AN IN VIVO EXPLORATION OF SKELETAL MECHANOSENSITIVITY AND ASSOCIATED FRAGILITY IN A CANADIAN COHORT OF WOMEN

by

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Abstract

The function of skeletal adaptation to mechanical load is to adjust the amount and distribution of bone tissue (geometry); such that stresses experienced within the bone are kept within certain physiological limits and fractures are prevented. Genetic, environmental or hormonal factors may cause heterogeneity in this adaptive response, altering geometry and consequently fragility. The purpose of this thesis was to explore the skeletal response to load in vivo, by evaluating stress at the hip under three different conditions: FRACTURE (Study 1), DIABETES (Study 2) and ESTROGEN deficiency (STUDY 3). We studied women 25 years of age or older who participated in the Canadian Multicentre Osteoporosis Study and had available Hip Structure Analysis (HSA) data from baseline dual energy x-ray absorptiometry (DXA) scans. Women were categorized according to fracture status (fracture or no fracture), diabetes status (diabetes or no diabetes) and estrogen use (current users or never users). We computed stress (megapascals=MPa) at the infero-medial margin of the femoral neck in a one-legged
stance using a 2-D engineering beam analysis. We used linear regression to determine associations between femoral neck stress and each categorical variable. Study 1 (n=2168) demonstrated higher stresses in postmenopausal women with fractures compared to women without fractures (10.57 ± 2.19 vs. 10.30 ± 2.03 MPa; p=0.0031). Study 2 (n=3665) demonstrated higher stresses in women with Type 2 Diabetes Mellitus compared to non-diabetic women (10.98 ± 2.33 vs. 10.57 ± 2.20 MPa; p=0.0194). Study 3 (n=2447) demonstrated higher stresses in postmenopausal women not on estrogen than in premenopausal women (10.66 ± 2.14 vs. 10.09 ± 2.01 MPa; p<0.0001), but no differences in stresses between postmenopausal women on estrogen and premenopausal women (10.16 ± 2.00 vs. 10.09 ± 2.01 MPa; p=0.6102). Since stress is an indicator of underlying geometry, and geometry should be adapted to prevalent loads, higher stress indicates weaker geometry and suggests an impaired modeling response in these three conditions. Compromised modeling has important clinical implications in terms of treatment selection, as individuals with reduced load sensitivity may respond best to metabolic agents that would improve modeling responses to load stimuli.
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CHAPTER 1: INTRODUCTION
1.1 Introduction

1.1.1 The burden of osteoporosis and fracture

Osteoporosis is characterized by a reduction in bone mass and a disruption of skeletal microarchitecture leading to an increased susceptibility to fracture with minimal trauma [1]. In Canada, approximately 1 in 4 women and 1 in 8 men over the age of 50 years have osteoporosis [2, 3]. The main consequence of osteoporosis is fractures, which typically occur at the hip, spine and wrist. While a fracture at any site can cause pain and disability, hip fractures are the most severe as they require hospitalization and can lead to premature death [1]. Indeed, osteoporosis is the cause of over 24 000 hip fractures each year, a figure that is expected to double by 2040 [4]. Individuals who sustain a hip fracture are plagued by many complications, including pain, disability, loss of independence and quality of life [5]. Furthermore, there is a 12-20% increased risk of mortality within the first year after a hip fracture [6].

While the incidence rates of osteoporotic fractures among Canadians are stabilizing, many people at high risk for fractures remain untreated. Recent data shows that 45-73% of Canadians who have sustained a low trauma fracture are not diagnosed or treated for osteoporosis [7]. Assuming demographic trends continue, fractures due to osteoporosis will continue to be a substantial source of morbidity and mortality among Canadians. Osteoporosis fractures are also costly. Annual economic implications of hip fractures in Canada are $650 million and are expected to rise to $2.4 billion by 2041 [5]. Preventing
osteoporosis may therefore be one way to reduce health care costs, as well as the burden of illness associated with osteoporosis.

1.1.2 Determining fracture susceptibility

Fractures occur when the stresses (force concentrations) from applied loads exceed the stress limits (material strength) of bone tissue [8]. Under any particular loading condition, stresses are generally determined by bone structural dimensions (geometry), while the material strength is a function of the composition of the bone tissue (i.e. tissue mineralization, orientation and properties of collagen fibers etc.). The ageing process is associated with changes in the amount of bone tissue and its distribution, resulting in alterations in bone geometry. It is also apparent that older bone tissue tends to be more brittle so that it fails more abruptly under load. However, material strength is not necessarily degraded with age [9, 10]. It is the interplay between these factors that ultimately determines the fragility of a bone and its susceptibility to fracture.

Clinical practice guidelines for the diagnosis and treatment of osteoporosis incorporate assessments of dual energy x-ray absorptiometry (DXA) measurements of bone mineral density (BMD) – bone mineral content/bone projected area. While low BMD is a strong and consistent risk factor for fracture [1, 11], many osteoporotic fractures occur in individuals who do not have osteoporosis by DXA criteria [12]. As aforementioned, the strength of a bone is determined by the geometry (size, shape and distribution of bone), as well as its material properties. Strength effects are not easily inferred from conventional DXA measures of BMD which do not completely capture underlying
geometric effects. For instance, if a bone’s projected area increases in size, BMD would paradoxically decrease even if there was no net change in bone mineral content. Moreover, increases in bone area would tend to improve structural rigidity despite reductions in BMD. Therefore, using BMD as a surrogate for bone strength has evident limitations.

There have been attempts to measure geometry more directly using Hip Structure Analysis (HSA). This method is advantageous because geometric information can be extracted easily from conventional DXA BMD scans. Bone geometry more accurately captures strength, and therefore may be more useful in clinical practice to identify those at risk for fracture.

1.1.3 Skeletal repair and adaptation to mechanical load

Bone tissue has the remarkable ability to continually repair and renew itself by the process of remodeling. More importantly for this thesis, bone tissue also has the ability to adapt its geometry to changes in mechanical loads imposed by activities of daily life through the process of modeling.

The function of bone’s adaptation to mechanical load is to adjust the amount and distribution of bone tissue (geometry) so that the bone is suited to resist the loading forces imposed by normal activities. This is accomplished by removing bone from surfaces within the bone where it is not needed and adding it to surfaces where it is needed. This implies that there is equilibrium between prevalent loading forces, or more
properly the stimuli they generate within bone and the structural geometry resulting from adaptation. The modeling stimulus is thought to be the minute deformations (strains) within bone tissue produced by loading forces. While not completely understood, the adaptive mechanism is currently believed to be initiated by signals generated by osteocytes perturbed by the deformation of the surrounding bone tissue. According to Frost’s Mechanostat theory (which describes this adaptive process) [13], if activity related strains exceed a certain upper threshold, the osteocytes generate a biochemical signal to initiate the formation of new bone on surfaces exceeding that threshold. Similarly, when activity generated strains are absent, or fall below a certain lower threshold, the osteocytes generate a signal to resorb (break down) excess bone on surfaces where strains are lowest. Once new bone is formed, strain levels are reduced below the upper threshold and bone formation ceases. Similarly, the resorption (break down) of bone in low activity conditions continues until strains increase above the lower set point. In this manner, bone adapts to the mechanical loading effects of activity by becoming stronger or weaker in absolute terms as activity levels change, but keeping strength at an appropriate level to withstand forces generated by current activity levels.

It is likely that the sensitivity of bone to loading may be enhanced or diminished and thus may vary between individuals. Systemic (hormones, vitamins, drugs, nutrients) and local (genes, cytokines, ligands, paracrine/autocrine changes) non-mechanical agents [14-16] have been suggested to work in synergy with the Mechanostat, thereby affecting its sensitivity [13]. Specifically, these agents are postulated to increase or decrease bone’s modeling and remodeling thresholds, such that adaptive changes are either blunted or
enhanced accordingly. This process, however, is difficult to observe without directly measuring strain levels with implanted strain gauges in vivo. Thus, it is difficult to gather direct evidence to investigate whether skeletal fragility is influenced by altered response to loading. Instead, we must infer these relationships mainly via use of cell culture or animal models. This thesis however, will approach the inability to measure strains by computing maximal stresses at the femoral neck in a one-legged stance. Stresses are proportional to strains and can be computed using engineering modeling given knowledge of estimated loads and geometry of the femur. Our conjecture is that differences in mechanosensitivity will be apparent as differences in maximal load stresses.

This thesis will explore Mechanostat sensitivity and consequential geometric alterations at the hip, through the presentation of three separate studies. Study 1 (FRACTURE) will examine associations between fracture frequency and stress, Study 2 (DIABETES) will examine associations between diabetes and stress and Study 3 (ESTROGEN) will examine associations between estrogen deficiency, exercise and stress. Following is a brief summary of literature in each of these topic areas followed by the rationale, objectives and hypotheses for each study. Background information as well as a more detailed review of literature in each of these areas will be presented in Chapters 2 and 3.

1.2 Study 1: FRACTURE

1.2.1 Summary and rationale for Study 1
Changes in bone geometry and strength throughout life are governed by bone modeling and remodeling patterns at endocortical, intracortical and periosteal surfaces of long bones [17]. After closure of the epiphyses and up until the third decade of life, bone remodeling occurs such that the amount of bone formed is the same as the amount of bone resorbed with no net bone loss. With advancing age there is a shift, and bone resorption starts to exceed bone formation such that there is an overall net loss of bone[17, 18]. This net loss is thought to be a result of age-related changes in nutritional, hormonal, genetic and environmental factors [19]. With less bone available to support loads, aging bone adapts by stimulating bone formation on the periosteal surface where strains experienced in the bone cross-section are highest. The addition of new bone via the modeling process increases the outer diameter (area) of the bone and allows for more of the bone material to be distributed further from the neutral axis. This adaptation effectively preserves bending strength, but paradoxically reduces BMD because BMD is a function of bone content over area. Of note, elastic stability (susceptibility to buckling), is also reduced with aging [20].

Several recent studies support the notion that bone structure is well-adapted to normal loading conditions but less well-adapted to atypical loading [21-23]. Fractures as a result of abnormal loads can partially explain fragility in aging individuals, but not completely. Genetic evidence suggests that there may be inherent differences in load sensitivity in individuals with fracture compared to those without fractures [24]. However, the work is preliminary, and this relationship has not been studied using a stress model. Study 1 was
therefore designed to explore the existence of heterogeneity in load response among older women with and without fractures

1.2.2 Objective and hypothesis for Study 1

Objective 1: To determine associations between the number of self-reported fractures, bone geometry parameters and stress at the femoral neck in a Canadian cohort of postmenopausal women.

Hypothesis 1: The number of self-reported fractures will be positively associated with stress. The number of self-reported fractures will be negatively associated with narrow neck (NN) BMD, cross-sectional area (CSA) and section modulus (Z) (see Appendix 3: Glossary of Key Terms).

1.3 Study 2: DIABETES

1.3.1 Summary and rationale for Study 2

Approximately 285 million people worldwide are affected by diabetes and this number is expected to rise to 438 million by 2030 [25]. In Canada, more than 9 million people are living with diabetes or prediabetes. Type 2 diabetes mellitus (T2DM) is the most common form accounting for 90-95% of diagnosed cases, and is often referred to as non-insulin-dependent diabetes, or adult onset diabetes. T2DM describes individuals who have insulin resistance [26]. The number of people with T2DM is increasing
dramatically in Canada due to our aging population, the rise in obesity and increased prevalence of sedentary lifestyles [25].

T2DM is associated with increased fracture risk, particularly in women more so than men [27, 28]. In a meta-analysis comparing five studies, the relative risk for hip fracture associated with type 2 diabetes was 1.38 (95% CI, 1.25-1.53) [28]. Several other studies have also confirmed these findings [29-32].

The increased hip fracture risk documented in women with T2DM has been deemed paradoxical given the fact that they tend to have higher bone mineral density (BMD), and low BMD is known to be an independent risk factor for fracture [33, 34]. While BMD is easily measured, it is fundamentally limited as a surrogate measure of bone strength [35], and does not adequately capture the fragility evident in women with T2DM. However, recent data suggests that this so-called paradox might be explained by an impaired skeletal response to load [36, 37].

While not widely appreciated, it is well established that bones continually adapt throughout life to prevalent loading conditions [13]. This homeostatic mechanism rooted in osteocyte signaling, serves to ensure that bones remain strong enough to resist the forces normally encountered during daily activities. Conceivably, the bones of diabetics might be more susceptible to fracture if they somehow fail to adapt adequately to loading forces, and thus are weaker than they should be. Data from a recent study by Garg and colleagues which examined femoral neck geometry in 5924 women enrolled in the
Women’s Health Initiative observational study, provided evidence that this might be the case [36]. The study demonstrated that diabetic women using insulin had larger BMD and bending strength at the proximal femur than non-diabetic controls, consistent with their larger body sizes. However, when these measures were adjusted for lean body mass, they were lower than in controls. The reason that this may suggest a deficiency in load adaptation is because physiologic skeletal loading forces are muscle generated, thus skeletal geometry normally scales in proportion to lean mass [38, 39].

The purpose of Study 2 was to continue that line of investigation to see if we can find more direct evidence supporting this intriguing explanation for the T2DM bone density paradox.

1.3.2 Objective and hypothesis for Study 2

Objective 2: To determine the association between diabetes status and stress at the femoral neck in a Canadian cohort of women.

Hypothesis 2: Stress will be negatively associated with diabetes status such that stresses are lower in non-diabetics compared to diabetics.

1.4 Study 3 : ESTROGEN

1.4.1 Summary and rationale for Study 3

Estrogen, the predominant female sex hormone, plays a role in almost all cells and tissues in the body [40]. Estrogens communicate their effects via two nuclear receptors: estrogen
receptor alpha (ERα) and estrogen receptor beta (ERβ). Estrogen is arguably the most important non-mechanical regulator of bone metabolism and is a key skeletal regulator throughout the lifespan.

Estrogen deficiency is a major factor in the bone loss associated with aging [41, 42]. The rapid decline in estrogen that occurs in women at menopause affects the bone remodeling process in several ways. First, it increases the rate of bone turnover [43]. Second, it induces a remodeling imbalance by prolonging the resorption phase (osteoclast apoptosis is reduced [44]) and shortening the formation phase (osteoblast apoptosis is increased [45] Section 2.1.2). It is conceivable that these observations are consistent with an attenuated load response.

Data from animal and cellular studies indicate that variations in estrogen availability mediate the skeletal response to mechanical load (mechanosensitivity) [46, 47]. There is a large body of literature to support the view that estrogen deficiency increases the strain threshold for bone to stimulate a formation response, effectively reducing bone’s sensitivity to mechanical load [48-50]. As such, the effects of exercise appear to be blunted with aging in women, as levels of estrogen decline after menopause [51]. Evidence for the beneficial effects of estrogen and exercise in postmenopausal women have been documented in several clinical studies [52-57]. Finally, Saxon and Turner argue that the estrogen receptors compete with one another such that signaling through ERα enhances mechanically induced bone formation at trabecular and endocortical
surfaces, and signaling through ERβ suppress periosteal bone formation [58], a characteristic exercise effect.

Interestingly, there is also data that opposes this view. Work by Jarvinen et al. suggests that estrogen and loading have independent and functionally specific effects on the skeleton that are based in evolutionary needs [59]. Specifically, this set of data suggests that estrogen secretion at puberty leads to packing of mechanically excess mineral into female bones for reproductive needs while simultaneously lowering the responsiveness of the female skeleton to mechanical load. They also suggest that the reverse occurs at menopause and that estrogen deficiency enhances skeletal response to load.

The combined effects of estrogen and exercise on skeletal geometry and strength in humans are still unclear. One point to consider is that the majority of studies in humans have examined BMD as their outcome measure. While an improvement in BMD is beneficial for bone, BMD is not a good surrogate measure to infer strength effects. The use of geometry-based analyses may help to explain the divergence in viewpoints. The purpose of Study 3 was to examine associations present between exercise, estrogen and stress using a geometry-based analysis.

1.4.2 Objectives and hypotheses for Study 3

Objective 3a: To determine the association between strenuous sports participation and stress at the femoral neck in a Canadian cohort of pre- and postmenopausal women.
Hypothesis 3a: Strenuous sports participation will be negatively associated with stress.

Objective 3b: To determine the association between estrogen availability and stress at the femoral neck in a Canadian cohort of pre- and postmenopausal women.

Hypothesis 3b: Premenopausal women will have lower stress than postmenopausal women not currently using estrogen supplements. There will be no difference in stress between premenopausal women and postmenopausal women currently using estrogen supplements.

1.5 Summary and Conclusions

These studies will explore Mechanostat sensitivity under three different conditions: fracture, diabetes and estrogen deficiency. An examination of these different conditions will offer novel insight into the skeletal response to mechanical load in vivo, and will provide a framework to further the development and implementation of therapies for the prevention and treatment of osteoporosis related fractures.
CHAPTER 2: BACKGROUND
2.1 Biomechanics of Bone

Bone is the primary structural element of the human body. It supports soft tissues, protects vital organs and provides points of attachment for skeletal muscles, necessary for locomotion. Bone is also unique among structural materials in that it is self-repairing and can alter its properties and geometry in response to changes in mechanical demand [60, 61]. This section will review the structure and composition of bone, the processes of bone modeling and remodeling as well as the basic biomechanical principles that govern bone’s adaptation to mechanical load.

2.1.1 Structure and composition of bone

Bone is a connective tissue which consists of cells embedded in a matrix called osteoid. Osteoid consists of a fibrous component (90% collagen and 10% amorphous ground substance) that gives bone its flexibility, and a mineral component (hydroxyapatite crystals and amorphous calcium phosphate) that gives bone its rigidity [62].

Whole bones are composed of two types of tissue: cortical bone and trabecular bone. Cortical bone comprises the diaphysis of long bones and the thin shell that surrounds the metaphyses. Trabecular bone in the metaphyses and epiphyses is continuous with the inner surface of the metaphyseal shell and exists as a three-dimensional, interconnected network of trabecular rods and plates. The trabeculae divide the interior into intercommunicating pores of varying dimensions, producing a structure of variable porosity and density. A network of rods produces low density, open cells, while a
network of plates can result in higher density, nearly closed cells [63]. The classification of bone tissue as cortical or trabecular is based on relative density – the ratio of specimen density to that of fully dense cortical bone (assumed to be 1.8 g/cc). The relative density of trabecular bone varies from 0.05 g/cc to about 0.7 g/cc corresponding to porosities that range from about 30-90%. The relative density of cortical bone ranges from about 0.7 g/cc to about 0.95 g/cc [62]. The classification is an anatomical distinction rather than a functional classification, in truth the porosity varies along a continuum from the densest cortical bone to the sparsest trabecular tissue.

2.1.2 Bone remodeling and modeling

Throughout the lifespan, bone continuously undergoes a process of renewal and repair termed bone remodeling. Bone remodeling is critical to repair microfractures or fatigue damage that occurs as a result of daily wear and tear on the skeleton. Bone modeling on the other hand, serves an adaptive rather than restorative function and is achieved by independent de-novo bone formation or resorption, depending on the directional change in stimulus [64]. Undoubtedly, the two processes are functionally linked.

There are 3 cell types responsible for the processes of bone remodeling and modeling: osteocytes that detect microdamage and/or loading conditions; osteoclasts that resorb or break down bone and osteoblasts that form new bone [65, 66]. Osteocytes originate from osteoblasts that remain trapped within the bone after it has been formed and are thought to control the overall processes of bone remodeling and modeling. Osteoclasts are multinucleated cells that differentiate from precursors in the monocyte/macrophage
lineage in response to coordinated expression of specific regulatory molecules including the receptor activator of nuclear factor kappa B (RANK) and its ligand RANKL [67]. Differentiation of osteoclasts is blocked by osteoprotegerin (OPG), which acts as a decoy receptor for RANKL. Osteoblasts differentiate from bone marrow stromal cells in response to activation of the transcription factor core binding factor A1 (Cbfa1) [68].

During bone remodeling, osteoclasts remove old or damaged bone by attaching to the surface of bone and forming a skirt (“ruffled border”) around its circumference. This process creates a pocket that the osteoclasts fill with hydrochloric acid and proteolytic enzymes that dissolve the mineral and protein of the bone beneath it. Subsequently, the osteoclasts migrate away from the area of bone undergoing resorption and undergo apoptosis. Osteoblasts then lay down the protein skeleton (osteoid). Later the osteoid becomes mineralized; calcium and hydroxyapatite begins to crystallize around the fibrils of collagen and the area becomes mature bone [65]. Bone remodeling can occur on trabecular or cortical bone surfaces and the resorption/formation cycle always occurs on the same surface. Bone remodeling has transient but important effects on bone geometry (size and shape).

During bone modeling, bone is formed where it is needed and resorbed where it is not needed. Because bones are cylindrical and act as levers, addition and removal of bone occurs on different surfaces (Section 2.2.3), unlike in the remodeling process where resorption is following by formation on the same surface. Osteocytes play a critical role
in this process as load sensors in this process to ensure that adaptation to changing load conditions is enabled.

