biology can be organized in pairs: osteoblasts and osteoclasts, resorption and formation, collagen and mineral, osteoid and hydroxyapatite, organic and inorganic, lightweight yet strong, rigid yet flexible, metabolic and structural, trabecular and cortical. While information in two's is mostly a coincidence and a creative writer could add some duality to any topic of discussion, the coincidental pairs mentioned above are useful for organizing information about bone, and that is especially true for its microarchitecture. Bone can be categorized into two subsets of tissue, cortical bone and trabecular bone. The organization of these tissues give bone a lightweight yet strong structure to be acted on as a lever by muscles, while

also accommodating the metabolic role of bone as a storage depot for calcium. The metabolic and structural functions of bone are reflected in trabecular and cortical bone respectively, although both tissues contribute to both functions[4] [25].

Cortical, or compact, bone is the outer most layer of bone; although, it is covered by a more superficial, well-vascularized membrane called the periosteum. Cortical bone has a specific organizational structure termed the haversian system and this system organizes cortical bone in concentric layers, or lamellae, around haversian canals which are occupied by blood vessels, lymph vessels, and nerves[5]. The outermost lamella is surrounded by a cement line that reflects the original deposition of mineral by osteoblasts after the haversian canal was initially formed by osteoclasts [25]; the cement line encapsulates what biologists term the functional unit of the haversian system, an osteon. The significance of this organizational structure is the maximization of space filled with bone matrix and mineral, leaving only what is necessary for nutrient supply via blood vessels. The compact structure is especially useful for resisting bending forces which is why the long bones, like the femur, have substantially higher cortical bone thickness compared to flat bones[6] [26], [27], furthermore, thickness increases in the diaphysis of long bones [28], where the magnitude of bending forces would be highest.

[4] Bone also serves a protective function, as vital organs are well encapsulated by the skeleton, but this would give bone tissue three functions (structural, metabolic, and protective) rather than two so this writer would subcategorize the protective role of bone under its structural function.

[5] Blood vessels span between harversian canals via volkmann's canals, providing blood supply to osteons between periosteal and endosteal surfaces.

[6] Cortical thickness of a vertebra is about .25mm [26] compared to 6.5mm [27] in the midshaft of the femur.

Trabecular, or cancellous, bone is the innermost portion of bone. Its microarchitecture is not as organized as cortical bone, but trabecular bone does form a lattice-like structure composed of trabeculae that aids in the distribution and absorption of external mechanical load [29]. Due to the lattice structure of trabecular bone, much of the inner cavity that is occupied by trabeculae is void of bone tissue and instead occupied by hemopoietic marrow, fat, or blood vessels. Specific to trabecular bone and its web-like structure, a large surface area of bone is available to the well-vascularized endosteum [7] and systemic circulation. The accessibility of trabecular bone allows systemic circulation to access a substantial portion of the bone surface for mineral exchange [30], the main metabolic role of bone tissue.

While the microarchitecture of cortical and trabecular bone differs, the bone tissue that is organized into either cortical or trabecular bone is similar, a combination of osteoid and hydroxyapatite. Osteoid is the organic portion of bone, a matrix initially laid by osteoblasts before mineralization and composed mostly of type I collagen fibers. Osteoid accounts for nearly 50% of bone by volume and about 40% by weight [31]. The organic matrix of type I collagen fibers is mineralized by the inorganic portion of bone tissue, hydroxyapatite. Hydroxyapatite, $Ca_{10}(PO_4)_6(OH)_2$, is the storage form of the minerals calcium (Ca^{2+}) and phosphate (PO_4^{3-}) in bone , accounting for the other half of bone by volume and about 60% by mass [31]. The combination of these two components contribute to the seemingly contradictory ability of bone to be both rigid yet flexible, and their relationship in bone tissue is commonly compared to the relationship

[7] The endosteum, while less fibrous, is similar to the periosteum; it provides a well vascularized membrane to link systemic circulation and the surface of bone. The endosteum also covers the endosteal surfaces of the haversian and volkmann canals.

between concrete and rebar. Unlike concrete and rebar, bone tissue is embedded with a host of living cells that facilitate the ongoing remodeling of bone, both for the purpose of calcium metabolism and for adapting to external load.

Osteoclast

Osteoclasts act as the main catabolic cells in bone, removing osteoid and mineral for the purpose of liberating calcium from the bone matrix to maintain homeostasis, or for removing damaged bone. Osteoclasts originate from hematopoietic, mononucleated precursors and upon activation of these pre-osteoclasts, they will combine to form a large multinucleated osteoclast cell [32]. Osteoclasts are characterized by their numerous nuclei, ruffled border, and the creation of a howship's lacuna [8]. Activation and regulation of osteoclasts is a topic of much discussion in bone biology as the deregulation of osteoclast via estrogen deficiency during menopause plays a large role in post-menopausal osteoporosis [33], [34]. In general terms though, osteoclasts are activated by macrophage colony stimulating factor (M-CSF) produced by stromal cells and RANKL expression on pre-osteoblasts. Once an osteoclast has attached to the bone surface via integrin proteins, a sealing zone is created and the ruffled border of the osteoclast initiates resorption by releasing protons and proteases for the purpose of breaking down collagen protein to release hydroxyapatite, and then breaking down hydroxyapatite to liberate calcium [32]. The fate of an osteoclast is apoptosis at the end of the resorption phase of remodeling.

[8] A Howship's lacuna is the cavity created during the resorption phase of bone remodeling by osteoclast, which in most cases is re-filled with osteoid and re-mineralized during the reversal phase.

Osteoblast

Osteoblasts are the main anabolic cell in bone tissue. They originate from stromal cells near the remodeling site that proliferate into pre-osteoblasts and then grow into mature mononuclear osteoblasts. As it pertains to the remodeling cycle, osteoblasts act to refill the area excavated by osteoclast with osteoid which is re-mineralized over a period of months or years [35]. Under non-remodeling conditions, or quiescence, proliferation of stromal cells into pre-osteoblasts is inhibited by sclerostin [36]. Without sclerostin inhibition, proliferation into pre-osteoblasts occurs along with release of M-CSF from stromal cells. The development of pre-osteoblast into mature osteoblasts is regulated in some part by signaling molecules produced by osteoclasts and factors released from the resorbed bone matrix [37]. Mature osteoblasts limit the development of new osteoclasts via osteoprotegerin (OPG) production [9] [38], refill the howship's lacuna with new osteoid, and assume one of three fates: development into osteocytes, development into bone-lining cells, or apoptosis.

Osteocyte

Osteocytes are terminally differentiated osteoblasts that are embedded in the bone matrix during the formation phase of the remodeling cycle. While an osteocyte occupies its own lacunae, or cavity, filopodial processes extended to other osteocytes via canaliculi, creating a network of mechanically sensitive cells that communicate strain between each other and with the bone surface. In cortical bone, the network of osteocytes is organized in circular lamellae around haversian canals; in trabecular bone, lamellae are organized from the deep center portion of the trabecula to the

[9] OPG inhibits activation of pre-osteoclast by blocking RANKL signaling.

superficial outer layer. In both cases, these networks allow strain and damage within the bone matrix to be communicated to the surface nearest the endosteum or periosteum. In a quiescent bone matrix, osteocytes secrete sclerostin which inhibits the initiation of a remodeling cycle [39]. However, if damage to the bone matrix or sufficient lack of mechanical loading occurs, osteocytes undergo apoptosis [39]; they no longer secrete sclerostin and release inflammatory factors upon death, the first step in recruiting osteoclast for bone resorption. Its not clear how damage to the bone matrix is relayed to the osteocyte in order to initiate apoptosis, but it is likely caused by physical damage to the osteocyte, its mechanosensing capacity, or a combination of the two [40]. It's worth mentioning that the likely mechanism for mechanosensing seems to be membrane bound mechanoreceptors responding to interstitial fluid flow between osteocyte cell walls and the walls of the lacuna and canaliculi they reside in [41], [42].

Bone Lining Cells

Bone lining cells are another set of further differentiated osteoblasts that line the border between the bone matrix and endosteum, or periosteum, of the quiescent bone matrix; however, actions of bone lining cells are not always clear in the literature. In the mid to late 80's bone lining cells were identified by their flat, elongated morphology and shown to prep the bone surface for interaction with osteoclasts [43], further, the canaliculi penetrating processes of lining cells were hypothesized to play a role in communication with osteocytes [44]. Research in the last twenty years has continued to reveal the functions of bone lining cells which have been shown to play a preparatory role in removing waste created by osteoclast before osteoblasts begin re-filling the Howship's lacuna [45] and that bone-lining cells are not terminally differentiated, able to

return to the morphology and action of osteoblasts [46]. Bone lining cells also demonstrate capacity to assist in the regulation of osteoclast via OPG production [47], which isn't surprising considering bone-lining cells are not terminally differentiated and osteoblasts also produce OPG. An especially interesting scientific finding regarding bone-lining cells is their role in creating the 'canopy' of the bone remodeling compartment (BRC) [48], as this finding hints at a direct link between the surface of the bone matrix and systemic circulation.

BMU and BRC

Bone molecular unit (BMU) was the common nomenclature for referencing the cells that are responsible for the maintenance and remodeling of the bone matrix before the year 2001. While use of BMU is still present in recent literature, it is now also common to have the area of remodeling referred to as the bone remodeling compartment (BRC). The use of the acronym BRC when referring to the area of ongoing remodeling is directly tied to the work of Hauge et. al. in 2001. Hauge demonstrated that cells expressing osteoblastic markers, or bone lining cells, were responsible for creating a canopy over the area of bone matrix undergoing a remodeling cycle [48]. While this work only confirmed the presence of this canopy in trabecular bone, Hauge et. al. [48] and other researchers [49] have suggested the canopy may exist in cortical bone as well, albeit without direct evidence. Additionally, Hauge mostly discussed the morphology of the BRC in relation to the remodeling phase of the underlying matrix; the paper had a comparatively small paragraph discussing the potential vascular implications. Vascular implications were expanded on in a review paper by a colleague named Erikson five years later [49].

Vascular Role in Bone Metabolism

Bone seems static to the uninitiated, but we are initiated [50], having an understanding of the metabolic activity of the cells that help direct mineral homeostasis, respond to mechanical stress and structural damage, and ultimately shape the microarchitecture of cortical and trabecular bone. The vascular role in the actions of bone cells has not yet been discussed in detail but have been hinted at in the above paragraphs. For cortical bone especially, there are hints towards the necessity of vasculature in bone construction and remodeling, as even when the need for compact, dense tissue is evident, space is made for blood vessels and the microarchitecture is oriented around them [51]. The link between bone and vasculature has been explored for at least 50 years [52], but has been especially active in the last 20 years, which is partly related to the work of Hauge and his colleges. While the hemopoietic origins of osteoclasts and osteoblasts have been recognized since 1986 [53] and 1991 [54] respectively, the identification of the BRC as a vascular space could help direct research in this area further. There are also some interesting relationships between angiogenic factors and bone [55]; for example, vascular endothelial growth factor (VEGF) plays a role in regulating both anabolic and catabolic portions of the remodeling cycle [56]. Of particular interest, changes in vascular morphology have been observed in correlation with bone morphology in response to loading and unloading [57], and aerobic exercise in mice [58].

Systemic Control of Bone Metabolism

The major storage site for calcium and phosphate is bone, but both minerals have widespread physiological functions. Calcium has significant roles in muscle

contraction, neural transmission and blood coagulation [59]; phosphate has roles in energy metabolism and cell regulation via protein phosphorylation [60]. Both minerals are transported around the body via systemic blood circulation and while both serum calcium and serum phosphate are regulated, direct maintenance of serum phosphate does not impact bone tissue and is instead directed at renal phosphate resorption [59]. Maintenance of serum calcium is directed at bone tissue, where low serum calcium prompts release of parathyroid hormone (PTH) into systemic circulation and high serum calcium prompts release of calcitonin [10]. PTH is produced by the parathyroid gland in response to low calcium concentrations in the blood, initiated by calcium sensing receptors without a calcium antagonist [59]. PTH acts on osteoblast, where high PTH concentrations reduce the capacity of osteoblasts to inhibit osteoclasts [59]; osteoclast activity increases, liberating calcium and phosphate into systemic circulation via the catabolism of hydroxyapatite. Along with attempting to increase serum calcium via de-inhibition of osteoclasts, PTH also acts on the kidneys to decrease calcium excretion [59], [60] and as a significant up regulator of the enzyme responsible for activating vitamin D [61]. While the direct effect of PTH on bone mineral is deleterious, its indirect effect as an activator of vitamin D spares bone mineral from resorption by increasing calcium absorption in the gastrointestinal tract. Vitamin D also spares bone by reducing osteoclast activation via RANKL [59], but the mechanism isn't yet clear [62]. Hormones not directly tied to mineral homeostasis also affect bone via systemic means. Although not considered to be majorly calciotropic, the following hormones are recognized: glucocorticoids, estrogen, growth hormone, thyroid hormones, and insulin [60]; in

[10] Calcitonin acts in inverse to PTH with respect to the bone, kidneys, and GI tract but does not seem to have a major effect in humans [34], [59]

general, all aforementioned hormones, except glucocorticoids, promote skeletal growth [59]. The impact of glucocorticoids on bone is typically seen as negative, especially when taken exogenously [63].

Measuring Bone

A large portion of the information used to outline bone biology in the above section of this paper is based on in vitro work, such as examining the effect of estrogen on osteoclast function in culture, or ex vivo work, such as histological imaging of trabecular bone to define the BRC. A large portion of the information to be presented in future sections of this paper will include in vivo and ex vivo measures of BMD and bone quality[11], as both are predictors of bone strength and fracture risk [16], [17], [19], [22], [64], [65]. Dual-energy x-ray absorptiometry (DXA) derived BMD will be especially common as much of the research examining the effects of exercise on bone are determined by comparing DXA scans between sedentary and active cohorts, or by comparing DXA derived BMDs pre and post exercise intervention to determine the intervention's efficacy. While not as common, quantitative computed tomography (QCT), high-resolution peripheral QCT (HR-pQCT), and Micro-CT (qCT) are also used to derive BMD, along with other image/model derived measures of bone quality. Additionally, trabecular bone score is a relatively new measure derived from DXA scans that attempts to estimate the quality of trabecular bone at the hip or spine [66]. DXA and CT derived measures of bone density and quality are outlined below (Table 5.1).

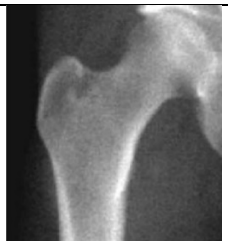
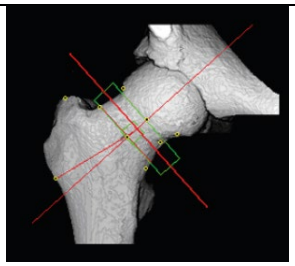
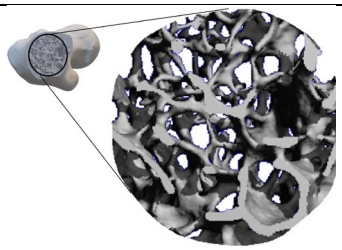
[11] Measures of bone quality are measures of morphology or mechanical properties that predict bone strength independently of mineral density.

2.3 Exercise and Bone

Early Research and General Relationships

Research on exercise and bone started around the 1970's and included research in rodents, pigs, and humans. Research in rodents and pigs were intervention based, determining the effects on bone before and after an exercise intervention [68], [69]. Early research in humans was cross-sectional, comparing sedentary controls to athletic populations [70], [71]. The aforementioned research demonstrated a link between exercise and bone that was generally positive [68]–[70], minus the examination of stress fracture in athletes [71]. Although, research examining stress fractures in various cohorts of athletes hinted at a relationship between exercise, bone, and loading, as stress fractures were not reported in swimmers [71]. Additionally, there was research comparing bone in the playing and non-playing arms of tennis athletes [72]; this research is commonly mentioned when describing the effects of exercise on bone as it demonstrates that the positive relationship between bone and exercise is specific to area of tissue being loaded. Research in exercise and bone continued into the 80's and 90's, when DXA became an especially prevalent measure of interest in exercise, not only for its ability to measure bone density but also body composition. Further, the specific loading factors that modulate bone, which are not necessarily tied explicitly to aerobic or resistance exercise, were better defined. These factors are outlined below (Table 2.2) and include strain magnitude, strain tensor, strain rate, cycle number, strain distribution, strain gradients, and fluid flow [73].

Table 2.1. Comparison of imaging methodologies for assessing bone mineral density and/or bone quality.

Sample Image/Model	Method	Measures	Benefits	Downsides
	DXA	aBMD (TBS)	-commonly used -relatively quick -very small radiation dose - (TBS provides an estimation of bone quality) -in vivo	-produces a 2D estimation of density -lacks estimation of bone quality (w/o TBS)
	QCT	vBMD Ct. Th	-volumetric BMD (less variation than areal) -some appreciation for bone quality -small radiation dose -in vivo	-doesn't provide much more than DXA
	HR-pQCT	vBMD Ct. Th Tb. Th Tb. Sp Tb. N	-volumetric BMD -substantially more appreciation for bone quality -decent resolution to assess trabecular morphology -in vivo	-Higher radiation dose limits scans to the peripheral bone tissues (limbs). -analysis is time consuming
	qCT	tBMD Ct. Th Tb. Th Tb. Sp Tb. N Tb. Conn.	-true measure of bone tissue density -higher resolution allows additional quality assessment of trabecula	-mostly ex vivo -high radiation dose limits in vivo testing to rodents - small samples (<100mm) -analysis is time consuming

DXA= dual-energy x-ray absoptiometry, TBS= trabecular bone score, QCT= quantitative computed tonometry, HR-pQCT= high-resolution peripheral QCT, qCT=micro CT, aBMD= areal bone mineral density, vBMD= volumetric bone mineral density, tBMD= tissue bone mineral density, Ct. Th= cortical thickness, Tb. Th= trabecular thickness, Tb. Sp= trabecular spacing, Tb. N= trabecular number, Tb. Conn.= trabecular connectivity

Note. Information for table was compiled partly with information from Donnelly 2011 [21], and Cambell and Sophocieous 2014 [67].

Table 2.2. Factors that describe the mechanical environment of bone.

	Description	Relationship to Bone	References
Strain Magnitude	Change in bone length or angle in response to load	Larger strain magnitudes are generally more osteogenic.	Qin et al., 1998
Strain Tensor	Direction and type of strain on bone (torsion versus compression)	Bone has variable responses to different directions and types of strain.	Rubin et al., 1996
Strain Rate	Change in bone length or angle per unit of time	Higher strain rates are generally more osteogenic.	Lanyon et al., 1984 Lamothe et al., 2005
Cycle Number	Number of times a strain is applied and subsequently removed	There is a limited number of cycles that will elicit a continued osteogenic response. (Changes inversely with strain magnitude)	Umemura et al., 1997 Robling et al., 2002 Gross et al., 2004
Strain Distribution	Variability in strain tensor	Higher variability in the types of strain leads to a higher osteogenic response.	Rubin et al., 1987
Strain Gradients	The variability in strain observed throughout a bone in response to overall strain	Sub-regions of bone under strain are paradoxical with respect to osteogenic response. Sub-regions near highly strained regions may benefit more than the higher strained area which may be related to fluid flow.	None listed
Fluid Flow	The change in flow of bone interstitial fluid in response to strain	Changes in intestinal fluid flow are connected to bone's response to loading, although the mechanism is not clear.	Rubin et al., 2006 Qin et al., 2003 Stevens et al., 2006

Note. Information and references for this table came from the section titled 'Regulation of bone morphology by mechanical stimuli' in:
S. Judex, J. Rubin, and C. T. Rubin, "Mechanisms of Exercise Effects on Bone Quantity and Quality," in Principles of Bone Biology, Two-Volume Set, 2008.

While the factors that describe the modulation of bone are useful when providing context for changes in bone density and morphology in response to loading, there is still a place for describing the effects of various types of exercise on bone. Regular physical activity (PA) and exercise provides significant benefits to health and wellness across the life span [11] and guidelines for PA are recommended to healthy adults [74], with adjustments when addressing older adults [24]. It is within the context of exercise being necessary for general health and wellbeing that we examine the effects of exercise on bone, examining how different modalities of exercise specifically affect bone and using that information to improve exercise guidelines more generally. For example, the American College of Sports Medicine released a position stand in 2004 on physical activity and bone health [75]. The position stand sought to examine the relationships between various modalities of exercise and bone, while also considering the mechanical mechanisms involved in bone remodeling. These findings are outlined in table 2.3, where both aerobic and resistance exercise are favorable when compared to sedentary behavior, and resistance exercise is especially effective at improving BMD. The findings from the 2004 position stand discussing bone were incorporated into future, more general, exercise guidelines for adults and older adults [24], [74].

