

DIETARY PROTOCOL FOR TREATING AGE-RELATED BONE LOSS

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Approval Signatures Page

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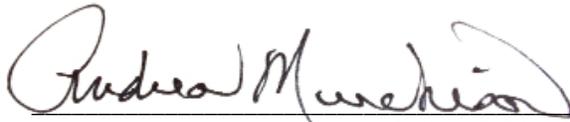
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TABLE OF CONTENTS

Chapter One Introduction	3
Chapter Two Methods	10
Chapter Three Osteoporosis Worldwide	12
Chapter Four The Calcium Problem	19
Chapter Five American Dietary Deficiency	42
Chapter Six Systemic Factors	50
Chapter Seven Natural Antiresorptive Agents	55
Chapter Eight Conclusion	62
References	65
Appendix A Glossary of terms	74
Appendix B Internal Review Board Approval Letter	78
Appendix C Book Excerpt: Foods Highest in Necessary Nutrients	79
Appendix D Book Excerpt: Herbal Antiresorptives	82
Appendix E Book Excerpt: Testing	86

ABSTRACT

Osteoporosis and subsequent worldwide fracture rates are steadily increasing due to aging populations combined with shifts in dietary paradigms. The US has arguably the highest fracture rate in the world, though in the land of plenty it is difficult to think that this could be due to dietary deficiency. Sales of supplements to prevent and treat osteoporosis such as calcium and more recently vitamin D, as standard recommendations of Western physicians, hovers near 1 billion dollars and very clearly is not stemming the tide of rising fracture rates. This investigation sets out to understand why, and to create a new dietary protocol to address the growing financial and emotional burden of osteoporotic fracture.

CHAPTER ONE: INTRODUCTION

This research came about from a desire to clearly understand the fundamental tools of medicine – nutrition. It became the basis for a science-of-food cookbook for age-related bone loss, *The Keep Your Bones Healthy Cookbook*, to be published by Chelsea Green in May 2016. The recipes were developed to target treatment and prevention of osteopenia and osteoporosis through examination of recent nutritional research. This work is an in-depth analysis of the science behind bone health, which provided the basis for the recipes. The book encapsulates the science, provides recipes, cooking tips, worksheets for a personal nutrition plan, and a chapter on speaking with your doctor. The book itself has a public health component in that it has the ability to reach lay people and help them directly improve their health, working alone or with their healthcare provider.

“With regard to healing the sick, I will devise and order for them the best diet, according to my judgment and means; and I will take care that they suffer no hurt or damage. Nor shall any man's entreaty prevail upon me to administer poison to anyone; neither will I counsel any man to do so.”

From the original Hippocratic oath, (late 5th Century BC)

Age-related bone loss, described as osteopenia and osteoporosis, affects approximately 50% of the US population over 50 years of age – a projected 61 million adults in the US by the year 2020. Worldwide, osteoporosis causes more than 8.9 million fractures annually, a fracture every 3 seconds. By 2050, the world wide incidence of hip

fracture is projected to increase an average of 270%. The cost of osteoporosis in the US is approximately 15 billion USD yearly. Worldwide costs of osteoporosis are projected at 131.5 billion USD by 2050.

The current understanding of osteoporosis is centered on hormone loss as the body ages coupled with a lack of dietary calcium and vitamin D. Beyond this, insufficient peak bone density at approximately age 30, smoking, medication, lack of exercise, and improper diet are also suggested as factors, however a complete understanding of the multiple factors remains to be formalized to effect treatment beyond pharmaceuticals for the general public.

In the last half of the last decade the World Health Organization (WHO) redefined its position on a definition of osteoporosis. It suggested a change from a diagnosis of "osteoporosis" to instead an analysis of multiple factors to find the total, individual 10-year fracture risk (AR-10). These factors were defined as: advanced age, prior fragility fracture, parental history of proximal femur fracture, low BMI, low bone mass, glucocorticosteroid treatment, rheumatoid arthritis, smoking, and overuse of alcohol (Czerwinski et al, 2007). This shift in understanding highlights the multifactorial nature of this malady that must be taken into consideration in the creation of a prevention or treatment protocol.

Untreated bone loss due to aging averages about 10% per decade of a person's life. This gradual loss is essentially equivalent in men and women, except for the approximately 10-year period of increased bone loss in women after menopause. This hormone loss does cause bone density decline, ordinarily between 5% and 10% - but this does not have to mean osteopenia or osteoporosis.

Osteoporosis rates vary widely between countries. Japanese osteoporosis rates were 40% of ours in the West on traditional diets. India also had lower rates of osteoporosis, as did China; however with the introduction of Western dietary practices their rates are climbing. This reveals two things: the fact that osteoporosis is not a necessary consequence of aging; and the recognition that some large-scale factors influence health of entire populations – certainly genetics and environment, but also dietary paradigms.

In 2012 the FDA revised its recommendations for the main class of pharmaceuticals, second- and third-generation bisphosphonates, for the treatment of age-related bone loss. They lowered the recommended time frame of treatment with these drugs to 3-5 years, and suggested effectiveness in long-term use only in patients with severe vertebral bone loss or history of vertebral fracture. They offered no additional recommendations for treatment. Additionally, it has been found that use of these pharmaceuticals can lead to “bisphosphonate fractures” of the femur and to deterioration of the mandible. These side effects are documented by the CDC and increasingly known by patients who are now seeking new therapies, however the pharmaceutical industry has little new to offer in terms of treatment.

Western physicians routinely recommend supplementing with calcium and more recently with the addition of vitamin D to increase bone density. Unfortunately the US Preventive Task Force does *not* recommend this supplementation because their research does not show this combination effectively reduces fractures. Additionally, there is ample evidence that high dose calcium supplementation plus intake of dietary calcium increases not only cardiovascular events but also overall mortality.

Although the wide disparity in osteoporosis rates by country indicate some level of dietary affect, combing the literature reveals very little information on *comprehensive* dietary approaches in treating osteoporosis – major websites, national and international societies do recommend diet and exercise, however their protocols are incomplete and often vague. Recent clinical research focuses on a particular nutrient or vitamin, or one aspect of lifestyle such as exercise that shows improvement in fracture outcomes. However a complete examination of exactly how and why nutrients – in combination and in context - affect bone deposition was not forthcoming in a literature search.

A number of new theories have been recently published such as the acid/ash balance, however even these are focused only on one influence rather than the complex interactions that define the function of our bodily systems, including our skeletal system.

The purpose of this study is three-fold: 1) To understand the nutritional biochemistry of bone loss and bone growth mechanisms; 2) To synthesize and integrate all of the most recent nutritional science; and 3) To create from this information a comprehensive, targeted nutrition plan for prevention and treatment of age-related bone loss.

This investigation is broken down into six parts:

1. Historic understanding of bone loss worldwide
2. Nutrients needed for bone health – the same information applies across all populations generally, and is a backdrop for understanding deficiencies affecting bone loss in high-risk countries such as the US.
3. Nutritional deficiencies in the US

4. Systemic factors affecting bone density
5. Effective non-pharmaceutical antiresorptive agents
6. Dietary protocol

Age-related bone loss, including osteopenia and osteoporosis, is asymptomatic until fracture. Additionally, bone density does not equate to fracture risk, which is the real measure of detriment in the case of age-related bone loss. Because of this, fracture rates were used for research purposes rather than osteopenia or osteoporosis rates.

CHAPTER TWO: METHODOLOGY

Although quantitative research is what is needed in order to translate TCM and complementary medicines into graspable concepts for Western readers, this research was too wide-ranging to enable a quantitative study. As the initial research attempts to grasp a snapshot of age-related bone loss in order to form a comprehensive dietary treatment for such, a multi-factor exploratory approach was needed. Rather than a comparison, this research approached the open question “is it possible to create an ideal dietary protocol for optimal bone health” and single-question quantitative assessment was not appropriate in this context.

To this end, the approach for the purposes of this document was rooted in Grounded Theory (Strauss & Corbin 1990), tending to Glaser’s Theoretical Sensitivity (Glaser 1998). This approach allowed for exploration of subject without attempts to define where the pertinent information would lie, and allowed the researcher to create the necessary structure based on the initial step of collecting a wide range of data and coding this data to understand patterns leading to a big picture.

Glaser’s approach is geared towards “theory generation”, or the equivalent, which by nature allows for the embrace of inductive reasoning. As this is not a social theory thesis, however, every effort was made to follow the trail of information for deductive results. There is however in this vein some element of what the researcher feels to be important through what can only be described as scientific instinct.

To help harness the breadth of literature needed to understand this query, previously published meta-analyses were used whenever possible. Meta-analyses were

chosen based on year published, and size of study pool, written in or translated into English, with human subjects. Unless no other information was found or meta-analysis was exceptional, years of meta-analyses used were no older than the year 2000, and study pool not less than 35. Individual studies were chosen according to year published (most recent), size of study population, and quality of translation if not originally published in English. If no human research was found, animal studies were used.

Articles were obtained using PubMed database as well as Nature database and google scholar searches, using key words, and eventually arranged in the following sections. When no information was found, general Google searches were conducted.

1. Bone loss worldwide
osteoporosis world statistics, fracture rate country
2. Nutrients needed for bone health
nutrients, bone, nutrient deficiency osteoporosis
3. Nutritional deficiencies for the US
Vitamin deficiency US, CDC, nutrient deficiency, mineral deficiency US
4. Systemic factors involved in bone loss in the US
Homocysteine, medication induced osteoporosis, stress osteoporosis, smoking osteoporosis, glucocorticoids, oxidative stress osteoporosis
5. Natural anti-resorptive agents– as dense nutrition, herbs used in osteoporosis treatment in Asia and the West
antiresorptive herb, herbal medicine osteoporosis, osteoblast, osteoclast

The IRB approval letter for this research is provided in Appendix A.

CHAPTER THREE: OSTEOPOROSIS WORLDWIDE

The first area of investigation was an overall view of osteoporosis in terms of worldwide fracture rates, and understanding how and why that was changing.

Two comprehensive meta-analyses were used. The first completed in 2011 used records as early as 1911, and contained data from then until 2009 (Dhanwal, 2011). The second used more recent data from 1950 through November 2011 (Kanis, 2012).

Worldwide fracture rates

Osteoporosis, and fracture rates, are changing rapidly. Looking at countries with historically low fracture rates gives some clues as to how diet might be impacting these numbers, as these cultures shift from traditional to more Western diets. Hip fracture was chosen as the reference as it is recognized as the most serious consequence of bone loss. With rising life expectancy worldwide, it is estimated that the incidence of hip fracture will rise from 1.66 million in 1990 to 6.26 million by 2050 (Cooper 1992).

In the first meta-analysis (Dhanwal, 2011) North America, Norway and Sweden showed the highest rates of hip fracture, with Japan showing the lowest, consistent with significantly lower rates historically in Asia (Cummings, 2002).

The second review (Kanis, 2012) showed differing results with Nigeria showing lowest and Sweden and Norway showing highest. However, the authors state that the data for Nigeria was slim and of poor quality. Additionally, this review separately coded

population types in the US, for example coding US Caucasians as high risk and the US black population as low risk.

A fracture risk map from the international osteoporosis foundation (Kanis, 2012) showing ten-year probability of a major osteoporotic fracture shows North America as a high-risk territory.

