BONE LOSS IN RELATION TO HYPOTHALAMIC ATROPHY IN ALZHEIMER'S DISEASE

Natalia Y. Loskutova
M.D. (Honors), Voronezh State Medical Academy, Voronezh, Russia

Submitted to the graduate degree program in Physical Therapy and Rehabilitation Science and the Graduate Faculty of the University of Kansas in partial fulfillment of the requirements for the degree of Doctor of Philosophy

_____
Jeffrey M. Burns, MD
(Co-chair)

_____
Lisa A. Stehno-Bittel, PT, PhD
(Co-chair)

_____
William M. Brooks, PhD

_____
David K. Johnson, PhD

_____
Patricia M. Kluding, PT, PhD

_____
Barbara P. Lukert, MD

_____
John A. Stanford, PhD

April 7, 2011
_____
Date of Dissertation Defense

The Dissertation Committee for Natalia Y. Loskutova
certifies that this is the approved version of the following dissertation:

BONE LOSS IN RELATION TO HYPOTHALAMIC ATROPHY IN ALZHEIMER'S DISEASE

_____
Jeffrey M. Burns, MD
(Co-chair)

_____
Lisa A. Stehno-Bittel, PT, PhD
(Co-chair)

April 25, 2011
_____
Date approved

ABSTRACT

Epidemiologic projections indicate that the incidence of Alzheimer's disease (AD) will increase dramatically in the coming decades due largely to the demographics of the disease and our aging population. Associated cognitive and physical decline greatly contributes to disability in older adults and places considerable burden on the health system, patients, and caregivers. Bone loss and increased risk of fractures are associated with AD, however little is known about mechanisms of this association. The body of presented work extends the literature on a relationship between bone loss and AD. Overall, the presented work provides initial evidence that accelerated bone loss observed in individuals in the early stages of AD may be partially due to distortion of central regulatory mechanisms by neurodegeneration. This is the first work to demonstrate that hypothalamic atrophy is related to bone loss and this relationship may be mediated by leptin-dependent mechanisms in humans in the early stages of AD.

The work in Chapter 2 assessed bone health in the earliest clinical stages of AD in comparison to non-demented aging and examined the relationship of bone mineral density (BMD) with cognitive performance and brain atrophy, both of which are used as surrogate markers of neurodegeneration. We tested the hypothesis that bone density would be lower in early AD and associated with brain atrophy and cognitive decline. The results of this cross-sectional study supported our hypothesis and found that BMD is reduced in men and women in the earliest clinical stages of AD and associated with brain atrophy and memory decline, suggesting that central mechanisms may contribute to bone loss in early Alzheimer's disease.

AD is associated with pathological changes in the hypothalamus, a key regulatory structure of bone remodeling. The aim of Chapter 3 was to extend previous findings of the association between BMD and neuroimaging markers of neurodegeneration by looking at global

and regional, hypothalamus specifically, measures of brain volume in early AD and non-demented aging. The results demonstrated that in early AD, low BMD was associated with low volume of gray matter in brain structures predominantly affected by AD early in the disease, including the hypothalamus, cingulate, and parahippocampal gyri and in the left superior temporal gyrus and left inferior parietal cortex. No relationship between BMD and regional gray matter volume was found in non-demented controls. These results suggest that central mechanisms of bone remodeling may be disrupted by neurodegeneration.

There is very limited guidance in the literature regarding useful and reliable techniques for studying hypothalamic anatomy using neuroimaging. In Chapter 4, we compared an automated neuroimaging technique – voxel-based morphometry (VBM) – to a "gold standard" manual method assessing volumetry of the hypothalamus. The atlas-based VBM volumetry showed promise as a useful tool for regional volumetry of the hypothalamus and has advantages over manual tracing as it is currently used. The results of this study provided guidance for method selection in future work.

In Chapter 5, we further examined the hypothesis that AD may influence bone density in cortical skeletal sites directly through atrophy of the hypothalamus, the major central regulatory structure of bone remodeling. We previously reported in cross-section that BMD was lower in those with early AD and suggested that brain atrophy, specifically of the hypothalamus, was associated with lower BMD in AD. We now examined if similar results were apparent in a two year longitudinal study to extend our previous finding of an association between hypothalamic atrophy and bone density. We also explore predictors of bone loss in AD and healthy aging. Our results demonstrate that bone loss may be accelerated in AD compared with non-demented controls. For AD participants, bone loss was associated with hypothalamic atrophy over two

years. Additionally, bone loss was associated with baseline levels of the vitamin D. For non-demented participants, bone loss was associated with age, female gender and decline in physical activity. Different predictors of bone loss may suggest that mechanisms of bone loss may differ in aging and AD and that neurodegeneration may contribute to bone loss in early AD. These results extend and strengthen the cross-sectional observations in Chapters 2&3.

The purpose of the work presented in Chapter 6 was to further extend previous observations by assessing the roles of leptin, growth hormone (GH) and insulin-like growth factor-1 (IGF-1) , two important regulators of hypothalamic control of bone remodeling, in mediating relationship between hypothalamic structural changes and bone loss in AD. We used a hypothetical model with statistical structural equation or path modeling to examine if leptin, GH, and IGF-I may mediate the relationship between hypothalamic structural changes. The model demonstrated that hypothalamic atrophy had a direct relationship with bone loss. There was no apparent association between baseline IGF-1 and leptin with bone loss but we observed changes in both leptin and IGF-1 over two years that were associated with hypothalamic atrophy. Leptin increased over two years in AD and increase in leptin was associated with hypothalamic atrophy. On the other hand, IGF-1 declined over two year and this decrease was associated with increase in leptin. These results suggest that it is conceivable that central regulatory mechanisms of bone mass may be disturbed by neurodegeneration leading to bone loss in participants in the early stages of AD.

In summary, this body of work demonstrates that bone density is reduced in women and men with early stages of AD and continues to decline over time, exceeding bone loss in non-demented older adults. While the causes of bone loss in AD remain unclear, the observed association of hypothalamic atrophy with bone loss suggests neurodegeneration may play a role

in bone loss observed in AD and highlights a need for further studies. This work also corroborates other studies on the importance of vitamin D and physical activity for bone health. The findings of this body of work are important because evidence that bone loss in AD is associated with the atrophy in regions involved in the central regulation of bone mass may be relevant to therapeutic strategies to prevent or treat bone loss in AD and neurodegenerative diseases.

Acknowledgments

I would like to first and foremost thank my parents, who as I have recently learned should be called "tiger parents." Mom and Dad, thank you for pushing me outside of my comfort zone, allowing me to grow up strong. Thank you for believing in my strength by teaching me responsibility. Thank you for providing comfort and support, a place to heal and share joy in your home, always.

I would like to thank my mentor, Dr. Jeff Burns for his three years of mentoring. I am very lucky to be able to learn from Dr. Burns and to be a part of his team. Thank you for providing a care-free and open-to ideas environment with just right proportions of independence and guidance.

I would also like to thank my committee members Dr. Lisa Stehno-Bittel, Dr. Bill Brooks, Dr. David Johnson, Dr. Patty Kluding, Dr. Barbara Lukert and Dr. John Stanford for their dedication, time and expertise. I would especially like to thank Dr. Brooks for the opportunity to disagree and argue and Dr. Lisa Stehno-Bittel for serving as my co-mentor.

I must also thank Dr. Irina Smirnova who suggested the Physical Therapy and Rehabilitation Science Department for my consideration and guided and supported me throughout my years in the program.

I would also thank the Physical Therapy and Rehabilitation Science Department, the faculty and students. It has been an incredibly enjoyable and wonderful learning experience.
I must thank everyone in the Alzheimer's and Memory Program for their support. I must especially thank Dr. Eric Vidoni for patiently and most elegantly handling every "Eric, I have a question…" situation.

This research would not be possible without dedication and support of all the study participants and support from the National Institutes of Health, University of Kansas Endowment Association and the University of Kansas. I especially would like to thank Dr. Nudo and internal advisory committee of the T32 Training Program in Neurological Rehabilitation for supporting my education and research.

Finally, my husband, Anatoly, and my daughter, Veronica, thank you! There are no words in both languages we speak to express how much I love you and treasure your company and support.

Table of Contents

List of Tables and Figures

Chapter 2

Chapter 3

Chapter 4

Chapter 5

Chapter 6

“One falling leaf is not just one leaf; it means the whole autumn”

D. T. Suzuki

Chapter 1

Introduction

1.1 Alzheimer's Disease

The prevalence of Alzheimer's disease (AD) is rising dramatically as the population ages and is now among the leading causes of death in older people (2008). AD is a progressive disorder that is characterized by accumulation of neurotoxic beta-amyloid protein (Aβ) plaques and neurofibrillary tangles in the brain and neurodegeneration. Plaques and tangles form predominantly in the entorhinal cortex, hippocampus, hypothalamus, basal forebrain and amygdala. Although the causal role of plaques and tangles in neuronal death and brain atrophy remains a matter of immense debate, the pathologic diagnosis of AD is associated with high tangle pathology (Braak stage) and high total percentage area of plaques in the hippocampus (Burton, Barber et al. 2009). Latter pathological changes in AD are associated with atrophy of medial temporal lobe and particularly the hippocampus (Jack, Dickson et al. 2002). Brain atrophy, a sensitive marker of neurodegeneration (Thompson, Hayashi et al. 2003), in AD progresses in a relatively stereotypical fashion, beginning in the limbic system (i.e., the posterior cingulate and the hippocampus), progressing to the temporal-parietal cortices followed by the frontal lobes and late in the disease the occipital lobe and sensorimotor cortices (Thompson, Hayashi et al. 2007).

Clinical manifestations of AD include memory loss, impaired ability to mentally manipulate visual information, language deterioration, poor judgment, confusion, restlessness, and mood swings. While cognitive decline is a clinical hallmark of AD, changes in physical health are also apparent during the course of AD and include increasing frailty, lower aerobic capacity, weight loss and gait and motor dysfunction (Kluger, Gianutsos et al. 1997; Barrett-Connor, Edelstein et al. 1998; Camarda, Camarda et al. 2007; Burns, Cronk et al. 2008). Eventually AD destroys cognition, personality, physical health and ability to function, greatly

contributes to disability in older adults and places considerable burden on the health system, patients, and caregivers.

1.2 Alzheimer's Disease and Bone Loss

Bone health is an important issue in aging and AD. Osteoporosis–related fractures are among the major health and socioeconomic concerns in aging. In the USA, 1.5-2 million fractures, including 325,000 hip fractures, occur annually with an estimated direct cost of $20 billion/year and predicted doubling in number of hip fractures within 30 years. Hip fractures in older adults result in greater morbidity, disability and premature death (18-33% of hip fracture patients die within a year). Patients with AD have two to three times higher incidence of fractures and are more likely to fall compared to cognitively healthy older adults. Importantly, the relationship between fractures and dementia is independent of falling (Weller and Schatzker 2004). Additionally, individuals with AD have poorer recovery (Buchner and Larson 1987; Weller 2000; Weller and Schatzker 2004) and higher mortality rates (van Dortmont, Douw et al. 2000) after suffering a hip fracture. For example, survival at 6 months after a hip fracture is up to 4 times lower in those with dementia (Nightingale, Holmes et al. 2001).

Bone mineral density (BMD) is a widely used proxy measure of bone health in clinical practice and research. BMD, an estimate of bone strength, accounts for 60-70% of bone strength. Regional (femoral neck, lumbar spine, Ward's triangle etc.) as well as total body BMD (Schott, Cormier et al. 1998) measures are strong predictors of bone fractures, and a determining factor in clinical diagnosis of osteoporosis (Ammann and Rizzoli 2003). Several studies in women suggest that low BMD is associated with poorer cognitive function and subsequent cognitive decline (Yaffe, Browner et al. 1999; Lui, Stone et al. 2003) and two times higher risk of

developing AD (Tan, Seshadri et al. 2005), suggesting that low BMD can be viewed as a risk factor for AD. The most recently published population study found that lower BMD and increased rate of bone loss were associated with increased risk of AD in both women and men (Zhou, Deng et al. 2010). However, there is little data on bone health in individuals with AD. In a study of women, BMD was reduced in AD compared to cognitively normal females of similar age, with decreasing BMD in the most severely demented (Sato, Honda et al. 2005). This evidence suggests that bone loss may be accelerated in AD and BMD may be predictive of AD progression. Despite the evident association between bone loss and AD, bone health in AD remains understudied and the mechanisms underlying the association between bone loss and AD are not well understood.

1.3. Bone Metabolism and Regulation of Bone Remodeling

Two distinct types of bone, cortical and trabecular, comprise the human skeleton and fulfill its functions. Cortical bone consists of concentric layers of mineralized collagen (matrix) with embedded osteocytes. Cortical bone comprises 80% of the human skeleton and forms shafts of long bones such as humerus, femur and tibia, and the surface of flat bones. Trabecular bone contains interconnecting trabeculae aligned along lines of stress and bone marrow filling out the spaces. Trabecular bone forms the ends of long bones and the inner parts of flat bones, such as pelvis, ribs, and vertebrae. Bone is a highly dynamic, metabolically active and responsive tissue. These qualities are attributed to actions of bone cells. Osteocytes “sense” molecular and mechanical signals and modulate mineral homeostasis and bone metabolism (or remodeling). Bone remodeling is maintained through a balance and tight coupling between bone resorption by

osteoclasts and bone formation by osteoblasts. In adulthood, bone remodeling is a continuous process of bone tissue turnover that occurs throughout the lifespan.

Multiple factors regulate or mediate this servo system and perturbations of this system can result in bone loss. The most important and well-studied major modulators of bone health are calcium, sex steroids, mechanical usage and vitamin D availability. Sex hormone levels are regulated by the hypothalamic-pituitary-gonadal (HPG) system and reproductive agenda. Withdrawal of estrogen leads to bone loss. Mechanical usage during physical activity translates to strain in the bone matrix and modulates bone remodeling. Although the molecular mechanisms of mechanical load and exercise are not defined, a reduction in bone strain due to immobilization can lead to severe bone loss, while strenuous physical activity can increase bone mass (Fredericson, Chew et al. 2007). In addition to major modulators, multiple local and systemic factors modulate bone health. Systemic growth factors and cytokines, such as insulin-like growth factor (IGF- 1) and tumor necrosis factor α (TNF-α) also regulate bone remodeling. Bone cells are able to release certain cytokines, growth factors, gap junction proteins and transcriptional factors that regulate bone formation and resorption. In addition to local regulatory function, bone-derived factors that regulate systemic metabolism have been identified. For example, osteocalcin, a hormone-like peptide that is produced by osteoblasts, is found to be involved in energy metabolism (Confavreux, Levine et al. 2009; Yadav, Oury et al. 2009). Octeocalcin regulates energy metabolism by positive effects on pancreatic β-cells proliferation, insulin secretion and sensitivity (Lee, Sowa et al. 2007).

1.3.1 Vitamin D is mainly synthesized in the skin in an inactive pre- D_3 form and is partially obtained from dietary origin. To be biologically active, D_3 has to be first converted to 25 hydroxy

vitamin D (25 OHD) by the liver-derived enzymes. 25 OHD is a reliable indicator of vitamin D status. The main function of its fully active form, 1, 25 $(OH)_2$, is to maintain plasma calcium and phosphorus levels at physiological levels by regulating intestinal calcium absorption, calcium storage via skeletal mineralization, and calcium renal reabsorption. The mechanism of action of the active 1, 25 $(OH)_2$ form is similar to other steroid hormones. Besides bone, vitamin D receptors (VDR) have been found in the pancreatic β-cells, various precursor cells, skin, thymus and brain, including hypothalamus (Eyles, Smith et al. 2005). Calcium deficiency because of poor diet, malabsorption or other pathology leads to bone loss via osteoclast-mediated bone resorption stimulated by vitamin D in the presence of PTH. Hyperparathyroidism and vitamin D deficiency can cause bone loss.

1.3.2 ***Central Regulation of Bone Remodeling****:* Superimposed upon local and systemic factors (Zaidi 2007), there is now clear evidence that the CNS directly regulates bone remodeling through the actions of the hypothalamus through two distinct pathways, the *neural* and *neurohumoral arms* (Harada and Rodan 2003; Takeda 2005).

The neural arm involves hypothalamic control of bone remodeling through sympathetic nervous system (SNS) output mediated by *leptin*. The SNS output from the leptinergic/peptidergic neurons in the ventromedial hypothalamus directly regulates bone remodeling through activation of β -2 adreno-receptors (β2AR) on the osteoblasts, resulting in reduced bone formation (Takeda and Karsenty 2008) and increased bone resorption secondary to the stimulation of pro-resorptive mechanisms (Elefteriou, Ahn et al. 2005). Recent evidence suggests that additional mediators of leptin's effects on bone may play a role in this mechanism. These include serotonin, CART (cocaine amphetamine regulated transcript), Neuromedin U and

neuropeptide Y. Leptin regulates production of these mediators in CNS including the hypothalamus. (Elefteriou, Ahn et al. 2005; Singh, Elefteriou et al. 2008; Yadav, Oury et al. 2009). There is evidence that sympathetic output from the hypothalamus via the neural arm has greater effects on regulation of bone remodeling than the actions of sex steroids (Elefteriou, Takeda et al. 2004; Pogoda, Egermann et al. 2006) or mechanical use (Martin, David et al. 2008).

Leptin, a cytokine-like 16-kDa protein that is synthesized mostly by white adipose tissue, is a key mediator of neural control of bone remodeling through the *neural arm*. Leptin appears to regulate a number of physiological functions such as feeding, fat storage and utilization, energy metabolism, and bone remodeling. The main effects are exerted by leptin acting in the brain. There is no evidence that leptin is produced in the brain (Elefteriou, Takeda et al. 2004); it enters the brain from the periphery in a unidirectional fashion (Takeda, Elefteriou et al. 2002). The actions of leptin are executed through binding of leptin to its receptor (Ob-R) that belongs to the family of class 1 cytokine receptors and exists in at least six isoforms. The Ob-R is present in the arcuate, dorsomedial and ventromedial hypothalamic nuclei, hippocampus, cortex and cerebellum (Harvey 2007) and many peripheral tissues. The role of leptin as a key regulator of bone remodeling is established in a series of animal experiments (Ducy, Amling et al. 2000; Patel and Elefteriou 2007). Central leptin acts on leptinergic/peptidergic neurons (different from the neuronal network involved in feeding) in the ventromedial hypothalamus. Through activation of SNS and β2AR on the osteoblasts, central leptin inhibits bone formation (Ducy, Amling et al. 2000; Sato, Takeda et al. 2001; Takeda and Karsenty 2008) and increases bone resorption secondary to the stimulation of pro-resorptive mechanisms (Elefteriou, Ahn et al. 2005).

Negative effects of high levels of both peripheral and central leptin on mostly cortical

bone (Martin, David et al. 2008) are consistently reported in animal models (Guidobono, Pagani et al. 2006; Martin, David et al. 2007). Additionally, high levels of central leptin dramatically decrease circulating IGF-1 and stimulate SNS in animals (Martin, David et al. 2008). The central hypothalamic relay is confirmed as the major regulatory mechanism of bone remodeling with peripheral levels of leptin modifying its activity (Elefteriou, Takeda et al. 2004). Importantly, observations in lipodystrophic mice that display a high bone mass phenotype and patients with congenital lipodystrophy that display low to undetectable levels of leptin and premature bone formation suggest that leptin's regulation of bone remodeling is preserved in humans (Elefteriou, Takeda et al. 2004). The role of leptin in bone remodeling in humans, however, needs to be further clarified. Current reports on association between leptin levels and BMD in humans are highly contradictory (Cock and Auwerx 2003) and range from negative (Oh, Lee et al. 2005; Maimoun, Coste et al. 2008) or no association (Di Carlo, Tommaselli et al. 2007; Peng, Xie et al. 2008) to positive relationship (Zoico, Zamboni et al. 2008; Oguz, Tapisiz et al. 2009), suggesting a clear need for further study.

Sympathetic nervous system (SNS) mediates the central leptin-dependent hypothalamic control of bone remodeling via β2AR in osteoblasts (*neural arm*). This mechanism was confirmed by data from various genetic animal model studies, discovery of β-2 adrenergic (but not β-1 or β-3) receptors on osteoblasts (Takeda 2005), and animal and human studies on β -blockers and adrenergic agonists. Animal studies demonstrated, for example, that: 1) leptin-deficient mice (*ob/ob*) have low sympathetic tone accompanied by high bone mass; 2) both stimulation of ventromedial hypothalamic neurons and central leptin infusions stimulate the SNS; 3) β2AR deficient mice have increased bone mass, bone formation rates, and numbers of osteoblasts and are resistant to central leptin infusions (Elefteriou, Ahn et al. 2005; Takeda and

Karsenty 2008). In line with these observations, use of β -blockers in mice and rats led to increased bone mass (Minkowitz, Boskey et al. 1991; Takeda, Elefteriou et al. 2002), while use of adrenergic agonists led to bone loss (Bonnet, Benhamou et al. 2005). Human studies have demonstrated a reduced fracture risk and increased BMD in β -blocker users (Turker, Karatosun et al. 2006; Wiens, Etminan et al. 2006; Meisinger, Heier et al. 2007). The SNS pathway is the only identified downstream signaling pathway for central leptin function in bone remodeling. Therefore, it is plausible that AD-related neurodegeneration, in particular affecting the hypothalamus, may disrupt central mechanisms regulating bone mass and contribute to bone loss in AD.

The neurohumoral arm involves hypothalamic control of the anterior pituitary hormones, such as thyroid stimulating hormone (TSH), follicle-stimulating hormone (FSH) and growth hormone (GH). TSH and FSH mediate bone remodeling directly through their receptors in bone cells and additionally, through secretion of thyroxin and sex hormones from target endocrine organs. GH and IGF-1 are anabolic hormones necessary in bone development and maintenance of bone mass in adulthood (Giustina, Mazziotti et al. 2008).

Growth hormone (GH) or somatotropin is a single-chain 191-amino-acid protein secreted by the anterior pituitary. Its secretion is primarily controlled by the hypothalamus. Hypothalamic GH-releasing hormone (GHRH) and somatotropin release-inhibiting factor (SRIF, somatostatin) directly regulate GH secretion. GH executes its anabolic action on bone mainly through IGF-1.

Insulin-like growth factor 1 (IGF-1) is a 70-amino acid hormone secreted by a wide range of tissues. The majority of IGF-1 is secreted by the liver under the control of GH. IGF-1 plays an important role in the stimulation of osteoblastic function, bone matrix collagen synthesis and bone formation. IGF-1 exerts a direct inhibitory effect on GH via long-loop feedback (Gahete,

Duran-Prado et al. 2009). Both GH and IGF-1 stimulate trabecular and cortical bone formation, however with greater effects on cortical bone (Murray, Adams et al. 2006).

In summary, while the central hypothalamic control of bone remodeling is established and well studied in animals, bone remodeling in conditions that directly affect brain in humans, such as neurodegeneration in AD, has not been investigated.

1. 4 The Hypothalamus

The hypothalamus, a part of the limbic system, has extensive connections within the brain and modulates a variety of regulatory processes including appetite, energy expenditure, sleep and wakefulness and stress responses, all of which are disturbed in AD (Chouinard, Lavigne et al. 1998; Volicer, Harper et al. 2001). Additionally, the hypothalamus plays a role in memory through connections with the hippocampal formation. Recent findings have confirmed that the hypothalamus is the main regulatory center in the central control of bone remodeling (Patel and Elefteriou 2007). However, bone remodeling in conditions that directly affect the hypothalamus is not well studied. AD is characterized by progressive brain atrophy that begins in the limbic system (Thompson, Hayashi et al. 2007). Evidence of AD-related limbic atrophy in the hypothalamus, however, is limited and to our knowledge has been reported in one cross-sectional study using manual tracing of the structure (Callen, Black et al. 2001). Measuring longitudinal changes in the hypothalamic volume in relation to function, such as maintaining bone mass, in patients with AD and non-demented elderly may provide evidence that hypothalamic atrophy may be one of the mechanisms of bone loss in AD.

1.5 The Hypothalamus: Changes in AD

Pathological changes occur in the hypothalamus in AD and include profound neuronal loss (de Lacalle, Iraizoz et al. 1993), abundant AD plaques and tau tangles (Standaert, Lee et al. 1991) and overall volume loss (Callen, Black et al. 2001). AD is associated with clinical symptoms referable to hypothalamic dysfunction including sleep and circadian rhythm disturbances(Wu and Swaab 2005), alterations in appetite and energy expenditure, and body wasting (Deshmukh and Deshmukh 1990; Ferrari, Arcaini et al. 2000). There is abundant evidence of AD-related dysfunction in both the neurohumoral and neural arms. Dysfunction of the hypothalamo-pituitary axis and activity of the SNS are reported in AD. For instance, GH and IGF-I levels (Alvarez, Cacabelos et al. 2007; Gomez 2008) are reduced in AD while interestingly a number of other hypothalamic factors are increased in AD, including corticotropin-releasing hormone (CRH)(Raadsheer, van Heerikhuize et al. 1995), cortisol (Peskind, Wilkinson et al. 2001; Rasmuson, Andrew et al. 2001), FSH (Bowen, Isley et al. 2000; Meethal, Smith et al. 2005), and TSH (de Jong, Masaki et al. 2007). Additionally, several studies demonstrate evidence of increased sympathetic activity (Pascualy, Petrie et al. 2000) including increased brain noradrenergic activity and elevated serum and cerebrospinal fluid levels of norepinephrine (Raskind, Peskind et al. 1984; Elrod, Peskind et al. 1997; Raskind, Peskind et al. 1999). Why increased activity has been observed in some aspects of the hypothalamic-pituitary axis in AD remains unclear but may be related to disturbed negative feedback (Elgh, Lindqvist Astot et al. 2006) while increased SNS activity may be related to overcompensation of remaining noradrenergic neurons in response to profound noradrenergic neuronal loss (Szot, Leverenz et al. 2000). New evidence suggests that leptin plays a role in AD pathology (Fewlass, Noboa et al. 2004) and is associated with cognitive function (Gunstad, Spitznagel et al. 2008; Holden,

Lindquist et al. 2008) and brain atrophy (Narita, Kosaka et al. 2008). Converging animal, clinical, pathological and neuroimaging data suggest that it is plausible that AD-related neurodegeneration, in particular affecting the hypothalamus, may disrupt central mechanisms regulating bone mass and contribute to bone loss in early AD.

1.6 Neuroimaging

Magnetic resonance imaging (MRI) based volumetric studies are increasingly being employed as surrogate outcome measures of disease. For example, hippocampal volume loss is considered a valid biomarker of AD neuropathology. Other sensitive markers of brain aging include whole brain volume as a marker of brain atrophy. The clinical importance of these measures is becoming increasingly clear given their association with reduced cognition, increased dementia risk, and strong relationships with age (Masdeu and Pascual 2008). The development of sensitive neuroimaging markers of brain aging and disease is a significant advance in understanding the mechanisms of brain aging and a foundation for future investigations of mechanisms of cognitive and centrally regulated physical function decline. High-resolution magnetic resonance imaging allows an in vivo assessment of structural brain changes. MRI, with employment of modern neuroimaging analysis tools, has the potential to capture structural changes in the hypothalamus.

Voxel-based morphometry (VBM) has gained substantial popularity due to its unique quality of utilizing digital information available for each voxel and transforming it to estimation of grey and white matter volumes. VBM is a powerful technique for systematic evaluation of the whole brain and statistical analysis that also allows measuring volumes of grey and white matter in semi-automated region-of-interest (ROI) approach. Optimized VBM with the Wake Forest

University Pickatlas (WFU) toolbox also automatically generates segmented atlas ROI templates and summarizes all the voxel values into grey or white matter volumes within each ROI (Good, Johnsrude et al. 2001). The advantage of VBM as an automated technique is that it provides a perfectly reproducible result on a given data set.

1.7 Potential Mechanisms for Relationship between Bone Mass and AD

Multiple factors have been postulated to explain the association between bone loss and cognitive decline, including estrogen exposure, apolipoprotein E4, depression and lifestyle factors such as physical activity, nutritional, dietary, and environmental factors. Overarching, however, is hypothalamic control over many of the factors that have been identified to modulate bone health. AD is associated with extensive hypothalamic structural damage and hypothalamic dysfunction, thus, it is plausible that hypothalamic damage could result in clinically evident bone loss, given the important role the hypothalamus plays in regulating bone mass. Additionally, AD exacerbates age related processes such as physical activity decline, hormonal changes and energy expenditure all found to contribute to bone loss. Therefore, the neurodegeneration and profound brain and hypothalamic atrophy may directly disrupt central neuronal network involved in regulation of bone remodeling and indirectly contribute to bone loss via negative effects on other hypothalamic functions (energy expenditure, gonadal function, PTH, vitamin D and Ca metabolism).

1.8 Significance of Present Work

Despite the fact that bone loss has been long associated with AD no studies have systematically assessed bone loss in AD and to date there has been no specific hypothesis that

comprehensively explains association of bone loss with AD. *The main hypothesis of this study is that neurodegeneration disrupts neural and neurohumoral mechanisms of central control of bone remodeling leading to bone loss secondary to a neurodegenerative disease, such as AD.* The fact that the hypothalamus, a key regulatory region in the central control of bone remodeling, is affected by AD pathology has been previously overlooked in attempts to explain the association between AD and bone loss. Thus, in this work we systematically evaluated bone health in early AD and used a novel assessment of neurodegeneration and atrophy of the brain and hypothalamus as one of the mechanisms of bone loss secondary to neurodegeneration. Additionally, bone loss was investigated in the association with progression of AD and brain atrophy. The results of the study contribute to a significant question on whether BMD and bone loss can be used as a predictor of AD progression.

Moreover, we are the first to test a theoretical model of mechanisms of bone loss in neurodegeneration in humans that brings together the hypothalamic neural and neurohumoral regulators of bone remodeling, clinical outcome measures of bone loss, neuroimaging markers of hypothalamic atrophy in a context of shared modifiable risk factors for AD and osteoporosis. Understanding the physiological mechanisms underlying bone loss and the role of neurodegeneration is relevant to developing effective preventive strategies and therapeutic interventions for bone loss in aging population affected by AD and other neurodegenerative diseases.

1.9 Specific Aims and Statement of Hypotheses

The main purpose of this project was to study effects of neurodegeneration (measured by neuroimaging markers) on central control of bone mass as a potential mechanism underlying the association between bone loss and AD. Three central aims directed this research.

Specific Aim 1: Characterize bone loss over two years in early AD.

Despite the fact that bone loss has been long associated with AD, no studies systematically assessed bone loss in AD and to date there has been no specific hypothesis that comprehensively explains association of bone loss with AD. Based on the evidence that, in addition to age (McCalden, McGeough et al. 1993), activation of central neural regulatory mechanisms has greater negative effects on cortical bone (Martin, David et al. 2008), we hypothesized that if systematically assessed, rates of bone loss at the skeletal sites of cortical bone, such as total body and legs, in AD would exceed bone loss in the control group. Bone loss at trabecular skeletal sites, such as the spine or pelvis, would be comparable between the groups. In the Chapter 2 we demonstrate that in a cross-sectional study low total body BMD (80% cortical bone) was observed in both men and women with AD. In a longitudinal study (Chapter 5), we demonstrate that not only was BMD lower at the baseline but the rates of bone loss were higher in AD at the skeletal sites of cortical bone (total body and legs), but not at the trabecular skeletal sites (the spine or pelvis).

Specific Aim 2: Assess the relationship of bone mass with neuroimaging markers of neurodegeneration including hypothalamic atrophy in a context of shared modifiable risk factors for AD and osteoporosis.

The evidence that AD is associated with brain pathology in the key regulatory regions in the brain including the hypothalamus has been previously overlooked in attempts to explain the association between AD and bone loss. We hypothesized that it is biologically plausible to see an association between bone loss and neuroimaging markers of neurodegeneration, including

hypothalamic atrophy. In the Chapters 3 and 4, we demonstrate that indeed, participants with AD had smaller hypothalamic volumes, suggestive of hypothalamic atrophy. Lower BMD was associated with lower total brain and hypothalamic volumes in early AD (Chapter 2&3) but not in controls. In the longitudinal assessment (Chapter 5), age, female gender and decline in physical activity were associated with bone loss in non-demented older group, while bone loss in AD was associated with vitamin D deficiency and atrophy of the hypothalamus.

AIM 3: Determine the relationship of bone mass with neural (leptin) and neurohumoral (growth hormone and IGF-1) mediators of hypothalamic control of bone remodeling and whether these relationships change in neurodegeneration.

Central nervous system (CNS) directly regulates bone remodeling through the actions of the hypothalamus via two distinct pathways, the neurohumoral (through the neurohormones) and direct neural (through SNS mediated by leptin) arms (Takeda 2005). Therefore, we hypothesized that accelerated bone loss will be associated with changes in mediators of hypothalamic control of bone remodeling driven by the hypothalamic volume loss in AD. In Chapter 6, hypothalamic atrophy and changes in levels of key hypothalamic mediators of bone metabolism were assessed in relation to bone loss using causal modeling statistical analysis. The model demonstrated that hypothalamic atrophy had a direct and a small indirect effect on bone loss. Hypothalamic atrophy was associated with increase in leptin in AD. Increase in leptin was associated with a decline in IGF-1 levels over two years in individuals with AD.

Five manuscripts based on the work presented in this dissertation have been published (2), submitted (1) or will be submitted for publication (2). The first manuscript was based on a

cross-sectional data with an aim of examining the bone density in early AD and its relationships with neuromaging and clinical marker of AD and comparing to non-demented controls (Chapter 2; published in the *Journal of Alzheimer's Disease*). The second manuscript utilized a smaller sub-sample of the data and looked at specific relationships between volumes of gray matter including the hypothalamus and bone density in participants with early AD and non-demented controls using neuroimaging analysis (Chapter 3; published in the *Journal of Alzheimer's Disease*). The third manuscript utilized randomly selected sub-sample of 40 participants to compare performance of two methods currently used in neuroimaging research for assessment of hypothalamic volume (Chapter 4; submitted to *NeuroImage*). The fourth manuscript utilized longitudinal data to look at rates and predictors of bone loss over two years in participants with early AD and non-demented controls (Chapter 5; to be submitted). The fifth manuscript is an exploratory study of potential mechanisms that may underlie the association of bone loss and hypothalamic atrophy in early AD using casual modeling statistical technique (Chapter 6; to be submitted).