Older Adults, Exercise and Bone

Decreases in bone mineral density and detriments in bone quality are associated with decreased bone strength, increased fracture risk, and share an inverse relationship with advanced age; this is especially true for post-menopausal women and men past the 7th decade of life. Age related losses in bone mass are well appreciated in the literature, and supplementation, dietary changes, and exercise are recommended in

Table 2.3. General relationships between bone and physical activity

Modality	Effect	Comments	References
Zero G/Bedrest	↓↓	Based on microgravity and long-term bed rest	Giangregorio et al, 2002
Sedentary	↔↓	Based on age related decreases in BMD	none
Aerobic Exercise	↔	Walking and/or moderate intensity aerobic exercise Running, stair climbing, jumping more effective (See below)	Cavanaugh et al., 1988 Nelson et al., 1991 Chow et al., 1987 Mussolino et al., 2001
Additional load		Provides additional benefit	Snow et al., 2000 Petit et al., 2002
Reduced Volume		May decrease benefit	Kelley et al., 2000
Resistance Exercise	↑	Weight training	Pruitt et al., 1992 Braith et al., 1996 Menkes et al., 1993
Increased intensity(%1RM)		May provide additional benefit	Maddalozzo et al., 2000
Increased Volume		May provide additional benefit	Cussler et al., 2003

Arrows indicate a decrease (↓), no change (↔), or improvement (↑) in bone density.
Note. Information and references are based on bone and exercise relationships in adults as they are presented in Kohrt et al., 2004.

older adults to improve, or at least attenuate, the age-related declines in BMD and bone quality [14]. Exercise is of interest as it is not only associated with improvements in BMD [75] and potential improvements in bone quality [76], but also with increased muscle strength and balance [24]. Further, there are additional benefits to overall well-being as exercise improves cardiovascular health [24] and maintains independence in older adults [77]. Considering the projected increase in osteoporosis prevalence over the next 30 years, the appreciated benefit of exercise on general health in older adults, and the positive relationship between bone and exercise, exploring the specific

relationship between exercise and bone health in older adults may provide additional perspectives for addressing the projected increase in the prevalence and treatment of osteoporosis and fractures, while maintaining or improving the general benefits of exercise to older adults.

The overall effect of exercise on bone in older adults is similar to the effects on the general population (Table 2.3); in fact, general relationships between bone and exercise are partly based on research in older adults [75]. There is a substantial amount of literature examining the effects of exercise on bone in older adults and a large portion of this research has been aimed at postmenopausal women [78], although research in men does exist [79]–[81]. The results from the literature have been compiled and summarized into a handful of reviews that examine the results generally, systematically, or via meta-analysis. The content of these reviews on bone and older adults include outcome measures for bone density and bone quality, address postmenopausal women and older adult men, and include the broader modalities of aerobic and resistance training along with more specific modalities such as whole-body vibration (WBV) [78], [82] and swimming [83]. These reviews are discussed below and outlined in Table 2.4.

Gómez-Cabello et al. conducted a systematic review on the effects of exercise in older adults in 2012 [77]. This review included fifty-nine controlled trails examining the effect of aerobic, resistance, combination, and WBV training on regional [12] and whole-body bone mass in older adults from the year 1988 to 2011, of which a large portion of the participants were post-menopausal women [13]. In general, Gómez-Cabello et al.

[12] Lumbar spine, femoral neck, distal wrist, etc.

[13] Research examining the effects of exercise on bone mass of older adult females was predominant compared to research in men. This was especially true for aerobic exercise and bone.

found a beneficial effect of exercise on bone mass in older adults. The specific effects of aerobic training on bone mass were varied, based on the systematic analysis, but benefits favored aerobic modalities with added loading or impact, (stair climbing, jogging, weighted walking) compared to unloaded, (walking, swimming). The portion of aerobic training studies reviewed by Gómez-Cabello et al. were especially biased toward post-menopausal women, with only one study examining the effects of walking, cycling, and arm ergometry over 4 months on men and women [84]; no effect on bone mass was found. The sex disparity for resistance and combination training in the review were less substantial, although sex differences in response to these training groups were not addressed. Resistance exercise had the general effect of improving bone mass in older adults, but the variability in combination training trials were more pronounced. Disparity in the effects of combination training in this review, similar to the disparity in aerobic training, were based on the variability in the loading or impact characteristics of the modalities implemented. For all interventions reviewed by Gómez-Cabello et al., (aerobic, resistance, and combination) no changes were found in whole-body BMD or BMC; any benefit found in bone mass was regional. The presence of regional bone mass changes in the absence of whole-body changes may hint at systemic exchange of mineral between areas of the skeleton, although this could just be related to the higher resolution of regional scans compared to whole body scans. Considering age-related changes in bone mass in sedentary individuals, this systemic review [78] provides clear evidence for the efficacy of exercise training for the purpose of preserving bone mass as even walking interventions generally provided an attenuation of age-related bone loss compared to sedentary controls.

Table 2.4. Reviews examining the effects of exercise on bone in older adults

Reference	Review Type	# of papers	Exercise Modality	Effect on Bone	Bone Measures	Comments
Gomez-Cabello et al., 2012	Systematic	59	AER AER+ RES COM WBV SED	↔↓ ↔↑ ↔↑ ↔ ↑ ↓	BMC aBMD	A large portion of the papers reviewed were in post-menopausal women (especially for aerobic training).
Mikhael et al., 2010	Systematic	2	WBV SED	↔ ↔	vBMD ALP NTX	Primary goal was to review effects on muscle morphology (limited bone)
Harding and Beck 2017	General	7	COM JMP	↔ ↑	aBMD vBMD CSA Ct.area/Tot.area	Examined exercise effects on bone geometry, some standard measures were mentioned
Bolam et al., 2013	Systematic	8	AER RES JMP SED	↔ ↔↑ ↔ ↔	aBMD	Male only
Kemmler et al., 2018	Systematic	8	AER RES JMP SED	↔ ↔↑ ↔ ↔	aBMD	Male only 4 out 8 references were also referenced in Bolam et. al., 2013
Simas et al., 2017	Systematic Meta-Analysis	11	SWM AER RES SED	↔ ↔↑ ↑ ↓	aBMD	Swimming compared to: AER, RES, and SED

Exercise Modalities: AER=aerobic training (walking), AER+=aerobic training with some weight being component, RES=resistance training, COM=both aerobic and resistance, WBV= whole-body vibration, JMP=jumping, SWM=swimming, SED=sedentary controls
Effect on Bone: ↓=detriment, ↔=no effect, ↑=improvement
Bone Measures: BMC=bone mineral content, BMD=bone mineral density, aBMD=areal BMD, vBMD=volumetric BMD, ALP=serum Alkaline Phosphate (bone formation), NTX= serum Urinary N-Telopeptide X (bone resorption), CSA=bone cross-sectional area, Ct.=cortical bone, Tot.=total bone
Note. When the scope of a review paper is broader than older adults and bone, data not derived from older adults or related to bone is not included in the table (when possible)

Whole-body vibration (WBV) training is a novel form of training specifically aimed at bone. Rather than inducing a large strain at a low frequency to induce bone growth (resistance training), whole-body vibration training aims to exploit the inverse relationship between cycle number and strain magnitude by promoting bone growth via

rapid, small magnitude strains [73]. The effects of WBV on bone in older adults have been examined in the literature, and both Gómez-Cabello et al. [78] and Mikhael et al. [82] included literature on WBV training in their systematic reviews on older adults and exercise. Mikhael's review only included two controlled trials examining the effects of WBV exercise on bone in older adults as the main purpose of the paper was to examine the effects of exercise on muscle morphology. For one study, both lumber spine aBMD and markers of bone formation (Serum Alkaline Phosphatase) and resorption (Urinary N-Telopeptide X) were measured before and after WBV training [84]; the other study in Mikhael's review measured vBMD of the tibia [85]. Neither study demonstrated significantly different changes in response to WBV compared to controls. The findings of the studies in Mikael's review contrast with those mentioned in Gómez-Cabello et al., although the number of studies reviewed by Gómez-Cabello et al. was substantially larger, ten vs two. The general finding of Gómez-Cabello et al. for the effects of WBV on bone in older adults, was positive, with 7 out of 10 studies [14] demonstrating a positive effect on bone in response to WBV training compared to no change or a negative effect in the control group. Gómez-Cabello et al. summarized the effects of WBV on bone in older adults as being similar to resistance training but the effects cannot be translated to males as none of the studies on WBV included men [78].

Improvements in technology have enhanced capacity to examine in vivo measures of bone quality in humans using the methodologies outlined in Table 2.2. As these measures provide additional context for fracture risk, there has been a push to further explore the effects of exercise on bone quality, in addition to BMD, in older

[14] Two of the three studies demonstrating a no effect for WBV in Gomez-Cabello et al. were the studies referenced by Mikhael el. al.

adults. While some progress has been made, there is still a substantial difference in the number of papers exploring bone quality [76] in response to exercise in older adults versus BMD or other measures of bone mass [78] [15]. The aforementioned disparity is evident in a recent review paper by Harding and Beck [76] that explored measures of bone geometry, or bone quality, in relation to exercise. While Harding and Beck's review was broader than bone geometry, exercise, and older adults, they did explicitly address the topic area with two papers that examined the effect of impact loading, or jumping, on bone geometry in older adults and five that addressed a combination of aerobic, resistance, and/or impact loading exercises. In general, the effects on bone geometry in response to exercise in older adults discussed by Harding and Beck [76] were comparable to the changes in bone mass addressed by Gómez-Cabello et al.[78]. In the case of jumping exercise, positive, non-significant trends were demonstrated in the cross-sectional area of the femur [86], and significant increases in the ratio of cortical bone area to total bone area were found at the distal tibia [87]. While Gómez-Cabello et al. did not specifically review jumping exercise, the results from combination and aerobic training that included jumping exercise do hint at the positive effect of jumping on bone mass. With respect to combination training, the results on bone geometry reported by Harding and Beck were similarly variable to the results on bone mass reported by Gómez-Cabello et al. as the variability in the modalities under the 'combination' umbrella is inconsistent. A final similarity between the two reviews was the

[15] Harding and Beck were only able to find 7 papers related to bone quality and exercise in older adults compared to the 59 papers addressing bone mass, exercise, and older adults referenced by Gómez-Cabello et. al.

dichotomy in research with respect to sex, as the lack of research examining bone mass in males is similar in research examining bone quality.

Two systematic reviews, also outlined in Table 2.4, attempted to address the above mentioned dichotomy in research by exploring the effects of exercise on bone in older adult males specifically [79], [81]. Both reviews were compiled from a small pool of viable research, each only including eight studies. The lack of literature in this area is further exemplified by the overlap in the two studies, where four out of the eight studies included Kemmler et al., 2018 were also addressed in Bolem et al., 2013 [88]–[91]. More concerning, only one study out of the twelve reviewed by the combined efforts of Kemmler et al. and Bolem et al. demonstrated a difference between exercised males and controls [91]. The one study that did demonstrate a positive effect on bone in older adult males only found a modest improvement in aBMD when implementing progressive resistance training with additional weight bearing impact activities. Compared to the other studies reviewed by Kemmler et al. and Bolem et al., there are no clear differences in modality that explain the singular benefits described by Kukuljian et al. nor are there clear explanations for the disparity between the general response of bone in older adult men[79], [81] compared to postmenopausal women [78].

The overall response of bone to exercise in older adults is generally positive. There are some clear benefits to be derived, at least with respect to attenuating age-related declines in BMD [78] and bone quality [76]. Small benefits are even found in non-load bearing exercise such as swimming, although aerobic exercise such as running provides additional benefit, with the largest benefit being derived from resistance training [83]. Of course, the above effects can only be confidently ascribed to

postmenopausal women, as research examining the effects of exercise on bone mass and quality in older adult males is extremely limited [79], [81] with only one study describing a positive relationship (compared to control) in response to progressive resistance training [91]; a result that isn't mirrored in other studies implementing resistance or impact exercise in older adult males[79], [81]. Future research exploring the effects of exercise on bone in older adults should attempt to add to the literature associated with older adult males and bone quality. Overall, research examining the effects of exercise on bone quality is lacking, more so in older adults, and even more so in older adult males. Additional research on bone quality in older males will not only address the lack of studies on the topic of exercise, bone, and older men, but may also provide some additional context for the lack of changes in bone mass in response to exercise described in this population. Considering the consistently positive effect of WBV training in postmenopausal women [16], a study comparing the effects of WBV training in males versus females on bone quality in older adults could help push science in this area further.

T2DM, Exercise and Bone

Type II diabetes mellitus (T2DM) is a chronic disease well appreciated for its negative effect on glucose regulation [92] as well as its social and economic cost [93]. The negative effects insulin insensitivity and hyperglycemia have on cardiovascular, metabolic, and neurological health have been clear for decades [92] but the relationship between T2DM and bone is only recently being elucidated [94], [95]. In general, individuals with T2DM have non-osteoporotic BMD but exhibit increased risk of fracture

[16] To my knowledge, there is still no research examining the effects of WBV training on bone in older adult males.

compared to the healthy population, especially at the hip [96], [97], a relationship now being referred to as ‘sweet bone’ [98]. Projections indicate the worldwide prevalence of diabetes is expected to increase by 50 percent from the year 2010 to 2030 [99], adding to the already substantial projected increased incidence of fracture discussed in the introduction of this review in the context of osteoporosis [1].

The effect of exercise on individuals with T2DM has been generally accepted as providing substantial benefits [100], and research in the last decade has provided additional evidence for the efficacy of combined aerobic and resistance training in this population [101]. While the effects of combination training don’t specifically translate from T2DM to bone, the generally positive relationship between bone and exercise in the normal adult population [15] does suggest a potential additional benefit in prescribing exercise to individuals with T2DM. In order to explore this relationship, original research examining the effects of exercise on bone in individuals with T2DM or animals expressing a T2DM phenotype were searched for using PubMed Central® and Google Scholar. The literature search produced eight original research studies spanning from the year 2004 to 2019 and included two articles on exercise, bone, and individuals with T2DM [102], [103], and six articles on exercise, bone, and rodents with T2DM [104]–[108].

De Luis Román et al., 2004 [102] and Daly et al., 2005 [103] were the first studies published examining the effects of exercise on bone in individuals with T2DM; of the eight research articles published in the topic area, De Luis Román et al. and Daly et al., are the only studies that examine human populations. While the publication dates and study cohorts are similar, no other commonalities exist between these original

research papers besides their description of a generally positive relationship between exercise and bone. De Luis Román et al. implemented a cross-sectional study to explore the relationships between BMD, dietary intake, and physical activity. The study included 92 males and females with T2DM; all participants tracked their diets for three days, described themselves as physically active or inactive, and had a measure of calcaneal BMD taken via a Pixi-Lumar x-ray scan. The main results presented in the study were the positive correlation between calcium intake and BMD, along with significantly higher calcaneal BMD in those who were considered physically active. While not an especially intricate study, De Luis Román et al., 2004 was the first to demonstrate a positive relationship between BMD and physical activity in individuals with T2DM, a result that translates to the normal adult population [75].

Research conducted by Daly et al., while published only a year after De Luis Román et al., was a substantially more involved study with a 12-month exercise intervention. A cohort of older adult, overweight individuals with T2DM were randomly assigned to two groups, resistance training plus weight loss (RT+WLoss) or weight loss (WLoss). Both groups followed a dietician prescribed diet plan with a slight caloric deficit for 6 months, while the RT+WLoss group also participated in a moderate to high intensity, whole-body resistance program. After 6 months, diet restrictions and exercise guidelines were made voluntary and the RT+WLoss group was provided the necessary equipment to continue exercising from home, if they chose to do so. DXA derived measures of whole-body, lumbar spine, and femoral neck BMD were taken at baseline, 6 months, and 1 year. Results from Daly et. al demonstrated a protective effect of resistance exercise on what was presumed to be weight loss derived reductions in bone

mineral density, but may have been an exercise induced reduction in the paradoxical relationship between BMD and fracture risk in individuals with T2DM, where T2DM produces an about or above average BMD that doesn't translate to a reduction in fracture risk [95]. The inclusion of an overweight, exercising control without T2DM could have provided additional context, but it's no surprise that this caveat wasn't considered, as the paradoxical relationship between BMD and fracture risk in individuals with T2DM had not yet been clearly described in the literature [95].

While the only literature examining the relationship between T2DM, bone, and exercise in humans are the two listed above, there are additional studies that examine the relationship via rodent populations. Research by Ikedo et al., 2019 is an especially recent contribution to the literature that provides further context for the relationship between bone, resistance exercise, and T2DM utilizing a cohort of naturally diabetic Otsuka Long-Evans Tokushima Fatty (OLETF) and non-diabetic Long-Evans Tokushima Otsuka (LETO)[17] rats to examine the effects of resistance training on bone [104]. All rats included in the study were resistance trained three days per week over a six-week period via electrically stimulated contraction of the right leg gastrocnemius. The left leg of each rat acted as sedentary control and after 6 weeks of resistance exercise, right and left tibias were dissected and analyzed using qCT and histology to determine differences between the exercised and non-exercised tibias across cohorts. Results from Ikedo et al. show non-exercised tibias from OLETF rats have higher BMD and bone area compared to LETO rats, but also have worse osteon morphology based on histology results, which may be related to increase fracture risk with T2DM. Further,

[17] Otsuka Long-Evans Tokushima Fatty (OLETF) rats and KK-Ay mice express a T2DM phenotype. (LETO) are commonly uses controls to OLETF rats.

comparisons between non-resistance trained and resistance trained tibias were consistent in both OLETF and LETO rats, with higher BMD and bone area, and better microarchitecture and osteon morphology in the resistance trained tibias.

Unlike resistance exercise, the effects of aerobic exercise on bone in T2DM completely lacks research in humans and only includes studies on rodents. Further, two of the five papers in this area examined the effect of voluntary aerobic exercise [107], [108], which likely wouldn't promote as large of a training stimulus compared to a more purposeful intervention. Still, there were some interesting findings. For example, Takamine et al., 2018 did not find any bone related differences in voluntarily exercising and non-exercising rats, but did report lower break force and a lack of stiffness in the femur of OLETF rats compared to LETO rats [108], perhaps relating to the relationship between increase fracture risk and BMD in individuals with T2DM. Minematsu et. al, 2016 also implemented a voluntary exercise intervention where OLETF rats allowed to exercise were compared to non-exercising OLETF and non-exercising LETO rats. The longer duration implemented in Minematsu's study showed higher BMD and BMC, along with better bone quality and mechanical properties in exercising OLETF rats compared to non-exercising OLETF groups, but this study would have been more comprehensive if there was a group of exercising LETO rats, as it would have allowed a more direct comparison of the effects of exercise on bone in populations with and without T2DM.

More purposeful aerobic exercise interventions were implemented by Ortinau et al, 2017 [106], Takagi et al., 2017 [105], and Pezhman et al., 2019 [109]. Ortinau et al. had OLETF rats exercise at a moderate intensity for one hour, five days per week for 12

weeks and compared these trained rats to sedentary OLETF and LETO rats. Differences between the trained and non-trained OLETF rats were substantial, with trained OLETF rats having denser bone, better measures of both trabecular and cortical bone morphology, and better mechanical properties. Differences between trained OLEFT rats and sedentary LETO rats were less substantial, and the lack of a trained LETO group did not allow for comparisons of the effects of exercise on OLETF and LETO rats. Takagi et al. also examined the effects aerobic exercise in rodents with T2DM , although KK-Ay mice, rather than OLETF rats, were used [105]. The unique contribution of this study is the comparison of higher intensity, lower duration aerobic exercise to lower intensity, longer duration exercise. KK-Ay mice trained with lower intensity, longer duration exercise had higher BMD than KK-Ay mice trained with higher intensity, lower duration exercise, the latter group having similar BMD compared to sedentary mice. While measures of bone quality were included in the analysis, no differences were found between groups. The last study examining the effects of structured aerobic exercise on bone in rodents with T2DM compared trained and non-trained, diabetic and non-diabetic rats after a 10-week swimming exercise intervention. While measures of bone density and morphology were not utilized in this study, Pezhman et al. did measure protein and gene expression associated with bone formation and resorption at the tibia. In general rodents, both diabetic and non-diabetic, trained with swimming exercise over 10 weeks had improved measures of bone formation vs resorption compared to non-trained, but the differences between the diabetic groups were blunted.

Overall, the literature examining the effects of exercise on bone in T2DM demonstrates a clear, positive relationship between exercise and bone in individuals or rodents with T2DM. It's not clear, however, whether resistance exercise or aerobic exercise provides a more substantial benefit on bone in T2DM, as no study to date has explicitly compared the two, and the body of research as a whole is not large enough to make general comparisons between aerobic and resistance exercise. If the effects of exercise on the general and older adult population were to be extrapolated to the population of individuals with T2DM, there would be an expectation of larger improvements in BMD in response to resistance exercise compared to walking or running, but it's not clear that BMD is the best consideration when recommending exercise to improve bone health in T2DM.

Considering that BMD is not as representative of fracture risk in T2DM [97], there is a need to utilize other measures of bone health when examining the effects of exercise on bone in humans with T2DM and to utilize direct measures of bone strength when examining rodents. For example, only two studies examine the relationship between bone, exercise and T2DM in humans, and the only measure of bone in both studies is BMD [18], a measure appreciated for its role in predicting bone strength but shown to be unreliable in doing so for diabetic individuals [97]. Future research on bone and exercise in human populations may benefit from including measures such as trabecular bone score (TBS) [19], as it has been shown to be predictive of fracture risk in type II diabetic populations [110] and requires little extra work when added to a standard

[18] The disordered relationship between BMD and fracture risk for T2DM had not yet been described in the literature.

[19] Discussed on pg. 12 and in Table 1.