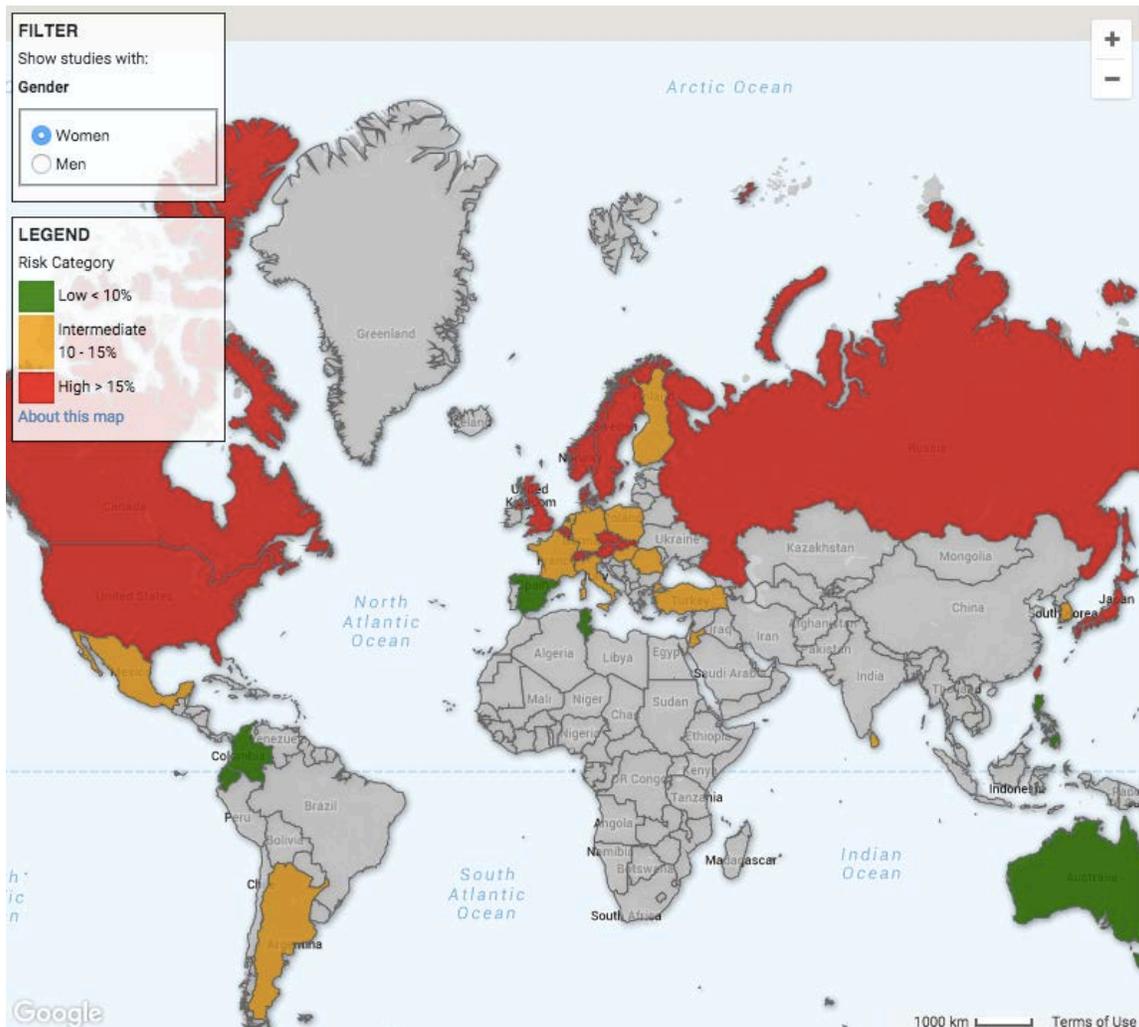


Figure 1. Ten-year probability of major osteoporotic fracture. [Source: International Osteoporosis Foundation]

Interestingly this image also shows Japan as a high-risk territory, which infers a steep rise in the risk of osteoporotic fracture since the early part of the last century (Ross, 1991).

A recent survey (Hagino *et al.* 2005) concluded that in the Japanese population aged 35 years or older incidence of hip fracture was 99.6 per 100,000 males and 368 per 100,000 females from 2004 to 2006. When incidence rates were compared with those from 30 years ago, the authors concluded that the incidence of hip fracture in the Japanese population is increasing. Historically, hip fractures of Japanese are less than half that of Caucasian Americans (Ross, 1991).

This change is thought to have three factors, the researcher believes – the ageing of the population, the move away from manual labor (exercise) and the move away from traditional diets towards more Western diets. It is projected that Asian countries will contribute more to the pool of hip fractures in coming years, by 2050 more than 50% of all osteoporotic fractures worldwide will occur in Asia (Cooper, 1992).

It is interesting to note that although the number of female Japanese tested as osteoporotic approximately corresponds to the US, the incidence of hip fracture in Japan appears to be much lower, approximately half (Fujita 1992). The researcher believes this indicates the measurement of bone density with which we define osteoporosis in the US is an incomplete picture.

Hip fracture rates in the US population are among the highest in the world. Data from a 20% sample of Medicare claims from 1985–2005 in patients ≥ 65 years were

examined (Brauer, 2009). The annual mean number of hip fractures was 957.3/100 000 for women and 414.4/100 000 for men.

On comparison with data from the US, the overall fracture rate in Canadian women was 30% lower than in US women in 2001.

Scandinavia reports the highest hip fracture numbers in Europe, and Switzerland the lowest. In Norway, the reported age-standardized annual incidence rate of hip fracture is 920/100 000 in women and 399.3/100 000 in men – just under that of the US. Switzerland reports 346/10000 women and 137.8/100 000 in men.

Few studies are available from Africa. Based on only the two known studies it is difficult to make a general statement about hip fracture incidence in Africa, but it seems that as in African-Americans, the hip fracture rates in the African population are much lower than in the Caucasian populations. Zebaze *et al.* conducted a study in Cameroon by documenting all patients aged 35 years and older admitted to the two main urban hospitals over a 2-year period with a diagnosis of fracture. Using the 1997 estimates of the population, the incidence of hip fracture was 57.1/100 000 women and 43.7/100 000 men.

Studies of hip fracture have also been done in both New Zealand and Australia and the fracture rates are comparable to that seen in Europe.

Fracture rates for Latin America are similar to that reported from the southern countries of Europe. Recently, hip fracture incidence has been reported from Rosario, Argentina. In the population over 65 years of age, the incidence was 646/100 000 for women and 345/100 000 for men.

In general people who live in latitudes further from the equator generally have a higher incidence of fracture (Dhanwal 2011).

Global nutrition dynamics

Global energy intake and related obesity levels are rapidly increasing, as are rates of other non-communicable diseases.

The rise in affluence of many populations since the end of World War II has given rise to widespread westernization. There is a worldwide shift in source of calories - diets appear to be shifting universally toward higher intakes of animal meats, partially hydrogenated fats, and sugars, and lower intakes of fiber (US Dietary Committee, Popkin 2006). This is combined with a general decline in physical activity. Degenerative diseases that were once mainly restricted to higher-income countries are now appearing throughout the world population (Anderson 1999).

Examples of adverse effects resulting from these lifestyle changes are well illustrated by the data from Hong Kong reported by Lau and Cooper (1999). These epidemiologists have linked the westernization of citizens of Hong Kong to increased hip fracture rates in both sexes over recent decades. Age-specific hip fractures in women in Hong Kong have increased steadily between 1966 and 1991 (Lau & Cooper, 1999).

Genetics

Considering such drastically different osteoporosis and/or age-related fracture rates raises the question of genetics. Is it likely that the alarmingly high rates of osteoporosis and fracture in the US are due to shared genes that are absent in the populations of countries with historically lower rates such as Japan?

Osteoporosis is a polygenic disorder, determined by multiple genes, each with relatively modest effects on bone mass and other determinants of fracture risk. These genes are clustered in three biological pathways: the estrogen endocrine pathway, the Wnt/beta-catenin signaling pathway and the RANK pathway. It is speculated that a minimum of 60% of peak bone mass (around age 30) is genetically determined, however even here it is impossible to dismiss dietary imbalance or excess, or the impact of physical exercise.

Population-based studies and case-control studies have identified polymorphisms in about 15 genes that have been associated with bone mass or osteoporotic fracture, including the vitamin D receptor (VDR) gene, estrogen receptor gene and collagen type I alpha gene. The individual contribution of these genes to the pathogenesis of osteoporosis is apparently small, however, reflected in the fact that the relationship between individual candidate genes and osteoporosis has been inconsistent in repeated studies (Stewart, 2000).

It seems that the genetic differences appear not so much in the genes themselves but in haplotypes. The VDR gene has been studied most extensively, and there is no doubt that allelic variations effect bone mineral density (BMD). The different alleles act the same across races, but frequencies of genotypes vary. The B allele *Bsm* I restriction fragment length polymorphisms (RFLPs) are associated with low BMD and high bone

turnover. This polymorphism is found in only 12% of Japanese women (1.4% homozygote BB), compared with 41% of Caucasians (16.7% homozygote BB) (Tokitan, et al, 2009). This indicates, in this single receptor scenario, much higher susceptibility to low BMD in Caucasian women.

However, it is documented that osteoporosis rates are rising in Japan over the last 40 years (as they are virtually everywhere), which shows that disease progression can occur in the Japanese regardless of genetic haplotype variation or tendency.

Although unquestionably genes play some part in the etiology of age-related bone loss, osteopenia, osteoporosis and the resultant fractures, it is indicated and equally accepted that diet and nutrition play a potentially more influential role, not only through direct affect but through epigenetic mechanisms. The percentage of responsibility of genes vs. environment is as yet un-definable, as this relationship is un-definable in all aspects of human development, though epigenetic effects of diet and lifestyle upon human health is beginning to be quantified. What is knowable at this moment, however, and what has been attempted here, is an understanding of the effect of diet on the mechanisms of bone health, which from a mechanistic angle are the same in all human bodies regardless of genotype. This understanding guides us towards the goal of a comprehensive understanding of the dietary factors involved in osteoporosis, and how to optimize their interactions to mitigate the effects of age on bone.

CHAPTER FOUR – THE CALCIUM PROBLEM, AND NUTRIENTS FOR BONE HEALTH

As calcium is the major component of bone, it has been generally accepted that calcium is the most necessary nutrient for strong bones, and therefore supplementation is the key. This is no doubt the impetus for the generalized recommendation of calcium supplementation by Western physicians for the prevention and treatment of osteoporosis, and for the overwhelming belief, in the researcher's experience speaking with patients, that calcium supplementation is the way to combat bone density loss.

Good data on the association of fracture rates with calcium intakes are available from Japan and clearly show that Japanese women have both less bone mineral and far fewer fractures than do American women (Fujita & Fukase, 1999). Their significantly lower calcium intake - 400-500 mg/day – is mainly as soybean products, small fish with bones, and vegetables. There is no appreciable intake of calcium supplements historically in Japan (Fujita, 1994).

Worldwide data raise serious questions about the relation between calcium intake and fractures. A large proportion of the worlds' population consumes low-calcium diets and, although quantitative data on the fracture rate in such populations are limited, it is obvious that these populations do not have excessive rates of fractures as would be expected if actual calcium requirements were far above their usual intake (Hegsted, 1986; Ross et al 1991). It seems clear that whatever the importance of calcium intake and bone mineral content may be, other important factors must be involved in determining the susceptibility to fractures.

In 2004 the US population spent 20.3 billion dollars on supplements (NIH). 61% of women over 60 take calcium supplements in the US (CDC) up from 28% in 1994. And yet, the US has one of the highest fracture rates in the world.

Even with the addition of vitamin D in recent years, the US preventive services Task Force concluded in 2013 that current evidence was insufficient to make a recommendation for post-menopausal women and this combined supplementation (Moyer et al, 2013). Looking at US fracture rates indicates that although this combination may be an improvement over calcium alone, the problem is still not being addressed.

Additionally, high dose calcium supplementation appears dangerous. A longitudinal (19 year) study of over 61,000 women indicate that high intakes of calcium in women are associated with higher death rates from all causes and cardiovascular disease but not from stroke (Michaelsson et al, 2013).

The association of calcium intake and mortality was especially strong when the study participant had a high level of calcium from diet combined with additional calcium supplementation. In this study there was no mention of Vitamin D, Vitamin K2 or other minerals, nutrients or trace elements.

Women with the highest intake of calcium (>1400 mg/day) and who used supplements had an all-cause risk for death 2.5 times higher than women who had similar total intakes but were not taking a supplement (Michaelsson et al, 2013).

Bone Histology

Osseous tissue consists of widely separated cells surrounded by large amounts of matrix. Compact bone is arranged in units called osteons or Haversian systems. Osteons contain blood vessels, lymphatic vessels, nerves, and osteocytes along with the calcified matrix. Osteons are aligned along lines of stress. Osteons are comprised of groups of osteoblasts, which secrete matrix and collagen fibers to build bone. Trabecular bone is the spongy bone that is comprised of collagen matrix and hydroxyapatite.

Mesenchymal precursor cells in bone marrow have the ability to differentiate into osteoblast, chondrocyte, adipocyte or myoblast lineages.

Osteochondroprogenitor cells are undifferentiated cells that arise as osteoblast lineage, and these cells can then differentiate into osteoblasts or chondrocytes, producing either bone or cartilage respectively.

Runx2 is a transcription factor that must be triggered for the precursor cell to differentiate into an osteoblast. Runx2 expression induces osteoblastic differentiation as well as triggering the expression of osteoblastic genes alkaline phosphatase, osteocalcin, osteopontin and collagen type 1.

Runx2 is promoted by retinoic acid, the active form of vitamin A in the body (Bergeron et al, 2006) and its interaction with bone morphogenetic proteins (BMPs).