Local (genes, cytokines, ligands, receptors, paracrine and autocrine effects, apoptosis etc.) as well as systemic (hormones, minerals, vitamins, drugs, nutrients etc.) nonmechanical agents work in synergy with mechanical cues to influence both the remodeling and modeling processes. Many of the factors which regulate these processes ultimately do so by influencing local expression of RANK, RANKL, OPG and Cbfa1 which together form a paracrine system that mediates osteoclast and osteoblast differentiation and function [69]. A more detailed explanation of the interplay between mechanical and non-mechanical agents in regards to bone remodeling and modeling is discussed in Section 2.2.2. The way in which bone geometry is altered in response to modeling and remodeling will be discussed in Section 2.2.3.

2.1.3 Basic biomechanical concepts

Fracture represents failure of bone tissue at the material level as well as failure of the whole bone at the structural level. Therefore, the prediction of failure or fracture is multifaceted and requires: characterization of tissue-level material properties; information on bone geometry; knowledge of the loads being applied; an analysis of the internal stresses (which incorporates geometric and loading information); and a comparison of the predicted stresses against the known strength properties at the tissue level [62]. If the predicted stresses under the assumed loading conditions exceed the material strength, the bone is at high risk of fracture under the assumed loading conditions and vice-versa.
In this section, the biomechanical concepts required to predict bone fracture under simplified loading conditions will be discussed. The simple case of axial loading in tension will be used to define stress, strain, modulus and strength and to contrast the material behavior of bone as a tissue with the structural behavior of whole bones. Axial and flexural loading will then be explained as ways to introduce important geometric concepts that can be used to characterize the geometry of long bones.

2.1.3a Axial loading (tension and compression)

When forces are applied to any solid object, the object is deformed from its original dimensions. Simultaneously, internal forces are produced within the object. The relative deformations created at any point are referred to as the strains. The internal force intensities (force/area) are referred to as the stresses at that point [70]. When a bone is subjected to forces, these stresses and strains are introduced throughout the structure and can vary in a complex manner. To avoid some of these complexities and demonstrate some important mechanical concepts, this section will describe a regular structure loaded under well-defined conditions. Similar specimens of regular geometry are used to determine the material properties of bone tissue.

In Figure 1a [62], a cylindrical bar of length L and a constant cross-sectional area (A) is shown subjected to pure tensile force (F). As load is applied, the cylinder begins to stretch. The mathematical relation for stretching of a cylinder is:

\[ \Delta L = \frac{FL}{AE} \]  

(1)
where \((\Delta L)\) is the elongation of the cylinder, \(L\) is the original unstretched length, \(A\) is the cross-sectional area, \(F\) is the force and \(E\) is the modulus (which describes whether the material is rigid or flexible). According to the simple relationship shown in equation 1, the elongation \((\Delta L)\) is directly proportional to the applied force and to the original length and inversely proportional to the cross-sectional area and to factor \(E\).

If a force-deformation curve is plotted to represent the structural behavior of the cylindrical bar, it would look like Figure 1b [62]. A cylinder of bone tested in tension would yield a linear region (elastic region) followed by a nonlinear region where “yielding” occurs and there is a permanent internal rearrangement of the structure that results in damage accumulation, even if loading does not continue to failure. After yielding, nonelastic deformation occurs until finally fracture results in the loss of load-bearing capacity of the cylinder. The load at which yielding occurs is referred to as the yield load, \(F_y\). The load at which failure occurs is called the ultimate or failure load, \(F_{ult}\). A force-deformation curve describes structural behavior since it reflects not only the material but also the geometry of the specimen.

To provide a standardized representation of the mechanical behavior of the material (as opposed to the behavior of the structure), a normalized curve known as a stress-strain curve can be plotted (Figure 1c) [62]. This normalizes the force-deformation relationship by dividing the applied force \((F)\) by the cross-sectional area \((A)\) and the deformation \((\Delta L)\) by the original length \((L)\). This internal force intensity is defined as stress \((\sigma)\), in
Newtons per squared meter (N/m$^2$) or Pascals (P). The ratio of the elongation to the original length is defined as the strain ($\varepsilon$). Note that strain is a nondimensional quantity. In a stress-strain curve, the slope of the linear elastic region is referred to as the elastic modulus (E). The material yields at a stress level known as the yield strength. Ultimately, the material fractures at a stress level known as the fracture strength.

Compressive loading is analogous to tensile loading except that the deformation $\Delta L$ is now a shortening of the cylinder. The magnitude of this shortening is still given by equation 1, with the associated compressive stress being given by $F/A$. By convention, compressive stresses are assumed to be negative (-) and tensile stresses are positive (+).

2.1.3b Bending (flexural loading)

In engineering, long slender structures that are designed to resist transverse or bending loads are referred to as beams. Beams are important in biomechanics because long bones function primarily as beam levers. A beam can be subjected to bending loads in a number of ways, the most common of which is the application of two sets of forces near the beam ends (Figure 2) [62]. This loading configuration known as four-point bending, subjects the central section of the beam to a constant bending moment (of $M = Fa$). The midspan deflection is given by the relationship shown in Figure 2 involving the geometric characteristics of the beam and the location of the applied forces (as expressed by $L$ and $a$), the magnitude of applied forces, the modulus E of the beam material, and a new quantity $I$, known as the areal moment of inertia. The areal moment of inertia expresses the characteristics of the distribution of the cross-sectional area in relation to a
transverse axis. It reflects, for example, the differences in bending resistance of a meter stick bent when it is held flat versus when it is held on edge.

There are similarities between the expressions used to characterize axial and flexural loadings (equation 1 and Figure 2). In both cases, the deformations are directly proportional to the applied forces and inversely to certain geometric features of the structure. Similarly, the deformations are inversely proportional to the material stiffness (E).

To visualize the stresses that occur in a beam during bending, one could imagine holding the ends of a meter stick and bending it so as to produce a convex surface on one side and a concave surface on the other side (Figure 3). The material on the convex side of the beam is subjected to tensile (stretching) strains and the material on the concave side is subjected to compressive strains. At some point between the two surfaces, called the neutral axis, the strains are zero (i.e. the original length of the beam is unchanged).

The linear variation in stress across the cross section is given by:

\[ \sigma = \pm \frac{M y}{I} \]  

(2)

where \( M \) is the bending moment at the cross section, \( y \) is the distance from the neutral axis, and \( I \) is the areal moment of inertia. The \( \pm \) sign is used to indicate that one surface of the beam is subjected to tensile stress (+) and one to compression (−). It is important to
note that the maximum stresses are experienced by the material on the surface of the beam. If the bending forces are increased until the beam begins to fracture, the fracture will be initiated at the surface of the beam where the stresses are highest.

Because long bones are slightly curved and are subjected to compressive loads applied at the joint surfaces, the most common loading situations in vivo is a combination of compressive and bending loads. Bending loads arise because the compressive loads do not act through the centre of the bone. The resulting stresses on a transverse section through a slightly curved bone can be found by summing the stresses caused by the compressive axial forces and by the bending stresses (Figure 4). As a result, even higher compressive stresses are created on the concave side of the bone, whereas the convex side experiences either reduced tensile stresses or even compressive stresses, depending on the magnitude of the axial force and its eccentricity (e), defined as the distance from the centre of the bone to the line of action of the applied compressive loads. The combined stress can be described by the following relationship:

(combined stress) = (compressive stress) ± (bending stress)

In mathematical terms, this can be written as:

\[ \sigma = -\frac{F}{A} \pm \frac{My}{I} \quad (3) \]
where F is the eccentrically applied axial load, A is the cross-sectional area, M is the bending moment at the cross section, y is the distance from the centre of the beam and I is the moment of inertia. Note that when y=0 (the neutral axis for pure bending), the bending stress does not contribute to the combined stress. Importantly, combined bending and axial compression loads ensure that stresses are always greatest at the outer cortical surface (largest value of y) and are always least at the neutral axis.

A detailed description of the specific geometry and strength parameters assessed in this thesis will be presented in the methods section (Chapter 4).

2.2 Mechanical Loading and Bone

Mechanical loading of the skeleton is essential for the development, growth and maintenance of strong bones [71]. Bone strength is plastic and can be modulated in adults, as evidenced by observational studies comparing athletes versus non-athletes and randomized controlled trials of the effects of exercise on bone size and strength [72, 73]. The following section will discuss how bone tissue adapts to mechanical load at both a cellular and whole bone level.

2.2.1 Cellular response to mechanical loading: mechanotransduction

Current research supports a four stage cell-mediated theory of mechanotransduction: 

*mechanocoupling* – the conversion of physiological loads applied to tissues into local mechanical signals experienced by bone cells; *biochemical coupling* – the process
whereby cells sense a load using mechanoresponsive structures and transform it into a biochemical response; *signal transmission* – the resultant downstream signaling within and between cells and *effector response* of osteoblasts and osteoclasts – the cellular outcomes that lead to build-up, remodeling or resorption of bone mineral and matrix [74]. The following sections will briefly review each of the steps involved in the mechanotransduction process.

*Mechanocoupling* describes the transduction of mechanical force into a local mechanical signal perceived by a sensor cell [75]. When loads are applied to bone, bone becomes deformed creating pressure gradients within fluid filled bone canaliculae and interstitial spaces that cause the tissue fluid to flow. This fluid flows past cell membranes and osteocytes, creating a fluid shear stress across the membrane [76, 77] and activating a cellular response. The two structures that detect bone loading are the osteocyte and the bone-lining cell [76, 78, 79].

*Biochemical coupling* refers to the transduction of a local mechanical signal into a biochemical signal ultimately leading to gene expression or protein activation [75]. Mechanical signals (strain signals) are detected by mechanoreceptors in the cell membrane such as a G-protein-linked mechanoreceptor or by transmembrane adhesion molecules. The local mechanical signal activates the G-protein-linked mechanoreceptor which causes an increase in intracellular calcium and the subsequent release of second messengers such as prostaglandins and nitric oxide. These second messengers can travel outside the cell and initiate both autocrine and paracrine signaling, or remain within the
cell and influence gene expression [77]. Within the cell, second messengers activate adhesion molecules (i.e. integrins or cadherins) which activate the actin cytoskeleton leading to restructuring which then activates the nucleoskeleton and finally DNA. Thus, mechanical signals can directly influence gene expression.

Signal transmission describes the transmission of the biochemical signal from the sensor cell to the effector cell. In the case of bone tissue, osteocytes and bone lining cells act as the sensor cells. These cells are interconnected with each other through functional gap junctions [80] and are responsive to mechanical loading stimuli in vivo [81]. Since neither bone lining cells or osteocytes can actively form or resorb bone, they signal to “effector cells” (osteoblasts or osteoclasts) before any change in bone structure can be initiated. The intermediaries for cell-to-cell communication between the “sensor” and “effector” cells in bone tissue are the prostaglandins and nitric oxide [82, 83].

The final step in mechanotransduction requires an effector – a component of bone that produces new or rearranged bone. The effector cells in bone tissue are osteoblasts and osteoclasts which form or resorb bone respectively when signaled following mechanical stimulation termed an effector response [75].

2.2.2 Frost’s Mechanostat theory

Skeletal adaptation to mechanical load in vivo can be described by the Mechanostat theory proposed by Harold Frost [84]. The theory hypothesizes that “bone’s biologic machinery would make healthy postnatal human load-bearing bones and their trabeculae
strong enough to keep typical peak voluntary mechanical loads from breaking them suddenly or in fatigue” [13]. In other words, bone functions to keep strains imposed by loads within a safe physiological range in order to prevent microdamage and fracture, and ultimately to maintain strength.

Frost likens bone’s response to load to a thermostat (“Mechanostat”), whereby specific physiological strain set points act as thresholds for the initiation of bone resorption or formation – termed modeling. Resorption serves to remove bone tissue from areas where it is not needed and formation serves to add bone tissues in areas where it is needed. The combined effects on bone strength according to Frost’s Mechanostat theory are described in Figure 5 [13]. The x-axis depicts typical peak bone strains from zero on the left, to fracture strain levels on the right (~25 000 microstrain). The locations of the remodeling (MESr), modeling (MESm) and microdamage (MESp) thresholds are also shown. The y-axis describes bone strength, increasing or decreasing about a horizontal axis representing no net gains or losses of strength. The lower dotted line curve suggests how disuse-mode remodeling would remove bone next to marrow when strains stay below the MESr range (50-100 microstrain), but otherwise would tend to maintain existing bone and its strength. The upper dashed line curve suggests how modeling drifts would begin to increase bone strength where strains enter or exceed the MESm range (1000-1500 microstrain). The dashed outlines suggest the combined modeling and remodeling effects on a bone’s strength. This curve was originally suggested by Carter in 1984 [85]. Beyond the MESp range (~3000 microstrain; bone’s yield point), woven bone formation usually replaces lamellar bone formation and microdamage starts to incur [48]. Bone fractures (Fx) at
approximately 25,000 microstrain, a value that likely decreases with age. At the top of the figure, four different strain windows are described: DW=disuse window; AW=adapted window; MOW=mild overload window; and POW=pathologic overload window.

The strain span between MESr (lower threshold for remodeling) and MESm (upper threshold for modeling) describes bone’s general biomechanical relation. In other words, the way in which bone adapts to the mechanical loading effects of physical activity by becoming stronger or weaker as activity levels change. For instance if activity related strains exceed the upper threshold (MESm), a biochemical signal is generated by the osteocytes to initiate the formation of new bone. Similarly, when activity generated strains are absent, or fall below a certain lower threshold, a signal to resorb excess bone is generated. Once new bone is formed, strain levels are reduced below the upper threshold and bone formation ceases. Similarly, the resorption of bone in low activity conditions continues until strains increase above the lower set point. In this manner, bone geometry continually adapts to changing load conditions to maintain strength.

It is important to note that other physiological processes work in synergy with the Mechanostat and thereby affect its functioning. For example, local (genes, cytokines, ligands, paracrine/autocrine changes) and systemic non-mechanical agents (hormones, vitamins, drugs, nutrients) can affect the way in which bones respond to mechanical stimuli [14-16]. Frost has broadly described a feedback loop that relates bone’s biologic machinery to such agents (Figure 6) [13]. In Figure 6, the boldface capitals denote the
mechanical feedback loop. CNS=central nervous system; PNS=peripheral nervous system; PNE=peripheral nerve endings; MC=muscle contraction forces; MU=mechanical usage; L=local non-mechanical agents; S=systemic non-mechanical agents. The “highways” include any mediators and modulators of strain-dependent signals, plus the threshold ranges that help control the modeling and remodeling bone strength functions. The mechanical feedback loop (m) concerns bone modeling by drifts, while the mechanical feedback loop (r) concerns bone remodeling. Italics signify local and systemic factors that could modulate the mechanically-dedicated message traffic without directly participating in it. One could view the Mechanostat as the combination of all of these responses to mechanical and non-mechanical agents of modeling.

2.2.3 Geometric remodeling of the cortex in response to mechanical load

Long bones function mainly as muscle actuated levers where forces during physical activity induce stresses within the bone that are mainly in a combination of bending and axial compression. The directions of bending and the magnitudes of the stresses vary dynamically depending on bone, the location where they are measured and the activity type. Since bending forces dominate, the maximum stress is always on the outer cortical surface while the minimum stress is at the neutral axis near the centre of the bone. Since strains are proportional to stresses, an increase in physical activity should cause strain stimuli to exceed the formation threshold on the periosteal surface, while a decrease in activity should cause strains to fall below the resorption threshold at the neutral axis (Figure 7) [64].
BMD is often used as a surrogate measure for bone strength in clinical studies. In the model described above, a decline in physical activity should produce a proportionate decline in BMD due to loss of BMC near the neutral axis. This change should be captured in standard BMD measurements. However, if we consider the effects of an increase in physical activity, the story is different. An increase in physical activity causes an increase in BMC, but since bone is added to the outer surface (via periosteal apposition), this causes an increase in region area. Since BMD is proportional to BMC over region area, the net improvement in estimated bone strength using BMD tends to be underestimated. The effects of exercise are thus better evaluated by looking at the geometry, specifically the cross-sectional area and section modulus [64, 86]. This is because their derivation is directly affected by addition or removal of bone from the surface margins. As aforementioned, a detailed description of the specific geometry and strength parameters assessed in this thesis will be presented in the methods section (Chapter 4).

2.3 Assessing BMD, Geometry and Strength in Vivo

The following section will review the common skeletal outcomes assessed in current clinical studies including BMD and bone geometry and stress and methods to assess these outcomes.

2.3.1 BMD and fracture risk
BMD refers to the average concentration of bone mineral (g) per unit of bone area (cm$^2$) or volume (cm$^3$). BMD can be reported in g/cm$^2$ or in g/cm$^3$ depending on the measurement technique used. BMD changes dramatically throughout the lifespan. During skeletal growth in childhood and adolescence, BMD increases until a peak accumulation is reached in the twenties (“peak bone mass”) [87]. Attainment of peak bone mass is determined primarily by genetic factors and to some extent, hormonal and environmental factors such as diet and exercise [87, 88]. Thereafter, BMD declines gradually with age, somewhat more quickly after menopause in women. Studies that use standard methods of measuring BMD find that, on average, women lose 1 to 3% of their BMD per year for 3 to 5 years after their last menstrual period. When at least 5 years have elapsed since menopause, the rate of BMD loss slows down for reasons that are still unclear [89]. Note that in the clinical setting, BMD decline is commonly equated with ‘bone loss’ while part of the decline is actually due to an increase in region area. Long bones generally increase in diameter with age [90], an effect that tends to make them more mechanically efficient since a larger diameter requires less material to achieve a given bending resistance than a narrower one.

Population-based studies strongly support the finding of low BMD as an independent predictor of fracture risk although it is only a surrogate measure of bone strength [91, 92]. The relationship between BMD and fracture risk is customarily quantified by the “relative risk per standard deviation decrease in BMD.” For example, a relative risk/standard deviation (RR/SD) of 1.5 means that a woman with a BMD that is 1 SD below the mean for her age has a 50% higher fracture risk than a woman with a BMD
that is average for her age. Among postmenopausal white women, the relationship between spine BMD and hip fracture is 1.4 to 1.6 RR/SD and the relationship between hip BMD and hip fracture is 2.6 RR/SD. That said, BMD and strength are related in a nonlinear fashion such that small changes in BMD can result in disproportionately larger changes in fracture risk [93].

2.3.2 Measuring BMD by DXA

BMD is most commonly assessed by DXA. DXA technology considers the body in two compartments, bone and non-bone, and uses low-dose x-ray beams at two distinct energy levels to separate the bone (mineral) from the non-bone soft tissues[94]. Advantages of DXA include the low level of radiation exposure, its accuracy and precision for measuring BMD, its ability to provide data regarding soft tissue composition as well as bone mineral, and its versatility to measure bone mineral at a range of sites in both the axial and appendicular skeleton. Limitations of BMD by DXA are primarily related to its planar nature. DXA is unable to measure bone volume, nor discriminate between trabecular and cortical bone compartments. Therefore strength estimates are not easily inferred from conventional DXA BMD measures. One final limitation with DXA technology is that it is unable to differentiate between calcium in bone and the calcification in blood vessels and joints, leading to an overestimation of BMD in older individuals, predominantly at the spine.

Clinical practice guidelines for the diagnosis and treatment of osteoporosis incorporate DXA measurements of BMD. Clinically, DXA is used to measure BMD at the hip,
lumbar spine and radius. Measurements are reported in two forms; the Z score and T score. A Z score is the number of standard deviations (SD) below (minus) or above (plus) the mean BMD value for people of the same age, gender and ethnicity [95]. A Z score of 0 means that the patient has a BMD value that is exactly at the mean for his or her age. A Z score of -2.0 means that the patient has a BMD at that site, by that method, that is 2 SDs below the mean value of others who are the same age. A T score is the number of SDs below the mean value of BMD for young (20-30 year old) adults [95]. A T score of 0 means that the patient has a BMD value that is exactly at the mean for young adults. A T score of -2.5 means that the patient has a BMD value at that site, by that method, that is 2.5 SDs below the average value found in healthy 20 to 30 year old adults. The World Health Organization (WHO) defines osteoporosis as a T score of -2.5 or lower at either the hip or the spine, however, there is no clear BMD threshold or cut point below which fracture risk increases. Moreover, most fractures occur in subjects with T-scores greater than -2.5 [96].

2.3.3 BMD versus bone strength

While BMD is easily measured and understood, it is important to remember that it is fundamentally limited as a surrogate measure of bone strength. Because low BMD is linked to osteoporotic fractures, it is broadly believed that the decline in BMD that occurs with aging represents a gradual reduction in mechanical strength that ultimately results in fragility. There are several issues with this line of thinking. As aforementioned, bone has the ability to keep strains imposed by daily loads
within a safe physiological range in order to prevent microdamage and fracture. This is accomplished by adding new bone in areas where strains are higher than some upper threshold value and removing bone in areas where strains are lower than some lower threshold value. Ultimately, this preserves strength, but only relative to the loading that is normally encountered. If BMD were a strength equivalent, this would mean that in young adulthood where BMD peaks, strength would also reach a peak. This is illogical, as strength should change in response to varying mechanical demands as they occur throughout the lifespan as it appears to do. A study evaluating structural trends in the aging femoral neck in adults, demonstrated that section modulus (an index of bending strength) remains nearly constant until the fifth decade in females and then declines at a slower rate than BMD thereafter [90]. Although there is net bone loss with age, the BMD trend tends to overestimate this bone loss. BMD is very good at identifying populations at risk for fracture, but poor at discriminating individual risk. Furthermore, most fractures occur in individuals who have osteopenia or normal BMD rather than osteoporosis [96].