DXA scan. Specific to research on bone, exercise and T2DM in rodents, the utilization of direct measures of bone strength, or mechanical properties, added additional clarity to the above-mentioned studies done by Ortinau et al., Takamine et al., and Minematsu et al., where differences in bone density or quality found in these studies were reinforced by appropriate changes in bone strength [106]–[108]. The study by Ikedo et al., while strengthened via the comparison of exercise on osteon morphology in OLETF and LETO rats [104], would have been more effective if there was a direct measure of the strength of the tibias.

Future research should also consider the effects of combined training on bone in T2DM. While a combination of resistance and aerobic exercise did not demonstrate improved bone health in older adults in comparison to resistance training or impact exercise [76], [78], the effects of combination training on bone in T2DM may provide additional benefits. Bone in T2DM is affected by the mechanical stimulation of exercise directly, and secondarily by the effects of exercise on improving insulin sensitivity and glucose regulation. A combination of resistance and aerobic exercise has been shown to be more effective, than aerobic or resistance exercise alone, at mitigating the systemic effects of T2DM based on more pronounced reductions in hemoglobin A_{1c}, and a reduction in the need for diabetic medications[101]. Fortunately, there is an ongoing clinical trial examining the effects of a two-year exercise intervention on TBS, where the exercise treatment is a combination of aerobic, resistance, and impact training. The trial is referred to as the SWEET-BONE study and its estimated completion date is the end of 2025.

Ketogenic Diet, Exercise and Bone

A ketogenic diet (KD) increases conversion of fat into ketones as an alternative to using blood glucose. This is accomplished by reducing carbohydrate consumption to levels low enough, and for long enough, to induce ketogenesis, the production of ketone bodies, which act as an alternative fuel source for most cells and tissues, minus red blood cells and the liver [111]. Adopting a KD is becoming increasingly popular, partly due to favorable impacts on blood glucose and body composition [111]. Further, there is research supporting the effectiveness of utilizing the effects of a ketogenic diet to avoid obesity and metabolic disease [112]; a 2005 study also examined the effectiveness of a ketogenic diet on individuals with T2DM with some favorable results on hemoglobin A_{1C} and weight reduction [112]. The original role of the ketogenic diet was in the management of epilepsy and was used in 'addition to' or as a 'proxy for' anti-convulsant medication [113]. While the positive effects of a KD were evident in epileptics, some negative effects were outlined, namely, a negative effect on mineral homeostasis.

The anti-convulsive effects of a KD were outlined in 1976 [113], and with short turn-around, another paper addressed some potential negative effects of a KD. Hahn et al., 1979 outlined the potential negative effects of the KD by comparing a group of control children to two groups of children receiving anti-convulsant medication, one on a KD and the other on a standard diet [114]. The results of this study showed controls to have bone mass nearly equal to age normal values and significantly higher than both the anti-convulsant group and the KD plus anti-convulsant group. The group of children on a KD and anti-convulsant drugs had the lowest bone mass. They also had a blood pH of 7.31, which is considered physiological acidosis and is tied to increased bone

resorption [115]. Hahn et al. was not specifically looking to discredit the KD, but simply noted that children taking anti-convulsive medication and adopting a KD should supplement with vitamin D [114]. Bergqvist et al., 2008 added to the literature on epileptic children and KD by reporting a worsening Z-score over a 15-month period, but again supplementation was suggested by the author as the diet was effective in addressing the symptoms of epilepsy [116]. More recent studies in 2017 [117] and 2019 [118] also examined the relationships between a KD, bone, and epilepsy, finding similar negative effects on BMD and BMC. The 2017 study also found a relationship between mobility and the KD where more mobile portions of the epileptic cohort had less deleterious effects on bone in response to a KD [117].

The major factor limiting the above studies is the lack of a non-epileptic control group. This isn't surprising as the research above was not aiming describe the negative impact of the KD in the normal population, but to share results for the purpose of improving outcomes for epileptic children. While a control group and a group of non-epileptic children would have provided additional context, there is also an ethical issue in prescribing, to children, a diet recognized for its positive effects on epilepsy but also for its deleterious effect on bone. However, there are studies on rodents that aim to provide more context to the bone loss observed in epileptic children. To date, there have been five studies on rodent bone in response to a KD [119]–[123]. The studies demonstrate a consistently negative effect of the KD on rodent bone, with both cancellous and cortical bone morphology being negatively impacted [120]. Further, the KD in rodents also seem to impact mineral digestion, reducing the amount of calcium absorbed compared to the amount of phosphorus [123], indicative of downstream

negative effects on bone metabolism, as detriments to blood calcium are the main driver of PTH production and resulting bone resorption [35].

Studies on non-epileptic, adult populations are sparse, with one study examining the effect of a ketogenic diet in healthy adults that measured a bone related parameter, which also happened to include a 12-week exercise intervention. McSwiney et al. examined the effects of a ketogenic diet and exercise training on body composition in endurance athletes [124]. After a 12-week KD and exercise intervention, there were no changes in whole-body BMD before and after the intervention, nor were there differences between the KD and control diet groups. Since the above-mentioned study was the only study to examine a KD, exercise, and a bone measure, it needed to be discussed in this literature review, but the study is far from optimized to examine the effects of a KD on bone, nor the relationship between a KD, bone, and exercise. Most importantly, with respect to the exercise effects, there wasn't a sedentary control group for comparison. The 12-week exercise duration also wasn't sufficient for measuring changes in bone, as research examining modulations in BMD or bone quality in humans typically span at least a 4-months to encompass the estimated time of a full remodeling cycle, not that changes in BMD are impossible over a smaller time window. Of course, the goal of McSwiney et al., was more related to examining the effects of a KD on athletic performance, and whole-body BMD was just included with their measures of general body composition.

The only other study to examine the effects of exercise and ketogenic diets on bone utilized serum markers to determine the acute effects of exercise. Heikura et. al. implemented a 3.5-week, well-controlled, dietary intervention, where endurance athletes

were fed a low carb, high fat (LCHF) or high carbohydrate (HCHO) diet [125]. Athletes maintained their standard training over the 3.5-week dietary intervention that ended in a single bout of exercise at 70% of VO_2 max, where serum markers of bone resorption (CTX) and bone formation (P1NP) were measured 2h prior to exercise, immediately before exercise, immediately after exercise, and 3h post exercise. Compared to baseline, pre-intervention levels, serum CTX was significantly higher and serum P1NP was significantly lower prior to exercise. P1NP did not change during exercise for either group, but area under the curve was lower for the LCHF group. Serum CTX rose significantly for the LCHF group in response to exercise and returned to pre-exercise levels 3h post. These data support a potentially negative effect of ketogenic diets on bone, where the acute catabolic effects of exercise are further exacerbated by a high fat, low carb diet.

The effect of a ketogenic diet on bone has been shown to be deleterious in rodents, and in epileptic children, but the effects of the KD on bone in healthy adults is certainly not clear. While Heikura et. al. did show that a ketogenic dietary intervention had negative implications with respect to acute exercise in endurance trained athletes, there is not enough evidence that a ketogenic diet would increase fracture risk in otherwise healthy humans [125]. Physiological blood acidosis in children with epilepsy [114] may partially explain the negative effects of a KD on bone in that population, but research examining blood pH in both athletes [126] and obese individuals [127] eating a ketogenic diet demonstrate values well within normal ranges and certainly not physiologically acidic. There may be some relationship between simply high fat consumption and a deleterious effect on bone, as there is research showing high fat,

non-ketogenic diets have a negative effect on rodent bone [128]–[130] , similar to a KD. Considering the popularity of KDs recently, there is efficacy in exploring the potential negative effects, not to stop people from adopting the diet, but perhaps prompting recommendations to supplement vitamins and minerals if research demonstrates a negative effect of the KD on bone in humans.

Conclusion

Improvements in both technology and our understanding of bone have substantially expanded both the capacity to and purpose for exploring this tissue. Exercise is uniquely positioned in bone research, as the 'leader board' for people killing is dominated by chronic disease, and osteoporosis prevalence is expected to continue growing, both problems being modifiable by exercise. The same is true for areas like T2DM as exercise both positively effects the impaired bone and the disease condition. There could be a similar case made for a KD, as the diet may not be ideal for BMD or bone quality, but exercise may play a protective role in bone while also improving general health and wellbeing. The relationship between exercise and bone is clearly positive and continued research in this area would be useful. Further research, just in the areas mentioned in this literature review, would provide substantial benefits toward reducing fracture risk, better managing the aging process, the treatment and management of osteoporosis, and mitigating the potential negative effects of a diet adopted to decrease weight and improve metabolic health.

2.4 Notes

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CHAPTER 3. BONE MINERAL DENSITY IN INDIVIDUALS WITH TYPE 2 DIABETES AFTER EXERCISE TRAINING: RESULTS FROM HART-D

3.1 Introduction

The glucose dysregulation associated with type II diabetes mellitus (T2DM) has far reaching effects; comorbidities such as cardiovascular disease and neuropathy are expected [1], but osteoporosis, typically diagnosed by low bone mineral density (BMD), is not commonly included, despite the fact that individuals with T2DM have higher than normal incidence of fracture [2], [3]. Bone mineral density and fracture-risk typically share an inverse relationship, as increased BMD is positively correlated with bone strength [4], [5], and stronger bone is less likely to break. However, BMD in individuals with T2DM overestimates bone strength as those with T2DM are shown to have a BMD slightly higher than the normal population but their bones are less resistant to fracture [3]. The lack of strength is thought to be related to poor bone quality [6], a set of metrics that examine the micro-architectural structure of bone [7]. The effect of T2DM on bone is now being referred to as ‘sweet bone’ [8], and while the cause is not yet discerned, there is clearly a rationale for exploring potential remedies for increased bone fragility in those with T2DM.

A combination of medication, supplementation, and lifestyle modification is suggested for reducing bone fragility and fracture risk in the normal population [9], but the atypical relationship between bone strength and BMD in ‘sweet bone’ introduces some complications in determining the clinical effect of treatment. In the normal population, treatment efficacy can be discerned via dual-energy x-ray absorptiometry and the resultant evaluation of BMD, but density evaluations in those with ‘sweet bone’

cannot confidently predict fracture risk [2], [3]. Still, the lack of literature on bone related interventions for individuals with T2DM leaves room for novel discovery, even in the context of outcome measures that may utilize BMD. The effect of exercise on individuals with T2DM is particularly interesting as exercise already has potent positive implications for the general metabolic dysregulation associated with diabetes [10], and in the context of the normal population, exercise is recommended in the prevention and treatment of osteoporosis [9].

Currently, literature examining the effectiveness of exercise interventions on 'sweet bone' is sparse, and most of the original research in this topic area utilize animal models [11]–[15]. When compared to control models, rodents with a T2DM phenotype were generally shown to have higher bone density and poorer bone quality; further, exercise was shown to potentially increase bone density, and improve bone quality [11], [13]. Original research using human subjects is especially limited, as there is currently only one study that examines the effects of an exercise intervention on bone in individuals with T2DM. Daly et. al. implemented a 12-month weight loss intervention in participants with T2DM, with or without resistance exercise [16]. Both groups demonstrated a loss of BMD, likely related to weight loss, but the resistance exercise group lost less bone density [16]. Further research in this area is needed as rodent models of T2DM do not necessarily represent human populations, and the literature examining humans would benefit from exercise specific interventions, as weight loss is a known negative modulator of BMD in the normal population [17].

HART-D, The Health Benefits of Aerobic and Resistance Training in Individuals with Type II Diabetes Study, was a large intervention trial aimed at determining the

modality dependent effects of exercise on metabolic health in individuals diagnosed with T2DM. Whole-body DXA scans were taken pre- and post-intervention on all participants of the study, as secondary outcomes required accurate measures of both fat and lean tissue. This ancillary analysis of the HART-D study aims to look further into the whole body DXA scans obtained during this trial and determine modality dependent effects of exercise on whole-body BMD. Specifically, the purpose of this analysis is to determine the effects of a 9-month aerobic, resistance, or combination exercise intervention on whole-body and whole-body derived regional measures of bone mineral density. We hypothesize that resistance and combination exercise over 9-months will increase whole-body and whole-body derived regional measures of BMD compared to no change with aerobic training and sedentary controls.

3.2 Methods

Prior to the start of the HART-D study, IRB approval was granted for the protocol, and all subjects gave written consent before screening. The full methods for the HART-D study have been previously published [10]; methods mentioned in this paper will be that which are pertinent to the ancillary analysis of BMD changes in response to the HART-D intervention.

Study Design

Sedentary (<20min aerobic exercise <3 days per week and no resistance exercise) men and women 30-75 years of age with T2DM (HbA_{1c} 6.5-11%) were recruited from the Greater Baton Rouge Area in Southern Louisiana. Exclusion criteria included: BMI >48 kg/m^2, fasting triglycerides >500mg/dL, blood pressure >160/100mmHg, medical history of stroke, advanced neuropathy, advanced

retinopathy, or any other serious medical condition contraindicated for exercise or that may limit adherence to the intervention. For the purpose of this ancillary analysis and to determine the efficacy of this intervention's ability to impact whole-body and whole-body derived regional measures of BMD, only compliant participants (at least 70% session completion with both baseline and follow-up data) were included (n=191). Participants included in the intervention, based on the aforementioned criteria, were randomized to either aerobic training (AER), resistance training (RES), combination training (COMB), or control (CON) groups. Baseline and follow-up measures were taken for whole body BMD, whole-body derived regional measures of BMD, Hemoglobin $A1_c$, VO_2 peak, and isokinetic strength. Subjects were randomized after baseline measures were taken.

Intervention

Individuals randomized to the CON group were offered voluntary stretching or relaxation courses once per week and asked to maintain their pre-randomization physical activity levels. For the exercise groups (AER, RES, and COMB), interventions were designed to require similar time allotments each week. All aerobic training (AER, COMB) was done via treadmill walking at a moderate to vigorous intensity, based on percentage of baseline VO_2peak, and was prescribed as volume in kilocalories per kilogram of bodyweight per week (KKW); weight was measured weekly and used to estimate caloric expenditure rate at treadmill speed and grade via standard equations [18]. Sessions per week for aerobic exercise ranged from 3-5 with no more than one session per day. Volume requirements for aerobic training (AER, COMB) were reduced by 33% only on weeks 12 and 24 for the purpose of subject recuperation. All resistance training (RES, COMB) included four upper body exercises (bench press, seated row,

shoulder press, and lat pull down), three lower body exercises (leg press, extension, and flexion), abdominal crunches, and back extensions. Each set was prescribed at a resistance that induced failure between 10-12 reps, with the prescribed resistance being increased when a participant completed the 12th rep on the final set of an exercise, two sessions in a row. The AER group was prescribed an aerobic exercise volume of 12KKW; the RES group completed 3 resistance training sessions per week with 3 sets for lower body exercises and 2 sets for all other exercises; the COMB group was prescribed an aerobic exercise volume of 10KKW and completed 2 resistance training sessions per week at one set for each resistance training exercise per session. Exercise prescriptions are generalized in Figure 3.1.

Figure 3.1. Exercise prescriptions across intervention groups.

Bone Mineral Density and Anthropometrics

Dual-energy x-ray absorptiometry scans were performed on a QDR 4500A whole-body scanner (Hologic Inc, Bedford Massachusetts) using standardized procedures for the purpose of determining whole-body muscle mass, fat mass, and bone mass per areal unit of volume (g/cm^2). After the scan was performed, analysis lines, shown in Figure 3.2, were placed according to standardized procedure for the purpose of deriving regional measures of lean mass, fat mass, and bone mass per areal volume (g/cm^2) for regional sections of the whole-body scan. Outcome measures for BMD included whole-body, thoracic spine, lumbar spine, pelvis, arm, rib, and leg. Regional density measures for arm, rib, and leg were a combination of the right and left sides, calculated by dividing the combined content by the combined area to determine the regional density. Weight was measured on a digital scale (GSE Scale Systems, Novi, Michigan) and height was measured using a standard stadiometer.

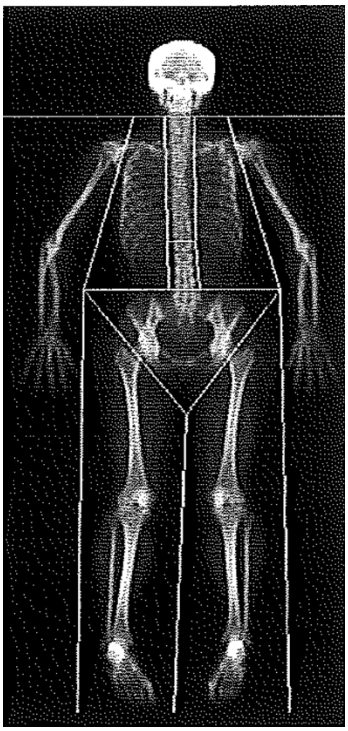

Figure 3.2. Analysis lines for whole-body derived regional measures of BMD.

Hemoglobin A1c

Hemoglobin A1c was determined using an automated glycosylated hemoglobin analyzer (DCA 2000+, Bayer, Dublin, Ireland). Measures were taken monthly via finger prick, but only the baseline and follow-up values for $HbA1_c$ were used in this ancillary analysis.

Isokinetic Strength

Isokinetic strength was determined as max torque at 60 degrees per second for knee flexion using a Biodex System 3 Dynamometer (Biodex Medical Systems, Shirley, New York). Each participant used their right leg to perform 3 sets of 5 maximal repetitions, and max torque (Nm) was determined as the highest score during the repetitions.

VO_2 peak

A graded maximal exercise test was conducted on a treadmill (Trackmaster 425, Carefusion, Newton, Kansas) with respiratory gas analysis via metabolic cart (ParvoMedics, Salt Lake City, Utah). Participants began the test at a self-selected walking speed at a 0% grade; grade was increased every 2 minutes until exhaustion, and the highest volume of oxygen consumption reported by the metabolic cart was used as VO_2peak ($ml \cdot kg^{-1} \cdot min^{-1}$).

Statistical Analysis

All data were analyzed with JMP version 15.1.0 (SAS Institute, Inc., Cary, NC) statistical software. Analysis of co-variance (ANCOVA) was used to determine baseline adjusted changes in whole-body and whole-body derived regional measures of BMD between groups. Post hoc comparisons were completed using a Student's t-test. Linear

regression (Pearson r) was used to determine relationships between baseline metrics of whole-body and whole-body derived regional measures of BMD in relation to age, baseline HbA1c, T2DM duration, baseline V02 peak, and baseline isokinetic strength. The same methodology was used to determine relationships between changes in whole-body and whole-body derived regional measures of BMD in relation to changes in HbA1c, VO2 peak, and isokinetic strength from baseline to follow-up. Paired t-tests were used to determine sex differences in baseline values, as well as sex differences in changes from baseline to follow-up. An alpha value of ≤0.05 was used to determine significance for all analysis.

3.3 Results

Baseline Characteristics

No significant differences were found between groups for baseline values of age, body weight, BMI, HbA1c, diabetes duration, VO_2 peak, peak torque, whole-body BMD, shown in Table 3.1, or whole-body derived regional measures of BMD. Analysis of baseline correlations showed that age was inversely correlated with baseline pelvis ($p=0.006$, $r=-0.20$) and rib BMD ($p=0.002$, $r=-0.22$), baseline VO_2peak was correlated with baseline whole-body ($p=0.02$, $r=0.17$), rib ($p<0.001$, $r=0.25$), arm ($p<0.001$, $r=0.035$), and leg BMD ($p<0.001$, $r=0.31$), and isokinetic leg strength was correlated with baseline whole-body ($p<0.01$, $r=0.34$), thoracic ($p<0.001$, $r=0.26$), pelvic ($p<0.001$, $r=0.28$), rib ($p<0.001$, $r=0.40$), arm ($p<0.001$, $r=0.55$), and leg BMD ($p<0.001$, $r=0.46$), shown in Figure 3.3. Sex comparisons showed females had significantly lower whole-body (1.18g/cm^2 vs 1.37g/cm^2), thoracic (1.15g/cm^2 vs 1.24g/cm^2), lumbar (0.97g/cm^2

vs 1.07g/cm^2), rib (0.69g/cm2 vs 0.76g/cm2), arm (0.76g/cm2 vs 0.88g/cm2), and leg BMD at baseline, $p<0.001$, shown in Figure 3.4.

Changes in Bone Mineral Density

Whole-body, thoracic, rib, arm, and leg BMD all increased from baseline to follow-up over 9 months (BMD change= 0.023±0.007 g/cm2, 0.034±0.013 g/cm2, 0.017±0.009 g/cm2, 0.016±0.009 g/cm2, and 0.022±0.008 g/cm2, respectively; mean±95%CI), shown in Figure 3.5; however, no group effects were found for changes in BMD (whole-body and all whole-body derived regional measures) after 9-months ($p\geq0.40$), shown in Figure 3.6. Changes in $HbA1_c$ were inversely correlated with changes in arm BMD ($p=.03$, $r=-0.16$) and changes in VO_2peak were inversely correlated with changes in thoracic BMD ($p=.04$, $r=-0.15$), Figure 3.6, and changes in pelvic BMD were found to be higher in females than males (+0.01g/cm2 vs -0.01g/cm2, $p=0.004$), shown in Figure 3.7.