Chapter 2 Preface

In Chapter 1, previously published work in the literature describing the relationship between bone health and AD was presented, largely from population studies and studies in severely demented women. While these studies provide important information to the field, some methodological limitations may drive the observed relationship. For example, AD and bone loss share numerous common risk factors. High level of these risk factors in the general population may simply increase the odds of co-occurrence of AD and bone loss. The relationship between AD and bone loss in severely demented may be potentially explained by malnutrition or reduced activity (e.g., bedridden state). Additionally, despite the evident relationship between poor bone health and AD, no studies have explored a relationship between bone density and brain structure and cognition.

In Chapter 2, we sought to explore the relationship between bone density and clinical and neuroimaging markers of AD in a cohort of community-dwelling older men and women without cognitive deficits and diagnosed with very early stages of AD, all of whom were otherwise relatively healthy.

Chapter 2

Bone Density and Brain Atrophy in Early Alzheimer Disease

Loskutova N, Honea R, Vidoni E, Brooks W, Burns J: "Bone Density and Brain Atrophy in Early Alzheimer Disease" 2009, Journal of Alzheimer's Disease 18, 777-785

2.1 ABSTRACT

Studies suggest a link between bone loss and Alzheimer's disease. To examine bone mineral density (BMD) in early Alzheimer's disease (AD) and its relationship to brain structure and cognition we evaluated 71 patients with early stage AD (Clinical Dementia Rating (CDR) 0.5 and 1) and 69 non-demented elderly control participants (CDR 0). Measures included whole body BMD by dual energy x-ray absorptiometry (DXA) and normalized whole brain volumes computed from structural MRI scans. Cognition was assessed with a standard neuropsychological test battery. Mean BMD was lower in the early AD group (1.11 ± 0.13) compared to the non-demented control group (1.16 ± 0.12, p=0.02), independent of age, sex, habitual physical activity, smoking, depression, estrogen replacement, and apolipoprotein E4 carrier status. In the early AD group, BMD was related to whole brain volume (b=0.18, p=0.03). BMD was also associated with cognitive performance, primarily in tests of memory (logical memory [b=0.15, p=0.04], delayed logical memory [b=0.16, p=0.02], and the selective reminding task - free recall [b=0.18, p=0.009]). BMD is reduced in the earliest clinical stages of AD and associated with brain atrophy and memory decline, suggesting that central mechanisms may contribute to bone loss in early Alzheimer's disease.

2.2 INTRODUCTION

The prevalence of Alzheimer's disease (AD) is rising dramatically as the population ages and is now among the leading causes of death in older people (2008). While cognitive decline is a clinical hallmark of AD, changes in physical health are also apparent during the course of AD and include increasing frailty, lower aerobic capacity, weight loss and gait and motor dysfunction (Kluger, Gianutsos et al. 1997; Barrett-Connor, Edelstein et al. 1998; Camarda, Camarda et al. 2007; Burns, Cronk et al. 2008).

Bone health is an important issue in AD given a higher risk of falls and increased incidence of fractures in individuals with AD compared to cognitively healthy older adults. Additionally, individuals with AD have poorer recovery (Buchner and Larson 1987; Weller 2000; Weller and Schatzker 2004) and higher mortality rates (van Dortmont, Douw et al. 2000) after suffering a hip fracture. While several studies in women suggest that low bone mineral density (BMD) is associated with higher risk of developing AD (Tan, Seshadri et al. 2005) and cognitive decline (Yaffe, Browner et al. 1999; Lui, Stone et al. 2003), there is little data on bone health in individuals with AD. In a study of women, BMD was reduced in AD compared to cognitively normal females of similar age, with decreasing BMD in the most severely demented (Sato, Honda et al. 2005), suggesting that bone loss may be accelerated in AD. Although this evidence suggests a link between AD and BMD (Tysiewicz-Dudek, Pietraszkiewicz et al. 2008) little is known about how early in the disease bone loss might occur and whether bone loss is observed in men with AD.

Multiple factors have been postulated to explain the association between bone loss and cognitive decline in women, including estrogen exposure, apolipoprotein E4, depression and lifestyle factors such as physical activity, nutritional, dietary, and environmental factors.

Accumulating evidence also suggests that the central nervous system directly regulates bone health through actions primarily orchestrated by the hypothalamus (Harada and Rodan 2003; Takeda 2005; Zaidi 2007), a structure affected early in the AD process (Raskind, Peskind et al. 1999; Callen, Black et al. 2001). However, no studies have evaluated bone health in relation to cognition and brain structure. The purpose of this study was to compare bone health in the earliest clinical stages of AD with non-demented aging and to determine whether BMD is associated with cognitive performance and brain atrophy, which is used as a marker of neurodegeneration. We hypothesized that bone density would be lower in early AD and that reduced BMD would be associated with brain atrophy and cognitive decline. Understanding the association of bone loss and AD, in the context of other BMD-modifying factors, may provide strategies for developing effective prevention and treatment of osteoporosis and could ease the burden associated with bone fractures in AD.

2.3 MATERIALS AND METHODS

Sample and recruitment

71 patients with early-stage AD (Clinical Dementia Rating (CDR) 0.5, n=57 and CDR 1.0, n=14) and 69 non-demented elderly control participants (CDR 0) were enrolled in the University of Kansas Brain Aging Project. Participants were recruited from a referral based memory clinic and by media appeals. Signed institutionally-approved informed consent was obtained prior to enrollment from all participants or their legal representatives and the informants who know the participants well and served as the collateral source. Participants with a history of neurologic disease other than AD, diabetes mellitus, ischemic heart disease, schizophrenia, major depression, contraindications to MRI, and a history of alcohol abuse were excluded from the

study. Clinical assessment methodology and clinical and neurological characteristics of the study participants have been presented previously(Burns, Cronk et al. 2008).

Whole body bone mineral density

Dual energy x-ray absorptiometry (DXA) (Prodigy fan-beam densitometer, Lunar Corp., GE Medical Systems, Madison, WI) was used to determine total skeleton BMD. We used whole body BMD as a measure of global bone health. Whole body BMD gives a comprehensive view of the whole skeleton (Blake, Herd et al. 2000) and is mostly determined by cortical bone (Szulc, Marchand et al. 2000). Cortical bone porosity and decline in mechanical properties are reported in aging (McCalden, McGeough et al. 1993) and associated with fractures. Cortical bone density decline is found to be more specific than trabecular BMD for the older population and may play an important role in bone fragility in elderly (Uusi-Rasi, Sievanen et al. 2007; Seeman 2008).

Magnetic resonance imaging

Structural MRI data were obtained at the Hoglund Brain Imaging Center with a 3.0 T Allegra MR scanner (Siemens Medical Solutions, Erlangen, Germany). T1 weighted magnetization prepared rapid gradient echo (MP-RAGE) sequence (1x1x1mm^3 voxels; TR = 2500ms, TE = 4.38ms, TI = 1100ms, FOV 256x256cm^2 with 18% oversample, flip angle 8 degrees) was performed for high resolution structural analysis. We used FMRIB Software Library (FSL; www.fmrib.ox.ac.uk/fsl) for computing whole brain volume. Each image was preprocessed and skull-stripped with Brain Extraction Tool (BET). Next, images were normalized by registration to the Montreal Neuroimaging Institute (MNI) average 152 template and then segmented into white, gray matter and CSF with FMRIB's Automated Segmentation

Tool. Since brain volumes differ between sexes, brain volumes were normalized to intracranial volume for each participant summing white and gray matter volumes and presented as percent of intracranial volume (% ICV). Image processing was conducted with Laboratory of Neuroimaging Pipeline (University of California, Los Angeles, www.pipeline .loni.ucla.edu). Normalized brain volumes were not related to sex (r=0.09, p=0.3). Normalized brain volume was used as a sensitive marker of brain atrophy(Battaglini, Smith et al. 2008).

Neuropsychological assessment

We administered a psychometric battery consisting of standard measures of memory (WMS-R Logical Memory I and II(Wechsler and Stone 1973), Free and Cued Selective Reminding Task (Grober, Buschke et al. 1988)), language (Boston Naming Test–15 item (Goodglass and Kaplan 1983)), working memory (WMS III Digit Span Forwards and Backwards (Wechsler and Stone 1973) WAIS Letter-number sequencing (Wechsler 1955)), executive function (Trailmaking A and B (Armitage 1946), Verbal Fluency [animals, fruits and vegetables](Hänninen, Reinikainen et al. 1994), and Stroop Color-Word Test(Stroop 1935)), and visuospatial ability (WAIS Block Design(Wechsler 1955)) as reported previously (Burns, Cronk et al. 2008). We created a composite measure of global cognition by first, converting individual test scores for each participant to z-scores using a reference sample of non-demented older adults (n=83) and then computing the mean of all z-scores for each participant. Mini-Mental State Examination (MMSE) was administered as a standard measure of global cognition (Folstein, Folstein et al. 1975).

Other potential modifiers

Habitual physical activity level was assessed using the Physical Activity Scale for the Elderly (PASE) (Washburn, McAuley et al. 1999). The PASE scores were collected from the study participants and their collateral sources. The modified Physical Performance Test (PPT) was administered as a physical frailty assessment (Burns, Cronk et al. 2008). Geriatric Depression Scale (GDS) was administered to study participants and their collateral source to assess depressive signs(Burke, Houston et al. 1989). Apolipoprotein E (ApoE) genotypes were obtained using restriction enzyme isotyping (Hixson and Vernier 1990). Self-reported tobacco history was collected along with demographic characteristics for each participant. Tobacco use for those with a present or past history of smoking was calculated in Pack Years. Participants were divided into three groups (never smoked, smoked more that 100 cigarettes previously but not in last 30 days, currently smoking) for statistical analysis. DXA was used to assess fat mass and percent body fat of the participants dressed in a hospital gown. Body weight in kg and height in cm were recorded and body mass index (BMI, kg/m^2) was calculated for each study participant. Total cholesterol, triglycerides, high-density (HDL) and low-density (LDL) lipoproteins levels were assessed in fasting venous blood samples as described previously (Burns, Donnelly et al. 2007).

Self-reported medication use was collected from the collateral source by a nurse clinician. Hormone-replacement therapy (HRT) medications (estrogen, testosterone and selective estrogen receptor modulators), thyroid hormone replacement, bisphosphonates, beta-blockers, vitamin D and calcium were considered as bone affecting. The total number of participants in each group that reported use of medications with primary or potential effects on bone health for two or more weeks prior to the study enrollment was recorded.

Statistical analyses

Statistical analyses were conducted using SPSS 16.0 software package (SPSS Inc., Chicago, Ill). Continuous variables were summarized as means ± standard deviation (SD) and categorical variables were summarized by percent. Independent samples t-tests were used for analyzing group differences in continuous variables and chi-square statistics were used for categorical data. The general linear model was used for assessing effects of dementia and additional potential modifiers (habitual physical activity (PASE), signs of depression (GDS), frailty (PPT), genetic component (ApoE4), use of bone affecting medications and tobacco history) on BMD, with age and sex included as primary covariates. We added an interaction term to examine whether the association of BMD and dementia was modified by sex (dementia*sex). An association of BMD with age was assessed with Pearson *r* coefficient of correlation. An association of BMD with past or present history of smoking (Pack Years) was assessed by partial correlation controlled for age and sex. We used a stepwise linear regression model to examine: 1) the association of BMD with whole brain volume, first controlling for age and sex followed by additional models assessing the effect of other covariates on the relationship between BMD and whole brain volume, and 2) the association of BMD with cognitive measures, controlling for age and sex and the effect of the potential modifiers on this association. Assumptions for linearity, normality, equal variance and homoscedasticity of errors were examined and adequately met. Statistical significance was tested at a=0.05.

2.4 RESULTS

Sample description

Demographic and clinical characteristics of the participants in the non-demented (n=69) and early AD groups (n=71) are summarized in **Table 1**. Early AD and non-demented groups were similar in age (mean age 74.1±6.8 years) and sex distributions. Average BMI, percent body fat, and serum levels of total cholesterol, triglycerides, HDL and LDL did not differ between groups. As expected, participants in the early AD group had mild global cognitive impairment, lower level of habitual physical activity (PASE), higher frailty scores (PPT) and more depressive signs (GDS). Participants with early AD were more likely to carry the ApoE4 allele than controls. Brain atrophy was evident in the early AD group. The early AD group had significantly smaller mean normalized whole brain volume than the non-demented group. Whole brain volume was significantly associated with MMSE score ($r=0.42$, $p<0.001$), our composite measure of global cognition ($r=0.47$, $p<0.001$), and dementia severity (CDR Box score, $r=-0.44$, $p<0.001$) after controlling for age and sex.

The groups did not differ in the use of medications with primary or potential bone affecting qualities. **Table 2** shows the percentage of participants reporting prolonged use of hormone replacement medications, bisphosphonates, thyroid hormone replacement, beta-blockers, vitamin D, and calcium supplements in each group.

Bone mineral density and dementia status

Mean whole body BMD was lower in the early AD group (1.11 ± 0.13) compared to the non-demented control group (1.16 ± 0.12, $p=0.02$). As expected, BMD decreased with age ($r=-0.21$, $p=0.01$) and was lower in females (1.08± 0.10) than in males (1.21± 0.12, $p<0.001$).

We next used the general linear model to assess the role of dementia status on BMD while controlling for age, sex, and additional covariates. After controlling for age and sex, individuals with AD had lower BMD than non-demented individuals (b1=-0.04, p=0.03). There was no interaction between dementia status and sex (dementia*sex, p=0.71) suggesting that AD-related BMD differences were similar in men and women (**Figure 1**).

Bone mineral density and additional modifiers

Additional modifiers (physical activity (PASE), frailty (PPT), signs of depression (GDS), genetic makeup (ApoE4) and use of bone affecting medications) were not related to BMD. There were no significant effects of smoking history on BMD (p=0.46) after controlling for age and sex and no dementia*smoking history interaction (p=0.75). There was no association between Pack Years and whole body BMD after controlling for age and sex in participants who are currently smoking and used to smoke in either early AD or non-demented groups.

Bone mineral density and whole brain volume

We specifically tested the hypothesis that brain atrophy was associated with BMD. First, we examined the association of BMD with whole brain volume in the entire cohort using stepwise linear regression analysis with age and sex as covariates. Higher BMD was associated with larger brain volumes, i.e. less brain atrophy (b=0.18, p=0.03). Age and sex accounted for 28.1% of the variance with whole brain volume explaining an additional 7.6% of the variance.

Given that group differences in brain volume and BMD may explain this relationship we next examined the association of brain volume with BMD in the early AD and non-demented groups separately. A scatterplot of whole brain volume and age- and sex-adjusted BMD is

presented in **Figure 2**. In early AD, whole brain volume was associated with BMD (b=0.3, p=0.004), explaining 8.8% of variance of BMD after controlling for age (non-significant) and sex (b=0.53, p<0.001, 26.2 % of variance of BMD explained). In the non-demented group, whole brain volume was not associated with BMD after controlling for age and sex.

Because bone health can be modified by multiple factors, we repeated a series of regression analyses to examine the influence of potential modifiers on the observed BMD - whole brain volume relationship. All models controlled for age and sex. Potential modifying factors such as habitual physical activity (PASE), frailty (PPT), depressive signs (GDS), Apolipoprotein E4 allele status (ApoE4 carriers vs. non-carriers), use of bone affecting medications and smoking history were each added to the linear regression model, but none attenuated the bone density-whole brain volume relationship.

Bone mineral density and cognitive performance

In the entire cohort, BMD was not associated with our composite measure of global cognition or MMSE performance. BMD was positively associated with performance on logical memory (b=0.15, p=0.04), delayed logical memory (b=0.16, p=0.02), and the selective reminding task (b=0.18, p=0.009) after controlling for age and sex. Addition of any of the potential modifying factors to the regression model did not attenuate the bone-density-cognitive performance relationship. Within-group analyses were conducted next to minimize the effect of group differences in BMD and cognitive performance. In the early AD group, BMD remained associated with logical memory (b=0.25, p=0.01), delayed logical memory (b=0.27, p=0.009), and the selective reminding task (b=0.25, p=0.02). Measures of dementia severity (CDR and

CDR box score) were not related to BMD. In non-demented participants, BMD was not associated with performance on any cognitive measures.

2.5 DISCUSSION

The results of the study suggest that BMD is reduced in the earliest clinical stages of AD in both men and women. Additionally, BMD was related to whole brain volume and memory performance in AD, with higher BMD associated with higher whole brain volume (less brain atrophy) and better memory performance. These results suggest that AD-related brain changes may affect bone remodeling or that bone loss and AD may share common biological mechanisms.

There is evidence that major factors contributing to bone loss are associated with AD though it remains unclear whether alterations in these factors are a cause or a consequence of AD. BMD has been viewed as a proxy for cumulative estrogen exposure in women and the BMD-cognition relationship has been interpreted as evidence for a link between estrogen and cognition (Yaffe, Browner et al. 1999). Although we did not specifically measure estrogen levels, our observation of reduced BMD in men with AD and more recent clinical trial evidence of an increased risk of dementia in estrogen users suggests alternative mechanisms may be responsible for the link between bone loss and AD (Shumaker, Legault et al. 2003).

Some epidemiological studies suggest dietary factors may play a role in AD risk (Scarmeas, Stern et al. 2006; Barberger-Gateau, Raffaitin et al. 2007). Though, the role of dietary protein and fat in bone health is still a matter of debate, animal (Mardon, Habauzit et al. 2008) and human (Ilich, Brownbill et al. 2003; Carlsson, Tidermark et al. 2005; Coin, Perissinotto et al. 2008) studies show that adequate energy and protein provision is essential for preservation of

skeletal health in elderly. Decreased food intake and changes in dietary patterns may occur in AD (Munoz, Agudelo et al. 2006; Smith and Greenwood 2008) and could potentially explain lower bone density in the AD group (Sanlier 2008). In addition to dietary deficiencies in microelements and nutrients, disturbances in microelement metabolism and exposure to high concentrations of some minerals are associated with both low bone mass (Lacka-Gazdzik, Klosa et al. 2005) and AD (Shcherbatykh and Carpenter 2007). High levels of trace elements in the brains of AD patients are associated with oxidative damage (Cornett, Markesbery et al. 1998), a potential etiologic mechanism of AD.

Although, diet was not assessed in the study participants (Bianco, Filippi et al. 2008), our early AD cohort was similar in BMI and percent body fat indicating that AD participants were not underweight (Sergi, Perissinotto et al. 2005). Serum levels of total cholesterol, triglycerides, HDL and LDL in the early AD group did not differ from non-demented controls, indirectly suggesting that their global nutritional status was similar to the non-demented older adults (Ibrahim, Eltom et al. 1994; Hrnciarikova, Hyspler et al. 2009).

The ApoE4 allele has also been associated with bone loss (Lindsay, Laurin et al. 2002; Zajickova, Zofkova et al. 2003). Our analyses were unchanged when controlling for ApoE4 carrier status suggesting that ApoE4 does not mediate this relationship, consistent with the findings of other studies (Efstathiadou, Koukoulis et al. 2004; Schoofs, van der Klift et al. 2004). Our data raise the possibility that the relationship between bone loss and Apoe4 in other studies of non-demented older women may have been driven by the likelihood of a higher prevalence of unrecognized, preclinical AD in the ApoE4 cohort.

Physical activity is an important modulator of bone mass, though its effect on bone mass may be attenuated in older adults (Kemmler, Weineck et al. 2004). Our early AD cohort

demonstrated significant reductions in levels of habitual physical activity and thus it is possible that AD-related behavioral changes may exacerbate age-related bone loss. Habitual physical activity level, however, was not associated with BMD and controlling for physical activity did not modify AD-related BMD differences or the relationship between BMD and brain volume. Smoking is another major lifestyle risk factor for osteoporosis and appears to have negative effects on bone mass at the major sites of osteoporotic fractures (Egger, Duggleby et al. 1996; Papaioannou, Kennedy et al. 2009). In our cohort, however, whole body BMD was not associated with smoking and controlling for smoking did not attenuate the relationship between brain atrophy and BMD in AD. We examined additional factors that may influence bone mass such as physical frailty (Kenny, Waynik et al. 2006; Buchman, Boyle et al. 2007), depressive signs (Diem, Blackwell et al. 2007; Wilson, Arnold et al. 2008), and hormone-replacement therapy including vitamin D and calcium supplement intake and we found no evidence that they influenced bone loss in the early stages of AD.

Another possible explanation of the current results is that AD-related neurodegenerative brain changes may directly affect central control of bone remodeling. Atrophy of the limbic system, including the hypothalamus, is prominent in AD (Callen, Black et al. 2001). The hypothalamus is a central regulator of a number of homeostatic metabolic processes including a major role in regulating bone mass (Zaidi 2007). Our results demonstrate the independent association of BMD with whole brain volume in early AD and suggest that neurodegenerative changes may influence bone mass in AD. Future studies should assess regional brain atrophy, including the limbic system, to examine the hypothesis that neurodegeneration may contribute to loss of bone mass in AD.

Limitations of the study include the cross-sectional design that reduces our ability to infer causal relationships among variables. Further longitudinal and interventional studies are necessary to establish cause and effect. Additionally, the sample size limits the power to fully examine the influence of covariates on BMD. We did not examine peripheral factors related to bone health including estrogen, calcium, and vitamin D serum levels, all of which have been linked to AD although we did take into account participant use of such therapies. Given that our study participants are a convenience sample, it remains possible that our results may be influenced by sampling bias. Additionally, our AD sample was confined to the earliest clinical stages of AD, with most of the demented subjects (80.3%) were in the very mild (CDR 0.5) stage, comparable to mild cognitive impairment. Although this very early AD group is one of the strengths of the study, it limits the range of dementia severity and may have reduced our power to resolve a relationship between BMD and dementia severity and other variables. Nevertheless, to our knowledge, this is the first study to suggest that bone loss is apparent in men and women in the earliest clinical stages of AD and that bone mineral density is related to brain structure and cognitive performance.

Table 2. 1 Sample characteristics

	Non-demented (n=69)	Early AD (n=71)	p-value
Age (years)	73.3 (6.9)	74.9 (6.6)	0.173
Education (years)	16.5 (2.7)	15.3 (3.4)	0.015
Females, n (%)	40 (58)	41 (57.7)	0.94
Bone mineral density (g/cm²)	1.156 (0.12)	1.112 (0.13)	0.02
Whole brain volume (% ICV)	78.0 (2.8)	75.3 (3.3)	<0.001
BMI (kg/m²)	25.7 (3.6)	25.0 (5.1)	0.24
Body fat (%)	36.7 (9.0)	36.6 (9.9)	0.95
Serum cholesterol (mg/dL)	182.8 (34.3)	188.7 (37.4)	0.33
Serum triglycerides(mg/dL)	111.3 (53.4)	120.1 (61.5)	0.37
HDL(mg/dL)	55.0 (13.1)	54.0 (13.4)	0.69
LDL(mg/dL)	105.5 (25.7)	111.6 (30.2)	0.21
MMSE	29.4 (0.8)	26.1 (3.5)	<0.001
ApoE4 carriers, n(%)	19 (27.9)	39 (58.2)	<0.001
Geriatric Depression Scale	0.83 (0.95)	1.72 (1.57)	<0.001
Physical activity (PASE)	125.27 (63.6)	98.00 (56.3)	<0.001
Frailty (PPT)	30.45 (3.4)	27.59 (3.8)	<0.001

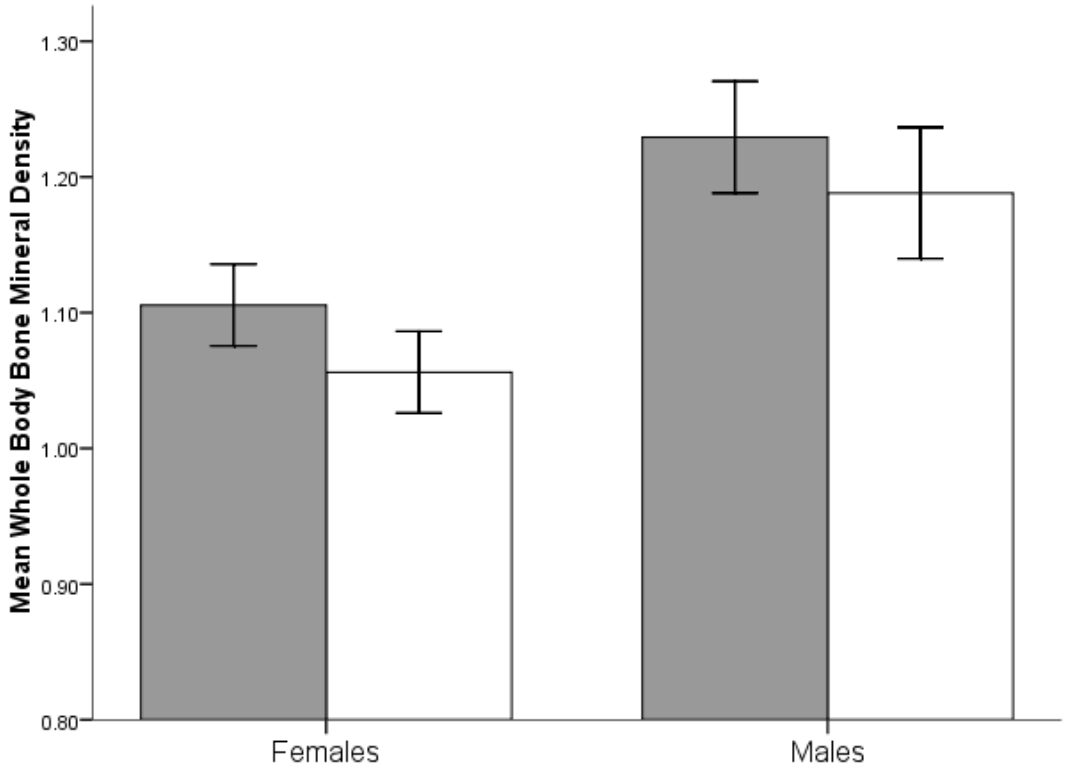
All data represent means (SD), unless otherwise noted. AD- Alzheimer's disease; % ICV- represents percent of intracranial volume; BMI- body mass index; HDL- high-density lipoproteins; LDL- low-density lipoproteins; MMSE- Mini-Mental State Examination; ApoE4- Apolipoprotein E; PASE- Physical Activity Scale for the Elderly; PPT- Physical Performance Test

Table 2. 2 Current medications

Medications	Non-demented (n=69)	Early AD (n=71)	p-value
Beta-blockers, n (%)	3 (4.3%)	9 (12.7%)	0.08
Thyroid hormone replacement, n (%)	12 (17.4%)	9 (12.9%)	0.44
Hormone replacement therapy, n (%)	9 (13%)	7 (9.9%)	0.55
Bisphosphonates, n (%)	12 (17.4%)	14 (19.7%)	0.72
Vitamin D, n (%)	29 (42%)	19 (26.8%)	0.06
Calcium, n (%)	34 (49.3%)	28 (39.4%)	0.16

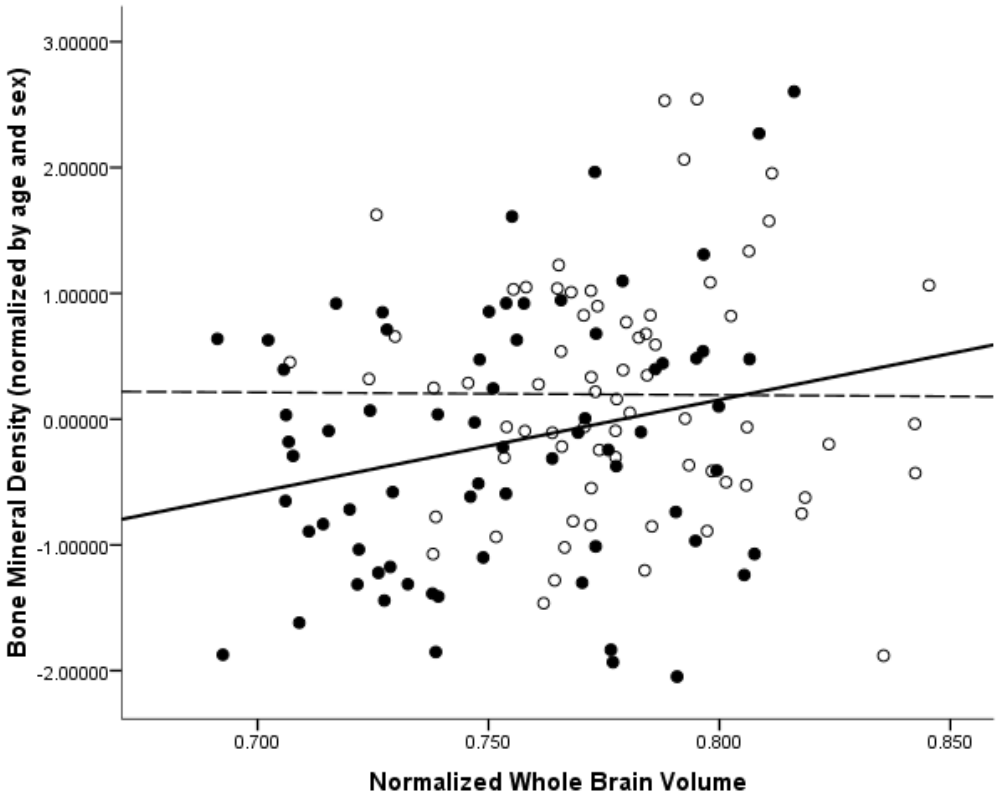
All data represent number (%) of participants in each group. AD- Alzheimer's disease

Figure 2. 1 Bone mineral density in non-demented and early AD groups



Bone mineral density (BMD, in g/cm^2) is reduced in males and females in the early stages of Alzheimer's disease (AD). Grey bars – non-demented group, white bars- early AD.

Figure 2. 2 Relationship between whole brain volume and whole body bone mineral density in the non-demented and early AD groups



Relationship between whole brain volume and whole body bone mineral density in the non-demented (dotted line) and early AD groups (solid line).

Larger normalized whole brain volumes (less brain atrophy) are associated with higher whole body bone mineral density. Open circles represent non-demented participants (CDR 0) and filled circles represent participants with early stages of AD (CDR 0.5 and 1). Normalized whole brain volume is in % of total intracranial volume.

Chapter 3 Preface

In Chapter 2, we demonstrated that BMD was reduced in the earliest clinical stages of AD in both men and women. Additionally, BMD was related to whole brain volume and cognitive performance in AD, with higher BMD associated with higher whole brain volume (less brain atrophy) and better cognitive performance. These results suggest that AD-related brain changes may affect bone remodeling or that bone loss and AD may share common biological mechanisms.

As discussed in Chapter 1, recent evidence allowed us to hypothesize that AD related structural changes in the hypothalamus may be one of the mechanisms of accelerated bone loss in AD. However, there have been no studies to investigate whether neurodegeneration of CNS and the hypothalamus specifically, is associated with bone loss.

In Chapter 3, we present a cross-sectional study examining the relationship of BMD with regional grey matter volume (as opposed to whole brain volume), including the hypothalamus, in a sample of participants with early AD and non-demented controls.

Chapter 3

Reduced limbic and hypothalamic volumes correlate with bone density in early Alzheimer's disease

Loskutova N, Honea R, Vidoni E, Brooks W, Burns J, 2010, Journal of Alzheimer's Disease 20(1):313-22

3.1 ABSTRACT

Accelerated bone loss is associated with Alzheimer's disease (AD). Although the central nervous system (CNS) plays a direct role in regulating bone mass, primarily through the actions of the hypothalamus, there is little work investigating the possible role of neurodegeneration in bone loss. In this cross-sectional study, we examined the association between bone mineral density (BMD) and neuroimaging markers of neurodegeneration (i.e. global and regional measures of brain volume) in early AD and non-demented aging. Fifty-five non-demented and 63 early AD participants underwent standard neurological and neuropsychological assessment, structural MRI scanning and dual energy x-ray absorptiometry. In early AD, voxel-based morphometry (VBM) analyses demonstrated that low BMD was associated with low volume in limbic grey natter (GM) including the hypothalamus, cingulate, and parahippocampal gyri and in the left superior temporal gyrus and left inferior parietal cortex. No relationship between BMD and regional GM volume was found in non-demented controls. The hypothesis-driven region of interest analysis further isolating the hypothalamus demonstrated a positive relationship between BMD and hypothalamic volume after controlling for age and gender in the early AD group but not in non-demented controls. These results demonstrate that lower BMD is associated with lower hypothalamic volume in early AD, suggesting that central mechanisms of bone remodeling may be disrupted by neurodegeneration.

3.2 INTRODUCTION

Epidemiologic projections indicate that the incidence of Alzheimer's disease (AD) will increase dramatically in the coming decades due largely to the demographics of the disease and our aging population. Associated cognitive and physical decline greatly contributes to disability in older adults and places considerable burden on the health system, patients, and caregivers. Bone health is an important issue in AD given that AD patients are at higher risk than cognitively healthy adults for osteoporosis, falls, bone fractures and poor post-fracture outcomes (Buchner and Larson 1987; Weller 2000). Bone mineral density is a strong predictor of bone fractures and accounts for 60-70% of bone strength (Ammann and Rizzoli 2003). We have previously demonstrated that BMD is reduced in both men and women in the earliest clinical stages of AD compared to non-demented older adults independent of habitual physical activity, smoking, depression, estrogen replacement, and apolipoprotein E4 carrier status. Moreover, low BMD was independently associated with lower whole brain volume and memory deficits, suggesting that degeneration of the central nervous system (CNS) may play a role in bone loss (Loskutova, Honea et al. 2009). However, it is unknown whether bone loss is related to volume loss in specific regions of the brain, such as the hypothalamus.