2.3.4 Measuring bone geometry

A method called Hip Structure Analysis (HSA) [97] was introduced to extract geometric strength information from archived hip DXA scans acquired in large research studies, and improves on the geometric limitations intrinsic assessing BMD by DXA. Since strength effects are not easily inferred from conventional DXA measures of BMD, there is growing interest in the clinical community in a more direct evaluation of bone strength in patients [35]. HSA is a partial solution because it not only measures BMD of the hip
bone, but also structural geometry of cross-sections traversing the proximal femur at specific locations. Because it is a 2D measurement however, HSA precision is insufficient for clinical use. The bone mass image is used directly from the DXA scan where pixel values are expressed in areal mass (g/cm²). The method employs the principle that a line of pixel values across the bone axis correspond to a cut plane traversing the bone at that location and contain some of the information about the cross section. The program analyzes the proximal femur at the narrow neck, intertrochanter and shaft. For each region, the distribution of the bone mass across the bone is extracted, then geometry [98]. An advantage of the HSA technique is that it can also be used in conjunction with other engineering analyses to estimate stress experienced within the bone.

2.3.5 Stress versus strain

As aforementioned, Frost’s Mechanostat Theory postulates that bone functions to keep strains imposed by loads within a safe physiological range in order to prevent microdamage and fracture, and ultimately preserve strength [13]. Central to this thesis is the idea that bone fragility may be influenced by an abnormal response to strain stimuli. If we could directly measure strain levels in bone tissue under a given load, we would be able to more accurately infer load response. While strain measurements are possible this can only be done via the use of implanted strain gauges, clearly not practical for large cohort investigations.
One way to get around this issue is to instead estimate stress, which is proportionally related to strain [62]. It is possible to estimate stresses for a particular bone cross-section using bone structural dimensions obtained from HSA and by estimating load magnitudes applied under physiologic directions. If one assumes that the structural geometry in any given individual is in equilibrium with prevalent loads, an assessment of the stresses generated under mechanically equivalent loads should be an index of mechanosensitivity. Such an index could potentially serve as a clinical indicator of fracture susceptibility and would be a useful measure. The method to estimate stress will be explained in detail in Chapter 4.
CHAPTER 3: REVIEW OF THE LITERATURE
3.1 Study 1: FRACTURE

3.1.1 Age-related changes in bone remodeling, geometry and strength

The geometric changes accompanying aging affect all bone surfaces, leading to decreases in cortical thickness, increases in cortical porosity and an overall reduction in bone mineral content and density [17]. These effects would seem to reduce the amount of mineralized bone tissue area that supports loads; however, it can be argued that modeling effects tend to distribute aging bone more efficiently, such that support is not necessarily compromised, at least with respect to normally encountered physiologic loads.

One of the most widely reported modeling effects is the general expansion of bone outer diameters with age. Expansion of the outer diameter has a non-linear effect on resistance to bending loads because bone added to the periosteal surface contributes by the square of the distance to the neutral axis to the areal moment of inertia. Effectively, a wider bone is more “mechanically efficient” in that it can maintain the bending strength of a narrower bone with a thinner cortex and less BMD. A study examining structural trends in the aging femoral neck in adults aged 20-99 who participated in the Third National and Nutrition Examination Survey (NHANES III), nicely highlighted this effect [90]. The study demonstrated that section modulus (an index of bending strength) at the narrow neck region remains nearly constant until the fifth decade in females, and then declines at a slower rate than BMD thereafter. The apparent mechanism for the discord between BMD and section modulus is a linear expansion in subperiosteal diameter which tends to
offset net loss of medullary bone mass. These results highlight that the loss of BMD with aging does not necessarily equate to reduced mechanical strength.

It is possible that age-related periosteal apposition may be less in women than in men, and could contribute to the increased fragility evident in women with aging; however, current findings are heterogeneous. Several large cohort studies support this theory. First, a cross-sectional study by Russo et al. involving 700 women and 600 men between the ages of 20-102 years of age, demonstrated clear sex-related differences in bone geometry and strength at the tibia with aging [99]. Specifically, periosteal diameter and cortical cross-sectional area increased more in men with age, while endosteal diameters increased similarly in both men and women. Consequently measures of bone bending strength were maintained in men but not in women because bone was distributed closer to the neutral axis. Similarly, a 7-year prospective study by Szulc et al. involving 1000 women between the ages of 31-89 years demonstrated that endocortical resorption at the radius increases with age, whereas periosteal apposition decreases so net cortical thickness declines overall [100]. The net effects are that a thinner cortex is displaced further from the neutral axis increasing bending strength in premenopausal women, preserving it in perimenopausal women but not in postmenopausal women.

In contrast, a study by Kaptoge et al. that examined age-related changes in hip geometry in a longitudinal cohort study, demonstrated that female sex was associated with having lower values of cross-sectional area, bending strength and periosteal diameter [101]. However, longitudinal analysis of rates of change revealed faster rates of subperiosteal
and endosteal expansion than men at the narrow neck and intertrochanteric regions. These findings suggest that perhaps other factors contribute to the increased fragility evident in older women compared to older men.

Another important age-related change is a distinct shifting of the centre of mass at the hip [20]. In normal bipedal walking, the sum of stresses from body weight and hip abductor muscles produces a net compressive stress gradient that is low at the supero-lateral cortex and high at the infero-medial cortex. Therefore, throughout life, bone is consistently forced to adapt at the infero-medial surface where stresses are highest. As bone mass decreases with age due to changes in remodeling, slow movement of the centre of mass away from the supero-lateral cortex allows for a lesser volume of bone tissue supero-laterally to sustain bending resistance. Older men and women have asymmetrical cortices that have infero-medial cortices, averaging 3.5 times the thickness of supero-lateral cortices by the 8th decade [102].

While preserving resistance to bending, asymmetry in the femoral neck cortex leads to increased elastic instability (resistance to compression). Elastic stability (buckling) is dependent on the width of the cortex – where a thinner cortex is more susceptible to buckle than a thicker one. Buckling becomes particularly important to consider with aging as the supero-lateral femoral neck cortex becomes quite thin. This effect was thoroughly explored in a study which used computed tomography to measure the distribution of bone in the femoral neck region of 77 cadavers from individuals aged 20-95 years [20]. The study demonstrated that a thin supero-lateral cortex may in fact be
the underlying cause of hip fracture in a sideways fall, as it is not as well-adapted to regular loading.

3.1.2 Adaptation to habitual versus non-habitual loading conditions

According to Wolff’s Law and the MechanoStat Theory [13], bone structure and strength should be well-adapted to the normal, habitual loads of daily life. In accidents, like a fall, or during different type of exercise, bone is exposed to a non-habitual loads. This brings us to the apparent contradiction that those who engage in sports for instance, have higher risks for accidents, but at the same time are better protected against fractures. Those who do not engage in sports have lower risks for accidents, but are less protected against their occurrence. This idea was coined “over-adaptation” and implies that skeletal adaptation occurs in response to habitual rather than abnormal or “error” loads [23].

Several recent studies also support the concept that bone structure is well-adapted to normal loading conditions but less well-adapted to atypical loading. A key study by Homminga et al. evaluated the trabecular load distribution in one osteoporotic and one healthy vertebra under normal loading conditions as well as atypical or “error” loading conditions [21]. The study demonstrated that the number of highly loaded trabeculae was not higher in the osteoporotic vertebra than in the healthy one under normal daily loads (8% and 9% respectively). The osteoporotic trabeculae were more oriented in the longitudinal direction, compensating for effects of bone loss and ensuring adequate stiffness for normal daily loading. However, the increased orientation did make the osteoporotic structure less resistant against collateral atypical or “error” loads. In this
condition, the number of overloaded trabeculae in the osteoporotic vertebra was higher than in the healthy one (13% and 4% respectively). Similar findings have also been observed at the proximal femur [22, 103, 104].

In contrast to the literature described above, findings from a study by Verhulp et al. that examined load distribution in the healthy and osteoporotic human proximal femur during a fall to the side, challenge this idea of over adaptation [105]. The study determined the contributions of trabecular and cortical bone to the strength of proximal femurs under habitual and non-habitual loading conditions. The study tested the hypothesis that the trabecular structure of osteoporotic bone is over-adapted to habitual loads. Distributions of maximal principal strain and effective strain in the entire model suggested that the contributions to bone strength of the trabecular and cortical structures were similar between healthy and osteoporotic femurs. Thus, the study concluded that the osteoporotic femur was not in fact “over-adapted.” This data lends us to think that perhaps it is not only adaptation that must occur, but that the degree of adaptation or sensitivity to load that is important.

3.1.3 Geometric differences in fracture versus non fracture cases

Not surprisingly, there are evident differences in femoral geometry between individuals with fractures and those without [101, 106]. Whether these differences can be ascribed to deviations in adaptive response to load and associated skeletal fragility are unclear.
Data from the cellular literature suggests that heterogeneity in skeletal load response exists. Klein-Nulend et al. demonstrated that osteocytes harvested from elderly individuals diagnosed with osteoporosis had smaller responses to strain stimuli in cell culture than non-osteoporotic individuals [107], suggestive of reduced mechanosensitivity among osteoporotic individuals. Further, the study did not demonstrate an age effect in strain sensitivity, suggesting that heterogeneity in load response may be a genetically inherent difference between individuals who have skeletal fragility and those that do not.

Results from a study by Jepsen et al. that sought to identify genes affecting bone strength, also support this line of thinking [24]. The study examined how genetic components regulate a phenotypic covariation network that was previously shown to accurately characterize the compensatory trait interactions involved in functional adaptation during growth. The study tested whether the growth processes specifying robustness and those specifying mechanical compensation were regulated by the same genes. Quantitative trait loci (QTLs) for robustness and morphologic compensation were found to regulate bone structure independently, indicating that each trait may be targeted separately to individualize treatments aiming to improve strength. Further, the study demonstrated that variation in morphologic compensation and tissue quality, not bone size determined femoral strength relative to body weight. Therefore it was concluded that an individual inheriting slender bones will not necessarily inherit weak bones unless the individual also inherits a gene that impairs compensation. That said, genetic differences may explain differences in functional skeletal adaptation in those with and without fractures.
Another interesting geometric difference noted among individuals with and without fractures is that outer diameter is typically higher in fracture cases. Wider bones were observed in hip fracture cases compared to fracture-free women in the Study of Osteoporotic Fractures [101] as well as in the Rotterdam Study [106]. These findings are intriguing and could indicate heterogeneity in modeling responses among individuals with and without fractures; however, this requires further exploration.

3.2 Study 2: DIABETES

3.2.1 Definition of diabetes mellitus

Diabetes mellitus describes a group of metabolic disorders characterized by chronic hyperglycemia with disturbances of carbohydrate, fat and protein metabolism, resulting from insulin secretion, insulin action or a combination of the two [26]. Symptoms of marked hyperglycemia include; polyuria, polydipsia, weight loss and blurred vision. Impairment of growth and susceptibility to certain infections may also occur. In its most severe forms, diabetes mellitus can cause ketoacidosis or a non-ketotic hyperosmolar syndrome, both of which are life-threatening [26]. Long-term complications of diabetes include retinopathy with potential loss of vision; neuropathy leading to renal failure; peripheral neuropathy with risk of foot ulcers, amputations; Charcot joints; and autonomic neuropathy causing gastrointestinal, genitourinary and cardiovascular symptoms as well as sexual dysfunction. Diabetic patients are also at increased risk for fracture [26, 108].
3.2.2 Prevalence and costs associated with diabetes mellitus

Approximately 285 million people worldwide are affected by diabetes and this number is expected to rise to 438 million by 2030 [25]. In Canada, more than 9 million people are living with diabetes or prediabetes, 10% with type 1 diabetes mellitus (T1DM) and 90% with type 2 diabetes mellitus (T2DM) [25] (Section 3.2.3). The number of people with T2DM is increasing dramatically due Canada’s aging population, the rise in obesity and increased prevalence of sedentary lifestyles [25]. Based on predictions from projection models, the prevalence of diabetes will be highest in the age groups between 55 years and 69 years and over the age of 80 years [109]. The total healthcare costs for individuals with diabetes in Canada are estimated to increase to greater than $8.14 billion by 2016 [109]. Personal costs of diabetes include a reduced quality of life, an increased likelihood of complications such as heart disease, stroke, kidney disease, blindness, amputation, sexual dysfunction and death. An estimated 41,5000 Canadians die each year from diabetes and Canadians living with diabetes are twice as likely to die prematurely compared to those without diabetes [25].

3.2.3 Classification of diabetes mellitus

There are three broad classifications of diabetes mellitus: (1) type 1 diabetes – which can be subdivided into immune mediated and idiopathic type 1 diabetes; (2) type 2 diabetes and (3) other diabetes – which encompasses; genetic defects of β-cell function, genetic defects in insulin action, diseases of the exocrine pancreas, endocrinopathies, drug or chemical induced diabetes, infections, uncommon forms of immune-mediated diabetes,
other genetic syndromes and gestational diabetes [26]. The discussion of diabetic classification will be limited to type 2 diabetes which is the focus of this thesis.

T2DM is the most common form of diabetes accounting for 90-95% of diagnosed cases, and is often referred to as non-insulin-dependent diabetes, or adult onset diabetes [26]. T2DM encompasses individuals who have insulin resistance (i.e. a diminished tissue response to insulin). Initially, and often throughout the duration of the disease, patients with this form of diabetes do not require insulin treatment to survive. Although the specific etiologies remain unknown, autoimmune destruction of β-cells does not occur and patients do not have any of the other explanatory causes of diabetes.

Most patients with T2DM are obese, and obesity in itself has been demonstrated to cause some degree of insulin resistance [110]. Individuals who are not obese by traditional weight criteria, may have an increased percentage of body fat distributed predominantly in the abdominal region. Ketoacidosis seldom occurs spontaneously in this type of diabetes and if it does occur, it is typically associated with the stress of another illness or infection. Type 2 diabetes frequently goes undiagnosed for many years because the hyperglycemia develops gradually and the associated symptomologies go unnoticed. Nevertheless, such patients are at increased risk of developing macrovascular and microvascular complications [26, 111]. Patients with type 2 diabetes have insulin levels that appear normal or elevated, but the higher blood glucose levels in these diabetic patients would be expected to result in even higher insulin values had their β-cell function been normal. Therefore, insulin secretion is
defective in these patients and insufficient to compensate for insulin resistance. Insulin resistance may improve with weight reduction and/or pharmacological treatment of hyperglycemia but is seldom restored to normal levels. The risk of developing type 2 diabetes increases with age, obesity and lack of physical activity. It occurs in individuals with hypertension or dyslipidemia and like type 1 diabetes, has a strong genetic predisposition [112].

3.2.4 Type 2 diabetes and fracture risk

Type 2 diabetes is associated with increased hip fracture risk, particularly in women more so than men [27, 28]. In a meta-analysis comparing five studies, the relative risk for hip fracture associated with type 2 diabetes was 1.38 (95% CI, 1.25-1.53) [28]. Several other studies have also confirmed these findings [29-32]. In studies that have included other fracture sites, most have also reported increased risk for all nonvertebral fractures or all fractures combined [29, 113]. However, data for specific sites is lacking and there is less certainty about findings. Of note, the Women’s Health Initiative Study did report that the risk of proximal humerus, foot and ankle fractures were increased among women with type 2 diabetes [29].

3.2.5 Type 2 diabetes, hip BMD and hip geometry

In cross-sectional studies of older adults, BMD at the hip measured by DXA is consistently elevated in type 2 diabetic patients compared to non-diabetic controls [28]. This finding is surprising given the fact that low BMD is known to be an independent predictor of fracture risk [91, 92] and that hip fracture risk is increased in these patients.
While this consistent finding has been deemed a paradox, recent data suggests that this so-called paradox might be explained by an impaired skeletal response to load [36, 37].

While not widely appreciated, it is well established that bones continually adapt throughout life to prevalent loading conditions [13]. This homeostatic mechanism rooted in osteocyte signaling, serves to ensure that bones remain strong enough to resist the forces normally encountered during daily activities. Conceivably, the bones of diabetics might be more susceptible to fracture if they somehow fail to adapt adequately to loading forces, and thus are weaker than they should be. Data from a recent study by Garg and colleagues which examined femoral neck geometry in 5924 women enrolled in the Women’s Health Initiative observational study, provided evidence that this might be the case [36]. The study demonstrated that diabetic women using insulin had larger BMD and bending strength at the proximal femur than non-diabetic controls, consistent with their larger body sizes. However, when these measures were adjusted for lean body mass, they were lower than in controls. The reason that this may suggest a deficiency in load adaptation is because physiologic skeletal loading forces are muscle generated, thus skeletal geometry normally scales in proportion to lean mass [38, 39]. Heterogeneity in modeling responses among diabetic women requires further exploration.

### 3.2.6 Cellular mechanisms for increased bone fragility in T2DM

The most convincing cellular mechanisms to explain increased bone fragility in T2DM, are low bone turnover and the accumulation of advanced glycation end products
(AGEs). There are a limited number of bone histomorphometry studies in humans to support a low turnover state in diabetes, however the available findings do concur [114]. Studies assessing bone turnover markers in humans have consistently reported lower or similar osteocalcin levels in those with both type 1 and type 2 diabetes [115-118]; however, findings for other markers such as bone-specific alkaline phosphatase, carboxy-terminal propeptide of type 1 procollagen and tartrate-resistant acid phosphatase have been heterogeneous [115, 117, 118]. Due to the fact that osteocalcin is the product of mature osteoblasts, whereas other markers are produced earlier, this pattern may indicate an effect of diabetes on later maturation of osteoblasts.

Bone resorption markers measured in urine have been reported as elevated [118], similar [117] and lower [117] in those with type 2 diabetes. More recent work has demonstrated lower levels of the resorption marker C-terminal cross-linking telopeptide of type I collagen measured in serum [116, 117].

Accumulation of AGEs, could also contribute to the low turnover state evident in T2DM. AGEs are formed as a result of non-enzymatic reactions between glucose and proteins, and are known to accumulate with both age and with diabetes [119]. AGEs have been shown to inhibit both proliferation and differentiation of osteoblasts [120, 121]. AGEs may reduce the ability of osteoclasts to resorb bone, presumably by decreasing the solubility of the bone [122], but results are not consistent [123].
Reduced bone turnover due to diabetes or indirectly due to the accumulation of AGEs could directly attenuate both formation and resorption responses to mechanical load, the critical process by which bone geometry is adjusted to preserve strength. Although unrelated to bone turnover or geometry, the presence of AGEs may also directly alter the material properties of bone collagen, increasing bone brittleness, which could also impair strength [124]. Cellular explanations for fragility in T2DM still require further investigation.

3.3 Study 3: ESTROGEN

3.3.1 Estrogen

Estrogen, the predominant female sex hormone, plays a role in almost all cells and tissues in the body [40]. Estrogens signal through two nuclear receptors: estrogen receptor alpha (ERα) and estrogen receptor beta (ERβ). ERα and ERβ are both expressed in most, if not all tissues, though usually at lower levels than found in reproductive tissues [125]. ERα and ERβ can bind to DNA at specific DNA motifs termed estrogen response elements (EREs), which are 13 base pair inverted palindromic sequences. Additionally, ERα can indirectly activate or repress transcription by binding to other DNA binding proteins. Estrogen can also have non-genomic effects inducing the phosphorylation of components of various signaling pathways or calcium regulation [126].

3.3.2 Estrogen, skeletal growth and the attainment of peak bone mass
Estrogen is one of the most important endocrine (nonmechanical) regulator of bone metabolism. During growth and adolescence, estrogen is responsible for the sexual dimorphism of the skeleton [42] and plays a critical role in the attainment of peak bone mass [41, 42]. During the prepubertal years, skeletal development is very similar between boys and girls; however, around the time of puberty these similarities diverge. Cross-sectional studies by Garn using geometric measurements made on the second metacarpal, demonstrate that cortical bone expands at both the periosteal and endocortical surfaces in boys and girls during the prepubertal years [127]. At puberty, periosteal apposition and endocortical resorption continue in males, but periosteal apposition decreases in females and endocortical apposition occurs causing the medullary cavity in post pubertal females to be reduced to the same size it was by the end of the first year. The idea that estrogen and androgens result in sexual dimorphism of skeletal geometry is also supported by the findings in several animal studies [128-130]. Evidence that estrogen plays a role in the attainment of peak bone mass stems from studies in females with hypogonadism, which demonstrate that late menarche [131, 132], premenopausal amenorrhea resulting from anorexia nervosa [133, 134], and a variety of other disorders [135] are all associated with low bone mineral density. Estrogen is also essential for the normal closure of growth plates [136], as individuals with estrogen deficiency during growth exhibit both delayed bone age and tall stature.