Table 3.1. Baseline participant characteristics

		Treatment Group			
	All	CON	AER	COMB	RES
n	191	32	49	56	54
Female (n)	120 (63)	21 (66)	32 (65)	36 (64)	31 (57)
Age (y)	57.3 (8.02)	58.6 (8.2)	56 (7.8)	56.7 (7.6)	58.4 (8.5)
Body weight (kg)	96.1 (17.4)	97.3 (21)	94.4 (14.7)	96.7 (19.3)	96.3 (15.7)
BMI (kg/m^2)	34.4 (5.8)	34.9 (6.3)	33.7(5.6)	34.7 (6.2)	33.9 (5.4)
HbA1c (%)	7.2 (1.1)	7.6 (1.5)	7.0 (0.9)	7.3 (1.2)	7.1 (1.0)
Duration of Diabetes (y)	7.3 (5.7)	7.1 (5.0)	7.5 (6.0)	7.0 (5.7)	7.7 (5.9)
VO_2 peak ($ml \cdot kg^{-1} \cdot min^{-1}$)	19.5 (4.4)	18.5 (3.9)	20.5 (5.3)	19 (3.4)	19.8 (4.5)
Whole Body BMD (g/cm^2)	1.19 (0.13)	1.19 (0.16)	1.20 (0.14)	1.18 (0.11)	1.19 (0.13)
Isokinetic Strength (Nm)	129.1 (45.7)	124.1 (40.6)	132.7 (46.2)	128.5 (48.8)	129.5 (45.8)

Data are n (%) or mean (SD).

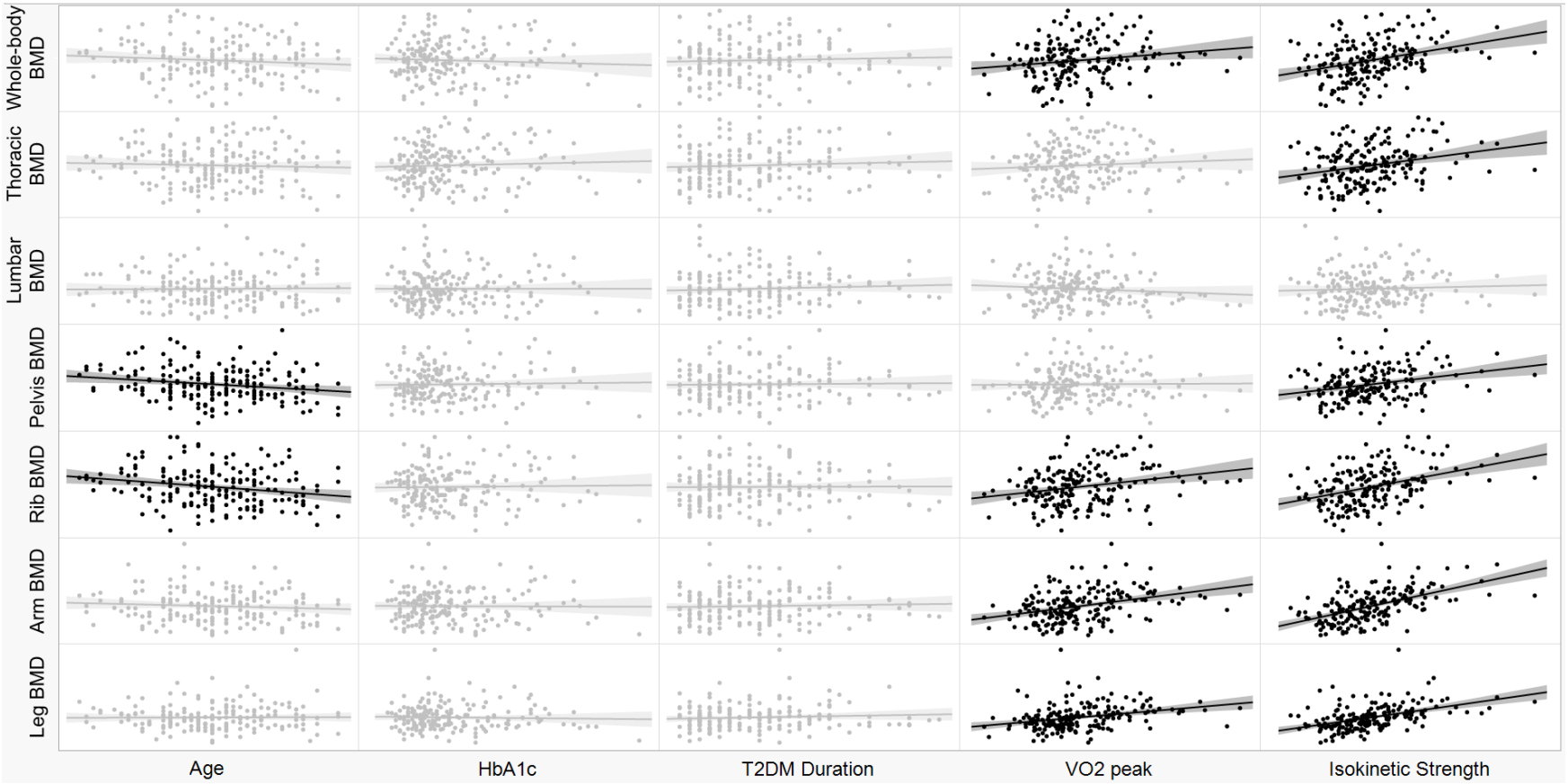

Figure 3.3. Baseline comparisons: BMD vs age, HbA1c, T2DM duration, VO_2 peak, & isokinetic strength. Highlighted relationships are significant ($p<0.05$). Age was negatively correlated to baseline pelvis and rib BMD (r= -0.20 and -0.22, respectively). VO_2 peak at baseline was positively correlated with baseline whole-body, rib, arm, and leg BMD (r=0.17, 0.25, 0.35, and 0.31, respectively). Isokinetic strength at baseline was positively correlated with baseline whole-body, thoracic, pelvis, rib, arm, and leg BMD (r=0.34, 0.26, 0.28, 0.40, 0.55, and 0.46, respectively).

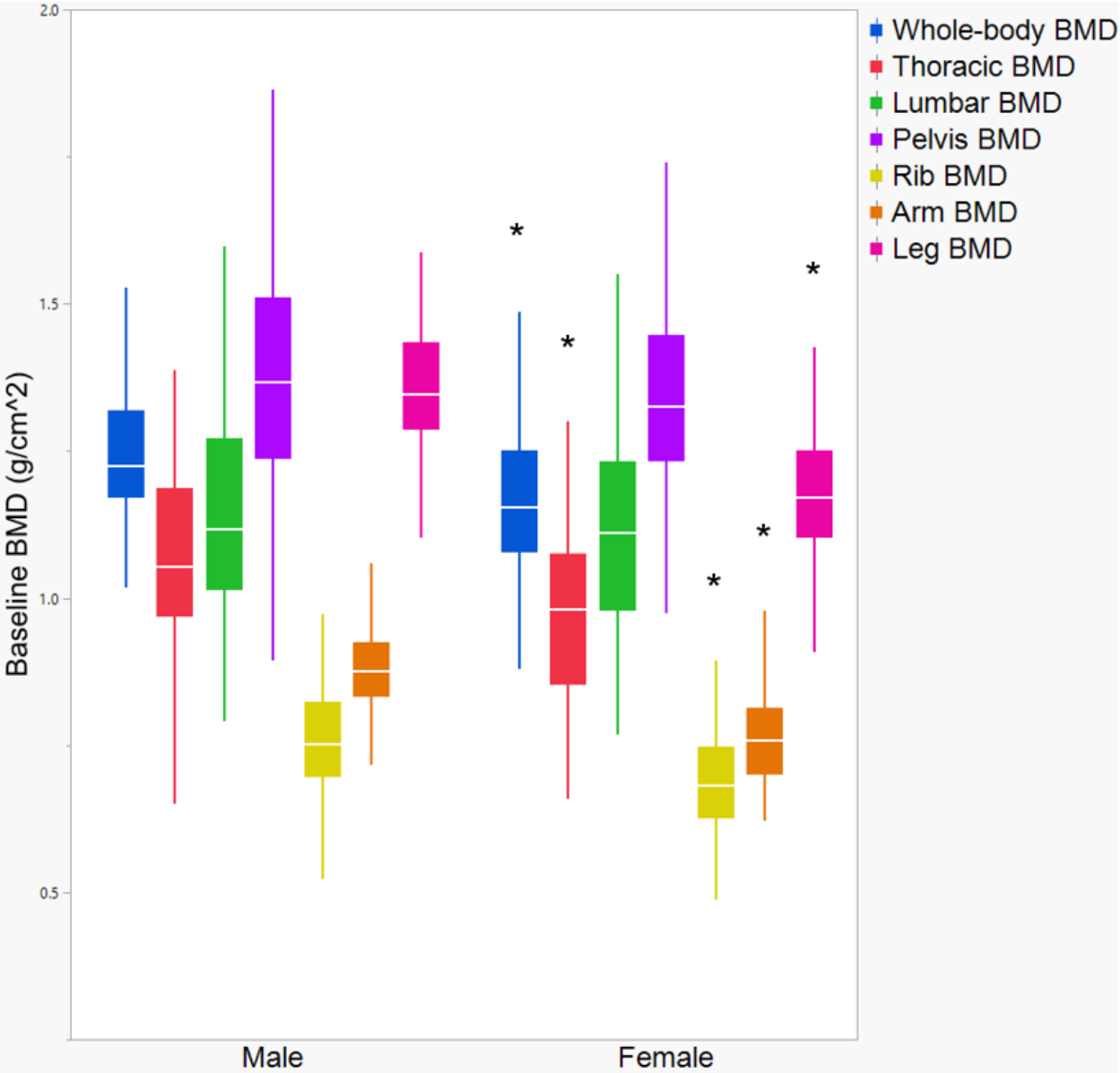

Fig 3.4. Baseline BMD sex differences. * indicates significant difference from corresponding male baseline BMD, p<0.001. Females had significantly lower whole-body, thoracic, rib, arm, and leg BMD.

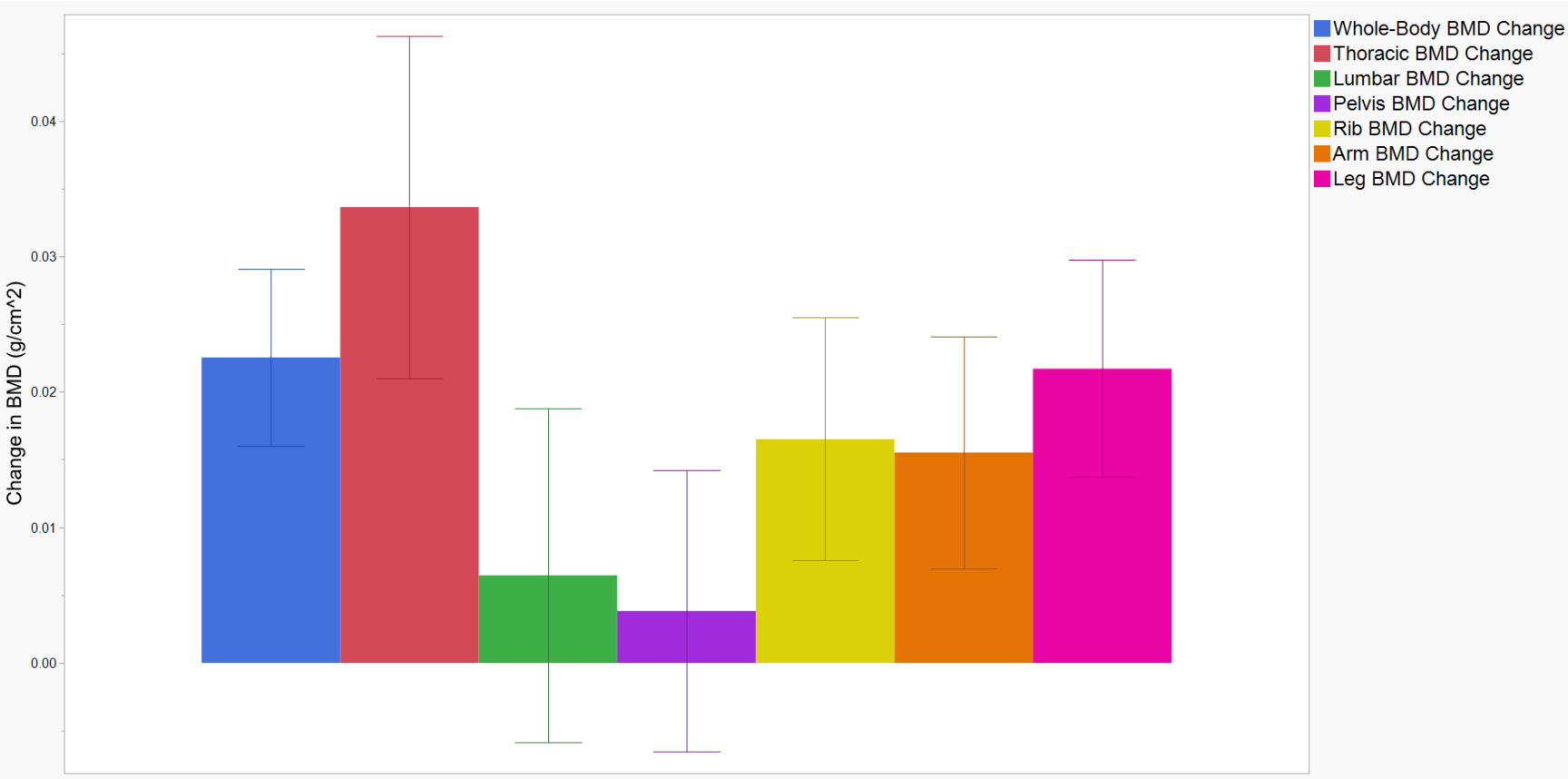

Figure 3.5. Overall whole-body and whole-body derived regional BMD changes from baseline to follow-up. Error bars are 95% confidence interval. Changes from baseline to follow up were significant for whole-body, thoracic, rib, arm, and leg BMD (BMD change=0.023±0.007 g/cm2, 0.034±0.013 g/cm2, 0.017±0.009 g/cm2, 0.016±0.009 g/cm2, and 0.022±0.008 g/cm2, respectively; mean±95%CI). This figure shows mean change for all participants (n=191) from baseline to follow-up.

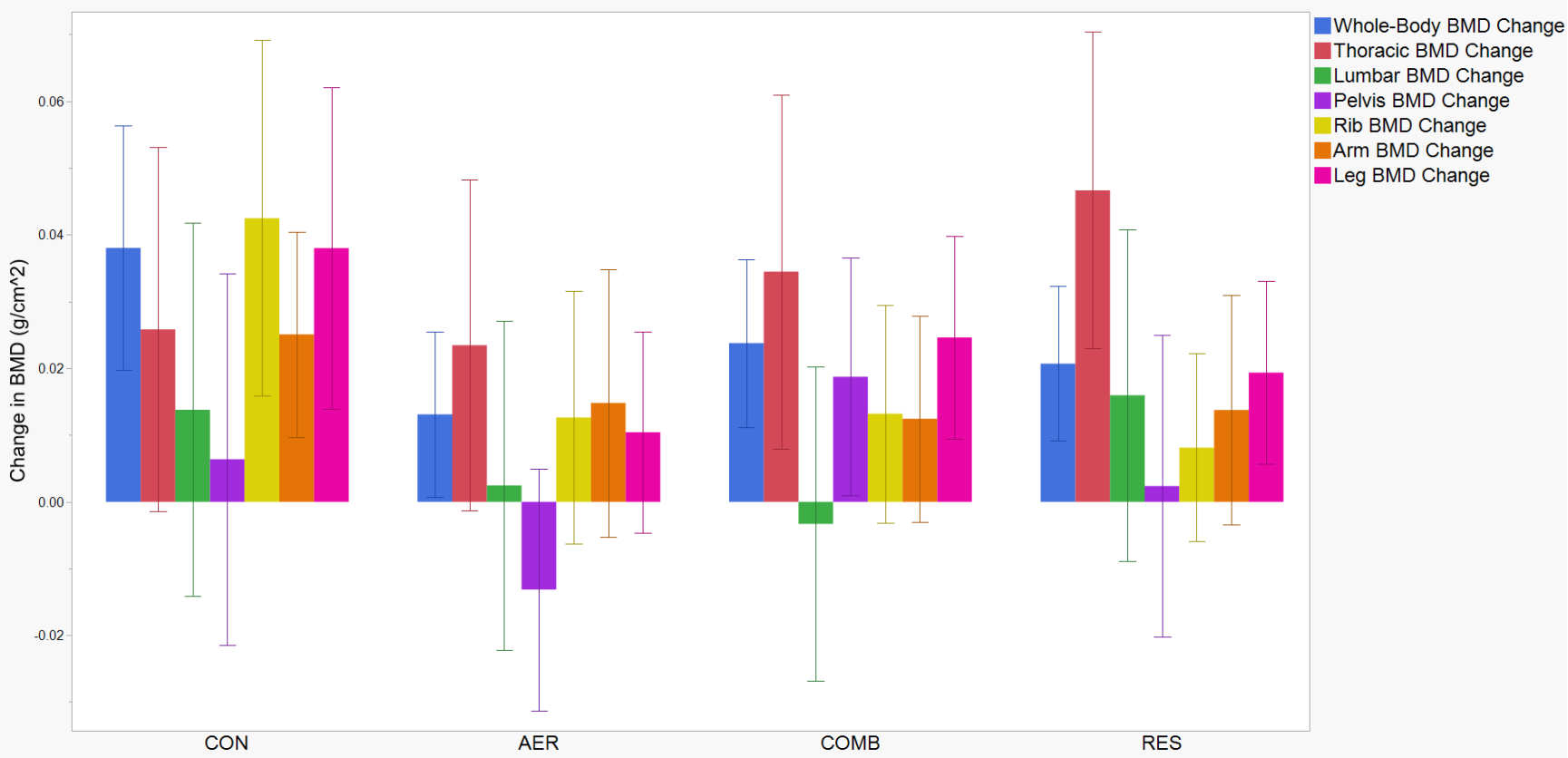

Figure 3.6. Whole-body and whole-body derived regional changes in BMD for each group after 9-months. Error bars are 95% confidence interval. No significant between group effects were found (p>0.40).

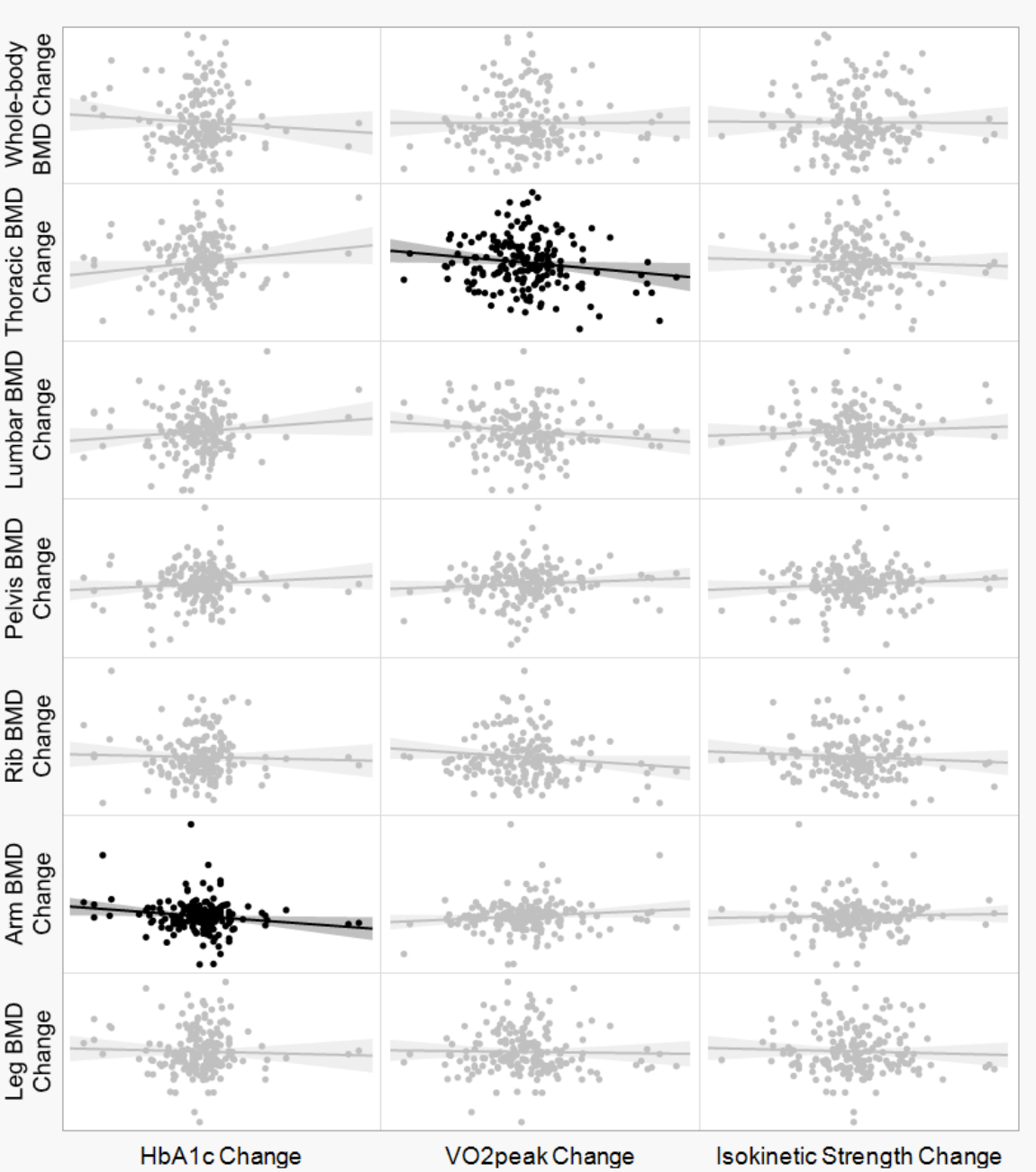

Figure 3.7. Change comparisons: BMD changes vs change in HbA1c, change in VO_2peak, and change in isokinetic strength. Significant correlations are highlighted, $p<0.05$. Arm BMD changes were inversely correlated with changes in HbA1c and thoracic BMD changes were inversely correlated with changes in VO_2peak ($r=-0.15$ and -0.16, respectively).