There is an inverse relationship between osteoblast and adipocyte differentiation, and in bone loss conditions, decreased osteoblast numbers correlate with increase adipocyte numbers. Here again retinoic acid inhibits adipocyte differentiation, favoring osteoblast lineage progression (Skillington et al, 2002). This differentiation is enhanced

through retinoic acid induction of alkaline phosphatase, osteocalcin and osteopontin (Sodek et al, 1995).

Bone Remodeling

Osteoblasts and osteoclasts work synergistically, regulating each other in the bone remodeling process. Immature osteoblasts secrete receptor activator of nuclear factor-kappaB ligand (RANKL), which binds to a receptor on the surface of the osteoclast precursor cells, activating maturity to osteoclasts. As they erode bone, the eroded bone releases growth factor signals such as insulin-like growth factor-1 (IGF-1) and transforming growth factor-beta (TGF-beta) that assist with maturation of osteoblasts. As osteoblasts mature, they secrete less RANKL and begin secreting osteoprotegrin (OPG), which prevents RANKL from activating osteoclasts, allowing bone formation to exceed bone breakdown. Estrogenic effects keep this balance in place.

Scaffold and Mineralization

Mineralization: calcium, phosphorus, magnesium, vitamin D, vitamin K2, manganese, boron, fluoride.

Scaffold: collagen, vitamin C, silicon, copper, zinc.

1. Mineralization

Calcium and Phosphorus combine to form hydroxyapatite

Calcium is absorbed in two ways in the intestine, actively and passively.

The first absorption site is considered active in that it requires the presence of 1,25Dihydroxyvitamin D₃ (1,25(OH)₂D₃) the hormonally active form of vitamin D. Passive paracellular absorption can happen throughout the entire length of the intestine but occurs predominantly in the ileum (Christakos et al 2011). Ileum absorption is passive in that it requires no assistance, however in the presence of a slowly absorbed simple sugar, such as lactose, absorption of calcium can double (Guegen, 2000).

It also appears that 1,25(OH)₂D₃ induces the expression of claudin-2 and claudin-12 in intestinal epithelial cells. Claudins increase junction ion permeability, thereby allowing increased calcium absorption in areas of passive absorption (Christakos et al, 2011).

The major function of vitamin D is to optimize intestinal calcium and phosphorus absorption for proper formation of the bone mineral matrix. It has recently been demonstrated that a minimum 25(OH)D level of 32 ng/mL is necessary for optimal protection from fracture. Without adequate vitamin D, the body absorbs no more than 10% to 15% of dietary calcium (Khazai et al, 2008). Intake to maintain minimum blood levels appears to be approximately 5,000iu/ day. This is confirmed in multiple studies, one by Veugelers et al, suggesting optimal levels of 50 nmol/L or more which is achieved when intake is approximately 8000 IU of vitamin D per day. Another study looked at traditional populations in East Africa and found mean levels of 50-171 nmol/L (Luxwolda et al, 2012).

Of all the nuclear receptors, the vitamin D receptor domain for binding DNA (DBD) is the most conserved domain of the nuclear receptor family (Lou and Tuohimaa,

2006). The researcher makes note of this with a mind towards establishing dominance in the hierarchy of nutrients to better understand ratios.

The presence of estrogen has also been seen to increase calcium absorption even in the absence of $1,25(\text{OH})_2\text{D}_3$, and it appears that prolactin may act with $1,25(\text{OH})_2\text{D}_3$ to increase active intestinal calcium absorption, which would make sense with the increasing need of calcium by the neonate.

Gut bacteria play a crucial role in proper calcium absorption. Healthy gut bacteria produce short-chain fatty acids (SCFAs), which increase the presence of proteins called calbindins that are necessary for calcium absorption across the enterocytes. Calbindin D-9k is activated by $1,25(\text{OH})_2\text{D}_3$. (Onishi, 2008)

Once the calcium has been properly absorbed, it must be bound with phosphorus in order to create hydroxyapatite, the mineralizing substance deposited on the bone matrix of trabecular bone. This is carried out primarily by osteocalcin (OC), a Gla protein secreted by osteoblasts and considered pro-osteoblastic. Osteocalcin is also implicated in calcium ion homeostasis (Hauschka 1989).

Crucial, however, is that osteocalcin undergo gamma carboxylation in order to function as a calcium binding protein. The presence of 3 vitamin K-dependent gamma carboxyglutamic acid residues is critical for osteocalcin's structure (Grundberg et al, 2012).

Osteocalcin is a vitamin K-dependent protein, with synthesis regulated by vitamin D metabolites (Skjodt et al, 1985). Magnesium is also implicated, however this interaction has yet to be elucidated (Alissa et al, 2014).

Under-carboxylated osteocalcin is used as a measure vitamin K status, and carboxylated osteocalcin is used as a marker of bone formation.

Menaquinones

Menaquinones are subtypes of vitamin K2. They appear to function very differently than vitamin K1. The carboxylation of osteocalcin is carried out by vitamin K2 subtypes (Yasui et al, 2006).

There appears to be little of this vitamin in the American diet. Vitamin K2 is a bacterial product, and is prevalent in fermented soybeans. The two subtypes that appear to influence bone strength are menaquinone-4 and menaquinone-7. Comparisons of BMD in post-menopausal women with and without menaquinone supplementation indicate strongly that menaquinones suppress the decrease of BMD as compared to controls (Iwamoto et al, 1999).

Of all the subtypes, menaquinone-7 (MK-7) has been studied most extensively in relation to bone density. Yamaguchi et al show that protein content, alkaline phosphatase activity, osteocalcin and DNA content in osteoblastic cells was significantly increased in the presence of MK-7 (Yamaguchi et al, 2001).

A meta-analysis carried out with Western studies supported the hypothesis that vitamin K2 plays a role in the prevention of fractures of postmenopausal women (Huang et al, 2015).

Other studies show increased bone strength without alteration in BMD. Iwamoto (Iwamoto et al 2014) and Shiraki both found that Vitamin K2 treatment effectively prevented the occurrence of new fractures, although the vitamin K2-treated group failed

to increase in LBMD. Shiraki also confirmed that vitamin K2 treatment enhances gamma-carboxylation of the osteocalcin molecule (Shiraki et al 2000).

There is increasing evidence that there are synergistic effects between vitamin D3 and vitamin K2. In investigating diabetic mice, Poon et al found that the combination treatment increased the levels of bone anabolic markers and bone formation transcription factors, with a greater magnitude of increase observed in osteoblasts of db/db mice. Combined vitamins K2 plus 1,25(OH)2D3 treatment significantly enhanced migration and the re-appearance of surface microvilli and ruffles of osteoblasts, returning them to function (Poon et al 2015).

Knappen et al showed for the first time clinically statistically significant protection of the vertebrae and the hip (femoral neck) against osteoporosis. After 3 years of supplementation of 180 mcg vitamin K2 as MK-7 daily, improvements in both bone-mineral content (BMC) and bone mineral density (BMD) were statistically significant in the vitamin K2 group. Moreover bone strength was statistically improved, demonstrating therapeutic benefits for the MK-7 group as compared to the placebo group. This data indicates that MK-7 supplementation helps postmenopausal women to prevent bone loss.

It is also widely accepted that there is a link between osteoporosis and vascular calcification (Hofbauer et al, 2007). Tissue calcification outside of the bone appears to be strongly positively effected by vitamin K2 administration, and the researcher suggests that the properties of vitamin K2 are a main correlative link between the two maladies.

Vitamin D and Vitamin A

The role of vitamin D in bone growth has been elucidated above. Vitamin A acts not only to trigger differentiation of osteoblasts, but functions later on in the bone remodeling process as a balance to the bone growth process promoted by vitamin D. Healthy bone metabolism cannot be maintained without the correct balance between vitamin D and vitamin A. Both osteoblasts and osteoclasts contain both receptors.

The very high rates of hip fracture seen in Sweden and Norway can be explained through this interactive balance. In high latitudes, the angle of the sun and absence of length of sunlight causes what is commonly known as the “vitamin D winter”, during which very little vitamin D is made in the skin. Combined with this, retinol intake appears to be approximately six-fold higher in these countries than elsewhere in Europe (Masterjohn, 2006). Melhus et al published the first study on vitamin A intake, bone mineral density (BMD) and hip fracture risk on women from the county of Uppsala, Sweden. They found that intakes of retinol exceeding 5,000 IU per day were associated with a 10 % decrease in BMD at the hip, and a doubling of the risk of hip fracture (Melhus et al, 1998). However, this is against the backdrop of exceptionally low vitamin D intake: The women averaged between 97 IU of vitamin D per day to 185 IU of vitamin D per day, consuming between one twentieth and one fortieth of what is suggested by recent research to maintain optimal serum levels of vitamin D.

In both the rat (Rhode and Deluca, 2005) and the human (Johansson and Melhus, 1998) vitamin A antagonizes the rise in serum calcium that is induced by vitamin D.

The ideal levels of vitamin A intake are as yet undetermined, however looking at history again can give clues. A mainstay traditional food of the northern latitudes is cod liver oil, which supplies approximately 10,000 iu of vitamin A (retinol) in a dose and 2-

5,000 iu Vitamin D. Vitamin A is complex however, in that retinol is the form the body uses, which is the form provided by animal products. Beta-carotene and beta cryptoxanthin are found in plants, and the conversion of these nutrients to retinol is required. 12x the amount of beta carotene is required, and 24x the amount of beta cryptoxanthin. So diet type will specify how much nutrient is needed.

In a study of 600 patients supplementation with 15,000iu/day of retinol slowed the loss of retinal function, without detriment even at a 12 year follow up (Hendler, 2008).

Influences on mineralization

Hormones

Osteoblast and osteoclast function, the functional cells of bone resorption and mineralization respectively, are interrelated. Bone turnover is continuous, and harmonious function of these two cell types ensures a balance of bone mineralization and breakdown. Estrogen appears to be the most direct and dominant factor in maintaining this equilibrium, and will promote this balance even in the presence of dietary deficiency.

In adults estrogenic affect on bone metabolism appears to be on inhibiting differentiation of osteoclasts through stimulating a decoy receptor osteoprotegrin (OPG). OPG intercepts the ligand for RANK, thereby eliminating the activation of the NF kappa b pathway that in bone activates osteoclastogenesis. Estrogen also appears to downregulate pro-inflammatory cytokines, which increase the creation of pre-osteoclast cells in bone marrow.

The most direct influences on calcium homeostasis outside of the bone are parathyroid hormone (PTH) and Calcitonin. Under normal circumstances and with normal serum mineral concentrations the balance of calcium in the blood and in the bones is functional both for the body processes requiring calcium and the bones. However in states of imbalance the body will either draw calcium from the bone (PTH) or put a stop to absorption and attempt direct deposition (Calcitonin). PTH is triggered through a delicate sensing mechanism when the body requires calcium. The presence of PTH triggers osteoclasts to begin to bone breakdown to increase serum calcium.

Phosphorus

Phosphorus is common in most all diets, and deficiency is not generally considered. However high intake of phosphorus is highly detrimental not only to bone density but has been implicated in vascular calcification, arterial sclerosis and cardiovascular diseases (Morimoto et al, 2014). The RDA from the US Dietary Committee is 700mg/day, and as phosphorus is common in meats, grains and dairy products, Americans have little trouble reaching this recommended allowance. However phosphorus in the form of phosphorus salts is a ubiquitous additive to processed food. It is thought that a diet high in processed foods will add approximately 1000mg of phosphorus to the daily diet. This phosphorus is almost entirely absorbed, compared to the approximately 60% absorption of naturally occurring mineral. Higher phosphorus intake is directly associated with higher serum PTH. Commercial cola beverages contain phosphoric acid, which has been shown to interfere with calcium absorption as well as trigger a release of PTH. This acute release of PTH draws calcium from the bone into the

bloodstream. In a large population-based cohort, consistent robust associations were observed between cola consumption and low bone mineral density in women (Tucker et al, 2006; Fitzpatrick & Heaney, 2003).

Sodium

Dietary sodium is another cause for concern in relation to calcium homeostasis. Once the body has achieved proper serum sodium, the excess is excreted. Every gram excreted pulls 26 mg of calcium. In other words, slightly more than 1 teaspoon of sodium will begin to pull circulating calcium into the urine. If there is insufficient serum calcium, PTH will be triggered and bone breakdown will begin. In 2004, the director of the National Heart, Lung, and Blood Institute estimated that reducing sodium levels in processed and restaurant foods by 50 percent would save 150,000 lives a year. Studies published in *Nutrient* magazine that looked at this relationship found that bone mineral density in adult women could be maintained by reducing sodium to 2,300 mg/day and calcium to 1000mg/day.

