The Effects of Acupuncture on Bone Mass Density and the Biochemical Mechanisms Behind It: A Research Synthesis

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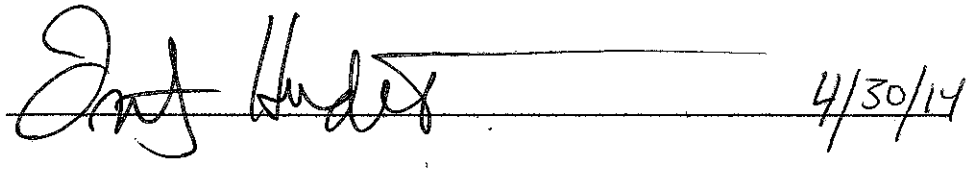

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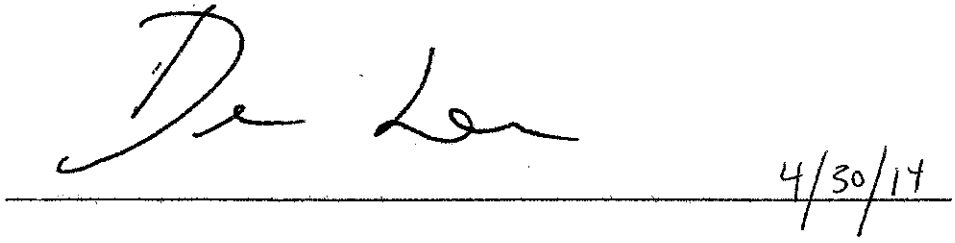

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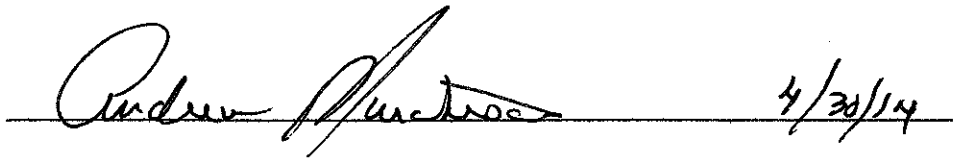

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Abstract

Osteoporosis affects everyone. According to the World Health Organization (WHO, 2004), 55 % of the population over 50 has osteoporosis, and 80% of them are women. This debilitating skeletal disorder accounts for most of the hip and vertebral fractures in post menopausal women. Osteoporosis causes 1.5 million fractures per year and accounts for 17 billion dollars a year in medical costs (OLPC, 2009). Acupuncture may be an alternative and complimentary treatment to western medicine. Acupuncture has a low incidence of side effects and can have a systemic effect on the body by affecting not just the bones but the whole body. This research synthesis will look at how acupuncture may help with this debilitating disorder by examining the biochemical chain reactions that acupuncture influences, and how those biochemical reactions effect bone mass density, bone health and bone maintenance. This study will help both patient and doctors understand the science behind acupuncture's effect on the bones. This research study found that acupuncture had a positive effect on osteoblasts, bone building cells biochemicals , as well as on estradiol and bone specific alkaline phosphates. It was found that acupuncture helps down regulate osteoclast, bone breakdown cells biochemicals; follicular stimulating hormone, TNF-α and osteocalin.

Acknowledgements

I dedicate this paper to my grandmother Marie Hansen whose crumbling spine and severe pain inspired me to do this project so that I could help my mom and others avoid osteoporosis. I thank my family dearly, for without all their love and support I would not be where I am today. You made it possible for me to complete my doctorate. Thanks for believing in me. I thank my friends, Ellisa Benson, Maria Andino and Tracy Carter, for putting up with me through this stressful process. To Chance, who knew when to stay and when to leave me alone, thanks for helping me relax. To all my patients, I express thanks for your support and putting up with my crazy schedule.

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Chapter 1: Introduction

Osteoporosis Definition

Osteoporosis affects everyone. According to the World Health Organization (WHO, 2004) 55 % of the population over 50 has osteoporosis, and 80% of them are women. This debilitating skeletal disorder accounts for most of the hip and vertebral fractures in post menopausal women. Osteoporosis causes 1.5 million fractures per year and is responsible for 17 billion dollars a year in medical costs (OLPC, 2009). The cause of this disorder is mostly genetic but can also be from life style choices, hormones, drugs, medications and nutrition (Fillit, Rockwood & Woodhouse, 2010).

It is unavoidable to have bone loss, but there are preventative measures one can take to slow down the progression and the inevitability of osteoporosis. A diet well rounded with adequate nutrition will build a strong base from which to lose from. Weight bearing exercises and movement throughout our lives, as well as fall prevention, adequate rest and a moderate life style will help keep bone loss to a minimum. If one is given a diagnosis of osteoporosis there are medications and treatments from both a western and eastern perspective that can help slow down further decline.

Osteoporosis is the compromise of our bone strength known as Bone Mass Density (BMD). As we age our bones become fragile due to increased osteoclasts (bone resorption) and decreased osteoblasts (bone formation) function, creating porous and weak bone structure.

This breakdown is due to a decrease in several hormones and vitamins. To have healthy bones there must be an adequate diet in vitamins and minerals to build and maintain bone health. Vitamins like D3, K2, magnesium, calcium, E, omega 3 and isoflavons are important in bone health. The two main substances, Calcium and Vitamin D, are very important in bone maintenance. Vitamin D is needed to help calcium absorption and to keep calcium serum levels optimal in the blood. If calcium levels drop, parathyroid hormone secretions initiate bone resorption and pull calcium from the bones to increase the plasma levels.

The most common hormone that affects bone maintenance is estrogen, which affects both women and men. Estrogen levels drop as we age, and this deficiency affects the bone building process by inhibiting the osteoblasts, and increasing osteoclasts, resulting in faster bone loss. Growth hormone is another important hormone in bone regulation. Growth hormone induces the production of Insulin like Growth Factor one (IGF-1). This hormone is important in cartilage expansion and linear growth especially in childhood. IGF-1 is also important throughout our lives for bone remodeling by recruiting osteoblasts to the bone for new bone formation. Those are just a few of the biochemical reaction that affect BMD. This capstone will explore biochemical reactions to see how acupuncture may maintain an optimal balance for healthy bones.

Most Western medicines are given when one has a low T-score -1.0 or lower. A T-score is your bone mass number compared to the peak bone mass of a healthy young adult. A normal T-Score is -1.0 or higher. Osteoporosis is indicated by a T-Score of -2.5 or lower. Osteopenia is indicated by a T-Score of -1.0 to -2.5, which is considered pre-osteoporosis. Both Eastern and Western medicines agree on exercise and diet plus supplements for prevention

and treatment of bone density loss. If those measures are not enough, then both Western and Eastern medicines have additional treatment options to offer. Some of Western treatments include medications such as, raloxifene, bisphosphonates, strontium ranelate, teriparaide, supplements, Hormone Replacement Therapy (HRT) and denosumab. Most of these medications have serious long term negative effects on the liver, kidneys and bones themselves, with other side effects of venous thrombosis, osteonecrosis of the jaw, hypocalcaemia, increased hot flashes, headaches, breast cancer, stroke and heart disease (Fillit et all., 2010). The side effects of these drugs can be worse than the original condition of osteoporosis. This is where acupuncture can help. Through the use of acupuncture, herbs and qi-gong exercises, one can improve bone health with fewer adverse effects on the body.

Traditional Chinese Medicine (TCM) uses acupuncture and electroacupuncture to stimulate body mechanisms that repair and heal. Medicinal herbs are used in the same manner treating the root causes of bone deficiency, and they have very few side effects. The problem is that Western doctors and patients tend to be skeptical of acupuncture due to not understanding how it works on the body. There are few studies on the biochemical effects of acupuncture. Therefore, this research synthesis will highlight the ground work that will lead to a greater understanding of acupuncture effects on the body and the biochemical pathways it influences to enhance bone health.

Research Question and Objective

This study will show how acupuncture may provide patients alternatives to and complement their existing bone health maintenance plans. The objective is to show how

acupuncture, especially electroacupuncture, effects the biochemical reactions of our bodies and influences the bones. It is hypothesized that regular treatments of electroacupuncture, which is the application of electrical current to acupuncture points, can increase bone mass density through stimulation of biochemicals like estradiol. Estradiol is a hormone that helps maintain bone health by preventing osteoblast apoptosis. Estradiol prevents bone building cells from dying off, and increases the cell death of osteoclasts, which are bone resorption cells. A look at estradiol and other biochemical markers like IGF-I, TNF-α, growth hormone, urinary calcium, testosterone, follicular stimulation hormone, osteocalcin, alkaline phosphate, gonadal steroids, parathyroid hormone, calcitonin, and deoxypridinoline will help to clarify acupuncture's affect on BMD. Acupuncture has a low incidence of side effects. Additionally, acupuncture offers the additional benefits of pain management of osteoporosis fractures and bone degradation, making it a viable option for patients and doctors. Through acupuncture and TCM principles an alternative treatment can be established for the treatment of osteoporosis.

This research synthesis will be of use to the health care practitioners, as it will show how acupuncture can help with osteoporosis. It will show the biochemical mechanisms of how acupuncture works and show scientific proof regarding its effectiveness. This study will provide to the acupuncture community further scientific basis for the treatment of their patients, and allow for further collaboration with Western medicine practitioners. The focus of the current study is to give the aging population as a whole, a possible alternative to current treatments of osteoporosis. This may be achieved through collaboration with Western medicine and traditional Chinese medicine proving patients more treatment alternatives.

It is also the author's goal to use this study to help build the ground work for future studies. This research has the potential to bridge Eastern and Western medicine.

Definition of Terms and Abbreviations

Angiotension II: A peptide hormone that causes vasoconstriction and raises blood pressure. (Fyhrquist, F., Metsarinne, K., Tikkanen, I., 1995)

Apoptosis: cell self destruction.

Aromatization: The process of an enzyme converting male hormone, testosterone to female hormone, estrogen. (Wisegeek, 2014)

Bone specific Alkaline-phosphate (ALP): Biomarker for bone formation.

Back shu: Any points on the urinary bladder channel line which is located on the back 1.5 inch lateral to the spine. They are each assigned to a zang fu (defined below) organ and accessing this point affects the qi (defined below) and function of the organ.

Channels: These are the network of lines that traverse the body and are accessed thru specific point on them. This is what acupuncture accesses. Each organ or zang fu (defined below) has its own channel bilaterally on the body. This network of channels are each linked to each other and either start on the extremities and travel to the trunk and head or start on the head and trunk and travel to the extremities.

Collagenase: Enzymes that breakdown collagen.

Deoxypyridinoline (Dpd): Biomarker for bone resorption measured from urine (Bilezikian, Raisz, Rodan, 1996).

DU: Acupuncture channel that runs up the spine. It is one of the eight extraordinary vessels that have an effect on the kidneys, brain, and zang fu.

Dynorphin: Opiate- like chemical in the brain that blocks pain signals along nerve fibers. (Venes, 2001)

Endorphins: Group of peptides in the brain and nervous system that have effect on physiological function, activating opiate receptors and reducing pain and stress. (WiseGeek, 2014)

Endomorphin -1: Endogenous opioid peptides that effect perception of pain, effect cardiovascular, respiratory and digestive functions. (WiseGeek, 2014)

Enkephalins: Neurotransmitters that help reduce pain. (WiseGeek, 2014)

Estradiol: Steroid produced by the ovaries (Davis, 2001). Has effect on BMD by stimulating osteoclast apoptosis and prevents osteoblast and osteocytic apoptosis (Takeda, 2005).

Follicular Stimulating Hormone (FSH): Hormone released from the anterior lobe of the pituitary that stimulates the maturation of the ovarian follicles in women and helps maintain spermatogenesis in men (Davis, 2001).

Gonadotrophin releasing hormone (GnRH): Hormone that is produced in the hypothalamus and causes the pituitary to release gonadotrophic substances, luteinizing hormone (LH) and follicle-stimulating hormone (FSH) (Davis, 2001).

IGF-1: Insulin-like growth factor 1 a hormone that effects cell growth and prevents cell death. (WiseGeek, 2014)

Mitogenic: To cause cell division.

Opioid: Pain reliever

Opioid antagonist: Has the reverse effect of an opioid

Osteocalcin: Gla-protein synthesized by osteoblast and incorporated into the bone matrix, a small amount is released and that is what is measured (Bilezikian et al. 1996).

Osteopenia: Low bone mass standard deviation of 1 to 2.5. Also known as pre-osteoporosis

Ovariectomized(OVX): One who has had their ovaries removed.

Oryzanol: A supplement substance taken from rice bran oil and used for cholesterol and menopause.

Parasympathetic nervous system: Part of the autonomic nervous systems that is responsible for rest and digests; increases gastric secretions, constricts pupils, slow heart rate and contraction of smooth muscle.