3.3.3 Estrogen, aging and postmenopausal bone loss

Estrogen deficiency is a major factor in the bone loss associated with aging and menopause [41, 42]. In women, estrogen levels decline greatly with age. The decline in
ovarian estrogen production (E1 and E2) is gradual, and commences several years before the last menstrual period [137]. At menopause, ovarian estrogen production ceases, reducing circulating estrogen levels by 50% or more [138, 139] compared to pre-menopausal levels.

Estrogen deficiency affects the bone remodeling process in several ways. First, it increases the rate of bone turnover [43]. Second, it induces a remodeling imbalance by prolonging the resorption phase (osteoclast apoptosis is reduced [44]) and shortening the formation phase (osteoblast apoptosis is increased [45]). As a consequence of these changes, the volume of the resorption cavity is increased beyond the capacity of the osteoblast to refill it and net bone loss ensues.

Due to these changes in bone remodeling, females undergo two distinct phases BMD loss: (1) an early, but transient accelerated phase that begins at menopause and (2) a slow, continuous phase that occurs thereafter [140]. During the accelerated phase, which occurs within the first 3-5 years after menopause, women lose 1-3% of their BMD per year for about 3 to 5 years after their last menstrual period; the rate is faster in the trabecular bone of the spine [141, 142]. Because estrogen inhibits bone resorption, the drop in estrogen during menopause leads to more aggressive bone resorption and faster BMD loss during the perimenopausal years. When several years have elapsed since menopause, the rate of BMD loss in the hip slows down then begins to accelerate again after age 70, reaching 1% or 2% per year in women older than 80 years of age [143, 144].
The causes of the slowing after menopause and acceleration of bone loss in elderly women are not clearly understood.

### 3.3.4 Estrogen and mechanical load

There are instances when bone’s sensitivity to loading may be enhanced or diminished. The following section will discuss the literature that has explored the role that estrogen plays in altering skeletal sensitivity to mechanical load.

#### 3.3.4a ERα and ERβ and mechanical load

Studies in cellular models suggest that estrogen influences the ability of the skeleton to detect mechanical signals. These signaling effects are relayed through the actions of ERα and ERβ. Estrogen receptors α and β are expressed in chondrocytes, osteoblasts and bone marrow stromal cells as well as in osteoclasts and their progenitors. Although the expression levels of ERα and ERβ are at least tenfold lower in bone cells than in reproductive tissues, interestingly, the levels do not vary between genders [145].

The constitutive absence of either ERα or ERβ is associated with a reduced osteogenic response to loading as compared to when both receptors are present [47]. This data is consistent with the theory that estrogens may decrease the set point of the Mechanostat, thereby increasing skeletal load sensitivity. Moreover, ERα number is decreased in osteocytes from bone biopsies of hormone-deficient women and decreased in osteocytes and osteoblasts from bone biopsies of men with osteoporosis [54], suggesting that estrogen receptor number and osteogenic response is dependent on estrogen sufficiency.
In contrast, Saxon and Turner argue that the estrogen receptors compete with one another. While signaling through ERα enhances mechanically induced bone formation at trabecular and endocortical surfaces, signaling through ERβ appears to suppress periosteal bone formation [58]. This suggests that estrogen may affect both upper and lower set points of the Mechanostat in opposing ways such that the upper threshold is decreased and the lower threshold increased.

3.3.4b Estrogen and exercise

Animal studies suggest that estrogen is a powerful inhibitor of periosteal bone formation and reduces endocortical bone resorption in ovariectomized rats [146, 147]. Mechanical loading on the other hand increases periosteal bone formation and in some instances endocortical bone formation [146-149]. When estrogen is given in conjunction to exercise, it reduces exercise-induced gains in periosteal bone formation but reduces endocortical bone resorption more than exercise alone [146, 147]. On trabecular surfaces, estrogen has been shown to augment the osteogenic response to exercise. Estrogen and exercise individually increase trabecular bone mass in ovariectomized rats and when combined the osteogenic response is enhanced [146, 147, 149], depending on when estrogen is administered. Early administration of estrogen suppresses the osteogenic response to mechanical loading, but estrogen given a few days later enhances the response [150]. This data suggests that the effect estrogen has on osteoblasts depends on the stage of cell differentiation.
Work by Jarvinen et al. suggests that estrogen and loading have independent and functionally specific effects on the skeleton that are based in evolutionary needs [59]. This interpretation suggests that estrogen secretion at puberty leads to packing of mechanically excess mineral into female bones for reproductive needs while simultaneously lowering the responsiveness of the female skeleton to mechanical load. They argue that the opposite occurs at menopause whereby an unpacking of this reproductive safety deposit causes an accelerated phase of bone loss. Their first experiment assessed whether there was a gender difference in the skeletal responsiveness to mechanical loading in growing rats and found that female bones have both higher BMD relative to the loads applied, but a significantly less prominent adaptive response to increased loading in comparison to males. Further, female rats that were ovariectomized had less dense bones with a significantly better responsiveness to increased loading than female rats with intact ovaries – in support of the evolutionary theory. It is critical to note that the endpoints used in these investigations were areal and volumetric BMD and not geometry parameters. BMD measures alone are inappropriate to infer strength effects due to load alterations.

A recent study which evaluated the contributions of locomotive loading and estrogen to the development of the ovariectomized rat femur diaphysis, demonstrated that estrogen-related gains were evident on endocortical surfaces, while loading-related gains were on periosteal surfaces. Interestingly, the anabolic effect of estrogen was mitigated when the locomotive loading was present. This finding underpins the apparent dominance of locomotive loading over endocrine control in terms of the macroscopic adaptation of
bone geometry and structure [151-153]. It should be noted that these results do not support the common notion that estrogen restricts periosteal apposition [154-157].

Several studies in humans support the theory that estrogen is beneficial for bone. Data from the Study of Osteoporotic Fractures (SOF) demonstrated that postmenopausal women currently on hormone therapy had mechanically stronger proximal femur geometry than never-treated women [53]. Similar effects have also been shown in clinical trials of women treated with estrogen [56] and the selective estrogen receptor modulator (SERM) raloxifene [158].

There have been few reported studies investigating the combined effects of estrogen and exercise on bone in postmenopausal women. Most have examined combination hormone replacement therapy. In a study by Kohrt et al., 11 months of weight-bearing exercise and HRT had independent and additive effects on lumbar spine BMD and a synergistic effect on total body BMD assessed by DXA [159]. The same group also reported decreased bone turnover in HRT and exercise plus HRT groups and a decrease in bone resorption with no detectable effect on bone formation in an exercise group [160]. Prince et al. also conducted a double-blind placebo-controlled exercise, calcium supplementation and HRT trial in postmenopausal women and found that although 2 year treatment involving exercise plus calcium attenuated forearm bone loss, those undertaking exercise plus continuous estrogen and progesterone treatment had an increase in BMD [57]. Cheng et al. also examined whether changes in bone mass distribution could be observed in postmenopausal women following HRT and/or high-
impact exercise and found that both HRT and exercise had local beneficial effects on bone mass [55]. Finally, a randomized trial examining the effects of jumping exercise on BMD in pre- and postmenopausal women demonstrated that pre- and postmenopausal women have different BMD responses to the same high-impact exercise [52]. Premenopausal women had significant increases in femoral BMD after 5 months of exercise training, whereas there was no improvement in femoral BMD among postmenopausal women, even after 12 months (consistent with the effects of periosteal apposition in the latter). Further studies are required to elucidate the relationship between estrogen, bone geometry and strength.
CHAPTER 4: METHODS
4.1 Study Subjects

For all three studies, we used data from individuals participating in the Canadian Multicentre Osteoporosis Study (CaMos). The CaMos cohort is made up of men and women, aged 25 years and older, residing within a 50km radius of one of the nine designated CaMos research centres (St. John’s, Halifax, Quebec City, Kingston, Toronto, Hamilton, Saskatoon, Calgary and Vancouver). The CaMos population was recruited at random by telephone between 1995 and 1997, and includes 2884 men and 6539 women (n=9423). A detailed description of the CaMos protocol is described elsewhere [161]. Our analyses were restricted to women who had available HSA data from baseline DXA scans.

4.2 Ethical considerations

All three studies were approved by the CaMos Design, Analysis and Publications Committee which permitted access to the CaMos dataset.

4.3 Study design and subject categorization

4.3.1 CaMos

CaMos is a national multi-centre 10 year prospective study (www.camos.org). The study was developed to assess the burden of osteoporosis and fracture in Canadian men and women, as well as fund research that identifies factors associated with osteoporosis and fracture that will lead to improvements in the diagnosis, prevention and treatment of osteoporosis.
All consenting CaMos participants completed an interviewer-administered questionnaire at baseline entry into the study, which captured information on: demographics, medical history, drugs and medications, fractures, reproductive history, family history, physical characteristics, tobacco and alcohol use, food intake, sunlight exposure, diet and physical activity patterns and mental health status. Participants then underwent BMD testing by DXA at the lumbar spine and hip and ultrasound testing of the shin and wrist. Those aged 50 years and older had spine X-rays taken to assess the prevalence of vertebral fractures. Participants were then mailed a follow up questionnaire annually and all testing was repeated at the end of the five and ten year periods.

We used information collected from the baseline questionnaire, as well as HSA data from baseline DXA scans to complete our analyses for the three studies. HSA data was not available from the five and ten year follow-up periods, so we were limited to cross-sectional investigations. We had available data from seven study centres (Vancouver, Calgary, Saskatoon, Hamilton, Kingston, Quebec City and Halifax centres) totaling 3728 women.

4.3.2 Study 1: FRACTURE

Our objective for Study 1 was to determine associations between the number of self-reported fractures, bone geometry parameters and stress at the femoral neck in postmenopausal women enrolled in CaMos. Study 1 was a cross-sectional design and we categorized postmenopausal women (n=2168) into 2 groups based on their number of
self-reported fractures at baseline: (1) no fractures or (2) one or more fractures. We did not discriminate by fracture type (severe trauma, minimal trauma, disease related) or location, as alterations in modeling responses should show effects systemically, rather than at specific sites [13].

4.3.2 Study 2: DIABETES

Our objective for Study 2 was to determine the association between diabetes status and stress at the femoral neck in a subgroup of women enrolled in CaMos. Study 2 was a cross-sectional design and consisted of a subgroup of 3665 women. We categorized these women into two groups based on the presence or absence of self-reported diagnosed T2DM.

4.3.3 Study 3: ESTROGEN

Our objectives for Study 3 were two-fold. Our first objective was to determine the association between strenuous sports participation and stress at the femoral neck in a pre- and postmenopausal women participating in CaMos. Our second objective was to determine the association between estrogen availability and stress at the femoral neck in the same subgroup. Study 3 was a cross-sectional design and included a subset of pre- and postmenopausal women between the ages of 25-92 years (n=2447). We categorized women by menstrual status (premenopausal or postmenopausal), estrogen use (current users and non-users) and exercise level (non-exercisers or exercisers). Premenopausal referred to women who were currently menstruating and postmenopausal referred to women over the age of 55 who had cessation of menses for one year or more. Women
who did not meet these criteria were excluded. Current users (postmenopausal E+) were postmenopausal women who were currently using estrogen therapy and never users (postmenopausal E-) were postmenopausal women who had never used estrogen therapy. Postmenopausal women who were previous, but not current estrogen users, were excluded from the analyses.

Exercise information was gathered from the physical activity portion of the CaMos questionnaire which consisted of 7 questions and asked generally about the amount of time participants spent in leisure physical activity, formal exercise or job-related activity. We used question 4 to determine our independent variable of interest for this study. Question 4 asked on average how many hours a week were spent on 3 different activity types: (1) strenuous sports (such as jogging, bicycling on hills, tennis, racquetball, swimming laps, aerobics); (2) vigorous work (such as moving heavy furniture, loading or unloading trucks, shoveling, weight lifting, or equivalent manual labour) and (3) moderate activity (such as housework, brisk walking, golfing, bowling, bicycling on level ground, gardening). Participants selected one of eight possible categorical answers: (1) never; (2) 30 minutes-1 hour per week; (3) 2-3 hours per week; (4) 4-6 hours per week; (5) 7-10 hours per week; (6) 11-20 hours per week; (7) 21-30 hours per week or (8) 31 or more hours per week. Exercise level was based on the number of self-reported weekly hours spent participating in strenuous sports. Non-exercisers were women who never engaged in strenuous sports (0 hours per week) and exercisers were women who engaged in 2 or more hours per week of strenuous sports.
4.4 Anthropometric measurements

For all three studies, height and weight were measured by the DXA technician at the time of the baseline BMD scan. Standing height was measured to the nearest 0.1 cm using a stadiometer. Body weight was assessed by scale and was recorded to the nearest 0.1 kg. Some participants in the CaMos cohort who did not have DXA scans, self-reported height and weight; however, in the case of our study DXA scans were necessary to collect HSA data, thus heights and weights were all measured.

4.5 Skeletal assessments

4.5.1 DXA and HSA

The left proximal femur of each participant was scanned using DXA at baseline entry into the CaMos study. Four types of DXA machines were used and varied according to the CaMos centre location (Hologic QDR4500; Hologic QDR2000; Hologic QDR1000; Lunar DPX). Study participants were scanned according to standard protocols for positioning and analysis by certified and trained technicians. Machines were calibrated daily according to manufacturer’s recommendations and daily monitoring was used to assess and correct longitudinal drift. All Lunar measurements were converted to equivalent Hologic values using standard reference formulas [162, 163]. Machines were cross-calibrated using measurements from an anthropomorphic phantom that was circulated and scanned in each center [161].
HSA was used to estimate bone geometry parameters from the DXA scans. The HSA method for extracting cross-sectional geometry from DXA bone mass image data is based on principles first described by Martin and Burr [164]. At a site where the cross-section is to be evaluated, a line of pixel values traversing the bone axis is extracted from the image. The resulting profile is a mass projection of the corresponding cross-section and can be used to describe its geometry relevant to scan plane stresses [35]. Current HSA algorithms average geometry over five parallel profiles. Three sites of the proximal femur are typically analyzed: (1) the narrow neck (NN), across the narrowest diameter of the femoral neck; (2) the intertrochanter (IT), along the bisector of the neck-shaft angle and (3) the shaft (S), 1.5 times the minimum neck width distal to the intersection of the neck and shaft axes. In all three of our studies, we restricted our analyses to the narrow neck region (narrowest diameter of the femoral neck), as it is the region most susceptible to fracture in a fall [165].

We used the HSA program to compute the following variables at the narrow neck region: (i) areal bone mineral density (aBMD in g/cm²); (ii) cross-sectional area (CSA in cm²) – an index of resistance to axial compressive forces and (iii) section modulus (Z in cm³) – an index of bending strength in the image plane. A detailed description of how each of these measures is derived can be found online at ftp://ftp.cdc.gov/pub/Health_Statistics/NCHS/nhanes/nhanes3/17a/hip_methods.pdf [98].

4.5.2 Stress analysis
The stress analysis at the femoral neck employed a mechanical model of the femur incorporating the forces acting on it and the parameters needed to define the geometry as shown in Table 1 and Figure 8. Note that this analysis was restricted to the frontal plane defined by the axes of the femoral neck and shaft, due to the limitations of the 2D DXA data. Resolved forces and geometry were calculated as follows:

\[
F_{Mx} = F_M \cos \theta \\
F_{My} = F_M \sin \theta \\
F_{Jx} = F_J \cos \phi \\
F_{Jy} = F_J \sin \phi \\
SL_x = SL \cos(90^\circ - \beta) \\
SL_y = SL \sin(90^\circ - \beta) \\
NL_x = NL \sin(180^\circ - \alpha - \beta) \\
NL_y = NL \cos(180^\circ - \alpha - \beta)
\]

All forces and moments were then balanced to achieve static equilibrium using equations (i) to (iii) (i.e. 
\[
\sum F_x = 0, \quad \sum F_y = 0, \quad \sum M = 0
\]

(i) 
\[
\sum F_x = 0 \\
F_{Jx} = F_M \cos \theta
\]

(ii) 
\[
\sum F_y = 0 \\
F_{Jy} = F_M \sin \theta + \frac{8}{9} W_1
\]

(iii) 
\[
\sum M = 0 \\
F_M = \frac{8}{9} \frac{W}{TL} \frac{SL \cos(90^\circ - \beta) - NL \sin(180^\circ - \alpha - \beta)}{\sin \theta}
\]

Note that the magnitude of the weight vector is assumed to be 8/9 body weight (1).

After computing \( F_{Jx}, F_{Jy} \) and \( F_M, F_J \) and its angle \( \phi \) were computed using equations (iv) and (v):
The internal forces in the neck resulting from the external forces $F_M$, $F_f$ and $W_1$ are shown in a femoral neck free-body diagram (Figures 9-11), where $M_s$, $P_s$ and $V$ are bending moment, axial load and shear force on the neck cross-section respectively. The small shear force was neglected in our calculations.

The bending moment $M_s$ and axial force $P_s$ were calculated using equations (vi) and (vii):

\[
P_s = F_f \cos(\theta - \alpha - \beta + 90^\circ) \tag{vi}
\]

\[
M_s = F_f \cdot d_x = F_f \sin(\theta - \alpha - \beta + 90^\circ) \cdot d \sin(180^\circ - \alpha - \beta) \tag{vii}
\]

Axial stress on the medial surface of the femoral neck was computed using a formula from simple engineering beam theory using equation (viii):

\[
F_f = \sqrt{F_{f_x}^2 + F_{f_y}^2} \tag{iv}
\]

\[
\theta = \tan^{-1} \frac{F_{f_y}}{F_{f_x}} \tag{v}
\]
$$\delta_{\text{Medial}} = \frac{M_S \cdot y_{\text{Medial}}}{I} + \frac{P_S}{A}$$

Where $M_S$ and $P_S$ are the bending moment and axial load on the neck cross-section $I$ and $A$ are the cross-sectional moment of inertia, and the cross-sectional area of the cross-section, and $y_{\text{Medial}}$ is the perpendicular distance between the medial surface, and the neutral axis. At the medial surface, the bending moment and axial load produce compressive stresses which are represented by a positive sign.

4.6 Power Calculations

All three studies were secondary data analyses. As such, power was determined based on the available data for our independent and dependent variables.

4.6.1 Study 1: FRACTURE

Power was determined based on the amount of HSA and fracture data that was available for women at baseline. The means and standard deviations of our dependent variables (medial stance stress; NN BMD; NN CSA; NN outer diameter; NN section modulus; NN radius of gyration) were incorporated into a standard statistical formula (two-sample Satterthwaite t test assuming unequal variances) to compute power for each of our desired comparisons between group means given our sample sizes. We completed separate power estimates for each of our comparisons between group means (Table 2).

4.6.2 Study 2: DIABETES
Power was determined based on the amount of HSA and diabetes data that was available for women at baseline. The mean and standard deviation of our dependent variable (medial stance stress) was incorporated into a standard statistical formula (two-sample Satterthwaite t test assuming unequal variances) to compute power for our desired comparison between group means given our sample size. (Table 3).

4.6.3 Study 3: ESTROGEN

Power was determined based on the amount of HSA and physical activity data that was available for women at baseline. The means and standard deviations of our dependent variable (medial stance stress) were incorporated into a standard statistical formula (two-sample Satterthwaite t test assuming unequal variances) to compute power for each of our desired comparisons between group means given our sample sizes. We completed separate power estimates for each of our comparisons between group means (Tables 4a and 4b).

4.7 Statistical Analyses

4.7.1 Study 1: FRACTURE

We used linear regression (SAS 9.3) to determine associations between stress, geometry parameters and number of fractures. Separate regression analyses were completed for each outcome variable (stress, BMD, CSA and Z). Models for BMD and bone geometry were adjusted for age, as well as body weight and height which were crude adjustments for load differences due to body size. The stress models were unadjusted as body weight
and height were incorporated into the stress computations and prior work from our group has demonstrated that stresses remain relatively stable with age [166]. We considered a p value of 0.05 statistically significant.

4.7.2 Study 2: DIABETES

We used linear regression (SAS 9.3) to determine the association between diabetes status and stress. We did not adjust this model for age, as previous work from our group has demonstrated that stresses remain relatively stable with age [166]. Further, we did not adjust for height or weight, because these variables were factored into our stress computations. We considered a p value of 0.05 statistically significant.