Considering the effects of phosphorus and sodium on bone density again highlights the importance of ratio in dietary intake.

Magnesium

About 60% of total body magnesium (Mg) is stored in the bone. Skeletal Mg resides on cortical bone either on the surface or in the hydration shell around the hydroxyapatite crystal. Osteoporotic women with demonstrated Mg deficiency have larger and better-organized crystals in trabecular bone than controls – larger crystal size as well as linearity impairs the ability of the bone to bear weight (Cohen, 1981). Apart

from a structural role in the crystals, Mg is essential to all living cells, including osteoblasts and osteoclasts.

In a study by Rude et al, using Mg deficient rats, serum 1,25-dihydroxy-vitamin D was significantly lower than in controls.

Mg deficiency showed a significant fall in both serum alkaline phosphatase and osteocalcin suggesting decreased osteoblast activity, however there was no difference in osteoblast numbers. Decreased bone volume and trabecular thickness were also noted (Rude et al, 2004). Increased bone resorption was suggested by an increase in osteoclast number over time compared with controls as well as surface of bone covered by osteoclasts and eroded surface (Rude et al, 2004).

Inadequate blood magnesium levels are known to result in low blood calcium levels, resistance to parathyroid hormone (PTH) action, and resistance to some of the effects of vitamin D (Rude et al, 2006). Low magnesium is correlated with strongly with diabetes, insulin resistance, and myocardial infarction. In a cross-sectional study of 11,686 middle-aged women; the lowest prevalence of metabolic syndrome was found in the group of women with the highest quintile of magnesium intakes (median intake, 422 mg/day). Smaller studies have looked directly at BMD and magnesium intake, showing improvement with 750mg/day (Stendig-Lindberg et al, 1993)

Ideal magnesium intake has yet to be undetermined. Looking to history we see early diets high in nuts, seeds, legumes and greens, all high in magnesium (Balter, 2012). It is suggested from these early diets that the ratio of magnesium to calcium was approximately 1:1 or higher (Dean, 2004).

The Interplay of Mg and vitamin K2 is also of significance. A Japanese study by Amizuka et al showed Mg-insufficient bone revealing fragility to mechanical stress despite normal or higher levels of bone mineral content (higher mineral content is most likely appearing due to the fact that Ca will “fill in” for Mg in the absence of Mg). This fragility stimulated osteoclastic bone resorption. In contrast, vitamin K(2) (MK-4) inhibited osteoclastic bone resorption stimulated by the Mg-insufficiency, thereby normalizing bone remodeling. The Mg-insufficiency caused an increased concentration of calcium, which resulted in an extremely-high purity of hydroxyapatite crystal and accelerated mineralization in bone. In contrast, MK-4 did not affect the calcium-concentration nor crystal purity, but repressed the mineralization accelerated by Mg-insufficiency. Thus, MK-4 appears to recover the "bone quality" lessened by the Mg-insufficiency by two mechanisms: controlling bone turnover and mineralization (Amizuka, 2005).

Japanese studies have used low dose supplementation (45mcg) and high dose supplementation (15,000mg) of vitamin K2 in studies and have seen positive effect in both cases, without any detrimental effects (Koitaya et al, 2013; Pucaj et al, 2011).

Trace Minerals

Boron

Supplementation has been studied in rats, with a study by Chapin et al showing boron increasing vertebral strength on compression significantly, by 5-10% in all dose

groups (Chapin et al 1997). All dose groups were significantly lower than what has been recognized as a toxic level.

There is a suggestion that boron may increase the efficacy or utilization of vitamin D, and boron can alleviate marginal vitamin D deficiency (Hunt, 2012). Boron has also been reported to increase the levels of steroid hormones (e.g. estrogen and testosterone) in serum by influencing their metabolism (Devirian & Volpe, 2003). Typical boron intake hovers around 1mg/day in the US. 3mg/day is where benefits begin to be found (Gallardo et al, 2004).

Manganese

Manganese-depleted diets demonstrate that osteoblast activity was impaired as was osteoclast activity. Manganese deficiency appears to slow bone resorption while at the same time produce brittle bone – which is then not broken down. The serum level of manganese in a group of osteoporotic postmenopausal women was significantly lower than age-matched controls (Strause 1987).

Blood calcium, phosphorus, and alkaline phosphatase levels were also elevated, which seems to indicate increased bone remodeling as a consequence of insufficient dietary manganese.

Fluoride

Fluoride is an integral part of bone and a necessary mineral in healthy bone formation. Fluoride stimulates osteoblast proliferation, and the formation of osteoid. Fluoride's high reactivity and small radius allow it to either displace the larger hydroxyl

(-OH) ion in the hydroxyapatite crystal to form fluoroapatite – which hardens tooth enamel - or to increase crystal density by entering spaces in the hydroxyapatite crystal thereby stabilizing bone mineral.

However an excess of fluoride is highly detrimental, in the long-term, to bone health. If too much fluoride is present continuously, this osteoid will not mineralize properly and this will become brittle bone susceptible to fracture. In a study by Chacra et al, it was found that though the osteoblasts in fluoride-treated bone are active, there are fewer highly secretory osteoblasts, suggesting that although fluoride is stimulative to osteoblastic precursors, it is toxic to individual osteoblasts at the same concentration (Chachra et al, 1999). In a recent study by Turner et al high dose fluoride treatment decreased bone strength in rabbits, even in the presence of normal mineralization. Overall the literature suggests that though it is necessary in trace amounts, over fluoridation has serious consequences on bone strength.

2. Scaffold

The bone matrix scaffold on which the hydroxyapatite is laid is predominantly collagen, and proper collagen formation requires a complete set of collagen peptides. Vitamin C must also be present as a cofactor in the cross linking of collagen fibrils, and zinc must be present as it stimulates the collagen synthesis in osteoblasts. Copper is also needed for collagen synthesis, and silicon appears to stabilize the collagen, assisting the vitamin C in formation of cross-links

Protein

Protein makes up about 50% of bone volume, and protein intake is necessary for bone strength. However the high protein content of Western diets is often cited as a risk factor for osteoporosis or bone fractures (Heaney 2007). High protein intakes have been shown to affect calcium homeostasis, resulting in increased calcium excretion, but findings regarding the effect of protein on calcium balance and bone health have been mixed.

Some studies suggest that, as a result of increased urinary calcium excretion with high protein intake, there is an increased risk of fractures or osteoporosis (Heaney 2007). As protein intake increases, there is an increase in urinary calcium. One estimate is that there is a 50% increase in urinary calcium associated with doubling protein intake or roughly 1 mg urinary calcium for every gram of dietary protein (Freskanich, 1996). However, studies in which diets provided 30% of energy as protein (181–214 g/d) found no increase in calciuria (Nuttall et al, 2006). In healthy adults, when protein intake was increased from 0.7 to 2.1 g / kg/ d, urinary calcium increased, but intestinal absorption of calcium increased as well (Kersttetter et al, 2003).

Increased calciuria does not necessarily translate to calcium loss, negative calcium balance, or reduced bone mass. To the contrary, several studies have observed a positive association between dietary protein intake and increased bone mineral content or decreased risk of fracture (Munger et al, 1999; Hannan et al, 2000). One study found that among premenopausal women, there was a significant positive association between

protein intake and bone mineral content, suggesting that dietary protein intake actually may be a determinant of the peak bone mass (Cooper et al, 1996).

However, at low protein intakes (<0.8 g /kg/ d) intestinal calcium absorption is reduced and levels of parathyroid hormone increase, causing the release of calcium from bone (Kerstetter et al, 2003).

Examining the mechanism, the researcher suggests that diets higher in protein, in the presence of adequate calcium, do not trigger PTH and do not negatively affect bone density. Again it is a question of ratios.

Protein intake provides structural peptides (amino acids) for the production of collagen, the bone matrix scaffold upon which mineralization occurs.

Collagen

The role of collagen in bone health is often overlooked. The DXA scan, which is the gold standard of bone density measurement and diagnosis of osteoporosis in the US considers mainly the density of the trabecular bone matrix but does not consider the health of the collagen matrix which supports it and gives bone its flexibility. Flexing under pressure could arguably be more important than density.

To this end the researcher proposes that BMD alone is not an accurate predictor of bone strength. Bone matrix consists of two parts – the mineralization that provides “density” and hardness, and the collagen scaffold that provides absorption of impact and flexibility. BMD is certainly a major parameter influencing bone strength, but the three-dimensional organization of the trabeculae and the health of the collagen scaffold must

also contribute to bone strength. Alterations of collagen properties can affect the mechanical properties of bone and increase fracture susceptibility. Several studies suggest that part of the large variation in bone strength may be related to differences in the quality of the collagenous matrix (Viguet-Carrin et al, 2005).

Type I collagen, the major organic component of bone matrix, undergoes cross-linking as part of a structural strengthening process. The quality of this cross-linking determines much of the strength of the matrix (Saito 2010). Collagen matrix undergoes a series of modifications that occur with aging, such as (non-enzymatic) glycation, which change the effectiveness of the scaffold. This reaction leads to the formation of advanced glycation end products (AGEs), which accumulate in bone tissue. A number of studies have looked at the effects of AGEs on bone.

The accrual of AGEs directly affects the quality of cross-linking, impairing formation of a structurally sound matrix (Viguet-Carrin, 2006).

Valcourt et al found that the resorption process was also markedly inhibited in the presence of AGEs (Valcourt et al, 2006). Once the impaired matrix is formed, AGEs also interfere with osteoclastogenesis and resorption, impairing the resorption of the faulty matrix. This impaired scaffold is retained in the bone. The accumulation of AGE crosslinks in cartilage results in increased stiffness and brittleness of the tissue (Verzijl et al, 2002). In addition to AGEs, racemization and isomerization appear to produce the smaller fibrils that may affect the proper mineralization of the collagen fiber, adding to decreased bone strength (Viguet-Carrin, 2006).

Vitamin C

Vitamin C is a necessary component in collagen synthesis, as evidenced in in-vitro studies showing vitamin C induced a dose-dependent increase in collagen type I deposits by normal human fibroblasts (Boyera, 1998). Vitamin C is known to stimulate procollagen, enhance collagen synthesis, and stimulate alkaline phosphatase activity, which is a marker for osteoblast formation (Morton, 2001). High vitamin C intake is associated with lower bone loss (Sahni et al, 2008). Morton et al report that a mean daily dose of 745mg provides beneficial effects on BMD in postmenopausal women. They also report that among current estrogen users, those also taking vitamin C had higher BMD levels at all sites (Morton et al, 2001). Stimulation of bone formation was also observed in ovariectomized mice (Zhu et al, 2012).

A study by Carr and Frei suggested raising the RDA to 120mg daily based on review of clinical and epidemiological evidence (Carr and Frei, 1999). This was updated by Frei et al. in 2012, based on analyses of randomized placebo controlled trials, to 200mg/day. However recent meta-analysis looking at disease prevention suggests a higher intake of 500mg/day (McRae, 2016).

Trace Minerals

Copper

Copper is required for the cross-linking of collagen and elastin, which are essential for the formation of strong and flexible connective tissue. It is the action of the lysyl oxidase (LOX) protein interacting with the copper that helps maintain the integrity of connective tissue in the bone, heart and blood (Turnland, 2006). “The role of copper in the metabolism of bone and connective tissue is so profound that one might be tempted to speculate that inadequate copper nutrition may be an important factor in the etiology of osteoporosis”. Copper supplementation increases the rate of bone healing (Dollwet 1988).

A study by Mahdavi-Roshan et al showed copper, magnesium, and zinc status of osteoporotic women were significantly lower than normal (Mahdavi-Roshan et al, 2015).

Silicon / Silica

Silicon improves bone matrix quality and facilitates bone mineralization. Increased intake of bioavailable silicon has been associated with increased bone mineral density (Reffitt, 2003). Silicon supplementation in animals and humans has been shown to increase bone mineral density and improve bone strength.

In vitro studies have demonstrated that silicon stimulates type 1 collagen synthesis and osteoblast differentiation (Reffitt, 2003). Studies in rats have demonstrated that silicon improves calcium incorporation in bone (Hott et al, 1993). The effects of silicon are seen primarily in the presence of estrogen (Macdonald et al, 2012).

In cases of bone generation through exercise, mineralization occurs in the electronegative areas that are generated by compression (Micalau et al, 2007). It is possible that silicon plays a role in the electrochemical process of mineralization.

Five reports have been published using the ovariectomized rat to study the effects of dietary silicon on bone metabolism. Hott et al. compared physiological levels to low levels of dietary silicon. The mineral deposition and bone formation rate was 30% greater in the group with silicon intake (Hott et al,1993).