Parathyroid hormone: Hormone secreted by the parathyroid gland that regulates blood levels of calcium and phosphorus (Davis, 2001)

Qi: A form of energy that is "Any of various dynamic phenomena of the body that are described in terms of the following functions: activity, warming, defensive, transformation, containment, strength, anger, disease." (Wiseman, Ye, 1998)

RANKL: Stands for Receptor activator of nuclear Kapp-B ligand, a protein that helps regulate osteoclasts activity. (WiseGeek - Densumab, 2014)

REN: Acupuncture channel that runs up the midline of the front of the body. It is one of the eight extraordinary vessels that has an effect on the local area of the body, has direct accesses to zang fu and is tonifing in nature. Effects the lower jiao, which includes reproduction,

urination; effects the middle jiao which include digestion; effects the upper jiao, which includes chest, heart lungs.

Substance P: Neurotransmitter that responds to pain, noxious stimuli, and anxiety, causes local tissue reaction (Venes, 2001).

Selective serotonin reuptake Inhibitors (SSRI): Class of drugs that are used in depression that inhibit the uptake of serotonin in the brain which allows for more serotonin to be available for neurotransmission which seems to improve mood.

Spicules: A term used to describe boney matrix in new bone formation.

Sympathetic nerve system: Part of the autonomic nervous system that reacts to stress and emergencies. This causes the body to go into fight or flight mode and causes increased heart rate, dilates pupils, vasoconstriction of skin and viscera, slowing peristalsis, secretion of epinephrine and norephinephrine.

TNF-α: Tumor necrosis factor alpha is a cytokine that stimulates osteoclast and promotes degradation of the matrix (Shevde, Bendixen, Dienger, Pike. 2000).

Trabecular bone: The inside of the bone, which is a network of osseous tissue that makes up spongy (cancellous) bone (Venes, 2001).

UMR-106 Cells: A clonal cell line of rat osteosarcome cells that can be ordered and used for experiments.

Yin – yang: Chinese concept of two complementary and opposing principles. Yin represents water, dark, night, female, heavy, downward, earth, autumn, winter and stasis. Yang represents fire, day, light, hot, male, upward, spring, summer, motion.

Zang Fu: Translated to mean viscera and bowels. These are the organs of the chest and abdomen. In TCM the viscera (heart, lung, spleen, liver and kidney) produce and store essence and bowels (stomach, small intestine, large intestine, gallbladder, and bladder) decompose food and convey waste (Wiseman, Ye, 1998).

Chapter 2 – Literature Review

This chapter will cover Western osteoporosis measurements, causes and treatments, western medicine, supplements and their effects, as well as TCM definitions, herbal medical treatments and their effect with a brief explanation of acupuncture and electroacupuncture.

Osteoporosis Measurements

A Dual Energy X-ray absorptiometry (DEXA) is the most common way to assess BMD. The results are expressed as a T-Score. T-score method is used for the assessment of the bone mass at several bone sites, the lumbar, the calcaneous and the hip. Those measurements are then compared to the peak bone mass of a healthy young adult. There is also a Z-score which is calculated from a comparison age group rather than peak bone mass of healthy young adults.

DEXA diagnosis is the standard used in the United States and is what most doctors use to determine medication and treatment protocols. According to World Health Organization (WHO, 2004) a T-score of -1.0 or greater is normal. A T-score between- 1.0 and -2.5 indicates osteopenia, and T-score below -2.5 indicates osteoporosis. Those with a T-score less than -2.5 are more susceptible to fractures. The WHO found that not everyone with a low T-score had fractures, so a system was developed to take into account other risks such as steroid therapy, genetics, life style, nutrition, and other diseases that contribute to the fracture risk. Assessing and diagnosing osteoporosis is crucial, since factures to the hip, wrist, ribs and vertebrae carry a high risk of complications that can lead to death.

Screening for biomarkers can also help determine bone degradation. The enzyme Cathepsin k breaks down to a type-1 collagen, an important constituent in bones, which when

detected can diagnose osteoporosis. Blood samples can be taken and levels of estradiol, IGF-1 (definition in glossary), testosterone, luteinizing hormone, osteocalcin and follicular stimulating hormone (FSH) are assessed. An increase in urinary excretion of C-Telopeptides, another type-1 collagen, can also be used to diagnosis osteoporosis (Yasuda, Kaleta, Bromme, 2005). Urinary samples can be taken to assess levels of deoxypyridinoline and calcium bone resorption markers. Bone histomorphometry is accomplished to assess bone volume, trabecular thickness, trabecular number, trabecular separation, mineralization rate, and bone formation rate. This is the process of cutting a cross section from the bones to assess the micro architecture of the bones. It has been found that a low circulation of 25 hydroxyvitamin D and an increase in circulating Parathyroid hormone (PTH) have been noted in older patients with osteoporosis. Recent studies have shown there are a number of gene substances that influence BMD and fracture risk. IGF-I, RANKL (definition in glossary), TNF-α (definition in glossary) and estrogen receptor gene are the most prevalent (Fillit et al., 2010). These genes substances, vitamin D and hormones have significant influence on the chemical reactions of the body and its associations with osteoclast/blast functions. Testing the levels of them can help determine osteoporosis risks. Testing is a crucial tool to help determine BMD. Once a picture of bone health is established, then corrective and preventative treatments can be established.

Osteoporosis Causes

Peak bone mass is achieved during the mid to late twenties. Thereafter, human start to lose bone mass between the ages of 35 and 40. Bone mass loss is due to a slow down of osteoblast, the formation of bone, and an increase or continued osteoclast, the breakdown/absorption of bone. While this is a natural process of aging, many factors can

speed it up and/or slowdown the bone building processes of our early lives. If early in our lives we do not build enough bone mass we will be at greater risk of osteoporosis later in life. Malnutrition, malabsorption, diabetes and steroid use in our early years can delay or stop osteoblast (OLPC, 2012). Malnutrition from poor diet with a deficiency in vitamins D, K, B12 and calcium can cause early osteoporosis. If one is not properly absorbing nutrients due to diseases like Crohn's disease, bulimia, anorexia, post gastric bypass surgery or bowel resection, there may be a vitamin deficiency with a slow or stopped bone formation. If one is inactive and doesn't do weight bearing activities, strong bones will not develop. Weight bearing exercises cause the osteoblast to consolidate at muscle attachment sites, thus creating denser bones. TCM implements Qigong exercises, which is purposeful movement to heal and maintain health. Tai Chi Chuan, a form of Qigong is a low impact weight bearing exercise, has been shown to slow the progression of bone loss in postmenopausal women (Yao, Dods & Brown, 2006). The earlier exercise is started, the greater BMD is likely to be present by age 35. Continued exercise will slow the loss of bone and keep the risk of fractures down.

There are other disorders and diseases that can affect BMD and can cause what is referred to as secondary osteoporosis. Hypogonadal states, deficiency of the ovaries or testes such as in menopause, can greatly affect BMD because it affects the hormone levels of estrogen, testosterone and glucocorticoids. These hormones are the building blocks and important components of the bone maintenance processes. Hypogonadal states include Turner's syndrome, andropause, hypothalamic amenorrhea, myeloma, oophorectomy, male hypogonadism and testectomy (OPLC, 2012). Endocrine disorders can also affect hormones and the osteoblast process; some of these would include Hypothyroidism, Cushing's syndrome,

hyperparathyroidism, diabetes, adrenal insufficiency, as well as pregnancy and lactation (OLPC, 2012). Rheumatologic diseases like rheumatoid arthritis, ankylosing spondylitis, lupus erthemotosus, and polyarticular juvenile arthritis can have a great affect on BMD (OLPC, 2012). Hematological diseases can get in the way of bone building process. Those diseases include leukemia, hemophilia, sickle cell, thalassemia, and lymphomas (OLPC, 2012).

Genetic disorders have an effect on the formation of bone and can lead to osteoporosis including glycogen storage diseases, osteogenesis imperfect, homocystinuria and hemochromatosis. Individuals with chronic obstructive pulmonary disorder (COPD) and Parkinson's have shown to have a higher than normal occurrence of osteoporosis (OLPC, 2012). Many medications can cause an increase in osteoclast and a decline in osteoblast (Fillit, Rockwood, & Woodhouse. 2010). The major medication group to be considered is steroids, which affect multiple chemical and hormonal reactions. We are not sure exactly where in the chemical chain the disruptions are caused. The drug phenytoin accelerates the metabolism of vitamin D, causing a lowered circulating plasma level of Vitamin D. Other drugs that have been shown to have osteoporosis causing effects are anticonvulsants, barbiturates, L-Thyroxin, aromatase inhibitors, methotrexate, anti-coagulates, proton pump inhibitors, thiazolidinediones and chronic lithium therapies (OLPC, 2012).

Western Osteoporosis Treatments

Most of the western osteoporosis medicines are given to patients who present a low T-score -1.to -2.5, osteopenia, so that fractures can be prevented. These treatments include

raloxifene, bisphosphonates, strontium ranelate, teriparaide, denosumab, Hormone Replacement Therapy (HRT) and supplements.

Raloxifene is a selective estrogen receptor modulator or SERM. It has bone building effects, but has also shown to have effect on the breast and endometrium (Fillit et al., 2010). It has shown to increase BMD on lumbar and femoral neck by 2% -3%, and it was shown to have reduced vertebral fractures by 30%- 50%. It has the added side-benefit of a decrease in breast cancer. However, it can also increase hot flashes and increase the risk of venous thromboembolisms (Fillit et al., 2010).

Oral bisphosphonates such as alendronate and risedronate, plus the commonly known actonal, boniva and fosamax have proven to increase BMD and decrease vertebral, hip and other fractures. Idandronate has shown to increase BMD and lower vertebral fractures and can be taken orally or intravenously. Zoledronate is an intravenous bisphosphonate that has shown a decrease in vertebral fractures by 70% and hip fractures by 41% (Fillit et al., 2010). It also has the effect of decreasing the mortality rate in patients with recent hip fractures. Zoledronate is also very effective in patients over the age of 75 (Fillit et al., 2010). The side effects of intravenous bisphosphontes is it may cause acute flu like symptoms for a few days after injection. Since it may also cause hypocalcaemia, calcium and Vitamin D are recommend. There can also be an increase in serious atrial fibrillation, but more studies are needed to prove this observation. Patients have shown to have osteonecrosis of the jaw when on high doses of Intravenous bisphosphonate, but not with low doses.

Strontium ranelate increases osteoblast and decreases osteoclast with studies showing a 12.7% increase in vertebral BMD with a 41% decrease in new fractures (Fillit et al., 2010s). This drug has good effects with people over the age of 80. Side effects include diarrhea and increase risk of venous thromboembolism; rarely a hypersensitivity reaction can cause rash, eosinophilia and systemic symptoms (Fillit et al., 2010).

Synthetic parathyroid hormone drugs include forteo and teriparatide. Teriparatide is a recombinant human parathyroid hormone 1-34 that is injected. Both parathyroid hormone 1-34 and 1-84 have shown to increase bone formation and reduce vertebral and non-vertebral fractures. Though they may have side effects of mild hypercalcemia, nausea, dizziness, and headaches, these drugs are expense, costing ten times more than bisphosphonate. As a result, it is given to those with severe osteoporosis, or those who cannot tolerate bishposponates (Fillit et al., 2010).

Hormone replacement therapy (HRT) has been recommended to postmenopausal women to help reduce vertebral, hip and other fractures, as well as colon cancer. HRT is not recommended any more due to the increased risk of breast cancer, coronary heart disease, stroke and thromboembolism. However, it is used for those that cannot tolerate other osteoporosis treatments (Fillit et al., 2010).

Denosumab is a monoclocal directed against receptor activator of nuclear factor-kappaB ligand (RANKL). RANKL signals regulators for the formation of osteoclasts, and densumab inhibits RANKL action on osteoclast and bone resorption. Denosumab has shown to increase BMD, and is given in twice yearly injections. Another new drug prolio is a monoclonal antibody

that inactivates osteoclast function. It is given to postmenopausal women with high fracture risk (Fillit et al., 2010). We still know little about the long term affects of these two drugs.

Most of the Western drugs are one sided in their treatment of osteoporosis and bone maintenance. They either only inhibit osteoclast or only increase osteoblast. The balance of osteoblast and osteoclast in bone maintenance is critical to strong yet flexible bones. The bones continually go through the maintenance of osteoclast breaking down bones to clean up and repair damage to old bones, and then the osteoblast comes through to repair and build bones. If there isn't a balance, and there is only increased osteoblast, the result is denser bones- but not flexible bones. If there is only increased osteoclast, the result is flexible bones—but not strong bones.

Osteoporosis and Supplements

Supplements, especially vitamin D, vitamin K, magnesium, calcium, vitamin E, omega 3 and isoflavons, can be taken at any point in life. However, they should always be taken with other osteoporosis therapy. They are positive additions to the diet. Calcium and vitamin D are the building blocks of bones and have shown a reduction in fractures, especially in patients in care facilities (Fillit et al., 2010). They should be taken together, since taken alone, they don't show the same decrease in hip fractures. Side effects of these supplements include abdominal bloating, changes in bowel activity, and other gastrointestinal symptoms. These symptoms can make compliance a problem. If there is a problem with absorption, which is especially high in an older population, pharmacological doses of Vitamin D or low doses calcitriol and alfacalcidol

should be given. A physician should then monitor the patient for hypercalcemia and renal function because a high dose of Vitamin D can cause these problems (Fillit et al., 2010).