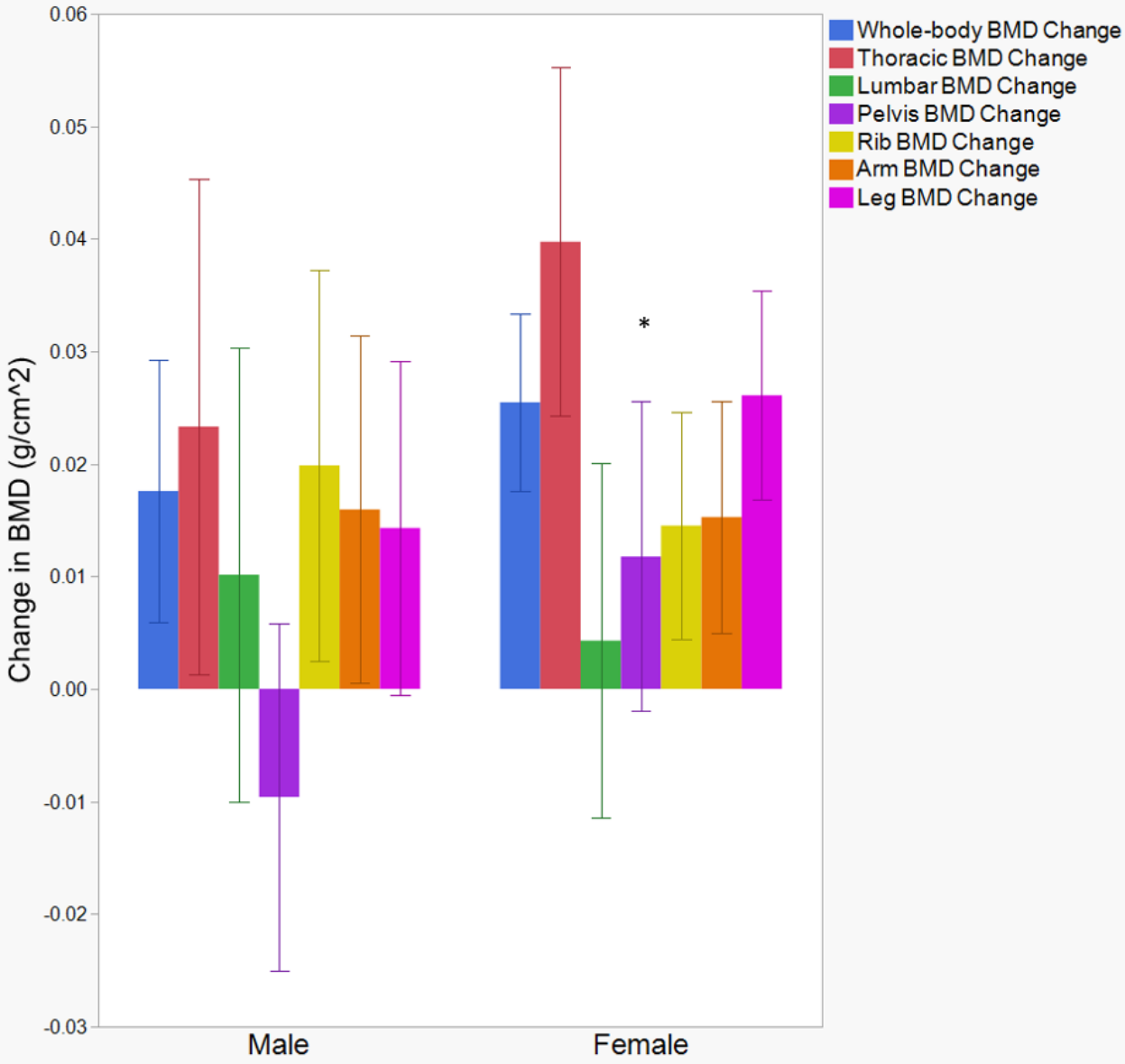

Figure 3.8. Gender differences for changes in whole-body and whole-body derived regional measures of BMD after 9-months. * indicates significant difference from corresponding male change in BMD, p<.05. Females had a significantly larger change in pelvic BMD after 9 months.

3.4 Discussion

The purpose of this ancillary analysis of the HART-D study was to determine the effects of a 9-month aerobic, resistance, or combination exercise intervention on whole-body and whole-body derived regional measures of bone mineral density. We

hypothesized that RES and COMB groups would increase whole-body and whole-body derived regional measures of BMD after 9-months compared to no change with AER and CON. The general rationale behind this hypothesis was that the anabolic effect of resistance exercise, part of the prescription for the RES and COMB groups, would increase BMD. Based on the results of this study, the hypothesis is incorrect as whole-body, thoracic, rib, arm, and leg BMD increased for the entire cohort, control group included (n=191) and there were no group interactions found for BMD changes. Baseline correlations and gender differences were in line with the literature: females had lower baseline values of BMD; strength was a positive predictor of baseline BMD, and fitness was a positive predictor of baseline BMD.

While dysregulation of bone in individuals with T2DM is suggested in the literature [8], it is not clear that BMD changes in response to exercise would deviate from what is generally expected in the normal population. Our data, however, does not show the differentiation we hypothesized. The sedentary control group stands out as especially odd, with significant positive changes in whole-body (0.038±0.018g/cm^2, mean change±95%CI) and leg (0.037±0.024g/cm2, mean change±95%CI) BMD over 9-months. Cross-sectional research examining relationships between age and BMD show individuals with T2DM have a higher BMD compared to age matched controls [3], but their BMD generally declines with age in a manner similar to the normal population [19]. Our data, however, suggest that BMD increases after 9-months in sedentary diabetics, along with those participating in an exercise intervention. There also does not seem to be any statistical anomalies in our data that would explain the unexpected changes, as the removal of outliers for whole-body BMD, thoracic, and leg BMD changes did not

adjust the significance of the outcome. Further, baseline differences between groups for potentially important metrics, in the context of BMD changes like age and baseline measures of BMD, had similar means and distributions between groups, Table 3.1. Also, worth mentioning, removal of the control group from analysis did not reveal any group effects between AER, RES, and COMB for BMD changes, and the results still showed a significant increase in whole-body, thoracic, and leg BMD from baseline to follow-up overall.

These results are somewhat limited by the regional measures of BMD, as they were derived from whole-body scans rather than individually imaged sections of each region. Scan specific methodology for areas such as the hip or lumbar spine would have improved resolution, reduced variability, and would more readily translate to clinical implications for changes; although, even with the potentially increased variability, our analysis shows significant differences from baseline to follow-up. Additionally, this ancillary analysis did not control for diet and medication. Both calcium and vitamin-D are positive modulators of bone and are modifiable in the diet [20], although no impetus for dietary modification with an emphasis on calcium and vitamin-D was given in the HART-D trial. Further, diabetic medications are a potential modulator of BMD as well. Example, metformin is commonly prescribed as a metabolic regulator in those with T2DM and is a dose dependent positive modulator of BMD [21].

While the results of this analysis were unexpected, the interpretation of these results are strengthened by other aspects of the HART-D study design. HART-D was a large, well-controlled clinical trial, even though it was designed for determining the modality dependent effects of exercise on metabolic outcomes rather than bone. The

training sessions were carried out and monitored by trained staff, and randomization algorithms maintained similar means and distributions across all of the baseline metrics, even those that would potentially impact bone, such as age, baseline values of strength, fitness, and baseline measures for BMD. While the results for this analysis were unexpected, there is no clear reason to suspect an issue with the data or data analysis beyond the limitations previously mentioned.

In conclusion, this ancillary analysis of the HART-D data does not simplify our understanding bone in individuals with T2DM and the increased BMD found in our control group adds more questions. There is room to explore further however, as BMD is not the only predictor of bone strength and there are other methodologies for measuring bone. Due the already understood dysfunction between BMD and fracture risk with T2DM, future research should include both serum markers of bone changes, such as CTX and P1NP, and should try to include assessments of bone quality, potentially via peripheral q-ct or even intermediate measures of bone quality such as DXA derived trabecular bone scores. It would also be interesting to see if the results in this analysis could be reproduced in other investigations, so the future use of DXA for determining whole- body and regional BMD could be included, even though BMD is not the best method of bone analysis for clinical outcomes in individuals with T2DM. There is exciting research currently happening in this area, as a large clinical trial, the Study to Weigh the Effect of Exercise Training on BONE quality and strength in type 2 diabetes, or ‘SWEET BONE’, is currently underway via the work of researchers in Italy [22]. Their study is specifically designed for determining changes in bone as a response to

exercise over a 2-year intervention on individuals with T2DM and may provide additional context on the relationship between exercise and bone in this population.

Funding: This work was funded by the National Institutes of Health (R01-DK068298).

3.5 Notes

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CHAPTER 4. CORTICAL AND TRABECULAR BONE MORPHOLOGY IN RESPONSE TO EXERCISE AND A KETOGENIC DIET

4.1 Introduction

The adoption of ketogenic diets has increased in popularity recently, and even though the adoption is partially related to ‘hype’ and popular culture, there is some merit to high fat, very low carbohydrate diets with respect to improving metabolic health and weight loss [1], [2]. While popular culture has played a large part in the recent adoption, high fat diets with zero carbohydrates were notably implemented in the 1970’s due to their efficacy in mitigating seizures in children suffering from epilepsy. The literature examining the health of epileptic youth adopting a ketogenic diet generally revealed favorable health outcomes, as the diet’s ketogenic effect, shifting from carbohydrate utilization to ketones, reduced the severity and number of seizures [3]. This was not without tradeoffs however, as a follow-up study revealed potentially negative effects on bone mineral density [4]. More recent investigations, in children with epilepsy adopting a ketogenic diet, supports the previous literature with negative implications for bone, based on decreases in bone mineral density [5], [6].

Research examining the interactions between ketogenic diets and bone without the added factor of epilepsy is lacking. Studies using rodent models have generally shown deleterious effects on bone in response to a ketogenic diet. Specifically, the adoption of ketogenic diets in rodents has been shown to negatively impact cortical bone, trabecular bone, the bone mechanical properties, and serum markers of bone formation and resorption [7]–[11]. Research in humans is also limited with only two studies, McSwiney et. al. and Heikura et al., examining the effects of a ketogenic diet on

humans with an outcome measure for bone, minus those examining individuals with epilepsy. Both studies had an exercise component in addition to the diet, and bone outcomes were ancillary to the original intent of the research. McSwiney et al., in a 2018 study, found no difference in whole-body bone mineral density after 12 weeks of exercise training, comparing two groups of endurance trained athletes, one group on a ketogenic diet and the other on a control diet over the 12-week exercise intervention [12]. In 2020, Heikura et. al, examined the acute effects of exercise on serum markers of bone formation and resorption after 3.5 weeks of a ketogenic diet vs a calorie matched control diet. Results showed that exercise dependent changes in markers of formation and resorption were less favorable in the ketogenic diet group and that some improvement was made with an acute reintroduction of carbohydrate [13].

The complement of exercise, in the context of a ketogenic diet, is an especially interesting addition to the above studies due to the typical nature of health minded lifestyle changes, as there is some chance for both exercise and diet modification to accompany each other. Further, exercise is generally expected to improve bone outcomes, while ketogenic diets seem to have a negative impact. In the interest of further exploring these interactions, the purpose of this study is to determine the effects of a ketogenic diet, 6 weeks, and aerobic exercise, 3 weeks, on cortical and trabecular bone morphology in mice. We hypothesize that a ketogenic diet will negatively impact trabecular bone morphology and the effect of exercise will improve trabecular bone morphology. No changes are expected for cortical bone.

4.2 Methods

Study Design

This analysis of bone outcomes was ancillary to a larger investigation related to metabolic outcomes [14]. Thirty-eight male C57BL/6Jmice were utilized for this study. Mice were divided into 2 groups (KSED/KEX or CSED/CEX) at 12 weeks of age and after 2 weeks, started a ketogenic or control diet, which was maintained for 6-weeks (20 weeks of age). 3-weeks into the dietary intervention, groups were subdivided into exercise (KEX and CEX) and non-exercise groups (KSED and CSED), where exercise groups participated in 1-hour of moderate to high intensity aerobic exercise on a treadmill 5 days per week for 3 weeks. At 20 weeks of age, mice were euthanized, and right femurs were collected; other tissues were collected for the primary analysis and are not discussed here. Femurs were analyzed via micro-quantitative tonometry for metrics of cortical and trabecular bone morphology. A visual representation of the design can be seen in Figure 4.1. This study design was approved as by the Institutional Animal Care and Use Committee at the Pennington Biomedical Research Center.

Animal Use and Handling

C57BL/6J male mice, sourced from Jackson Laboratories (Stock #000664; Bar Harbor, ME), were obtained at 12 weeks of age. Mice were group-housed at room temperature with a 12:12 hour light and dark cycle. Mice were maintained by veterinary staff and researchers; they were allowed ad libitum access to food and water throughout the study. 2-weeks prior to randomization, mice were scanned via nuclear magnetic resonance spectroscopy (NMR) (Bruker Minispec LF50 Time; Billerica, MA) to ensure

similar body compositions between the groups receiving a ketogenic diet (KSED and KEX) vs the groups receiving a matched control diet (CSED and CEX). Mice were euthanized at 20 weeks of age at 9:00am via peritoneum injection of ketamine/xylazine/acepromazine (16mg/mL, 0.8 mg/mL and 0.32mg/mL) and femurs were collected immediately after.

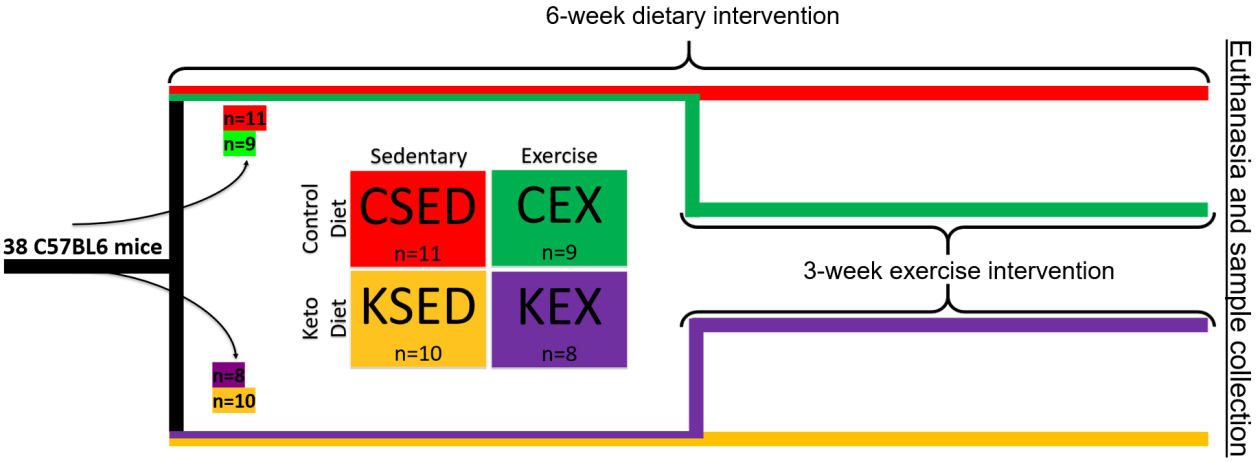

Figure 4.1. Study design. Thirty-eight mice are split into two groups and received a control (n=20) or a ketogenic diet (n=18) for 6-weeks. 3-weeks into the dietary intervention the two groups are subdivided into exercise (CEX, n=9; KEX, n=8) and non-exercise groups (CSED, n=11; KSED, n=10). Euthanasia and sample collection occurred within 24hrs of the last exercise session, 6-weeks after starting the dietary intervention, and at 20-weeks of age for the mice.

Dietary Intervention

Mice were separated into dietary groups (KSED+KEX or CSED+CEX) based on NMR derived body composition at 12 weeks of age. At 14 weeks of age, Control mice (CES+CEX) were provided a low-fat diet, percentage of total calories= 16% protein, 12% fat, and 72% carbohydrate (TestDiet #5TJS, St. Louis, MO), and keto mice (KSED+KEX) were provided a high-fat diet, percentage of total calories=16% protein, 84% fat (NPKD; TestDiet #5TJQ, St. Louis, MO). Food and water were provided *ad*

libitum, except during exercise. Dietary intervention lasted for 6 weeks (14 weeks of age to 20 weeks of age), shown in Figure 4.1. NMR was used to measure fat and lean mass in grams 2 weeks and immediately prior to intervention and each week of the intervention. Change scores were calculated using the difference between week 0 and week 6 for fat and lean mass.

Exercise Intervention

Two and a half weeks into the dietary intervention all mice (n=38) were habituated on an Exer 3/6 treadmill (Columbus Instruments; Columbus, OH) for 3 days at a 10° incline for 17 min with speed increasing every 5 minutes (0, 5, 10, and 15m/min for 5, 5, 5, and 2 minutes, respectively). The habituation protocol did not reveal any poor responders so mice being fed a control or ketogenic diet were randomly subdivided into non-exercise (CSED+ KSED) and exercise (CEX+KEX) groups. Starting at week 3 of the dietary intervention (17 weeks of age), mice in the exercise groups (CEX+ KEX) began training for 5 days/week, 1hr/d at a 10% incline. Speed was increased in stages for each exercise session and weekly to maintain relative intensity with adaptations, shown in Table 4.1 with better detail. Mice were monitored throughout the exercise bouts and motivated via light tapping with a brush and light electrical shock to ensure exercise bouts were completed. Blood lactate was measured (Lactate Plus Meter; Nova Biomedical, Waltham, MA) via tail vein immediately post exercise on weeks 1 and 3 to estimate intensity; both groups showed an average blood lactate level between 4-5 mM, suggesting the stimulus was sufficient to be considered moderate to vigorous intensity.

Table 4.1. Exercise training protocol

	Week 4			Week 5			Week 6		
Stage	**m/min**	**min**	**m**	**m/min**	**min**	**m**	**m/min**	**min**	**m**
1	10	10	100	12	10	120	14	10	140
2	12	10	120	14	10	140	16	10	160
3	14	10	140	16	10	160	18	10	180
4	16	10	160	18	10	180	20	15	300
5	18	10	180	20	10	200	22	15	330
6	20	10	200	22	10	220			
Total		60	**900**		60	**1020**		60	**1110**

Speed, duration, and distance are shown as m/min, min, and m, respectively. All exercise was at a 10° incline.

Bone Morphology

Right femurs were harvested at 20-weeks and were cleaned, wrapped in phosphate-buffered saline soaked gauze, wrapped in foil and stored at -20°C until analysis. Prior to scanning, femurs were thawed at room temperature, and once thawed, placed upright in a tube with the greater trochanter top side; styrofoam was used to secure the femur within the tube. The cortical and trabecular regions of interest were scanned using a Scano μ-CT 40 (Scano Medical AG, Brüttisellen, Switzerland) at 6 μm voxel resolution, 55kVp, 145uA, and 200 millisecond integration time. Using the scano medical software, femur lengths were measured from the proximal most portion of the femoral head to the distal most portion of the femur. The cortical scan area (100, 6 μm slices) was centered on the mid-point of the femur encompassing a 600μm length of the diaphysis. The trabecular scan area (150, 6 μm slices) began one slice proximal to the last visible portion of the fabella and extended proximally for 900μm. All cortical scans were contoured via automated scrips but were checked visually. All cortical scans differentiated bone tissue from other tissues at a threshold of 306 mg HA/cm^3. Outcome measures for cortical bone included: bone volume (mm^3), cortical thickness (mm), and cortical bone mineral density (BMD) (mg HA/cm^3). Trabecular scans were contoured

manually every 5 slices and transformed to contour the slices in between. All trabecular scans differentiated bone tissue from other tissues at a threshold of 217 mg HA/cm3. Outcome measures for trabecular bone included: trabecular bone volume (mm^3), trabecular thickness (mm), trabecular spacing (mm), trabecular number ($1/mm^{-1}$), connection density (mm^{-3}), and trabecular BMD (mg HA/cm^3). Cortical and trabecular regions of interest along with 1-slice illustrations of contours are shown in Figure 4.2.