4.7.3 Study 3: ESTROGEN

We used linear regression (SAS 9.3) to determine associations between strenuous sports participation and stress between exercisers and non-exercisers. We also used general linear models to compare stresses between the premenopausal and postmenopausal E+ groups as well as the premenopausal and postmenopausal E- groups. We examined these comparisons in both exercise groupings. As stresses have been shown to remain relatively stable with age [166], we did not further adjust for age in our statistical models. We did however adjust for body mass index (BMI), as a crude adjustment for load differences due to body composition (skeletal usage). An alpha of 0.05 was considered statistically significant.
CHAPTER 5: MANUSCRIPT STUDY 1: FRACTURE
“Heterogeneity in skeletal load adaptation points to a role for modeling in the pathogenesis of osteoporotic fracture”
Introduction

There are a multitude of factors that influence fracture risk [167]. One factor that has not been well studied or understood is bone modeling – the ability of the skeleton to adapt its geometry (size and shape) to changes in mechanical demands [13]. Modeling differs from the remodeling process in that it serves an adaptive rather than restorative function, and is achieved by independent de-novo bone formation or resorption, depending on the directional change in mechanical stimulus [48]. Modeling occurs in response to minute deformations (strains), produced by loading forces within bone tissue during daily activities.

It is possible that not all individuals respond equally to mechanical stimuli, nor generate equivalent modeling responses. Some individuals may require higher loads to stimulate an adaptive response in bone geometry, whereas others may be more sensitive to load stimuli such that geometry adapts at smaller loads. A bone that is hyposensitive to load stimuli should be intrinsically weaker than normal and independent of other factors should be more likely to fracture when exposed to trauma. Heterogeneity in modeling responses may be due to genetic, environmental or hormonal factors [46, 107, 168] and could explain differences in fracture risk independent of bone mineral density (BMD). If we could measure strains in vivo, we should expect to see that under comparable loading conditions, the maximum strains in the bones of those with deficient loading response are higher than normal. Unfortunately bone strains cannot be measured non-invasively but strains are proportional to stresses (force concentrations). Stresses can be computed from
bone geometry data available by non-invasive means. In this study, we computed load stresses for the medial (thickest) cortex at the femoral neck in a one legged stance. This is a location where the femur is highly adapted to resist large forces generated during ambulation.

The purpose of our study was to determine if heterogeneity in modeling responses could help to explain differences in fracture prevalence among postmenopausal women participating in the Canadian Multicentre Osteoporosis Study (CaMos). We hypothesized that postmenopausal women with a history of fractures would have higher stresses and that this would also be evident as compromised bone geometry at the femoral neck compared to postmenopausal women without fractures.

Materials and Methods

Study participants
We conducted a secondary data analysis in postmenopausal women 50 years of age or older, with cessation of menses for one year or more, who were enrolled in CaMos and had available HSA data from baseline dual energy x-ray absorptiometry (DXA) scans of the proximal femur (n=2168). CaMos is a national multi-centre 10 year prospective study (www.camos.org), developed to assess the burden of osteoporosis and fracture in Canadian men and women. The CaMos cohort was recruited at random by digit dialing between 1995 and 1997, and includes 2884 men and 6539 women. A detailed description of the CaMos protocol has been documented elsewhere [161]. Informed consent was
obtained from all individuals participating in CaMos. This sub-study was approved by the CaMos Data Analysis and Planning committee.

*Anthropometric measurements*

Height and weight were measured by the DXA technician at the time of the DXA scan. Standing height was measured to the nearest 0.1 cm using a stadiometer. Body weight was assessed by balance or digital scale and was recorded to the nearest 0.1 kg [161].

*Categorization by number of fractures*

We categorized women into 2 groups based on their number of self-reported fractures at baseline: (1) no fractures or (2) one or more fractures. We did not discriminate by fracture type (severe trauma, minimal trauma, disease related) or location, as alterations in modeling responses should show effects systemically, rather than occur at specific sites.

*Hip Structure Analysis and bone geometry*

The purpose of the study was to report stresses generated by an engineering analysis using femur geometry generated here from conventional DXA scans of the hip by the HSA method. A thorough explanation of the HSA technique has been described previously [97], and while three sites of the proximal femur are typically analyzed, we only evaluated the narrow neck (NN) region (narrowest diameter of the femoral neck). In addition to stresses we also report bone mineral density (BMD), cross-sectional area (CSA; an index of resistance to compressive loads) and section modulus (Z; an index of
resistance to bending loads) all at the NN region. Note that the NN region does not have
an exact counterpart in the conventional BMD analysis and thus cannot be exactly
compared. However the NN region is typically overlapping or more proximal to the
wider conventional femoral neck region.

Stress analysis
We computed stress on the infero-medial margin of the femoral neck cross-section at its
narrowest point using a mechanical model depicting forces acting on the femur (Figure
8), and engineering beam theory that incorporated dimensions and geometry from DXA
scans of the left proximal femur using the HSA method. This site was selected as it is
normally loaded under compression, adapted to upright walking [169] and relatively well
preserved in both men and women with age [20]. A detailed description of the derivation
of medial femoral neck stance stress is described in Section 4.5.2.

Statistical analysis
We used linear regression (SAS 9.3) to determine associations between stress, geometry
parameters and number of fractures. Separate regression analyses were completed for
each outcome variable (stress, BMD, CSA and Z). Models for BMD and bone geometry
were adjusted for age, as well as body weight and height which were crude adjustments
for load differences due to body size. The stress models were unadjusted as body weight
and height were incorporated into the stress computations and prior work from our group
has demonstrated that stresses remain relatively stable with age [166]. A p value of 0.05
was considered statistically significant.
Results

Demographics of postmenopausal women by number of fractures

Characteristics of the subgroup are outlined in Table 5. Almost all women were Caucasian (99%). The majority (1248) reported no self-reported fractures (58%) and 920 (42%) reported one or more fractures. Women who reported fractures were slightly older than those without fractures (p=0.0002) but there were no difference between fracture groups in weight, height or physical activity levels.

Number of fractures, stance stress and bone geometry

Postmenopausal women with one or more fractures had 2.6% higher stress than postmenopausal women with no fractures (Figure 12). Postmenopausal women with one or more fractures also had significantly lower NN BMD (4.2%), CSA (3.9%) and Z (9.6%) than postmenopausal women without fractures (Figure 13).

Discussion

We demonstrated that when standing on one leg, postmenopausal women with a history of one or more fractures generated higher stress on their medial femoral neck cortices than postmenopausal women without fractures. We believe that this is indicative of more poorly adapted femoral neck bone geometry as it is evident in their lower BMD values and weaker indices of bending and axial strength. As stance mode represents a habitually loaded mechanical condition that is adapted to upright walking and that the medial cortex
where we measured is relatively well preserved with age [20, 169]. These findings are consistent with our hypothesis that altered skeletal load sensitivity and consequential modeling responses could explain differences in fracture prevalence.

If all individuals responded equally to mechanical stimuli, regardless of body size and physical activity level, their skeletal geometry should be adapted such that prevalent loads generate equivalent tissue stresses [13]. Alternatively, heterogeneity in load response should alter the equilibrium between normally encountered loads and maximum stresses experienced within bone tissue. An individual with decreased sensitivity to load should have geometrically weaker bones such that a given maximal load results in a higher maximum stress while the opposite would be true for those with greater load sensitivity. If we assume that traumas are equally distributed throughout the population, those with relatively weaker bones should therefore be more susceptible to fractures, all else equal.

Our stress results are consistent with this theory that heterogeneity in skeletal load response exists and that a reduced adaptive response to load can contribute to increased fragility. This idea of impaired adaptation to load is also supported by cellular data demonstrating that osteocytes harvested from osteoporotic individuals have smaller formation responses to strain stimuli in cell culture than non-osteoporotic individuals [107].
Our geometry results provide a clear mechanical explanation for the differences in maximal stresses noted between postmenopausal women with and without fractures. Underlying the higher stresses in postmenopausal women with fractures, we demonstrated lower BMD, and smaller indices of resistance to axial (CSA) and bending forces (Z) independent of body size, than postmenopausal women without fractures. These are the geometric changes we would expect in the presence of reduced load sensitivity.

Our study had several limitations. One important point to consider is that geometry measurements reflect structural strength, and that strength scales with the size of an individual, as well as with an individual’s level of skeletal usage or loading [35]. We know that long bones function as muscle-actuated levers. Since muscles tend to operate with a mechanical disadvantage or as inefficient levers, muscle forces dominate loads to which the skeleton must adapt [170-172]. Therefore, a measure of muscle size such as total body lean mass would be a good surrogate to account for differences in skeletal usage between individuals. Unfortunately we did not have this information available, but did adjust for height and weight as a crude method of scaling for body size.

Another limitation is that there is evidence to suggest that adaptation not only influences the structural geometry, but also the material properties of bone tissue [24] which cannot be evaluated using DXA or other non-invasive methods. Adaptation effects may therefore be greater than depicted in our analyses. It should also be noted that the stress analysis is derived using geometry from DXA data which has inherent limitations.
Bending properties derived from HSA are only evaluated in the image (frontal) plane and are highly sensitive to error due to variations in the projected femur dimensions when positioned inconsistently [35]. However, although the HSA method is imprecise, it is most robust in large cohort studies like this one where precision effects are minimized by statistical power.

Our study demonstrates heterogeneity in modeling responses among Canadian postmenopausal women with and without fractures. Women with fractures appear to have reduced sensitivity to load, evidenced by higher levels of stress within the bone and evident in size-independent compromises in underlying bone geometry. Collectively, these findings suggest an important role for modeling in the pathogenesis of bone fragility in postmenopausal women. Compromised modeling has important clinical implications in terms of treatment selection, as individuals with altered load sensitivity may respond best to anabolic bone agents such as parathyroid hormone or nitrates, which would improve formation responses to load stimuli.
CHAPTER 6: MANUSCRIPT STUDY 2: DIABETES
“Evidence for impaired skeletal load adaptation among Canadian women with type 2 diabetes mellitus”
**Introduction**

Diabetes mellitus and osteoporosis are two common health problems affecting millions of women worldwide. Approximately 20% of women over the age of 50 years have osteoporosis [2] and 13.5% over the age of 65 years have diabetes [173]. Type 2 diabetes mellitus (T2DM) is the most common form of diabetes, accounting for 90-95% of cases [26], and is associated with an increased risk of hip fracture, more so in women than men [27, 28]. This increased hip fracture risk has been deemed paradoxical, given the fact that women with T2DM tend to have higher bone mineral density (BMD) compared to non-diabetic controls [33, 34], and low BMD is known to be an independent risk factor for fracture [174]. However, recent data suggests that this so-called paradox might be explained by an impaired skeletal response to load [36, 37].

While not widely appreciated, it is well established that bones continually adapt throughout life to prevalent loading conditions [13]. This homeostatic mechanism rooted in osteocyte signaling, serves to ensure that bones remain strong enough to resist the forces normally encountered during daily activities. Conceivably, the bones of diabetics might be more susceptible to fracture if they somehow fail to adapt adequately to loading forces, and thus are weaker than they should be. Data from a recent study by Garg and colleagues which examined femoral neck geometry in 5924 women enrolled in the Women’s Health Initiative observational study, provided evidence that this might be the case [36]. The study demonstrated that diabetic women using insulin had larger BMD and bending strength at the proximal femur than non-diabetic controls, consistent with
their larger body sizes. However, when these measures were adjusted for lean body mass, they were lower than in controls. The reason that this may suggest a deficiency in load adaptation is because physiologic skeletal loading forces are muscle generated, thus skeletal geometry normally scales in proportion to lean mass [38, 39].

The purpose of this paper is to continue that line of investigation to see if we can find more direct evidence supporting this intriguing explanation for the T2DM bone density paradox. The approach is as follows. One would expect that skeletal adaptation ensures that the maximum bone strains (minute deformations) in normal bones do not exceed some normal value when physiologic forces are applied during physical activity. Abnormally deficient adaptation would result in weaker bone so that equivalent physical activity generates higher than normal strains within bones. Unfortunately it is not currently possible to measure bone strains by non-invasive means, but strains are proportional to stresses (force concentrations) and stresses can be computed from knowledge of the loading forces, the bone dimensions (geometry) and a suitable engineering method. We used an engineering beam analysis incorporating bone geometry derived from dual energy x-ray absorptiometry (DXA) scans by the Hip Structure Analysis (HSA) method, to determine if stresses at the femoral neck region of the proximal femur were higher in women with T2DM compared to non-diabetic controls.

**Materials and Methods**
**Study participants**

We conducted a secondary data analysis in a subset of women 25 years of age or older who participated in the Canadian Multicentre Osteoporosis Study (CaMos) and had available HSA data from baseline DXA scans of the left proximal femur (n=3665). CaMos is a national multi-centre 10 year prospective study (www.camos.org), developed to assess the burden of osteoporosis and fracture in Canadian men and women. The CaMos cohort was recruited by random digit dialing between 1995 and 1997, and includes 2884 men and 6539 women (n=9423). A detailed description of the CaMos protocol has been described elsewhere [161]. Informed consent was obtained from all individuals participating in CaMos. This sub-study was approved by the CaMos Data Analysis and Planning committee.

**Categorization by diabetes status**

We categorized women into two groups based on the presence or absence of self-reported T2DM.

**Anthropometric measurements**

Height and weight were measured by the DXA technician at the time of the DXA scan. Standing height was measured to the nearest 0.1 cm using a stadiometer. Body weight was assessed by balance or digital scale and was recorded to the nearest 0.1 kg.

**Dual Energy X-Ray Absorptiometry**
The details of areal BMD measurements obtained by DXA in CaMos have been published in detail elsewhere [161]. In the current study, we used BMD measures obtained at the femoral neck region.

*Hip Structure Analysis*

We used the HSA method to extract cross-sectional geometry of the left proximal femur from baseline DXA scans. A thorough explanation of the HSA technique has been described previously [97]. While three sites of the proximal femur are typically analyzed with HSA, we only evaluated the narrow neck (NN) region (narrowest diameter of the femoral neck), as it is the region most susceptible to fracture in a fall [165].

*Stress analysis*

We computed stress on the infero-medial margin of the femoral neck cross-section at its narrowest point using a mechanical model depicting forces acting on the femur (Figure 8), and engineering beam theory that incorporated dimensions and geometry from DXA scans of the left proximal femur using the HSA method. A detailed description of the derivation of medial femoral neck stance stress is described in Section 4.5.2.

*Statistical analysis*

We used linear regression models (SAS 9.3) to determine the association between diabetes status and stress. We did not adjust this model for age, because prior work from our group has demonstrated that stresses remain relatively stable with age [166]. Furthermore, we did not adjust for height or weight, because these variables were
factored into our stress computations, accounting for body size differences. We considered a p value of 0.05 statistically significant.

Results

Participant demographics are outlined in Table 6. There were 3501 women without diabetes (95.5%) and 164 with T2DM (4.5%). Nearly all women were of White ethnicity (>99% in each group). Women with diabetes were older, weighed more and had a higher body mass index than those without diabetes. Stresses computed at the infero-medial margin of the femoral neck were 3.9% higher in T2DM women than in non-diabetics (Figure 14).

Discussion

Consistent with our hypothesis, we found that femoral neck stresses were higher in women with T2DM compared to non-diabetic women. Since stress is an indicator of underlying geometry, and geometry should be adapted to prevalent loads, higher stress indicates weaker geometry among women with T2DM. As aforementioned, weaker geometry has been documented in two other studies of women with T2DM and is in line with our current findings [36, 37]. The previously mentioned study by Garg and colleagues which examined 5924 women enrolled in the Women’s Health Initiative Observational study, demonstrated that after adjustment for total body lean mass, diabetic women on insulin had significantly lower BMD, cross-sectional area (an index of
compressive strength) and section modulus (an index of bending resistance) at the femoral neck than non-diabetics [36]. Lower composite strength indices at the femoral neck were also reported among perimenopausal diabetic women compared to non-diabetic controls in the Study of Women’s Health Across the Nation [37] after adjustment for body mass index as well as several other confounders.

Since skeletal geometry is continually adjusted via the cellular process of modeling in response to changing load conditions, one potential explanation for weaker geometry and higher stress among women with T2DM is a diminished adaptive response to load. The theory behind this line of thinking is Frost’s Mechanostat Theory [13], which postulates that bone adapts to mechanical loads to keep strength at an appropriate level to withstand forces generated by current activity levels. If all individuals responded equally to mechanical stimuli, regardless of body size and physical activity level, their skeletal geometry should be adapted such that prevalent loads generate equivalent tissue stresses. Alternatively, heterogeneity in load response should alter the equilibrium between normally encountered loads and maximum stresses experienced within bone tissue. An individual with decreased sensitivity to load, as we postulate is the case among women with T2DM, should have geometrically weaker bones such that a given maximal load results in a higher maximum stress. The opposite would hold true for those with greater load sensitivity. If we assume that traumas are equally distributed throughout the population, those with relatively weaker bones should therefore be more susceptible to fractures, all else equal. Decreased sensitivity to load may therefore be one explanation for the fragility evident in women with T2DM.
Of note, an attenuated response to load is also in line with cellular mechanisms thought to contribute to increased fragility in T2DM. Advanced glycation end products (AGEs), which are formed as a result of non-enzymatic reactions between glucose and proteins are known to accumulate in diabetes [119]. AGEs have been shown to inhibit both proliferation and differentiation of osteoblasts [120, 121]. This could directly impair the bone formation response to load and essentially contribute to the weakened skeletal geometry observed in diabetics. Although unrelated to a geometry explanation, the presence of AGEs may also directly alter the material properties of bone collagen, increasing bone brittleness and further impairing strength [124].

Our study had several limitations. Of critical importance is the recognition that increased hip fracture risk among patients with T2DM is undoubtedly multifactorial and likely cannot only be explained by an impaired load response [175]. Diabetic complications such as peripheral neuropathy may increase risk for falls which can lead to fractures [176]. Decreased levels of physical activity, common in T2DM may also increase the risk for falls, as well as decrease total body lean mass fraction which is protective for bone [176, 177]. Further renal insufficiency, and lower calcium and vitamin D intake may lead to detrimental skeletal alterations [175]. Another limitation is that there is evidence that adaptation not only influences the structural geometry, but also the material characteristics of bone tissue which cannot be evaluated using DXA methods [24]. Adaptation effects may therefore be greater than depicted in our analyses. It should be noted that the stress analysis is derived using geometry from DXA data which has inherent limitations. Bending properties derived from HSA are only evaluated in the
image (frontal) plane and are highly sensitive to error due to variations in the projected femur dimensions when positioned inconsistently [35]. However, although the HSA method is imprecise, it is most robust in large cohort studies like this one where precision effects are minimized by statistical power.

In conclusion, our study demonstrated that femoral neck stresses were higher in women with T2DM compared to non-diabetic women. Higher stress indicates weaker skeletal geometry for a given load, and suggests an impaired adaptive response to load may exist in women with T2DM. Among other factors, impaired modeling could contribute to an increased fracture risk in women with T2DM and sheds light on the so-called T2DM and BMD paradox. Further longitudinal analyses are required in larger cohorts, to pin point the geometrical changes that lead to increased stress and the changes evident with progressive diabetes severity.
CHAPTER 7: MANUSCRIPT STUDY 3: ESTROGEN
“Estrogen influences the skeletal response to exercise in pre- and postmenopausal women”
Introduction

Bone tissue has the remarkable ability to model its geometry (size and shape) and perhaps its material properties [24] to changes in mechanical loads imposed by normal physical activities of daily life. Modeling serves an adaptive function, and is achieved by independent de-novo bone formation or resorption depending on the directional change in stimulus [64]. It is generally agreed that modeling stimuli take the form of minute deformations (strains) produced within bone tissue by loading forces. According to Frost’s Mechanostat Theory [13], if activity related strains exceed a certain upper threshold, osteocytes respond by generating biochemical signals initiating the formation of new bone. Once enough new bone is accrued, strain levels are reduced below the upper threshold and bone formation ceases. Similarly, when activity generated strains are absent, or fall below a certain lower threshold, osteocytes generate a signal to resorb excess bone. The resorption of mechanically unnecessary excess bone continues until strains increase above the lower threshold. In this manner, bone adapts to the mechanical loading effects of physical activity by becoming stronger or weaker as activity levels change. At steady state, there should be equilibrium between structural geometry and prevalent loading forces.

Estrogen, the predominant female sex hormone, is intimately involved in the mechanical control of bone homeostasis [49, 50, 149, 178, 179]. Data from animal and cellular studies indicate that variations in estrogen availability mediate the skeletal response to mechanical load (mechanosensitivity) [46, 168]. Specifically, it has been postulated that
estrogen deficiency increases the strain threshold for bone to stimulate a formation response, effectively reducing bone’s sensitivity to mechanical load [48-50]. The effects of exercise may therefore be blunted with aging in women, as levels of estrogen decline after menopause [51].

Although strains cannot be assessed in vivo, they are generally proportional to load stresses, and stresses for a particular bone cross-section can be computed from knowledge of bone structural dimensions and load magnitude. In a one-legged stance where stresses are maximal, load can be estimated using body weight. In individuals who are inactive, body weight serves as a fairly reliable index of maximal loads normally encountered at the hip. In active individuals however, the magnitude of the maximal loads encountered at the hip during various types of activities well exceeds that of body weight. For instance, during fast walking, jogging and going up and down stairs ground reaction forces have been shown to reach magnitudes of 5-6 times body weight [180-182]. Due to the fact that we cannot measure the maximal encountered loads during exercise, we would therefore expect stance mode stresses to appear lower in those who are more active. This divergence however, allows us to use estimated stresses as an indicator of load sensitivity. Individuals with reduced sensitivity should have weaker bones evidenced by higher stresses and vice versa.