Although silicon supplementation is associated with increased bone mineral density, the exact mechanism for this action has not been identified (Price et al, 2013). Serum measurements of bone turnover have been inconsistent, while markers of bone matrix formation are consistently increased. This may indicate that silicon improves mineralization without affecting the rate of bone formation or bone loss. There may also be an effect on collagen that improves bone strength independent of mineral density (Spector et al, 2008).

Diets containing more than 40 mg/day of silicon have been positively associated with increased femoral bone mineral density compared to dietary intake of less than 14 mg/day (Jugdaohsingh et al, 2004). In a North American epidemiological study, none of the postmenopausal women achieved 40 mg/day of dietary silicon intake (Jugdaohsingh et al, 2004).

Zinc

Zinc treatment enhances bone formation by stimulating osteoblast proliferation, bone marker protein alkaline phosphatase activity and collagen synthesis (Seo et al, 2010). Cell proliferation was stimulated even at low zinc treatment. Zinc appears to enhance the effects of vitamin K2 on bone calcium content, showing an appreciable increase in alkaline phosphatase activity (indicating bone formation) and calcium content in normal rats (Ehara 1996).

CHAPTER FIVE: AMERICAN DIETARY DEFICIENCY

Nutrient Deficiency

The CDC Second Nutrition Report is a report on nutrition and deficiency in the US. It uses the US Dietary Committee RDAs (Table 1) as a reference, and interprets the National Health and Nutrition Examination Survey (NHANES) data.

Table 1. Recommended RDAs from the Dietary Guidelines Advisory Committee

Nutrient	RDA
Calcium	1,200 mg
Vitamin D3	400 iu
Magnesium	400 mg
Phosphorus	1000 mg
Protein	50 g
Vitamin A	5,000 iu
Vitamin C	60 mg
Zinc	8 mg

The 2006 CDC report shows over 10% of the population is deficient in vitamin B6, over 8% vitamin D, 6% vitamin C, less than 1% vitamin A.

This is compared with the much more dire report from the Environmental Working Group (EWG).

The EWG interpreted the NHANES data, with most recent information from 2011. Vitamin D is found to be deficient in 95% of the US population over 19 years of age, magnesium in 61%, calcium in 49% and vitamin C in 43%.

From the EWG report:

“Some American adults get too little vitamin D, vitamin E, magnesium, calcium, vitamin A and vitamin C. More than 40 percent of adults have dietary intakes of vitamin A, C, D and E, calcium and magnesium below the average requirement.

Some Americans get too much Vitamin A while others get too little. On one side, more than half of American adults and American teenagers have low dietary vitamin A intake. On the other side, at least 13 percent of children 8 and younger ingest vitamin A in amounts exceeding the tolerable upper intake level set by the Institute of Medicine.”

The NHANES does not report data on key nutrients for bone health: vitamin K2, and trace minerals including silicon.

The US Dietary Guidelines Advisory Committee (DGAC) also interpreted the NHANES data and found that while diet quality varies somewhat by the setting where food is obtained, overall, independent of where the food is prepared or obtained, the diet quality of the U.S. population does not meet recommendations for fruit, vegetables, dairy, or whole grains, and exceeds recommendations, leading to overconsumption, for the nutrients sodium and saturated fat, and the food components refined grains, solid fats, and added sugars.

The DGAC found that several nutrients are under-consumed and the Committee characterized them as “shortfall nutrients”: vitamin A, vitamin D, vitamin E, vitamin C, folate, calcium, magnesium, fiber, and potassium. They went on to say that obesity and chronic diseases with a nutritional origin are “very common” in the US.

Hormone deficiency – estrogen and phytoestrogens

Decline of estrogen is a well-accepted primary cause of bone density loss. However as highlighted previously, lower BMD does not equate to fracture risk, and more to the point decline of estrogen happens in all women, but not all women suffer osteoporosis or fracture. One possible piece of this puzzle is the intake of dietary phytoestrogens, which have been repeatedly shown to function as a weak estrogenic compound in the body, including the repression of osteoclasts.

The anti-resorptive effects of estrogen on bone metabolism are thought to be mediated through modulation of osteoblastic lineage cells. Receptor activation of nuclear factor-kappaB ligand (RANKL) is the essential factor for osteoclast formation and activation of this pathway enhances bone resorption. By contrast, osteoprotegerin (OPG), which is produced by osteoblastic lineage cells acts as a decoy receptor that neutralizes RANKL and prevents bone loss. 17 beta-estradiol was found to stimulate OPG mRNA and protein secretion in a human osteoblastic cell line through activation of the estrogen receptor (ER)-alpha. The assumption by this researcher is that phytoestrogens would have an effect less than but similar to estrogen. In a study done in 2004, Kanno *et al.* investigated the effects of coumestrol and “other phytoestrogens” on osteoclast differentiation and found an inhibitory effect on the differentiation of osteoclasts.

Another study published in 2014 by Huh et al. used formononetin, a phytoestrogen found in red clover. Similarly they found that this substance blocked osteoclast activation. Note that one pharmaceutical, denosumab, a human monoclonal antibody, is directed towards this pathway with some success but with side effects.

Phytoestrogen

Many studies report the positive effects of phytoestrogens on osteoblastogenesis, osteoblastic differentiation or activation, or repressing osteoclastogenesis.

Ming et al reviewed previous studies on genistein (from soy) and icariin (from Epimedium/Yin Yang Huo). Genistein has dual functions on bone cells, able to inhibit bone resorption activity of osteoclasts and stimulate osteogenic differentiation and maturation of bone marrow stromal progenitor cells (BMSCs) and osteoblasts. Genistein is an estrogen receptor (ER)-selective binding phytoestrogen, with a greater affinity to ERbeta. Genistein enhances osteoblastic differentiation and maturation by activation of ER pathways, and inhibits osteoclast formation and bone resorption through inducing osteoclastogenic inhibitor osteoprotegerin (OPG) and blocking NF-kB signaling; though in a single study Yamaguchi et al evaluated the effects of both estrogen and genistein on the NF-kB pathway, and found that 17beta estradiol suppressed this inflammation pathway but genistein did not (Yamaguchi et al 2006).

Icariin isolated from Epimedium herb (Yin Yang Huo), stimulates osteogenic differentiation of BMSCs and inhibits bone resorption activity of osteoclasts. Interestingly it appears from these studies that Icariin has no estrogenic activity, though it

appears to be more potent than genistein in promoting osteogenic differentiation and maturation of osteoblasts. (Zhang et al, 2006)

The existence of a prenyl group has been suggested to be the reason why icariin is more potent than genistein in osteogenic activity. It is suggested that the prenylflavonoids may represent a class of flavonoids with a higher osteogenic activity (Jia 2012).

Atkinson et al looked at a daily dose of 26 mg biochanin A, 16 mg formononetin, 1 mg genistein, and 0.5 mg daidzein for 1 year in a double-blind, randomized, placebo-controlled trial; 177 completed the trial. Bone density, body composition, bone turnover markers, and diet were measured at baseline and after 12 months. Bone-specific alkaline phosphatase and N-propeptide of collagen type I, both bone formation markers, were significantly increased in the intervention group compared with placebo in postmenopausal women (Atkinson et al, 2004).

Vierek et al assessed the effects of genistein on OPG mRNA and protein production in osteoblasts obtained from healthy donors. Genistein increased OPG mRNA levels and protein secretion by osteoblast cells by up to six-fold. Pre-treatment with genistein partially prevented the inhibitory effects of the glucocorticoid dexamethasone on OPG mRNA and protein production. The stimulation of OPG mRNA levels by genistein was not affected by a protein synthesis inhibitor (cycloheximide) so was shown to be due to enhancement of OPG gene transcription. This suggests that the genistein is capable of upregulating the production of OPG in osteoblasts. Thus, dietary sources of

phytoestrogens may help to prevent bone resorption and bone loss by enhanced osteoblastic production of OPG (Vierek et al).

Rickard et al investigated genistein compared to 17beta estradiol (E(2))and raloxifene. Both genistein and E(2) increased the endogenous gene expression of the progesterone receptor (PR) and alkaline phosphatase (AP), but inhibited osteopontin (OP) gene expression and interleukin-6 (IL-6) protein levels. Raloxifene had no effect on these bone markers. Genistein, but not raloxifene, also mimicked E(2) action in the osteoblast/ERbeta cells increasing PR gene expression and inhibiting IL-6 production. These findings demonstrate that genistein behaves as a weak E(2) agonist in osteoblasts (Rickard et al, 2003).

An examination of the human studies in vivo present a range of effective dose levels, starting at about 45mg/day, and appear to be dose-dependent with positive effects seen up to 85g or higher (Setchell and Lydeking-Olsen, 2009). There is however a wide range of effect seen in Western studies, and this appears to be due to the presence of the proper gut bacteria. Only approximately 45% (Setchell and Lydeking-Olsen, 2009) of the Western post-menopausal participants have the proper bacteria to transform soy isoflavones genistein and daidzein into equol. Equol has a higher affinity for the estrogen receptor, and those who were equol metabolizers were the ones in which BMD improved.

Phytoestrogen deficiency in the US

Isoflavone levels quantified from the NHANES 1999–2004 subsamples were 4 to 50 times lower than levels observed in Japanese men and women (Adlercreutz et al.,

1991; CDC, 2005; Uehara et al., 2000); Japanese women (Arai et al., 2000); postmenopausal Chinese women (Zheng et al., 1999); Singaporean women (Chen et al., 1999; Seow et al., 1998); and Japanese women living in Hawaii (Maskarinec et al., 1998).

Supplementing an omnivorous U.S. diet over a three month period with 60 grams of soy powder for female subjects increased isoflavone levels by more than thirteen-fold. (Albertazzi et al., 1999).

Estrogen and exercise

The positive effects of exercise on bone density are undisputed, and impact exercise is an integral and necessary part of building bone mass. The effects of exercise on bone can be amplified in the presence of estrogen or estrogenic compounds.

The osteocyte network throughout bone senses mechanical force. It transmits an electric current through the osteocyte network to influence the activity of osteoblasts and osteoclasts. Mechanical stress on bone causes the piezoelectric effect, which triggers osteoblastogenesis.

Osteocyte death increases after estrogen loss and/or lack of exercise for long periods. When there's no stress osteoblastogenesis is halted as osteoclastogenesis is triggered.

Kondoh et al showed that ER alpha in osteocytes regulates trabecular bone formation in female mice. Trabecular bone mineral density of female, but not male ERalpha knockout mice was significantly decreased. Bone formation parameters in these

mice were significantly decreased while osteoclast parameters appeared unchanged. This suggests that ERalpha in osteocytes exerts osteoprotective function by positively controlling bone formation (Kondoh et al, 2014).

CHAPTER SIX: SYSTEMIC FACTORS INFLUENCING BONE HEALTH

There are a number of system-wide factors that can influence nutrient absorption and override nutrient effectiveness. These factors are noted briefly in this section.

Inflammation

This exploration previously discussed the integral role of the NF-kB/RANKL pathway, which is directly responsible for osteoclast differentiation and activation, and osteoblast suppression.

Homocysteine

High homocysteine appears to correlate to higher incidence of hip fracture. Women with high homocysteine had nearly a doubled risk of hip fracture (McClellan et al, 2004). This was confirmed by Bahtiri et al which found high homocysteine levels to be an independent risk factor for osteoporosis (Bahtiri et al, 2015).

The B complex of vitamins regulate homocysteine. Although there is very little-to-no B vitamin deficiency listed in the reviews of the NHANES data, it is important to note the relatively high rate of occurrence of the MTHFR mutation, in which the body cannot methylate folate. In these instances methylated folate supplementation is necessary.

Stress

Cortisol release reduces calcium absorption in the intestines, slowing bone formation (Cohen, 2012). Collagen can be a direct target of chronically elevated cortisol, as I

interferes with cross link formation. Cortisol also attacks the periosteum, resulting in an inhibition of osteoblast formation (Cohen 2012).

Medications:

Selective Serotonin Reuptake Inhibitors (SSRIs)

There is evidence that SSRIs affect bone metabolism (Kerbage et al, 2013) through the effects of serotonin produced in the gut on bone mass. High levels of serotonin outside of the brain (approximately 95% of serotonin is produced in the gut and circulates in platelets) lead to lower bone density (Warden 2008).

Glucocorticoids

Synthetic glucocorticoids are often used to treat inflammatory conditions. Though glucocorticoids do decrease inflammation, they also directly affect bone turnover. When used for more than a week, glucocorticoids begin to impair the adrenal glands, suppress the immune system, and induce bone loss. The presence of glucocorticoids alter the differentiation of osteoblasts, tipping the scale towards adipocyte formation instead. If taken extensively they trigger glucocorticoid-induced osteoporosis.