Vitamin D3 is very important for bone health as well as general health. There are two forms of vitamin D important to humans, D2 ergocalciferol and Vitamin D3 choecalciferol (Setright, 2011). Vitamin D2 is not naturally occurring in the body and is in most supplements, but D3 is natural and a more potent form of vitamin D. Both are converted in the liver to Hydroxycholecalciferol (25-(OH) D3) through photochemical reactions. Then the parathyroid stimulates the kidneys to convert it to 1,25 dihydorxycholecalciferol (1,25-(OH) D3), the active form of vitamin D, when blood calcium levels are low (Liska et al. 2004). Vitamin D3 helps to keep blood levels of calcium and phosphorus at optimal levels (Setright, 2011). To maintain an optimal level of D3 one needs adequate sun exposure from early morning or late afternoon sun. At these times the best ultra violet-B rays are generated. Those violet-B rays allow the skin to convert 7 –dehydrocholestrol to Calcidiol (25-hydroxyvitain D), the active form of vitamin D (Setright et al., 2011). However, excessively large doses of Vitamin D can cause toxicity and calcification of soft tissue.

Omega 3 fatty acids have been shown to increase bone strength in ovarianectomized rats (Yao et al., 2006), but studies in humans are limited and more are needed.

Magnesium is an important mineral in bone health. Deficiency of magnesium is associated with slowed skeletal growth and osteoblast formation, leading to weak and fragile bones. Magnesium serves as a mitogenic for bone cell growth. A deficiency of magnesium may affect the magnesium channel that is important for osteoblast function (Rosanoff, Weaver &

Rude, 2011). Magnesium deficiency then affects the crystal formation and can result in larger, more perfect crystals, which in turn weakens bones (Rosanoff et al., 2011).

Calcium is another important mineral for bone health. There are two main sources of calcium, carbonate and citrate. Carbonate has higher levels of elemental calcium but is harder to break down. Circulating calcium levels can be low due to many factors. Howver, in today's society a lower Ph in intestines and stomach is a primary cause. With a lowered Ph level, food will not breakdown, and subsequently will cause a decrease in nutrients and calcium from being released and absorbed, consequently causing compensatory responses such as hyperparathyroidism.

Hyperparathyroidism is a reaction to low levels of serum calcium. In response the parathyroid activates mechanisms to increase calcium levels by initiating osteoclastic bone resorption to put more calcium into the blood (Liska et al., 2004), which then leads to weaker bones. Those with GERD, those who use a proton pump inhibitor, and chronic users of acid reducers should monitor and increase their calcium intake due to a lower stomach Ph and decreased calcium absorption (Wright, 2008). Other factors that could impair calcium absorption include excess dietary fat, excess dietary fiber, caffeine, phytic acid, oxalic acid, cocoa and ethanol, which cause an increase in fecal excretion of calcium (Liska et al., 2004). Excessive protein may cause excessive renal excretion of calcium and cause a calcium imbalance and a higher bone loss (Liska et al., 2004). To increase solubility and absorption of calcium, include Vitamin D, hydrochloric acid, ascorbic acid, citric acid, glyscine and lysine in the diet.

Vitamin A, specifically retinoic acid, can be detrimental to bone health. Too much vitamin A can cause a suppression of osteoblast function and stimulate osteoclast function. Retinol can also antagonize vitamin D's ability to maintain calcium levels (Genaro & Martini 2004). According to Genaro and Martine (2004) bone mass density is optimal with intake of 2000 to 2800 IU/day. It was found that those who took a dosage higher or lower than this incurred a lower bone mass density (Ribaya-Mercado & Blumberg, 2007). Ribaya-Mercado and Blumberg (2007) also noted that an increase of 1 mg per day increased fracture risk by 68%. As we age our serum retinol levels increase and should be monitored especially in men, those on oral contraceptive and estrogen (Genaro & Martini, 2004).

Vitamin E, which can be found in lipid- rich plant products, is known for its antioxidant functions. There are two main types of vitamin E, tocopherols (the commonly know and studied one), and tocotrienols, which is starting to be studied, and seems to be a more potent antioxidant. Vitamin E becomes part of the cellular membrane and inhibits the oxidation of lipids; tocotrienols has a more potent scavenging of the chain-propagating peroxyl radicals in liposomeres (Wong & Radhakrishnan, 2012), reduce DNA damage, sister chormatid exchange frequency, and urinary 8-hydroxy-2'- deoxyguanosine levels (Wong & Radhakrishnan, 2012). These antioxidative effects can help those with osteoporosis because those with this disease usually have low levels of antioxidants and higher levels of reactive oxygen species (Wong & Radhakrishknan, 2012). Wong et al. (2012) also noted that rats given dexmethaosone had an increase in bone calcium content and also concluded that those given palm gamma-tocotrienol, could receive help with glucocorticoid induced bone damage.

Vitamin K2 a fat soluble vitamin, menaquinone 4, is synthesized by bacteria in the gut from other forms of Vitamin K. Vitamin K carboxylates the calcium binding protein osteocalcin's glutamic acid to gamma-carboxyglutamic acid, which is part of the calcium uptake in bone formation. While both K1 and K2 have this effect, K2 has been found to be more potent (Iwamoto, Takeda & Sato, 2011). Serum biomarker of osteoclacin is a good marker of bone quality (Iwamoto et al., 2011). K2 seems to have a synergistic effect with bisphosphonates causing a decrease in facture rates (Plaza & Lamson, 2005).

Phytoestrogenic is a nutraceuitcal plant derivative that binds with estrogen receptors and can modulate estrogen action and may affect bone metabolism through increased calcium absorption and production of Insulin growth factor 1 (IGF-1). IGF-1 is known to increase osteoblast activity. Studies conflict on the effectiveness of phytoestrogenic soy isoflavones effects on increasing bone mass.

As we have shown, vitamins are very important to bone health and maintenance. Therefore, a diet with adequate intake is vital. Most people do not receive enough essential vitamins from their diet, and as a result supplements should be incorporated.

TCM Osteoporosis Definition

In TCM osteoporosis is considered an imbalance of Yin, Yang, Qi and Blood. In the "Plain Questions" in the Nei Jing it states: "Calm Yin and steady Yang result in good vitality. The separation of Yin and Yang results in the depletion of vitality" (Xu, Lawson, Krass, Ryan, 2005). This means as we age we disrupt this balance and deplete our vitality though our life style choices, stress, overwork, excesses sex, and improper diet. Creating this imbalance of yin and

yang directly leads to depletion of the zang-fu organs and the structures of the body including our bones.

Looking at the different TCM organ systems, an imbalance can be created by a spleen deficiency. The spleen is in charge of post heaven Qi and the transformation and transportation of Gu Qi, the nutrients of the body, it is in charge of digestion and nutrients separation. Looking at it from a Western point of view, if we are not digesting and absorbing our nutrients like vitamin K, calcium and magnesium, we are beginning to cause a chain reaction of deficiency of bone building nutrients, leading to osteoporosis. In TCM the kidneys control the bones, store essence, are in charge of growth and development, regulate water circulation, supply the marrow and determine the condition of the bones. It can then be predicted that if one is kidney deficient, the person is more likely to have osteoporosis. For women and for men to a lesser degree, a depletion of kidney Yin is the major cause of osteoporosis. A deficiency of Yin leads to an excess of Yang, leading to excess fire, which dries up the water causing the bones to shrink and shorten. In a study out of Australia, 40 menopausal women had their bone structures and function assessed (Xu et al., 2005). The study results showed a strong relationship between Kidney deficiency patterns and a low bone formation marker, osteocalcin, with higher bone resorption marker, urinary Pyridum (Xu et al., 2005). The study highlighted an imbalance of Yin and Yang leading to a depletion of vitality causing aging and hence depletion of the mind, body and the bones.

As we age. we naturally lose our vitality and pre-heaven Qi. Pre-heaven Qi is the qi we get from our parents at conception, and it determines our constitution. Pre-heaven Qi is then supported and nourished throughout our lives by our post heaven Qi, which is made up of the

food ingested, and processed by the spleen organ system. If pre and post heaven Qi are strong, we will have strong growth and development, and will be able to stave off illness and keep from aging rapidly. Both of pre and post heaven essence can be used up by a life of overwork, excessive sex, poor and/or over consumption of food. The recommendation is that leading a moderate life will help one live healthier and longer. Looking at this model in regard to bones, it is evident that those born with weak pre-heaven Qi will have weak, fragile bones and retarded growth. On the other hand, those born with strong pre-heaven Qi, but who lead lives of overindulgence and overwork or poor diet, will experience early aging and weak, fragile bones. TCM believes in preserving your essence to live a long healthy life.

TCM Herbal Treatments

Herbs can be a viable alternative to medications for helping to slow and reverse bone loss. A study done in China looked at several Chinese herbs and tested them to see how they effected osteoblast cell proliferations (LI, Wang, Jiang, Gao, & Zhao, 2001). It was found that many of TCM Kidney tonifying herbs have osteoblast cell stimulation. In TCM, Yang is considered the functional component. Therefore, it makes sense that Yang tonic herbs would increase the function of cell building osteoblasts. A prepared aqueous and alcohol extract of herbal solutions was added to UMR 106 (definition in glossary) cells, and many were found to increase proliferation of the cells (LI, Wang, Jiang, Gao, & Zhao, 2001). UMR-106 cells have osteoblast functions, which relates to an increase in bone density. The herbs that showed the most cell activity were from the kidney tonifying herb category. As was stated earlier, the kidneys are in charge of growth and development and determine the condition of the bones. Most kidney yang herbs strengthen the sinews and bones.

Herbs that rated highest in increasing osteoblast function had some sort of essence or vitality enhancing function. The herb that had the most effect on the UMR106 cells was an alcohol extract of the herb Zhi He Shou Wu, which is a blood tonic herb that goes to the kidney and liver channels, with the functions of nourishing yin, blood and essence. Its functions include helping with low back and extremity pain. The low back is an area of the body associated with the kidney organ system in TCM. Pain in the low back is associated with kidney deficiency. Xiao Moa is a kidney herb that goes primarily to the kidney channel. It has the function of tonifying the kidney yang, and warming the gate of vitality. It is very good for weak bones and sinews due to cold.

Fu Pen Zi goes to the kidney and liver channels, it augments Yin and secures essence with a kidney Yang tonification that helps with low back pain. Making an alcohol solution of Fu Pen Zi is a positive addition to a TCM treatment of osteoporosis. This is just a small sample of the herbs tested. However, with their multifunction's and minimal to zero side effects, herbs are an effective alternative to western pharmaceticals.

The individual herbs disucssed above can have a profound effect on bones. However, when these herbs are combined into a group as a formula, they can have a systemic effect on the whole body. A study done by Azizi, H.Liu, Du, Wang, Bahrami-Taghanaki, Esmaily, Azizi, H., and Xue in 2011 studied the formula Kun Bao Wan combined with acupuncture in comparison with hormone therapy, and found it as effective as estrogen therapy. The results of Azizi et al.'s 2011 study showed an improvement in menopausal symptoms, increased estradiol, and lowered FSH. The patients not only had systemic improvement in quality of life, but also fewer hot flashes, an increase in estradiol and the lowering of FSH, all resulting in improved bone

health. This study shows that acupuncture and an herbal formula could be a viable alternative to some Western drugs. Herbals are also noted to have less side effects and fewer adverse reactions.

The ingredients in the formula, Kun Bao Wan, used in Azizi et al.'s (2011) study, have multiple effects on the bones. Below are the ingredients and a breakdown of their functions in the formula. Kun Bao Wan contains: Fructus ligustri lucidi, Radix paeoniae lactiflorae, Radix paeoniae lactiflorac, Radix rehmanniae, Fructus rubi, Radix angelicae sinensis, Semen cuscutae chinensis, Radix et caulis jixueteng, Lycii fructus, Semen zizyphi spinosae, Radix scutellariae baicalensis, Flos chrysanthemi morifolii, Herba ecliptae, Radix adenophonrae, Folium mori albai, Radix cynanchi baiwei, Anemarrhena rhizome, Radix polygoni multiflori, Herba dendrobii, Lycii cortex, Colla plastrum testudinis, Tuber ophiopogonis japonica, Concha margaritifera and honey.

Fructus ligustri lucidi, radix paeoniae lactiflorae, radix rehmanniae all nourish the liver and kidney yin. A deficiency of yin effects estradiol and causes a shirinking and shortening of our bones. Flos chrysanthemi morifolii and radix scutellariae baicalensis are said to subdue liver yang, which helps with anger and the rush of heat to the head and upper body, helping with subduing hot flashes, and menopause symptoms.

Fructus ligustri lucidi and radix paeoniae lactiflorae have been found to improve calcium balance and help prevent osteoporosis through the TCM mechanism of nourishing the kidney yin and improving digestion for the assimilation of nutrients. Fructus ligustri lucidi improves

calcium balance, modulates the calciotropic hormone levels and increases vitamin D-dependant calcium transport in OVX rats (Azizi et al., 2001).

Therefore, when all these herbs are combined one can see how herbal medicine could be a great help for not just bone health but the whole person by dealing with the other symptoms of yin deficiency like hot flashes, anger, mood changes and digestion. The above paragraphs have addressed just a few of the herbs that could be beneficial to osteoporosis and should be further looked into for the great benefits they may have for osteoporosis treatments.