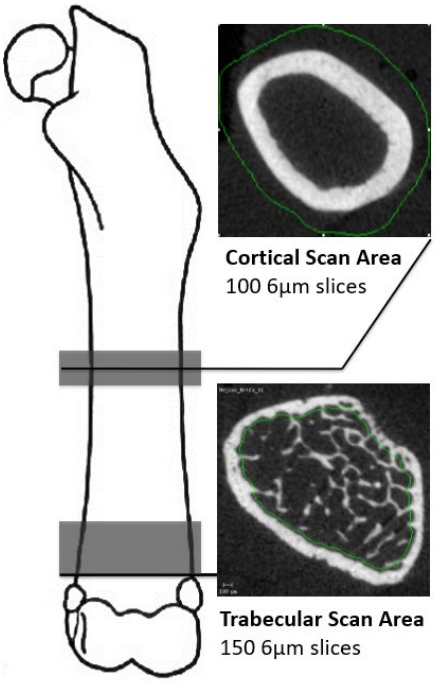

Figure 4.2. Cortical and trabecular regions of interest.

Statistical Analysis

All data were analyzed with JMP version 15.1.0 (SAS Institute, Inc., Cary, NC) statistical software. Analysis of variance (ANOVA) was used to determine group effects at baseline (2 weeks prior and 0 weeks prior) for body composition. Two-way analysis of variance was used to determine diet and exercise effects along with diet and exercise interactions in relation to bone outcomes and for week 0 adjusted changes in fat and

lean mass from week 0 to week 6. If a significant effect or interaction was found, post hoc comparisons were completed using a Student's t-test to determine between group differences. Linear regression (Pearson r) was used to determine relationships between bone outcomes and changes in lean and fat mass from week 0 to week 6. An alpha value of ≤0.05 was used to determine significance for all analysis.

4.3 Results

Cortical and Trabecular Bone

Table 4.2 shows mean±95% confidence interval data by group for all bone outcomes and p-values for diet effects, exercise effects, diet*exercise interactions (means are contrasted by group when an effect or interaction is significant). An exercise effect was found for trabecular thickness, $p=0.002$. Post-hoc analysis showed CEX to have significantly thicker trabeculae compared to CSED and KSED, and KEX had significantly thicker trabeculae than KSED, $p<0.05$. An interaction between diet and exercise was found for trabecular BMD, $p=0.038$. Post-hoc analysis showed CEX had significantly higher trabecular BMD compared to all other groups (KEX, CSED, and KSED), $p<0.05$. No other significant effects or interactions were found for bone outcomes. Figure 4.3 shows a simplified visualization for all bone outcomes by group. Figure 4.4 and Figure 4.5 show trabecular thickness and trabecular BMD by group, respectively.

Body Composition and Correlations

Table 4.3 shows mean±95%CI data by group for all body composition outcomes. Pre-randomization (2 weeks prior to intervention) fat and lean mass showed no significant differences between groups, but significant differences, were found at week 0

(post randomization and immediately prior to intervention), shown in Table 4.3. An effect of exercise and diet was found for changes in weight (week 0 to week 6), p=0.03 and p=001, respectively. Student's t-test showed sedentary mice (CSED+KSED) had larger increases in weight compared to exercising mice (CEX+KSED) and mice on a ketogenic diet (KSED+KEX) showed larger increases in weight. An effect of exercise and diet were shown for changes in fat mass (week 0 to week 6), p=0.017 and 0.003, respectively. Student's t-test showed sedentary mice (CSED+KSED) had larger increases in fat mass compared to exercising mice (CEX+KSED) and mice on a ketogenic diet (KSED+KEX) showed larger increases in fat. An effect of exercise was shown for changes in lean mass (week 0 to week 6), $p<0.0001$. Student's t-test show exercising mice (CEX+KEX) lost significantly more lean mass than sedentary mice (CSED+KSED), $p<0.05$.

Trabecular BMD was inversely correlated with changes in fat mass and weight, r=-0.33 and -0.38, respectively; $p<0.05$. No other correlations were found between bone outcomes and changes in fat or lean mass.

Table 4.2 Bone outcomes

	Group				Effects (p-value)		
	CSED	**CEX**	**KSED**	**KEX**	**Ex**	**Diet**	**Ex*Diet**
Cortical							
BV	0.52±0.02	0.53±0.02	0.53±0.02	0.52±0.02	0.97	0.86	0.33
Thickness	0.18±0.008	0.19±0.005	0.18±0.003	0.18±0.004	0.85	0.32	0.49
BMD	1123±8.42	1131±9.3	1129±8.8	1131±9.3	0.33	0.54	0.48
Trabecular							
BV	0.31±0.04	0.33±0.04	0.32±0.04	0.32±0.04	0.48	0.99	0.71
Thickness	0.045 ± 0.001^{bc}	0.048 ± 0.002^{a}	0.045 ± 0.002^{c}	0.047 ± 0.002^{ab}	**<0.01**	0.42	0.76
BMD	754 ± 10.7^{b}	777 ± 11.8^{a}	759.46 ± 11.2^{b}	758 ± 12.5^{b}	0.08	0.24	**0.04**
Number	4.5±0.15	4.5±0.17	4.5±0.16	4.7±0.18	0.54	0.35	0.13
Spacing	0.21±0.007	0.21±0.008	0.21±0.008	0.20±0.009	0.32	0.64	0.21
Con Den	157±14	159±16	156±15	158±17	0.86	0.88	0.99

Values shown as mean±95% confidence interval. When an effect or interaction is significant, p-value is bolded and superscript letters within rows show significant differences between means. BV=bone volume (mm3), thickness (mm), BMD=bone mineral density (mg HA/cm^3), Number (1/mm^{-1}), Spacing (mm), Con Den=Connection Density (mm^{-3}).

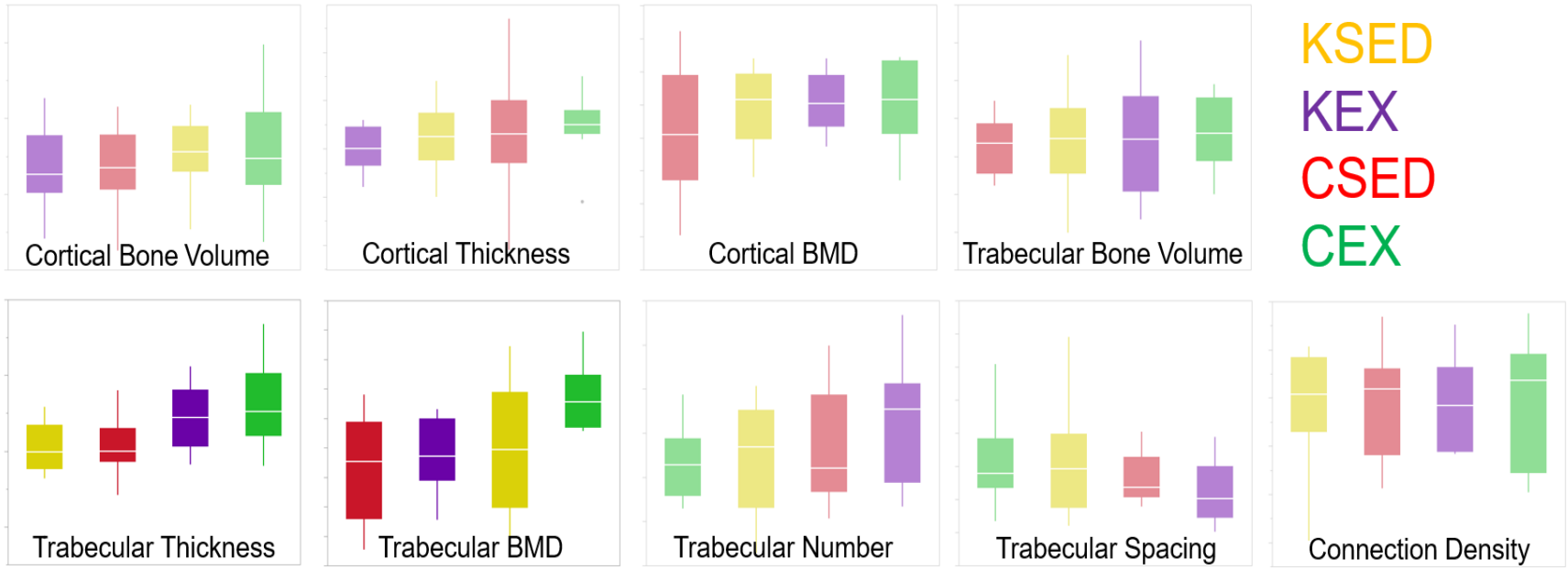

Figure 4.3. Simplified group distributions for bone outcomes. Groups are ordered by means from least to greatest for each outcome, except for trabecular spacing. Highlighted graphs indicate significant effects or interactions (trabecular thickness & trabecular BMD), $p<0.05$, and are shown in greater detail in figures 4.4 and 4.5. Faded graphs show no significant effects, interactions, or group differences, $p>0.05$. Means and 95% confidence intervals are shown in table 4.2.

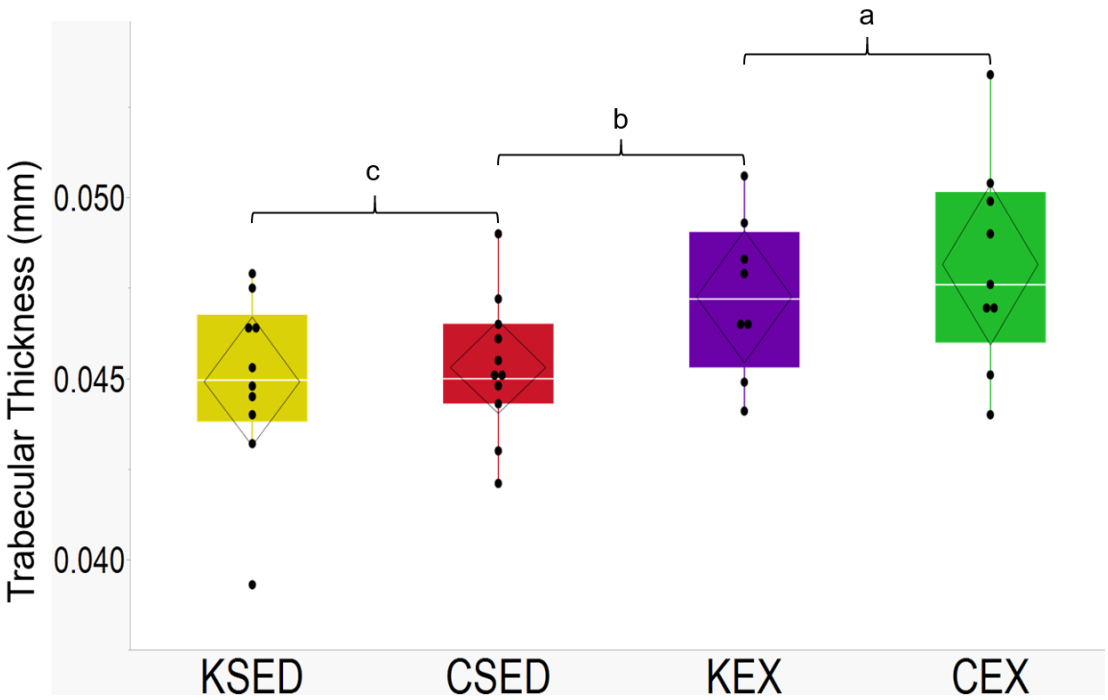

Figure 4.4. Changes in trabecular thickness by group. ◊ indicates mean and 95% confidence interval. A significant effect of exercise was found, p=0.002. Groups not connected by the same letter are significantly different.

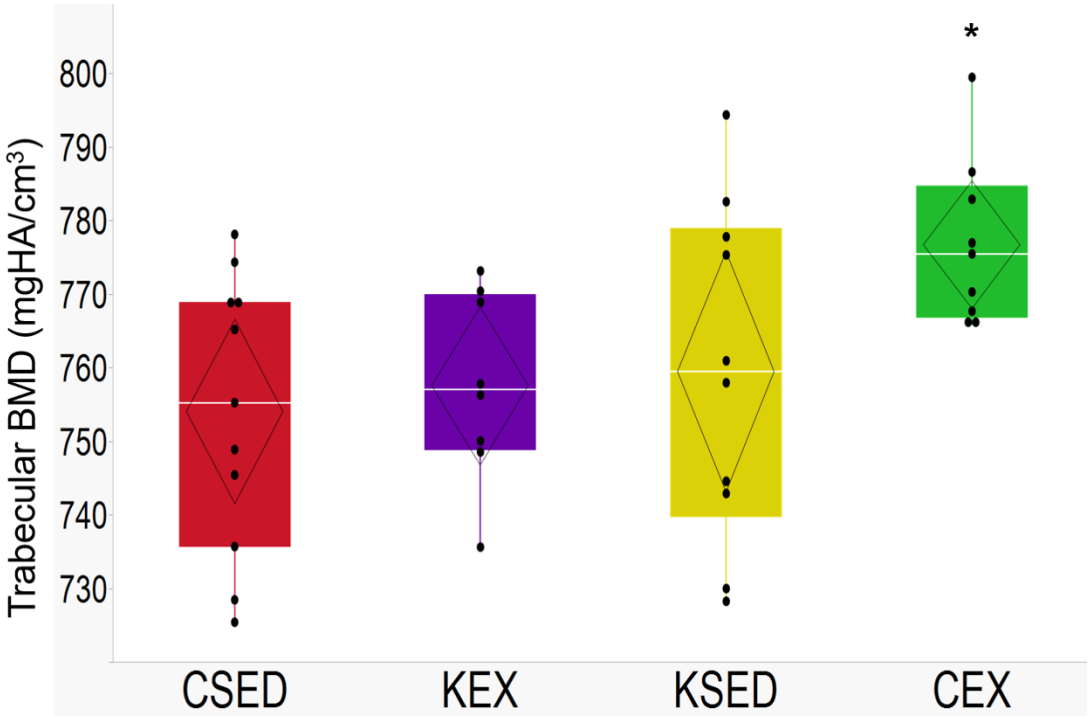

Figure 4.5. Changes in trabecular bone mineral density (BMD). ◊ indicates mean and 95% confidence interval. A significant interaction between exercise and diet was found, p=0.038. * indicates a significant difference from other groups.

Table 4.3 Body composition outcomes

	Group				Effects (p-value)		
	CSED	**CEX**	**KSED**	**KEX**	**Group**		
Week -2							
Weight	28.9±1.6	30.3±2.9	29.8±1.8	29.0±1.9	0.68		
Lean mass	18.8±1.1	19.0±1.4	19.0±1.1	18.2±0.004	0.75		
Fat mass	3.1±0.9	4.0±1.7	3.2±1.0	3.3±1.7	0.60		
Week 0							
Weight	30.1±2.8	30.8±2.4	32.0±2.0	31.7±2.8	0.59		
Lean mass	19.2±0.8	20.3±1.3	20.5±1.1	20.0±1.4	0.26		
Fat mass	3.6±0.9^{c}	4.1±1.0^{bc}	5.4±1.0^{a}	5.4±0.8^{ab}	**0.01**		
Change from 0 to 6 weeks					**Ex**	**Diet**	**Ex*Diet**
Weight	1.78±1.86^{b}	0.09±2.05^{b}	5.02±1.95^{a}	2.32±2.18^{ab}	**0.03**	**0.01**	0.61
Lean mass	-0.33±0.52^{a}	-1.49±0.58^{b}	-0.37±0.55^{a}	-1.94±0.61^{b}	**0.0001**	0.38	0.47
Fat mass	1.81±1.02^{b}	0.61±1.13^{b}	3.71±1.07^{a}	2.18±1.20^{ab}	**0.02**	**0.003**	0.76

Values shown as mean±95% confidence interval. When an effect or interaction is significant, p-value is bolded and superscript letters within rows show significant differences between means. Week -2 is pre-randomization, 2 weeks prior to the start of the dietary intervention, with mice at 12 weeks of age. Week 0 is immediately before the start of the intervention, with mice at 14 weeks of age.

4.4 Discussion

The purpose of this study was to determine the effects of a 6-week ketogenic diet interceded by 3 weeks of aerobic exercise on mice. We hypothesized that a ketogenic diet would negatively impact trabecular bone morphology and that exercise would improve trabecular bone morphology. No changes were expected for cortical bone morphology. Based on the results found here, the hypothesis is not explicitly correct. Two-factor ANOVA revealed no diet effects for bone outcomes, and a 6-week ketogenic diet did not result in significant bone morphology differences between CSED and KSED. The three-week exercise intervention, starting 3 weeks into the dietary intervention, was shown to improve trabecular thickness (CEX/KEX vs CSED/KSED) and there was an interaction between exercise and diet for trabecular BMD. Based on the post-hoc analysis, there does seem to be a general trend toward a ketogenic diet limiting the potential improvements in bone that would otherwise be expected with exercise. Improvements in trabecular thickness were blunted, although not significantly, in the KEX group compared to CEX as trabecular thickness in the CEX group was significantly higher than both the KSED and CSED groups, while trabecular thickness in the KEX group was only significantly higher than the KSED group (Figure 4.4) and improvements in trabecular BMD found in the CEX group, were not found in the KEX group (Figure 4.5). While the hypothesis was incorrect, these results do trend toward what was expected.

In comparison to other literature examining the effects of exercise and ketogenic diets on bone, this is the only study to look at the effects of an intervention with dietary and sedentary controls. McSwiney et al. examined the effects of a ketogenic diet vs a

control diet on BMD over a 12-week exercise training intervention but did not have a sedentary control group [12]. Like our study, McSwiney et al. did not find an effect of diet on bone, although it is worth mentioning that their study was on endurance trained humans, rather than mice. Heikura et. al. also examined the effects of exercise and a ketogenic diet on bone in humans, but bone outcomes were serum markers for formation and resorption in response to a 3.5-week ketogenic diet and an acute exercise bout [13]. In short, Heikura et al.'s research demonstrates negative implications for bone in response to a ketogenic diet, both at rest and acutely in response to exercise.

The current studies examining the effects of ketogenic diets on bone without an exercise component only utilize rodents, but their results are in-line with the negative implications suggested by Heikura et al. Studies that examine the effects of a ketogenic diet on bone unanimously demonstrate a negative effect of a ketogenic diet on bone parameters: morphology, density, serum markers, and mineral homeostasis [7]–[11]. Considering the clear relationships between ketogenic diets and bone shown in the literature, the lack of a significant ketogenic diet effect on bone in our study is unexpected. All studies cited above explicitly stated *ad libitum* access to food and water, except for one, and none tracked total caloric intake. Further, all studies used a very low-carb, high fat diet although macronutrient composition did vary to small degrees. The most apparent disparity between this study and others examining bone outcomes in response to a ketogenic diet is the length of intervention, as most of the studies utilized a 12-week dietary intervention [7], [9], [10]. The shorter, 6-week period implemented in

our study may explain the lack of significant changes in bone outcomes related to the dietary intervention.

The lack of a longer intervention is a significant limitation, although it is worth mentioning that this study was designed for metabolic outcomes and bone outcomes were an ancillary consideration, after the protocol had started. Another considerable limitation is an unexpected weight change from the initial body composition measures to the second body composition measures, immediately before the start of the intervention. Table 4.3 outlines the group differences at week 0 which show the mice in the ketogenic diet groups to have higher fat mass than the controls. This difference was not present in the initial body composition measurement which was used to initially randomize mice into ketogenic and control diet groups. This disparity was likely caused by technician error, where mice in the ketogenic groups were given *ad libitum* access to both a chow and ketogenic diet for 3 days leading up to the week 0 body composition measure. The group differences in fat mass are noted, because there are relationships between fat mass and bone generally [15], and our own analysis found a negative correlation between fat mass and trabecular BMD. Strengths of the study are somewhat inherent to most studies utilizing rodent models, as the variability of factors not associated with the intervention were well controlled.

In conclusion, 3 weeks of aerobic exercise interceding a 6-week dietary intervention improved bone morphology, based on increased trabecular thickness and trabecular BMD. While there were no explicit diet effects found, there does seem to be a reduction in potential exercise derived benefits to bone. Future research in the area of exercise and bone in relation to a ketogenic diet should utilize a longer duration

intervention to match other studies examining outcomes related to bone morphology or density. Further, there is a clear need to pursue more research in this area with human subjects as the adoption of ketogenic diets are becoming more commonplace and there is clearly a negative implication for bone, which could potentially be abated with exercise.

Funding: This work was supported in part by National Institutes of Health (NIH) - COBRE (NIH 3P30-GM118430) and NIH-NORC (NIH 2P30-DK072476) center grants, as well as NIH 1R01DK103860 (R.C.N.).

4.5 Notes

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CHAPTER 5. TRABECULAR BONE SCORE AND BONE MINERAL DENSITY: ANCILLARY DATA FROM THE REALPA STUDY

5.1 Introduction

Bone mineral density (BMD), most commonly measured via dual-energy x-ray absorptiometry (DXA), has largely defined fracture risk since the World Health Organization established the criteria for diagnosing osteoporosis in 1994 [1]. The WHO's criteria for diagnosing osteoporosis was based on a preponderance of epidemiological evidence suggesting a link between age related bone loss and fracture incidence [2]. Since then, the scientific literature has grown substantially and there is a clear interest in attenuating age-related bone loss via lifestyle intervention, with physical activity being strongly recommended for fracture prevention [3]. Exercise, specifically load-bearing exercise, has been shown to attenuate or even reverse age-related losses in BMD [4]–[6]. Exercise can also help maintain or improve general parameters of fitness and health, but more than 80 percent of older adults in the US do not meet recommendations for physical activity [7] and the prevalence of osteoporosis is expected to continue growing for the next 10-20 years [8].