Proton Pump Inhibitors (PPIs)

A prospective study by Ozdil et al showed that long term treatment with a PPI results in a significant reduction in bone density (Ozdil et al 2013), confirming the

positive correlation found through a systematic study review by Tetsuhide et al (Tetsuhide et al 2010).

Selenium, zinc, copper, and iron (and magnesium) all require the presence of adequate stomach acid for absorption. Inhibited stomach acid production appears to result in decreased mineral absorption and decreased nutrient absorption overall.

In June 2015, Stanford University researchers data-mined 16 million health records and found that taking this type of proton pump inhibitor increases the chances of heart attack by 16 to 21 percent. The FDA drug safety communication recently recommended treatment “for the shortest duration possible”, not to exceed three 2-week treatments per year.

Gut Dysbiosis

Gut dysbiosis is increasingly an issue in the US, and appears to correlate with a lowered diversity of gut flora. The direct effects of impaired gut flora population and population health was discussed in relation to the formation of calbindin proteins that enable the transport of calcium through the intestinal lining. On a larger systemic scale, gut health also impacts absorption of all nutrients, as well as generation of hormones. The effects of impaired gut health are exceptionally wide ranging and hugely influential on human health.

Advanced glycation endproducts (AGEs)

Advanced glycation endproducts (AGEs) were previously explored as a detrimental factor in the aging of the collagen bone matrix. Their effects however are systemic and they interfere with any and all tissue types in the body. Foods subjected to high heat, such as many processed foods, contain high levels of AGEs.

These glycotoxins form when sugar attaches to protein, as when starchy foods such as potatoes and grains, or red meats, are cooked in the absence of water at very high temperatures.

AGEs create oxidative stress and inflammation and are implicated in the present epidemic of diabetes and cardiovascular disease. Diabetics form AGEs as high blood sugar levels cause sugar to stick to the protein in cell membranes, and it appears that these AGEs cause the side effects of diabetes such as nerve damage, blindness, and kidney damage. AGEs can damage any and every tissue in the body. AGEs disturb bone remodeling during aging as discussed, and this process is accelerated in the presence of diabetes.

The higher the cooking temperature, the more AGEs are formed. They do not form when food is cooked in or with water. Cooking with water prevents sugars from binding to proteins.

Heterocyclic Amines (HCAs)

HCAs are formed when creatines and amino acids react together with heat. HCAs are genotoxic, causing mutations in DNA.

Polycyclic Aromatic Hydrocarbons (PAHs)

PAHs include compounds formed by the incomplete burning of organic matter (including foods) at temperatures in excess of 392 degrees F (200 °C).

With all three of these chemicals, temperature is the most important factor. Problems begin at 212 F (100 °C), with highly toxic HCAs forming at about 572 degrees F (300 °C). As with AGEs, the advice is to slow cook, use indirect heat and cooking methods with water such as poaching, stewing, braising, or steaming.

Green tea and other polyphenol-rich plants inhibit the formation of AGEs.

CHAPTER SEVEN: NATURAL ANTIRESORPTIVE AGENTS

This section reviews the medicinal plants displaying antiosteoporosis properties including their relevant active constituents and method of action when possible. The plants reported here are commonly used in traditional medical systems, including Traditional Chinese Medicine, and have demonstrated clinical effectiveness against osteoporosis due to their nutritional and phytoestrogen content.

The most comprehensive review was provided by Jia et al, which showed 76 medicinal plants reported in ethnopharmacological studies for potential benefits in osteoporosis treatment (Jia et al, 2012). A second meta-analysis of high-quality RCTs shows an overwhelmingly positive effect on lumbar spine density but not femoral neck, and suggest that over 12 months of treatment increases density in the hip (Wang et al, 2013).

Plants in this section were chosen by the researcher based on three criteria: high antiresorptive quality; knowledge about the plant by the researcher, and considering creation of a complex formula balancing all aspects of increasing bone density/creating positive state bone metabolism.

Epimedium – Yin Yang Huo

Yin Yang Huo is one of the most frequently used herbs in TCM antiosteoporosis formulas (Jia et al, 2012).

Flavonoids including icariin, epimedin B, and epimedin C are the main antiosteoporotic constituents. These possess an estrogen-like activity and modulate bone metabolism through the ER pathway activating estrogen-dependent osteoblastic activity (Mok, 2010), and may improve the development of osteoblasts by promoting the ALP (alkaline phosphatase) activity through regulating the expression of IL-6, OPG, and RANKL (Zhang et al, 2006).

Flavonoids extracted from epimedium inhibited bone resorption and stimulated bone formation, therefore preventing osteoporosis without hyperplastic effect on the uterus in the ovariectomized rat (Zhang et al, 2006).

***Psoralea corylifolia L.* -Bu Gu Zhi**

Extracted from both the fruit and the seed, two isoflavones corylin and bavachin stimulate bone formation and have potential antiosteoporotic activity (Xin et al, 2010). Bavachalcone inhibits osteoclastogenesis by interfering with the Akt signaling pathways during differentiation. Both corylin and bavachin have been shown to stimulate osteoblastic proliferation (Tang et al, 2011).

Though phytoestrogens are generally thought to prefer binding to ER beta (Kuiper et al, 1998), bakuchiol has a three-fold higher binding affinity for ER alpha. However bakuchiol appears to have no uterotrophic activity. It demonstrated oestrogenic activity in the in vitro assays, and reduced postmenopausal bone loss by increasing ALP, Ca concentrations, and bone mineral density (Park et al, 2008).

Psoralen, a coumarin-like derivative extracted from fruits of *P. corylifolia* L., has been reported to possess stimulatory effect on local new bone formation in vivo, and promote osteoblast differentiation by upregulation of expressions of osteoblast-specific marker genes including type I collagen, osteocalcin and enhancement of ALP activity (Tang et al, 2011).

***Pueraria lobata* - Ge Gen**

High levels of genistein and daidzein present in pueraria increase the BMD and bone mineral content in ovariectomy and orchidectomy rats and mice, without exhibiting estrogenic action in the uterus (Wang et al, 2003).

Isolated isoflavonoid Puerarin caused a significant increase in cell viability, ALP activity and mineral nodule formation in osteoblasts through activation of the Akt pathway (Zhang et al, 2007).

***Urtica dioica* - Qian Ma – Stinging Nettle**

When ovariectomized rats were treated with *Urtica dioica* (200mg/kg) it normalized all elevated serum calcium, phosphorus, bone specific alkaline phosphatase and osteocalcin levels and increased femur as well as uterine weight. BMD and bone volume were also increased. (Gupta et al, 2014).

***Salvia miltiorrhiza* – Dan Shen**

Tanshinones isolated from *S. miltiorrhiza* appear to reduce the formation of osteoclasts; Tanshinone IIA suppresses bone turnover in vivo without stimulating osteoblast ALP activity, and suppresses osteoclast formation by inhibiting the expression of genes (c-fos and NFATc1) induced by binding of RANKL, in a similar fashion to estrogen. Salvianolic acid A, the bioactive component, effectively prevents bone loss due to long-term administration of prednisone in rats. It protects bone from glucocorticoid-induced bone marrow malfunction by stimulating osteogenesis in bone marrow (Cui et al, 2009).

Salvianolic acid B, another bioactive component, also prevents glucocorticoid induced bone loss through positive effects on differentiation. It stimulates bone marrow stromal cell (MSC) differentiation to osteoblasts and increases osteoblast activities, whilst decreasing glucocorticoid-associated adipogenic differentiation, through regulating the expression of Runx2 (and other genes) in MSC (Cui et al, 2012).

***Drynaria fortunei* (Kunze) J. Sm. – Gu Sui Bu**

Traditional Chinese and Korean formulas to treat osteoporosis usually contain the rhizome of *Drynaria fortunei*. In recent study therapeutic effects on osteoporosis and bone fracture in the ovariectomized rat model were found, with enhanced bone formation through induction of BMP-2 and ALP, accumulation of bone matrix proteins such as type I collagen, up-regulated Runx2 and osteocalcin expression (Jeong et al, 2004). The flavonoids in *Drynaria* rhizome, include naringin and neoeriocitrin. Naringin is the main

active ingredient, which inhibits the retinoic acid-induced osteoporosis in rats, increases BMP-2 expression and induces bone formation. It was also found to enhance the proliferation of osteoblasts through osteogenic differentiation of human bone mesenchymal stem cells (BMSCs) (Wang et al, 2011).

***Morinda officinalis* - Ba Ji Tian**

In mice with impaired locomotion (sciatic neurectomy), the root extracts significantly suppressed the decrease in hind limb thickness, tibia failure load, BMD, tibia calcium and phosphorus contents, while showing an increase in serum osteocalcin. The anthraquinones isolated from *Morinda officinalis* appear act as both a suppressor of bone resorption and an enhancer of bone formation in vivo (Seo et al, 2005). They have been shown to have inhibitory effects on osteoclastic bone resorption and decrease the formation of bone resorption pits. They also enhance the apoptosis of osteoclasts and improve the ratio of OPG and RANKL in osteoblasts. These findings show that the anthraquinone compounds from *M. officinalis* are inhibitors of bone resorption (Wu et al, 2009).

***Taraxacum officinale* – Pu Gong Ying -Dandelion leaves**

Dandelion is not traditionally used in osteoporosis in TCM, however in Western herbal traditions it is, as the leaves are a good source of silicon, magnesium, calcium and boron. Dandelion also increases calcium absorption. Inulin, a naturally occurring soluble fiber in

dandelion, feeds the healthy probiotic bacteria in the intestines, and has a beneficial effect on blood sugar levels.

***Trifolium pretense L.* – Red Clover**

Red clover contains four detectable potentially estrogenic isoflavones: daidzein, genistein, formononetin, and biochanin A. In a study by Occhiuto et al extracts of *Trifolium pretense* were examined in ovariectomized rats. Treatment significantly increased bone mineral content, mechanical strength of the tibia, femoral weight, femoral density and prevented the rise of serum alkaline phosphatase levels. In addition, the treatment significantly reduced the number of osteoclasts compared with the ovariectomized control rats (Occhiuto et al, 2007).

***Equisetum arvense* - Horsetail**

Horsetail's predominant constituent is silicon, and this is responsible for the majority of the plant's healing properties. One study was found with 122 women taking horsetail or horsetail and calcium, but it was poorly designed. The necessity of silicon was discussed above.

***Eucommia ulmoides* - Du Zhong**

Treatment with *E. ulmoides* extract (DZCE) at higher doses (300 to 500 mg/kg/day) was found to be able to significantly prevent ovariectomized-induced decrease in biomechanical quality of the femur. The mechanical changes were associated with the prevention of a further BMD decrease and with improvements in microarchitecture. DZCE dose-dependently inhibited total BMD decrease in the femur caused by ovariectomy, which was accompanied by decreased levels of bone turnover markers.

It was concluded that 16 weeks of DZCE treatment improved bone biomechanical quality through modifications of BMD, and trabecular microarchitecture without hyperplastic effect on uterus (Zhang R et al, 2016).

Maitake mushroom

Maitake is the only mushroom containing high levels of vitamin D through normal growth without requiring additional UV exposure. 1 cup of fresh maitake contain about 800iu of vitamin D, in a combination of D2 and D3.

CHAPTER EIGHT: CONCLUSION – A PROTOCOL

There is no doubt that nutrient deficiency in the US contributes to the near epidemic proportions of bone density loss and resulting fracture rates. And by extension, the move towards Western dietary practices is affecting fracture rates worldwide. The researcher proposes the revised RDA, presented in Table 2, for increased bone health, both to improve the microarchitecture of the bone and to improve flexibility.

The researcher also proposes that a method be standardized to test bone flexibility as well as density, as risk for fracture is no doubt a more useful tool than simply knowing how much bone matrix has been created or lost. To that end the FRAX scale, as developed by the WHO, is an improvement on the DXA scan alone.

Thirdly, the researcher proposes that since the presence of estrogen so strongly assists not only some element of nutrient viability in terms of bone health but also assists in densifying bone through exercise, that bone health is assessed earlier than it is presently, so the most effective preventive measures can be optimized.

For most nutrients, there is a range of values rather than a fixed amount because the concept of *ratio* is so vitally important to health. The research into proper nutrient levels is evolving, and the researcher proposes these new intake levels as a starting point for further research. See Appendices B and C for application of the new RDAs in an expanded protocol.