Bone mass is maintained locally by the balance between bone resorption by osteoclasts and bone formation by osteoblasts. Multiple factors modulate this servo system and perturbations of this system can result in bone loss. The most important and well-studied regulators of bone health are calcium and vitamin D availability, sex steroids, and mechanical usage. Recent work, however, indicates that the CNS directly regulates bone remodeling through the actions of the hypothalamus through two distinct pathways, the neurohumoral and neural arms (Harada and Rodan 2003; Takeda 2005). Briefly, the neurohumoral arm involves hypothalamic control of the

anterior pituitary hormones, such as growth hormone (GH), thyroid stimulating hormone (TSH) and follicle-stimulating hormone (FSH) (see (Zaidi 2007) for a full review). GH executes its anabolic action on bone through insulin-like growth factor 1 (IGF-1). TSH and FSH receptors are present in bone cells and receptor activation stimulates bone resorption. The neural arm involves hypothalamic control of bone remodeling through sympathetic nervous system (SNS) output. SNS output from the leptinergic/peptidergic neurons in the ventral hypothalamus directly regulates bone remodeling through activation of beta-2 adreno-receptors on the osteoblasts, resulting in reduced bone formation (Takeda and Karsenty 2008). There is evidence that sympathetic output from the hypothalamus via the neural arm may be more important than actions of sex steroids in the regulation of bone remodeling (Elefteriou, Takeda et al. 2004; Pogoda, Egermann et al. 2006).

Clinical, neuropathological, and neuroimaging data together suggest that the hypothalamus is affected in AD and undergoes neuronal loss (de Lacalle, Iraizoz et al. 1993), profound plaque and tangle formation (Saper and German 1987; Standaert, Lee et al. 1991) and overall atrophy (Callen, Black et al. 2001). However, there have been no studies to investigate whether neurodegeneration of CNS, and the hypothalamus specifically, is associated with bone loss. We hypothesized that atrophy of the hypothalamus and loss of hypothalamic neurons associated with AD may be one of the mechanisms of accelerated bone loss in AD. Thus, the aim of this cross-sectional study was to examine the underlying neural substrate of an association between BMD and brain volume and establish specific correlations of regional grey matter (GM) with bone loss in early AD and non-demented aging using voxel-based morphometry (VBM) analysis.

3. 3 MATERIALS AND METHODS

Sample and recruitment

71 patients with early-stage AD (Clinical Dementia Rating (CDR) 0.5 (n=57) and CDR 1 (n=14)) and 69 non-demented elderly control participants (CDR 0) were enrolled in the University of Kansas Brain Aging Project. Of these, 16 non-demented and 6 early AD participants were excluded for excessive head motion. The final sample included 63 patients with early-stage AD (Clinical Dementia Rating (CDR) 0.5, (n=49) and CDR 1 (n=14)) and 55 non-demented elderly control participants (CDR 0). Participants were recruited from a referral based memory clinic and by media appeals. All participants had standard neurological and neuropsychological assessment, structural MRI scanning and dual energy x-ray absorptiometry. Mini-Mental State Examination (MMSE) was administered as a standard measure of global cognition (Folstein, Folstein et al. 1975). The absence or presence and severity of AD were determined by a standard clinical evaluation that included the CDR (Morris 1993). Detailed clinical assessment methodology and clinical and neurological characteristics of the study participants have been presented previously (Burns, Cronk et al. 2008). Participants with a history of neurologic disease other than AD, diabetes mellitus, ischemic heart disease, schizophrenia, major depression, alcohol abuse, contraindications to MRI, and use of antipsychotic and other investigational medications were excluded from the study. Signed institutionally approved informed consent was obtained prior to enrollment from all participants or their legal representatives.

Whole body bone mineral density

Dual energy x-ray absorptiometry (DXA; Prodigy fan-beam densitometer, Lunar Corp., GE Medical Systems, Madison, WI) was used to determine total skeleton BMD. We used whole body BMD, mostly determined by cortical bone (Szulc, Marchand et al. 2000), as a measure of global bone health that gives a comprehensive view of the whole skeleton(Blake, Herd et al. 2000). Cortical bone porosity and decline in mechanical properties are reported in aging (McCalden, McGeough et al. 1993) and associated with fractures. Cortical bone density decline is found to be more specific than trabecular BMD loss for the older population and may play an important role in bone fragility in elderly (Uusi-Rasi, Sievanen et al. 2007; Seeman 2008).

Magnetic resonance imaging and voxel-based morphometry

Structural MRI data were obtained at the Hoglund Brain Imaging Center with a 3.0 T Allegra MR scanner (Siemens Medical Solutions, Erlangen, Germany). T1-weighted magnetization prepared rapid gradient echo (MP-RAGE) sequence (1x1x1mm^3 voxels; TR = 2500ms, TE = 4.38ms, TI = 1100ms, FOV 256x256cm^2 with 18% oversample, flip angle 8 degrees) were collected and processed for voxel-based morphometry (VBM) analysis. Every scan was examined for image artifacts and gross anatomical abnormalities. Sixteen non-demented and six demented subjects were excluded for movement artifact or inhomogeneity that distorted brain matter. We used MRIcro® software to reconstruct raw Dicom images. Data analysis for 55 non-demented and 63 AD subjects was performed using the VBM5 toolbox (http://dbm.neuro.uni-jena.de), an extension of the SPM5 algorithms (Wellcome Department of Cognitive Neurology, London, UK) running under MATLAB 7.1 (The MathWorks, Natick, MA, USA) on Linux. The VBM5 toolbox extends and enhances the unified segmentation approach

implemented in SPM5 (Ashburner and Friston 2005) by using a generative model that integrates tissue classification, image registration and MRI inhomogeneity bias correction. The approach allows for more accurate classification of brain tissues in presence of excess atrophy or abnormal morphology (Meisenzahl, Koutsouleris et al. 2008; Wilke, Holland et al. 2008). Images were then modulated and saved using affine registration plus non-linear spatial normalization (Wilke, Holland et al. 2008), resulting in final tissue maps of grey matter (GM), white matter (WM) and cerebro-spinal fluid (CSF) and smoothed with a 10 mm FWHM Gaussian kernel before statistical analysis. Additionally, for each study participant total GM, WM, CSF and whole brain volumes (GM plus WM) in cm^3 were computed using the normalized tissue maps of each study participant. Finally, total hypothalamic volumes in cm^3 were derived from small volume correction (SVC) analysis in VBM.

Statistical analyses

Statistical analyses were conducted using SPSS (Version 16, SPSS Inc., Chicago, Ill). Continuous variables were summarized as means ± standard deviation (SD) and categorical variables were summarized by percent. Independent samples t-tests were used for analyzing group differences in continuous variables and chi-square statistics were used for categorical data. Effect size for total hypothalamic volume was computed using G*Power3 free software (Faul, Erdfelder et al. 2007). Partial correlations controlling for age and gender were used to examine an association between BMD, CDR score, MMSE and brain volumes. Statistical significance was tested at a=0.05.

Imaging Statistics

To analyze brain images in SPM5, we used a linear regression model with independence between subject groups, unequal variance, no grand mean scaling, and centered covariates on the overall mean. We used absolute threshold masking set at 0.10 to restrict each analysis to one tissue type. First, the relationship of BMD to brain volume was assessed across all voxels within each group using multiple regression analysis, with age and gender as confounding variables. The relationship between BMD and regional GM was considered significant at $p<0.001$ uncorrected. We then examined the relationship of BMD with hypothalamic volumes using the small volume corrections (SVCs). The bilateral hypothalami were derived from the Wake Forest University Pickatlas (http://www.fmri.wfubmc.edu) (Maldjian, Laurienti et al. 2003). These hypothalamic volumes of interest (VOIs) were pre-selected based on our hypothesis that the hypothalamic control of bone remodeling is affected in AD(Zaidi 2007). To correct for multiple comparisons in SVC analyses, results were considered significant at $p<0.05$ FWE. The hypothalamic volumes were extracted for each participant from the previous automated step and used for visualization purposes in graphic representations and secondary correlation analyses within AD group. All analyses were covaried for age and gender. The *x*, *y*, *z* coordinates of the areas of significant correlation were obtained from the analyses and reported with reference to the Montreal Neurological Institute (MNI) standard space within SPM5 after conversion to the standard space of Talaraich and Tournoux using custom software (Honea, Meyer-Lindenberg et al. 2008).

3.4 RESULTS

Sample description

Demographic and clinical characteristics of the participants in the non-demented (n=55) and early AD groups (n=63) are summarized in **Table 1**. Early AD and non-demented groups were similar in age (mean age 73.9±6.4 years) and gender distribution. As expected, participants with AD had mild deficits in global cognition (MMSE 26.1±3.7 vs. 29.5± 0.7 in non-demented, $p<0.001$). The groups did not differ in the use of anti-osteoporotic medications and vitamin D and calcium supplements as has been reported in details previously (Loskutova, Honea et al. 2009).

As we previously reported in a larger group of these participants (Loskutova, Honea et al. 2009), mean whole body BMD was lower in the early AD group (1.11 ± 0.13) compared to the non-demented control group (1.16 ± 0.12, $p=0.03$). As expected, BMD decreased with age ($r=-0.22$, $p=0.02$) and was lower in females (1.08± 0.10) than in males (1.21± 0.12, $p<0.001$). The early AD group had smaller mean normalized whole brain ($p<0.001$) and grey matter volumes ($p<0.001$) than the non-demented group. White matter volumes did not differ between groups ($p=0.42$).

Bone density and brain structure

We first assessed the relationship of BMD with regional brain volumes on a global level (across the entire grey matter) followed by SVC analyses confined to the hypothalamus using VBM neuroimaging analyses. Given the group differences in brain volume and BMD, we assessed the non-demented and early AD groups separately to avoid correlations driven by these group differences. All analyses were controlled for age and gender.

In the early AD group, analysis of regional GM volume adjusted for age and gender showed that BMD was significantly associated with large clusters of GM areas in the left superior temporal gyrus and left inferior parietal cortex, with the peak voxels in the left inferior parietal lobule (BA 40), and in the limbic GM with peaks in the cingulate, parahippocampal gyri, and the hypothalamus (**Figure 1**). All clusters for the voxel-wise analyses ($k>100$, $Z>3.4$ and p uncorrected <0.001) are presented in **Table 2**. There were no significant inverse correlations between BMD and regional GM volumes. We did not observe any significant relationship between BMD and GM volume of any area in the non-demented group.

Bone density and hypothalamic volume

We specifically tested the hypothesis that hypothalamic volume was associated with BMD. The hypothesis-driven SVC isolating the hypothalamus demonstrated a significant positive relationship between BMD and the hypothalamus after controlling for age and gender in the early AD group (**Figure 2**). The significant cluster for each result was extracted using the VOI function in SPM5 and the mean volume per cluster for each individual was used to plot the results for visualization purposes.

Secondary analyses of the hypothalamic volumes derived for each individual demonstrated a difference between groups ($p<0.001$, effect size $d=0.61$) with lower volumes in AD ($0.57 cm^3 \pm 0.10$) compared to non-demented controls ($0.65 cm^3 \pm 0.12$). We did not observe any relationship between BMD and hypothalamic volume in the non-demented group ($r=0.15$; $p=0.3$; **Figure 3**). Total hypothalamic volume was associated with BMD ($r=0.34$, $p=0.007$) in AD. The magnitude of this association was moderate in the CDR 0.5 sub-group (very mild AD; $r=0.33$, $p=0.03$) and larger in the CDR 1 sub-group (mild AD; $r= 0.59$, $p=0.04$) of AD patients

(**Figure 4).** There was, however, not a significant CDR * hypothalamic volume interaction (p=0.38) for AD participants perhaps related to the small CDR 1 sample size (n=14).

3.5 DISCUSSION

In our previous work, we demonstrated a relationship between bone mineral density and lower whole brain volume in the earliest clinical stages of AD and proposed a hypothesis that neurodegenerative changes may influence bone mass in AD via alteration in the central regulatory mechanism of bone remodeling controlled by the hypothalamus (Loskutova, Honea et al. 2009). This study extends the prior study by identifying specific regional relationships between BMD and grey matter volume, including the hypothalamus, that are present in early AD but not non-demented controls.

Our data suggest that hypothalamic volume is reduced in individuals in the early clinical stages of AD compared to non-demented older adults, consistent with prior studies demonstrating lower hypothalamic volume in AD (Callen, Black et al. 2001). Furthermore, by using VBM neuroimaging analyses we have demonstrated that hypothalamic volume is associated with whole body BMD, with lower hypothalamic volumes correlated with reduced BMD in the AD group. Additionally, we have demonstrated that lower grey matter volume in other areas predominantly affected in AD correlates with BMD reductions. These data suggest that AD-related neurodegeneration, in particular affecting the hypothalamus, may disrupt central mechanisms regulating bone mass and contribute to bone loss in early AD.

Brain atrophy in AD

The hypothalamus, a part of the limbic system, has extensive connections within the brain and modulates a variety of regulatory processes including appetite, energy expenditure, sleep and wakefulness and stress responses, all of which are disturbed in AD (Chouinard, Lavigne et al. 1998; Volicer, Harper et al. 2001). Additionally, the hypothalamus plays a role in memory through connections with the hippocampal formation. Brain atrophy, a sensitive marker of neurodegeneration, progresses in a relatively stereotypical fashion through the course of AD, beginning in the limbic system (i.e., the posterior cingulate and the hippocampus), progressing to the temporal-parietal cortices followed by the frontal lobes and late in the disease the occipital lobe and sensorimotor cortices (Thompson, Hayashi et al. 2007). Evidence of AD-related limbic atrophy in the hypothalamus is limited but has been reported using manual tracing of the structure (Callen, Black et al. 2001). We used automated VBM-based volume computation to assess hypothalamic volume and found evidence of hypothalamic volume loss in AD with a similar effect size as that previously reported. Thus, although our hypothalamic volumes were slightly larger than the previous manual tracing studies (Callen, Black et al. 2001), our results using automated estimation of the hypothalamic volume corroborate previously reported findings of smaller hypothalami in AD, suggesting that atrophy is present in the hypothalamus in AD.

Bone density and brain structure

Our results demonstrate that BMD is associated with hypothalamic volume in AD, with smaller hypothalamic volumes associated with lower BMD independent of age and gender. This relationship was observed in AD participants only and not in non-demented controls, suggesting the relationship of BMD with hypothalamic volume may be AD-specific. Our findings of

associations between BMD and lower GM volumes of the parietal and temporal cortices and limbic system, areas of the brain that are preferentially affected in the early stages of the disease (Honea et al., ADAD, 2009 in press), further suggests this relationship is specific to AD. These findings extend prior observations associating BMD with cognitive decline (Lui, Stone et al. 2003) and an increased risk of AD (Yaffe, Browner et al. 1999; Tan, Seshadri et al. 2005). We interpret these findings as suggesting either the presence of a pathological mechanism common to both bone loss and limbic atrophy or that hypothalamic atrophy in AD may contribute to bone loss via alterations in central regulatory mechanisms of bone remodeling.

Pathological changes occur in the hypothalamus in AD and include profound neuronal loss (de Lacalle, Iraizoz et al. 1993), abundant AD plaques and tau tangles (Standaert, Lee et al. 1991) and overall volume loss (Callen, Black et al. 2001). AD is associated with clinical symptoms referable to hypothalamic dysfunction including sleep and circadian rhythm disturbances (Wu and Swaab 2005), alterations in appetite and energy expenditure, and body wasting (Deshmukh and Deshmukh 1990; Ferrari, Arcaini et al. 2000). Thus, it is plausible that hypothalamic damage could result in clinically evident bone loss, given the central role the hypothalamus plays in regulating bone mass. The hypothalamus regulates bone mass through both a direct neural pathway and through the neurohumoral arm (Takeda 2005). Although, this study does not assess direct measures of hypothalamic dysfunction, there is abundant evidence of AD-related dysfunction in both the neurohumoral and neural arms. Dysfunction of the hypothalamo-pituitary axis and activity of the SNS are reported in AD. For instance, GH levels (Gomez 2008) are reduced in AD while interestingly a number of other hypothalamic factors are increased in AD, including corticotropin-releasing hormone (CRH) (Raadsheer, van Heerikhuize et al. 1995), cortisol (Rasmuson, Andrew et al. 2001); (Peskind, Wilkinson et al. 2001), FSH

(Bowen, Isley et al. 2000; Meethal, Smith et al. 2005), and TSH (de Jong, Masaki et al. 2007). Additionally, several studies demonstrate evidence of increased sympathetic activity (Pascualy, Petrie et al. 2000) including increased brain noradrenergic activity and elevated serum and cerebrospinal fluid levels of norepinephrine (Raskind, Peskind et al. 1984; Elrod, Peskind et al. 1997; Raskind, Peskind et al. 1999). Why increased activity has been observed in some aspects of the hypothalamic-pituitary axis in AD remains unclear but may be related to disturbed negative feedback (Elgh, Lindqvist Astot et al. 2006) while increased SNS activity may be related to overcompensation of remaining noradrenergic neurons in response to profound noradrenergic neuronal loss (Szot, Leverenz et al. 2000). Thus, further study should examine if hypothalamic structural change in AD alters neurohumoral systemic mediators of bone remodeling such as the pro-resorption factors (cortisol, FSH, and TSH, and sympathetic output) and bone formation factors (GH).

Additionally, common factors, such as vitamin D deficiency, have been linked to both AD (Oudshoorn, Mattace-Raso et al. 2008; Annweiler, Allali et al. 2009; Masoumi, Goldenson et al. 2009) and bone loss (Holick 2006; Holick 2007; Holick 2007). Recent discovery of vitamin D receptors in rodent(Jirikowski, Kauntzer et al. 2009) and human hypothalamus (Eyles, Smith et al. 2005) indicates that vitamin D may affect hypothalamic function or serve as central neuroactive substance. The possibility that vitamin D deficiency contributes to AD pathology, hypothalamic dysfunction and subsequent bone loss should be explored in the future studies.

It is important to recognize that the cross-sectional design of the study limits our ability to examine temporal development and cause-effect relationship between BMD and regional brain volumes. Additionally, the role of AD severity in this relationship should be more precisely defined in future studies. In addition, given that our study participants are a convenience sample,

it remains possible that our results may be influenced by sampling bias. Our data suggest that the association between BMD and total hypothalamic volume may be accentuated in more advanced AD, with a larger relationship observed in more advanced AD (CDR 1 vs. CDR 0.5). Although, our very early AD group is one of the strengths of the study, the relatively narrow range of dementia severity limits our ability to examine bone loss - regional brain volume relationship in more advanced stages of AD.

In conclusion, to our knowledge, this is the first study to demonstrate that lower BMD is associated with lower hypothalamic volume in early AD, suggesting that central mechanisms may contribute to AD-related bone loss. Bone loss in AD is an increasingly important clinical problem that may thus require different treatment approaches and preventive strategies as central mechanisms of bone remodeling may be disrupted by neurodegeneration.

Table 3. 1 Sample characteristics

	ND n=55	AD n=63	p-value
Age (years)	73.2 (6.3)	74.4 (6.5)	0.30
Females (n)	32	39	0.68
MMSE	29.5 (0.7)	26.1 (3.7)	<0.001
BMD (g/cm^2)	1.16 (0.12)	1.11 (0.13)	0.03
WBV (cm^3)	945.6 (87.2)	861.4 (93.6)	<0.001
GMV (% of ICV)	43.0 (2.1)	40. 7 (2.4)	<0.001
WMV (% of ICV)	34.8 (1.8)	34.5 (2.3)	0.42
Total Hypothalamic volume (cm^3)	0.65 (0.12)	0.57 (0.10)	<0.001

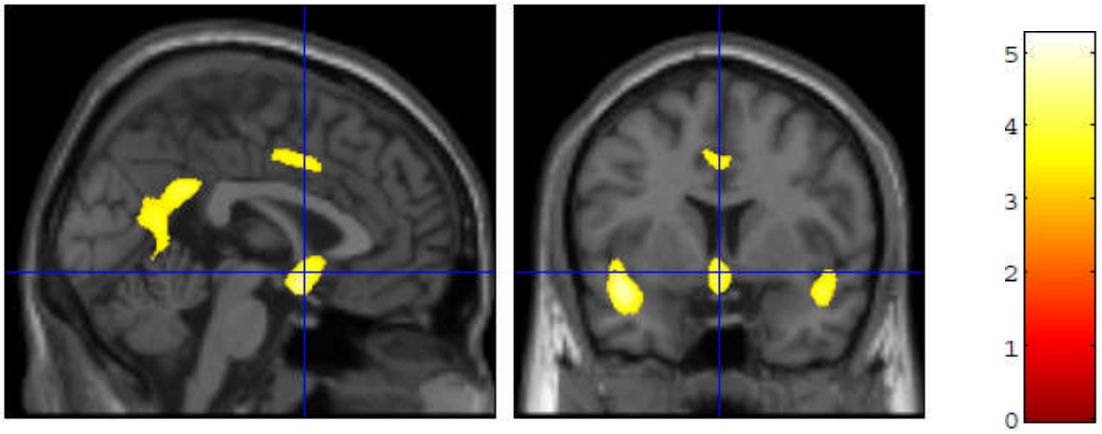
MMSE- Mini-Mental State Examination ; BMD- whole body bone mineral density; WBV- normalized whole brain volume in cm^3; ICV- total intracranial volume; GMD-grey matter volume; WMV- white matter volume.

Table 3. 2 Positive correlations between BMD and grey matter volume in the participants with AD with adjustment for age and gender

Cluster	K	Anatomical Region	Talairach Region	Talairach coordinates in MNI space			Z	P Value	
				x	y	z		FWE	uncor
1	17864	Parietal	L Inferior Parietal lobule, BA,40	-60	-47	22	4.74	0.03	<.001
		Temporal	L Superior temporal gyrus	-43	11	-15		0.11	<.001
2	3750	Limbic	L Precuneus Posterior Cingulate, BA23	-5	-57	12	4	0.42	<.001
3	2222	Limbic	L Parahippocampal Gyrus, BA 34	-12	1	-20	4.31	0.16	<.001
		Subcortical	Hypothalamus	-1	3	-15	4.11	0.3	<.001
4	1509	Limbic	R Cingulate Gyrus	2	-1	42	3.54	0.9	<.001
		Limbic	L Cingulate Gyrus, BA 32	-5	6	43	3.49	0.93	<.001
		Frontal	R Medial Frontal Gyrus, BA 9	8	27	34	3.48	0.94	<.001
5	390	Occipital	L Middle occipital gyrus	-49	-69	-15	3.45	0.95	<.001
6	143	Limbic	R Parahippocampal/ Fusiform	22	-44	-13	3.43	0.96	<.001

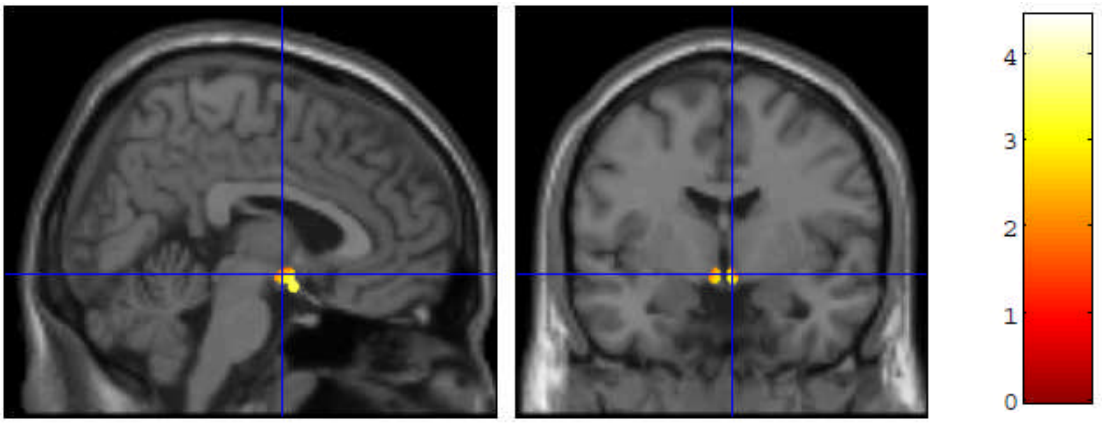
Only clusters for the voxel-wise analysis with k>100, Z>3.4 and p uncorrected <0.001 uncorrected are listed; K-cluster size; L-left; R- right; BA-Brodmann area; MNI- Montreal Neurological Institute; FWE- Family-Wise Error; uncor-uncorrected p value.

Figure 3. 1 Whole brain VBM results in AD



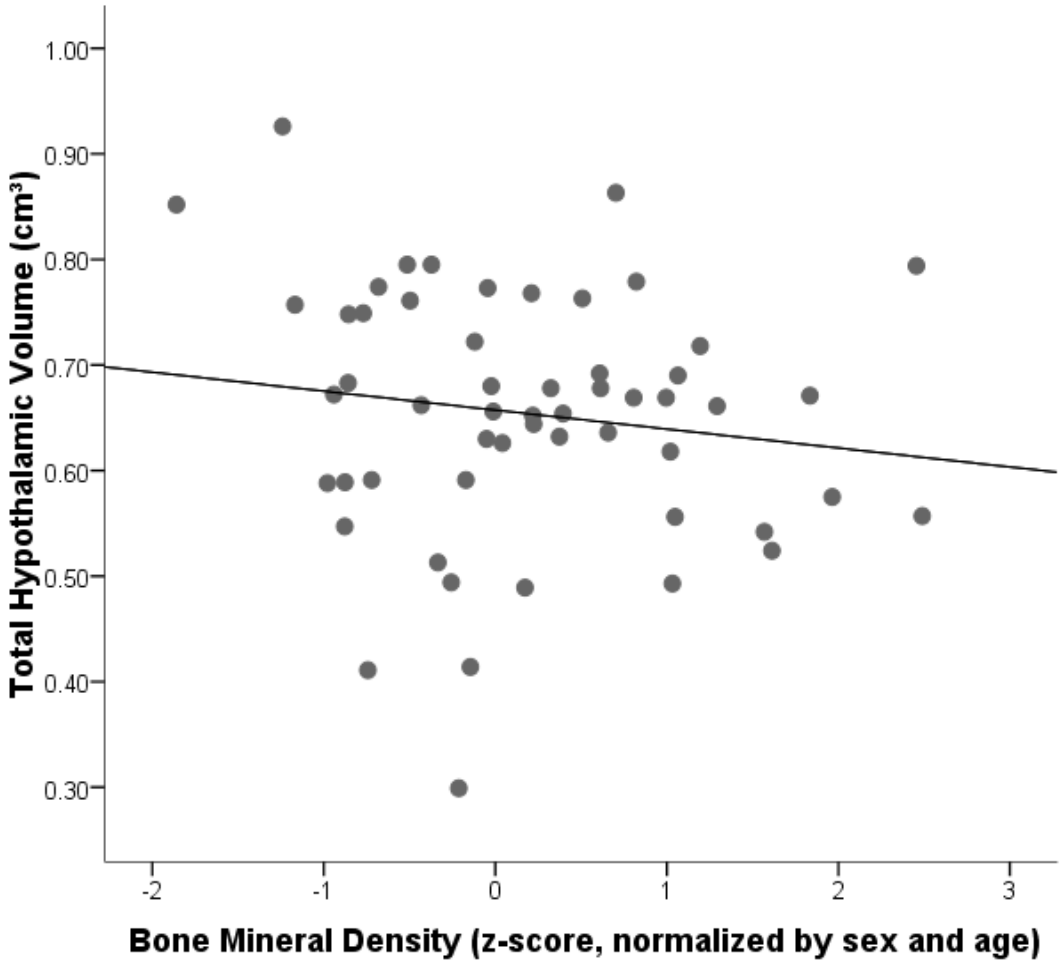
Areas in yellow indicate regions where grey matter volume correlates with bone mineral density in AD participants. Identified regions include the left superior temporal gyrus, left inferior parietal cortex (peak voxels in the left inferior parietal lobule [BA 40]), and limbic GM (peaks in the cingulate, parahippocampal gyri, and the hypothalamus). Analyses were controlled for age and sex and reported as significant at $p<0.001$ uncorrected (see Table 2). The coronal image is presented in neurological orientation (left is left).

Figure 3. 2 Hypothalamic volume correlates with BMD in AD



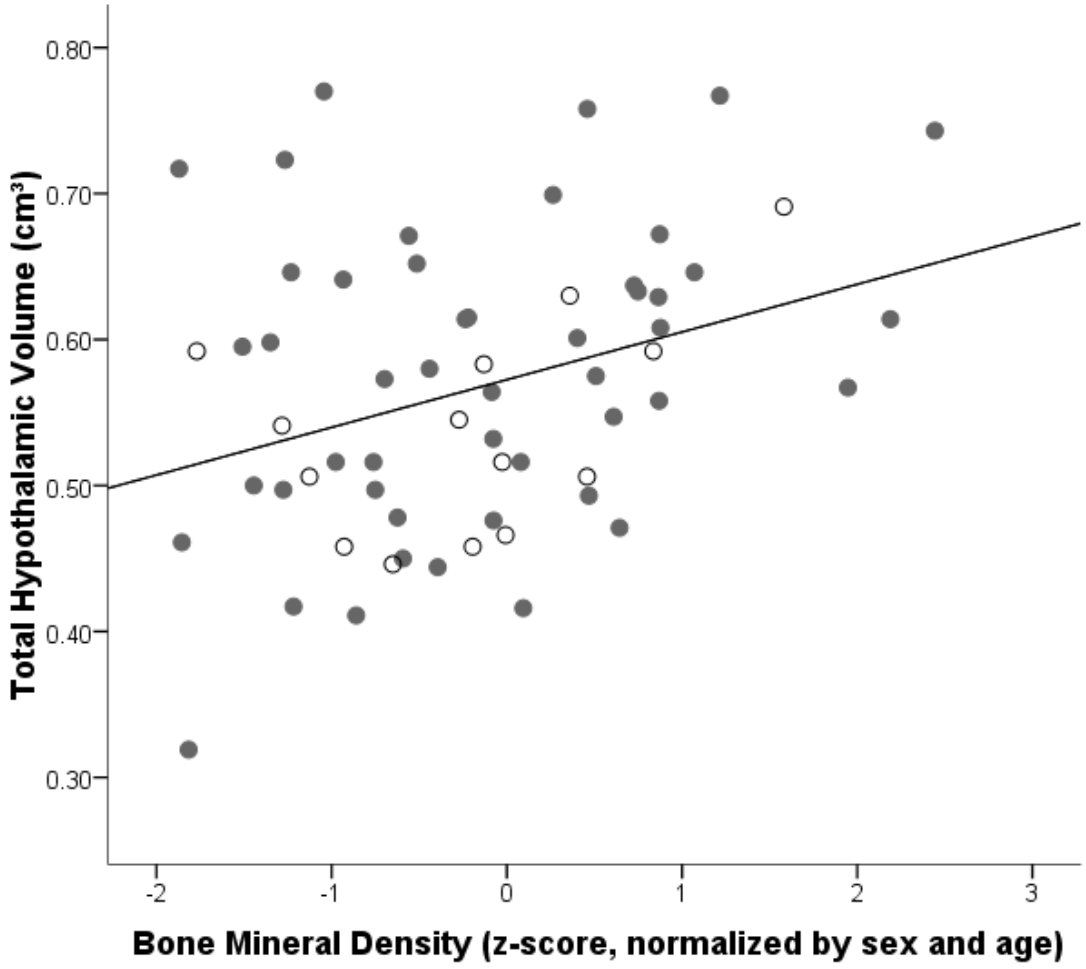
Small volume correction analyses confined to the hypothalamus demonstrate a significant positive correlation between whole body bone mineral density and the hypothalamic volume after controlling for age and sex ($p<0.05$ FWE) in the early AD group. The coronal image is presented in neurological orientation (left is left).

Figure 3. 3 Hypothalamic volume and BMD in non-demented



Insignificant relationship between whole body bone mineral density and total volume of the hypothalamus (b0=0.79, b1=-1.2, p=0.3) in non-demented group.

Figure 3. 4 Hypothalamic volume and BMD in AD



Positive relationship between whole body bone mineral density and total volume of the hypothalamus (b0=-1.93, b1=3.4, p=0.007) in the early AD group (clinical dementia rating (CDR) 0.5 (filled circles) and CDR 1.0 (open circles).

Chapter 4 Preface

In Chapter 3 we demonstrated, by using voxel-based morphometry (VBM) automated neuroimaging analysis that hypothalamic volume was associated with whole body BMD, and lower hypothalamic volumes correlated with reduced BMD in the AD group. VBM is a powerful technique for systematic evaluation of the whole brain and statistical analysis. This method has gained substantial popularity in neuroimaging studies.

Generally in the neuroimaging field, manual volumetry, or manual tracing of a brain structure by a trained human operator, is regarded as a "gold standard" while automated methods are often referred to as a "black box". There is legitimate concern particularly in regards to face, or anatomical, validity of automated methods. Given that VBM also allows measuring volumes of different brain regions including the hypothalamus we were interested in testing and comparing its performance with "gold standard" manual volumetry of the hypothalamus in application to AD research. Chapter 4 presents the results of a cross-sectional study that was designed to provide guidance for method selection in future neuroimaging studies of the hypothalamus.

Chapter 4

Cross-sectional estimation of the hypothalamic volume by atlas-based VBM compared to manual tracing in application to Alzheimer's disease

Loskutova N, Honea R, Brooks W, Burns J (to be submitted to *NeuroImage*, 2011).

4.1 ABSTRACT

The hypothalamus is a key brain structure that modulates a variety of neuroendocrine and regulatory processes including appetite, energy expenditure, sleep, wakefulness, stress responses and mood and memory, all of which are disturbed in Alzheimer's disease (AD). Clinical and pathologic evidence suggests that the hypothalamus might be affected by AD, however few studies used neuroimaging to assess anatomical changes in the hypothalamus. In this study, we compared the hypothalamic volumes estimated by voxel-based morphometry (VBM) and manual volumetry for 19 participants with early AD and 19 non-demented controls. Manual tracing was performed on unmanipulated T1-weighted images (intra-rater ICC for the hypothalamus was 0.89). The correlation between manual (adjusted for the head size) and atlas-based VBM volumetry total hypothalamic volumes was $r= 0.57$ $p<0.001$. Atlas-based VBM hypothalamic volumes were larger than manual volumes (614.0±104.0 vs. 333.4±49.9 mm3). Total hypothalamic volume was larger in nondemented vs. AD when using atlas based VBM methods ($p<0.001$), but no group differences were observed with manual tracing ($p=0.42$). Clinical measures of memory and cognitive performance correlated with hypothalamic volumes extracted by atlas-based VBM but not with manual volumetry results. Atlas-based VBM shows promise as a useful tool for regional volumetry of the hypothalamus, as it correlated with clinical measures, detected group differences and had fair agreement with manual tracing of the hypothalamus in a cross-sectional study.