Along with the larger body of literature examining the effects of exercise on BMD, there is a growing body of literature examining the effects of exercise on bone morphology. While bone morphology measures provide additional context in relation to bone strength and health, the equipment necessary for these measures is significantly more expensive, less common, and have less general utility than DXA. Trabecular bone score (TBS) is a newer metric for estimating the trabecular architecture of the lumbar spine or hip [9] and may serve as a useful middle-ground for assessing bone

morphology via the data available in the lumbar spine or hip scans obtained from DXA. Studies examining the utility of TBS show increased capacity for fracture prediction compared to BMD alone [10], but there are a limited number of studies examining the relationships between physical activity and TBS [11]–[15], an even smaller number examining the effects of exercise on TBS [16]–[20], and only two studies examining the effects of exercise on TBS in older adults [16], [20]. Of the four studies examining the effects of exercise on TBS, three show a positive relationship [16], [17], [19] and one shows no effect [18]; further, it's not clear how the populations used in two of the studies, individuals post bariatric surgery [17] and amphetamine users [18], translate to the normal population. More succinctly, TBS responds similarly to exercise compared to BMD, but there is room to expand our scientific understanding, especially in the context of older adults.

REALPA is an NIH funded pilot study meant to determine the effects of resistance exercise combined with low intensity breaks in sedentary activity on health in older adults. While the study's specific aims centered on skeletal muscle and cardiometabolic health, whole-body and lumbar spine scans were taken via DXA, and the effects of this novel intervention on BMD and TBS are worth exploring. The purpose of this ancillary analysis is to determine the impact of resistance exercise with low intensity breaks in sedentary activity on whole-body BMD, lumbar spine BMD, and lumbar spine TBS. The hypothesis is that resistance exercise will increase lumbar spine BMD and TBS compared to baseline; however, the difference between resistance exercise with low-intensity exercise breaks in sedentary activity (REALPA) will not be

significantly different compared to resistance exercise with no aerobic exercise (RE) or standard aerobic exercise (RE+AE).

5.2 Methods

Study Design

Physically inactive community-dwelling older adults, 65-80 years, males and females with a body mass index (BMI) between 18.5-34.9 kg/m^2 were included in the Pennington Biomedical Research Center's (PBRC), and Louisiana State University's IRB approved study (REALPA, NCT03771417). Prior to randomization into intervention groups, subjects were screened and had their physical activity and sedentary time quantified. Before and after the intervention, subjects were assessed using VO_2 peak testing, strength testing, a whole-body DXA scan, a lumbar spine DXA scan, and a blood draw to determine blood-derived markers of bone turnover. After pre-intervention testing, subjects were randomized into 1 of 3 groups (RE, RE+AE, or REALPA). All groups participated in resistance exercise 2 times per week for the 16-week intervention; however, those randomized to RE+AE also participated in moderate intensity aerobic exercise 3 times per week and those who were randomized to REALPA participated in low-intensity aerobic exercise 6 times per day, 5 days per week; Those randomized to RE participated in resistance training only. Figure 5.1 provides additional detail for the study design.

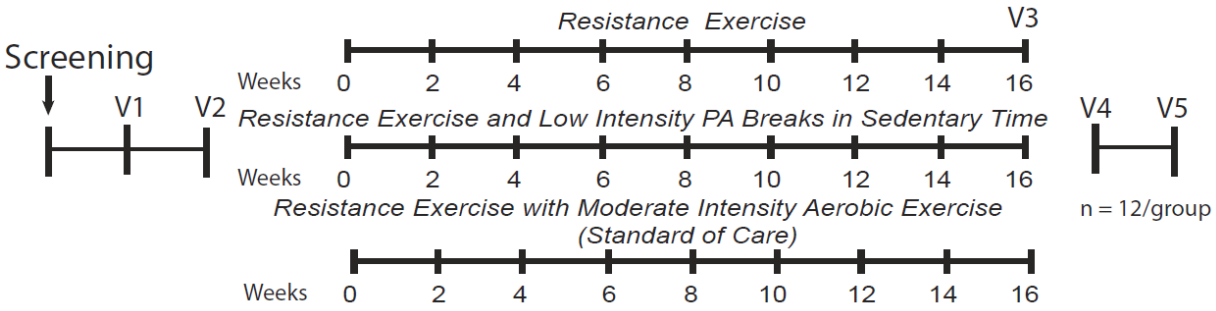

Figure 5.1. Overall study design.

Screening

Prior to intervention, subjects filled a PARQ+ and medical history questionnaire along with multiple physical activity and function questionnaires. During this time subjects also had height and weight measured along with resting vitals: heart rate, blood pressure, and EKG. Once inclusion criteria were determined, subjects had their physical activity and sedentary time quantified via a wrist-worn and another thigh-worn activity monitor (Actigraph, GT9X). These monitors were worn for 24h/d for 7 days (±3 days) prior to randomization to intervention groups; standard algorithms were used to determine physical activity and sedentary levels. Subjects who had greater than or equal to 100 min per week of moderate to vigorous-intensity physical activity were excluded.

Whole-body and Lumbar Spine BMD and TBS

Whole-body and whole-body derived regional measures of fat, muscle, and bone mass were assessed using a whole-body dual energy X-ray scanner (Hologic, Horizon A). Each subject fasted overnight (>10 hours) and were required to wear light weight clothes without zippers (e.g., t-shirt and shorts) or other DXA approved attire for each scan. They were asked to remove all metal-containing objects from his/her body, and to

lie down on the table. Subjects were carefully positioned on the table according to Hologic guidelines, and his/her legs were secured at the feet. The subjects were asked to remain completely still while the scan was in progress. Lumbar BMD and TBS were also assessed using a whole-body dual-energy X-ray scanner (Hologic, Horizon A) and the guidelines for a lumbar spine scan were followed as described by Hologic. The Trabecular Bone Score was calculated for the lumbar spine via software (TBS iNsight) from each subject's lumbar spine scan. Lumbar spine BMD and TBS are presented as lumbar vertebrae(L1-4), but measures of L2-4 and L1, L2, L3, and L4 were also measured and analyzed. Scans were taken on visit 1 and visit 4, figure 5.1.

Blood-Derived Markers of Bone Formation and Resorption

Blood was taken via the antecubital vein in the morning on fasted participants before and after the intervention (visit 1 and visit 4, figure 5.1). Samples were drawn into 5mL EDTA plasma tubes, temporarily stored in ice, and centrifuged within an hour at 500RCF at 4°C for 10 minutes. Post-centrifugation, 300µl aliquots were stored at -80°C. Beta C-terminal telopeptide of type 1 collagen (β-CTX) was measured using an enzyme-linked immunosorbent assay (ELISA; NOVUS Biologicals) as a marker of bone resorption. Procollagen type 1 Intact N-terminal propeptide (P1NP) was measured using an enzyme-linked immunosorbent assay (ELISA; NOVUS Biologicals) as a marker of bone formation.

VO_2 peak

VO_2 peak was measured using an incremental treadmill protocol with indirect calorimetry (Parvo-Medics TrueOne) based on ACSM guidelines. Subjects were

initially asked to walk at 1.5 miles per hour at 0% grade for 5 minutes. Following this initial warm-up, subjects began walking at a self-selected brisk walking speed at a 0% grade. Thereafter the speed remained constant, and the grade increased by 2% every 2 minutes until volitional exhaustion. Once volitional exhaustion was reached, a 5-minute cooldown starting at 1.5 mph and 0% grade was initiated. Heart rate was continuously assessed using a 12 lead EKG, while blood pressure and ratings of perceived exertion (RPE, Borg 6-20) were assessed every 2 minutes. VO_2 peak testing was done on the screening visit and visit 3, figure 5.1.

Strength Testing

Before strength testing began, subjects walked on the treadmill for 5 minutes to warm-up. Strength (peak torque, N◦m) of the knee extensor muscles was measured using isometrically (0°/s) isokinetic dynamometry (Biodex System 3) at 60°/s. Prior to each test, the subjects were provided with a short period of practice so that they could acclimate to the testing protocol. During each test, the subjects were asked to work as hard as they could for each repetition.

Intervention

Subjects were randomized to one of three 16-week interventions: RE, REALPA, or RE+AE (Figure 5.1). The RE group completed supervised RE (2 x/wk). The REALPA group completed a supervised RE (2 x/wk) and regular unsupervised low intensity breaks in sedentary time (5 d/wk, 6x10 min breaks/d at 2 METS (~30-40% VO2 peak), with ~1 bout per hour. Text, email, and app-based reminders were used to promote adherence. The RE+AE group completed supervised RE (2 x/wk) and calorically matched moderate intensity AE (3 d/wk, 50 min/session at 4 METS (~60-75% VO2

peak). The RE component for all 3 groups consisted of 3 sets of 10-12 repetitions to failure for a total of 8 exercises targeting the large muscle groups (Table 5.2). The AE component for the RE+AE group consisted of treadmill walking at ~4 METs for 50 min (table 5.2). All supervised exercise sessions included a 5-minute warm-up and cool-down on either a bike or treadmill. Up to 2 weeks were added to a subject's exercise intervention, to allow for the make-up of missed exercise sessions and provide flexibility for scheduling study visits. To reduce the risk of injury and muscle soreness, intensity and volume of the exercise prescriptions were progressively increased to the targeted training intensities over the first month of the intervention. Prior to each training session, resting blood pressures and heart rates were assessed. Heart rate and perceived exertion was also monitored during exercise sessions via heart rate monitors (e.g., Polar) and subjective scales (RPE, Borg 6-20) throughout the supervised sessions.

Statistical Analysis

All data were analyzed with JMP version 15.1.0 (SAS Institute, Inc., Cary, NC) statistical software. Baseline measures in, table 5.3, were compared across groups with one-way ANOVA. Outcome measures were assessed via repeated-measures analysis of variance (RMANOVA). If significant effects were found, post-hoc analysis was done using student's t test. Correlations between baseline BMD, TBS, bone turnover markers, and other parameters (age, weight, isometric strength, and VO_2peak) were assessed via linear regression (Pearson r); the same was done for changes from pre to post intervention. Paired t-tests were used to determine sex differences for baseline bone outcomes and for changes in bone outcomes. Alpha levels for significance were set at $p \leq 0.05$.

Table 5.1. Intervention protocols

	Mon	Tue	Wed	Thu	Fri
A. Resistance Exercise (RE)		• Leg Press • Leg Extension • Leg Curls • Chest Press • Lat Pulldowns • Shoulder Press • Triceps Extension • Biceps Curls 3 Sets 10-12 Reps to Failure		• Leg Press • Leg Extension • Leg Curls • Chest Press • Lat Pulldowns • Shoulder Press • Triceps Extension • Biceps Curls 3 Sets 10-12 Reps to Failure	
B. LPA Breaks in Sedentary Time (ST)	6x10 min bouts at 2 METS	6x10 min bouts at 2 METS	6x10 min bouts at 2 METS	6x10 min bouts at 2 METS	6x10 min bouts at 2 METS
C. Aerobic Exercise (AE)	1 x50 min bouts at 4 METS		1 x50 min bouts at 4 METS		1 x50 min bouts at 4 METS
The RE group will complete supervised RE (2x/wk) (A). The REALPA breaks in ST group will complete supervised RE (2x/wk) and regular unsupervised LPA breaks in ST (5x/wk) (A+B). The RE+AE group will complete supervised RE (2x/wk) and calorically matched moderate intensity AE (3x/wk) (A+C).					

5.3 Results

Baseline Characteristics, Correlations, Sex Differences

Baseline characteristics are shown in table 5.2; no significant differences were found between groups for bone outcomes and the other parameters presented in the table based on one-way ANOVA. Baseline correlations between TBS and BMD are

shown in figure 5.2. Positive correlations were found between whole-body BMD and lumbar spine BMD, whole-body BMD and lumbar spine TBS, and lumbar spine BMD and TBS (r= 0.9, 0.8, and 0.9, respectively; $p<0.0001$). Bone outcomes were also compared to age, weight, isometric strength, and VO_2peak, shown in figure 5.3. Bodyweight was shown to be positively correlated to whole-body BMD, lumbar spine BMD, and lumbar spine TBS (r= 0.7, 0.7, and 0.6, respectively; $p<0.01$). Isometric strength was shown to be positively correlated to whole-body BMD and lumbar spine TBS (r= 0.6 and 0.5, respectively; $p<0.05$). Females were shown to have significantly lower baseline whole-body BMD (0.96g/cm^2 vs 1.16g/cm^2), lumbar spine BMD (0.91g/cm^2 vs 1.15g/cm^2), and lumbar spine TBS (1.27 vs 1.41), $p<0.05$.

Changes and Correlations, Pre vs Post

No significant effects were found pre vs. post or across groups for whole-body BMD, lumbar spine BMD, or lumbar spine TBS, shown as change from pre to post in table 5.3. While not presented as baseline values for table 5.2 or as changes from pre to post in table 5.3, the same analysis was done on whole-body derived regional measures of BMD (thoracic spine, pelvis, ribs, arm, and leg) and on the BMD and TBS of individual vertebrae (L1, L2, L3, L4, and L2-L4); no significant effects were found. Correlations between bone outcomes (whole-body BMD, lumbar spine BMD, and lumbar spine TBS) only found a significant correlation between lumbar spine BMD and TBS, r=0.5 and $p<0.05$; figure 5.4 shows changes in lumbar spine BMD and TBS for each participant (n=18), ordered by BMD changes. Changes in lumbar spine TBS were also correlated with changes in weight, r=-0.5 and $p<0.05$. No other correlations were

found in relation to bone outcomes. No sex differences were found for bone outcome changes from pre to post ($p>0.7$).

Blood-derived Markers of Bone Turnover

Due to high variability between replicates and known issues determined post-assay, data for CTX and P1NP are not shown. Models were run with and without high variability replicates, all showed no change from pre to post and no differences between groups, but the data is not considered reliable. Other literature has shown no change in response to exercise in older adults undergoing weight loss [20].

Table 5.2. Baseline characteristics

		Treatment Group		
	All	RE	RE+AE	REALPA
n	18	5	7	6
Female (n)	11 (61)	3 (60)	4 (57)	4 (67)
Age (y)	70.4 (4.3)	71.3 (3.3)	70.3 (5.6)	69.7 (4.0)
Body weight (kg)	79.5 (9.9)	81.8 (9.9)	79.6 (7.0)	77.5 (13.7)
BMI (kg/m^2)	28.5 (3.3)	30.6 (4.4)	27.6 (2.9)	27.7 (2.3)
Isometric Strength (Nm)	152 (40)	149 (36)	162 (47)	143 (41)
VO_2 peak ($ml \cdot kg^{-1} \cdot min^{-1}$)	20.2 (5.4)	19.3 (6.7)	22.0 (4.3)	18.9 (5.7)
Lumbar Spine BMD (g/cm^2) L1-L4	1.00 (0.19)	0.94 (0.23)	0.99 (0.17)	1.07 (0.20)
Lumbar Spine TBS L1-L4	1.32 (0.11)	1.28 (0.16)	1.33 (0.04)	1.34 (0.13)
Whole Body BMD (g/cm^2)	1.04 (0.13)	1.02 (0.15)	1.04 (0.12)	1.05 (0.15)

Data are n (%) or mean (SD).

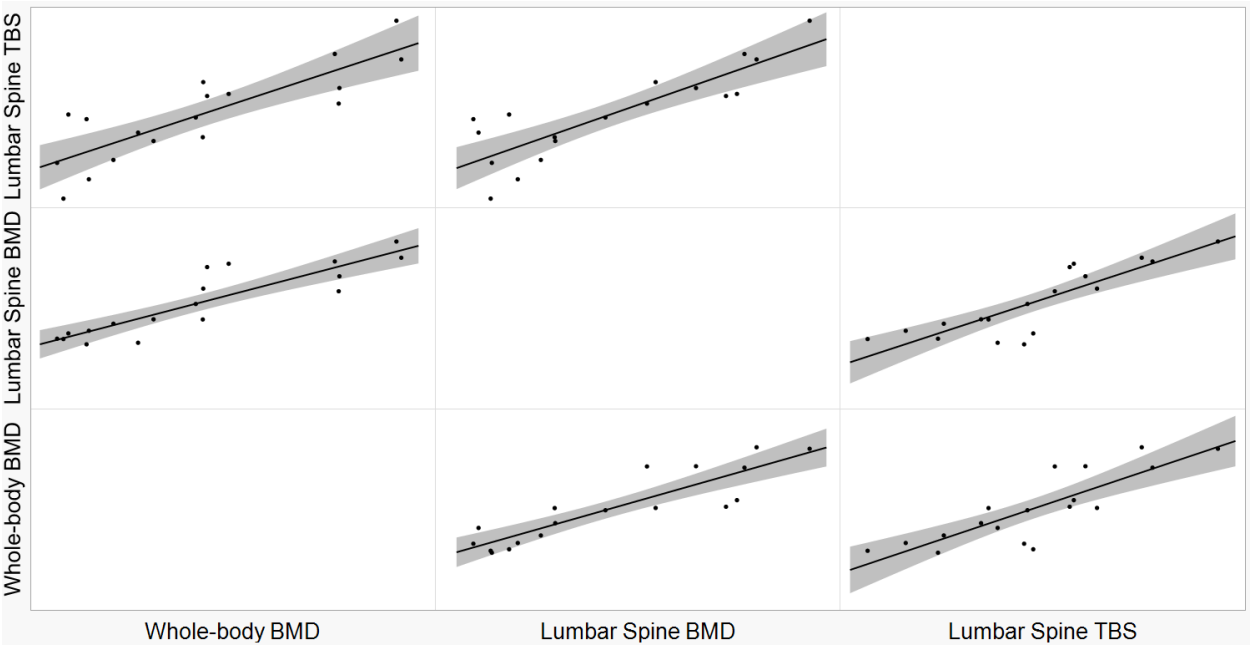

Figure 5.2. BMD and TBS correlations at baseline. Positive correlations were found between whole-body BMD and lumbar spine BMD, whole-body BMD and lumbar spine TBS, and lumbar spine BMD and TBS (r= 0.9, 0.8, and 0.9, respectively; $p<0.0001$)

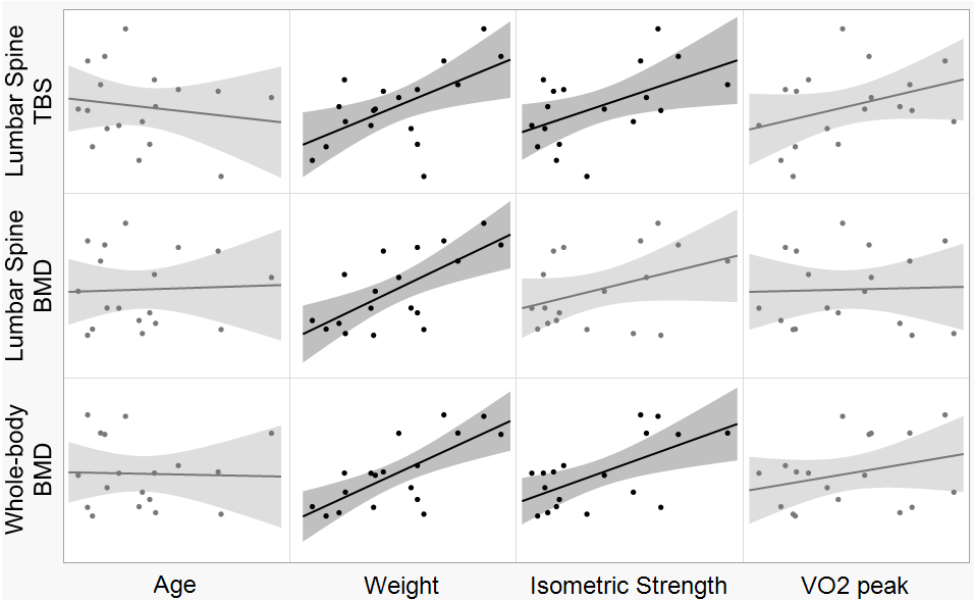

Figure 5.3. BMD and TBS correlations with age, weight, strength, and VO_2 peak. Body weight was shown to be positively correlated to whole-body BMD, lumbar spine BMD, and lumbar spine TBS (r= 0.7, 0.7, and 0.6, respectively), and isometric strength was shown to be positively correlated to whole-body BMD and lumbar spine TBS (r= 0.6 and 0.5, respectively); $p<0.05$.

Table 5.3. Changes from pre to post

	All (n=18)	Treatment Group RE (n=5)	RE+AE (n=7)	REALPA (n=6)	Effect (p-value) Pre/Post	Group
Body weight (kg)	0.30±1.08	0.10±2.03	-0.05±1.71	0.86±1.85	0.56	0.83
Lean Mass (kg)	**1.39±0.85**	**1.70±1.59**	1.05±1.34	1.43±1.45	**<0.01**	0.84
Fat Mass (kg)	-1.05±1.12	-1.62±2.11	-0.95±1.78	-0.57±1.92	0.06	0.34
Isometric Strength (Nm)	**16.8±10.5**	17.6±19.7	13.0±16.6	**19.7±18.0**	**<0.01**	0.79
VO_2 peak ($ml \cdot kg^{-1} \cdot min^{-1}$)	0.64±1.07	0.51±2.13	-0.43±1.62	1.85±1.75	0.22	0.75
Lumbar Spine BMD (g/cm^2) L1-L4	0.006±0.013	0.017±0.024	0.006±0.020	-0.005±0.022	0.31	0.60
Lumbar Spine TBS L1-L4	0.011±0.016	0.015±0.031	0.024±0.026	-0.005±0.030	0.16	0.69
Whole Body BMD (g/cm^2)	-0.003±0.007	-0.003±0.013	-0.004±0.011	-0.004±0.011	0.29	0.91

Data are shown as change scores ± 95% confidence interval. Change scores that represent a significant difference from pre- to post-means are bolded. Significant p-values are bolded. No significant effects were found for bone outcomes; there was no differences in bone outcomes after 4-months of resistance training.