Table 2. New Recommended Daily Amounts (RDA) intake of nutrients for bone health

Nutrient	RDA
Calcium	800 - 1,000 mg
Vitamin D3	5000 iu
Magnesium	800 - 1,500 mg
Phosphorus	700 -1,000 mg
Zinc	10 mg
Copper	2.5 mg
Silicon	> 40 mg
Boron	3 mg
Vitamin K2	80 - 300 mcg
Protein	50 - 150 g
Vitamin A (retinol)	10,000 - 15,000 iu
Vitamin C	300 - 600 mg
Phytoestrogen	> 50 mg

The dietary protocol that resulted from this research was employed with one osteoporotic patient with a history of 20 years of continuous bone loss. She had no history of pharmaceutical use for osteoporosis nor had ever used hormone replacement therapy (HRT). After employing the protocol loss of bone was halted, suggesting that the proposed protocol has potential for improving bone health. However, more research is needed to determine the effects of this new recommended protocol in improving bone structure and strength, and the researcher suggests the following future directions:

1. Revised RDA dietary protocol tested in the rat model (the murine model does not appear to equate to human bone density loss as well as the rat model).
2. Revised RDA dietary protocol tested in human trials
3. Examine the revised RDA on atherosclerosis/calcified plaque deposition

4. Is it possible to convert the (American) gut bacteria to equol production by consumption of properly prepared soy products, and how quickly.
5. US studies using properly prepared soy for phytoestrogen content with silicon on osteoporosis with and then without exercise
6. Clinical trial on herbal vinegar for osteoporosis, with and then without exercise

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APPENDIX A

Glossary of Terms

Akt Pathway - part of the signaling cascade in osteoblast differentiation and maturation.

Alkaline Phosphatase (ALP) - a bone specific marker of osteoblast activity

Bisphosphonate – Pharmaceuticals to halt bone resorption.

Bone Density/Bone Mass – Weight per unit volume in the bone, used as a diagnostic in osteoporosis..

Bone Marrow Stromal Progenitor Cells (BMSCs) -

Bone Matrix – trabecular bone; collagen scaffold for hydroxyapatite mineralization.

Bone mineral density (BMD) test – DXA or DEXA scan employed to understand level of bone loss, or bone growth.

Bone morphogenic proteins (BMPs) - a group of growth factors inducing the formation of bone, cartilage, and osteoblast differentiation.

Bone mineralization – the action of osteoblasts in laying down hydroxyapatite on the collagen scaffold to densify bone.

Bone remodeling - The natural continuous turnover of bone matrix and mineral that involves first an increase in osteoclastic activity/resorption and then bone osteoblastic activity/formation.

Bone resorption – the work of osteoclasts, breaking down old or injured bone

Bone turnover – the same as bone remodeling

Calbindin – protein that binds calcium to enable it to be absorbed in the intestine, depending on vitamin D.

Calcitonin - Hormone secreted by the thyroid gland, stopping bone calcium from being dissolved into the bloodstream when serum levels are sufficient. Also occasionally prescribed for osteoporosis.

Cholecalciferol - Vitamin D3. A secosteroid. Also called Calcitriol.

Chondrocytes – a cell that secretes collagen matrix, then embeds in it.

Claudins – proteins at tight junctions that control the flow of molecules between epithelium, such as in the intestinal epithelium.

Collagen - Type of protein that forms fibers and forms much of the structure of the body, including bones. There are varying types in the body, mostly type 1 in bones.

DXA or **DEXA** - Dual Energy X-ray Absorptiometry. Most used diagnostic test for osteoporosis in the US. Uses X-Rays at low intensity to measure density of bone mineral.

Hydroxyapatite – calcium and phosphorus, bone mineral

Osteoblasts – Bone building cells that secrete the structural components that build bone.

Osteocalcin – protein required for calcium binding in the formation of hydroxyapatite; vitamin D, vitamin K2 dependent.

Osteoclasts – Bone breakdown cells responsible for bone resorption.

Osteocytes – Osteoblasts that have worn out embed themselves in bone and relay information about mechanical stress on the bone.

Osteopontin – protein that anchors osteoclasts in the mineral matrix, and initiates their activation to begin bone resorption.

Osteoprotegerin (OPG) – osteoclastogenesis inhibitory factor; a decoy receptor for RANKL stimulated by estrogen in order to suppress the production of osteoclasts (by inhibiting differentiation binding of RANKL and therefore osteoclast differentiation).

Parathyroid hormone (PTH) - Regulates the level of calcium in the blood by triggering bone breakdown to release minerals. A synthetic parathyroid hormone, teriparatide, is sometimes used to treat osteoporosis.

Periosteum - Membrane that covers the outside of bones (except for the ends of long bones).

Prenylflavonoids – plant compounds with phytoestrogenic (and adaptogenic) properties that may be increased in activity compared to flavonoids.

Proteoglycans – proteins heavily bound with sugars, components of connective tissue including bone.

Raloxifene - A Selective Estrogen Receptor Modulator; an agonist at bone and antagonist at breast and uterus. Sold under the brand name Evista.

RANK/RANKL – Receptor activator of Nuclear factor κ B/ and its ligand, key regulator of osteoclast differentiation and activation.

Resorption - Dissolving of bone mineral out of the bone and into the bloodstream, carried out by osteoclasts.

Retinoic Acid – the active form of Vitamin A in the human body.

Runx2 – transcription factor triggering osteoblastic differentiation

Selective Estrogen Receptor Modulators (SERMs) – competitive partial agonists of estrogen receptor (ER), producing estrogenic or antiestrogenic effects depending on tissue type.

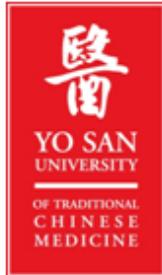
Steroid-induced osteoporosis - Osteoporosis resulting from use of glucocorticosteroids over a long period of time.

T-score - A statistical measure of bone density, indicating the number of standard deviations in difference from the mean of bone densities of 30-year old people of the same sex. The main indicator in diagnosis of osteoporosis today in the US.

Trabecular bone – Spongy Bone – containing bone matrix measured for density.

APPENDIX B

IRB Approval Letter



November 20, 2015

Laura Kelly
11965 Venice Blvd, Suite 305
Los Angeles, CA 90066

Dear Laura,

Your research proposal, Dietary Protocol for Treating Age-Related Bone Loss, has been approved, with no additional recommendations effective through March 31, 2016.

Should there be any significant changes that need to be made which would alter the research procedures that you have explained in your proposal, please consult with the IRB coordinator prior to making those changes.

Respectfully,

Ed Mervine,
IRB Coordinator

13315 W Washington Blvd, Los Angeles 90066

APPENDIX C

Foods highest in the necessary nutrients for bone health

For calcium (RDA 800 to 1,000 mg per day)

Source	Quantity of Calcium provided	Notes
Almonds, 1 cup	350mg	Soak, sprout and dehydrate before eating - or buy sprouted almonds.
Bok choy, 1 cup	70 mg	Good eaten raw
Savoy cabbage, 1 cup	50 mg	Good eaten raw
Broccoli raab/rapini/broccolini, 1 cup	60 mg	Good eaten raw; relatively low in oxalic acid.
Sardines, salmon (with bones) canned, per serving	115 mg	Many canned sardines proudly announce <i>bones free</i> —which is not good for your bones! Read the label.
Collard greens, 1 cup	175 mg	
Milk, yogurt, mozzarella chesse, 1 cup	100 mg	100 mg is the amount that will be absorbed; the actual amount present is 350 mg.
Turnip greens, 1 cup	100 mg	Boil for five minutes to release oxalic acid. Add a splash of apple cider vinegar to release calcium

For magnesium (RDA 1,000 to 1,500 mg per day)

Source	Quantity of Magnesium provided	Notes
Dark chocolate, 70 % minimum, 1 cup	440 mg	
Pumpkin and squash seeds, ½ cup	325 mg	
Soy beans, 1 cup	150 mg	Soy beans must be properly prepared and the process is complex.
Swiss chard and spinach, 1 cup	160 mg	Boil for at least 3 minutes.

White beans (cannellini beans), French beans, 1 cup 90 mg

For Phosphorus (RDA 1000 mg)

Source	Quantity of Phosphorus provided	Notes
Beans and lentils, 1 cup	450 mg	Sprouted
Brazil nuts, 1 cup	964 mg	
Pork chop, 1 chop	550 mg	Any cuts of pork or beef are a good source
Pumpkin and squash seeds, 1 cup	1550 mg	
Romano cheese, 1 ounce	215 mg	
Salmon, 1 filet	1000 mg	Choose wild when possible, Farming practices vary; farmed salmon can be low in nutrients

For boron (RDA unknown)

Source	Quantity of Boron provided	Notes
Almonds	trace	Should be sprouted
Avocado	trace	
Bananas	trace	
Beans	trace	Should be sprouted
Chickpeas	trace	Should be sprouted and cooked
Oranges	trace	
Pears	trace	
Walnuts	trace	

For silica (RDA unknown)

Source	Quantity of Silica provided	Notes
Beer	trace	
Horsetail	trace	An herb
Jerusalem artichoke	trace	
Millet	trace	
Oats	trace	Must be properly prepared

For vitamin D (RDA 5,000 iu)

Source	Quantity of Vitamin D provided	Notes
Cod liver oil, 1 teaspoon	500 iu	
Fish roe (fish eggs), 1 tablespoon	Up to 8,000 iu	Often sold in supermarkets in small jars labeled as fish eggs. You don't have to buy the expensive kind to get the bone health benefit.
Maitake mushrooms, 1 cup	1,200 iu	Also called hen of the woods; not the same as chicken mushrooms
Pastured egg yolk, from 1 egg	50 iu	
Salmon/oily fish/swordfish, mackerel, smoked salmon, per serving	600 iu	
Shiitake, portobello, and other mushrooms sunned for 2 days	400 iu	

For Vitamin K2 (RDA 80-300 mcg, higher if therapeutic)

Source	Quantity of Vitamin K2 provided	Notes
Goose liver pate, 100 g	370 mcg	
Gouda cheese, 1 ounce	20 mcg	
Natto, 1 tablespoon	450 mcg	
Pastured eggs, 1	35 to 80 mcg	Amount varies depending on the chickens' diet and lifestyle
Pastured butter and milk	20 to 40 mcg	Amount varies depending on the animals' diet and lifestyle
Pasture-raised organ meats and dark meat chicken	60 mcg	

For Phytoestrogens (RDA 50-150 mg)

Source	Quantity of Phytoestrogens provided	Notes
Red clover and other herbs, ½ cup	1,322 mg	Prepare in bone vinegar
Soy, as milk, tofu, or fermented soy products, ½ cup	128 mg	Must be properly prepared
Alfalfa, flaxseed, hops, lentils, mung beans, pomegranates, sesame seed	Lesser amounts	

APPENDIX D

Herbal Nutrition – Quick start

Nettle Infusion

If you were going to do only two things to prevent and treat osteoporosis, use homemade bone vinegar on greens and drink this nettle tea. Both are herb infusions.

Nettle tea is a gift from traditional medicines. Think of it as a liquid vitamin and calcium pill with a very big difference: nettle infusion not only gives you energy – consume a glass for a week and see – but it provides nearly everything you need to strengthen bones: calcium, magnesium, iron, vitamins B, C A, D and K, protein, trace minerals, potassium, zinc, copper, sulphur and boron. Nettle is more nutritious than blue-green algae.

As do most whole nutritious foods, nettle supports general health too. It nourishes the adrenal glands. It is an aid for many complaints from weak nails to allergies. In traditional medicine men take nettle as an antidote to frequent nighttime urination. In traditional cultures pregnant and lactating women take nettle tea as a tonic, as the tea's iron content can treat blood loss and/or anemia.

Nettle infusion provides energy and a rich source of calcium. And, since the vitamins and minerals that calcium needs for absorption and transport are integral, nettle tea is safe, effective calcium supplementation. For bone strengthening, drink a half cup/4 ounces every day.

(Serves 4)

2 oz. dried nettle

2 quarts boiling water

Fill the mason jar with 2 oz. dried nettle stalks and leaves. Fill jar with boiling water. Cover and brew overnight.

Next day strain out the plant material and, still using gloves, squeeze the herbs to get all the good stuff. Refrigerate. It will taste good for two days. If it goes sour, it makes a great plant food.

Caution: Nettle (especially fresh) has tiny hairs that release the same irritating substance ants do when they bite, so when handling fresh or dried nettle stalks always wear rubber gloves and use tongs.