4.2 INTRODUCTION

Alzheimer's disease (AD), the most common cause of dementia, is associated with pathological and functional changes in the hypothalamus (Saper and German 1987; Standaert, Lee et al. 1991; Chouinard, Lavigne et al. 1998; Pascualy, Petrie et al. 2000; Volicer, Harper et al. 2001). The hypothalamus, the ventral-most part of the diencephalon, contains at least 12 defined nuclei and has extensive connections within the brain. The hypothalamus regulates a variety of neuroendocrine, autonomic and somatic functions and is involved in memory and mood processing. Senescence and AD-related decline are associated with dysregulation and decline in hypothalamic function with subsequent decline in pituitary, gonadal and adrenal functions, however, cause – effect relationship between hypothalamic dysfunction and AD remains unclear (Gil-Bea, Aisa et al. ; Atwood, Meethal et al. 2005).

Aging is associated with decline in the somatotropic axis and mild partial growth hormone (GH) deficiency (Giordano, Lanfranco et al. 2005). In AD, profound pathological changes such as neuronal loss and plaque and tangle formation are reported in most of the hypothalamic nuclei (Swaab, Goudsmit et al. 1992). Clinical symptoms of hypothalamic dysfunction and alterations in hypothalamic-pituitary axis have been consistently reported. Stimulation of GH release by hypothalamic GH- releasing hormone and GH-secretagogues has been suggested as a way to delay frailty in older adults (Giordano, Bonelli et al. 2008; Hersch and Merriam 2008). Development of future AD treatments targets neurohormones and peptides, such as leptin, GH, GH secretagogues, and insulin-like growth factor I, that either act through the central hypothalamic mechanisms or are regulated by the hypothalamic and pituitary hormones (Carro and Torres-Aleman 2004; Vitiello, Moe et al. 2006; Gomez 2008; Signore, Zhang et al. 2008; Tezapsidis, Johnston et al. 2009). There is increasing interest in hypothalamic

neuroendocrine axis in aging and AD, although it remains unclear whether anatomical changes in the hypothalamus such as AD related atrophy underlie hypothalamic dysfunction.

High-resolution magnetic resonance imaging (MRI) allows an in vivo assessment of structural brain changes. MRI has the potential to capture structural changes in the hypothalamus. However, very few studies have used neuroimaging to assess anatomical changes in the hypothalamus in AD (Loskutova, Honea et al. ; Callen, Black et al. 2001; Copenhaver, Rabin et al. 2006). Hypothalamic imaging studies may be more difficult due to challenges in subcortical segmentation (classification of brain regions) (Fischl, Salat et al. 2002). Manual, semi-automated and fully automated approaches have been used in segmentation and volumetry of subcortical grey matter. Manual volumetry is still the most widely used method of hypothalamic volume estimation, although the well-recognized shortcomings of the method are numerous. Publically available automated methods (FIRS/FSL, FreeSurfer) developed for subcortical segmentation are limited in the number or regions that can be studied and do not separate the hypothalamus from the larger ventral diencephalon region of interest (ROI). Others (for example, Classifier Fusion and Labeling (CFL) or Profile Active Appearance Model (AAM)) are not freely available and require substantial technical and computational resources and expertise. Finally, automated methods, although highly reliable, are often questioned in regards to their face, or anatomical, validity. Concisely, there is little guidance in the literature for those interested in using neuroimaging for studying the hypothalamus and hypothalamic dysfunction in AD.

Voxel-based morphometry (VBM) has gained substantial popularity due to its unique quality of utilizing digital information available for each voxel and transforming it to estimation of grey and white matter volumes. VBM is a powerful technique for systematic evaluation of the

whole brain and statistical analysis that also allows measuring volumes of grey and white matter in semi-automated region-of-interest (ROI) approach. Optimized VBM with Wake Forest University Pickatlas (WFU) toolbox also automatically generates segmented atlas ROI templates and summarizes all the voxel values into grey or white matter volumes within each ROI (Good, Johnsrude et al. 2001). The WFU Pickatlas incorporates a broad range of ROI templates including hemisphere, lobar, anatomical label, tissue-type and Brodmann area atlases (Maldjian, Laurienti et al. 2003; Maldjian, Laurienti et al. 2004). Publically available atlas-based VBM volumetry is suggested to be unbiased, objective, reliable, and comparable to manual tracing approach within the capabilities of most research groups (Gonoi, Abe et al.). The advantage of VBM as an automated technique is that it provides perfectly reproducible result on a given data set. This approach, however, does not require an *a priori* hypothesis or extensive training and knowledge of anatomy, and therefore is one of the main concerns. It is believed that small brain structures are particularly vulnerable to brain distortions introduced by necessary image manipulations in VBM. Nevertheless, VBM has been widely and successfully used for detecting volume differences in small areas of the brain affected by AD pathology. Unfortunately, very few studies have utilized VBM to study the volume, anatomy and function of the hypothalamus in AD (Whitwell, Petersen et al. 2007; Whitwell, Weigand et al. 2007).

It has been recommended that VBM and manual volumetry should be used in tandem; with VBM used as first-pass strategy for hypothesis generation which is followed by manual volumetry to test a specific hypothesis about certain anatomical regions (Giuliani, Calhoun et al. 2005). We previously demonstrated that VBM detected hypothalamic volume differences between cognitively normal older controls and participants with early AD (Loskutova, Honea et al.). The whole brain analysis was followed by the ROI analysis using WFU Pickatlas and small

volume correction to test a specific hypothesis regarding relationships between hypothalamic volume and function. The purpose of the present study was to test and compare manual volumetry of the hypothalamus and ROI analysis using VBM WFU Pick atlas in application to AD research. Specifically, the goals of the study were:

1. To address the lack of quantitative validation of volumes of the hypothalamus by comparing atlas-based VBM volumetry with manual volumetry obtained from unmanipulated images.
2. To assess the application of hypothalamic volumetry with hypothalamic structural changes in AD and cognitively normal aging.
3. To assess the potential of the VBM WFU Pickatlas for regional volumetry of the hypothalamus by assessing face, or anatomical, validity of the VBM hypothalamic ROI.

4.3 METHODS

Sample and recruitment

Hypothalamic volumes estimated by atlas-based VBM volumetry were available for 63 participants with early stages of AD (Clinical Dementia Rating, CDR 0.5-1.0) and 55 non-demented elderly controls (CDR 0) as a part of the University of Kansas Brain Aging Project (BAP) as previously reported (Loskutova, Honea et al.). The power analysis showed that a total of 30 participants was sufficient to detect group differences in the hypothalamic volume obtained by atlas-based VBM volumetry between AD and non-demented aging. To account for possible exclusions, a random sample of 20 participants with early stages of AD and 20 non-demented elderly controls (total N=40) was selected from the available BAP pool for the purpose of this study. All participants had standard neurological and neuropsychological assessment, structural

MRI scanning and dual energy x-ray absorptiometry. Mini-Mental State Examination (MMSE) was administered as a standard measure of global cognition (Folstein, Folstein et al. 1975). The absence or presence and severity of AD were determined by a standard clinical evaluation that included the CDR and CDR sum of boxes score (Morris 1993). A psychometric battery including standard tests for memory, executive function and visuospatial ability was administered to every participant. Scores from all the tests in the psychometric battery were converted to z scores (higher scores representing better performance) and global cognitive (Global Cognitive index) and memory performance (Global Memory index) scores were computes as a mean of all z scores for each participant. Detailed clinical assessment methodology and clinical and neurological characteristics of the study participants have been presented previously (Burns, Cronk et al. 2008). Participants with a history of neurologic disease other than AD, diabetes mellitus, ischemic heart disease, schizophrenia, major depression, alcohol abuse, contraindications to MRI, and use of antipsychotic and other investigational medications were excluded from the study. Signed institutionally approved informed consent was obtained prior to enrollment from all participants or their legal representatives.

Magnetic resonance imaging and voxel-based morphometry

Structural MRI data were obtained at the Hoglund Brain Imaging Center with a 3.0 T Allegra MR scanner (Siemens Medical Solutions, Erlangen, Germany). T1-weighted magnetization prepared rapid gradient echo (MP-RAGE) sequence (1x1x1mm^3 voxels; TR = 2500ms, TE = 4.38ms, TI = 1100ms, FOV 256x256cm^2 with 18% oversample, flip angle 8 degrees) were collected and processed for VBM analysis. Every scan was examined for image artifacts and gross anatomical abnormalities. We used MRIcro® software to reconstruct raw

Dicom images. Data analysis was performed using the VBM5 toolbox (http://dbm.neuro.uni-jena.de), an extension of the SPM5 algorithms (Wellcome Department of Cognitive Neurology, London, UK) running under MATLAB 7.1 (The MathWorks, Natick, MA, USA) on Linux. Images were modulated and saved using affine registration plus non-linear spatial normalization (Wilke, Holland et al. 2008), resulting in final tissue maps of grey matter (GM), white matter (WM), and cerebrospinal fluid (CSF) and smoothed with a 10 mm FWHM Gaussian kernel. Additionally, for each study participant, total intracranial volume (TICV), total GM, WM, CSF, and whole brain volumes (GM plus WM) in cm^3 were computed using the normalized tissue maps of each study participant. Finally, the bilateral hypothalami were derived using ROI approach and the Wake Forest University Pickatlas toolbox (http://www.fmri.wfubmc.edu) (Maldjian, Laurienti et al. 2003) from the final grey matter tissue maps of each participant. Total hypothalamic volumes were computed as a sum of right plus left and reported in mm^3. The detailed procedure for MRI acquisition and VBM processing for the larger group of participants, 40 of whom were randomly selected for the current study, has been previously reported (Loskutova, Honea et al.).

Manual Tracing protocol

Manual tracing was performed on unmanipulated T1-weighted images using the ITK-SNAP software (version 1.9.10, www.itksnap.org) (Yushkevich, Piven et al. 2006). Before tracing, images were checked for alignment to AC-PC plane, rotation and tilt. Images were initially acquired in aligning to AC-PC plane minimizing the need for re-alignment and manipulations of the images. Thirty-eight images passed quality control and were included in the study. Two images were excluded based on midbrain artifacts that distorted the hypothalamus.

All traces were drawn blind to participants' group assignment by the same rater (NL). We used the tracing protocol and procedure manual previously outlined and published by Callen et al.(Callen, Black et al. 2001) but restricted it to the hypothalamus only. The hypothalamus was traced on enlarged coronal views with reference to sagittal and axial planes starting where individual columns of fornices appear (anterior to the mamillary bodies) and ending at the decussation of the anterior comissure. Medial boundaries were defined by the medial brain surface. Lateral boundaries were defined as a line connecting superior - lateral edge of the optic tracts with superior - lateral edge of the fornix. Inferior boundary was defined by the inferior brain surface and superior boundary was defined as a horizontal line from the medial brain surface to the bottom of the fornix. Traces included most of the anterior and tuberal regions and a portion of most anterior-inferior mamillary region and excluded the mamillary bodies and lateral hypothalamus. Detailed anatomical boundaries and operational instructions of the hypothalamic tracing protocol can be found elsewhere(Callen, Black et al. 2001). For each image, the total volume of the hypothalamus was estimated within demarcated area by the ITK-SNAP and reported in mm^3. Intra-rater reliability was assessed after repeated tracing of all selected images within one week interval by the same rater (NL). The volumetric results of the second tracing were used for further analyses.

Head size correction

Volumes obtained by the manual tracing were corrected by the total intracranial volume in order to correct for preexisting differences in head size in further statistical analyses. Total intracranial volume (TICV) computed by VBM was used for consistency of reference used by both VBM and manual methods. Average TICV for all participants was 1513.4 cm^3 and did not

differ between early AD and non-demented groups (p=0.82). The correction was made by dividing participant's uncorrected hypothalamic volumes obtained from manual volumetry by the TICV of each participant and multiplying the ratio by the average TICV of the sample. The group average TICV was selected based on no difference between AD and ND in our sample, which is consistent with previous findings.

Statistical analyses

Statistical analyses were conducted using SPSS (Version 17, SPSS Inc., Chicago, Ill). Continuous variables were summarized as means ± standard deviation (SD) and categorical variables were summarized by ratio. Independent samples t-tests and Mann-Whitney U test were used for analyzing group differences in continuous variables and chi-square statistics were used for categorical data. Bland & Altman test was performed using SPSS and custom syntax. Pearson, Spearman's rho and partial correlations were used to examine the associations between hypothalamic volumes of two methods and their associations with demographic and clinical variables. ANOVA was used to assess gender differences in hypothalamic volumes. Statistical significance was tested at a=0.05.

Agreement between automated atlas-based VBM and manual methods

The agreement between automated atlas-based VBM volumetry and manual methods was tested using:

1. Volumes

First, we used Pearson correlation coefficient to assess the strength of a relation between hypothalamic volumes obtained by atlas-based VBM volumetry and manual volumetry.

Additionally, we tested the group differences in the hypothalamic volumes estimated by each method and correlated hypothalamic volumes with demographic and clinical measures in AD and non-demented aging. We expected to find reduction in hypothalamic volume in AD and that hypothalamic volumes will correlate with clinical measures of dementia severity. This approach is widely used in reliability correspondence between methods in neuroimaging (Babalola, Patenaude et al. 2009).

2. Bland-Altman analysis

Next, we used Bland-Altman analysis to assess whether agreement between two methods is sufficient (Bland and Altman 1986). Bland-Altman analysis provides information about whether two measurements are comparable, or interchangeable, without considering either one as the gold standard. The results of Bland-Altman analysis are summarized in graphic representation and as the mean difference *d*, standard deviation of difference *s*, and the limits of agreement (*d-1.96s and d+1.96s*) between hypothalamic volumes obtained by atlas-based VBM volumetry and manual volumetry. Coefficient of reproducibility for two methods was computed as CR = 100*SD (diff)/Mean (averages).

Literature search

We performed a PubMed index search using following key words: “hypothalamus”, “volume” or “volumetry” and “manual”, “VBM” or “voxel-based morphometry”. The search was restricted to research reports of MRI human studies published in English. The results and methodology sections of relevant records were assessed for reports of the actual hypothalamic

volume and the method used. The results of eight articles included in the literature review are reported in Table 2.

4.4 RESULTS

Sample characteristics:

The final sample included 19 participants with early AD (17 participants with CDR 0.5 and 2 with CDR 1.0, average Sum of Boxes score = 2.95±1.1) and 19 non-demented controls (CDR 0). The groups did not differ in body composition, education, age and gender distribution. As expected, early AD group had lower MMSE scores (27.0. ± 3.2) compared to the non-demented controls (29.4 ± 0.7, p=0.002) and poorer Global Cognitive and Global Memory performance (Table 1). Whole brain volume was lower in AD (881.5 ± 82.5) compared to non-demented controls (943.3 ± 93.3, p=0.04) with no difference in the TICV between the groups (p=0.82).

Intra-rater reliability of manual volumetry:

In the analysis of intra-rater reliability of the tracing protocol, the ICC for the repeated hypothalamic tracings of all 38 images in the study was 0.89, Spearman-Brown split half coefficient was 0.90 and coefficient of variation was 8.5%.

Agreement between atlas-based VBM volumetry and manual volumetry

1. Volumes

In the overall group, atlas-based VBM volumetry yielded larger total hypothalamic volume (614.0±104.0 mm^3) than manual volumetry (333.4±49.9 mm^3). In the analysis of

agreement between the methods, the correlation between manual hypothalamic volume and hypothalamic volume estimated by VBM was r= 0.49, p=0.002. The correlation between total hypothalamic volumes of two methods improved after adjusting manual results for TICV (r= 0.57 p<0.001) (Figure 1). Correlation between atlas-based VBM and manual tracing was better in the non-demented r=0.71, p<0.001 than in AD r=0.42, p=0.08.

Bland-Altman Analyses

In the Bland-Altman plot, the mean difference *d* and standard deviation of difference *s* for hypothalamic volumes obtained by atlas-based VBM volumetry and manual volumetry was 277.5 mm^3 ± 85.83. The limits of agreement (*d*-1.96*s* and *d*+1.96*s*) for atlas-based VBM volumetry and manual volumetry were between 105.8 and 449.15 mm^3. The differences fell within limits of agreement that suggests acceptable degree of agreement between VBM and manual volumetry in overall sample and within groups. The coefficient of reproducibility for the two methods was 18.1 %. Atlas-based VBM volumes were systematically larger than volumes produced by manual tracing. There was a trend towards larger differences between methods for larger hypothalami and better agreement for smaller volumes (Figure 2).

Group differences and clinical and demographic measures

In the manual tracing protocol, although the absolute volumes of the hypothalamus were smaller in AD, there was no difference between AD and non-demented groups (340.1 ± 52.5 vs. 326.7±47.7, p=0.42, Table 1). Adjusting for TICV did not change the results (p=0.52). The mean hypothalamic volumes measured by atlas-based VBM volumetry were smaller in AD than in non-demented (560.5 ± 69.2 vs. 667.5 ± 106.8, p=0.001).

The hypothalamic volumes yielded by both methods did not correlate with total brain volume and age. There was no difference in hypothalamic volumes between men and women for either method.

The hypothalamic volumes measured by atlas-based VBM volumetry correlated with Global Cognitive Index ($r= 0.39$, $p=0.02$) and Global Memory Index ($r= 0.48$, $p=0.003$) and a trend was observed with MMSE ($r= 0.30$, $p=0.08$), after controlling for age and gender. There was no correlation between total manual hypothalamic volume adjusted for TIVC and any of the global cognitive measures.

Face validity

Visual inspection showed accurate hypothalamic ROI placement after inspection by a trained expert for all participants demonstrating good anatomical validity of the automated atlas-based VBM hypothalamic ROI, as the example in Fig 3 demonstrates.

4.5 DISCUSSION

We examined hypothalamic volumes derived from an atlas-based VBM approach and manual tracing in a mixed sample of participants with early stages of AD and non-demented controls. The results of our study demonstrate that the two methods showed an acceptable degree of agreement, although hypothalamic volumes measured by atlas-based VBM volumetry were systematically larger by a mean of 277.5 mm^3 than manual volumetry. The discrepancy between the two methods was not affected by the presence of AD, which suggests that these methods have comparable agreement in neurodegenerative disease and normal aging. In addition, atlas-

base VBM, but not manual volumetry, was able to detect group differences and correlated with clinical measures of cognitive performance.

Post-mortem data suggest that the hypothalamus is affected by AD pathology and is a site of abundant plaque and tangle formation and neuronal loss (Standaert, Lee et al. 1991; Swaab, Goudsmit et al. 1992). Clinical studies have reported various signs of hypothalamic dysfunction in patients with AD that may be due to pathological changes in the hypothalamus in AD (Elgh, Lindqvist Astot et al. 2006). We, therefore, hypothesized that pathological processes may be manifested as hypothalamic volume loss in AD and can be detected by neuroimaging volumetry. Atlas-based VBM volumetry detected differences in hypothalamic volume between early AD and non-demented groups while manual methods did not, suggesting that VBM is more sensitive to disease-related differences even in a relatively small sample. Numerous previous reports show that VBM is highly sensitive to brain volume changes in various disease conditions. It is further supported by the correlations of VBM derived volumes with clinical measures of cognitive and memory functions in our study. These correlations were not observed using manual volumetry of the hypothalamus. Our inability to detect hypothalamic differences with manual tracing may be explained by either insufficient power or that hypothalamic volume loss in AD occurs in neuronal populations not captured by manual ROI (Swaab, Goudsmit et al. 1992).

Manual volumetric approaches are regarded as a gold standard due to high anatomical or face validity. In manual volumetry, the MRI image is considered a true analogue of the neuroanatomy and accurate anatomic volume of a brain structure can be calculated by manual tracing on the MRI image of the brain *in vivo* (Bobinski, de Leon et al. 2000). For the automated methods such as VBM, the main concern in the literature is its questionable face validity. However, whether questionable face validity refers to possible displacement of an ROI due to

extensive image manipulations or inability to capture true anatomical volume of the structure remains unclear. Upon visual inspection of the hypothalamic ROI in the atlas-based VBM the placement was anatomically correct mostly covering the anterior, tuberal and anterior-most portion of mammillary regions of the hypothalamus.

According to the postmortem studies, the true volume of the hypothalamus is approximately 3.6 cm^3 (3600mm^3), or 0.3-0.5 % of the whole brain volume(Hofman and Swaab 1992). In our study, however, the region of interest in manual tracing was considerably smaller than the autopsy-defined anatomical volume of the hypothalamus. Since the manual tracing protocol that we used was limited to the anterior and tuberal regions of the hypothalamus, we expected somewhat smaller manual volumes. Such dramatic discrepancy with autopsy-defined hypothalamic volume, however, questions the ability of this method to serve as a gold standard in volumetric neuroimaging studies of the hypothalamus. Interestingly, most of the published neuroimaging studies we reviewed to determine the range of the hypothalamic volume used manual tracing approaches and none resulted in the hypothalamic volumes in its autopsy-defined anatomical range (Table 2). It is worth noting that numerous *in vivo* neuroimaging volumetric studies of the hippocampus have reported hippocampal volumes corresponding to its autopsy-defined anatomical volume range of 6.0-7.0 cm^3 (Walker, Highley et al. 2002) with both, manual and automated approaches (Geuze, Vermetten et al. 2005; Tae, Kim et al. 2008). Our results suggest that excellent anatomical validity of manual volumetry cannot be assumed for all brain structures, unless tested. Although the manual tracing is considered a gold standard in volumetric studies, a fundamental shortcoming of this method - the discrepancy with autopsy-defined anatomical volume of the hypothalamus should be addressed before it can be used as a reference or a gold standard method in future studies. Our results indicate that together with several other

properties of this approach, such as operator-induced error, time required for individual tracing of each set of brain slices, lower power in detecting group differences in smaller samples, and inconsistency of tracing protocols represent methodological limitations in using manual tracing for studying the hypothalamus in cross-sectional and potentially longitudinal studies.

The VBM approach has some advantages over manual tracing such as absence of contamination of the results by intra- or inter-rater errors, higher reproducibility of the results due to use of more standardized ROIs provided by WFU Pickatlas, and greatly reduced operator's time required to analyze a certain region. Additionally, the atlas-based VBM volumetry may have better applications in large-scale research due to higher sensitivity and power in detecting group differences than the manual tracing of the hypothalamus, and potentially other small subcortical structures. Results of our study should be interpreted with caution, however, due to well-recognized sensitivity of VBM to intra- and inter-scanner variations, difference in scanning protocols and contrast non-uniformity that may affect the GM probability of deep structures and final volumetric results (Huppertz, Kroll-Seger et al. ; Tardif, Collins et al.). This issue is especially important in longitudinal studies due to reported susceptibility of longitudinal VBM to false positives (Thomas, Marrett et al. 2009). Thus, longitudinal performance of atlas-based VBM of the hypothalamus and other deep structures needs to be addressed in future studies.

In conclusion, atlas-based VBM showed acceptable degree of agreement with manual volumetry, demonstrated good anatomical validity and better performance in detecting group differences and correlations with clinical measures of cognitive performance in a cross-sectional study. The atlas-based VBM volumetry shows promise as a useful tool for regional volumetry of the hypothalamus and has advantages over manual tracing as it is currently used.

Table 4. 1 Sample characteristics

	ND (n=19)	**AD (n=19)**	**P value**
Age, (y)	72.9 (6.0)	76.3 (6.2)	0.10
Gender (F/M)	12/7	12/7	1.0
Education (y)	16.5 (2.2)	15.0 (3.4)	0.11
MMSE	29.4 (0.7)	27.0 (3.2)	0.002
Global Cognitive Index (z)	0.04 (0.43)	-1.61 (1.16)	<0.001
Global Memory Index (z)	0.09 (0.79)	-1.82 (1.01)	<0.001
Body Mass Index (kg/m^2)	25.8 (3.7)	25.8 (3.1)	0.98
Gray Matter Volume (VBM), (cm^3)	501.7 (45.0)	450.4 (48.0)	0.002
Whole Brain Volume (VBM), (cm^3)	943.3 (93.3)	881.5 (82.5)	0.04
Hypothalamic Volume (VBM), (mm^3)	667.5 (106.8)	560.5 (69.2)	0.001
Hypothalamic Volume (Manual), (mm^3)	340.1 (52.5)	326.7(47.7)	0.42
Hypothalamic Volume (Manual normalized, mm^3)	343.3 (69.2)	329.10 (58.1)	0.52
Total Intracranial Volume (cm^3)	1520.5 (171.3)	1508.5 (150.3)	0.82

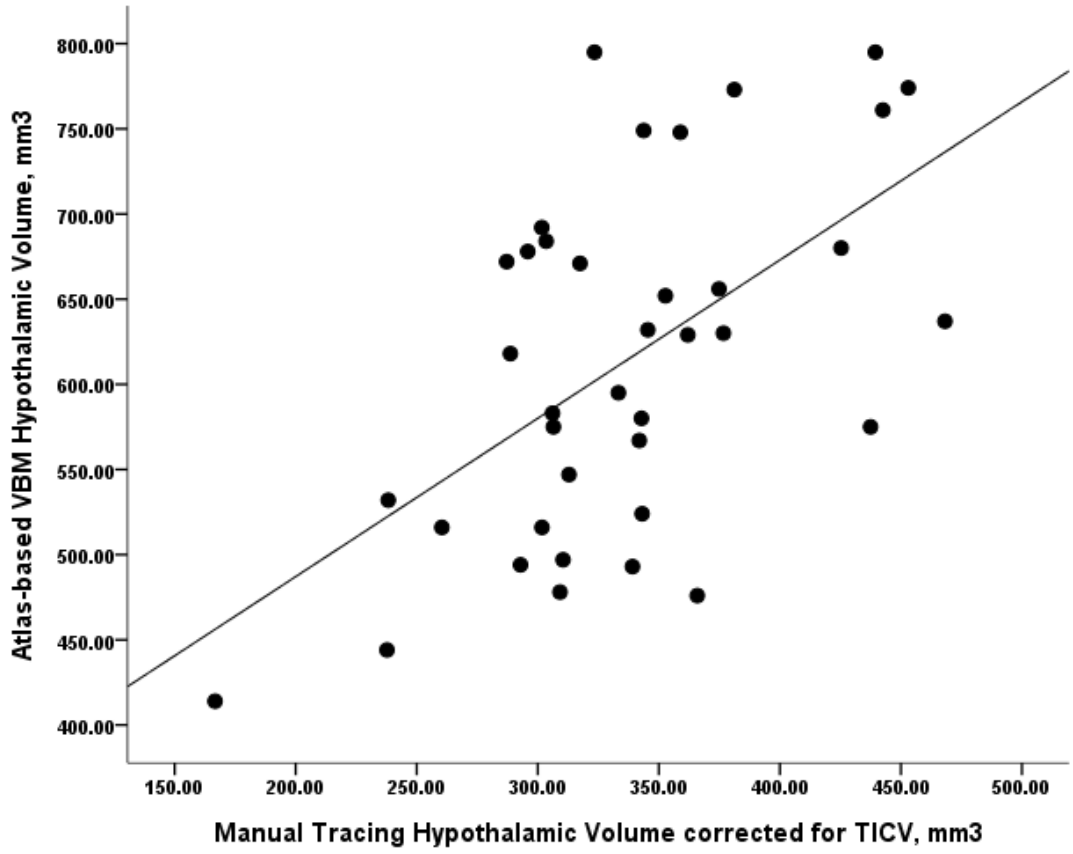
All data represent means (SD), unless otherwise noted. ND- non-demented controls, AD- Alzheimer's disease; MMSE- Mini-Mental State Examination; VBM – voxel-based morphometry**,** Manual normalized – normalized to total intracranial volume.

Table 4. 2 Summary of literature review

	Sample size	Controls Hypothalamic Volume	Study group Hypothalamic Volume	Condition	Method	Intra-rater Reliability / ICC	Group difference p value
Politis et al., 2008(Politis, Pavese et al. 2008)	N=18	728.5 ± 96.02 mm^3	609.89± 126.81 mm^3	Symptomatic Huntington's	Manual(Politis, Pavese et al. 2008)	Not reported	0.06
Koolschijn et al., 2008(Koolschijn, van Haren et al. 2008)*	N=44	1040± 140 mm^3	920 ± 140 mm^3	Schizophrenia	Manual(Pol, Cohen-Kettenis et al. 2006)	0.91	P<0.05
Goldstein et al., 2006 (Goldstein, Seidman et al. 2007)*	N=136	780 ± 160 mm^3 920 ± 110 mm^3	830 ± 170 mm^3 1000 ± 120 mm^3	Schizophrenia	Manual(Goldstein, Seidman et al. 2007)	Not reported (Inter- rater 0.81)	0.03
Callen et al., 2004 (Callen, Black et al. 2004)	N=80	298.6 ± 43.2 mm^3	266.5± 54.3 mm^3	AD	Manual(Callen, Black et al. 2001)	>0.90	0.002
Buskova et al., 2006 (Buskova, Vaneckova et al. 2006)*	N= 35	798 ± 930 mm^3	696± 920 mm^3	Narcolepsy/ cataplexy	In-house combination of VBM and manual		<0.01
Peper et al., 2010(Peper, Brouwer et al.)*	N=85	Healthy children Girls 1010± 90 mm^3 Boys 1050 ± 120 mm^3			Manual (Pol, Cohen-Kettenis et al. 2006)	0.86	N/A
Piquet et al., 2010(Piguet, Petersén et al.)	N=32	Anterior:156±39 mm^3 Posterior:193±43 mm^3	Anterior: 104 ± 22 - 119±32 mm^3 Posterior:104±46 - 149±52 mm^3	Fronto-temporal dementia	Manual (Piguet, Petersén et al.)	0.94	Anterior NS Posterior 0.04
Hulshoff Pol et al., 2006(Pol, Cohen-Kettenis et al. 2006)*	N=29	F: 1000 ± 50 mm^3 M: 1050± 180 mm^3	F: 930± 150 mm^3 M: 1070± 130 mm^3	Transsexual	Manual (Pol, Cohen-Kettenis et al. 2006)	0.86	NS

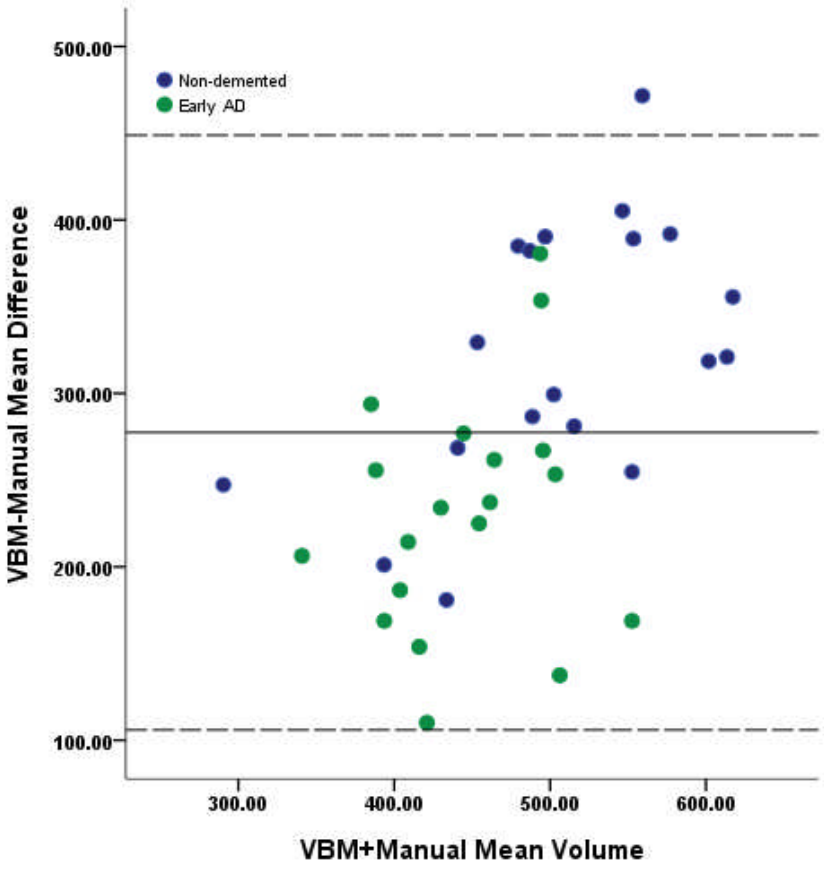
For articles denoted by * original reported units (ml or cc^3) were converted to mm^3. AD-Alzheimer's disease; ICC- intra-class correlation coefficient; F-females; M-males; NS – not significant; N/A – not applicable.

Figure 4. 1 **Correlation between methods**



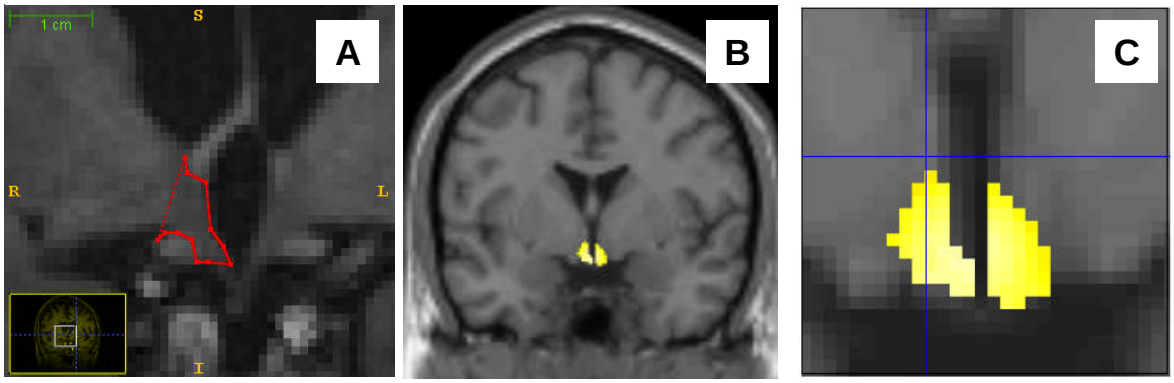
Fair correlation between hypothalamic volumes obtained by atlas-base VBM and manual volumetry ($r= 0.57$ $p<0.001$); TICV – total intracranial volume.