Electroacupuncture

Bone loss can potentially be slowed, or in some cases turned around, with acupuncture. Acupuncture is the insertion of filiform needles into specific points in the body to effect change. When manually stimulated by a practitioner, the procedure is called simple needling (SN) or regular acupuncture. When electrical stimulation is incorporated it is referred to as electroacupuncture. Electroacupuncture (EA) has shown great effect in many conditions. and therefore could be helpful for osteoporosis. According to Mayor (2007) electrical currents through chemical, physical and thermal influences, effect the body at the cellular, tissue, segmental and systemic levels. Electric current stimulates nerves to elicit action potentionals and causes a chain reaction of chemical processes in the body. The tissue is stimulated by current that is delivered percutaneously in either high or low frequency. This continuous stimulation from electrical currents may be why EA seems to be more effective than manual acupuncture on influencing changes in the body.

The frequency of electrical stimulation is measured in hertz (Hz), cycles per second. Low frequency is 10 hertz or less, and high frequency is 50 to 100 hertz. Peripheral nerve fibers and nociceptive C fibers respond well to low frequency, around 2 hertz. Low frequency EA has longer lasting effect on pain. It modulates activity in the brain, lowers blood pressure and can mediate μ and 8 opioid peptide receptors (Mayor, 2007, pg 68). Endorphins and endomorphin-I are released, and enkephalin synthesis is stimulated in the brain at 2 hertz (Mayor, 2007). A frequency between 15 and 20 hertz can block pain signals and may influence non-opioid mechanisms (Mayor, 200). A high Frequency of 100 hertz, has effects on substance P and angiotension II, and can help with opioid withdrawal. Dynorphins in the brain are mediated by both 15 and 100 hertz.

The intensity of the electrical stimulation has an effect on treatment with EA as well. This is measured in milli ampere (mA), the strength of the stimulation. Strong stimulations have been shown to activate the sympathetic nervous system, raise blood pressure and heart rate and have a potentiating effect on some forms of cardiac arrhythmia (Mayor, 2007, pg 67). Strong stimulations can induce longer lasting pain relief and can help spasmodic conditions. Howver, it can exacerbate inflammation. Less intense frequency stimulates the parasympathetic nervous system causing relaxation, reducing blood pressure, moderating arrhythmia and effecting neurogenic pain (Mayor, 2007, pg 67). High Frequency with strong intensity is good for traumatic pain and electro-anesthesia, while low frequency with strong stimulation is good for paralysis, early stroke issues and numbness. One can mix and match frequencies and intensity for different effects. Alternating between high and low frequency, called dense and dispersing, is effective for chronic pain, muscle weakness, stroke, tissue

regeneration, inflammation, edema, multiple sclerosis, and chronic Bell's palsy. Duration of treatments is twenty to thirty minutes. For pain one would do two to three, twenty minute treatments a week. According to Mayor (2007) L-dopa, antihistamines and caffeine may counteract EA effects, whereas selective serotonin reuptake inhibitors (SSRI) may improve EA effects. This observation may be due to the fact that L-dopa, antihistamine and caffeine suppress the opioid effects, thus counter acting the effectiveness of EA. The enhancement of EA effects from SSRI, may be due to their stimulation of the parasympathetic system. Both EA and SSRI may cause relaxation.

As mentioned, low frequency acupuncture stimulates endogenous opioid and enkephalin, providing pain relief as well as increasing blood flow to the needle sites, initiating histamine response and a surge of mast cells to promote the body's nature healing mechanisms. So when a case of a post-menopausal woman who was treated for serious back pain due to osteoporosis resolved after nine treatments we can see why. With seven months of continuous treatments the patient showed an increase in her BMD as well (Xu, Lawson, Kras, Ryan, 2005). The following points where used every time DU20, DU14, DU9 and DU4.

- DU20 is the sea of marrow, the meeting point of all Yang Qi of the body and has a profound effect on regulating the Yang Qi; that could be seen as the builder of function in the body, and according to the classics treats "one hundred diseases".
- DU14 is the sea of Qi and it clears Yin heat, which as we know shrinks bones.

- DU9 tonifies the middle Jiao allowing for better absorption of nutrients and it also clears kidney heat.
- DU4 clears heat, tonifies the kidneys, benefits the lumbar spine, influence Ming Men, is good for kidney essence deficiency and has strong effect on the spine and lumbar.
- They also needled 4 to 6 of the following points: REN4 to nourish Yin, blood, kidneys and benefits Yuan QI.
- REN 6 a sea of QI point, tonifies Qi, Yang and strengthens Yuan Qi.
- UB23 is the Back shu of the kidneys.
- UB32 strengthens the back and the kidneys.
- KD13 is the meeting point of kidney channel. All of these points affect the kidneys and through that influence the bones.
- GB39 is the influential point of marrow and is a very good point for BMD.
- ST36 nourishish the middle jiao, digestion, Qi and blood, UB20 the back shu of the spleen and SP6 which tonifies the spleen and the blood, all effect the middle Jiao and the gastrointestinal track, thereby influencing the building of blood and the control of nutrient absorption (Xu et al., 2005).

As seen from these few examples TCM has many alternatives for the treatment of osteoporosis. However, scientific proof is still needed to account for its actions. It is the hope of this author that this study's mapping of the effects of acupuncture biochemical chain reactions will give acupuncturists, MDs and their patients a firm grounding in the science of acupunctures effects.

Chapter 3 - Method

This chapter describes the methods used to complete this research synthesis project. This section addresses research design, data sources, search terms, data collection procedures, study limitations and data analysis. This research was a qualitative study of available research studies using grounded theory method to create a resource on osteoporosis and acupuncture's effect on the biochemical pathways of the body. This review will be used to show the scientific effectiveness of acupuncture, in order to facilitate the creation of effective treatments, and to also establish the ground work for further clinic trails and studies.

Research Design

This study is a qualitative research synthesis using grounded theory. "Grounded theory is an attempt to develop theories from an analysis of the patterns, themes and common categories discovered in observational research. Grounded theory combines theory and research (Crossman, A., 2012). This study investigated many different systems to see if and what biochemicals are involved in acupuncture, and if these biochemicals then had an effect on bones. The biochemicals were then looked at to see what chain reactions they effected and how that related to bone maintenance. Data and i\Information on the biochemical pathways from different systems studied were examined for the purpose of linking the theory regarding of the effects of acupuncture on biochemical actions of the body to illustrate how acupuncture may increase bone mass density and bone health. The current study used as whole body approach. We are not just disjointed parts. Every chemical chain reaction has a cascading effect on the entire body. By treating the person as a whole, you can clear up multiple

problems. The approach of the current study is to bring together pieces to create a whole picture. This holisitic approach will examine research on animal and human subjects of different organ systems in an attempt to qualify acupunctures biochemical effects.

Sampling and Sampling Procedures

Research syntheses data were complied through articles found from medical journals of both Western and Traditional Chinese Medicine. Online research databases included Pubmed, EBSCOhost, Google scholar and UCLA medical library database. This research was conducted on the principle investigator's home computer, Yo San university library and UCLA Biomed library. The search used the following words and phrases: Osteoporosis, electroacupuncture, electro stimulation, acupuncture, alternative therapies, bone health, bone mass density, post menopausal, menopause, chemical markers, TGF-1, growth hormone, estrogen, hormone replacement therapy, anterior pituitary, RANKL, calcium, hypothalamus, TNF-α, follicular stimulating hormone, beta-adrenergic receptors, parathyroid hormone, vitamin D, calcitonin, glucocortcoids, osteoprotegerin.

This study used research data from both human and animal studies. Articles from 1990 to present were included. Articles from all countries were accepted. Only peer review articles were used. Information needed included; names of points, measurable chemical reactions, types of subjects and measurable outcomes. If an abstract indicated the presence of these factors, then the article was located in full text and analyzed, with data entered onto an article abstraction form.

Instrumentation and Data Collection Procedures

An article abstraction form was used to organize and summarize the information from each article. The data collected from each article on each form was then used to create graphs to chart the information an data. The information was then compiled to generate a summary theory regarding acupuncture's impact on bone health and bone maintenance through the biochemicals reactions. The information/data gathered on the article abstraction form included the following factors:

- Number and type of subject: human or animal, male or female.
- Length of study
- Type of treatment: acupuncture, electroacupuncture, herbs, embedding, moxa.
- What system was studied; menopause, bone, gynecological, arthritis, immune.
- Measurement of biochemicals was recorded on whether they increased or decrease noting number values.
- Biochemical markers looked at were: estradiol, follicle stimulating hormone, osteocalcin, calcitonin, parathyroid hormone (PTH), alkaline phosphates, noepinphrin, calcium, phosphorus, MMp-13, IGFI, IGF-II, deoxypyridinoline (Dpd), TNF-α, testosterone, luteinizing hormone (LH)
- Bone histomrphometry looked at: trabecular thickness, trabecular number, trabecular separation, marrow cavity, number of nodes, node terminus, bone ash weight, bone volume, BMD femur, tibia, vertebra, bone formation rate, Mineral apposistion, mineral surface, energy to failure rate, and load max.

- Also looked at was body weight, uterine weight, heart rate.
- Conclusions of the studies researchers
- Conclusions of this researcher

The article abstraction form also included the following treatment information.

- What were the comparison groups: Model- normal control group, Control group that had same operations as treatment groups. And what were the treatment groups acupuncture (ACU), electroacupuncture (EA), herbs, western medicine or different points used groups.
- Points used.
- Needle type
- Type of manipulation: regular acupuncture, electroacupuncture, moxa, embedding needles, sham devices
- Type of EA – hertz and ampere measurements
- The length of needle retention.
- The frequency of treatments: Dailey, weekly, biweekly.
- Length of the study: one day to 6 months

All the above data items were charted, assembled and analyzed to provide for the potential generation of further theory regarding the impact of acupuncture on bone health.

Data Analysis

In this section the process of data abstraction and analysis will be described. The use of article abstraction form was the start of data analysis and coding. All of the article abstraction

forms were compiled, assessed and charted to find common themes. Then through the understanding of bone mechanisms. the information was put together to show how acupuncture helped to increase bone health and maintenance. By looking at and charting the different biochemical effects, a picture was developed as to how acupuncture treatment has a cascading effect on the bones and on the person as a whole. Tables were used to chart, link and organize the information pulled from research articles.

First, the articles were analyzed and grouped by the system studied: bone, neurological, gynecological, menopause, immune and cartilage (Appendix D, Table 1). This initial analysis helped the researcher to discern trends of organ systems. This analysis also allowed the researcher to see which systems had a greatest effect on bone chemicals.

The data were then organized into type of study, whether animal or human. This categorization helped the researcher to see the trends of subjects used. This analysis is reflected in Appendix E Table. Appendix E Table was used to help make Graph 18, amount of Hertz (Hz) used in EA studies on page 105. This table breaks down the amount of hertz used in EA studies on animal and humans.

Among the the major factors studied in this project was which biochemical markers were tested for and analyzed. These data were assembled in Appendices F & G Table – Biomarkers Screened For. This table shows which markers were tested, whether there was an increase or decrease in the marker, and which system was being studied. Biochemical markers measured included: osteocalcin, calcitonin, TGF-I, estradiol,blood pressure, heart rate, TNF-α, noepieprine, cartilage damage, deoxypyridinoline, Alkaline-phosphate, tartrate-resistant acid

phophatase, testosterone, BMD, bone ash weight, trabecular measurements, luteinizing hormone, follicular stimulating hormone (FSH), mature vaginal epithelia, gonadotrophin releasing hormone (GnRH), corticotrophin releasing hormone (CRH), aromatase activity. Once all of this information was gathered, the researcher was able to analyze which markers were most often screened, and that information was used to report in chapter four, "Results." Appendix F & G Tables were also used to make Table 1 - estradiol Study Results Summary, and Table 5 - FSH Study Results Summary, in Chapter Four.

The current study also looked at the design of studies. It was noted if the study used acupuncture or electroacupuncture, or other methods like moxa, tuina, acupressure or embedding needles, which system was being studied, i.e. the bone system or other organ systems. This information/data are shown in Appendix H Table – Methods of Treatment. This information allowed the researcher to trace which methods were being studied, and if they were having the effects on biochemical chains. This data provided the content for Graph 19 – type of needling in Chapter Four.

Method of treatment was charted and organized in Appendix I Table - Treatment Details of TCM. This table shows which method was used, acupuncture, EA, embedded needles, moxa, tuina or ear seeds; which points were used, the needle type or instrument type used, length of needle retention, number of treatments and the length of the study. The Appendix I Table is important for discerning what type of acupuncture was affecting the body, and what frequency of treatments was effective so they can be reproduced and time lines of treatment for results could be discerned. This information is of importance for laying the ground work for future studies and putting together treatment protocols. Appendix I Table 5 treatment details and

abstraction forms provided data for Graphs 7, 8, 9, 12 and 13 ("frequency of treatments," "needle retention" and "treatment lengths"). Points were taken from Appendix I Table to make Graphs 1, 2, 3, 4, 5, 6, 10, 11, 14 and 15 ("points used for biomarkers").