Nutrition Information per serving

428mg calcium, 800iu vitamin A, 50 mg magnesium, boron, copper, zinc, silica

Bone Building Calcium-Rich Vinegar

We've trumpeted the benefits of natural apple cider vinegar in earlier chapters. Now here's another vinegar that packs a bone-health punch and helps you reap even more bone-building vitamins and minerals from greens, bone broths, and even grains.

This vinegar is brewed with calcium-rich herbs that are so common they can be found in your backyard. Take a look around and you will see a stash of calcium-rich herbs - suddenly you will see these weeds in a new light!

Just one tablespoon of Bone Building Vinegar equals 350 to 400 mg of calcium. (800 -1,000 mg/day is recommended for menopausal and postmenopausal women). To get that much calcium from food, you'd need to eat: 1 tablespoon of blackstrap molasses; 1 cup cooked turnip greens, kale, broccoli, bok choy or mustard greens; 2 cups cooked collard greens; 2 tablespoons of almond butter or tahini. This vinegar is one of bone health's closest friends.

Organic apple cider vinegar is the base for infusing the bone-building herbs. Use ingredients that are available near you and in season. You can find fresh herbs at your local farmer's market, or you can order from the suppliers listed below. We do not recommend foraging for yourself unless you have certified professional training in and knowledge of herbology.

Ingredients

You will need a selection of any five of these bone-building herbs:

dandelion leaves	alfalfa
stinging nettle	parsley
horsetail	comfrey
red clover	raspberry leaves
hounds tongue	blackberry leaves
motherwort	thimbleberry leaves
mugwort	sage
mint	amaranth leaves
wild arugula	lambsquarter
chickweed	kale
shepherd's purse	cabbage
oatstraw	

If you want to boost the bone building properties add 0.35 of an ounce of each of a few specific Chinese herbs: Gu Sui Bu and Xu Duan work together to build healthy bone. Bu Gu Zhi and Du Zhong also assist in bone building and repair. Yin Yang Huo (Epimedium) is an effective bone strengthening herb on it's own. These herbs are available without a prescription from Chinese herbal pharmacies or from some of the suppliers listed in the Resources section. All can be safely added to your bone-building brew.

Making the Vinegar

Fill the jar with fresh well-snipped herbs - you'll need a lot. Pour in apple cider to cover the herbs. Seal and label the jar with the date. Put the jar in a dark cupboard well away from any exposure to direct sunlight. Wait 6 weeks.

Aim for at least a tablespoon/day. Put on salads, in stir-fry, season beans or grains. A good preventive home remedy is drinking a teaspoon of bone-building vinegar in any amount of filtered water in the morning. Not only does it provide a substantial calcium supplement in fully natural form; grandmothers past have said it relieves conditions from minor arthritic pain to acid reflux.

Laura's brew contains dandelion, horsetail, nettle, red clover, raspberry leaves, mugwort (*Ai Ye*), Epimedium (Yin Yang Huo), Gu Sui Bu, Xu Duan, Bu Gu Zhi, Du Zhong, and licorice.

Bone Shrub

In the 17th century Shrubs were all the rage. They started life as medicinal cordials that arrived in England from Italy where they were first produced during the Renaissance, to "...renew the natural heat, recreate and revive the spirits, and free the whole body from the malignity of diseases" according to an anonymous text of the time. Shrubs are a concoction of fruit, vinegar, and sparkling water, sometimes combined with alcohol. Drinking shrub is similar to the old adage to that taking a teaspoon of apple cider vinegar in the morning cures many ills.

Some shrub cordials were a bright yellow hue and contained flecks of gold leaf, and so took their name from the 'cordial virtues' of the rays of the sun, which some alchemists thought the medicines contained. And in a way the alchemists were right – the plants use the sun's rays to produce the enormous and varied benefits they provide to us as foods.

By the 19th century, typical American recipes for shrubs used vinegar poured over fruit—traditionally berries— left to infuse anywhere from overnight up to several days. Then the fruit would be strained out and the remaining liquid would be mixed with a sweetener such as sugar or honey and reduced to create a syrup. The sweet-and-sour syrup could be mixed with either water or soda water and served as a soft drink, or it could be used as a mixer in cocktails. Shrubs eventually fell out of popularity with the advent of home refrigeration.

Making a shrub is an ideal way to take your bone-building vinegar. Contemplate the difference between enjoying this healthful tonic and popping an industrially made supplement, while you sit in the sun absorbing your vitamin D and sipping this tart fruity gift from nature. The herbs in the bone-building vinegar you'll use to make a shrub contain all the vitamins and trace minerals you need for bone health. Nettle alone contains over 400 mg of calcium in a cup. Your tasty, fruity shrub will provide you with all of the base nutrition you need to foster healthy bones. Better by far than any supplement in pill form!

How to Make Shrub

Making shrub is not a precise process, we encourage you to adapt and explore, and make this as simple or as complex as you like.

The basic idea is to take whatever fruit rinds and fruit scraps you have on hand, put them in any jar with a clean, tight-fitting cover and pour bone building vinegar over them.

Add a teaspoonful of your ginger bug - the ginger goes well with the herbs in the bone vinegar. Let the brew sit, covered, at room temperature for a few days.

Strain the liquid through a sieve, mashing the fruit pulp to get the fruit flavor. Add 2 Tbsp. of honey or sugar.

The resulting syrup can be enjoyed by adding it to some sparkling water and an ice cube.

Use a Tablespoon of the fruity sweet vinegar, mixed with sparkling water or something sweeter if you like.

The Sweet Hibiscus Tea, an ice cube and some fizzy water is lovely. You can also put it in our homemade soda. Our bone vinegar has a lovely spice to it, which goes really well with the ginger beer.

APPENDIX E

Testing: how to use testing to help with strengthening your bones

1. Nutrition Evaluation

In order to build strong bone, you must have the building blocks. A test called a nutrition evaluation will give you information on your levels of vitamins and minerals so you can start to see where your deficiencies lie. The latest analysis by the Center for Disease Control¹ reports that over half of the US is deficient in vitamin D and magnesium, and a large portion of the population is vitamin A deficient as well as vitamin B6 deficient. A separate analysis by the Environmental Working Group² showed even more severe deficiencies - 95% of the population is vitamin D deficient, 60% magnesium deficient, and around half the population deficient in calcium and vitamin C. There is not yet any measuring of vitamin K2.

If you are approaching the menopause or a scan shows osteopenia or osteoporosis, speak with your healthcare provider about a series of more specific tests that profile your bone *turnover*, which you will find at the end of this worksheet. The results provide additional clues for you and your health care provider as to how rapidly you are losing bone.

Nutrient Testing List

Ideally you want to know your levels for these nutrients:

Calcium	Vitamin B1	Manganese
Phosphorus	Vitamin B6	Boron
Magnesium	Vitamin B9 (Folate)	Omegas
Vitamin D	Vitamin B12	Iron
Vitamin C	Zinc	Silica
Vitamin A	Copper	Sulfur

2. Bone Density Testing

When bone breaks down, whether in the natural course of bone turnover or because the body calls for calcium, the process is called bone resorption. For reasons of bone density, bone flexibility and overall bone health it is important to know whether new growth keeps pace with breakdown. Three types of tests provide important and different information about how well your bones are keeping up with balanced turnover.

¹http://www.cdc.gov/nutritionreport/pdf/4Page_%202nd%20Nutrition%20Report_508_032912.pdf

² <http://www.ewg.org/research/how-much-is-too-much/appendix-b-vitamin-and-mineral-deficiencies-us>

DEXA (or DXA) scans

In the US, the most widely used test in the US is the DEXA scan. The results are called T scores.

Using enhanced x-ray technology DEXA measures the density of your bone compared to the density of the average healthy 30 year old at peak bone mass. Typically physicians recommend the test for women 65+ whose estrogen, and often bone density, have declined. If the test shows osteopenia or osteoporosis, doctors typically recommend a DXA scan every two years.

Laura's thoughts about DXA scans

Since estrogen plays a central role in bone health right from puberty, and menopause typically begins well before age 65, if DXA is the scan of choice I suggest DXA scans earlier while there is still time to strengthen bone that shows very little loss - or to get started on a bone health diet for those whose bones suggest significant dietary deficiency.

Scanning when the bones are already osteoporotic turns many doctors and patients toward pharmaceutical treatment - though at this writing the bisphosphonates treatment period is just three to five years. After that it's back to square one.

Estrogen plays a vital role in creating bone able to resist aging³. If the age of first DXA scan were lowered to, for example, 5 years before a woman's menopausal transition⁴, then there would be ample time to strengthen bone against future loss and reduce the chance of relying on pharmaceuticals.

Preventive scanning would no doubt save a great deal of money worldwide.

The However - Bone Density, Bone Flexibility and Vitamin K2

When we speak about bone health, we've become accustomed to equating bone health with bone density. Clearly, low bone density is the best correlative we have for fracture. But does increasing bone density always result in lessening of fracture? Bisphosphonates raise bone density, and theoretically this would eliminate fracture.

³ There is a correlation between high bone density and breast cancer. Women who have naturally higher bone density have increased rates of breast cancer. The link is no doubt at least in part to high natural estrogen. The rule to now has been a lot of calcium and lately with vitamin D, but it seems vitamin K2 is not yet in the loop. We don't know how the bone density/breast cancer relationship would change were vitamin K2 central to the regimen, as K2 also appears to play a part in cancer prevention. However, we do know that adding phytoestrogens weakens the body's response to natural estrogen, which could be highly beneficial in these cases.

⁴ Age of menopause is strongly genetically linked, so absent other factors like health problems and medications, the literature suggests that in most cases you will reach menopause near the age your mother did.

However denser bones can also become increasingly brittle and likely to fracture.

Studies suggest that taking Vitamin K2, soy isoflavones and similar potential modulators of bone density may not affect density but fracture rates decrease. According to Medscape⁵

"If the increase in BMD (bone mass density) by the bisphosphonates was solely responsible for the reduction in fracture risk, a 1-SD (standard deviation - the marker by which your bones are compared to the young person who has not lost any bone density) increase in BMD should produce an approximate 50% reduction in fracture risk. In reality, small increases in BMD are associated with markedly reduced fracture risk."

Taken together this suggests that factors other than bone density may decrease fracture risk. I suspect that one of these is flexibility, which at the moment we have no standard tools to measure.

FRAX - Fracture Risk Assessment⁶

The WHO fracture risk assessment tool FRAX appears to be better than lumbar spine and femoral neck T scores (DXA scans) for assessing fracture risk.

Online, the World Health Organization Collaborating Center for Metabolic Bone Diseases at the University of Sheffield, UK has the FRAX tool that you can use to assist with fracture assessment, and discuss with your doctor.

Click *Calculation Tool*, choose your geographic region fill and in the boxes. There is another calculation tool on the right to assist with weights and measures.

The FRAX tool considers bone density, but it is not limited to, and repeatedly performs better than bone density alone.

3. Specific Tests for bone turnover

In addition to tests that measure overall density and assess fracture risk, other types of tests measure markers of bone turnover.

These tests are extremely useful as a baseline, especially if you take them before you undertake to strengthen your bones naturally. Taking the tests annually will give you an idea how effective your bone health strategies are. These tests can be a great window into the functioning of your body in relation to bone and how the steps you take are affecting it. Here is a brief on the tests your healthcare provider can order. If you are

⁵ http://www.medscape.com/viewarticle/472681_2

⁶ <http://www.shef.ac.uk/FRAX/>

already taking bisphosphonates or other pharmaceuticals for osteoporosis, your healthcare provider might already be using these tests.

Osteocalcin (serum N-terminal)

Osteocalcin, also known as bone gla protein (BGLAP), is a bone-specific protein that has proven to be a sensitive and specific marker of osteoblast activity. Activated osteocalcin requires the presence of both vitamins D3 and K2.

Alkaline Phosphatase (bone-specific)

Alkaline phosphatase in serum has been used for more than fifty years to monitor bone metabolism and it is still the keystone marker. Alkaline phosphatase, an enzyme, attaches to the surface of osteoblasts.

Hydroxyproline-Containing Peptides

Urinary hydroxyproline values rise after menopause and fall when antiresorptive drugs such as hormones or bisphosphonates are taken. They will also fall as turnover trends towards creating bone density.

Propeptide type 1 collagen

Type 1 collagen peptides in the blood are a marker of bone formation.

CTX

Elevated levels indicate increased bone resorption. They will drop as your bone breakdown stops.

Once you have spoken with your doctor about results of your nutritional evaluation and other tests your doctor may have ordered, you're ready to begin using the worksheets and gradually make cooking for bone health part of your lifestyle. As you set out, please keep in mind that the timings are suggested; and while they are realistic, please feel free to tweak the timings to suit the realities of your lifestyle and personal or family needs.