Figure 4. 2 Bland-Altman agreement between methods



Bland-Altman mean difference plot for hypothalamic volume demonstrates acceptable agreement between methods in overall sample and within groups. Atlas-based VBM volumes were systematically larger than volumes produced by manual tracing with a trend towards larger differences between methods for larger hypothalami and better agreement for smaller volumes.

Figure 4. 3 Face validity



Assessment of face validity. A: Example of manual tracing of the hypothalamus on one slice of an individual image. The same procedure was repeated for the opposite side on each slice (4-6 slices in total) within predefined boundaries. The total volume of the hypothalamus was calculated in mm^3 within demarcated area.

B and C: Colored area represents group average bilateral hypothalamic ROI from WFU Pickatlas overlaid on the standard MNI brain in the initial larger sample for which hypothalamic volumes were available. Forty atlas-based VBM volumetry results were randomly selected and 38 included in current report: right - enlarged image.

Chapter 5 Preface

In the previous chapters we demonstrated that: (i) BMD was reduced in the earliest clinical stages of AD in both men and women, (ii) BMD was related to whole brain volume and memory performance in AD, with higher BMD associated with higher whole brain volume (less brain atrophy) and better memory performance; (iii) hypothalamic volume was associated with whole body BMD, with lower hypothalamic volumes correlated with reduced BMD in the AD group by using VBM neuroimaging analyses; and (iv) established automated atlas-based VBM volumetry as a method of choice in studying the hypothalamic volume.

The findings in Chapters 2&3 suggest that AD-related brain changes specifically in the hypothalamus are associated with bone health in AD. Bone loss and brain atrophy, however, are dynamic and change over time. Findings from cross-sectional studies are meant to indirectly approximate longitudinal changes but their limitation to one time point restricts the ability to strongly conclude that a longitudinal relationship exists. Thus, we sought to extend our cross-sectional findings by assessing if similar relationships would be observed over a two-year time course. Longitudinal relationships between bone loss and hypothalamic atrophy and shared risk factors for AD and osteoporosis have not been examined in early AD.

In Chapter 5, we present results of a two-year longitudinal study which extends previous findings of an association between hypothalamic atrophy and bone density and explores predictors of bone loss in AD.

Chapter 5

Different Predictors Suggest Different Mechanisms of Bone Loss in Alzheimer's Disease Compared to Aging

Loskutova N, Honea R, Burns J (to be submitted to *Osteoporosis International*, 2011).

5.1 ABSTRACT

Objective: To assess the relationship of bone loss with brain atrophy and other osteoporotic risk factors including vitamin D levels, body composition and physical activity in Alzheimer's disease (AD) and aging.

Design: Two-year longitudinal case-control study

Participants: Individuals without dementia (Clinical dementia Rating, CDR 0, n=64) and with early stage AD (CDR 0.5 or 1, n=58) in the University of Kansas Brain Aging Project.

Main outcome measures: Participants were evaluated with dual energy x-ray absorptiometry (DXA), brain magnetic resonance imaging (MRI), neuropsychological testing and Physical Activity Scale for the Elderly (PASE) questionnaire at baseline and two-year follow-up. Body composition, brain atrophy, bone mineral density (BMD), physical activity, and baseline levels of 25 (OH)D (vitamin D) were measured.

Results: Bone loss was accelerated in AD compared with non-demented controls for the total body and legs. Vitamin D levels were lower and prevalence of vitamin D deficiency was higher in AD. Hypothalamic volume was lower in AD at both time points. For AD participants, bone loss was associated with baseline levels of the vitamin D and hypothalamic atrophy. For non-demented participants, bone loss was associated with age, female gender and decline in physical activity.

Conclusion: Risk factors associated with bone loss in non-demented aging were different in AD and suggest that neurodegeneration and vitamin D deficiency may contribute to bone loss in early AD.

5.2 INTRODUCTION

Alzheimer's disease (AD) and bone loss are two of the most common conditions associated with aging. Bone loss is associated with a higher prevalence of factures, in particular hip fractures (Weller and Schatzker 2004). Several large population-based studies reported an association between hip fractures and AD, placing dementia among the risk factors for fractures (Friedman, Menzies et al. 2010). On one hand, individuals in all stages of AD have increased incidence of fractures (Melton, Beard et al. 1994; Sato, Kanoko et al. 2004). Fractures in AD are associated with poorer recovery and up to four times higher mortality rate at 6 months compared to cognitively healthy older adults (van Dortmont, Douw et al. 2000; Weller 2000; Xie, Brayne et al. 2008). Lower bone mineral density (BMD) has also been reported in both men and women with AD (Sato, Honda et al. 2005; Loskutova, Honea et al. 2009). On the other hand, population studies suggest that low bone density, one of the major predictors of bone fractures (Ammann and Rizzoli 2003), and increased rates of bone loss are associated with higher risk of AD (Lui, Stone et al. 2003; Tan, Seshadri et al. 2005; Zhou, Deng et al. 2010). This evidence suggests that bone loss may develop years before the AD clinical diagnosis occurs and persists as dementia progresses. The cause - effect relationship between dementia and fractures remains unknown. There is a growing body of evidence that risk factors for bone loss including age, decline in physical activity, impaired nutritional status and deficiency in essential vitamins and microelements or genetic predisposition are also associated with AD (Friedman, Menzies et al. 2010). These factors are independently associated with both AD and bone loss but the causal relationship between AD and decline in bone health remains imprecisely defined.

Accumulating evidence suggests that the central nervous system directly regulates bone health through actions primarily orchestrated by the hypothalamus (Harada and Rodan 2003;

Takeda 2005; Zaidi 2007), a structure affected early in the AD process (Raskind, Peskind et al. 1999; Callen, Black et al. 2001). The central hypothalamic mechanisms are mediated by leptin, a fat derived hormone, and appear to affect cortical bone predominantly (Guidobono, Pagani et al. 2006; Murray, Adams et al. 2006; Martin, David et al. 2007; Martin, David et al. 2008). Therefore, we hypothesize that AD may influence bone density in cortical skeletal sites directly through pathological changes in the hypothalamus, the major central regulatory structure of bone remodeling. We previously reported lower BMD in early AD and suggested that brain atrophy, specifically of the hypothalamus, was associated with lower BMD in AD in a cross-sectional study. The purposes of this study were 1) to extend previous cross-sectional findings of an association between hypothalamic atrophy and bone density and 2) to explore predictors of bone loss in AD in a 2-year longitudinal study. Understanding the association between bone loss and AD in the context of shared risk factors will help to identify modifiable risk factors and interventions for reducing bone fractures in a high-risk population of older adults with dementia.

5.3 METHODS

Study participants

Data for this study were collected within the University of Kansas Brain Aging Project, a case-control study to evaluate the relationship between lifestyle factors and brain aging. Community dwelling participants 65 years and older residing in the Greater Kansas City Metropolitan Area were recruited from a referral based memory clinic and by media appeals. Signed institutionally-approved informed consent was obtained prior to enrollment from all participants or their legal representatives.

Seventy-one patients with early-stage AD (Clinical Dementia Rating (CDR) 0.5, n=57 and CDR 1.0, n=14) and 69 non-demented elderly control participants (CDR 0) were enrolled in the University of Kansas Brain Aging Project. All participants had standard neurological and neuropsychological assessment, structural MRI scanning and dual energy x-ray absorptiometry (DXA) at the baseline and follow-up after two years (mean follow-up time 2.1 [SD 0.2] years) of study enrollment. Blood samples were collected between 8 and 10 am after overnight fasting, processed and stored frozen at -80°C. Mini-Mental State Examination (MMSE) was administered as a standard measure of global cognition (Folstein, Folstein et al. 1975). The absence or presence and severity of AD were determined by a standard clinical evaluation that included the CDR and CDR sum of boxes score (Morris 1993). A psychometric battery including standard tests for memory, executive function and visuospatial ability were administered to every participant.

Baseline clinical and demographic characteristics of these individuals and detailed clinical assessment methodology have been reported previously (Burns et al., 2008; Honea et al., 2009)(Loskutova, Honea et al. 2009). Participants with a history of neurologic disease other than AD with the potential to impair cognition (i.e., Parkinson disease), current or past history of diabetes mellitus (defined as a clinical diagnosis, use of an ant-diabetic agent, or 2-hour post-load serum glucose > 199), recent history of cardiovascular disease (e.g. diagnosis of congestive heart failure, acute coronary artery event or angina in the 2 years previous to the baseline evaluation), major depression, use of investigational medications, significant visual or auditory impairment, systemic illness that may have impaired completion of the study, current or past history of alcoholism, and MRI exclusions (e.g. pacemakers) were excluded from the study at the baseline. Participant who did not return for the follow-up DXA assessment and non-demented

participants converted to AD at the follow-up clinical evaluation were excluded from this study. The final sample included 58 participants with early AD and 64 non-demented older controls.

Bone density

The BMD was measured for the total body by dual energy x-ray absorptiometry (DXA, Prodigy fan-beam densitometer, Lunar Corp., GE Medical Systems, Madison, WI). Regions of interest predominantly composed of either cortical (the legs and total body) or trabecular (the spine and pelvis) bone obtained from total body BMD measurements were also assessed. The precision of DXA equipment was assessed by two repeated measurements after repositioning by the same technician in 15 volunteers (74±11 years old, M/F=7/8) in the KUMC Precision Assessment of DXA Bone Mineral Density and Total Body Fat Percentage Testing study. Coefficients of variation (CV) were: 0.9 % for the total body and legs, 2.0% for the pelvis and 3.1% for the spine. The stability of the DXA equipment was checked according to manufacturer's instructions. BMD measures in absolute values (g/cm^2) at the baseline and two years were obtained and absolute bone loss in g/cm^2 and percent bone loss were computed.

Magnetic resonance imaging and hypothalamic atrophy

Structural MRI data were obtained at the Hoglund Brain Imaging Center with a 3.0 T Allegra MR scanner (Siemens Medical Solutions, Erlangen, Germany). T1-weighted magnetization prepared rapid gradient echo (MP-RAGE) sequence (1x1x1mm3 voxels; TR = 2500ms, TE = 4.38ms, TI = 1100ms, FOV 256x256cm2 with 18% oversample, flip angle 8 degrees) were collected and processed for VBM analysis. Every scan was examined for image artifacts and gross anatomical abnormalities. We used MRIcro® software to reconstruct raw

Dicom images. Data analysis was performed using the VBM5 toolbox (http://dbm.neuro.uni-jena.de), an extension of the SPM5 algorithms (Wellcome Department of Cognitive Neurology, London, UK) running under MATLAB 7.1 (The MathWorks, Natick, MA, USA) on Linux. Images were modulated and saved using affine registration plus non-linear spatial normalization (Wilke, Holland et al. 2008), resulting in final tissue maps of grey matter (GM), white matter (WM), and cerebrospinal fluid (CSF) and smoothed with a 10 mm FWHM Gaussian kernel. Additionally, for each study participant, total intracranial volume (TICV), total GM, WM, CSF, and whole brain volumes (GM plus WM) in cm^3 were computed using the normalized tissue maps of each study participant. Finally, the bilateral hypothalami were derived using ROI approach and the Wake Forest University Pickatlas toolbox (http://www.fmri.wfubmc.edu) (Maldjian, Laurienti et al. 2003) from the final grey matter tissue maps of each participant (Loskutova, Honea et al.). Total hypothalamic volumes were computed as a sum of right plus left and reported in cm^3. Same procedures were performed for the baseline and follow-up brain images. The hypothalamic atrophy was computed as difference in hypothalamic volumes between evaluations in cm^3.

Habitual physical activity level

We used the Physical Activity Scale for the Elderly (PASE) questionnaire to assess participants' level of physical activity. In individuals with mild dementia (Vidoni et al, in review), similar to non-demented older adults (Dinger, Oman et al. 2004), the PASE has been shown to be valid and reliable measure of habitual physical activity level. The PASE estimates levels of habitual physical activity based on 7 day reports of the frequency and duration of

participating in a variety of physical activities. The information provided by the study participants was used in the analysis. Change in the PASE score between visits was calculated.

Baseline vitamin D levels

Baseline serum level of 25-OHD (vitamin D) were obtained using the DiaSorin Liaison analyzer for 25-OH Vitamin D TOTAL assay using chemiluminescent immunoassay (CLIA) technology at the Clinical and Translational Research Center Core Lab at The Children's Hospital, University of Colorado Denver, Aurora, Colorado. Repeatability of the assay coefficient of variation in serum samples is 3.0%.

Statistical analysis

Statistical analyses were conducted using SPSS (PASW statists 18, SPSS Inc., Chicago, Ill). Continuous variables were summarized as means ± standard deviation (SD) and categorical variables were summarized by ratio. Independent samples t-tests and Mann-Whitney U test were used for analyzing group differences in continuous variables and chi-square statistics were used for categorical data. Pared t-test, repeated measures ANOVA or Wilcoxon Signed Rank exact test for two-related samples (longitudinal) two-tailed were used to determined change in measured variables over two years as deemed appropriate. Pearson and Spearman correlations were used to investigate the univariate relationships between predictors and bone density. Stepwise backward elimination linear regression was used next for multivariate analyses. All variables that correlated at a trend level ($p<0.10$) in the univariate analyses were included in the stepwise multiple regression analysis.

5.4 RESULTS

Bone loss is accelerated in AD

The baseline and follow-up characteristics for participants in the study are presented in Table 1. There was no difference in age and gender distribution between the groups at baseline and follow-up. BMD declined from baseline in both groups ($p < 0.05$) with greater bone loss in AD compared to the non-demented controls in total body (2.10% in AD vs. 0.95% in non-demented, p=0.03, *Figure 1*) and leg BMD (3.18% in AD vs. 1.68% in ND, p=0.03). There was no difference in the baseline BMD or rate of bone loss in the spine, trunk, or pelvis between the groups. Since gender is the main determinant of bone density and our sample was mixed, we next looked at the rate of bone loss in men and women with and without dementia separately. Significant decline in total BMD was observed in both men and women with AD ($p < 0.05$) and non-demented women ($p < 0.05$). BMD in non-demented men was unchanged (p=0.16) over two years. When compared within genders (*Figure 2*), women with AD lost more total body BMD than women without dementia (2.82% vs. 1.96%) over two years although this difference was not significant (p=0.16). Men with AD lost more total body BMD over two years (decline by 1.18%, p=0.003) than men without dementia. The total body BMD loss values were smaller in respect to the LSC (0.9*2.77=2.6%) in all but women with AD.

Decline in physical activity in both groups

The average baseline level of physical activity of the control group was comparable to that reported for community dwelling elderly persons in the US (Washburn, McAuley et al. 1999) (PASE score =130.7±55.5). Baseline habitual physical activity level was lower in AD

(102.8±76.5 p=0.02) than in non-demented. Both groups declined over two years and there was a trend to greater decline in physical activity in AD vs. non-demented (p=0.07).

Lower levels of vitamin D in AD

In the overall cohort 23.4% or participants were vitamin D deficient (<20 ng/ml) and 21.8% had sufficient levels of vitamin D (≥ 30 ng/ml), consistent with population studies (Holick 2007). As shown in *Figure3*, a higher prevalence of vitamin D deficiency was observed in the early AD group (N=17 (36.2%) in AD vs. N=7 (12.3%) in non-demented, p<0.05). Baseline serum level of vitamin D (25 OHD) was lower on average in early AD compared to controls (24.1±8.7 vs. 27.4±8, p=0.04).

Whole brain and hypothalamic atrophy

As expected, whole brain atrophy rate was higher in AD (2.2% vs. 1.2% in non-demented per year, p<0.001). Whole brain atrophy however did not correlate with bone loss in either group. Hypothalamic volumes were smaller in AD group at baseline (0.65 ±0. 10 cm^3 vs. 0.72 ± 0.12 cm^3 in non-demented, p=0.001) consistent with previously reported results (Loskutova, Honea et al.), and follow-up (0.54 ± 0. 82.1 cm^3 in AD vs. 0.58 ± 0.78 cm^3 in non-demented, p=0.006). Hypothalamic atrophy correlated with whole brain atrophy (r= 0.21, p=0.05) in the overall group but not when analyzed separately in non-demented and AD. Hypothalamic atrophy (volume difference between gender and age adjusted T1 and T2 hypothalamic volume) correlated with total BMD loss in AD group at a trend level r=0.29, p=0.07, but not in non-demented (r = - 0.005, p=0.97).

Changes in body composition

Groups were similar in measures of adiposity: BMI, percent body fat, and waist-to-hip circumference ratio between the groups at baseline. Over two years however, the AD group had a slight increase in the percent body fat (34.9 ± 11.1 vs. 34.0 ± 10.4 at baseline, $p<0.05$) and waist-to-hip circumference ratio (0.91 ± 0.07 vs. 0.87 ± 0.08 at baseline, $p<0.001$) while no change was found in non-demented controls.

Predictors of bone loss

We correlated changes in total body BMD with baseline measures of vitamin D, demographic characteristics, baseline levels of physical activity, two-year change in physical activity, adiposity, whole brain atrophy, and hypothalamic atrophy. Analyses were conducted within groups (AD and non-demented) due to group differences in bone loss across groups. Next, all variables that correlated with bone loss at a trend level ($p<0.10$): age, gender, and change in physical activity, hypothalamic atrophy and baseline levels of vitamin D were entered in a backward linear regression analysis. Backward linear regression analysis demonstrated (Table 2) that in the non-demented group, decline in the total body BMD was related to decline in physical activity ($\beta = 0.23$, $p = 0.09$), age ($\beta = -0.31$, $p = 0.02$) and female gender ($\beta = -0.32$, $p = 0.02$), together explaining 28% of total variance in bone loss.

In the AD group, decline in the total body BMD was associated with lower baseline vitamin D level ($\beta = -0.41$, $p = 0.02$, Figure 4) and hypothalamic atrophy ($\beta = 0.30$, $p = 0.06$, Figure 5), which together explained 23.1% of total variance in bone loss.

5.5 DISCUSSION

In non-demented aging, we found as expected that the canonical triad of age, female gender and physical decline predicted rate of bone loss, consistent with the well-established literature demonstrating these three factors as the major predictors of bone loss with age (Gudmundsdottir, Oskarsdottir et al. ; Bakhireva, Barrett-Connor et al. 2004; Havill, Mahaney et al. 2007). In early AD, however, bone loss was observed in both men and women and was not related to physical activity or age. Rather, bone loss in AD was associated with baseline vitamin D level and hypothalamic atrophy suggesting that mechanisms influencing bone loss in AD may be different form bone loss in normal aging.

Vitamin D availability is one of the major modulators of bone health (Holick 2006). It has been suggested that circulating vitamin D levels above 20 ng/ml are necessary to support normal bone health in the general population (Holick 2007). Our findings suggest vitamin D deficiency (circulating 25 (OH) vitamin D level below 20 ng/ml) is more prevalent in AD than non-demented controls. Low levels of Vitamin D have been associated with AD (Buell, Dawson-Hughes et al. ; Kipen, Helme et al. 1995; Sato, Asoh et al. 1998; Oudshoorn, Mattace-Raso et al. 2008), although whether vitamin D influences AD risk or, alternatively, AD related changes influence vitamin D levels or its metabolism remains unknown. Our data suggest that even though on average both groups fell into vitamin D insufficient category (circulating 25 (OH) vitamin D level between 20 and 30 ng/ml) the observed lower Vitamin D in AD may be clinically relevant given its linear association with BMD loss in this group of participants. Recent evidence indicates that in addition to its bone benefits in aging (Holick 2006), vitamin D has a positive effect on muscle mass, physical performance, cognition, inflammation, and neuronal survival (Adams and Hewison 2010; Nimitphong and Holick 2011). Therefore, our

observations may have therapeutic relevance as our data suggest vitamin D replacement may be relatively more important in AD, in particular AD men, though further randomized controlled trials will be necessary to determine this.

Bone loss in AD was observed despite an increase in measures of adiposity over two years. This is interesting given well-described associations between higher BMD in obesity and protective effects of fat on bone mass (Reid 2010). Why bone loss was observed in the presence of increasing adiposity is unknown. Bone loss in AD was not explained by actual changes in adiposity measures, suggesting that mechanisms mediating relationships between fat and bone may be different in AD and obesity. Several potential mechanisms could explain our observations. Leptin, a hormone produces by adipose tissue, is involved in regulation of bone remodeling and energy metabolism (Takeda, Elefteriou et al. 2002). Levels of leptin correlated highly with fat mass with increase in fat leading to elevated levels of leptin (Solin, Ball et al. 1997). Leptin via central regulatory mechanisms orchestrated by the hypothalamus inhibits bone formation (Elefteriou, Takeda et al. 2004). The precise mechanisms of bone loss in neurodegeneration however are still unclear and need further exploration. Previously published reports on pathological changes and atrophy in the hypothalamus, high levels of leptin and increased sympathetic tone in AD (Gil-Bea, Aisa et al. ; Standaert, Lee et al. 1991; Swaab, Goudsmit et al. 1992; de Lacalle, Iraizoz et al. 1993; Pascualy, Petrie et al. 2000; Callen, Black et al. 2001) together with our observations of a relationship between the hypothalamus atrophy and bone loss suggest a possibility that disruption of central hypothalamic regulatory mechanisms of energy expenditure and bone mass may be one of the potential mechanisms of bone loss in AD.

The current study is limited by its observational nature and future interventional studies will be necessary to define the mediating mechanisms of the relationship between bone density and AD. The participants in the study were a convenient sample of Caucasian race, which limits generalizebility of our findings. Also, the relatively small sample size could limit the power to observe group differences or relationships between important factors. Nevertheless, our data extend previous work and suggest that bone loss is accelerated in AD and hypothalamic atrophy and vitamin D insufficiency may contribute to bone loss in early AD.

Table 5. 1 Sample characteristics

	Non-demented (n=64)		Early AD (n=58)		Between group difference		
	baseline	2 years	baseline	2 years	baseline	2 years	Δ
Age,y	73.8 ± 6.8		75.4 ± 6.3		ns		
Gender, F/M	34/29		33/25		ns		
Vitamin D, ng/ml	27.5 ± 8.0		24.1 ± 8.7		*		
MMSE	29.4 ± 0.8	29.2 ± 1.3t	26.2 ± 3.1	22.4 ± 7.2***	***	***	***
Physical Activity	130.7 ± 55.5	121.8 ± 58.5	103.4 ± 75.9	75.6 ± 64.4**	*	***	t
BMI	26.2 ± 3.8	25.8 ± 3.8*	25.8 ± 3.8	25.9 ± 4.3	ns	ns	t
Body fat, %	33.6 ± 8.8	33.5 ± 8.9	34.0 ± 10.4	34.9 ± 11.1*	ns	ns	*
Waist/hip circumference	0.87 ± 0.10	0.88 ± 0.08	0.87 ± 0.08	0.91 ± 0.07***	ns	t	*
Bone density, g/cm^2					Δ p value		
Total body	1.16 ± 0.11	1.15 ± 0.12**	1.12 ± 0.12	1.10 ± 0.13***	*		
Legs	1.26 ± 0.16	1.24 ± 0.18***	1.21 ± 0.17	1.17 ± 0.18***	*		
Spine	1.09 ± 0.18	1.09 ± 0.20	1.05 ± 0.18	1.06 ± 0.18	ns		
Pelvis	1.09 ± 0.14	1.06 ± 0.17***	1.06 ± 0.14	1.04 ± 0.14*	ns		

Data represent means ± SD unless otherwise stated; AD – Alzheimer's disease; MMSE – Mini Mental State Exam;

BMI – body mass index; *P≤ 0.05; **P≤0.001; ***P≤0.0001, ^{t}P<0.10 – trend; ns P>0.10

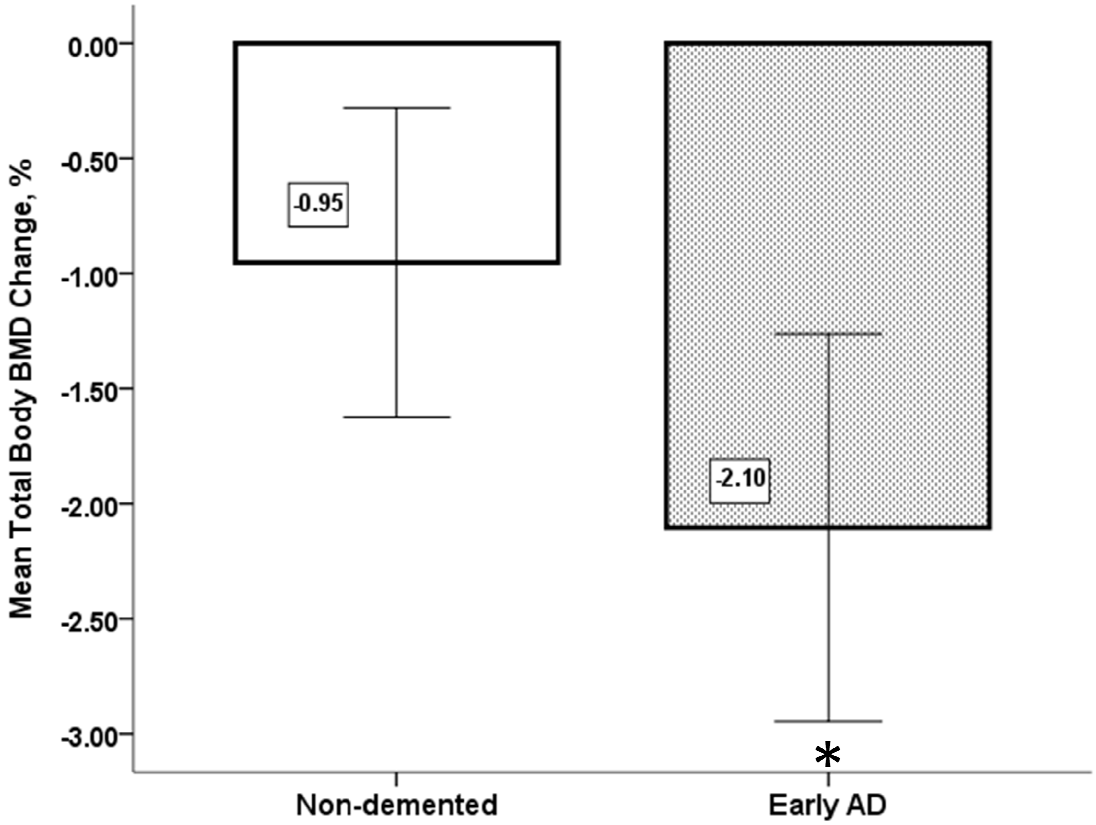
Table 5. 2 Predictors of bone loss

	Bone loss, ND (g/cm^2)	Bone loss, AD (g/cm^2)
Age	-0.31*	-
Gender	-0.32*	-
Δ Physical Activity	0.23^t	-
Baseline Vitamin D	-	-0.41*
Δ Hypothalamic Volume	-	0.30^t

Predictors of bone loss (standardized coefficients, β) in aging and early Alzheimer's disease

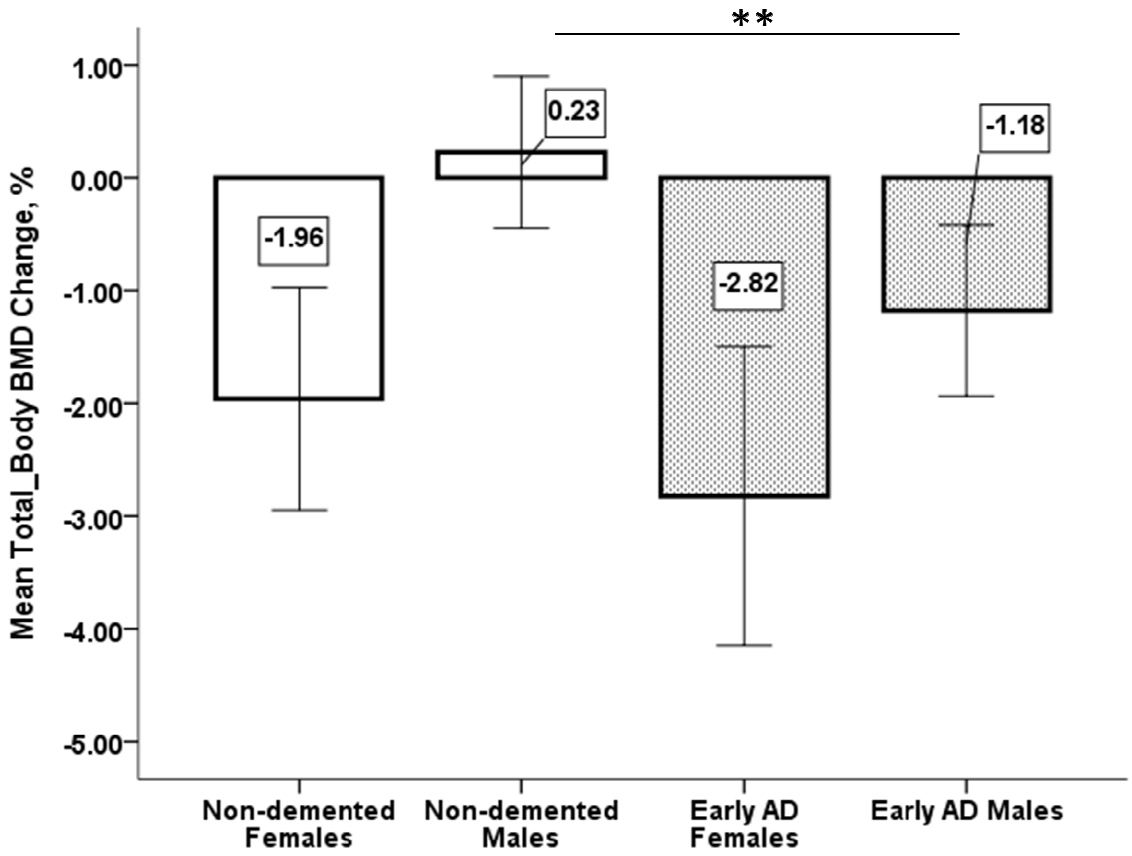
*P≤ 0.05; ^{t} P<0.10 – trend

Figure 5. 1 Two-year bone loss



Bone loss (percent from the baseline) is accelerated in AD compared to non-demented aging (*p=0.03).

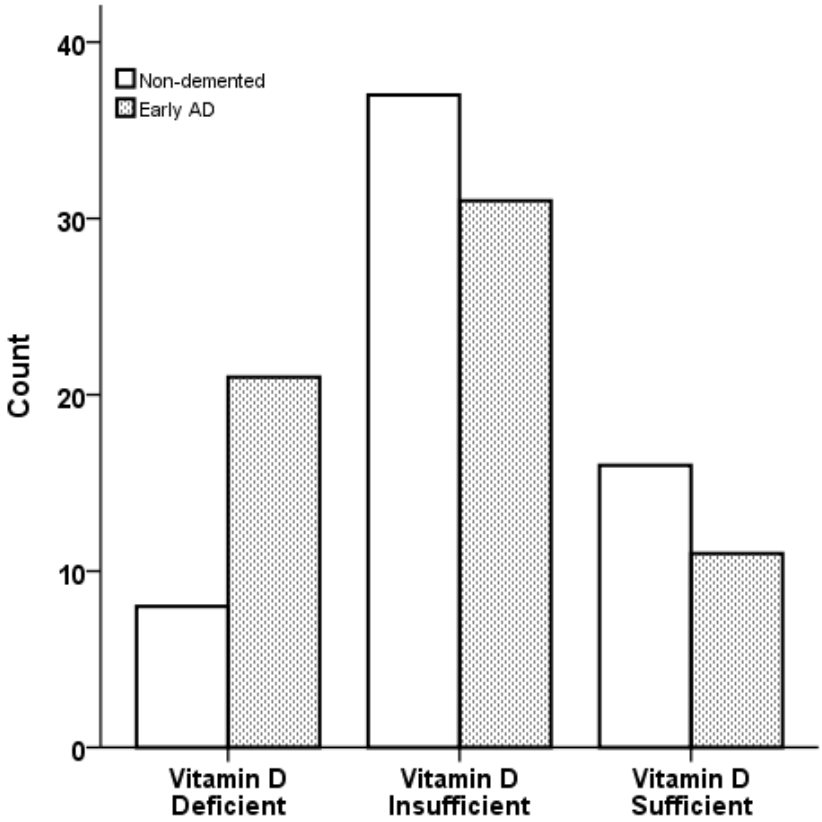
Figure 5. 2 Bone loss in women and men with and without AD



Bone loss in women and men with and without AD; bone loss in men is accelerated in AD.

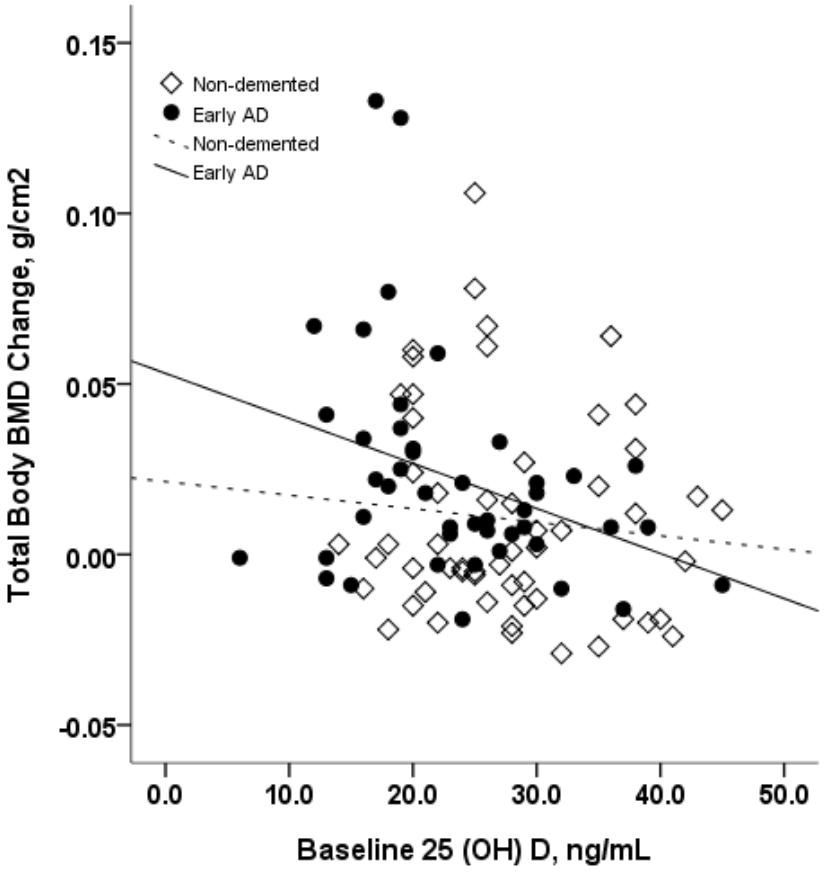
Greater bone loss was observed in men with AD compared to non-demented men (**p=0.003).