The article abstraction form was used to record the biochemical value changes. These data were recorded in Tables 2 thru 19 ("the measurement results for the biomarkers").

The final tables provide data regarding the results of the studies and how they can be related to the bone mechanisms and BMD and shown in Appendix J Table. Studies were reviewed and the conclusions of the studies were noted. This table looked at the number of study subjects, which systems where studied and bio-markers measured. The treatment interventions show what each study's breakdown of test groups were. As an example: group one: acupuncture only, group two: acupuncture and X drug, Group 3: X drug only, Group four: control group. Then the results were recorded to reflect whether the study produced the intended results, and did the studies show a change in biomarkers and what was that effect. All this information was used to trace the links and common threads of acupuncture's effect on the biochemistry of the body, and how it can affect bone health and maintenance.

Limitations

The limitations of this study included limited articles on acupuncture treatments for osteoporosis and bone health. There were many studies that measured biochemicals, but not all the same biochemicals. As a result, there is a lack of multiple studies that compare the same measurements. There was also a lack of articles in general that dealt with biochemical measurements. Many of the articles were hard to find because they were in Chinese and had

not been translated to English. As for the articles themselves, most were on an animal subjects, which is a good starting point. If human subject were used, they were small scale studies lacking description of points, diagnoses used, treatment strategies, treatment length and treatment time. Human studies tended to compare true acupuncture points to sham points. Despite these limitations a comprehensive picture emerged. With the application of the grounded theory approach the researcher was able to discern important patterns and potential theories from all areas of acupuncture.

Chapter 4 - Results

This chapter articulates the results of the current study with focus on the biochemical and bone formation markers observed in studies involving acupunctgure. Studies performed on both animal and human subjects were reviewed. Treatment details were examined for acupuncture points used, frequency of treatments, length of treatments and type of treatments.

Estradiol and Estrogen Results

Estrogen and Estradiol have an effect on BMD by stimulating osteoclast apoptosis and prevent osteoblast and osteocytic apoptosis (Takeda, 2005). Estradiol was the biochemical most studied and measured that relates to bone maintenance and health. The focus of the estradiol systems studies was split between menopause / gynecological disorders and osteoporosis / bone maintenance studies. Sixteen of the seventeen studies showed an increase in estradiol. Ten studies used electroacupuncture. Six studies used regular acupuncture, and one study used catgut implantation. Within the seventeen studies that measured estradiol, nine were on animal subjects, and eight were on human subjects. Results of improvement in estradiol levels were similar between animal and human studies. This data is shown in the following Table 1 (Estradiol study results).

Table 1 - Estradiol study results

Biochemical	Study number	Change	System studied	Type of subject
Estradiol	1	Decreased	Bone	Animal
	5	Increased	Cartilage	Animal
	6	Increased	Bone & menopause	Human
	7	Increased	Menopause	Animal
	13	Increased	Menopause	Animal
	14	Increased	Menopause	Human
	15	Increase	Menopause	Human
	16	Increased	Gynecology	Human
	17	Increased	Menopause	Human
	19	Increased	Gynecology	Animal
	20	Increased	Menopause	Human
	24	Increased	Bone	Human
	25	Increased	Bone	Animal
	26	Increased	Bone	Human
	27	Increased	Bone	Animal
	28	Increased	Bone	Animal
	30	Increased	Bone	Animal

Estradiol Animal Studies. The following information is also shown in Table 2 (Animal studies measurement results on estradiol).

Study#1: (Electroacupuncture promotes insulin-like growth factors system in ovariectomized osteoporosis rats, by Feng, Y., Lin, H., Zhang, Y., Wu, X., Wang, T., Liu, Y. & Tan, Y. 2008) This study used female rats that were forced into menopause by having ovariectomy.

There were five groups with ten rats in each group. The groups were as follows: the control group was left intact, meaning they had no surgery on their ovaries and had no treatments done to them (Control), the sham operation group had the fat removed from around the ovaries and had no other treatment (Sham). The last three groups had their ovaries removed, model group (OVX -model), an estrogen group (OVX-E) and electroacupuncture group (OVX-EA). The treatments; the model group had no treatments, the estrogen group was given nilestriol .9 ml/week/per 100g weight of rats, and the EA group received bilateral GB39 and DU4 had 30 minute daily treatments for four weeks. The size of needles used were .22mm diameter and 10 mm length, with EA applied at a frequency of 2 Hz and 1mA of intensity.

Study #1 results showed a decrease in estrogen in the OVX-EA and OVX-Model groups. Estrogen levels in the model and sham groups showed no difference in estrogen levels and estrogen group showed an increase in estrogen, in the OVX - Model and OVX-EA groups, estrogen levels were markedly lower than model intact and sham control groups but showed an increase in IGF-1 levels, as did the estrogen group, but not as much as the OVX-EA group. The OVX-EA group did show an increase in BMD even though there wasn't an increase in estradiol, this could be due to the increase in IGF-1 levels. IGF-I gene has an effect on bone remodeling by recruiting pre-osteoblast cells to the remodeling surface (Takeda, 2005).

Study #5: (Effects of electro-acupuncture on oestrogen levels, body weight, articular cartilage histology and MMP-13 expression in ovariectomised rabbits by Qin, Y., He, J., Xia, L., Guo, H., He, C.,2013) This study used 24 rabbits, divided into four groups. The groups were a normal control group (Control) that received no treatment and no ovariectomy, the other three groups were forced into menopause by having their ovaries removed, and then divided into

three groups; a model group that received to treatment (OVX-model), estrogen groups (OVX-E) that received estrogen tablets orally .625mg/day, and an electroacupuncture group (OVX-EA) that were needle at UB18, UB 20, UB 23, ST36 and SP6 with stimulation of 10Hz, 1.5mA electroacupuncture applied to UB23 and ST36. The study used .3 mm by 25mm needles insertion depth was 10-15mm. All treatments were received five days a week for two weeks.

Study #5 results showed that estrogen levels of the OVX groups, were lower at the beginning of the treatments and at 8 weeks after ovariectomy, compared the normal control group, by about 10 points. After two weeks of treatment the OVX-EA and OVX-E groups had levels only a few points below the OVX-model group, and the OVX-model group was just over 10 points lower than the control group. The OVX-EA was higher in estradiol than OVX-E group. The effect on MMp-13 was promising. The treatment groups were higher than the OVX- model group but lower than the control group. The OVX-E group had slightly lower numbers than the OVX-EA group at end of the study.

Body weights of the rabbits at the beginning of treatment were same in all the OVX groups. The OVX groups were all higher than the control group. After treatment the OVX-model group was the highest. The OVX-EA had the lowest body weight of all the groups even lower than the control group. The OVX-E and control group were the same. This shows that EA systemic effect can help maintain a healthy body weight in OVX or menopausal patient.

The cartilage samples were assessed and the OVX groups had more damage than the control group, but the treatment groups had much less damage, and OVX-E group had less damage than the OVX-EA group. The OVX-E group seemed to help with lower MMp-13 and

cartilage damage better than OVX- EA, but OVX-EA had fewer side effects than the estrogen therapy group, plus the numbers were not that different between the two groups, only a few points different and when compared to the OVX-model group. This showed that estradiol and EA could help regulate MMp-13 damage.

Study #7: (The evaluation of electroacupuncture on ovariectomized rats: Implications of modern scientific mechanisms on acupuncture curing woman perimenopausal syndrome by Zhao, H and Chen, B. .2005) This study used 58 female rats and divided them into five groups. The first two groups were left intact, one received no treatment (INT) and the other (INT-EA) received same EA treatment as the OVX-EA group. The other three were forced into menopause by having their ovaries removed; the three OVX groups are as followed; the OVX-model received no treatment, OVX-EA received EA on the specific points REN3, REN4, SP6 and Zi gong xue,, the last group was the OVX-Sham group which received EA needling at Hua Tuo Jia Ji at Thoracic 3-5. Electroacupuncture was done for 30 minutes a day for three days at 3Hz at 1-2mA intensity, and then six hours after treatments the rats were sacrificed and the blood, liver, brain and abdominal adipose were taken for measurements.

Study #7 estradiol levels were lower in OVX groups compared to the Model - INT group. Estradiol in OVX-EA group was significantly higher than the other OVX groups. OVX- EA treatment group, was higher than the OVX sham treatment group. This study also applied EA true treatment to intact model as well and showed no change in estrogen level over the no-treatment model (data not shown).

Mature vaginal epithelia were decreased significantly in the OVX rats compared to the INT rats, but OVX-EA had twice folded increased epithelium compared to the OVX-model and OVX-Sham, and the INT-EA showed no change. GnHR was higher in OVX groups compared to INT, the OVX-EA GnHR was lower than OVX-model and INT-EA GnHR was lower than the INT group. Corticosterone level was increased in OVX-EA group compared to all other groups. Testosterone was higher in the INT groups and lowest in OVX groups with OVX-EA levels being the lowest of all the groups. Extragonadal aromatization in subcutaneous abdominal adipose (SA adipose) and the liver tissues aromatization was significantly higher in the OVX-EA group compared to all other groups, the INT and INT-EA groups were the same level and only slightly higher than the OVX-sham in SA adipose aromatase activity, whereas aromatization in the Liver of the OVX-sham and INT-EA were the same.

Study #13: (Electroacupuncture enhances extragonadal aromatization in ovariectomized rats, by Zhao, h., Tain, Z., Cheng, L., Chen, B., 2004) This study used 60 female rats that were divided into five groups. The groups were as follows; the intact control (INT) group was left intact and received no treatment, the model-EA group was left intact and received EA on REN4, REN3, Unilateral SP6 and bilateral Zigongxue for 30 minutes a day for three days, the OVX-model group received overiaectomy and no treatment. OVX-EA group received ovariaectomy and EA on REN4, REN3, unilateral SP6 and bilateral Zi gong xue for 30 minutes a day for three days. OVX-ShamEA received ovariaectomy and EA on Hautoujiaji Thoracic 1 through Lumbar 5 vertabrea, unilateral SJ5 for 30 minutes a day for 3 days. EA was applied at 3Hz with 1-2mA intensity.

Study #13 estradiol levels were also lower in the OVX groups as is expected but OVX-EA group was significantly higher than the other OVX groups. These two studies EA levels were almost five times higher than OVX group but only half as high as intact model.

Vaginal smears showed decrease in mature epithelia cells in the OVX-model group compared to the INT group, and the OVX-EA showed much higher number of cells than the other OVX groups. Corticosterone level was highest in the OVX-EA group compared to the other groups but not significantly. The aromatase activity was much higher in the OVX-EA groups over all the groups in both the SA adipose and liver tissues, the liver of OVX-EA was significantly higher in aromatase activity than all other groups, and interestingly the aromatase activity in the liver was the same in the INT-EA and the OVX groups. The SA adipose ratio was higher in the OVX groups than in the INT and OVX-model was higher than OVX-EA, with no real difference in the INT groups thought INT-EA was slightly higher. The mRNA expression in SA adipose was higher in the OVX groups with OVX-EA level the highest and the mRNA expression in the liver was lower in the OVX compared to the INT, with OVX-EA was higher than OVX-model.

Study #19: Acupuncture normalizes dysfunction of hypothalamic-pituitary-ovarian axis by B. Chen (1997) used 18 female rats divided into four groups. The groups were as follows; OVX-model group received ovariectomy and no treatment, the OVX-EA received ovariectomy and 30 minutes of EA of 3Hz one time a day for three day at REN4, REN3, SP6 and Zigongxue. Then 3days of sham points houttuojiaji and SJ5 EA 3Hz, the model groups (INT) were intact rats and received no treatment, the last group INT-EA was was left intact and received 30 minutes of EA of 3Hz 1x a day for three day of REN4, REN3, SP6 and Zigongxue followed by 3days of sham points houttuojiaji and SJ5 EA 3Hz.

Study #19 EA increased estradiol levels 6.11 pg/ml but was still lower that the intact model group by 6.79pg/ml. It was found the estradiol receptor level decreased in OVX groups, the corticosterone levels increased less in OVX-EA over OVX group, the adrenal weight were higher in OVX-EA rats and an increase in vaginal epithelium cells in the OVX-EA rats after treatment was applied. This study found there was no difference in the levels between the true acupuncture points and sham points in either OVX or INT groups.

Study #25: (Effect of electroacupuncture on the biochemical indices of bone and bone collagen metabolism and TNF-alpha in osteoporosis model rats without ovaries by Bao, SY., Zhang, SJ., Lin, WJ., Chen, JF.,2010) This study used 60 rats divided into five groups. The groups were as follows; Model intact group that had no treatment, the four remaining groups all had their ovaries removed and EA applied once a day for 6 days a week for twelve weeks, EA group received EA at UB20, UB21, UB23, UB24, EAT tonification group received EA at REN4 and ST36, EAD the blood stagnation group received EA at UB23, UB17, UB11, the EATD the blood stagnation and tonification group received EA at REN4, ST36, UB23, UB17 andUB11.