The purpose of the current study was to use menopause as a model of estrogen deficiency to study the effects of exercise at the hip. We hypothesized that computed stresses on the medial cortex of the femoral neck in a one-legged stance would be higher in non-
exercisers compared to exercisers, higher in postmenopausal women than in premenopausal women, that estrogen supplementation would reverse this effect and that loading would elicit a greater adaptive response in the premenopausal women as compared to postmenopausal women.

**Materials and methods**

*Study participants*

We used a subset of women 25 years of age or older who participated in the Canadian Multicentre Osteoporosis Study (CaMos). CaMos is a national multi-centre 10 year prospective study (www.camos.org), developed to assess the burden of osteoporosis and fracture in Canadian men and women, as well as fund research that will lead to improvements in the diagnosis, prevention and treatment of osteoporosis. The CaMos cohort was recruited at random by telephone between 1995 and 1997, and includes 2884 men and 6539 women (n=9423). A detailed description of the CaMos protocol is documented elsewhere [161]. Our sub study included a subset of pre- and postmenopausal women who had available Hip Structure Analysis (HSA) data from baseline dual energy x-ray absorptiometry (DXA) scans (n=2447).

*Categorization by menstrual status, estrogen use and exercise level*

Women were categorized by menstrual status (premenopausal or postmenopausal), estrogen use (current users and non-users) and exercise level (non-exercisers or exercisers). Premenopausal referred to women who were currently menstruating and
postmenopausal referred to women over the age of 55 who had cessation of menses for one year or more. Current users (postmenopausal E+) were postmenopausal women who were currently using estrogen therapy and never users (postmenopausal E-) were postmenopausal women who had never used estrogen therapy. Postmenopausal women who were previous, but not current estrogen users, were excluded from the analyses. Exercise level was based on the number of self-reported weekly hours spent participating in strenuous sports (defined as activities such as jogging, bicycling on hills, tennis, racquetball, swimming laps and aerobics). Non-exercisers were women who never engaged in strenuous sports (0 hours per week) and exercisers were women who engaged in 2 or more hours per week of strenuous sports.

**Anthropometric measurements**

Height and weight were measured by the DXA technician at the time of the DXA scan. Standing height was measured to the nearest cm using a 0.1 stadiometer. Body weight was assessed by balance or digital scale and was recorded to the nearest 0.1 kg.

**Hip Structure Analysis (HSA)**

We used the HSA method to extract cross-sectional geometry of the left proximal femur from baseline DXA scans. In this technique, at a site where the cross-section was evaluated, a line of pixel values traversing the bone axis was extracted from the image. The resulting profile was a mass projection of the corresponding cross-section and was used to describe its geometry relevant to scan plane stresses [35]. Current HSA algorithms average geometry over five parallel profiles. While three sites of the proximal
femur are typically analyzed with HSA, we only evaluated the narrow neck (NN) region (narrowest diameter of the femoral neck), as it is the region most susceptible to fracture in a fall [165]. A thorough explanation of the HSA technique and associated geometric variables has been described previously [97].

**Stress analysis**

As depicted in Figure 8, a one-legged stance was simulated assuming an abductor force (muscular) acting on the superior aspect of the greater trochanter ($F_M$), a joint force passing through the femoral head ($F_J$) and a ground reaction force through the knee ($W_1$), adjusted to achieve static equilibrium (vector sums of forces equal zero). Stress on the infero-medial margin of the femoral neck cross-section at its narrowest point was computed using a mechanical model and engineering beam theory that incorporated dimensions and geometry from DXA scans of the left proximal femur using the HSA method. A detailed description of the derivation of medial femoral neck stance stress is described in Section 4.5.2.

**Statistical analysis**

We used linear regression (SAS 9.3) to determine associations between strenuous sports participation and stress between exercisers and non-exercisers. We also used general linear models to compare stresses between the premenopausal and postmenopausal E+ groups as well as the premenopausal and postmenopausal E- groups. We examined these comparisons in both exercise groupings. As stresses have been shown to remain relatively stable with age [166], we did not further adjust for age in our statistical models.
We did however adjust for body mass index (BMI), as a crude adjustment for load differences due to body composition. An alpha of 0.05 was considered statistically significant. As our sub study was a secondary data analysis, power was limited by the amount of HSA and physical activity data that was available at baseline entry into the CaMos study. Participants with missing data were excluded.

Results

Participant demographics

Participant demographics are outlined in Table 7. There were 2156 non-exercisers (88%) and 291 exercisers (18%). Almost all women were of White ethnicity (99%; data not shown). Exercisers were slightly younger than non-exercisers across menstrual and estrogen groupings. Postmenopausal never users (postmenopausal E-) were older than postmenopausal users (postmenopausal E+) in both exercise groupings. Non-exercisers were heavier (weight) and had larger BMIs than exercisers across menstrual and estrogen groupings, but height was similar. The majority of exercisers participated in between 2 and 6 hours per week of strenuous sports.

Stress

Stresses were lower in exercisers compared to non-exercisers across all menstrual and estrogen groupings (Figure 15a). After adjustment for BMI, stresses remained lower in exercisers compared to non-exercisers in premenopausal women, but were no different in the postmenopausal E+ or postmenopausal E- groups (Figure 15b). In both exercisers
and non-exercisers, there were no detectable differences in stress between the premenopausal group and postmenopausal E+ group; however, stresses were higher in the postmenopausal E- group than in the premenopausal group (Figure 16a). This association remained present even after adjustment for BMI (Figure 16b).

Discussion

Consistent with our hypothesis regarding exercise effects, we found that femoral neck stresses computed for a body weight load were lower in exercisers than in non-exercisers regardless of menopausal status or estrogen use. Also consistent with our hypothesis regarding the effects of estrogen, we found that stresses were significantly higher in postmenopausal women not on estrogen than in premenopausal women, regardless of exercise level. Further, we found that postmenopausal women on estrogen had stress levels that could not be statistically distinguished from corresponding levels in premenopausal exercisers and non-exercisers. Non-exercising postmenopausal women not taking estrogen (postmenopausal E-) had femoral neck stresses that were significantly higher than all other subgroups, but those who did exercise reduced their stresses to levels similar to postmenopausal exercisers taking estrogen.

Clearly estrogen plays an important role in mediating skeletal sensitivity to mechanical load, although the role is arguably complex. It has been postulated that skeletal sensitivity to load is enhanced in the presence of estrogen and diminished in its absence [46, 183]. Exercise and estrogen are thought to have a synergistic effect on bone through their effects on estrogen receptor-α. Exercise induces bone formation through activation
of estrogen receptor-α, which is also up-regulated by estrogen therapy [184]. Moreover, estrogen receptor-α is a transcription factor for osteoblasts [140], regulating gene expression when activated by estrogen or other ligands. Estrogen receptor-α is also found in osteoclasts and osteocytes and its activation leads to osteoblast proliferation and differentiation [185], promotion of osteocyte survival and osteoclast apoptosis [140]. Estrogen receptor-α number has also been shown to be decreased in osteocytes from bone biopsies of hormone-deficient women [54], suggesting that estrogen receptor-α number, and therefore exercise effects, are dependent on estrogen sufficiency.

Our finding that estrogen restores stresses to premenopausal levels is in line with evidence from other clinical studies. Data from the Study of Osteoporotic Fractures (SOF) demonstrated that postmenopausal women currently on hormone therapy had mechanically stronger proximal femur geometry than never-treated women [53]. Similar effects have also been shown in clinical trials of women treated with estrogen [56], combined hormone therapy [186] and the selective estrogen receptor modulator (SERM) raloxifene [158].

We know that exercise should improve body muscularity such that exercisers should have a greater relative proportion of lean mass than non-exercisers. We did not have information on total body lean mass, so we attempted to adjust for this confounding effect by crudely adjusting for BMI. Doing so did not change the associations between exercise and stress levels in premenopausal women, but made the associations disappear in both postmenopausal estrogen (postmenopausal E+) and non-estrogen users.
(postmenopausal E+). Consistent with the cellular evidence [184] our findings suggest estrogen and exercise have a synergistic effect in premenopausal women which is not apparent in postmenopausal women in our study. This may be due to the fact that exercise levels were apparently greater and in a larger proportion of our population in premenopausal women than we observed after the menopause. Clearly this effect will require further study. We might also find that the synergistic benefits of exercise and estrogen may persist after the menopause if examined in a larger cohort of women with greater statistical power. In line with the latter, the associations between stress, menopausal and estrogen status remained significant even after adjustment for BMI.

While our results support the view that estrogen enhances skeletal sensitivity to mechanical load, it should be noted that an alternative view exists [151]. The alternative paradigm defends that estrogen drives deposition of extra mineral into growing female bones during puberty to prepare the female skeleton for pregnancy- and lactation-induced bone loss [187, 188]. Subsequently, it is argued that the sensitivity of female bones to mechanical loads during this time period is reduced. On the flip side, the withdrawal of estrogen at menopause is thought to promote a reverse unpacking of bone mineral along with an increase in skeletal load sensitivity. This line of thinking is based on observations from several studies in rats [59] as well as pooled data from cohort studies [189, 190] based primarily on BMD and BMC observations. These measures do not reflect load responses, therefore, the use of a geometry based analysis in the present study may account for the differences in our findings.
Our study had several limitations. Since this investigation was conducted with cross-sectional data, no causal relationships among any of the variables can be assumed. Another limitation was the quantification of exercise level by self-report, which is subject to recall bias, particularly with regard to exercise time and intensity, which are often overestimated [191]. Further, self-reported measures tend to be only modestly correlated with objective measures of exercise, thus they may not be sufficient to detect true bone effects [192, 193]. Another limitation is that there is evidence that adaptation not only influences the structural geometry, but also the material characteristics of bone tissue [24] which cannot be evaluated using DXA methods. Adaptation effects may therefore be greater than depicted in our analyses. Finally, it should be noted that the stress analysis is derived using geometry from DXA data which has inherent limitations. Bending properties derived from HSA are only evaluated in the image (frontal) plane and are highly sensitive to error due to variations in the projected femur dimensions when positioned inconsistently [35]. However, although the HSA method is imprecise, it is most robust in large cohort studies like this one where precision effects are minimized by statistical power.

There are few studies that provide insight about the skeletal sensitivity to mechanical load in vivo. Overall, our findings are consistent with the theory that skeletal sensitivity to mechanical load is enhanced in the presence of estrogen and diminished in its absence. This is in line with cellular data [46, 54, 183] as well as several other investigations in humans [56, 158, 159, 186, 194-196]. Further prospective studies are required to
examine the specific bone geometry adaptations that contribute to the changes in stress that occur in women across the menopause.
CHAPTER 8: DISCUSSION AND CONCLUSIONS
8.1 Introduction

The purpose of this thesis was to explore skeletal mechanosensitivity in vivo, by evaluating geometric alterations at the hip under varied conditions. Study 1 (FRACTURE) evaluated associations between fracture prevalence and stress. Study 2 (DIABETES) evaluated associations between diabetes and stress and Study 3 (ESTROGEN) evaluated associations between estrogen deficiency, exercise and stress. The following section will review the main objectives and hypotheses of this thesis and discuss whether or not the studies presented in Chapters 5-7 satisfied these objectives and hypotheses.

8.1.1 Study 1: FRACTURE

The purpose of Study 1 was to determine if heterogeneity in modeling responses could explain differences in fracture prevalence among postmenopausal women participating in the Canadian Multicentre Osteoporosis Study (CaMos). The concept for this study stemmed from genetic, hormonal and environmental data [46, 107, 168] suggesting that not all individuals respond equally to mechanical stimuli nor generate equivalent modeling responses and if this is so, that heterogeneity in load response could help explain variations in skeletal fragility.

Our study objective was to determine associations between the number of self-reported fractures, bone geometry parameters and stress at the femoral neck in a Canadian cohort of postmenopausal women. The thinking here is that individuals who have experienced a fracture should be more likely to have geometrically weaker bones, evidenced by higher
stresses and compromised bone geometry. We therefore hypothesized that the number of self-reported fractures would be positively associated with stress and negatively associated with NN BMD, CSA and Z.

Consistent with our hypotheses, we found that postmenopausal women with one or more fractures had significantly higher stress, and lower NN BMD, CSA and Z than women without fracture (Figures 12 and 13). These results remained the same even after adjustment for relevant confounders when appropriate.

Specifically, we explored the confounding effects of age, weight and height in our models. Age is known to be negatively associated with BMD, CSA and Z so we adjusted for age in the geometry models [90, 194]. We did not adjust the stress model for age, as prior work from our group has demonstrated that stresses remain relatively stable with age [166]. It is also important to consider that geometry measurements reflect structural strength, and that strength scales with the size of an individual, as well as with an individual’s level of skeletal usage or loading. A larger stature naturally necessitates larger bones and a lesser stature smaller bones. Disuse or inactivity leads to weaker bones and increased usage or activity leads to stronger bones. To account for size effects we adjusted for weight and height in the bone geometry models. We did not adjust for weight or height in the stress models as these two variables were incorporated into our stress calculations. We did not have lean body mass information which could reflect skeletal usage, so were unable to appropriately adjust for this confounder. A thorough discussion of these confounders will be presented in Section 9.1.5. Collectively, our
results suggest that heterogeneity in modeling responses to skeletal load exist, and may explain differences in fracture prevalence.

Revisiting theoretical concepts, if all individuals responded equally to mechanical stimuli, regardless of body size and physical activity level, their skeletal geometry should be adapted such that prevalent loads would generate equivalent bone strains at habitually loaded skeletal sites. Alternatively, heterogeneity in load response should alter the equilibrium between the normally encountered load and maximum strains that the load induces within the bone. For example, an individual with increased load sensitivity should have geometrically stronger bones so that a given maximal load produces a smaller maximum strain and vice versa. If we assume that traumas are equally distributed throughout the population, those with relatively weaker bones should be more susceptible to fractures, all else equal. Since stresses are proportional to strains, stresses can be used to identify differences in load sensitivity in vivo. Examination of the geometry parameters can aid in the interpretation of potential adaptation mechanisms.

We chose to examine skeletal load response at the infero-medial margin of the femoral neck, in a cross-section where fractures occur but paradoxically in a location in the cross-section where it is strongest and where fractures are not likely to initiate. As discussed in Section 3.1.1, this is a site that is normally loaded under compression, adapted to upright walking [169] and relatively well preserved with age in both men and women [20]. Therefore, if skeletal response to mechanical load were equal in all individuals, we would not expect to see higher stress levels in those who fracture. Since we did observe higher
stress levels in women with fractures, we believe that this is highly suggestive of a deficient load response in these populations.

As aforementioned (Section 3.1.3), data from the cellular and genetic literature also supports heterogeneity in skeletal load response. A study by Klein-Nulend et al. demonstrated that osteocytes harvested from elderly individuals diagnosed with osteoporosis had smaller formation responses to strain stimuli in cell culture than non-osteoporotic individuals [107], suggestive of reduced mechanosensitivity among osteoporotic individuals. While this study does not completely explain why individuals without diagnosed osteoporosis might fracture, importantly the study did not demonstrate an age effect in strain sensitivity. Again this is consistent with our inability to show an age effect on load stress in previous work [166]. Collectively, these data do suggest that heterogeneity in load response may be a genetically inherent difference between individuals who have skeletal fragility and those who do not. Work by Jepsen et al. also suggests that sensitivity to load may be genetically determined and does vary among individuals [24].

Our geometry findings help to provide a more complete picture of how an altered load response condition might be manifested. Our study demonstrated that women with more fractures had smaller indices of resistance to axial and bending forces, cross-sectional areas and section moduli, independent of body size than women without fractures. If we think back to Frost’s Mechanostat Theory [13], this implies a compromised response to load on the outer surface of bone where strains are highest (increased upper threshold),
and a consequential reduced periosteal formation response [64]. These are the geometric changes we would expect in the presence of reduced load sensitivity.

To conclude, Study 1 demonstrates heterogeneity in modeling responses among Canadian postmenopausal women with and without fractures. Women with fractures appear to have reduced sensitivity to load, evidenced by higher levels of stress within the bone and evident in size-independent compromises in underlying bone geometry. Collectively, these findings suggest an important role for modeling in the pathogenesis of bone fragility in postmenopausal women. Compromised modeling has important clinical implications in terms of treatment selection, as individuals with altered load sensitivity may respond best to pharmacologic agents that would improve formation responses to load stimuli. Future directions for this area of research will be discussed in Section 9.2.

8.1.2 Study 2: DIABETES

In cross-sectional studies of older adults, BMD at the hip measured by DXA is consistently elevated in type 2 diabetic patients compared to non-diabetic controls [28]. This finding is surprising given the fact that low BMD is known to be an independent predictor of fracture risk [91, 92]. While this consistent finding has been deemed a paradox, recent data suggests that this so-called paradox might be explained by an impaired skeletal response to load [36, 37]. The purpose of Study 3 was to provide further evidence supporting this intriguing explanation for the T2DM bone density paradox.
Our objective was to determine the association between diabetes status and stress at the femoral neck in a Canadian cohort of women. We hypothesized that stress would be negatively associated with diabetes status. Consistent with this hypothesis, we found that stresses were higher in women with T2DM than in non-diabetics (Figure 14). Since stress is an indicator of underlying geometry, and geometry should be adapted to prevalent loads, higher stress indicates weaker geometry among women with T2DM. One explanation for weaker geometry is an impaired modeling response to load in the diabetic condition. Among other factors, this could explain why fracture risk is increased among women with T2DM despite the consistent finding that BMD tends to be higher in these patients [33, 34].

Support for impaired remodeling has also been documented in two other clinical studies. As aforementioned, a study by Garg and colleagues which examined 5924 women enrolled in the Women’s Health Initiative Observational study, demonstrated that after adjustment for total body lean mass, diabetic women on insulin had significantly lower BMD, cross-sectional area (an index of compressive strength) and section modulus (an index of bending resistance) at the femoral neck than non-diabetics [36]. Lower composite strength indices at the femoral neck were also reported among perimenopausal diabetic women compared to non-diabetic controls in the Study of Women’s Health Across the Nation [37] after adjustment for body mass index as well as several other confounders.
The theory behind an impaired adaptive response to load stems from Frost’s Mechanostat Theory [13]. As described in Section 2.2.2, the Mechanostat postulates that bone adapts to mechanical loads to keep strength at an appropriate level to withstand forces generated by current activity levels. If all individuals responded equally to mechanical stimuli, regardless of body size and physical activity level, their skeletal geometry should be adapted such that prevalent loads generate equivalent tissue stresses. Alternatively, heterogeneity in load response should alter the equilibrium between normally encountered loads and maximum stresses experienced within bone tissue. An individual with decreased sensitivity to load, as we postulate is the case among women with T2DM, should have geometrically weaker bones such that a given maximal load results in a higher maximum stress. The opposite would hold true for those with greater load sensitivity. If we assume that traumas are equally distributed throughout the population, those with relatively weaker bones should therefore be more susceptible to fractures, all else equal. Decreased sensitivity to load may therefore be one explanation for the fragility evident in women with T2DM and is essentially what we confirmed with our findings.

Of importance, an attenuated response to load is also in line with cellular mechanisms thought to contribute to increased fragility in T2DM. Advanced glycation end products (AGEs), which are formed as a result of non-enzymatic reactions between glucose and proteins are known to accumulate in diabetes [119]. AGEs have been shown to inhibit both proliferation and differentiation of osteoblasts [120, 121]. This could directly impair the bone formation response to load and essentially contribute to the weakened skeletal
geometry observed in diabetics. Although unrelated to geometry, the presence of AGEs may also directly alter the material properties of bone collagen, increasing bone brittleness and further impairing strength [124].

Accumulation of AGEs, could also contribute to the low turnover state that has been documented in T2DM, which could also perpetuate a reduced formation response [114]. Studies assessing bone turnover markers in humans have reported lower osteocalcin levels in those with both type 1 and type 2 diabetes mellitus [115-118]. Due to the fact that osteocalcin is the product of mature osteoblasts, this pattern may indicate an effect of diabetes on later maturation of osteoblasts which could also impair the formation response to mechanical load, essentially affecting geometry.

Of further importance is the recognition that increased hip fracture risk among patients with T2DM is undoubtedly multifactorial and likely cannot only be explained by impaired load response alone [175]. It should be noted that diabetic complications such as peripheral neuropathy and vestibular dysfunction may increase risk for falls [176, 197]. Decreased levels of physical activity, common in T2 diabetics may also increase the risk for falls, as well as decrease total body lean mass fraction which is protective for bone [176, 177]. Further renal insufficiency, and lower calcium and vitamin D intake may lead to detrimental skeletal alterations [175].