Figure 5. 3 Vitamin D status in non-demented and AD



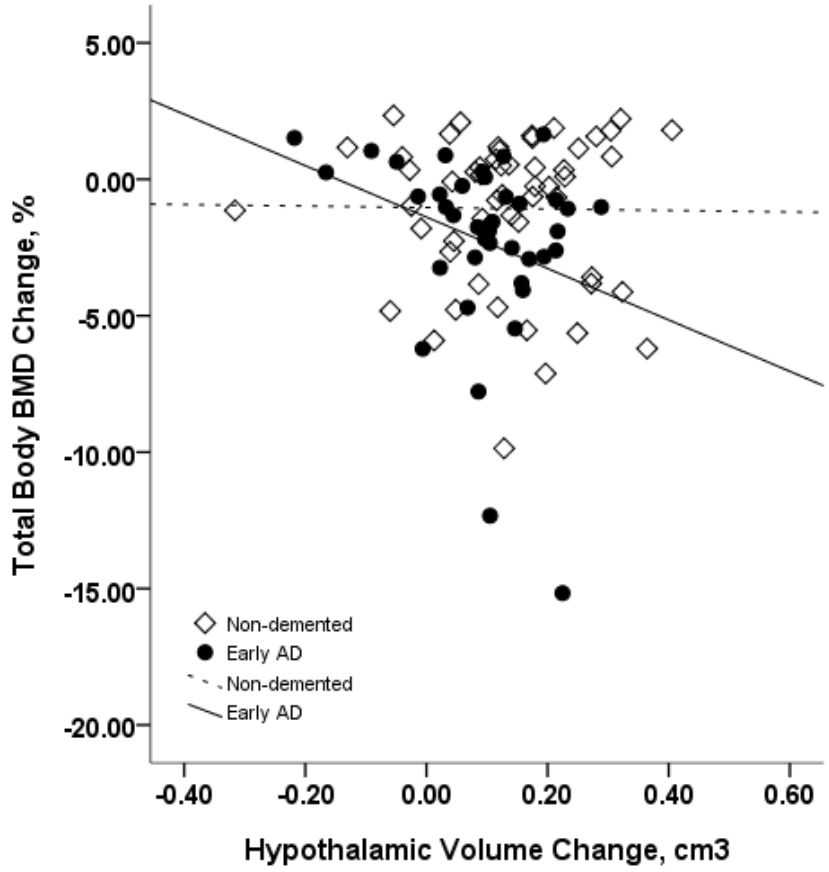
Prevalence of vitamin D deficiency is higher and adequate levels of vitamin D (sufficiency, 25 OH D >30ng/ml) are lower in AD. Higher numbers of participants with AD were vitamin D deficient (25 OHD <20 ng/ml) when compared to non-demented. More non-demented participants were either vitamin D sufficient or had intermediate vitamin D levels (25 OH D between 20 and 29 ng/ml, $p<0.05$ for all).

Figure 5. 4 Correlation of bone loss with vitamin D



Relationship between baseline vitamin D levels and total body bone loss in the non-demented (dotted line) and early AD groups (solid line). Higher vitamin D levels are associated with less bone loss in AD but not in non-demented controls.

Figure 5. 5 Correlation of bone loss and hypothalamic atrophy



Relationship between baseline hypothalamic atrophy levels and total body bone loss (%) in the non-demented (dotted line) and early AD groups (solid line). Greater hypothalamic atrophy is associated with higher percent bone loss from the baseline in AD but not in non-demented controls.

Chapter 6 Preface

In the previous chapter we reported that hypothalamic atrophy and vitamin D levels were associated with bone loss in early AD but not in non-demented aging. We also discussed several potential mechanisms to explain these relationships including the one that is the main focus of this work – involvement of central regulatory mechanisms mediated by neural (leptin) and neurohumoral (GH-IGF-1) factors. In Chapter 6 we extend our previous finding of an association between hypothalamic atrophy and bone loss and explore roles of leptin and IGF-1 as potential mediating factors between AD related hypothalamic atrophy and bone loss.

Chapter 6

Role of Central Hypothalamic Mechanisms in Bone Loss in Alzheimer's Disease: Testing a Path Model

Loskutova N, Burns J (to be submitted, 2011)

6.1 ABSTRACT

Aim: To test a model that assesses the role of hypothalamic atrophy in bone loss in Alzheimer's disease (AD) and potential mediators through neural (leptin) and neurohumoral (insulin-like growth factor –1, IGF-1) mechanisms.

Background: Animal studies provide strong evidence that the CNS directly regulates bone remodeling through the actions of the hypothalamus via two distinct pathways, the neural (mediated by leptin) and neurohumoral (mediated by neurohormones and growth factors) arms. The impact of AD on central regulatory mechanisms on bone mass is not known.

Methods: The proposed model was developed using a conceptual framework based on previously established evidence to assess the role of hypothalamic atrophy in bone loss. The data were collected as a part of the University of Kansas Brain Aging Project, a two year observational study of older adults with early stage AD and non-demented controls. Demographic characteristics and measures of bone density, body composition, and hypothalamic volume, serum levels of leptin, growth hormone, vitamin D and IGF-1 were collected. Data analysis included descriptive statistics, correlation techniques and path analysis.

Results: Hypothalamic atrophy had a direct effect on bone loss. The indirect effect was small due to absence of independent associations between IGF-1 or leptin with bone loss. Leptin increased over two years in AD and the increase in leptin was associated with both hypothalamic atrophy and declines in IGF-1.

Conclusions: The results of our study suggest that central regulatory mechanisms of bone mass may be disturbed by neurodegeneration and contribute to bone loss in participants in the early stages of AD. Further studies are needed to explore the role of brain atrophy in bone loss.

6.2 INTRODUCTION

Alzheimer's disease (AD) and bone loss are two of the most common conditions associated with aging. Low bone mineral density (BMD) and increased rates of bone loss are associated with cognitive decline and a higher risk of developing AD (Yaffe, Browner et al. 1999; Tan, Seshadri et al. 2005; Zhou, Deng et al. 2010). Individuals with all stages of AD have lower bone density and an increased incidence of fractures (Melton, Beard et al. 1994). Fractures in AD are associated with poorer recovery (van Dortmont, Douw et al. 2000) and up to four times higher mortality rate at 6 month compared to cognitively healthy older adults who suffer fractures (Nightingale, Holmes et al. 2001). Bone loss may occur years before the onset of cognitive manifestations and a clinical diagnosis of AD and appears to persist as dementia progresses. Multiple factors have been postulated to explain the association between bone loss and AD, including shared common risk factors, such as age, estrogen exposure, genetic predisposition, and low physical activity, nutritional, dietary, and environmental factors (Friedman, Menzies et al. 2010). These factors are independently associated with both AD risk and bone loss although the causal relationship between AD and decline in bone health remains unclear.

Animal studies provide strong evidence that the CNS directly regulates bone remodeling through the actions of the hypothalamus via two distinct pathways, the neural and neurohumoral arms (Harada and Rodan 2003; Takeda 2005). Briefly, the neural arm involves hypothalamic control of bone remodeling through increased sympathetic nervous system (SNS) output mediated by leptin. Leptin's actions are mediated by its receptor in the the ventro-medial hypothalamus that regulates SNS output. Activation of the SNS reduces osteoblast proliferation and bone formation via β2 adrenoreceptors on osteoblasts. The neurohumoral arm involves

hypothalamic control of the anterior pituitary hormones, such as growth hormone (GH) and its executive mediator insulin-like growth factor-1 (IGF-1). IGF-1 plays an important role in the stimulation of osteoblastic function, bone matrix collagen synthesis and bone formation (Murray, Adams et al. 2006).

The evidence of AD-related dysfunction in both the neurohumoral and neural arms is abundant. Dysfunction of the hypothalamo-pituitary axis and activity of the SNS are reported in AD. For instance, GH and IGF-1 levels (Alvarez 2007; (Gomez 2008) are reduced in AD. Profound deficits have been reported in the IGF-1 receptors and signaling in the postmortem AD brains (Steen 2005, Moloney 2008). Additionally, several studies demonstrated evidence of increased sympathetic activity (Pascualy, Petrie et al. 2000) including increased brain noradrenergic activity and elevated serum and cerebrospinal fluid levels of norepinephrine (Raskind, Peskind et al. 1984; Elrod, Peskind et al. 1997; Raskind, Peskind et al. 1999). Current reports on association between leptin levels and BMD in humans however, are highly contradictory (Cock and Auwerx 2003) and range from negative (Oh, Lee et al. 2005; Maimoun, Coste et al. 2008) or no association (Di Carlo, Tommaselli et al. 2007; Peng, Xie et al. 2008) to positive relationships (Zoico, Zamboni et al. 2008; Oguz, Tapisiz et al. 2009), suggesting a clear need in further studies.

We previously demonstrated that low bone density and bone loss in AD were associated with atrophy of the hypothalamus and proposed a hypothesis that AD-related hypothalamic structural change alters neural and neurohumoral regulatory systems of bone remodeling and contributes to bone loss in early AD. The purpose of this study was to extend previous observations by assessing the roles of leptin and GH-IGF-1 in mediating relationship between hypothalamic structural changes and bone loss in AD.

6.3 DATA COLLECTION AND METHODS

Study participants and demographics

Data for this study were collected within the University of Kansas Brain Aging Project, a case-control, 2-year longitudinal study assessing the role of lifestyle factors in brain aging. Community dwelling participants 65 years and older residing in the Greater Kansas City Metropolitan Area were recruited from a referral based memory clinic and by media appeals. Signed institutionally-approved informed consent was obtained prior to enrollment from all participants or their legal representatives.

Seventy-one patients with early-stage AD (Clinical Dementia Rating (CDR) 0.5, n=57 and CDR 1.0, n=14) and 69 non-demented elderly control participants (CDR 0) were enrolled in the University of Kansas Brain Aging Project. All participants had standard neurological and neuropsychological assessment, structural MRI scanning and dual energy x-ray absorptiometry (DXA) at baseline and two years later (mean follow-up time 2.1 [SD 0.2] years). Blood samples were collected between 8 and 10 am after overnight fasting, processed and stored frozen at -80°C. Mini-Mental State Examination (MMSE) was administered as a standard measure of global cognition (Folstein, Folstein et al. 1975). The absence or presence and severity of AD were determined by a standard clinical evaluation that included the CDR and CDR sum of boxes score (Morris 1993). A psychometric battery including standard tests for memory, executive function and visuospatial ability was administered to every participant.

Baseline clinical and demographic characteristics of these individuals and detailed clinical assessment methodology have been reported previously as part of a larger cohort (Burns et al., 2008; Honea et al., 2009). Participants with a history of neurologic disease other than AD with the potential to impair cognition (i.e., Parkinson disease), current or past history of diabetes

mellitus (defined as a clinical diagnosis, use of an ant-diabetic agent, or 2-hour post-load serum glucose > 199), recent history of cardiovascular disease (e.g. diagnosis of congestive heart failure, acute coronary artery event or angina in the 2 years previous to the baseline evaluation), major depression, use of investigational medications, significant visual or auditory impairment, systemic illness that may have impaired completion of the study, current or past history of alcoholism, and MRI exclusions (e.g. pacemakers) were excluded from the study at the baseline. Participant who did not return for the follow-up DXA assessment and non-demented participants converted to AD at the follow-up clinical evaluation were excluded from this study. The final sample included 57 participants with early AD and 64 non-demented individuals.

Bone density and body composition

We used dual energy x-ray absorptiometry (DXA, Prodigy fan-beam densitometer, Lunar Corp., GE Medical Systems, Madison, WI) to assess body composition and total body BMD. The precision of DXA equipment was assessed by two repeated measurements after repositioning by the same technician in 15 volunteers (74±11 years old, M/F=7/8) in the KUMC Precision Assessment of DXA Bone Mineral Density and Total Body Fat Percentage Testing study. Coefficient of variation for the total body BMD was 0.9 %. The stability of the DXA equipment was checked according to manufacturer's instructions. BMD measures in absolute values (g/cm^2) at the baseline and two years were obtained. Bone loss was measured as an absolute bone loss in g/cm^2 (baseline minus follow-up measures, higher positive values = more loss) and Δ BMD in percent as $\Delta \text{ BMD} = (\text{BMD1} - \text{BMD0}) / \text{BMD0} \times 100\%$, where BMD0 is a baseline measure and BMD1 is a follow-up measure (higher negative values = more loss).

Magnetic resonance imaging and hypothalamic atrophy

Baseline and follow-up whole brain structural MRI data were obtained at the Hoglund Brain Imaging Center with a 3.0 T Allegra MR scanner (Siemens Medical Solutions, Erlangen, Germany). T1-weighted magnetization prepared rapid gradient echo (MP-RAGE) sequence (1x1x1mm3 voxels; TR = 2500ms, TE = 4.38ms, TI = 1100ms, FOV 256x256cm2 with 18% oversample, flip angle 8 degrees) were collected and processed for VBM analysis. Every scan was examined for image artifacts and gross anatomical abnormalities. We used MRIcro® software to reconstruct raw Dicom images. Data analysis was performed using the VBM8 toolbox (http://dbm.neuro.uni-jena.de), an extension of the SPM8 algorithms (Wellcome Department of Cognitive Neurology, London, UK) running under MATLAB 7.2 (The MathWorks, Natick, MA, USA) on Linux. Images were modulated and saved using affine registration plus non-linear spatial normalization (Wilke, Holland et al. 2008), resulting in final tissue maps of grey matter (GM), white matter (WM), and cerebrospinal fluid (CSF) and smoothed with a 10 mm FWHM Gaussian kernel. Additionally, for each study participant, total intracranial volume (TICV), total GM, WM, CSF, and whole brain volumes (GM plus WM) in cm^3 were computed using the normalized tissue maps of each study participant. Finally, the bilateral hypothalami were derived using ROI approach and the Wake Forest University Pickatlas toolbox (http://www.fmri.wfubmc.edu) (Maldjian, Laurienti et al. 2003) from the final grey matter tissue maps of each participant. Total hypothalamic volumes were computed as a sum of right plus left and reported in cm^3. Same procedures were performed for the baseline and follow-up brain images. The detailed procedure for MRI acquisition and VBM processing for the baseline brain images of study participants has been previously reported (Loskutova, Honea et

al.). The hypothalamic atrophy was computed as difference in hypothalamic volume in cm^3 (baseline minus follow-up, higher positive values=more atrophy).

Blood sampling and analysis

Venous blood samples were collected at the baseline and follow-up visits between 8 and 10 am after overnight fasting, processed and stored frozen at -80°C. Baseline serum level of 25-OHD were obtained using the DiaSorin Liaison analyzer for 25-OH Vitamin D total assay using chemiluminescent immunoassay (CLIA) technology. Repeatability of the assay coefficient of variation in serum samples is 3.0%. Total serum IGF-1 levels were determined using human IGF-1 ELISA kit (Diagnostic Systems Laboratories, Inc. Texas, USA) with sensitivity of15 ng/ml and precision of 5.0%. Leptin was determined by radioimmunoassay (RIA, Beckman Coulter, Inc., California, USA). The sensitivity of the essay is 0.5 ng/ml and precision is 5.9%. The Growth Hormone (GH, HGH) levels were determined by the Human Growth Hormone ELISA kit (Diagnostic Systems Laboratories, Inc. Texas, USA) with sensitivity of 0.03 ng/ml and precision of 5.0%. All analyses were done at the Clinical and Translational Research Center Core Lab and The Children's Hospital, University of Colorado Denver, Aurora, Colorado. Change (Δ) in leptin and IGF-1 was computed as an absolute change in ng/ml (baseline minus follow-up). For Δ leptin higher negative values = more increase in leptin levels; for Δ IGF-1 higher positive values = more decline in IGF-1 levels over two years.

Statistical Analysis

Statistical analyses were conducted using SPSS (PASW statists 18, SPSS Inc., Chicago, Ill). Continuous variables were summarized as means ± standard deviation (SD) and categorical

variables were summarized by ratio. Independent samples t-tests and Mann-Whitney U test were used for analyzing group differences in continuous variables and chi-square statistics were used for categorical data. Pared t-test, repeated measures ANOVA or Wilcoxon Signed Rank exact test for two-related samples (longitudinal) two-tailed were used to test change in measured variables over two years as deemed appropriate. Pearson and Spearman correlations were used to investigate the univariate relationships between variables.

Path analyses and hypothetical model

Development of the model of effects of neurodegeneration on central control of bone remodeling via neural and neurohumoral hypothalamic regulatory arms was based on a series of animal studies confirming such biological regulatory mechanisms (Takeda 2005), evidence of hypothalamic structural damage and dysfunction in AD and our prior observations of an association between hypothalamic atrophy and bone loss. The hypothetical model was tested by using path analysis. Path analysis is closely related to multiple regression analysis and referred to as causal modeling technique. The causal assumptions (what causes what) are shown in the path diagram by arrows. It is best suited for testing a complex *a priori* theoretical model and can be used to explain the interrelationships among a set of observed variables, as well as to evaluate the relative importance of each variable in the model in a certain observed variable, through the use of direct and indirect effects. This type of analysis allows exploring direct and indirect relationships even when one variable may not have a direct effect, but it may have an indirect effect. We tested the basic model of the hypothalamic control of bone mass in non-demented aging and in AD (Figure 1). The hypothetical model included direct effects of hypothalamic

atrophy on bone loss and indirect effects mediated by changes in neural (leptin) and neurohumoral (GH-IGF-1) regulatory arms of hypothalamic control of bone remodeling.

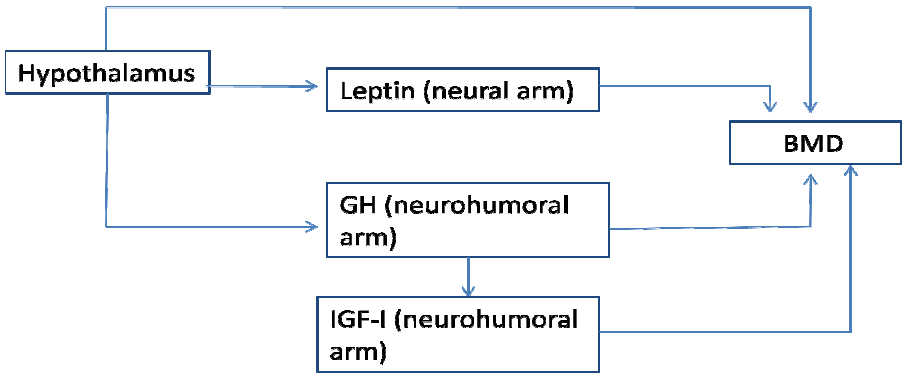


Figure 6. 1 Basic hypothetical model of the relationship between hypothalamic atrophy and bone loss; GH-growth hormone; IGF-1- insulin-like growth factor-1

The analysis was done using the Amos™ 17.0, an extension of SPSS statistical software (SPSS Inc., Chicago, Ill). The goodness-of-fit, standardized (β-values), and p-values are reported within each model. For relationships with known statistical and biological directionality, significance level set as one-tailed $p \leq 0.05$ was considered significant. The indicator of good fit of a model included a chi-squire goodness - of - fit, comparative fit (CFI) and normed fit (NFI) indices > 0.9, root mean square error of approximation (RMSEA) <0.05 and Hoelter's N >200.

6.4 RESULTS

Sample characteristics

The baseline and follow-up characteristics for participants in the study are presented in Tables 1 and reported in details previously (Chapter 5). There was no difference in age and gender distribution, between the groups at the baseline and follow-up. As previously reported,

BMD declined from baseline in both groups with greater bone loss in AD compared to the non-demented controls in total body BMD (2.10% in AD vs. 0.95% in non-demented, p=0.03).

There was no difference in the adiposity measures (BMI or percent body fat) at baseline between the groups. Over two years, however, the AD group had a slight increase in the percent body fat (34.8% ± 11.14 at follow-up vs. 34.0% ± 10.42 at the baseline, $p<0.05$). Change in percent body fat (Δ body fat) over two years differed between group ($p<0.05$). There was no difference between the groups at baseline in measures of serum leptin, total IGF-1, and growth hormone. Levels of IGF-1 declined slightly but equally over two years in both groups. Leptin levels in the control group remained unchanged over two years (13.1± 10.3ng/ml at baseline vs. 13.3 ± 10.0ng/ml at follow-up, p=0.84). In the AD group leptin levels increased significantly (14.9 ± 11.5 ng/ml vs. 21.9 ± 19.8 ng/ml at follow-up, $p<0.001$).

BMD and bone loss correlates

The relationships among body composition variables, serum levels of IGF-1, leptin, growth hormone and vitamin D, hypothalamic volume and bone density were analyzed using Pearson's correlation coefficients. Baseline and change over time correlates of total body BMD and ΔBMD are presented in the tables 2&3. Due to significant correlation between leptin and body fat, the levels of leptin were corrected for total fat mass in further analysis.

Path analysis

Based on the correlation matrix and the theoretical relationships among variables we tested the hypothetical model using simultaneous regression models in path analysis. As reported in our previous study, low baseline vitamin D levels (25-OHD) were associated with bone loss in

AD (Chapter 5). This relationship is clinically and biologically important, thus baseline vitamin D levels were added to the model. No relationship was observed between baseline growth hormone levels and the dependent variable (bone density or bone loss) or mediating independent variables (leptin, IGF-1 or vitamin D), therefore GH was excluded from the initially proposed model. The variables of interest met the normal distribution assumption and were entered into the initial full model based on the theoretical or clinical importance and for assessment of covariances and possible indirect effects. We compared path coefficients to explore relative effects of variables in the model on bone loss. We started with the model that tested direct effects of all four independent variables (hypothalamic atrophy, vitamin D, Δ leptin, and ΔIGF-1) on bone loss and additionally tested covariances in a stepwise manner. We found that hypothalamic atrophy and vitamin D had direct effects on bone loss while leptin change and IGF-1 change did not. Additionally, correlations existed between 1). hypothalamic atrophy and leptin change and 2). leptin change and change in IGF-1.

Next, we adjusted the model (Figure 2) and investigated whether hypothalamic atrophy had any indirect effect on bone loss through either changes observed in the neural arm (leptin change) or neurohumoral arm (IGF-1 change) independently.

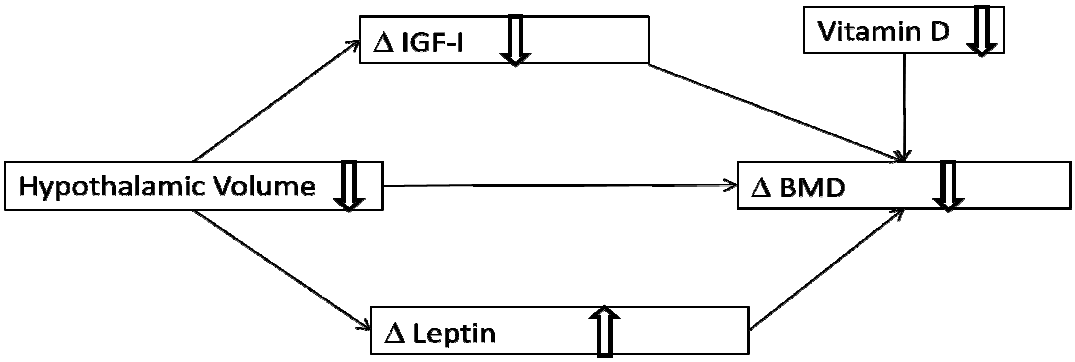


Figure 6. 2 Intermediate hypothetical model of the relationship between hypothalamic atrophy and bone loss; IGF-1- insulin-like growth factor 1, vitamin D was also included in the model.

As a result, no indirect effect of hypothalamic atrophy on bone was observed through either IGF-1 change or leptin change alone. Vitamin D demonstrated a direct effect only and did not mediate any other relationships in the model. Interestingly, in our model leptin change did not have a direct effect on bone loss, however, it independently correlated with change in IGF-1 and hypothalamic atrophy. Therefore, we tested an alternative biologically plausible model that examined the potential role of changes in leptin and IGF-1 on bone loss (Figure 3). The final model examined four predictors of bone loss (%, dependent variable, 2. Δ BMD). The predictors (or independent variables) included in the model were: 1. Hypothalamic Atrophy, 3. Vitamin D, 4. Δ Leptin, and 5. Δ IGF-1.

For the AD group, in this model 20 % of the total variance in bone loss (Δ BMD) was explained. Hypothalamic Atrophy had a direct effect (β = - 0.28, p=0.001) and a small indirect effects (β = -0.02) through both leptin and IGF-1 simultaneously on bone loss (Δ BMD). Indirect effects are obtained by multiplying the effects along each indirect path. Total effect of Hypothalamic Atrophy on bone loss (Δ BMD, [the direct effect of hypothalamic atrophy (β = -0.28) added to the indirect effect (β = -0.02) via modifying independent variables]) was (β =-0.30). Effects of Vitamin D were direct and independent of other independent variables in the model (β =0.29). In the model, Hypothalamic Atrophy explained 5% of the total variance in Δ Leptin. Increase in leptin (Δ Leptin) explained 17 % in total variance in decline in IGF-1 (Δ IGF-1). Relationship between change in IGF-1(Δ IGF-1) and bone loss (Δ BMD) was not significant, however. The alternative model provided a good fit for the data ($\chi 2$ = 2.0, df =5, p= 0.85; CFI = 1.0; NFI = 0.93; RMSEA <0.001 CI = 0.00-0. – 0.10; Hoelter's N = 313). None of the tested models fit the data in the non-demented group.

6.5 DISCUSSION

In our previous work, we demonstrated a relationship between bone loss and hypothalamic atrophy in the earliest clinical stages of AD in cross-sectional and longitudinal studies (Loskutova, Honea et al. 2009) and (Chapter 5). No association between hypothalamic volume and bone density or loss was observed in non-demented controls. Here, we tested a hypothesis that neurodegenerative changes in AD may influence bone mass via alteration in central regulatory mechanisms of bone remodeling. We observed moderate effects of hypothalamic atrophy on bone loss (total β=-0.30) with a direct effect due to unexplained factors and found evidence of small and incomplete indirect mediation through leptin and IGF-1. As expected, more atrophy in the hypothalamus was associated with: 1). accelerated bone loss and 2). increase in lepin over two years. The observed increase in leptin was also associated with decline in IGF-1, consistent with previous studies (Milewicz, Krzysiek et al. 2005).

Genetic and pharmacological manipulations in animal models are converging with clinical and epidemiological observations in humans to suggest the importance of central regulation of bone homeostasis through hypothalamic control via both neural pathways and hypothalamo-pituitary hormonal mechanisms (Takeda 2005). Leptin acts on the leptinergic/peptidergic neurons in the ventro-medial hypothalamus (VMH) to activate sympathetic nervous system (SNS) output. SNS output activates β -2-adrenoreceptors on the osteoblasts and results in decreased proliferation and bone formation. Additional mediators of leptin's effects on bone have also been identified and include serotonin, CART (cocaine amphetamine regulated transcript), Neuromedin U and neuropeptide Y (Wei and Ducy 2010). Peripheral circulating leptin levels may serve as a regulation threshold that initiates central cascade mechanisms through a variety of signaling mechanisms to negatively influence bone

formation via central regulatory control in the ventro-medial hypothalamus(Martin, David et al. 2008). The neurohumoral arm is an additional mechanism through which the hypothalamus controls bone remodeling via regulation of neurohormones and growth factors. Leptin influences levels of IGF-1 with numerous studies reporting an inverse relationship between leptin and IGF-1(Milewicz, Krzysiek et al. 2005; Martin, David et al. 2007). Various unobserved mechanisms involved in hypothalamic control of bone mass may explain the direct effect of hypothalamic atrophy and some of the unexplained variance in bone loss in AD.

Our initial understanding of separate neural and neurohumoral/hormonal mechanisms led us to hypothesize that the relationship between hypothalamic atrophy and bone loss would be mediated by two separate paths: through decline in IGF-1 and through increase in leptin. Our results however suggest that that relationship between hypothalamic volume loss and bone loss was not mediated by either an increase in leptin or decline in total IGF-1. Our results rather imply that hypothalamic atrophy was associated with an increase in leptin levels over two years that may have mediated a decline in IGF-1. In our study, the peripheral leptin levels increased significantly in AD participants but not in non-demented controls over two years. Levels of IGF-1 declined over time and, consistent with previous literature, were related to increases in leptin (Milewicz, Krzysiek et al. 2005). Why increased level of leptin were observed in our study remains unclear, but may be related to abnormal functioning of hypothalamus regulated timekeeping and clock genes (e.g. nocturnin) that are found to be involved in shifting differentiation of mesenchymal stem cells towards adipogenesis in aging (Kawai, Green et al. ; Kawai, Green et al. ; Kirkland, Tchkonia et al. 2002; Moerman, Teng et al. 2004).

Decline in IGF-1 however was not related to bone loss, which is interesting given well-described effect of IGF-1 on bone mass (Yakar, Courtland et al.). Lack of an association between

IGF-1 and bone has been reported in several studies. For example, in a study of 123 postmenopausal women IGF-1 did not have a direct effect on measured parameters of bone density or bone turnover (Martini, Valenti et al. 2001). We speculate that in our study this could be due to several possible mechanisms. It is possible that our model is missing an important link that mediated a relationship between bone and peripheral total IGF-1. Since we only measured total IGF-1 (a complex of free IGF-1 and its binding proteins), imbalance in the IGF-1 system components perhaps is more important in assessing anabolic effects of IGF-1 (Mohan, Farley et al. 1995). Highly elevated levels of IGF-1 binding proteins have been reported in AD (Tham, Nordberg et al. 1993). Reduced activity of IGF-1 may be due to increased levels of IGFBP. Thus, bone loss may still be observed regardless of relatively high levels of total IGF-1. Alternatively, IGF-1 resistance has been reported in AD (Salehi, Mashayekhi et al. 2008), which may explain observed relationships between greater bone loss and relatively high levels of IGF-1.

The results of our study should be interpreted with caution due to exploratory nature of the study. Our study was based on synthesis of animal literature and statistical modeling. Statistically modeled hypothetical causation may not be necessarily correct in a biological model and is most likely an oversimplification. Additionally, we did not measure levels of free IGF-1 in serum and levels of leptin or IGF-1 in the CSF and our conclusions were based on peripheral levels of leptin and IGF-1. Our measures were taken once in the morning and did not assess differences in fluctuations of these hormones. Much variance in bone loss remained unexplained by potential involvement of central regulatory mechanisms within our model, additional or alternative mechanisms may contribute to these relationships in the very early stages. We did not account for other potential mechanism such as change in sex hormones, actual activity of SNS,

detailed nutritional status and effects of other local and systemic mediators of bone remodeling. Another limitation of our study is relatively small sample size that does not allow us to explore the effects of gender on observed relationships.

This study extends previous findings by suggesting that bone loss secondary to AD may be mediated by neurodegenerative changes (atrophy) in the hypothalamus. Additionally, these changes may in turn initiate a cascade of events including increase in peripheral levels of leptin and decline in IGF-1. However the model of independent neural and neurohumoral arms that contribute to bone loss in individuals with AD is perhaps oversimplified. The co-dependent relationships among brain, bone, fat and metabolism should be further investigated in normal and pathological conditions in humans and animal models to gain a better understanding of these interrelationships and to provide a basis for clinical applications.

Altogether, we are the first to explore the potential effects of hypothalamic atrophy on bone and possible mechanisms of bone loss secondary to AD. We conclude that it is conceivable that central regulatory mechanisms of bone mass may be disturbed by neurodegeneration leading to bone loss in participants in very early stages of AD.