Study #25 results showed a decrease in Dpd, creatinine, ALP, osteocalcin, TNF-α and calcium over the model intact groups. All the EA treatments showed an increase in estradiol, but EADT was significantly better than all the other treatment groups in decreasing ALP, osteocalcin TNF-α and calcium.

Study #27: (Effect of electroacupuncture on plasma estrin and bone mineral density in ovariectomized rats, by Wei, YF., Liu, YL., Zhang, SH., Wang, ZO., Liu, Y., Wang, HC., Yao, JF., Li, F. and Wang, CH.,2007) This study used 32 rats divided into 4 groups. The groups were as

follows; normal control group that had no treatment or surgeries, OVX-model which had their ovaries removed but had no other treatments, OVX-EA had their ovaries removed and had EA at 1-3 Hz at .7-1mA for 20 minutes every day for eight weeks at ST36 and SP6, OVX-E group had their ovaries removed and was give daily dose of 5% nilestriol 5mL for eight weeks. Study 27 increase BMD and estradiol to similar effect as the OVX-E group.

Study #28: (Effect of electroacupuncture on bone mineral density, oestradiol level and osteoprotegerin ligand expression in ovariectomized rabbits by He, J., Yang, L., Qing, Y. and He, C., 2013) This study used 21 rabbits divided into three groups. The groups were as follows; Normal control group with no treatment and intact, the last two groups had their ovaries removed, OVX-model had no other treatment, OVX-EA received EA at 10Hz, 2mA stimulation to St35, UB20, UB23 fro 20 minutes a day for 14 days. This study increased BMD and estrdiol over the OVX-model group and decreased the osteoprogerin ligand express.

Study #30: (Effect of acupuncture on bone metabolism and serum estradiol level in ovariectomy-induced osteoporosis rats by Ma, J., Yun-guang, H. and Zhang, DH., Ma et al., 1999) This study used 40 rats divided into 4 groups. The groups were as follows; a sham operation group that had the fat removed from their ovaries but received no other treatment, the other three groups all had their ovaries removed, the OVX-model group received no other treatments, OVX-ACU received manual stimulation acupuncture once a day for 30 minutes for 60 day at UB11, UB23, UB20, OVX-DES group received 22.5microg/ml of diethylstilbestrol once a day for 60 days. This study showed that OVX-ACU group had similar results to OVX-DES group, an increase in estradiol, and uterus weights with decrease in ALP, tartate-resistant acid phosphates and gla protein over the sham model group.

Studies #25, #27, #28 and #30 all showed increases in estradiol over OVX-model groups stated as P values for each study: study #27 P<.05, study #28 P=.012 and study #29 P<.01.

Table 2 - Animal studies Measurement results on Estradiol pg/ml

Study #	Model Int.	Control	OVX – No TX	OVX-sham TX	OVX-EA	OVX-EST		
1	106.7	91.87	47.37		41.23	97.93		
5 At 0 week	41.05		39.18		45.92	39.21		
5 at 8 weeks	38.24		27.67		29.55	28.35		
5 at 10 weeks	40.75		29.01		42.55	41.26		
7 *	58		3	5	28			
13*	60		5	5	27			
19 Before	18.00				5.47			
19 After	18.34				11.58			
25					↑ over OVX no tx			
27					↑P<.05			
28					↑P=.012			
30					↑P<.01	↑P<.01		

*Estimate from graph

Estradiol Human Studies. The following information is shown in Table 3 human studies measurement results on estradiol.

Study #6: (Effect of acupoint cat-embedding on the quality of life, reproductive endocrine and bone metabolism of postmenopausal women by Chen, G., Xu, T., Zhang, J., Liu, S. and Guo, Z.,2010) This study was done on postmenopausal women split into two groups. The groups were as follows; the first group received two daily capsules one of Fufuchun which contained .625ug of Ethiyl and .25mg Provera as well as a daily capsule of vitamins, which contained vitamin A 500U, vitamin D2 500U, vitamin E 50 Mg and calglucon 150mg. The second group received catgut embedding once every two weeks with retention of 1-2 days, every patient in this group received UB23, SP6, and REN4 plus a supplemental point depending on symptoms of UB18, ST36 and UB20. Points were embedded with 3/0 catgut at 1.5 cm in length, a .35X50mm needle was used to acquire Da Qi then help thread the catgut into the points. This study used a group of childbearing normal cycled women as comparison.

Study #6 estradiol levels were markedly increased in both groups but the supplement group was more, at .14nmol/L in catgut and .19nmol/L compared to levels in normal childbearing women at .58nmol/L. Supplement group increased by .07 nmol/L were as catgut only increased by .02nmol/L.

In study #6, the quality of life scale was improved in both groups, general health, emotional role, metal health, was better in ACU group and Vitality was better than control group. Testosterone was increased in both groups and ACU group increased markedly more than the supplement group, FSH and LH decreased in both groups, osteocalcin and calcitonin was lowered in both groups but ACU groups had lowest levels, the PTH was much lower in both groups with ACU group slightly more than supplement group, and alkaline phosphatase was remarkably lowered in both groups.

Study #14: The effect of acupuncture on postmenopausal and reproductive hormones: a sham controlled clinic trial by Sunay, D., Ozdiken, M., Arslan, H., Seven, A. and Aral, Y. (2011) used 53 postmenopausal women split into two groups. The first group received needle acupuncture with .25mmX25mm needles inserted and manipulated to achieve Da qi sensation and retained for 20 minutes, the second groups received sham acupuncture with sham needles, a blunt needle with O ring taped to the skin to avoid puncturing the skin, the sham group also had 20 minute "retention" time. Both groups received twice a week treatments for 5 weeks for a total of 10 sessions, this study also notes that the acupuncturist had 6 years of experience.

Study #14 estradiol levels were increased significantly after the first treatment in ACU group and slightly more after the tenth treatment two weeks later. The sham group lowered estradiol slightly after the first treatment but it rebounded back up over base line by one point after the last treatment.

Study #14 also noted that the menopausal rating scale was reduced significantly in the ACU group over the sham group. FSH was lowered 5 points after the first treatment and 2 more points by the last treatment in the ACU group, and the sham group only lowered FSH by one point. The ACU group LH was lowered 4 points after the first treatment and stayed the same through to the last treatment, the LH in the sham group raised by one point over the treatments.

Study #15: (Acupuncture for treatment of climacteric syndrome – a repot of 35 cases by Xiaoming, S., Yuanhoa, D., Li, Y., Yang, X., Hong, Y., Guiru,H., Yongtie, G. and Xuemin, S., 2005) This study used 65 female patients with an average age of 48 with climacteric (menopausal)

syndrome divided into two groups, and acupuncture group and a medication group. The medication groups received oral oryzanol .01g-.02g, vitamin B1 .01-.02g and vitamin E .5-1g daily for 4 weeks, the second group received acupuncture according to their individual diagnosis: the first diagnosis type was kidney and liver Yin deficiency as the main cause with accompanied syndrome of Liver-fire or excesses heart fire. The second diagnosis was kidney Yang deficiency as the main cause with accompaniment of Spleen-yang or insufficiency of both heart and spleen yang. Both diagnosis received D16, D20, R6 and UB23. Then points were selected from the following according the patient condition; UB20, UB18, K3, ST36, PC6, LIV3, and Sishencong. The treatments were 6 days a week for four weeks, all treatments were 15-20 minutes long with two manipulations during the session, reinforcing / reducing needling was done on all points with UB23 only receiving reinforcing twisting of the needle.

For Study #15 the acupuncture groups had a total effective rate of 97.14%, with 14 cases cured, 16 with marked improvement, 6 with improvement and 1 failure. The supplement group had a total effective rate of 83.33% with 3 cured cases, 8 cases markedly improved, 14 cases improved and 5 cases failures. Estradiol increased markedly in the acupuncture group to 14.09 pg/ml compared with the supplement group of a 1.15 pg/ml decrease.

Study #16: (Electroacupuncture modulated reproductive hormone levels in patients with primary ovarian insufficiency: Results from a prospective observational study, by Zhou, K., Jiang, J., Wu, J. and Liu, Z.,2013) This study was a case study of 11 human female subjects with primary ovarian insufficiency. All subjects received EA treatments 5 times a week for 4 weeks and then 3 times a week every other day for 2 months. They received two point prescriptions rotated every other treatment; prescription one was bilateral UB23, and prescription two was

REN4, Bilateral ST25 and ST29, the treatments were 20 minutes long with EA applied at 20Hz with 1-4 mA intensity, the needles used were .45mmX125mm for UB23 and .30mmX40mm for REN4, ST25 and ST29.

Study #16 showed estradiol was increased by an average of 190.47 pmol/L over the base line of 33.35pmol/L, the level peaked at 232.82 pmol/L after treatment and then maintained that high level, and it only dropped slightly to 217.53 pmol/L at the 3 month follow-up tests. The FSH levels decreased by 43.71U/L on average and the level remained about the same through the rest of treatment with a follow up level of 39.8U/L. The LH levels deceased by 15.02U/L on average and maintained that low level through treatments and during the 3 month follow-up.

Study #17: (Acupuncture for hot flushes in perimenopausal and postmenopausal women: a randomized, sham controlled trial, by Kim, D., Jeong, J., Kim, K., Rho, J., Choi, M., Yoon, S., Choi, S., Kang, K., Ahn, H. and Lee, M., 2011) This study used 54 menopausal women with average age of 51, that were divided into two groups, true acupuncture and sham acupuncture points. The true acupuncture group points were PC6, HT7, HT8, LI4, ST36, SP6, REN4, the sham group received needles below SP9, above LI11 and 8 points around umbilicus, 2cm above and below. 3 and 6 cm lateral to umbilicus. Both groups received 20 minutes treatment two times a week for four weeks and then one time a week for three weeks, the needles used were .30mmX30mm and they were stimulate to achieve Da Qi.

In Study #17 the true ACU group had normal results with 18pg/ml increase in estradiol and the sham group had significant decrease in estradiol with a -21.2 pg/ml.

Study #20: (Menopause-related symptoms: traditional Chinese medicine vs hormone therapy, by Azizi, H., Liu, Y., Du, L., Wang, C., Bahrami-Taghanaki, H., Esmaily, H., Azizi, H. and Xue, X.,2011) This study used 57 peri/post menopausal women with average age of 48, they were split into 3 groups and received treatments for 2 months. The groups were as follows; A Chinese herbal medicine group (CHM) received the Chinese herbal formula Kun Bao Wan, 5g twice a day, the CHM-ACU group received herbal formula Kun Bao Wan, 5g twice a day plus acupuncture; this group received 10 sessions of acupuncture over the 2 month trail, using points used to tonify kidney and liver Yin and subdue raising Yang: UB23, UB15, K3, SP6, LIV3, LU7, K6, REN4, HT6, K7 and LI4. The last group received hormone replacement therapy (HRT), this was a combination of conjugated estrogen and medroxy progesterone acetate.

In study #20, estradiol increased in all groups, with increases of CHM group to 26.9pg/mL, HRT to 27.82pg/mL, and CHM+ACU to 20.42pg/mL. ACU+CHM had the lowest increase; CHM and HRT were almost the same. The Kupperman index was used to assess physical symptoms of patient; the Kupperman index score decreased in the CHM groups by 8.59, 11.13 in HRT and 14.55 in the CHM+ACU group.

Study #24: (Effect and safety evaluation of catgut implantation at acupoint for levels of bone metabolism and free radicals in postmenopausal women, by Chen, GZ., Xu, YX., Zhang, JW., Liu, SH. and Gou, ZY., 2010) This study used 65 female postmenopausal women split into two groups. Group one received catgut implantation at SP6, UB23, REN4 plus syndrome differentiation points once every two weeks, the second group received oral Fufuchun capsule once a day. This study showed that catgut had an effective rate of 93.9% and Fufuchun groups

had effective rate of 96.9%, but catgut groups had less adverse reaction than hormone Fufuchun group.

Study #26: (Effects of warm needle moxibustion on bone mass density and biochemical indexes of bone metabolism in patients of postmenopausal osteoporosis, by Zhao LH., Nong ZN., Zhong, X., Pang, Y., Liang, JS. Li, XD. and Ye, FW., 2008) This study used 40 postmenopausal women split into two groups. The first group used warm needle moxibusion (ACU) on UB11, UB18, UB23, ST36, GB34, every other day for three months, medication group received oral administration of caltrate with vitamin D2. This study showed an increase in BMD in ACU group but not the medication group, and an increase in estradiol in both groups. Osteocalcin and calcium/creatinine levels decrease in both groups.

Study #24 and study #26 manifested increase in estradiol levels. However, study #24 did not do as well as the herbal formula treatment. It is interesting that acupuncture did not perform as well as herbal medicine and as shown in study #20 where the combination of acupuncture and herbal medicine actually performed worse than the herbal medicine on its own.