To summarize, Study 2 demonstrated that femoral neck stresses were higher in women with T2DM compared to non-diabetic women. Higher stress indicates weaker geometry
for a given load and suggests an impaired adaptive response to load may be present in women with T2DM. Among other factors, impaired modeling could contribute to an increased fracture risk in women with T2DM and sheds light on the so-called T2DM and BMD paradox. Further longitudinal analyses are required in larger cohorts, to pin point the geometrical changes that lead to increased stress and the changes evident with progressive diabetes severity. Future directions will be discussed in Section 9.2.

8.1.3 Study 3: ESTROGEN

Our first objective (Objective 3a) was to determine associations between strenuous sports participation and stress at the femoral neck. We hypothesized that strenuous sports participation would be negatively associated with stress indicating a benefit of exercise. Our results supported this hypothesis. We found that femoral neck stresses computed for a body weight load were lower in exercisers than in non-exercisers regardless of menopausal status or estrogen use (Figure 15a,b).

Our second objective (Objective 3b) was to determine associations between estrogen availability and stress at the femoral neck. We hypothesized that premenopausal women would have lower stress than postmenopausal women not currently using estrogen supplements. We further hypothesized that there would be no difference in stress between premenopausal women and postmenopausal women currently using estrogen supplements. Our results fully supported these hypotheses. We found that stresses were significantly higher in postmenopausal women not on estrogen than in premenopausal women, regardless of exercise level. Further, we found that postmenopausal women on
estrogen had stress levels that could not be statistically distinguished from corresponding levels in premenopausal exercisers and non-exercisers (Figure 16a,b). Non-exercising postmenopausal women not taking estrogen (postmenopausal E-) had femoral neck stresses that were significantly higher than all other subgroups, but postmenopausal women (E+/E-) who did exercise, reduced their stresses to levels similar to premenopausal non-exercisers.

We know that exercise should improve body muscularity such that exercisers should have a greater relative proportion of lean mass than non-exercisers and that this should affect skeletal stress (Section 9.1.5). We did not have information on total body lean mass, so we attempted to adjust for this confounding effect by crudely adjusting for BMI (Figures 15b and 16b). Doing so did not change the associations between exercise and stress levels in premenopausal women, but weakened the associations in both postmenopausal estrogen (postmenopausal E+) and non-estrogen users (postmenopausal E+) (Figure 15b). Consistent with the cellular evidence [184] our findings suggest estrogen and exercise have a synergistic effect in premenopausal women which is not apparent in postmenopausal women in our study. This may be due to the fact that exercise levels were apparently greater and in a larger proportion of our population in premenopausal women than we observed after the menopause. Clearly this effect will require further study. We might also find that the synergistic benefits of exercise and estrogen may persist after the menopause if examined in a larger cohort of women with greater statistical power. In line with the latter, the associations between stress, menopausal and estrogen status remained significant even after adjustment for BMI (Figure 16b).
Estrogen clearly plays an important role in mediating skeletal sensitivity to mechanical load, although the role is arguably complex. Our findings from Study 3 are in support of the view that sensitivity to load is enhanced in the presence of estrogen and diminished in its absence. This view has been supported by a variety of cellular evidence [46, 183].

Exercise and estrogen are thought to have a synergistic effect on bone through their effects on estrogen receptor-α. Exercise induces bone formation through activation of estrogen receptor-α, which is also up-regulated by estrogen therapy [184]. Moreover, estrogen receptor-α is a transcription factor for osteoblasts [140], regulating gene expression when activated by estrogen or other ligands. Estrogen receptor-α is also found in osteoclasts and osteocytes and its activation leads to osteoblast proliferation and differentiation [185], promotion of osteocyte survival and osteoclast apoptosis [140]. Estrogen receptor-α number has also been shown to be decreased in osteocytes from bone biopsies of hormone-deficient women [54], suggesting that estrogen receptor-α number, and therefore exercise effects, are dependent on estrogen sufficiency.

Our finding that estrogen restores stresses to premenopausal levels is also in line with findings from several other clinical studies. Data from the Study of Osteoporotic Fractures (SOF) demonstrated that postmenopausal women currently on hormone therapy had mechanically stronger proximal femur geometry than never-treated women [53]. Similar effects have also been shown in clinical trials of women treated with estrogen [56], combined hormone therapy [186] and the selective estrogen receptor modulator (SERM) raloxifene [158].
While our results support the view that estrogen enhances skeletal sensitivity to mechanical load, the alternative paradigm [151] suggests the opposite. The alternative view is based on observations from several studies in rats [59] as well as pooled data from cohort studies [189, 190] based primarily on BMD and BMC observations. The use of a geometry based analysis in our current study should account for the differences in our findings.

There are few studies that provide insight about the skeletal sensitivity to mechanical load in vivo. Overall, our findings are consistent with the theory that skeletal sensitivity to mechanical load is enhanced in the presence of estrogen and diminished in its absence. This is in line with cellular data [46, 54, 183] as well as several other investigations in humans [56, 158, 159, 186, 194-196]. Further prospective studies are required to examine the specific bone geometry adaptations that contribute to the changes in stress that occur in women across the menopause.

8.1.4 Summary and Conclusions

The purpose of this thesis was to explore variations in skeletal adaptation to mechanical load at the hip in vivo. Our research idea was based on Frost’s Mechanostat Theory [13], which suggests that there should be equilibrium between loading forces experienced by bone tissue, and the structural geometry resulting from adaptation.
As aforementioned, systemic (hormones, vitamins, drugs, nutrients) and local (genes, cytokines, ligands, paracrine/autocrine changes) non-mechanical agents [14-16] are thought to work in synergy with the Mechanostat, thereby affecting its sensitivity [13]. Specifically, these agents are postulated to increase or decrease bone’s modeling and remodeling thresholds, such that adaptive changes are either blunted or enhanced accordingly. This process, however, is difficult to observe without directly measuring strain levels with implanted strain gauges in vivo. Instead, we generally infer these relationships mainly via the use of cell culture or animal models.

This thesis was novel because we approached the inability to measure strains by computing maximal stresses at the femoral neck in a one-legged stance. Since stresses are proportional to strains, they can be computed using engineering modeling given knowledge of estimated loads and geometry of the femur. Our conjecture was that differences in load sensitivity would be apparent as differences in maximal load stresses, and that differences in maximal load stresses might explain differences in tissue fragility.

We explored three different clinical scenarios in which we hypothesized that load sensitivity would be attenuated: fracture (Study 1), T2DM (Study 2) and estrogen deficiency (Study 3). The results from our investigations suggested that variations in load sensitivity do exist and are attenuated in each of these conditions. Further, we were able to demonstrate a geometry-based mechanistic explanation to explain increased load stresses. Since stress is an indicator of underlying geometry, and geometry should be
adapted to prevalent loads, higher stress indicates weaker geometry and suggests an impaired modeling response in each of the three conditions studied.

Modeling is one factor that can potentially contribute to fracture risk; however, research to date has given this process little attention. The bulk of research on the causes of osteoporotic fragility has focused primarily on the restoration of balance between formation and resorption in the remodeling process. This is because there is consistent data to suggest that remodeling is imbalanced by aging and osteoporosis [17, 19]. The findings from this thesis provide convincing evidence that modeling may play an equally important role in the pathogenesis of bone fragility as remodeling imbalance. Compromised modeling has important clinical implications in terms of treatment selection, as individuals with reduced load sensitivity may respond best to metabolic agents that would improve modeling responses to load stimuli. Further research should continue to examine the role that altered modeling can play in contributing to skeletal fragility. Section 9.2 will continue a discussion of future directions in this area of research.
CHAPTER 9: LIMITATIONS AND FUTURE DIRECTIONS
9.1 Limitations

There are a number of limitations with this work, many of which were highlighted in the previous three chapters. The following section will elaborate on six of the common limitations across studies including: the use of a cross-sectional study design, the estimation of geometry from HSA, the body weight estimation of load in a one-legged stance, the assessment of physical activity, scaling for body size and skeletal usage and the power analysis.

9.1.1 Cross-sectional study design

One of the limitations of this thesis was the cross-sectional design of each of the three studies. Although CaMos is a national longitudinal study, longitudinal HSA was not available, therefore we were limited to use of the baseline data set and cross-sectional analyses. This thesis assessed a variety of different associations. Study 1 (FRACTURE) determined associations between fracture frequency, age at first fracture and bone geometry and stress at the hip. Study 2 (DIABETES) determined associations between diabetes status, femoral neck BMD and stress at the hip. Study 3 (ESTROGEN) determined associations between strenuous sports participation, estrogen availability, the combination of the two and stress at the hip. Since we examined associations, no causal relationships among any of these variables can be assumed. For example, we cannot conclude that diabetes causes increase stress at the hip, we can only report an association. Prospective studies are required to determine causation, which would be the next step in this program of research. These three studies however, were an important first step toward directing future prospective work.
9.1.2 Estimating geometry from HSA

Another limitation of this thesis was the estimation of geometry parameters using the HSA method. The HSA method makes several assumptions and has inherent limitations within its methodology [35].

DXA scanners were designed to measure BMD, and have excellent precision for doing so. However, they were not designed to measure geometry. Based on data from several multicenter clinical trials, geometry precision is approximately 1.5 to 2 times worse than conventional BMD on the same scans [198]. In practice, this means that changes in geometry must be larger to be statistically detectable.

The proximal end of the femur is irregular in shape and it rotates about the acetabulum which is not located on its long axis. Small changes in femur rotation therefore have a large effect on the projected dimensions from which the geometry is extracted. The technologist performing the scan cannot see the femur to position it and must use palpation of bony features to guess an individual’s degree of anteversion and determine how much to rotate the leg so that the plane of the neck-shaft angle is parallel to the scan table. Even the most seasoned technologist will have trouble consistently positioning dissimilar patients and with the same patient scanned years apart. Because the DXA image is two-dimensional and bone cross-sections are not axially symmetric, improper positioning (rotation) will affect parameters evaluated in the image plane such as CSMI and section modulus. Fortunately, the inconsistencies caused by positioning errors tend to average out in large research studies with many subjects. Since the CaMos cohort is
quite large, even when subdivided for our sub-studies, we can assume that this issue was minimized.

Another limitation of HSA is that DXA scan images are often noisy and blurred making edge margins difficult to locate precisely. Image quality problems tend to be exacerbated with the fastest scan modes as well as in heavier patients. Again, this should be minimized in large cohort studies where scan modes as well as patient size varies.

A more elusive limitation of HSA is that average tissue mineralization is assumed to be that of the average adult. This is a reasonable assumption, as average tissue mineralization does not fluctuate much through adult life. However, in those with reduced tissue mineralization such as children and patients with osteomalacia, HSA will underestimate geometry. In those who have increased tissue mineralization, such as patients on some forms of bisphosphonate therapy [199], geometry will be overestimated. As our sub-studies examined older adults, we did not run the risk of underestimating geometry from reduced tissue mineralization. However, since there were individuals on bisphosphonate therapy, we would expect that in select cases geometry might have been overestimated. This effect should have been minimized overall because of our large sample sizes.

9.1.3 Using body weight to estimate load in a one-legged stance

As previously noted, we really do not know the magnitude of the forces that an individual has experienced that have stimulated adaptation to their current femur geometry. In each
of our three sub-studies, we estimated stresses in a one-legged stance mode where they are maximal and used body weight to estimate load. We are banking on the fact that the forces on the hip should be some multiple of body weight in normal ambulation. That multiple however increases, in active individuals depending on the type and intensity of activities. For instance, during fast walking, jogging and going up and down stairs ground reaction forces have been shown to reach magnitudes of 5-6 times body weight [180-182]. Due to the fact that we could not measure the maximal encountered loads during exercise, we would therefore expect stance mode stresses to appear lower in those who are more active. While this is technically a limitation of our methodology, the divergence still allowed us to use estimated stresses as an indicator of load sensitivity. We expected individuals with reduced sensitivity to have weaker bones evidenced by higher stresses and vice versa.

9.1.4 Physical activity assessment

Another limitation of this thesis was the quantification of exercise level by self-report via questionnaire in Study 3 (Chapter 7). Self-reported physical activity is subject to recall bias, particularly with regard to exercise time and intensity, which are often overestimated [191]. Further, self-reported measures tend to be only modestly correlated with objective measures of exercise, thus they may not be sufficient to detect true bone effects [192, 193].

A better way to classify activity levels in Study 3 would have been to use a measurement technique such as pedometers or accelerometers which provide more objective
estimations of caloric expenditure [94]. Unfortunately, these techniques were not widely used when the CaMos study was designed. Moreover, doing this sort of assessment in the large sample size of the CaMos cohort would have made this task extremely costly.

A secondary limitation with our assessment of physical activity in Study 3 was the inability of the questionnaire to identify activity type, particularly to assess loads experienced at the hip. The questionnaire asked participants about the number of hours spent per week in three broad activity categories: (1) strenuous sports; (2) vigorous work and (3) moderate activity. The definitions for each of these categories was vague and extremely subjective (Section 4.3.3). While the information obtained from the responses to these questions provided a reasonable overview of activity frequency, the type of activity and its consequences in femur loading were lost in this method of classification.

It should be noted that activity type which reflects site-specificity of a given load, is extremely important when assessing the skeletal response to activity. For example, while running and cross-country skiing are comparable in terms of activity intensity [200], running only loads the lower limbs, whereas cross-country skiing loads both upper and lower body segments. Although most types of exercise provide some form of skeletal loading through the hip, it should be noted that specific classification of activity type would have improved our prediction of loads acting at the hip. Furthermore, it should be mentioned that bone is known to respond to activity that is dynamic rather than static, shorter rather than longer in duration, and novel rather than typical [201, 202]. These subtleties were also beyond the scope of the questionnaire used.
9.1.5 Scaling for size and skeletal usage

It is important to consider that geometry measurements reflect structural strength, and that strength scales with the size of an individual, as well as with an individual’s level of skeletal usage or loading. A larger stature naturally necessitates larger bones and a lesser stature smaller bones. Disuse or inactivity leads to weaker bones and increased usage or activity leads to stronger bones. These variations are starkly reflected in geometry measurements.

Although scaling for size and usage is critical, the way to account for it in research studies is not entirely clear. In terms of size effects, geometric measurements at the hip should be adjusted for height, but there is known heterogeneity in the way that bone linear dimensions scale with height. Measured bone lengths would have been preferred but some generalized heterogeneity was addressed by use of different formulas for scaling femur length from height in men and women and in different racial groups. In terms of usage effects, the synergy evident between muscle and bone is key. We know that long bones function as muscle-actuated levers. Since muscles tend to operate with a mechanical disadvantage or as inefficient levers, muscle forces dominate loads to which the skeleton must adapt [170-172]. This effect is apparent in observations that section moduli scale better with lean mass than with body weight [194, 203] and that exercise effects are mostly evident in the section modulus [86, 204]. That said, total body lean mass assessed by DXA is the preferred way to account for usage scaling over body weight.
In all three studies, we adjusted for height in our analyses of geometry parameters to scale for body size. Since we did not have information on muscle force, we were unable to account for differences in muscul arity. Given body lean mass, this could have permitted a more objective muscul arity adjustment since lean mass is mostly muscle. In Study 3, since our focus was to determine exercise effects, in addition to adjusting for weight we further adjusted for BMI as a crude index of lean mass fraction. While these adjustments were not ideal, they served the purpose. Collecting total body lean mass data was not an option in the CaMos study design given its time constraints and budgetary restrictions.

9.1.6 Power Analysis

As discussed earlier, this thesis was comprised of three studies which were sub datasets from a large Canadian national cohort study entitled the Canadian Multicentre Osteoporosis Study. As such, our power was determined based on available data for our questions of interest. The complete CaMos cohort includes 6539 women and was powered appropriately; however, our sub-studies were much smaller and were underpowered to detect associations for some of our comparisons (Tables 2-4).

For Study 1, we were underpowered to detect associations between number of fractures and section modulus (Table 2; p=0.658). In Study 2 we were underpowered to detect associations between stress and diabetes status (Table 3; p=0.593). Since the power of a statistical test is defined as the probability that the test will reject a false null hypothesis
when the null hypothesis is false, or essentially that the statistical test will detect an effect when there is an effect to be detected, power is only a concern if no significant effect is found. We found significant associations in both Studies 1 and 2 so power was not limiting here.

For Study 3, we were underpowered to detect associations between non-exercisers and exercisers in the postmenopausal E+ group (Table 4a; p=0.537). We did not find a significant association between non-exercisers and exercisers in this group in unadjusted models (Figure 15a; p=0.0648) nor in the models adjusted for BMI (Figure 15b; p=0.4460). Since we were underpowered, it is possible that a significant association was present but undetectable. Clinically, we would have expected exercisers in the postmenopausal E+ group to have significantly lower stress than non-exercisers, in line with what we found in the other groupings.

In Study 3, we were also underpowered to detect associations between premenopausal and postmenopausal E+ women in both non-exercise and exercise groupings (Table 4b), and did not find significant associations between groups in the unadjusted models (Table 16a p=0.6102; p=0.1394) nor in the models adjusted for BMI (Table 16b p=0.8067; p=0.2243). Again, because we were underpowered, it was possible that we concluded that no association was present when there may have been one. Had we found significance, this would have slightly modified our clinical interpretation of the findings to state that estrogen therapy partially restores the skeletal response to exercise in postmenopausal women, but not fully.
9.2 Future Directions

This thesis explored Mechanostat sensitivity and geometric alterations at the hip through the presentation of three cross-sectional studies. The following section will review the findings of each study and suggest directions for future work.

Study 1 (FRACTURE) evaluated associations between fracture prevalence, stress and hip geometry (Chapter 5). In this study, we demonstrated that postmenopausal women with more fractures had higher stress and less well adapted femoral neck bone geometry than postmenopausal women with fewer or no fractures. These findings are in line with the theory that women who fracture have reduced mechanosensitivity, a compromised adaptive response to load and increased skeletal fragility. In summary, Study 1 suggested that an altered adaptive response to load is present among individuals who fracture, and points to an important role for modeling in the pathogenesis of bone fragility.

The next logical step in the program of research for Study 1 would be to conduct a prospective cohort study in women examining the same stress and geometry parameters, ideally including lean mass data to better characterize forces on the femur for the stress calculation. A prospective cohort study would allow us to determine whether or not fracture prevalence is caused by altered geometry.

The idea that modeling is impaired in fracture patients has important clinical implications in terms of treatment selection. Individuals with altered mechanosensitivity may respond
best to anabolic agents such as parathyroid hormone or nitrates [205, 206], that would improve response to load stimuli. An interesting study would be one that evaluates the geometric response to anabolic versus anti-resorptive treatment in fracture patients, as well as their combined effects with an exercise intervention.

Study 2 (DIABETES) evaluated associations between diabetes status and stress (Chapter 6). We demonstrated that femoral neck stresses were higher in women with T2DM compared to non-diabetic women. Higher stress indicates weaker geometry for a given load and suggests an impaired adaptive response to load may be present in women with T2DM. Among other factors, impaired modeling could contribute to an increased fracture risk in women with T2DM and sheds light on the so-called T2DM and BMD paradox.

Further prospective studies are required to determine a causal relationship between diabetes and compromised skeletal geometry which would explain altered mechanosensitivity. An interesting prospective study would be one that compares geometry changes in diabetics and non-diabetics across different exercise tertiles. A subsequent study could also evaluate the treatment effects of anabolic agents such as parathyroid hormone or nitrates [205, 206] in diabetic patients, alone or in conjunction with an exercise intervention. Further longitudinal analyses are also required to identify changes in modeling that occur with progressive diabetes severity and insulin-use.
Study 3 (ESTROGEN) evaluated associations between estrogen deficiency, exercise and hip geometry (Chapter 7). We demonstrated that femoral neck stresses were lower in exercisers than in non-exercisers regardless of menopausal status or estrogen use. We also found that stresses were significantly higher in postmenopausal women not on estrogen than in premenopausal women, regardless of exercise level. Further, we found that postmenopausal women on estrogen had stress levels that could not be statistically distinguished from corresponding levels in premenopausal exercisers and non-exercisers. Non-exercising postmenopausal women not taking estrogen had femoral neck stresses that were significantly higher than all other subgroups, but those who did exercise reduced their stresses to levels similar to postmenopausal exercisers taking estrogen.

Further prospective studies are required to examine the causal effects of estrogen and exercise on bone in women across the menopause. Additionally, studies that examine the specific bone geometry adaptations that contribute to the observed changes in stress, would more clearly define the actions of estrogen and exercise on endosteal and periosteal bone surfaces. Finally, exercise classification could be better defined by the use of objective measures of physical activity such as pedometers or accelerometers, or by incorporating an exercise intervention.

To conclude, this thesis provided novel insight into the skeletal response to mechanical load in vivo under three different conditions (fracture, diabetes and estrogen deficiency). Specifically, our findings provided evidence that modeling is impaired in these three conditions which could explain alterations in skeletal fragility. From a clinical
standpoint, compromised modeling has important implications in terms of treatment selection, as individuals with reduced load sensitivity may respond best to metabolic agents that would improve modeling responses to load stimuli. While prospective studies are warranted, this thesis provided an important framework to direct future investigations in this area. Ultimately, further studies examining mechanosensitivity in vivo, will contribute to a better understanding of skeletal fragility, and to the development and implementation of new therapies for the prevention and treatment of OP related fractures.
Table 1. Parameters used in the derivation of medial femoral neck stance stress.