Table 6. 1 Sample characteristics at baseline and change over two years

	Baseline		Change over time (Δ, baseline minus follow-up)		p-value	
	Non-demented (n=64)	Early AD (n=58)	Non-demented (n=63)	Early AD (n=58)	baseline	Δ
Age, y	73.8 ± 6.8	75.4 ± 6.3			ns	
Gender, F/M	34/30	33/25			ns	
Vitamin D, ng/ml	27.5 ± 8.0	24.1 ± 8.7	-	-	*	-
MMSE	29.4 ± 0.8	26.2 ± 3.1	0.3 ± 1.1	3.6 ± 5.4	***	***
Growth Hormone, ng/ml	0.90 ± 0.94	0.85 ± 1.14	-	-	ns	-
Insulin-like Growth Factor-1, ng/ml	118.9 ± 49.7	116.9 ± 44.3	35.7 ± 33.5	30.3 ± 30.9	ns	ns
Leptin, ng/ml	12.6 ± 10.0	14. 8 ± 11.4	- 0.2 ± 6.0	- 7.3 ± 11.8	ns	***
BMI, kg/m^2	26.2 ± 3.8	25.8 ± 3.8	0.4 ± 1.3	-0.8 ± 1.8	ns	t
Body Fat, %	33.6 ± 8.8	34.0 ± 10.4	0.04 ± 2.3	- 1.1 ± 4.3	ns	*
Waste/hip Circumference	0.87 ± 0.10	0.87 ± 0.08	-0.2 ± 0.08	-0.3 ± 0.06	ns	*
Total Bone Density, g/cm^2	1.16 ± 0.11	1.12 ± 0.12	0.01 ± 0.03	0.02 ± 0.03	t	*
Total Bone Density, %	-	-	-0.95 ± 2.7%	-2.1 ± 3.1%	-	*

Data represent means ± SD unless otherwise stated; AD – Alzheimer's disease; MMSE – Mini Mental State Exam;

BMI – body mass index; *P≤ 0.05; **P≤0.001; ***P≤0.0001, ^{t}P<0.10 – trend; ns P>0.10

Table 6. 2 **Univariate correlates of bone density at baseline**

	BMI	Lean Mass	Fat Mass	25OHD	Leptin	HGH	IGF-1	HV
ND BMD	0.26*	0.70***	0.15	0.18	-0.25^{t}	-0.21	0.31*	0.01
AD BMD	0.32*	0.64***	0.08	0.12	-0.07	-0.05	0.13	0.25^{t}

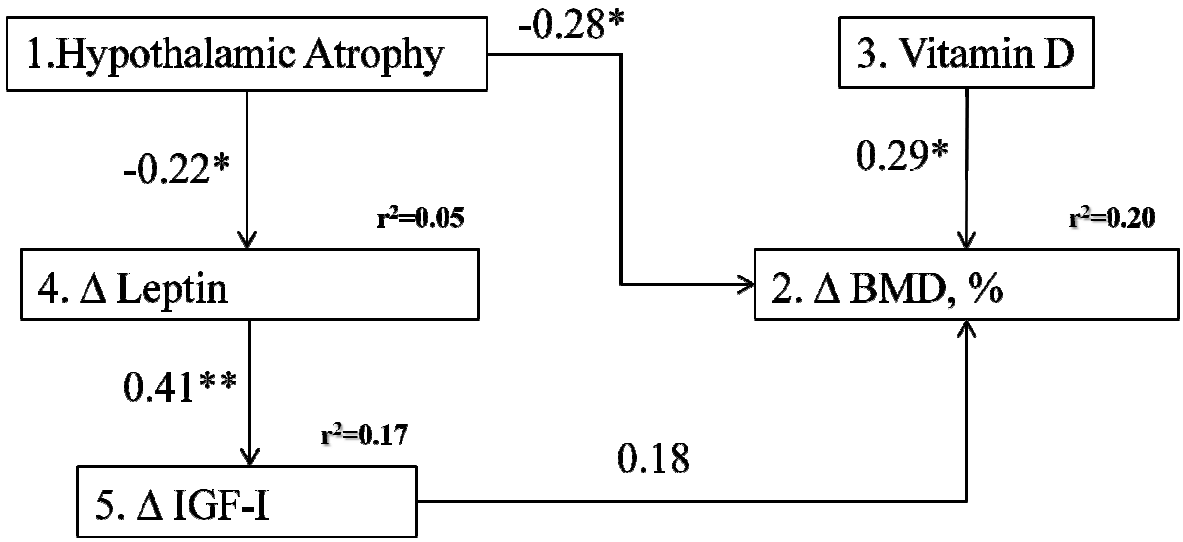
ND non-demented; BMD – total body bone mineral density; AD – Alzheimer's disease; BMI – body mass index (kg/m^2); Fat and lean masses in kg; 25OHD- serum levels of vitamin D; HGH – human growth hormone; IGF-1 – total serum insulin-like growth factor 1; HV – hypothalamic volume in cm^3; *P≤ 0.05; **P≤0.001; ***P≤0.0001, ^{t}P<0.10 – trend.

Table 6. 3 Univariate correlates of percent change in bone density from baseline

	ΔBMI	ΔLean Mass	ΔFat Mass	Baseline Vitamin D	ΔLeptin	Baseline HGH	ΔIGF-1	ΔHV
ΔND BMD	-0.09	-0.12	-0.11	-0.10	-0.23	-0.18	0.007	-0.01
ΔAD BMD	-0.004	-0.08	-0.02	0.33*	-0.08	-0.2	0.22	-0.29^{t}

ND non-demented; BMD – total body bone mineral density; AD – Alzheimer's disease; BMI – body mass index (kg/m^2); Fat and lean masses in kg; 25OHD- serum levels of vitamin D; HGH – human growth hormone; IGF-1 – total serum insulin-like growth factor 1; HV – hypothalamic volume in cm^3; *P≤ 0.05; ^{t}P<0.10 – trend; Δ - for all but BMD = absolute change over two years.

Figure 6. 3 Final results of path analysis



Final hypothetical model of the relationship between hypothalamic atrophy and bone loss; BMD – bone mineral density; IGF-1- insulin-like growth factor 1; the errors indicate the direction of hypothetical causation; β coefficients displayed along the errors; regression coefficients indicate proportion of total variance explained by a hypothetical cause.

Chapter 7

Discussion and Conclusions

7.1 Summary of findings

The body of presented work extends the literature on a relationship between bone loss and AD. Overall, the presented work provides evidence that accelerated bone loss observed in individuals in the early stages of AD may be partially due to disruption of central regulatory mechanisms by neurodegeneration. This is the first work to demonstrate that hypothalamic atrophy is related to bone loss and this relationship may be mediated by leptin-dependent mechanisms in humans with early stages of AD. Potential mechanisms of this association and possible explanations of why bone loss is observed in AD will be discussed. This work has applications for clinical practice in AD and aging and provides a basis for future studies.

Chapter 2. Bone Density and Brain Atrophy in Early Alzheimer Disease

The purpose of work in Chapter 2 was to compare bone health in the earliest clinical stages of AD with non-demented aging and to determine whether BMD is associated with cognitive performance and brain atrophy, which is used as a marker of neurodegeneration. We hypothesized that bone density would be lower in early AD and that reduced BMD would be associated with brain atrophy and cognitive decline. In this cross-sectional study, we found that BMD is reduced in men and women in the earliest clinical stages of AD and associated with brain atrophy and memory decline, suggesting that central mechanisms may contribute to bone loss in early Alzheimer's disease.

Chapter 3. Reduced Limbic and Hypothalamic Volumes Correlate With Bone Density in Early Alzheimer's Disease.

The aim of the cross-sectional study in Chapter 3 was to extend previous findings of the association between BMD and neuroimaging markers of neurodegeneration by assessing both global and regional measures of brain volume in early AD and non-demented aging, with a specific focus on the hypothalamus. We demonstrated that in early AD, low BMD was associated with low volume of gray matter in brain structures predominantly affected by AD early in the disease, including the hypothalamus, cingulate, and parahippocampal gyri and in the left superior temporal gyrus and left inferior parietal cortex. No relationship between BMD and regional gray matter volume was found in non-demented controls. These results suggest that central mechanisms of bone remodeling may be disrupted by neurodegeneration.

Chapter 4. Cross-sectional estimation of the hypothalamic volume by atlas-based VBM compared to manual tracing in application to Alzheimer's disease.

There is very limited guidance in the literature regarding neuroimaging studies of the hypothalamic anatomy and useful methods to do so. VBM, is used for the global assessment of the entire brain, and also allows measuring volumes of different brain regions including the hypothalamus. In the work presented in Chapter 4, we tested and compared the performance of VBM methods with the "gold standard" manual volumetry of the hypothalamus. The atlas-based VBM volumetry showed promise as a useful tool for regional volumetry of the hypothalamus and has advantages over manual tracing as it is currently used. The results of this study provided guidance for method selection in our future studies.

Chapter 5. Different predictors suggest different mechanisms of bone loss in AD and aging.

In the work presented in Chapter 5, we tested the hypothesis that AD may influence bone density in cortical skeletal sites directly through pathological changes in the hypothalamus, the major central regulatory structure of bone remodeling.. We previously reported lower BMD in the early AD group and suggested that brain atrophy, specifically of the hypothalamus, was associated with lower BMD in AD in a cross-sectional study. Considering often-seen lack of associations between cross-sectional and longitudinal studies, the purposes of the study presented in Chapter 5 were: (i) to extend previous findings of a direct association between hypothalamic atrophy and bone density and (ii) to explore predictors of bone loss in AD in a longitudinal study. We found that bone loss was accelerated in AD compared with non-demented controls for the total body and legs. For AD participants, bone loss was associated with baseline levels of the vitamin D and hypothalamic atrophy. For non-demented participants, bone loss was associated with age, female gender and decline in physical activity. Different predictors of bone loss in AD suggest that mechanisms of bone loss may differ in cognitively-normal aging and AD and that neurodegeneration may contribute to bone loss in early AD.

Chapter 6. Role of Central Hypothalamic Mechanisms in Bone Loss in Alzheimer's Disease: Testing a Path Model

The purpose of the work presented in Chapter 6 was to extend previous observations by assessing the roles of leptin and GH-IGF-1 in mediating a relationship between hypothalamic structural changes and bone loss in AD. Using a hypothetical model with statistical structural equation or path modeling, we demonstrated that hypothalamic atrophy had a direct effect on bone loss. IFG-1 and leptin were not independently associated with bon loss although

hypothalamic atrophy was associated with observed increases in leptin over two years in the AD group. Additionally, increased leptin over two years was associated with declines in IGF-1. The results of our study suggest that it is conceivable that central regulatory mechanisms of bone mass may be disturbed by neurodegeneration in participants in very early stages of AD. Further studies are needed to explore the role of brain atrophy in bone loss.

7.2 Overview of Possible Mechanisms of Bone Loss in AD

The association between bone loss and AD is well-documented. Nevertheless, bone health in AD remains understudied and the mechanisms underlying the association between bone loss and AD are not well understood. Multiple factors have been postulated to explain the association of bone loss with cognitive decline and AD. Despite increasing evidence of a key role that the hypothalamus plays in the regulation of bone mass (Ducy, Amling et al. 2000), the hypothalamic atrophy due to AD has never been previously considered among potential mechanisms. The direction of bone-AD relationship is unclear. Two scenarios are possible: (i) common pathological mechanisms drive plaque and tangle formation, cognitive decline, metabolic, hormonal and body changes in AD and (ii) loss of essential neurons due to AD results in imbalance of the centrally regulated functions including energy metabolism and bone loss. Given that AD is associated with extensive hypothalamic structural damage and hypothalamic dysfunction; it is plausible that hypothalamic damage could result in clinically evident bone loss. However, gaps remain in our knowledge about mechanisms of bone regulation and central control of bone metabolism. The results of our study provide evidence that suggests it is plausible that atrophy of the hypothalamus may lead to bone loss in AD and possibly to other clinical symptoms of hypothalamic dysfunction.

7.3 Summary of Finding in Relation to Theories of Brain-Bone Connection

We humans consider ourselves superior to any other species in the world. However, we are still subject to the basic principle of biology. *No life without energy.* This is as important in humans as in any other species. Just like most living organisms, we obtain our energy from glucose.

Here we will introduce the current state of knowledge and theories on central regulatory mechanisms of bone and energy metabolism and discuss several potential mechanisms of bone loss. The first part is mostly concerned with *how* the brain regulates bone. The second part will discuss some theories on *why* the brain regulates bone. As we discuss different aspects of this study, the current state of knowledge, and theories in the field, we will try to make sense of the question: *Why do we highlight the importance of energy and glucose?*

As discussed throughout the body of this work, several lines of evidence in animals and humans strongly suggest that leptin, a fat-derived hormone, plays an important role on bone metabolism (Ducy, Amling et al. 2000; Elefteriou, Takeda et al. 2004). A key role of leptin is in regulating feeding and energy metabolism through regulating appetite and energy expenditure via hypothalamic mechanisms in arcuate (ARC) nucleus have been discovered long before its effects on bone (Zhang, Proenca et al. 1994; Farooqi and O'Rahilly 2009). Initially, animal studies suggested that leptin acts on the leptinergic/peptidergic neurons in VMH that lead to activation of SNS, β-2-adrenoreceptors on the osteoblasts and decreased proliferation, thus, decreased bone formation. This pathway was described as neural arm of hypothalamic control of bone remodeling and was believed to be independent of feeding mechanisms and hypothalamo-pituitary hormonal mechanisms (neurohumoral arm) (Takeda, Elefteriou et al. 2002). More recent studies suggested that instead of acting directly on hypothalamic neurons leptin signals to

its receptors expressed on serotonergic neurons in the brainstem, thus utilizing serotonin as secondary messenger to exert its own effect on VMH and ARC hypothalamic neurons (Yadav, Oury et al. 2009). This actions cause decrease in serotonin production and results in less inhibition of neurons in VMH and increased sympathetic activity. Serotonin is also found to stimulate IGF-1 release (Schaeffer and Sirotkin 1997). It may be speculated then that decrease in serotonin may cause reduction in IGF-1 and subsequent bone loss via complimentary mechanisms. Reported often inverse relationship between leptin and IGF-1 may be also mediated by this mechanism. Important though, fat derived leptin also negatively regulates insulin secretion by a direct effect on pancreatic cells (Covey, Wideman et al. 2006; Tuduri, Marroqui et al. 2009). Bone-derived hormone osteocalcin in turn regulates energy metabolism by positive effects on pancreatic β -cell proliferation, insulin secretion and sensitivity (Lee, Sowa et al. 2007). Leptin, through discussed earlier mechanisms, stimulates sympathetic tone which decreases octeocalcin bioactivity (Hinoi, Gao et al. 2008), thus regulating insulin secretion though yet another mediators – bone (Hinoi, Gao et al. 2009). It may be speculated therefore that the primary purpose of leptin-dependent central control of bone metabolism is to again regulate energy metabolism. Why would there be such an obvious redundancy in the brain regulation of energy metabolism?

No life without energy? Or should we say – *no life without energy in the brain?* In 2004 Achim Peters and the group proposed "The Selfish Brain" theory (Peters, Schweiger et al. 2004; Peters 2011). In short, "The Selfish Brain" theory states that the ability of the individual to maintain its homeostatic energy balance greatly depends on the ability of the brain to competently demand energy from the body. An average size human needs 200g of glucose every day. Earlier theories wrongly hypothesized that this energy is equally distributed between the

brain and the body. The brain however consumes 2/3 of the daily glucose supply (Reinmuth, Kogure et al. 1968). In stress conditions, for example, the brain demands are even higher. “The Selfish Brain” theory proposes that the energy is distributed within the organism on a competitive basis between the brain and the body. Most importantly, the brain prioritizes the regulation of its own energy content and sufficiency, demanding energy from the body and its deposits when needed. Under conditions of brain energy deprivation, existing central hypothalamic mechanisms of energy metabolism regulation are able to suppress insulin production and guarantee sufficient glucose supply for the brain (Mattson 2002).

In application to Alzheimer’s disease, this theory ties together with the data from physical anthropology that demonstrated that AD shares close similarities with syndromes of deprivation or starvation such as decrease in metabolic rate, reduction of anabolic hormone levels, activation of hypothalamo-pituitary-adrenal axis, and insulin resistance (Heininger 2000; Reser 2009). These syndromes represent metabolic reduction program or simply thrifty ways to minimize energy expenditure and support supply of energy to the brain – the most vital organ in the human body.

The results of our studies do not directly answer either *how* or *why* questions. They are in line with the enormous body of evidence that bone loss in AD may be driven by hypothalamus - dependent mechanisms. Moreover, our results allude to the possibility that bone loss via metabolic mechanisms in AD may be either a “side effect”, or even one of the mechanisms of allocation of energy recourses to meet damaged brain’s demands for energy.

This author concludes that future studies on body changes, including bone loss in AD, should not only explore *how it happens* but also the *why it happens* aspects of the problem.

7.4 Clinical Implications

Clinicians need to pay attention to bone issues in women and men at risk for AD and those who have already been diagnosed. In the absence of a cure for AD, the goal for the clinicians, patients and caregivers is to prolong independence and delay institutionalization in patients with AD. While cognitive deterioration is the major concern, co-morbidities definitely contribute to the burden of caring for demented older people. Hip fracture is one of the common devastating events in the elderly that greatly impacts independence of patients and puts additional stress on caregivers. As the western societies progressively age, vitamin D deficiency reaches epidemic levels and exercise becomes optional, we will face a dramatic increase in a number of older people with dementia and hip fracture.

This work contributes to understanding the mechanisms that relate AD and bone loss. If years from now we that are we can target modifiable common mechanisms and risk factors by specific interventions, we would be able to influence development or reduce severity of both conditions and promote healthy aging. If it turns out that one condition influences the occurrence of the other, identifying mediating mechanism may provide strategies to decrease burden of co-morbidities. While important discoveries are still in the future, results of our study once again emphasize the importance of physical activity and vitamin D in bone health.

7.5. Limitations

Study Design and Sample Size

The observational nature of this work did not allow us to manipulate important predictor variables to assess cause-effect relationship. However, observational data provides an important

step in generating hypotheses and to justify and guide clinical trial development on bone loss in AD. This study allowed us to begin to examine important variables that relate to bone density and bone loss in AD. For example, a well-described protective effect of fat mass on bone was not supported by the results of our study though the study is limited by a relatively small sample size or short follow-up time that may influence our power to observe relationships. The study is also limited in its ability to study gender differences. The floor-effect of some of the measures (perhaps, physical activity or brain atrophy measures) may limit our ability to fully examine the influence of covariates on BMD. We did not examine environmental (diet) and several peripheral factors related to bone health including sex hormones or calcium, although we did take into account participant use of such therapies. Given that our study participants are a convenient sample composed primarily of Caucasians, it remains possible that our results may be influenced by sampling bias and have limited generalyzability. Additionally, our AD sample was confined to the earliest clinical stages of AD, with most of the demented subjects (80.3%) were in the very mild (CDR 0.5) stage, comparable to mild cognitive impairment. Although this very early AD group is one of the strengths of the study, it limits the range of dementia severity and may have reduced our power to resolve a relationship between BMD and dementia severity and other variables.

Bone Density Measures

In our work, the total body BMD loss values were smaller in respect to the least significant change (0.9*2.77=2.6%) in all but women with AD. While, significant difference was still observed in bone density and rates of bone loss between non-demented and AD groups, it could take less time to see these differences if CV for total body reliability is lowered to widely

reported 0.5% for the same densitometer version. It should be considered in designing future studies and accounted for by either reducing the CV or increasing the length of the study.

Neuroimaging

Neuroimaging analytic choices for studying the hypothalamus are limited due to challenges in subcortical segmentation (classification of brain regions). The VBM approach allows for more accurate classification of brain tissues in presence of excess atrophy or abnormal morphology. While use of this approach is increasing in popularity and has many advantages over other methods, its applications in longitudinal studies have been only emerging. Several issues with this approach have been brought to attention of researchers including well-recognized sensitivity of VBM to intra- and inter-scanner variations, difference in scanning protocols and contrast non-uniformity that may affect final volumetric results especially in a longitudinal study. Results of our neuroimaging study should be interpreted with caution, although we checked every original image for artifacts, movement, contrast issues as well as tissue maps after being processed by VBM. Images that had artifacts which could not be corrected by remedy strategies were excluded from the analysis. Additionally we validated the VBM method against the "gold standard" manual tracing and concluded that manual tracing of the hypothalamus does not have a superior performance for the purposes of our work.

Medications

Participants who reported taking bone affecting medications were not excluded from the data analysis. Self-reported medication records were collected. Hormone-replacement therapy medications (estrogen, testosterone and selective estrogen receptor modulators), thyroid hormone

replacement, bisphosphonates, beta-blockers, vitamin D and calcium were considered as bone affecting. While low bone density was perhaps the reason for taking most of these medications, the number of participants receiving the medications was small and the proportion of these individuals in both groups was equal. Additionally, these medications did not affect group average rates of bone loss over two years.

Statistical Path Modeling

The path analyses cannot test real-world directionality in relationships. The directions of arrows in a structural equation model are based on the researcher's hypotheses of causality within a system. The choice of variables and pathways limits the ability of the models to recreate relationships in natural biological systems and oversimplifies the reality. Not surprisingly, several models that represent the concept may fit the data equally well. This approach is useful in understanding relationship patterns in complex systems. The ability of this approach to data analysis to distinguish between direct and indirect paths, allows a better understanding of complex concepts and identification of influential modifiers.

7.6 Future directions

The regulatory mechanisms of bone metabolism and bone loss are extremely complex. Many factors involved in regulation of bone homeostasis are pleiotropic and have profound multiple effects. The effects may include regulating each other or functioning beyond the bone metabolism. The fascinating fact about hormonal systems is that they are also regulated by a negative feedback loop. As discussed throughout this body of work, many factors have already been identified in the central regulatory mechanisms on bone remodeling. The pathway expands

with every new fact. It is apparent that we know only a small portion about central regulatory mechanisms of bone remodeling and we know very little about the role of the hypothalamus in bone regulation in humans. The results of this body of work suggest that the hypothalamic mechanisms are involved in bone loss in individuals with AD. Based on observations by other researchers and results of this work we propose several directions in future studies.

Observational studies on mechanisms of bone loss.

The hypothesis that neurodegeneration in AD may disrupt central regulatory mechanism of bone remodeling was proposed and tested in human studies for the first time. Attention to this possibility is emerging in different clinical conditions. For example, recent review on bone loss in patients with spinal cord injuries suggested that disruption of the spinal cord tracts may also lead to "neurogenic" bone loss (Qin, Bauman et al. 2010). Although, it seems logical that processes regulated by CNS would be affected by neuronal death in critical brain structures, this hypothesis is difficult to test in humans. Bone loss in AD is a significant clinical problem. As an extension for this work, future studies should characterize other potential mediators of central mechanisms of remodeling (e.g. serotonin or sympathetic nervous system output) in relation to bone mass and bone loss in humans and whether these relations change in neurodegenerative diseases, such as AD. Better characterizing a relationship between bone loss and peripheral total and free IFG-1 may be important. IGF-1 is an anabolic factor that is responsive to physical activity (Rojas Vega, Knicker et al. 2010), thus is potentially modifiable by treatment interventions. Likewise, identifying other modifiable factors associated with bone loss may inform future clinical trials and lead to new intervention.

Hypothalamic atrophy and dysfunction

Mounting evidence supports that AD is associated with multiple signs of hypothalamic dysfunction. Therefore, in the context of proposed hypothesis, the association between hypothalamic structural damage and its functions should be explored. Future studies may assess whether clinical observations of sleep, mood and appetite disturbances, abnormal sexual behaviors, and impaired energy metabolism and thermoregulation in AD (Wu and Swaab 2007; de Medeiros, Rosenberg et al. 2008; Di Iulio, Palmer et al. 2010) will be associated with hypothalamic atrophy.

Clinical trials: Exercise for bone health

Although we did not find an association between habitual physical activity and bone loss in AD we think that was due to extremely low levels of engagement in the strenuous physical activities, the most beneficial for bone health. Perhaps, individuals with AD reduce these challenging activities in the pre-clinical stages (Scarmeas, Luchsinger et al. 2009). This question is important and should be investigated in the future studies.

Physical activity provides great benefits for general health. The data on effects of exercise on bone health in older adults is contradictory. In older men and women, reports range from clinical trial observations that several exercise interventions (including resistance training or regular walking by itself) have no benefits for bone health, to others concluding that for these interventions to be effective, they should be fast, intense and use heavy loads (see (Guadalupe-Grau, Fuentes et al. 2009) for full review), suggesting a need for further studies to address this inconsistency. While clinical trials for effects of exercise on cognitive function in AD are

emerging, up to date no studies have looked at the effects of exercise intervention on bone health in individuals with AD.

Therefore, future clinical trials should investigate the effects of physical activity as a potential preventive or therapeutic strategy on bone health in AD. The activities that utilize high-impact or odd-impact (e.g. jumps, tennis, soccer, golf and dance) are found to be the most effective for bone health (Nikander, Kannus et al. 2009). These are also high risk activities for adverse events, so they cannot be used as sports, but rather the main components should be incorporated in safe interventions. Considering cognitive deficits, frailty and increased risk for falls, in patients with AD, future studies should focus on designing and testing effective, easy to comprehend, safe exercise interventions enjoyable for both genders.

Clinical trials: Vitamin D supplementation for bone health in AD

Vitamin D is one of the main factors related to bone health. Our results demonstrated that prevalence of vitamin D deficiency is higher in AD. Low levels of vitamin D were also associated with increased bone loss. Recent systematic review on vitamin D supplementation in relation to bone health in older adults reported that numerous clinical trials provide fair evidence that vitamin D supplementation increases levels of 25 OH D and improves bone health (Cranney, Weiler et al. 2008). Similar systematic review in AD however identified only 3 clinical trials on effects of combination of risedronate sodium (Actonel®, a bisphosphonate used to treat osteoporosis) and vitamin D with several for risk of fractures in severely demented women residing in nursing homes (Iwamoto, Sato et al. 2009). This suggests a clear need for more high – quality trials of vitamin D supplementation in relation to bone health outcomes in women and men with AD.

Neuroimaging

Considering that applications of modern neuroimaging techniques in the longitudinal studies are just emerging, it is important to test new and improved techniques in studying the hypothalamus in order to identify the ones that are less affected by imaging artifacts, time between brain scans and different scanners.

Based on our results, the current methods can also be improved by, first, creating a manual hypothalamic mask using MRI brain images and validating results with autopsy-defined volume and shape of the hypothalamus, similar to study design by Bobinski et al. (Bobinski, de Leon et al. 2000). Next, utilizing the mask in automated approaches will allow fast and reliable assessment of structural changes in AD and other condition associated with hypothalamic dysfunction.

7.7 Summary of conclusions

The body of presented work extends the literature on a relationship between bone loss and AD. Overall, the presented work provides initial evidence that accelerated bone loss observed in individuals in the early stages of AD may be partially due to distortion of central regulatory mechanisms by neurodegeneration. This is the first work to demonstrate that hypothalamic atrophy is related to bone loss and this relationship may be mediated by leptin-dependent mechanisms in humans in the early stages of AD. The findings of this work indicate a need for future studies to determine a role of neurodegeneration in centrally regulated body functions and mechanisms of physical decline, including bone loss in AD.

References

(2008). "2008 Alzheimer's disease facts and figures." Alzheimers Dement **4**(2): 110-133.

Adams, J. S. and M. Hewison (2010). "Update in vitamin D." J Clin Endocrinol Metab **95**(2): 471-478.

Alvarez, A., R. Cacabelos, et al. (2007). "Serum TNF-alpha levels are increased and correlate negatively with free IGF-I in Alzheimer disease." Neurobiol Aging **28**(4): 533-536.

Ammann, P. and R. Rizzoli (2003). "Bone strength and its determinants." Osteoporos Int **14 Suppl 3**: S13-18.

Annweiler, C., G. Allali, et al. (2009). "Vitamin D and cognitive performance in adults: a systematic review." Eur J Neurol **16**(10): 1083-1089.

Armitage, S. G. (1946). "An analysis of certain psychological tests used in the evaluation of brain injury." Psychological Monographs **60**: 1-48.

Ashburner, J. and K. J. Friston (2005). "Unified segmentation." Neuroimage **26**(3): 839-851.

Atwood, C. S., S. V. Meethal, et al. (2005). "Dysregulation of the hypothalamic-pituitary-gonadal axis with menopause and andropause promotes neurodegenerative senescence." J Neuropathol Exp Neurol **64**(2): 93-103.

Babalola, K. O., B. Patenaude, et al. (2009). "An evaluation of four automatic methods of segmenting the subcortical structures in the brain." Neuroimage **47**(4): 1435-1447.

Bakhireva, L. N., E. Barrett-Connor, et al. (2004). "Modifiable predictors of bone loss in older men: a prospective study." Am J Prev Med **26**(5): 436-442.

Barberger-Gateau, P., C. Raffaitin, et al. (2007). "Dietary patterns and risk of dementia: the Three-City cohort study." Neurology **69**(20): 1921-1930.

Barrett-Connor, E., S. Edelstein, et al. (1998). "Weight loss precedes dementia in community-dwelling older adults." J Nutr Health Aging **2**(2): 113-114.

Battaglini, M., S. M. Smith, et al. (2008). "Enhanced brain extraction improves the accuracy of brain atrophy estimation." Neuroimage **40**(2): 583-589.

Bianco, V., F. Filippi, et al. (2008). "Diet assessment and the screening for osteoporosis: a survey in a healthy menopause population." Minerva Ginecol **60**(4): 299-306, 306-210.

Blake, G. M., R. J. Herd, et al. (2000). "The effect of weight change on total body dual-energy X-ray absorptiometry: results from a clinical trial." Osteoporos Int **11**(10): 832-839.

Bland, J. M. and D. G. Altman (1986). "Statistical methods for assessing agreement between two methods of clinical measurement." Lancet **1**(8476): 307-310.

Bobinski, M., M. J. de Leon, et al. (2000). "The histological validation of post mortem magnetic resonance imaging-determined hippocampal volume in Alzheimer's disease." Neuroscience **95**(3): 721-725.

Bonnet, N., C. L. Benhamou, et al. (2005). "Severe bone alterations under beta2 agonist treatments: bone mass, microarchitecture and strength analyses in female rats." Bone **37**(5): 622-633.

Bowen, R. L., J. P. Isley, et al. (2000). "An association of elevated serum gonadotropin concentrations and Alzheimer disease?" J Neuroendocrinol **12**(4): 351-354.

Buchman, A. S., P. A. Boyle, et al. (2007). "Frailty is associated with incident Alzheimer's disease and cognitive decline in the elderly." Psychosom Med **69**(5): 483-489.

Buchner, D. M. and E. B. Larson (1987). "Falls and fractures in patients with Alzheimer-type dementia." Jama **257**(11): 1492-1495.

Buell, J. S., B. Dawson-Hughes, et al. "25-Hydroxyvitamin D, dementia, and cerebrovascular pathology in elders receiving home services." Neurology **74**(1): 18-26.
Burke, W. J., M. J. Houston, et al. (1989). "Use of the Geriatric Depression Scale in dementia of the Alzheimer type." J Am Geriatr Soc **37**(9): 856-860.
Burns, J. M., B. B. Cronk, et al. (2008). "Cardiorespiratory fitness and brain atrophy in early Alzheimer disease." Neurology **71**(3): 210-216.
Burns, J. M., J. E. Donnelly, et al. (2007). "Peripheral insulin and brain structure in early Alzheimer disease." Neurology **69**(11): 1094-1104.
Burton, E. J., R. Barber, et al. (2009). "Medial temporal lobe atrophy on MRI differentiates Alzheimer's disease from dementia with Lewy bodies and vascular cognitive impairment: a prospective study with pathological verification of diagnosis." Brain **132**(Pt 1): 195-203.
Buskova, J., M. Vaneckova, et al. (2006). "Reduced hypothalamic gray matter in narcolepsy with cataplexy." Neuro Endocrinol Lett **27**(6): 769-772.
Callen, D. J., S. E. Black, et al. (2004). "The influence of sex on limbic volume and perfusion in AD." Neurobiol Aging **25**(6): 761-770.
Callen, D. J., S. E. Black, et al. (2001). "Beyond the hippocampus: MRI volumetry confirms widespread limbic atrophy in AD." Neurology **57**(9): 1669-1674.
Camarda, R., C. Camarda, et al. (2007). "Movements execution in amnestic mild cognitive impairment and Alzheimer's disease." Behav Neurol **18**(3): 135-142.
Carlsson, P., J. Tidermark, et al. (2005). "Food habits and appetite of elderly women at the time of a femoral neck fracture and after nutritional and anabolic support." J Hum Nutr Diet **18**(2): 117-120.
Carro, E. and I. Torres-Aleman (2004). "Insulin-like growth factor I and Alzheimer's disease: therapeutic prospects?" Expert Rev Neurother **4**(1): 79-86.
Chouinard, J., E. Lavigne, et al. (1998). "Weight loss, dysphagia, and outcome in advanced dementia." Dysphagia **13**(3): 151-155.
Cock, T. A. and J. Auwerx (2003). "Leptin: cutting the fat off the bone." Lancet **362**(9395): 1572-1574.
Coin, A., E. Perissinotto, et al. (2008). "Predictors of low bone mineral density in the elderly: the role of dietary intake, nutritional status and sarcopenia." Eur J Clin Nutr **62**(6): 802-809.
Confavreux, C. B., R. L. Levine, et al. (2009). "A paradigm of integrative physiology, the crosstalk between bone and energy metabolisms." Mol Cell Endocrinol **310**(1-2): 21-29.
Copenhaver, B. R., L. A. Rabin, et al. (2006). "The fornix and mammillary bodies in older adults with Alzheimer's disease, mild cognitive impairment, and cognitive complaints: a volumetric MRI study." Psychiatry Res **147**(2-3): 93-103.
Cornett, C. R., W. R. Markesbery, et al. (1998). "Imbalances of trace elements related to oxidative damage in Alzheimer's disease brain." Neurotoxicology **19**(3): 339-345.
Covey, S. D., R. D. Wideman, et al. (2006). "The pancreatic beta cell is a key site for mediating the effects of leptin on glucose homeostasis." Cell Metab **4**(4): 291-302.
Cranney, A., H. A. Weiler, et al. (2008). "Summary of evidence-based review on vitamin D efficacy and safety in relation to bone health." Am J Clin Nutr **88**(2): 513S-519S.
de Jong, F. J., K. Masaki, et al. (2007). "Thyroid function, the risk of dementia and neuropathologic changes: The Honolulu-Asia Aging Study." Neurobiol Aging.
de Lacalle, S., I. Iraizoz, et al. (1993). "Cell loss in supraoptic and paraventricular nucleus in Alzheimer's disease." Brain Res **609**(1-2): 154-158.

de Medeiros, K., P. B. Rosenberg, et al. (2008). "Improper sexual behaviors in elders with dementia living in residential care." Dement Geriatr Cogn Disord **26**(4): 370-377.
Deshmukh, V. D. and S. V. Deshmukh (1990). "Stress-adaptation failure hypothesis of Alzheimer's disease." Med Hypotheses **32**(4): 293-295.
Di Carlo, C., G. A. Tommaselli, et al. (2007). "Longitudinal evaluation of serum leptin and bone mineral density in early postmenopausal women." Menopause **14**(3 Pt 1): 450-454.
Di Iulio, F., K. Palmer, et al. (2010). "Occurrence of neuropsychiatric symptoms and psychiatric disorders in mild Alzheimer's disease and mild cognitive impairment subtypes." Int Psychogeriatr **22**(4): 629-640.
Diem, S. J., T. L. Blackwell, et al. (2007). "Depressive symptoms and rates of bone loss at the hip in older women." J Am Geriatr Soc **55**(6): 824-831.
Dinger, M. K., R. F. Oman, et al. (2004). "Stability and convergent validity of the Physical Activity Scale for the Elderly (PASE)." J Sports Med Phys Fitness **44**(2): 186-192.
Ducy, P., M. Amling, et al. (2000). "Leptin inhibits bone formation through a hypothalamic relay: a central control of bone mass." Cell **100**(2): 197-207.
Efstathiadou, Z., G. Koukoulis, et al. (2004). "Apolipoprotein E polymorphism is not associated with spinal bone mineral density in peri- and postmenopausal Greek women." Maturitas **48**(3): 259-264.
Egger, P., S. Duggleby, et al. (1996). "Cigarette smoking and bone mineral density in the elderly." J Epidemiol Community Health **50**(1): 47-50.
Elefteriou, F., J. D. Ahn, et al. (2005). "Leptin regulation of bone resorption by the sympathetic nervous system and CART." Nature **434**(7032): 514-520.
Elefteriou, F., S. Takeda, et al. (2004). "Serum leptin level is a regulator of bone mass." Proc Natl Acad Sci U S A **101**(9): 3258-3263.
Elgh, E., A. Lindqvist Astot, et al. (2006). "Cognitive dysfunction, hippocampal atrophy and glucocorticoid feedback in Alzheimer's disease." Biol Psychiatry **59**(2): 155-161.
Elrod, R., E. R. Peskind, et al. (1997). "Effects of Alzheimer's disease severity on cerebrospinal fluid norepinephrine concentration." Am J Psychiatry **154**(1): 25-30.
Eyles, D. W., S. Smith, et al. (2005). "Distribution of the vitamin D receptor and 1 alpha-hydroxylase in human brain." J Chem Neuroanat **29**(1): 21-30.
Farooqi, I. S. and S. O'Rahilly (2009). "Leptin: a pivotal regulator of human energy homeostasis." Am J Clin Nutr **89**(3): 980S-984S.
Faul, F., E. Erdfelder, et al. (2007). "G*Power 3: a flexible statistical power analysis program for the social, behavioral, and biomedical sciences." Behav Res Methods **39**(2): 175-191.
Ferrari, E., A. Arcaini, et al. (2000). "Pineal and pituitary-adrenocortical function in physiological aging and in senile dementia." Exp Gerontol **35**(9-10): 1239-1250.
Fewlass, D. C., K. Noboa, et al. (2004). "Obesity-related leptin regulates Alzheimer's Abeta." Faseb J **18**(15): 1870-1878.
Fischl, B., D. H. Salat, et al. (2002). "Whole brain segmentation: automated labeling of neuroanatomical structures in the human brain." Neuron **33**(3): 341-355.
Folstein, M. F., S. E. Folstein, et al. (1975). "Mini-mental State: A practical method for grading the cognitive state of patients for the clinicians." J.Psychiatr.Res. **12**: 189-198.
Fredericson, M., K. Chew, et al. (2007). "Regional bone mineral density in male athletes: a comparison of soccer players, runners and controls." Br J Sports Med **41**(10): 664-668; discussion 668.