Table 3 - Human Studies Measurement Results on Estradiol

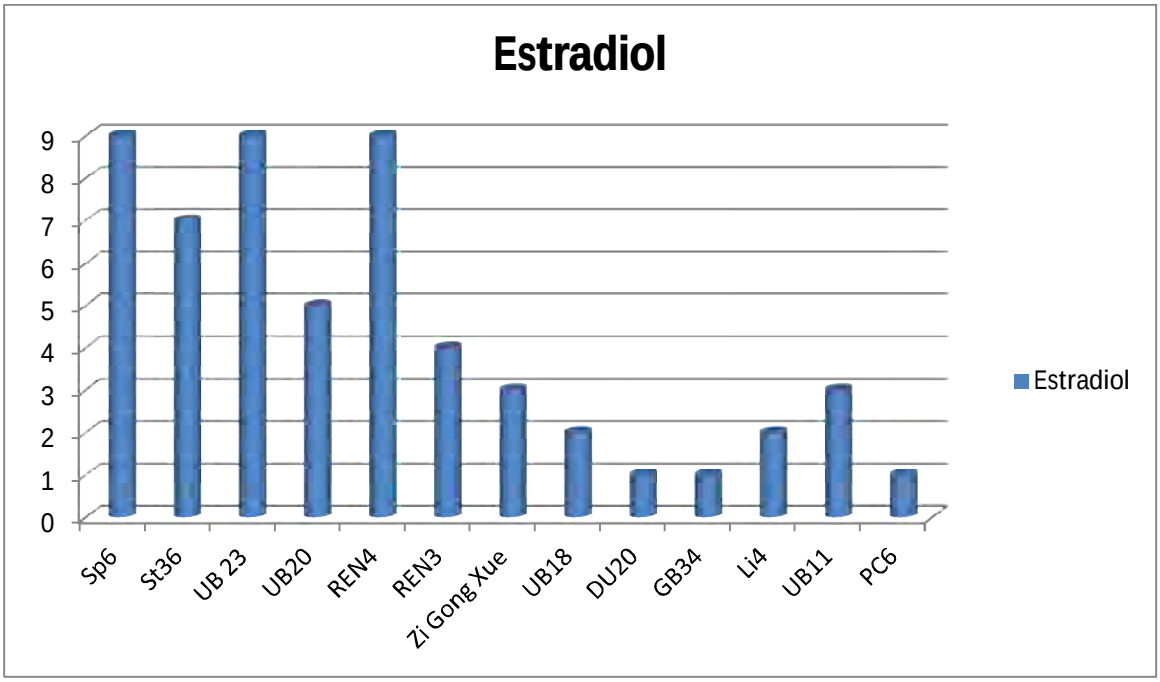
Study #	Model Normal childbearing	EA	sham	ACU	ACU Herbs	HRT	Catgut	Supplement
6 - before	.58						.12nmol/L	.12 nmol/L
after							.14nmol/L	.19 nmol/L
14 Before			17.9	14.2				
14 after 1st tx			16.9	20.9				
14 after last tx			18.8	22.9				

15 Before				38.14pg/ml				40.12
15 after				52.23				38.97
16 base line		33.35						
16 after tx		223.82						
16 3 month follow up		217.53						
17 before			52.0	34.9pg/ml				
17 mean change			-21.2	18.0				
20 before tx					55.58 pg/ml	14.72		49.23 herb
20 after tx					76.01	42.55		76.13
20 difference					20.42	27.83		26.9
24							↑but < herb	
26		↑ P< .01						

The following Table (Table 4 Treatment details of estradiol studies) shows the treatment details for estradiol studies. This table provides a breakdown of type of treatment used, points used, length of treatment and study.

Estradiol points. The following Graph 1, Points for estradiol, shows how many studies used which points for the study of estrdiol measurements. SP6, UB23 and REN4 are the most commonly used for estradiol studies.

Graph 1 - Points used for Estradiol



Follicular Stimulating Hormone

Follicular stimulating hormone (FSH) stimulates osteoclast through modulation of TNF-α. Studies have shown that maintaining a lower level of FSH keeps the expression of cytokines that influence osteoblast production in check; but higher numbers increase osteoclast and therefore increase bone breakdown. Studies show that if FSH levels are higher in pre-menopause women bones become fragile due to trabecular perforation, which affect the micro architecture rather than the bone mass and that make bones fragile (Colaianni, Cuscito, Colucci. 2013). Table 4 (FSH study results summary) shows which studies measured for FSH and their results.

Table 4 – FSH Study Results Summary

	Study #	Measurement	System studied	Subject type
Follicular stimulating hormone	6	Decreased	Repo & Bone	Human
	14	Decreased	Menopause	Human
	15	Decreased	Menopause	Human
	16	Decreased	Gynecology	Human
	17	Decreased	Menopause	Human
	20	Decreased	Menopause	Human
	21	Decreased	Gynecology	Human

This section will describe Table 5 – Measurement outcomes for FSH. All studies were done on human subjects.

Study #6 by Chen et al. (2010), which used catgut implantation and provera/supplement capsule, showed that both groups lowered FHS levels with catgut implantation slightly better than provera/supplement capsule group, -5.24 IU/L and -5.35 IU/L respectively.

Study #14 by Zhao et al. (2004), used true acupuncture verse a sham device acupuncture showed that both groups lowered FSH levels, but the true group was better than the sham group with a -6.50IU/mL compared to the sham at a -1.70 IU/mL

Study #15 by Xiaming et al. (2005), compared acupuncture group to a medication/supplement group, and showed a significant FSH decrease, -6.65 IU/mL in the ACU group compared to the supplement/control group which had an increase of 1.20 IU/mL.

Study #16 by Zhou et al. (2013), a case studies series on women with primary ovarien insufficiency, who had EA treatments, showed a significant decrease in FSH levels,- 43.73 IU/L and then only had a slight increase of 3.91 IU/L at the three month follow up, which kept the levels at a lower level.

Study #17 by Kim et al. 2011, used true and sham acupuncture where the true acupuncture group decreased FSH by -8.7mIU/L and sham group actually increased FSH by 3.5 mIU/L.

Study #20 by Azizi et al. (2011), decreased FSH levels in all groups, CHM by -8.84IU/L, CHM+Acu by -14.18IU/L and HRT had the greatest decrease with a -33.4IU/L. HRT was the best at lowering FSH in this study, but unlike in the estradiol measurements for Azizi et al. study number 20, CHM+Acu had greater effect in lowering FSH level than CHM alone.

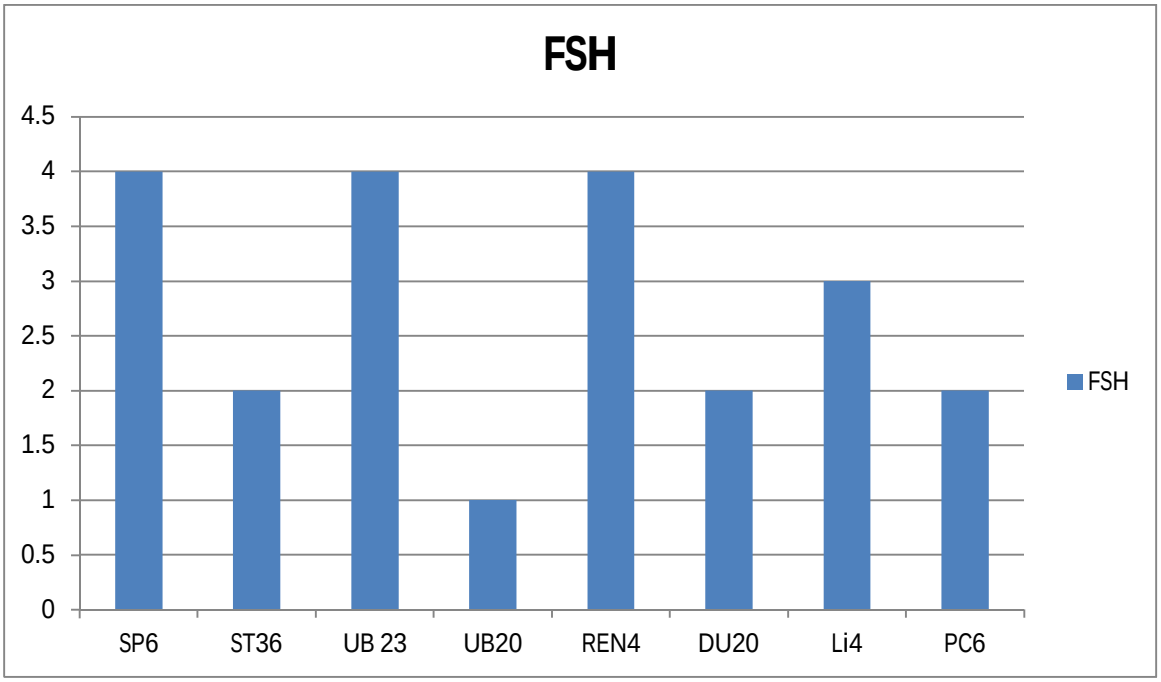
Study #21; True and sham acupuncture produced similar frequency of ovulation and improved LH to FSH in women with polycystic ovary syndrome (PCOS), by Pastore, L., Williams, C., Jenkins, J. and Patrie, J. (2011) used 84 PCOS women. They were divided into two groups; true acupuncture and sham acupuncture, first they received two treatments a week for four weeks and the once a week for 4 weeks for a total of 8 weeks and 12 sessions, the true acupuncture group received EA bilaterally on UB23, UB28, SP6, SP9, and manual acupuncture on PC6, SJ5 and D20, the sham group received a sham device placed on skin at points on extremities chosen to avoid standard acupuncture meridians and acupuncture points. This study showed FSH levels of the true ACU group decreased by -.40 mIU/mL and the sham device group went up by .3 mIU/mL.

Table 5 - Measurement outcomes for FSH – All Studies on Human Female

Study #	Model – Normal	Control	ACU	EA	Sham	Supplement	Catgut implantation
6 Before	3.90 IU/L					30.92 -HRT	30.03
After						25.57	24.79
14 Before			87.1 IU/mL		64.8		
after 1st treatment			82.7		63.0		
after last treatment			80.6		63.1		
15 Before			27.34			27.81 IU/L	
after			20.69			29.01	
16 Before				89.10 IU/L			
After				45.37			
Follow-up				49.28			
17 Before			65.8 mIU/mL		72.8		
Difference			-8.7		3.5		
20 Before		HRT 80.78 IU/L	+ Herbal formula 55.40			Herbal formula 55.93	
After		48.37	41.29			47.08	
Difference		-33.40	-14.18			-8.84	
21 Before				5.2mIU/L	5.5		
After				4.8	5.8		
Follow-up				4.7	5.7		

Treatment details for FSH. Graph 2 shows the points used for FSH studies and how many studies used them. The most common of these points were SP6, UB23, REN4 and LI4.

Graph 2 - Points used for FSH



Urinary Calcium

Urinary calcium is used to measure calcium in the serum. Excess calcium in the urine is a sign of excess bone resorption. When serum blood level is low the parathyroid will release parathyroid hormone that will pull calcium from the bones to bring the serum calcium levels back to normal in the body; this in the long run will contribute to osteoporosis. So having a high level of urinary calcium should be further investigated for other disease conditions that contribute to bone loss.

The following Table 6 shows the measurement outcomes of urinary calcium results, t outcomes of which will be discussed.

Study #3: (Preventative and therapeutic effects of acupuncture on bone mass density in osteopenic ovariectomized rats by Zhang, W., Kanehara, M., Ishida, T., Guo, Y., Wang, X., Li, G., Zhang, B., Kondo, H. and Tachi, S.,(2004) This study used 35 female rats that were divided into four groups, three of the groups were forced into menopause by having their ovaries removed these are the OVX groups. The groups broke down as follows; a control group that had the fat removed from their ovaries and had no treatment, an OVX-model group that had their ovaries removed but received no other treatment, OVX-ACU-A group that had bilateral acupuncture on SP6 and STt36, OVX-ACU B group had acupuncture on UB20 and UB23. The acupuncture groups received 15 minute of acupuncture, 5 days a week for 16 weeks and the needles were manually manipulated for 1 minute at the first and last minute of each treatment, using .22mmX.8mm needles.

In study #3, the calcium excretion was highest in the OVX-model group over the OVX-ACU groups and the control group at the midpoint of the study, by the end of the study both ACU groups were significantly lower in urinary calcium excretion than both the control and OVX-model group, with OVX-ACU-B group slightly lower than OVX-ACU-A group.