<table>
<thead>
<tr>
<th>Parameter Name</th>
<th>Description</th>
<th>Symbol</th>
<th>Unit</th>
<th>Derivation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neck shaft angle</td>
<td>The angle between the axes of the neck and shaft.</td>
<td>$\alpha$</td>
<td>Radians</td>
<td>Computed in HSA</td>
</tr>
<tr>
<td>Theta</td>
<td>Angle of the resultant muscle force vector with the horizontal.</td>
<td>$\theta$</td>
<td>Radians</td>
<td>Assumed to equal 70°</td>
</tr>
<tr>
<td>Null</td>
<td>Angle of the joint force vector with the horizontal.</td>
<td>$\varnothing$</td>
<td>Radians</td>
<td>Derived</td>
</tr>
<tr>
<td>Beta</td>
<td>Angle of the femoral shaft with the vertical.</td>
<td>$\beta$</td>
<td>Radians</td>
<td>Assumed to equal 10°</td>
</tr>
<tr>
<td>Neck length</td>
<td>Distance from the user defined centre of the femoral head to the intersection of the neck and shaft axes.</td>
<td>NL</td>
<td>Metres</td>
<td>Computed in HSA</td>
</tr>
<tr>
<td>Shaft length</td>
<td></td>
<td>SL</td>
<td>Metres</td>
<td>Computed using a standard forensic formula (2)</td>
</tr>
<tr>
<td>Neck to trochanter length</td>
<td>Neck to trochanter length (assumed to be horizontal).</td>
<td>TL</td>
<td>Metres</td>
<td>Computed in HSA</td>
</tr>
<tr>
<td>Neck distance</td>
<td>Neck to studied cross-section length (at the narrowest point in the neck).</td>
<td>d</td>
<td>Metres</td>
<td>Computed in HSA</td>
</tr>
<tr>
<td>Body weight</td>
<td>Body weight</td>
<td>W</td>
<td>Newtons</td>
<td>Computed from DXA scan</td>
</tr>
</tbody>
</table>
Table 2. Power calculations for Study 1: FRACTURE.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Fracture Group</th>
<th>n</th>
<th>Variable (SD)</th>
<th>Alpha</th>
<th>Power</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean Medial Stance Stress</td>
<td>No fractures</td>
<td>1248</td>
<td>10.30 (2.03)</td>
<td>0.05</td>
<td>0.833</td>
</tr>
<tr>
<td></td>
<td>1 or more fractures</td>
<td>920</td>
<td>10.57 (2.19)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NN BMD</td>
<td>No fractures</td>
<td>1248</td>
<td>0.75 (0.15)</td>
<td>0.05</td>
<td>0.998</td>
</tr>
<tr>
<td></td>
<td>1 or more fractures</td>
<td>920</td>
<td>0.72 (0.14)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NN Cross Sectional Area</td>
<td>No fractures</td>
<td>1248</td>
<td>1.88 (0.37)</td>
<td>0.05</td>
<td>0.995</td>
</tr>
<tr>
<td></td>
<td>1 or more fractures</td>
<td>920</td>
<td>1.81 (0.34)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NN Section Modulus</td>
<td>No fractures</td>
<td>1248</td>
<td>0.91 (0.20)</td>
<td>0.05</td>
<td>0.658</td>
</tr>
<tr>
<td></td>
<td>1 or more fractures</td>
<td>920</td>
<td>0.89 (0.19)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
**Table 3.** Power calculations for Study 2: DIABETES.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Diabetes Status</th>
<th>n</th>
<th>Variable (SD)</th>
<th>Alpha</th>
<th>Power</th>
</tr>
</thead>
<tbody>
<tr>
<td>Medial Stance Stress</td>
<td>No Diabetes</td>
<td>3501</td>
<td>10.57 (2.20)</td>
<td>0.05</td>
<td>0.593</td>
</tr>
<tr>
<td></td>
<td>Diabetes</td>
<td>164</td>
<td>10.98 (2.33)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
**Table 4a.** Power calculations for Study 3: ESTROGEN. Comparisons for non-exercisers vs. exercisers.

<table>
<thead>
<tr>
<th>Menopausal Status</th>
<th>Exercise Category</th>
<th>n</th>
<th>Mean Medial Stance Stress (SD)</th>
<th>Alpha</th>
<th>Power</th>
</tr>
</thead>
<tbody>
<tr>
<td>Premenopausal</td>
<td>Non-Exercisers</td>
<td>428</td>
<td>10.09 (2.01)</td>
<td>0.05</td>
<td>0.982</td>
</tr>
<tr>
<td></td>
<td>Exercisers</td>
<td>142</td>
<td>9.31 (1.97)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Postmenopausal E+</td>
<td>Non-Exercisers</td>
<td>610</td>
<td>10.16 (2.00)</td>
<td>0.05</td>
<td>0.537</td>
</tr>
<tr>
<td></td>
<td>Exercisers</td>
<td>75</td>
<td>9.70 (1.79)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Postmenopausal E-</td>
<td>Non-Exercisers</td>
<td>1118</td>
<td>10.66 (2.14)</td>
<td>0.05</td>
<td>0.857</td>
</tr>
<tr>
<td></td>
<td>Exercisers</td>
<td>74</td>
<td>9.99 (1.80)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 4b. Power calculations for Study 3: ESTROGEN. Comparisons for menopausal status.

<table>
<thead>
<tr>
<th>Exercise Category</th>
<th>Menopausal Status</th>
<th>n</th>
<th>Mean Medial Stance Stress (SD)</th>
<th>Alpha</th>
<th>Power</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-Exercisers</td>
<td>Premenopausal</td>
<td>428</td>
<td>10.09 (2.01)</td>
<td>0.05</td>
<td>0.086</td>
</tr>
<tr>
<td></td>
<td>Postmenopausal E+</td>
<td>610</td>
<td>10.16 (2.00)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Premenopausal</td>
<td>428</td>
<td>10.09 (2.01)</td>
<td>0.05</td>
<td>0.998</td>
</tr>
<tr>
<td></td>
<td>Postmenopausal E-</td>
<td>1118</td>
<td>10.66 (2.14)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Exercisers</td>
<td>Premenopausal</td>
<td>142</td>
<td>9.31 (1.97)</td>
<td>0.05</td>
<td>0.311</td>
</tr>
<tr>
<td></td>
<td>Postmenopausal E+</td>
<td>75</td>
<td>9.70 (1.79)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Premenopausal</td>
<td>142</td>
<td>9.31 (1.97)</td>
<td>0.05</td>
<td>0.717</td>
</tr>
<tr>
<td></td>
<td>Postmenopausal E-</td>
<td>74</td>
<td>9.99 (1.80)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 5. Study 1: FRACTURE. Characteristics of postmenopausal women by fracture group.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Fracture Group</th>
<th>Mean (SD)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No fractures (n=1248)</td>
<td>1 or more fractures (n=920)</td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>65.5 (8.5)</td>
<td>66.9 (8.9)</td>
<td>0.0002</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>68.8 (13.4)</td>
<td>69.5 (13.3)</td>
<td>NS</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>159.3 (6.0)</td>
<td>159.3 (6.5)</td>
<td>NS</td>
</tr>
<tr>
<td>Number of kilocalories expended per week</td>
<td>4202.9 (3165.4)</td>
<td>4151.3 (2986.2)</td>
<td>NS</td>
</tr>
<tr>
<td>Medial femoral neck stance stress (MPa)</td>
<td>10.30 (2.03)</td>
<td>10.57 (2.19)</td>
<td>0.0031</td>
</tr>
<tr>
<td>Adjusted Mean (SE)†</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NN bone mineral density (g/cm²)</td>
<td>0.75 (0.004)</td>
<td>0.72 (0.004)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>NN cross sectional area (cm²)</td>
<td>1.88 (0.009)</td>
<td>1.81 (0.010)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>NN section modulus (cm³)</td>
<td>0.91 (0.005)</td>
<td>0.89 (0.006)</td>
<td>0.0042</td>
</tr>
<tr>
<td>Frequency (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ethnicity</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>1240 (99.4)</td>
<td>920 (100)</td>
<td></td>
</tr>
<tr>
<td>Black</td>
<td>8 (0.6)</td>
<td>0 (0)</td>
<td></td>
</tr>
<tr>
<td>1st fracture trauma type</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Severe trauma</td>
<td>Not applicable</td>
<td>362 (39.3)</td>
<td></td>
</tr>
<tr>
<td>Minimal trauma</td>
<td>Not applicable</td>
<td>549 (59.7)</td>
<td></td>
</tr>
<tr>
<td>Disease related</td>
<td>Not applicable</td>
<td>6 (0.7)</td>
<td></td>
</tr>
<tr>
<td>Unknown</td>
<td>Not applicable</td>
<td>3 (0.3)</td>
<td></td>
</tr>
</tbody>
</table>

Kg=kilograms; cm=centimeters; MPa=megapascals; g=grams; NN=narrow neck.
† adjusted for age, weight and height
Table 6. Study 2: DIABETES. Subject characteristics by group.

<table>
<thead>
<tr>
<th>Diabetes group</th>
<th>No diabetes (n=3501)</th>
<th>Type 2 diabetes (n=164)</th>
<th>Mean (SD)</th>
<th>p value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>61.2 (12.5)</td>
<td>66.8 (11.0)</td>
<td>61.2 (12.5)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>68.3 (13.6)</td>
<td>75.1 (15.5)</td>
<td>68.3 (13.6)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>159.8 (6.4)</td>
<td>159.1 (5.1)</td>
<td>159.8 (6.4)</td>
<td>0.1792</td>
</tr>
<tr>
<td>Body mass index</td>
<td>26.8 (5.0)</td>
<td>29.6 (16.5)</td>
<td>26.8 (5.0)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Medial stance stress (MPa)</td>
<td>10.57 (2.20)</td>
<td>10.98 (2.33)</td>
<td>10.57 (2.20)</td>
<td>0.0194</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Frequency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethnicity</td>
</tr>
<tr>
<td>White</td>
</tr>
<tr>
<td>Black</td>
</tr>
</tbody>
</table>

*Comparison between groups: MPa=megapascals
**Table 7.** Study 3: ESTROGEN. Subject characteristics by group.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Premenopausal (n=570)</th>
<th>Postmenopausal E+ (n=685)</th>
<th>Postmenopausal E- (n=1192)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Non-Exercisers (n=428)</td>
<td>Exercisers (n=142)</td>
<td>Non-Exercisers (n=610)</td>
</tr>
<tr>
<td>Age (years)</td>
<td>44.7 (7.3)</td>
<td>42.1 (8.4)</td>
<td>65.0 (6.9)</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>69.6 (17.0)</td>
<td>66.1 (11.8)</td>
<td>68.1 (12.5)</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>162.2 (6.0)</td>
<td>162.7 (6.0)</td>
<td>159.5 (5.9)</td>
</tr>
<tr>
<td>Body Mass Index (kg/m²)</td>
<td>26.6 (6.3)</td>
<td>25.0 (4.4)</td>
<td>26.8 (4.6)</td>
</tr>
<tr>
<td>Frequency (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hours per week of strenuous sports</td>
<td>0 (428) (100)</td>
<td>610 (100)</td>
<td>1118 (100)</td>
</tr>
<tr>
<td>2-3</td>
<td>92 (64.79)</td>
<td>43 (57.33)</td>
<td>45 (60.81)</td>
</tr>
<tr>
<td>4-6</td>
<td>35 (24.65)</td>
<td>22 (29.33)</td>
<td>21 (28.38)</td>
</tr>
<tr>
<td>7-10</td>
<td>12 (8.45)</td>
<td>9 (12.00)</td>
<td>4 (5.41)</td>
</tr>
<tr>
<td>11-20</td>
<td>3 (2.11)</td>
<td>1 (1.33)</td>
<td>2 (2.70)</td>
</tr>
<tr>
<td>21-30</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>2 (2.70)</td>
</tr>
</tbody>
</table>
FIGURES
Figure 1. Material versus structural behavior: (a) compressive loading of a cylinder of trabecular bone (with length L and cross-sectional area A) results in a deflection $\Delta L$; (b) plot of force versus deflection defines structural behavior because specimen geometry influences the stiffness $AE/L$ and the ultimate load $F_{ult}$; (c) plot of stress versus strain defines material (or tissue-level) behavior because the effects of geometry have been eliminated [62].
Figure 2. Flexural (bending) loading of a beam. The midspan deflection is inversely proportional to flexural rigidity $EI$ [62].

\[ \delta = \frac{F}{EI} \frac{a}{24} \left( 3L^2 - 4a^2 \right) \]

\begin{itemize}
  \item $E =$ ELASTIC MODULUS
  \item $I =$ MOMENT OF INERTIA
  \item $EI =$ FLEXURAL RIGIDITY
\end{itemize}
Figure 3. Stresses associated with flexural loading of beams. Tensile stresses are generated on the convex surface and compressive stresses on the concave surface. The unstressed central region is referred to as the neutral axis [62].
Figure 4. Stresses caused by eccentric axial compressive loading of a slightly curved beam. Because of the eccentricity of the applied load, stresses related to axial compression and bending are combined, resulting in reduced tensile stresses on the convex surface and increased compressive stresses on the concave surface [62].
Figure 5. Combined modeling and remodeling effects on bone strength according to Frost’s Mechanostat theory. The x-axis depicts typical peak bone strains from zero on the left, to fracture strain levels on the right. The locations of the remodeling (MESr), modeling (MESm) and microdamage (MESp) thresholds are also shown. The y-axis describes bone strength, increasing or decreasing about a horizontal axis representing no net gains or losses of strength. The lower dotted line curve suggests how disuse-mode remodeling would remove bone next to marrow when strains stay below the MESr range but otherwise would tend to maintain existing bone and its strength. The upper dashed line curve suggests how modeling drifts would begin to increase bone strength where strains enter or exceed the MESm range. The dashed outlines suggest the combined modeling and remodeling effects on a bone’s strength. Beyond the MESp range microdamage starts to incur and bone fractures at Fx. At the top of the figure, four different strain windows are described: DW=disuse window; AW=adapted window; MOW=mild overload window; and POW=pathologic overload window [13].
**Figure 6.** A feedback loop describing local and systemic non-mechanical agents that work in synergy to the Mechanostat and thereby affect its functioning. The boldface capitals denote the mechanical feedback loop. CNS=central nervous system; PNS=peripheral nervous system; PNE=peripheral nerve endings; MC= muscle contraction forces; MU=mechanical usage; L=local non-mechanical agents; S=systemic non-mechanical agents. The “highways” include any mediators and modulators of strain-dependent signals, plus the threshold ranges that help control the modeling and remodeling bone strength functions. The mechanical feedback loop (m) concerns bone modeling by drifts, while the mechanical feedback loop (r) concerns bone remodeling. Italics signify local and systemic factors that could modulate the mechanically-dedicated message traffic without directly participating in it [13].
Figure 7. Typical strain distribution in a bone cross section (a) and changes in this strain distribution with increased (b) and reduced (c) physical activity [64].
Figure 8. Forces acting on the femur in a one-legged stance. $F_M$ is the resultant force exerted by the hip abductor muscles on the greater trochanter; $F_J$ is the joint reaction force applied by the pelvis on the femur, and $W_1$ is the reaction force at the knee which is equal to the body weight minus the weight of the lower leg ($\approx 8/9$ of Body Weight ($W$)).
Figure 9. Free body diagram of the femur. In (a) forces are shown in the original directions. In (b) forces are resolved in the x-y directions. $\alpha$: Neck- Shaft Angle (NSA); $\theta$: Angle of the resultant muscle force vector with the horizontal; $\Phi$: Angle of the joint force vector with the horizontal; $\beta$: Angle of the femoral shaft with the vertical; $NL$: Neck Length; $SL$: Shaft Length; $TL$: Neck to Trochanter Length (assumed to be horizontal); $d$: Neck to studied Cross-Section Length (at the narrowest point in the neck).
**Figure 10.** Femur internal forces resulting from the external forces $F_M$, $F_J$ and $W_1$ (shown in Figure 8). $M_s$, $P_S$ and $V$ are bending moment, axial load and shear force on the cortical cross-section respectively.
Figure 11. Free-body diagram of the femoral neck. Bending moment = $M_S = F_{J2} \cdot d_x = F_J \sin(\theta - \alpha - \beta + 90^\circ) \cdot d \sin(180^\circ - \alpha - \beta)$ and axial force = $P_S = F_{J1} = F_J \cos(\theta - \alpha - \beta + 90^\circ)$. 
Figure 12. Study 1: FRACTURE. Stress in megapascals by number of fractures in postmenopausal women. Models are unadjusted. *p<0.01
Figure 13. Study 1: FRACTURE. Bone geometry variables by number of fractures in postmenopausal women. Models are adjusted for age, weight and height. BMD=bone mineral density; CSA=cross-sectional area; Z=section modulus. **p<0.0001; *p<0.01.
Figure 14. Study 2: DIABETES. Stress levels in non-diabetic women versus women with T2DM. Models are unadjusted. *p<0.05.
Figure 15a. Study 3: ESTROGEN. Stress levels in exercisers versus non-exercisers by menopausal and estrogen status. Unadjusted means and standard deviations.
Figure 15b. Study 3: ESTROGEN. Stress levels in exercisers versus non-exercisers by menopausal and estrogen status. Means adjusted for body mass index and standard errors.
**Figure 16a.** Study 3: ESTROGEN. Stress levels in premenopausal, postmenopausal E+ and postmenopausal E- women by exercise group. Unadjusted means and standard deviations.
Figure 16b. Study 3: ESTROGEN. Stress levels in premenopausal, postmenopausal E+ and postmenopausal E- women by exercise group. Means adjusted for body mass index and standard errors.
APPENDIX 1: GLOSSARY OF KEY TERMS
<table>
<thead>
<tr>
<th>Term</th>
<th>Abbreviation</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bone Mineral Content</td>
<td>BMC</td>
<td>Mineral content of bone tissue (g)</td>
</tr>
<tr>
<td>Bone Mineral Density</td>
<td>BMD</td>
<td>Density of bone tissue.</td>
</tr>
<tr>
<td><strong>Dual Energy X-Ray Absorptiometry</strong></td>
<td>DXA</td>
<td>Scanning technique that measures BMD typically at the spine, hip, and radius.</td>
</tr>
<tr>
<td>Bone geometry</td>
<td></td>
<td>Bone structural dimensions or size and shape parameters of bone.</td>
</tr>
<tr>
<td>Hip Structure Analysis</td>
<td>HSA</td>
<td>Program used to extract geometric strength information from hip DXA scans.</td>
</tr>
<tr>
<td>Narrow Neck</td>
<td>NN</td>
<td>Narrowest diameter of the femoral neck.</td>
</tr>
<tr>
<td>Narrow Neck BMD</td>
<td>NN BMD</td>
<td>Areal BMD (g/cm²) calculated as: ( \text{NN BMD} = (\text{NN CSA}/\text{NN Width}) \times 1.05 ).</td>
</tr>
<tr>
<td>Narrow Neck Cross Sectional Area</td>
<td>NN CSA</td>
<td>Equivalent to the amount of cortical bone surface area (cm²) in the cross-section after excluding all trabecular and soft tissue spaces. Computed as sum of pixel values in profile * (pixel spacing/1.05).</td>
</tr>
<tr>
<td>Narrow Neck Outer Diameter</td>
<td>NN OD</td>
<td>Outer diameter (cm) of the bone computed as the blur-corrected width of the mass profile.</td>
</tr>
<tr>
<td>Narrow Neck Section Modulus</td>
<td>NN Z</td>
<td>Indicator of bending strength for maximum bending stress in the image plane.</td>
</tr>
<tr>
<td>Strain</td>
<td></td>
<td>Relative tissue deformation.</td>
</tr>
<tr>
<td>Stress</td>
<td></td>
<td>Tissue internal force intensity (force/area).</td>
</tr>
<tr>
<td>Medial Stance Stress</td>
<td>Stress</td>
<td>Measured in megapascals.</td>
</tr>
<tr>
<td>Mechanostat Theory</td>
<td></td>
<td>Theory by Harold M. Frost that describes skeletal adaptation to mechanical load.</td>
</tr>
<tr>
<td>Type 2 Diabetes Mellitus</td>
<td>T2DM</td>
<td>Non-insulin-dependent diabetes or adult onset diabetes. Characterized by insulin resistance.</td>
</tr>
</tbody>
</table>

\( \text{†Mineral density value taken from Martin RB, Burr DB, J Biomechanics 1984;17:195-201. It assumes the density of mineral is } \sim 3 \text{ gm/cm}^2 \text{ and that mineral occupies } \sim 35\% \text{ of fully mineralized bone; thus mineral density } = 0.35 \times 3.0 = 1.05 \)
REFERENCES


[98] Beck TJ. Hip Structural Analysis (HSA) Program (BMD and structural geometry methodology): as used to create NHANES III dataset. 2002.


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