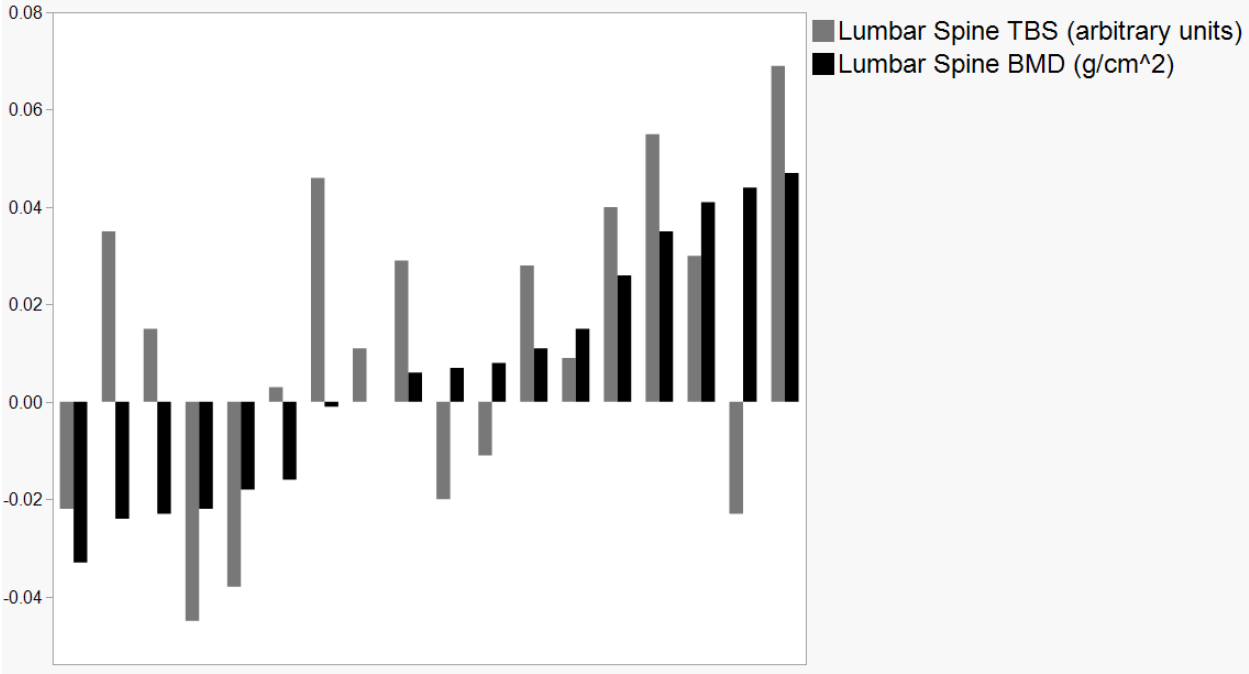

Figure 5.4. Changes in lumbar spine BMD and TBS for all participants (n=18) ordered by ascending BMD. Changes in lumbar spine BMD and TBS are significantly correlated, r=0.5 and p<0.05.

5.4 Discussion

The purpose of this ancillary analysis was to determine the effects of resistance exercise with low-intensity physical activity breaks in sedentary time (REALPA) on whole-body BMD, lumbar spine BMD, and lumbar spine TBS. It was hypothesized that lumbar spine BMD and TBS would increase in response to resistance exercise (RE, RE+AE, REALPA), but no significant differences would be found between groups. Four-months of resistance training did not result in any changes to whole-body BMD, lumbar spine BMD, and lumbar spine TBS. Correlations at baseline were in-line with the literature; weight and muscle strength were positively correlated with BMD, and sex differences showed female participants had lower BMD at baseline. Considering the typical response of bone to resistance exercise, a lack of change in bone outcomes

after a resistance training-based intervention is interesting. Systematic reviews examining exercise dependent changes in bone density for older adults show that larger magnitude changes in bone density are typically found when resistance exercise is implemented, compared to aerobic or combination [4], [5], [21]. Many of the studies on exercise derived changes in BMD implement longer interventions, which may partially explain the lack of differences found here. Although, there are at least two studies that implemented a similar, 16-week, resistance training intervention and found either no change in BMD, Ryan et al. [22], or modest improvements, Menkes et al [23]. Both studies utilized a similar training stimulus, but Menkes et al. used males while Ryan et al. used women, and both studies had an average participant age of about 60 years, while the average participant age for this ancillary analysis was 70 years. The differences in age and sex may explain the disparities in BMD changes for our study and the others mentioned.

Changes in TBS were comparable to BMD in this study. At baseline, there were strong correlations between TBS and BMD, which is expected as both are shown to predict fracture risk, but with differences in the specific parameters measured. Change scores for BMD and TBS were similarly related, although the correlation was only significant for lumbar spine. Figure 5.4 shows a waterfall plot for both lumbar spine BMD and TBS; it is meant to highlight the potential variability between the measures, but the relationship is still clear as a large increase or decrease in BMD for an individual is rarely accompanied by a large change for TBS in the opposite direction. Even so, the aforementioned generalizations should be taken lightly as significant differences were not found from pre to post for BMD nor TBS. While previous literature has indicated that

TBS predicts fracture risk independently of BMD and with greater accuracy in combination [10], TBS of the lumbar spine did not provide any additional context in this 16-week resistance training intervention in relation to bone changes. This is not to say that measures of TBS are meant to respond to potential interventions at a more rapid rate, as TBS is an estimation of the trabecular microstructure [9], which has not been shown to be more or less static compared to BMD over time. Two other studies have examined the effects of exercise on TBS in older adults, with one showing increases in response to a high-intensity, 20-week, jumping intervention [16] and another showing no change after an 18-month weight-loss and exercise intervention [20].

This study is initially limited by its ancillary nature. Because bone outcomes were not considered prior to design, the duration of the intervention was substantially shorter than what is typical when examining the effects of exercise on bone. For example, Gomez-Cabello et. al. reviewed 14 papers related to bone changes in response to resistance exercise and of those studies, the two shortest interventions were 16-weeks and showed mixed results, while the average intervention length for the studies reviewed was 10 months, and generally showed improvements in bone density [4]. This study is also limited by the lack of a control group, an intentional decision for this pilot study. The lack of a control group has clear implications for determining the effects of exercise after 4-months, versus the maintenance of sedentary habits, but we chose not to enforce poor habits in an already at-risk population of sedentary older adults for a pilot study. The last substantial limitation, in the context of this ancillary analysis, is the lack of a larger participant pool. This study had originally intended to recruit 10-12 participants per group (n=36, total), but due to the COVID-19 pandemic and its

substantial risk to the older adult population, we temporarily suspended the study. While the above limitations are substantial, the results of this study are strengthened by the novel nature of measuring the TBS of older adults participating in an exercise trial, as there has only been two other studies to do so [16], [20].

In conclusion, a 4-month resistance exercise intervention with low-intensity physical activity breaks in sedentary time (REALPA) did not significantly change whole-body BMD, lumbar spine BMD, or lumbar spine TBS. Still, it is not clear that outcomes were unfavorable compared to potentially sedentary behavior, as it is possible that the addition of a sedentary control would have shown reductions in BMD and/or TBS over the 16-week period. Due to TBS's novel nature, the general benefits of exercise to fitness and health, and the well-appreciated relationship between bone density and fracture risk, future research should implement a similar intervention with a longer duration and the potential addition of hip derived TBS.

Funding: The REALPA was funded in part by a grant from the National Institute of Aging (1R21AG058181).

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CHAPTER 6. CONCLUSION

The purpose of this dissertation was to determine the effects of exercise on bone in relation to other potential modifiers: individuals with type II diabetes mellitus (T2DM), rodents on a ketogenic diet, and older adults participating in a novel modulation of their sedentary time. The first study, chapter 3, was an ancillary analysis of a large exercise intervention trial on individuals with T2DM. After 9-months, the entire cohort (n=191), sedentary control group included, showed an increase in whole-body, thoracic spine, rib, arm, and leg bone mineral density (BMD). The lack of between group differences, especially between the control and exercise groups, was surprising as we hypothesized whole-body and regional measures of BMD would increase for the resistance and combination exercise groups, but not for the sedentary and aerobic groups. While these results were unexpected, this research is one of only two studies that examine the relationship between bone and individuals with T2DM. Future research should utilize longer interventions, measures of bone that do not depend on density, and interventions specifically aimed to modulate bone. Ongoing research in Italy is already addressing the suggestions mentioned above.

Chapter 4 demonstrated a well-understood relationship between exercise and bone, where mice that exercised 5d/wk at a vigorous intensity for the 3-weeks prior to euthanasia had more robust trabecular morphology than non-exercising mice. Further, the general effect of a ketogenic diet on bone was also demonstrated in this study, as mice eating a ketogenic diet and exercising did not demonstrate similar improvements in trabecular bone. Though the effects of a ketogenic diet on bone in exercising mice did not reveal any differences compared to non-exercising mice, both ketogenic and control

diets, future research should expand the length of intervention to see if the additional adaptation window would allow for more group specific differences to develop. Additionally, there is a clear need for further research in the area of ketogenic diets and exercise in humans, as the adoption of ketogenic diets and physical activity in conjunction with each other is not necessarily uncommon when the overall goal is to improve metabolic health and reduce weight. Rodent models are useful analogues for humans, but explicit outcomes for human subjects would provide additional clarity.

Chapter 5 added to the already substantial body of literature on the effects of exercise on bone density in older adults; however, the addition of trabecular bone score (TBS) along with the more well understood metric of BMD adds to the current literature. Further, the combination of resistance exercise and multiple daily bouts of low-intensity physical activity had some potential to modify bone in a novel way. The results for the intervention did not show any changes for BMD in response to resistance exercise, with or without low-intensity bouts of exercise though out the day and the same was true for TBS. While the use of blood derived markers of bone formation and resorption did not provide useful context to the lack of BMD and TBS changes in our research; future research should utilize these metabolic markers along with more static measures like BMD and TBS to widen our understanding of the measurable factors related to bone.

In conclusion, the general relationships between bone density and exercise are clear, but novel modulators of bone such as T2DM and ketogenic diets need further research to be better understood. With respect to measuring bone, methodologies such as TBS and blood derived markers of bone turnover would benefit from continued research to increase evidence of validity in various populations and in response to

various interventions. Measures like TBS may provide additional clarity to bone changes but could be especially useful in populations that have abnormal BMD, such as individuals with T2DM. Markers of bone turnover in blood could provide useful context of overall bone metabolism and may give enough evidence for the efficacy of an intervention as a modulator of bone, without needing to carry out long duration pilot trials to pursue larger funding.

APPENDIX. STUDY APPROVAL FORMS

1.1 IRB Stamped and Approved HART-D Protocol

IRB #PBRC26046 06/19/2009

Health Benefits of Aerobic and Resistance Training in individuals with type 2 diabetes (HART-D)

Timothy S. Church, MD, PhD, MPH, Principle Investigator
Conrad P. Earnest, PhD, Co-Investigator
William T. Cefalu, MD, Co-Investigator

Project Summary

The goal of the proposed study, Health Benefits of Aerobic and Resistance Training in individuals with type 2 diabetes (HART-D), is to compare the effect of resistance training alone (RT), resistance in combination with aerobic training (AT+RT), and aerobic training alone (AT) to stretching and relaxation (SR) on hemoglobin A1C (HbA1C), in initially sedentary women and men with type 2 diabetes (T2D). Although it is generally accepted that regular exercise provides substantial health benefits to individuals with T2D, the exact exercise prescription in terms of type (AT versus RT versus AT+RT) still remains largely unexplored, particularly in regard to week-to-week glucose control as assessed by HbA1C.

There is a need for more adequately powered and well-controlled studies to examine the effects of RT, AT and AT+RT on HbA1C in individuals with T2D. With the incidence of T2D expected to increase dramatically in the coming years, it is essential to have a better understanding of the relative benefits of various exercise interventions. This information can help better formulate exercise recommendations for patients with T2D as well as potentially provide more exercise options, which is important given the small percentage of individuals with TD2 who regularly exercise.

The study group will be sedentary women and men with T2D, aged 30 to 75 years. We will randomly assign 300 individuals to an aerobic exercise training only group (AT; n=87), a resistance training only group (RT; n=87), a combination of aerobic plus resistance training (AT+RT; n=87), or a stretching and relaxation group (SR; n=40). The SR individuals will complete one or more 45 minute sessions with a trained exercise professional that focus on increasing flexibility and reducing stress. This group is intended to serve as a control group, as we do not expect stretching to have measurable effects on HbA1C. The AT individuals will participate in 3 or 4 training sessions each week for 9 months progressing to a total energy expenditure of 12 kcal/kg/week (KKW), which is an exercise dose consistent with the current public health recommendations for physical activity for individuals with T2D.[1,2] The target exercise intensity will be 50%-80% of baseline VO_2 max. The RT group will participate in 3 sessions per week (9 exercises, 2-3 sets each), which focuses on large muscle groups. This RT regimen is based on the studies that most successfully improved HbA1C in individuals with T2D. Individuals in the AT+RT group will complete 10 KKW of aerobic training and a reduced resistance-training regimen of 2 sessions per week (9 exercises, 1 set of each). The AT+RT regimen represents the exercise recommendations of the American College of Sports Medicine (ACSM) and the American Diabetes Association (ADA).[3,4] All participants will complete a one hour consultation with a Certified Diabetes Educator (CDE) following randomization, during which, participants will be provided with educational materials and general guidelines for healthy living. Participants will also complete monthly sessions with the CDE, during which they will receive further instruction and guidance.

Simply stated, we wish to compare the effect of resistance training alone, resistance in combination with aerobic training, and aerobic training alone to stretching exercise on HbA1C, in initially sedentary women and men with T2D. The primary outcome measure is HbA1C, an integrated measure of blood glucose control over the past 8-12 weeks. Other outcomes of interest include homeostasis model assessment (HOMA), resting blood pressure, C-reactive protein (CRP), total body fat, and lean muscle mass as measured by DEXA, cardiorespiratory

1.2 IACUC Study Modification Approval

Form Version 2.2

Pennington Biomedical Research Center

Protocol Amendment Form

Protocol Number:	941	Submitted Date:	04/22/2016 08:48 AM
Principal Investigator:	Robert Charles Noland	Approved Date:	04/22/2016
Protocol Title:	Exploration of the role of carnitine octanoyltransferase in preventing lipid-induced insulin resistance.		

SECTION A: Modifications being proposed

A.1: Number of animals **(addition of greater than 5% requires full committee review)**

- Proposed modification and how does this differ from the original protocol:

  Requesting 40 additional C57BL6/J mice. The original protocol has approval for 2976 mice, thus the addition of 40 additional mice represents <1.5% of the original mouse total.

- Provide justification for the additional animals:

  We are proposing to perform a study comparing metabolic adaptations in mice fed a chow diet vs. a ketogenic diet that have either remained sedentary or been exercise trained (justification for experiment is stated below in Section B). As described in our original protocol, 10 mice per group provides statistical power between 0.8-0.95 for all groups. Since there are 4 groups in the proposed study and we require 10 mice per group, we are requesting an additional 40 C57BL6/J mice to complete this study.

A.3: The modification, addition or deletion of a procedure

- Proposed modification and how does this differ from the original protocol:

  Due to the nature of the ketogenic diet, we are proposing weekly monitoring of ketone levels in the blood via tail vein collection over the course of 8 weeks. A NovaMax Plus ketone meter would be used which requires 0.3uL per test strip. This type of monitoring is identical to how we obtain glucose and lactate readings from the tail vein and these procedures are already approved in the original Protocol 941.

A.15: Other modifications not listed above

- Proposed modification and how does this differ from the original protocol:

  We are proposing to put 20 C57BL6/J mice on a high fat ketogenic diet (Test Diet 5TJQ, Purina) for 8 weeks while 20 control mice will be fed a chow control diet (Test Diet 5TJS). At week 4 of the dietary intervention half of each dietary group will be placed on a 4 week endurance exercise training protocol. The endurance exercise training protocol is already approved in Protocol 941.

SECTION B: Justification for the modification(s):

We have exercise trained mice on a low fat standard chow diet in order to determine whether endurance exercise training alone is sufficient to enhance peroxisomal function and carnitine octanoyltransferase (CrOT) activity. The results from these studies have been somewhat marginal; however, this is not necessarily unexpected since it seems likely that peroxisomal function and CrOT activity would not be driven unless a significant lipid stimulus were also present. Moreover, there is also evidence that consumption of carbohydrate in the post-exercise period negates adaptations in lipid metabolism pathways. In order to address whether a lipid stimulus facilitates greater peroxisomal adaptations and CrOT function in response to exercise training we are proposing to put mice on a high fat ketogenic diet. This diet provides 84% of kcal/g from fat and 16% from protein, while 0% is provided by carbohydrate. While the composition of this diet may not represent one that would be adopted by humans, we feel it provides an ideal model to determine whether exercise plus a high lipid environment will result in greater adaptations in peroxisomal function and CrOT activity without the confounding effects of carbohydrate that could limit adaptations in lipid metabolism pathways. To achieve this goal we intend to put 20 C57BL6/J mice on a high fat ketogenic diet, while 20 mice will remain on a standard chow diet containing 16% of energy from protein, 12% from fat and 72% from carbohydrate. We are proposing to monitor ketones in the blood every week to not only validate that the ketogenic diet is working, but also to ensure the safety of the animals. Half of each group will be subjected to a 4 week endurance exercise training regimen and peroxisomal adaptations and CrOT activity will be measured at the end of the study period.

SECTION C: Additional Personnel

- **Personnel**

Name	Email	Phone	Role
John Brown	john.brown@pbrc.edu	225 763-2796	Student Worker • Restraint Monitoring • Blood Collection • Animal Breeding • Animal Breeding Records and Reports • Behavioral Experiments • Routine Noninvasive Procedures • Routine Animal Colony Management • Anesthesia • Euthanasia
Matthew Scott	mscot26@lsu.edu	225 763-2796	Graduate Student

Veterinary Review

☐ Significant amendment: **Requires full committee review**

IACUC Chair signature: _____ Date: _____

☐ Significant amendment: **Requires new protocol submission**

1.3 REALPA IRB Approval Letter for REALPA

PENNINGTON BIOMEDICAL RESEARCH CENTER LSU

IRB Certificate of Approval

FWA # 00006218

Date of Approval: **June 20, 2018**
Study Expiration Date: **June 5, 2019**
Submission Type: **Initial**
Review Frequency: **12 months**
Number of Subjects Approved: **45**
Review Type: Expedited Approval of Board Requested Revisions
Approval Status: **Approved**

Principal Investigator: **Brian Irving, Ph.D.**
IRB # **2018-026-LSU REALPA Breaks in Sedentary Time Pilot Study**
Title: **Resistance Exercise and Low-Intensity Physical Activity Breaks in Sedentary Time to Improve Skeletal Muscle and Cardiometabolic Health in Older Adults–REALPA Breaks in Sedentary Time Pilot Study**
Sponsor: **National Institute on Aging (NIA)**

Approval Includes: **Irving IRB IFC Ia V3 Clean 061118 (Informed Consent (Part IA)), Irving IRB Protocol V3 Clean 061118 (Protocol), Irving IRB IFC Ib Clean 061118 (Informed Consent (Part IB)), HIPPA_061118 (HIPAA), IPAQ Eldelry (Questionnaire), SLUMS Mental Health Status (Questionnaire), Pittsburgh Fatigability Scale (Questionnaire), Sedentary Behavior Questionnaire (Questionnaire), PROMIS Physical Function Questionnaire (Questionnaire)**

Clinicaltrials.gov responsibility: **Principal Investigator**

This study is required by law to be registered on clinicaltrials.gov within 21 days after the 1st subject enrolls. The International Committee of Medical Journal Editors (ICMJE) requires that all clinical trials be entered into a public registry as a condition of consideration for publication.

Investigators and study staff must comply with the Human Research Protection Program policies and procedures that apply to IRB members and staff, which can be found at www.pbrc.edu/HRPP

Signed Wednesday, June 20, 2018 1:47:48 PM ET by Rhode, Paula Ph.D.

6400 Perkins Road, Baton Rouge, Louisiana 70808-4124 • Phone: (225) 763-2693 • irb@pbrc.edu

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VITA

Matthew Scott was born in southern Louisiana in November of 1989. He graduated from Chalmette High School in 2008 and began his college career at Louisiana State University in fall of the same year. Although Matthew's interest in music led him to college, he changed his focus to kinesiology in pursuit of scientific knowledge in physiology and exercise. He completed his bachelor's degree in 2012, his master's degree in 2014 and now his doctoral degree, all here at LSU. Since beginning his graduate studies, Matthew married his wife Kristina, had two kids, and built a life alongside his academic pursuits. Upon graduation, Matt plans to continue in academia or pursue some other work that allows him to continue enjoying his life with family and friends nearby.