Friedman, S. M., I. B. Menzies, et al. (2010). "Dementia and Hip Fractures." Geriatric Orthopaedic Surgery & Rehabilitation **1**(2): 52-62.
Gahete, M. D., M. Duran-Prado, et al. (2009). "Understanding the multifactorial control of growth hormone release by somatotropes: lessons from comparative endocrinology." Ann N Y Acad Sci **1163**: 137-153.
Geuze, E., E. Vermetten, et al. (2005). "MR-based in vivo hippocampal volumetrics: 1. Review of methodologies currently employed." Mol Psychiatry **10**(2): 147-159.
Gil-Bea, F. J., B. Aisa, et al. "HPA Axis Dysregulation Associated to Apolipoprotein E4 Genotype in Alzheimer's Disease." J Alzheimers Dis.
Giordano, R., L. Bonelli, et al. (2008). "Growth hormone treatment in human ageing: benefits and risks." Hormones (Athens) **7**(2): 133-139.
Giordano, R., F. Lanfranco, et al. (2005). "Somatopause reflects age-related changes in the neural control of GH/IGF-I axis." J Endocrinol Invest **28**(3 Suppl): 94-98.
Giuliani, N. R., V. D. Calhoun, et al. (2005). "Voxel-based morphometry versus region of interest: a comparison of two methods for analyzing gray matter differences in schizophrenia." Schizophr Res **74**(2-3): 135-147.
Giustina, A., G. Mazziotti, et al. (2008). "Growth hormone, insulin-like growth factors, and the skeleton." Endocr Rev **29**(5): 535-559.
Goldstein, J. M., L. J. Seidman, et al. (2007). "Hypothalamic abnormalities in schizophrenia: sex effects and genetic vulnerability." Biol Psychiatry **61**(8): 935-945.
Gomez, J. M. (2008). "Growth hormone and insulin-like growth factor-I as an endocrine axis in Alzheimer's disease." Endocr Metab Immune Disord Drug Targets **8**(2): 143-151.
Gonoi, W., O. Abe, et al. "Age-related changes in regional brain volume evaluated by atlas-based method." Neuroradiology **52**(10): 865-873.
Good, C. D., I. S. Johnsrude, et al. (2001). "A voxel-based morphometric study of ageing in 465 normal adult human brains." Neuroimage **14**(1 Pt 1): 21-36.
Goodglass, H. and E. Kaplan (1983). Boston Naming Test scoring booklet. Philadelphia, Lea & Febiger.
Grober, E., H. Buschke, et al. (1988). "Screening for dementia by memory testing." Neurology **38**: 900-903.
Guadalupe-Grau, A., T. Fuentes, et al. (2009). "Exercise and bone mass in adults." Sports Med **39**(6): 439-468.
Gudmundsdottir, S. L., D. Oskarsdottir, et al. "Risk factors for bone loss in the hip of 75-year-old women: a 4-year follow-up study." Maturitas **67**(3): 256-261.
Guidobono, F., F. Pagani, et al. (2006). "Different skeletal regional response to continuous brain infusion of leptin in the rat." Peptides **27**(6): 1426-1433.
Gunstad, J., M. B. Spitznagel, et al. (2008). "Serum leptin levels are associated with cognitive function in older adults." Brain Res **1230**: 233-236.
Hänninen, T., K. J. Reinikainen, et al. (1994). "Subjective memory complaints and personality traits in normal elderly subjects." Journal of the American Geriatrics Society **42**: 1-4.
Harada, S. and G. A. Rodan (2003). "Control of osteoblast function and regulation of bone mass." Nature **423**(6937): 349-355.
Harvey, J. (2007). "Leptin: a diverse regulator of neuronal function." J Neurochem **100**(2): 307-313.
Havill, L. M., M. C. Mahaney, et al. (2007). "Effects of genes, sex, age, and activity on BMC, bone size, and areal and volumetric BMD." J Bone Miner Res **22**(5): 737-746.

Heininger, K. (2000). "A unifying hypothesis of Alzheimer's disease. IV. Causation and sequence of events." Rev Neurosci **11 Spec No**: 213-328.
Hersch, E. C. and G. R. Merriam (2008). "Growth hormone (GH)-releasing hormone and GH secretagogues in normal aging: Fountain of Youth or Pool of Tantalus?" Clin Interv Aging **3**(1): 121-129.
Hinoi, E., N. Gao, et al. (2009). "An Osteoblast-dependent mechanism contributes to the leptin regulation of insulin secretion." Ann N Y Acad Sci **1173 Suppl 1**: E20-30.
Hinoi, E., N. Gao, et al. (2008). "The sympathetic tone mediates leptin's inhibition of insulin secretion by modulating osteocalcin bioactivity." J Cell Biol **183**(7): 1235-1242.
Hixson, J. E. and D. T. Vernier (1990). "Restriction isotyping of human apolipoprotein E by gene amplification and cleavage with HhaI." J Lipid Res **31**: 545-548.
Hofman, M. A. and D. F. Swaab (1992). "The human hypothalamus: comparative morphometry and photoperiodic influences." Prog Brain Res **93**: 133-147; discussion 148-139.
Holden, K. F., K. Lindquist, et al. (2008). "Serum leptin level and cognition in the elderly: Findings from the Health ABC Study." Neurobiol Aging.
Holick, M. F. (2006). "The role of vitamin D for bone health and fracture prevention." Curr Osteoporos Rep **4**(3): 96-102.
Holick, M. F. (2007). "Optimal vitamin D status for the prevention and treatment of osteoporosis." Drugs Aging **24**(12): 1017-1029.
Holick, M. F. (2007). "Vitamin D deficiency." N Engl J Med **357**(3): 266-281.
Honea, R. A., A. Meyer-Lindenberg, et al. (2008). "Is gray matter volume an intermediate phenotype for schizophrenia? A voxel-based morphometry study of patients with schizophrenia and their healthy siblings." Biol Psychiatry **63**(5): 465-474.
Hrnciarikova, D., R. Hyspler, et al. (2009). "Serum lipids and neopterin in urine as new biomarkers of malnutrition and inflammation in the elderly." Nutrition **25**(3): 303-308.
Huppertz, H. J., J. Kroll-Seger, et al. "Intra- and interscanner variability of automated voxel-based volumetry based on a 3D probabilistic atlas of human cerebral structures." Neuroimage **49**(3): 2216-2224.
Ibrahim, S. A., A. M. Eltom, et al. (1994). "Correlation of some biochemical parameters with clinical features of protein energy malnutrition." East Afr Med J **71**(2): 77-83.
Ilich, J. Z., R. A. Brownbill, et al. (2003). "Bone and nutrition in elderly women: protein, energy, and calcium as main determinants of bone mineral density." Eur J Clin Nutr **57**(4): 554-565.
Iwamoto, J., Y. Sato, et al. (2009). "Prevention of hip fractures by exposure to sunlight and pharmacotherapy in patients with Alzheimer's disease." Aging Clin Exp Res **21**(4-5): 277-281.
Jack, C. R., Jr., D. W. Dickson, et al. (2002). "Antemortem MRI findings correlate with hippocampal neuropathology in typical aging and dementia." Neurology **58**(5): 750-757.
Jirikowski, G. F., U. W. Kauntzer, et al. (2009). "Distribution of vitamin D binding protein expressing neurons in the rat hypothalamus." Histochem Cell Biol **131**(3): 365-370.
Kawai, M., C. B. Green, et al. "Nocturnin: a circadian target of Pparg-induced adipogenesis." Ann N Y Acad Sci **1192**: 131-138.
Kawai, M., C. B. Green, et al. "A circadian-regulated gene, Nocturnin, promotes adipogenesis by stimulating PPAR-gamma nuclear translocation." Proc Natl Acad Sci U S A **107**(23): 10508-10513.

Kemmler, W., J. Weineck, et al. (2004). "The effect of habitual physical activity, non-athletic exercise, muscle strength, and VO2max on bone mineral density is rather low in early postmenopausal osteopenic women." J Musculoskelet Neuronal Interact **4**(3): 325-334.
Kenny, A. M., I. Y. Waynik, et al. (2006). "Association between level of frailty and bone mineral density in community-dwelling men." J Clin Densitom **9**(3): 309-314.
Kipen, E., R. D. Helme, et al. (1995). "Bone density, vitamin D nutrition, and parathyroid hormone levels in women with dementia." J Am Geriatr Soc **43**(10): 1088-1091.
Kirkland, J. L., T. Tchkonia, et al. (2002). "Adipogenesis and aging: does aging make fat go MAD?" Exp Gerontol **37**(6): 757-767.
Kluger, A., J. G. Gianutsos, et al. (1997). "Motor/psychomotor dysfunction in normal aging, mild cognitive decline, and early Alzheimer's disease: diagnostic and differential diagnostic features." Int Psychogeriatr **9 Suppl 1**: 307-316; discussion 317-321.
Koolschijn, P. C., N. E. van Haren, et al. (2008). "Hypothalamus volume in twin pairs discordant for schizophrenia." Eur Neuropsychopharmacol **18**(4): 312-315.
Lacka-Gazdzik, B., Z. Klosa, et al. (2005). "The impact of selected metallic elements in the environment on the occurrence of osteoporosis in the voivodeship of Silesia." Ortop Traumatol Rehabil **7**(4): 433-439.
Lee, N. K., H. Sowa, et al. (2007). "Endocrine regulation of energy metabolism by the skeleton." Cell **130**(3): 456-469.
Lindsay, J., D. Laurin, et al. (2002). "Risk factors for Alzheimer's disease: a prospective analysis from the Canadian Study of Health and Aging." Am J Epidemiol **156**(5): 445-453.
Loskutova, N., R. A. Honea, et al. "Reduced limbic and hypothalamic volumes correlate with bone density in early Alzheimer's disease." J Alzheimers Dis **20**(1): 313-322.
Loskutova, N., R. A. Honea, et al. (2009). "Bone Density and Brain Atrophy in Early Alzheimer's Disease." J Alzheimers Dis.
Lui, L. Y., K. Stone, et al. (2003). "Bone loss predicts subsequent cognitive decline in older women: the study of osteoporotic fractures." J Am Geriatr Soc **51**(1): 38-43.
Maimoun, L., O. Coste, et al. (2008). "No negative impact of reduced leptin secretion on bone metabolism in male decathletes." Eur J Appl Physiol **102**(3): 343-351.
Maldjian, J. A., P. J. Laurienti, et al. (2004). "Precentral gyrus discrepancy in electronic versions of the Talairach atlas." Neuroimage **21**(1): 450-455.
Maldjian, J. A., P. J. Laurienti, et al. (2003). "An automated method for neuroanatomic and cytoarchitectonic atlas-based interrogation of fMRI data sets." Neuroimage **19**(3): 1233-1239.
Mardon, J., V. Habauzit, et al. (2008). "Influence of high and low protein intakes on age-related bone loss in rats submitted to adequate or restricted energy conditions." Calcif Tissue Int **82**(5): 373-382.
Martin, A., V. David, et al. (2007). "Opposite effects of leptin on bone metabolism: a dose-dependent balance related to energy intake and insulin-like growth factor-I pathway." Endocrinology **148**(7): 3419-3425.
Martin, A., V. David, et al. (2008). "Impaired energetic metabolism after central leptin signaling leads to massive appendicular bone loss in hindlimb-suspended rats." J Bone Miner Res **23**(12): 2040-2047.
Martini, G., R. Valenti, et al. (2001). "Influence of insulin-like growth factor-1 and leptin on bone mass in healthy postmenopausal women." Bone **28**(1): 113-117.

Masdeu, J. C. and B. Pascual (2008). "Neuroimaging of disorders leading to dementia." Curr Neurol Neurosci Rep **8**(6): 443-444.
Masoumi, A., B. Goldenson, et al. (2009). "1alpha,25-dihydroxyvitamin D<formula>_{3}</formula> Interacts with Curcuminoids to Stimulate Amyloid-beta Clearance by Macrophages of Alzheimer's Disease Patients." J Alzheimers Dis.
Mattson, M. P. (2002). "Brain evolution and lifespan regulation: conservation of signal transduction pathways that regulate energy metabolism." Mech Ageing Dev **123**(8): 947-953.
McCalden, R. W., J. A. McGeough, et al. (1993). "Age-related changes in the tensile properties of cortical bone. The relative importance of changes in porosity, mineralization, and microstructure." J Bone Joint Surg Am **75**(8): 1193-1205.
Meethal, S. V., M. A. Smith, et al. (2005). "The gonadotropin connection in Alzheimer's disease." Endocrine **26**(3): 317-326.
Meisenzahl, E. M., N. Koutsouleris, et al. (2008). "Structural brain alterations at different stages of schizophrenia: A voxel-based morphometric study." Schizophr Res **104**(1-3): 44-60.
Meisinger, C., M. Heier, et al. (2007). "Beta-blocker use and risk of fractures in men and women from the general population: the MONICA/KORA Augsburg cohort study." Osteoporos Int **18**(9): 1189-1195.
Melton, L. J., 3rd, C. M. Beard, et al. (1994). "Fracture risk in patients with Alzheimer's disease." J Am Geriatr Soc **42**(6): 614-619.
Milewicz, T., J. Krzysiek, et al. (2005). "Age, insulin, SHBG and sex steroids exert secondary influence on plasma leptin level in women." Endokrynol Pol **56**(6): 883-890.
Minkowitz, B., A. L. Boskey, et al. (1991). "Effects of propranolol on bone metabolism in the rat." J Orthop Res **9**(6): 869-875.
Moerman, E. J., K. Teng, et al. (2004). "Aging activates adipogenic and suppresses osteogenic programs in mesenchymal marrow stroma/stem cells: the role of PPAR-gamma2 transcription factor and TGF-beta/BMP signaling pathways." Aging Cell **3**(6): 379-389.
Mohan, S., J. R. Farley, et al. (1995). "Age-related changes in IGFBP-4 and IGFBP-5 levels in human serum and bone: implications for bone loss with aging." Prog Growth Factor Res **6**(2-4): 465-473.
Morris, J. C. (1993). "The Clinical Dementia Rating (CDR): current version and scoring rules." Neurology **43**(11): 2412-2414.
Munoz, A. M., G. M. Agudelo, et al. (2006). "[Nutritional condition in patients with Alzheimer-type dementia from the neurosciences' group, Medellin 2004]." Biomedica **26**(1): 113-125.
Murray, R. D., J. E. Adams, et al. (2006). "A densitometric and morphometric analysis of the skeleton in adults with varying degrees of growth hormone deficiency." J Clin Endocrinol Metab **91**(2): 432-438.
Narita, K., H. Kosaka, et al. (2008). "Relationship Between Plasma Leptin Level and Brain Structure in Elderly: A Voxel-Based Morphometric Study." Biol Psychiatry.
Nightingale, S., J. Holmes, et al. (2001). "Psychiatric illness and mortality after hip fracture." Lancet **357**(9264): 1264-1265.
Nikander, R., P. Kannus, et al. (2009). "Targeted exercises against hip fragility." Osteoporos Int **20**(8): 1321-1328.
Nimitphong, H. and M. F. Holick (2011). "Vitamin D, neurocognitive functioning and immunocompetence." Curr Opin Clin Nutr Metab Care **14**(1): 7-14.

Oguz, S., O. L. Tapisiz, et al. (2009). "Is leptin a significant predictor of bone mineral density in postmenopausal Turkish women?" Rheumatol Int **29**(4): 393-396.
Oh, K. W., W. Y. Lee, et al. (2005). "The relationship between serum resistin, leptin, adiponectin, ghrelin levels and bone mineral density in middle-aged men." Clin Endocrinol (Oxf) **63**(2): 131-138.
Oudshoorn, C., F. U. Mattace-Raso, et al. (2008). "Higher serum vitamin D3 levels are associated with better cognitive test performance in patients with Alzheimer's disease." Dement Geriatr Cogn Disord **25**(6): 539-543.
Papaioannou, A., C. C. Kennedy, et al. (2009). "Risk factors for low BMD in healthy men age 50 years or older: a systematic review." Osteoporos Int **20**(4): 507-518.
Pascualy, M., E. C. Petrie, et al. (2000). "Hypothalamic pituitary adrenocortical and sympathetic nervous system responses to the cold pressor test in Alzheimer's disease." Biol Psychiatry **48**(3): 247-254.
Patel, M. S. and F. Elefteriou (2007). "The new field of neuroskeletal biology." Calcif Tissue Int **80**(5): 337-347.
Peng, X. D., H. Xie, et al. (2008). "Relationships between serum adiponectin, leptin, resistin, visfatin levels and bone mineral density, and bone biochemical markers in Chinese men." Clin Chim Acta **387**(1-2): 31-35.
Peper, J. S., R. M. Brouwer, et al. "HPG-axis hormones during puberty: a study on the association with hypothalamic and pituitary volumes." Psychoneuroendocrinology **35**(1): 133-140.
Peskind, E. R., C. W. Wilkinson, et al. (2001). "Increased CSF cortisol in AD is a function of APOE genotype." Neurology **56**(8): 1094-1098.
Peters, A. (2011). "The selfish brain: Competition for energy resources." Am J Hum Biol **23**(1): 29-34.
Peters, A., U. Schweiger, et al. (2004). "The selfish brain: competition for energy resources." Neurosci Biobehav Rev **28**(2): 143-180.
Piguet, O., Å. Petersén, et al. "Eating and hypothalamus changes in behavioral-variant frontotemporal dementia." Annals of Neurology: n/a-n/a.
Pogoda, P., M. Egermann, et al. (2006). "Leptin inhibits bone formation not only in rodents, but also in sheep." J Bone Miner Res **21**(10): 1591-1599.
Pol, H. E. H., P. T. Cohen-Kettenis, et al. (2006). "Changing your sex changes your brain: influences of testosterone and estrogen on adult human brain structure." Eur J Endocrinol **155**(suppl_1): S107-114.
Politis, M., N. Pavese, et al. (2008). "Hypothalamic involvement in Huntington's disease: an in vivo PET study." Brain **131**(Pt 11): 2860-2869.
Qin, W., W. A. Bauman, et al. (2010). "Evolving concepts in neurogenic osteoporosis." Curr Osteoporos Rep **8**(4): 212-218.
Raadsheer, F. C., J. J. van Heerikhuize, et al. (1995). "Corticotropin-releasing hormone mRNA levels in the paraventricular nucleus of patients with Alzheimer's disease and depression." Am J Psychiatry **152**(9): 1372-1376.
Raskind, M. A., E. R. Peskind, et al. (1984). "Norepinephrine and MHPG levels in CSF and plasma in Alzheimer's disease." Arch Gen Psychiatry **41**(4): 343-346.
Raskind, M. A., E. R. Peskind, et al. (1999). "Patterns of cerebrospinal fluid catechols support increased central noradrenergic responsiveness in aging and Alzheimer's disease." Biol Psychiatry **46**(6): 756-765.

Rasmuson, S., R. Andrew, et al. (2001). "Increased glucocorticoid production and altered cortisol metabolism in women with mild to moderate Alzheimer's disease." Biol Psychiatry **49**(6): 547-552.
Reid, I. R. (2010). "Fat and bone." Arch Biochem Biophys **503**(1): 20-27.
Reinmuth, O. M., K. Kogure, et al. (1968). "Total cerebral blood flow and metabolism in human brain stem disease." Neurology **18**(3): 280-281.
Reser, J. E. (2009). "Alzheimer's disease and natural cognitive aging may represent adaptive metabolism reduction programs." Behav Brain Funct **5**: 13.
Rojas Vega, S., A. Knicker, et al. (2010). "Effect of resistance exercise on serum levels of growth factors in humans." Horm Metab Res **42**(13): 982-986.
Salehi, Z., F. Mashayekhi, et al. (2008). "Insulin like growth factor-1 and insulin like growth factor binding proteins in the cerebrospinal fluid and serum from patients with Alzheimer's disease." Biofactors **33**(2): 99-106.
Sanlier, N. (2008). "The effect of body composition on blood lipids, leptin, bone mineral density, and nutrition in females." Saudi Med J **29**(11): 1636-1642.
Saper, C. B. and D. C. German (1987). "Hypothalamic pathology in Alzheimer's disease." Neurosci Lett **74**(3): 364-370.
Sato, M., N. Takeda, et al. (2001). "Association between serum leptin concentrations and bone mineral density, and biochemical markers of bone turnover in adult men." J Clin Endocrinol Metab **86**(11): 5273-5276.
Sato, Y., T. Asoh, et al. (1998). "High prevalence of vitamin D deficiency and reduced bone mass in elderly women with Alzheimer's disease." Bone **23**(6): 555-557.
Sato, Y., Y. Honda, et al. (2005). "Vitamin K deficiency and osteopenia in elderly women with Alzheimer's disease." Arch Phys Med Rehabil **86**(3): 576-581.
Sato, Y., T. Kanoko, et al. (2004). "Risk factors for hip fracture among elderly patients with Alzheimer's disease." J Neurol Sci **223**(2): 107-112.
Scarmeas, N., J. A. Luchsinger, et al. (2009). "Physical activity, diet, and risk of Alzheimer disease." JAMA **302**(6): 627-637.
Scarmeas, N., Y. Stern, et al. (2006). "Mediterranean diet and risk for Alzheimer's disease." Ann Neurol **59**(6): 912-921.
Schaeffer, H. J. and A. V. Sirotkin (1997). "Melatonin and serotonin regulate the release of insulin-like growth factor-I, oxytocin and progesterone by cultured human granulosa cells." Exp Clin Endocrinol Diabetes **105**(2): 109-112.
Schoofs, M. W., M. van der Klift, et al. (2004). "ApoE gene polymorphisms, BMD, and fracture risk in elderly men and women: the Rotterdam study." J Bone Miner Res **19**(9): 1490-1496.
Schott, A. M., C. Cormier, et al. (1998). "How hip and whole-body bone mineral density predict hip fracture in elderly women: the EPIDOS Prospective Study." Osteoporos Int **8**(3): 247-254.
Seeman, E. (2008). "Structural basis of growth-related gain and age-related loss of bone strength." Rheumatology (Oxford) **47 Suppl 4**: iv2-8.
Sergi, G., E. Perissinotto, et al. (2005). "An adequate threshold for body mass index to detect underweight condition in elderly persons: the Italian Longitudinal Study on Aging (ILSA)." J Gerontol A Biol Sci Med Sci **60**(7): 866-871.
Shcherbatykh, I. and D. O. Carpenter (2007). "The role of metals in the etiology of Alzheimer's disease." J Alzheimers Dis **11**(2): 191-205.

Shumaker, S. A., C. Legault, et al. (2003). "Estrogen plus progestin and the incidence of dementia and mild cognitive impairment in postmenopausal women: the Women's Health Initiative Memory Study: a randomized controlled trial." Jama **289**(20): 2651-2662.
Signore, A. P., F. Zhang, et al. (2008). "Leptin neuroprotection in the CNS: mechanisms and therapeutic potentials." J Neurochem **106**(5): 1977-1990.
Singh, M. K., F. Elefteriou, et al. (2008). "Cocaine and amphetamine-regulated transcript may regulate bone remodeling as a circulating molecule." Endocrinology **149**(8): 3933-3941.
Smith, K. L. and C. E. Greenwood (2008). "Weight loss and nutritional considerations in Alzheimer disease." J Nutr Elder **27**(3-4): 381-403.
Solin, M. S., M. J. Ball, et al. (1997). "Relationship of serum leptin to total and truncal body fat." Clin Sci (Lond) **93**(6): 581-584.
Standaert, D. G., V. M. Lee, et al. (1991). "Molecular features of hypothalamic plaques in Alzheimer's disease." Am J Pathol **139**(3): 681-691.
Stroop, J. (1935). "Studies of interference in serial verbal reactions." J Exp Psychol **18**: 643-662.
Swaab, D. F., E. Goudsmit, et al. (1992). "The human hypothalamus in development, sexual differentiation, aging and Alzheimer's disease." Prog Brain Res **91**: 465-472.
Szot, P., J. B. Leverenz, et al. (2000). "Tyrosine hydroxylase and norepinephrine transporter mRNA expression in the locus coeruleus in Alzheimer's disease." Brain Res Mol Brain Res **84**(1-2): 135-140.
Szulc, P., F. Marchand, et al. (2000). "Cross-sectional assessment of age-related bone loss in men: the MINOS study." Bone **26**(2): 123-129.
Tae, W. S., S. S. Kim, et al. (2008). "Validation of hippocampal volumes measured using a manual method and two automated methods (FreeSurfer and IBASPM) in chronic major depressive disorder." Neuroradiology **50**(7): 569-581.
Takeda, S. (2005). "Central control of bone remodeling." Biochem Biophys Res Commun **328**(3): 697-699.
Takeda, S., F. Elefteriou, et al. (2002). "Leptin regulates bone formation via the sympathetic nervous system." Cell **111**(3): 305-317.
Takeda, S. and G. Karsenty (2008). "Molecular bases of the sympathetic regulation of bone mass." Bone **42**(5): 837-840.
Tan, Z. S., S. Seshadri, et al. (2005). "Bone mineral density and the risk of Alzheimer disease." Arch Neurol **62**(1): 107-111.
Tardif, C. L., D. L. Collins, et al. "Regional impact of field strength on voxel-based morphometry results." Hum Brain Mapp **31**(7): 943-957.
Tezapsidis, N., J. M. Johnston, et al. (2009). "Leptin: a novel therapeutic strategy for Alzheimer's disease." J Alzheimers Dis **16**(4): 731-740.
Tham, A., A. Nordberg, et al. (1993). "Insulin-like growth factors and insulin-like growth factor binding proteins in cerebrospinal fluid and serum of patients with dementia of the Alzheimer type." J Neural Transm Park Dis Dement Sect **5**(3): 165-176.
Thomas, A. G., S. Marrett, et al. (2009). "Functional but not structural changes associated with learning: an exploration of longitudinal voxel-based morphometry (VBM)." Neuroimage **48**(1): 117-125.
Thompson, P. M., K. M. Hayashi, et al. (2003). "Dynamics of gray matter loss in Alzheimer's disease." J Neurosci **23**(3): 994-1005.
Thompson, P. M., K. M. Hayashi, et al. (2007). "Tracking Alzheimer's disease." Ann N Y Acad Sci **1097**: 183-214.

Tuduri, E., L. Marroqui, et al. (2009). "Inhibitory effects of leptin on pancreatic alpha-cell function." Diabetes **58**(7): 1616-1624.
Turker, S., V. Karatosun, et al. (2006). "Beta-blockers increase bone mineral density." Clin Orthop Relat Res **443**: 73-74.
Tysiewicz-Dudek, M., F. Pietraszkiewicz, et al. (2008). "Alzheimer's disease and osteoporosis: common risk factors or one condition predisposing to the other?" Ortop Traumatol Rehabil **10**(4): 315-323.
Uusi-Rasi, K., H. Sievanen, et al. (2007). "Age-related decline in trabecular and cortical density: a 5-year peripheral quantitative computed tomography follow-up study of pre- and postmenopausal women." Calcif Tissue Int **81**(4): 249-253.
van Dortmont, L. M., C. M. Douw, et al. (2000). "Outcome after hemi-arthroplasty for displaced intracapsular femoral neck fracture related to mental state." Injury **31**(5): 327-331.
Vitiello, M. V., K. E. Moe, et al. (2006). "Growth hormone releasing hormone improves the cognition of healthy older adults." Neurobiol Aging **27**(2): 318-323.
Volicer, L., D. G. Harper, et al. (2001). "Sundowning and circadian rhythms in Alzheimer's disease." Am J Psychiatry **158**(5): 704-711.
Walker, M. A., J. R. Highley, et al. (2002). "Estimated neuronal populations and volumes of the hippocampus and its subfields in schizophrenia." Am J Psychiatry **159**(5): 821-828.
Washburn, R. A., E. McAuley, et al. (1999). "The physical activity scale for the elderly (PASE): evidence for validity." J Clin Epidemiol **52**(7): 643-651.
Wechsler, D. (1955). Manual: Wechsler Adult Intelligence Scale. New York, Psychological Corporation.
Wechsler, D. and C. P. Stone (1973). Manual: Wechsler Memory Scale. . New York, Psychological Corporation.
Wei, J. and P. Ducy (2010). "Co-dependence of bone and energy metabolisms." Arch Biochem Biophys **503**(1): 35-40.
Weller, I. and J. Schatzker (2004). "Hip fractures and Alzheimer's disease in elderly institutionalized Canadians." Ann Epidemiol **14**(5): 319-324.
Weller, I. I. (2000). "The relation between hip fracture and alzheimer's disease in the canadian national population health survey health institutions data, 1994-1995. A cross-sectional study." Ann Epidemiol **10**(7): 461.
Whitwell, J. L., R. C. Petersen, et al. (2007). "Patterns of atrophy differ among specific subtypes of mild cognitive impairment." Arch Neurol **64**(8): 1130-1138.
Whitwell, J. L., S. D. Weigand, et al. (2007). "Focal atrophy in dementia with Lewy bodies on MRI: a distinct pattern from Alzheimer's disease." Brain **130**(Pt 3): 708-719.
Wiens, M., M. Etminan, et al. (2006). "Effects of antihypertensive drug treatments on fracture outcomes: a meta-analysis of observational studies." J Intern Med **260**(4): 350-362.
Wilke, M., S. K. Holland, et al. (2008). "Template-O-Matic: a toolbox for creating customized pediatric templates." Neuroimage **41**(3): 903-913.
Wilson, R. S., S. E. Arnold, et al. (2008). "Change in depressive symptoms during the prodromal phase of Alzheimer disease." Arch Gen Psychiatry **65**(4): 439-445.
Wu, Y. H. and D. F. Swaab (2005). "The human pineal gland and melatonin in aging and Alzheimer's disease." J Pineal Res **38**(3): 145-152.
Wu, Y. H. and D. F. Swaab (2007). "Disturbance and strategies for reactivation of the circadian rhythm system in aging and Alzheimer's disease." Sleep Med **8**(6): 623-636.

Xie, J., C. Brayne, et al. (2008). "Survival times in people with dementia: analysis from population based cohort study with 14 year follow-up." BMJ **336**(7638): 258-262.
Yadav, V. K., F. Oury, et al. (2009). "A serotonin-dependent mechanism explains the leptin regulation of bone mass, appetite, and energy expenditure." Cell **138**(5): 976-989.
Yaffe, K., W. Browner, et al. (1999). "Association between bone mineral density and cognitive decline in older women." J Am Geriatr Soc **47**(10): 1176-1182.
Yakar, S., H. W. Courtland, et al. "IGF-1 and bone: New discoveries from mouse models." J Bone Miner Res **25**(12): 2267-2276.
Yushkevich, P. A., J. Piven, et al. (2006). "User-guided 3D active contour segmentation of anatomical structures: significantly improved efficiency and reliability." Neuroimage **31**(3): 1116-1128.
Zaidi, M. (2007). "Skeletal remodeling in health and disease." Nat Med **13**(7): 791-801.
Zajickova, K., I. Zofkova, et al. (2003). "Apolipoprotein E 4 allele is associated with low bone density in postmenopausal women." J Endocrinol Invest **26**(4): 312-315.
Zhang, Y., R. Proenca, et al. (1994). "Positional cloning of the mouse obese gene and its human homologue." Nature **372**(6505): 425-432.
Zhou, R., J. Deng, et al. (2010). "Association between Bone Mineral Density and the Risk of Alzheimer's Disease." J Alzheimers Dis.
Zoico, E., M. Zamboni, et al. (2008). "Relation between adiponectin and bone mineral density in elderly post-menopausal women: role of body composition, leptin, insulin resistance, and dehydroepiandrosterone sulfate." J Endocrinol Invest **31**(4): 297-302.