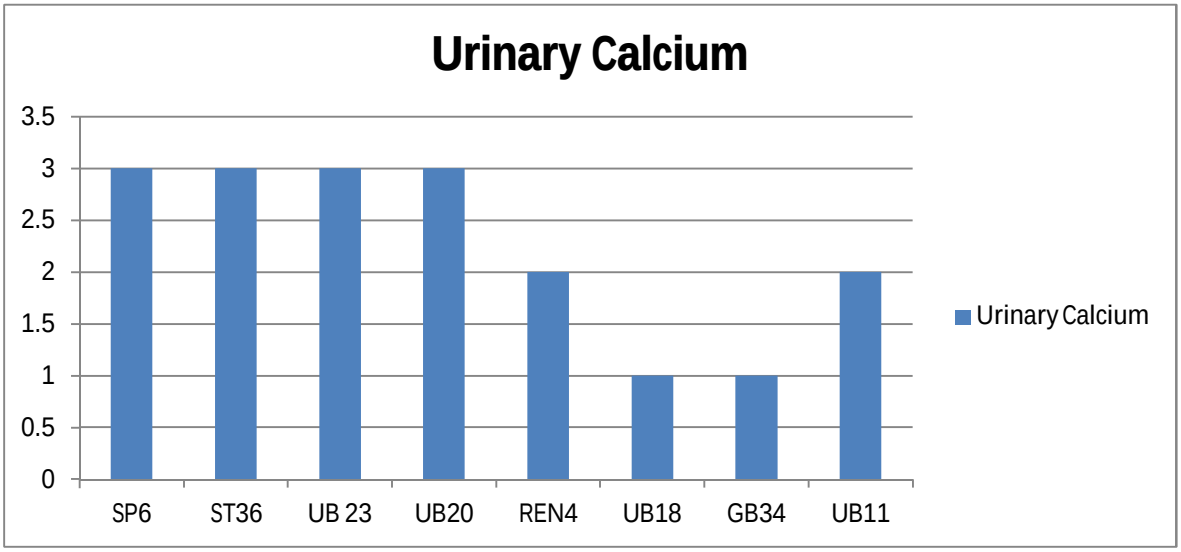
In studies #25 by Bao et al. (2012), which compared EA at different point selection, and study #26 by Zhoa et al. (2008), that compared supplement to warm needling moxa, both showed that the needling groups had a greater decrease in urinary calcium excretion compared to the model.

Table 6 - Urinary Calcium Measurements

Study #	Sham Model	OVX control	OVX –ACU A	OVX – ACU B	EA	ACU
3 after 11 week	3.8	5	4	4.2		
After 15 week	3.5	4	2.7	2.5		
25					↓ compared to model	
26						↓P<.05

Treatment details for Urinary Calcium. Graph 3 shows the points used for urinary calcium studies. The most common points are SP6, ST36, UB23, UB20 and UB11.

Graph 3 - Urinary Calcium Points



Osteocalcin

Osteocalcin is a biomarker for bone formation. This biomarker shows that osteoblasts are active. Osteocalcin is a marker for the new osteocyte formation, so a lower level means lower bone formation. This section will discuss the detail of Table 7 measurement outcome for osteocalcin. Study results showed a decrease in osteocalcin over the OVX model and an increase over normal controls groups.

Study #2: (B-Blockers and other analogous treatments that affect bone mass and sympathetic nerve activity in ovariectomized rats, by Zhang, W., Kanehara, M., Zhang, Y., Wang, X. and Ishida, T.,2007) This study used 43 female rats that were divided into five groups. The first group had a sham operation (Sham) were the fat was removed from their ovaries and received no other treatment for a control. The other four groups had their ovaries removed and were divided as follows: a Model group (OVX-Mod) that received no treatments; Propranolol group (OVX-Pro) that received 2.8 mg/day of propranolol hydrochloride; a needling group (OVX-N) that received bilateral needling 15 minutes a at SP6 and PC6, with manual manipulation of about 2 Hz (120 turns/minute) using .22mm by 10mm needles; and a Ethonol Fructus Citri Sarcodactylis group (OVX-Fcs) that receive an ethanol 0.9 g/kg extract of that herb. All treatments were given 5 days a week with weekends off for 36 weeks. This study showed that acupuncture increased osteocalcin over the OVX-Pro estrogen therapy and OVX-Fcs herbal medicine groups, but was lower than OVX-model group and higher than sham control group.

Study #11: (The more efficacious acupoints of zusanli and sanyinjiao than that of

non-acupoints on bone mass in osteopenic ovariectomized rats, by Zhang, W., Kanehara, M., Zhang, Y., Yu, Z., Zhang, G., Yang, Y., Sun, Y., Zhang, J. and Ishida, T., 2005). This study used 40 female rats split into five groups. Four of the groups received ovarianectomies and the fifth group had a sham operation of fat removal from around the ovaries and received no other treatments. The OVX-model group received no treatment. The last three groups all received acupuncture treatments for 15 minutes with reinforcing/reducing balancing method, using .22mmx10mm needles, five times a week for 23 weeks; OVX N-A received acupuncture on ST36 and SP6, OVX N-B received needling to the opposite side of ST36 and SP6. OVX- N-C group received needling at ST36 and SP6 with the needles made to touch the bone.

The results in study #11 showed that OVX-N-A, the true acupuncture group which used the appropriate needling depth, was more effective in raising osteocalcin, than the OVX-N-B, off channel group and the OVX-N-C, true acupuncture that touched the bone group. The OVX-N-C group had the lowest osteocalcin levels of the OVX groups and was closest to normal sham control group.

Study #25 by Bao et al. (2012), that compared different acupuncture points selection showed a decrease in osteocalcin over the model group with the study sating a P-value of $P<.01$, as did study 30 by Ma el al. (2005), which compared acupuncture to HRT.

In the human subject groups, osteocalcin had similar result as animal subjects with a lower level over OVX model groups. Study 6 by Chen el al., 2010 using showed that acupuncture actually slowed the decline of osteocalcin; the HRT group had a decrease of 1.55ng/mL whereas catgut implantation had a 1.34 ng/mL decrease. Study 26 using warm

needle moxa treatments by Zhao et al. (2008), showed a decline on both OVX model and ACU groups with the study author stating a P-value of p<.01.

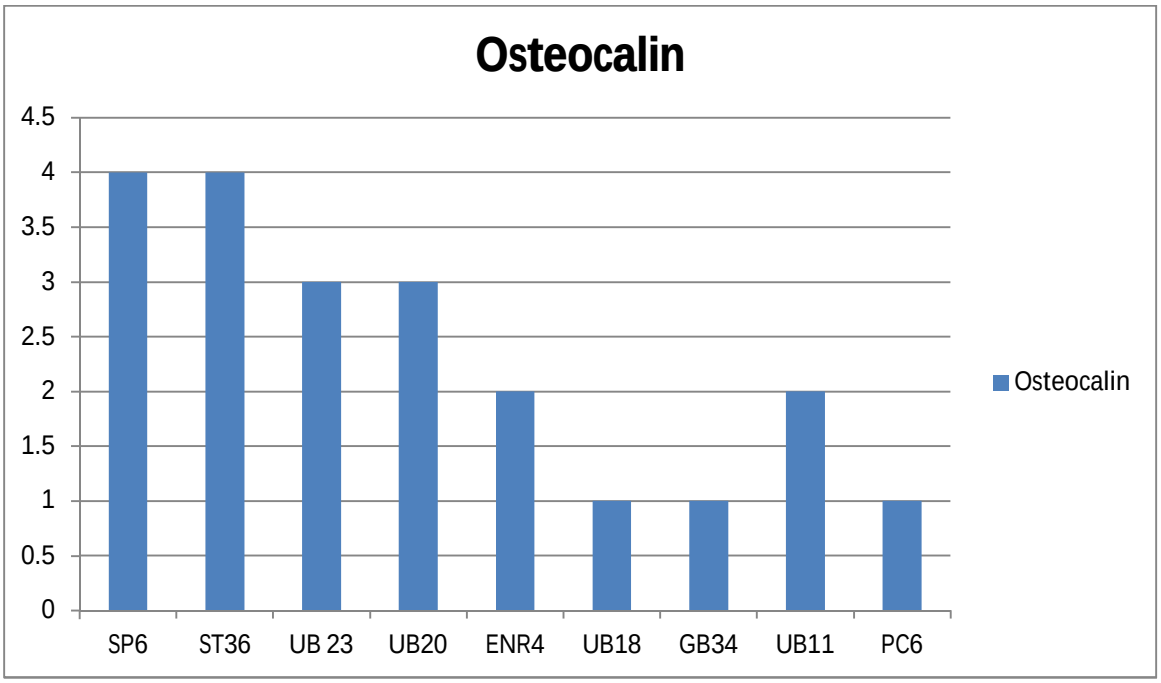
Table 7 – Measurement Outcomes for Osteocalcin

Study #	**Control Normal**	**Model OVX**	**OVX ACU-A**	**OVX- ACU-**	**OVX- ACU-C**
11* - animal	.6	.7	.75	.65	.62
	Control Normal	**Model OVX**	**HRT - estrogen**	**Herb**	**ACU**
2 –animal	200ng/ml	451	350	349	390
	Normal	**Supplement**	**Catgut Implant.**	**ACU**	
25 – animal				EA ↓ P<.01 over model	
30 – animal			↓P<.01		
6 – human Before		10.12 ng/mL	9.41		
After		8.57	8.07		
26 human		↓P<.01		↓P<.01	

* Estimate from graph

Points for osteocalcin. Graph 4 below shows the points used for osteocalcin. Points used are the same power houses of SP6, ST36, with new points of UB11 and UB20. UB11 is the meeting point for the bones and the point of sea of blood. Then UB20 is the back shu for spleen helpful or post heaven Qi, nutrient assimilation and digestion of bone building materials.

Graph 4 -Points for Osteocalcin



TNF-α

Tumor necrosis factor alfa modulates osteoclast activity by stimulating existing osteoclast. TNF-α activates existing osteoclast minimally on its own, but with RANKL (defined in glossary). It has a synergistic effect that increases activation of new and existing osteoclast. Levels of TNF-α are produced in excess in post menopause, that coupled with a decrease in estrogen, which down-regulates RANKL induced osteoclast differentiation (Shevde, Bendixen, Dienger, Pike., 2000). Therefore, bone resorption is increased. TNF-α can also inhibit the expression of IGF-1. IGF-I recruits pre-osteoblast cells to the remodeling surface of the bones (Takeda, 2005). TNF-α up-regulates osteoclast resorption and inhibits osteoblast formation. By lowering TNF-α balance can be restored in bone health.

This section will discuss the measurement outcomes shown on Table 8 for TNF-α which is shown below.

Study #8: (Effect of Electro-acupuncture on tumor necroses factor-α and vascular endothelial growth factor in peripheral blood and joint synovial of patients with rheumatoid arthritis, by Ouyang, B., Gao, J., Che, J., Zhang, Y., Li, J., Yang, H., Hu, T., Yang, M., Wu, Y. and Ji L.,(2011) This study used a total of 63, male and female human subjects with an average age of 49. Patients were divided into two groups, an EA group and regular needling acupuncture group, both groups treatments were received every other day for 10 times a course, and 3 courses in total were given, for a total of 2 months of treatments, they used needles sized .25mmX25mm or .30mmX40mm, and needle were retained for 30 minutes. Each group received the following treatments; 3-5 points were selected from the following support the Yang points including Du20, LI11, SJ6, ENR4, ST36, GB34, GB39, SP6, UB23, UB20 ect. Local Ashi points were selected each time as well for both groups. The EA group received 15 minutes of electro stimulation to these local points with continuous wave from and intensity to the patient comfortably. Then patients were switched to prone position and points needled on dorsal side of the body. The EA groups received EA on UB 20 and UB23 for 15 minutes. The simple needling (SN) group received thrusting, lifting and twisting method to stimulate Da Qi, with needle retention for 30 minutes after Da qi is achieved.

Study #8 showed blood levels of TNF-α were significantly lowered in both groups but the blood level was lower in the EA group -7.53 ng/L over the simple needling group -5.89 ng/L. There was no difference between the two groups in the synovial measurements, but both had significantly lowered TNF-α, EA at -6.87ng/L and ACU at -6.39 ng/L.

Study #12: (Electroacupuncture at acupoint ST36 reduces inflammation and regulated immune activity in collagen-induced arthritic mice, by Yim, Y., Lee, H., Hong, K., Kim, Y., Lee, B., Son, C. and Kim, J.,2006). This study used 50 male rats that had arthritis induced through collagen injections. They were then divided into 5 groups; normal, model, needle retention, EAI and EAII. Normal group had nothing done to them. The control group had injection and was held in needling device but had no needles. Needle retention group had ST36 needle, but no EA was applied. EAI and EAII had ST36 needled for 15 minutes 3 times a week. Electrical-stim was applied to the needle at 2 Hz of continuous wave with intensity of 6-7mA. The second electrode was attached to ST41 area but not needled. The EAI treatments lasted for five weeks and EAII treatments lasted for nine weeks.

Study #12 reduced TNF-α over the OVX-model in all ACU and EA groups but only EA lowered it over the normal group. OVX-model group was highest at 1380 pg/ml. Regular acupuncture of ST36 was lower than OVX-model at 1250 pg/ml but higher than the normal group which level was 750 pg/ml. EA groups were the lowest with EA1 525 pg/ml and EA2 at 450pg/ml. Both EA groups needled ST36 at 2 Hz, EA2 at nine weeks in length had better lowering results than EA1, which was only five weeks in treatment length.

Study #23: (Effects of some acupoints (DU-14, II-11, ST-36 and SP-6) on serum TNF-α and hsCRP levels in healthy young subjects, by Karatay, S., Akcay, F., Yildirim, K., Erdem, F. and Alp, F.,2011). This study used 90 healthy human subjects with a combination male and female between the ages of 20-30. They were divided into five point groups: each point was one group, DU14, LI11, ST36, SP6, and a sham point, 1.5 cun lateral to UB56. Each group was

needled in their point three time a week for 30 minutes, for 2 weeks, for a total of 6 sessions. No manipulations of the needle were done.

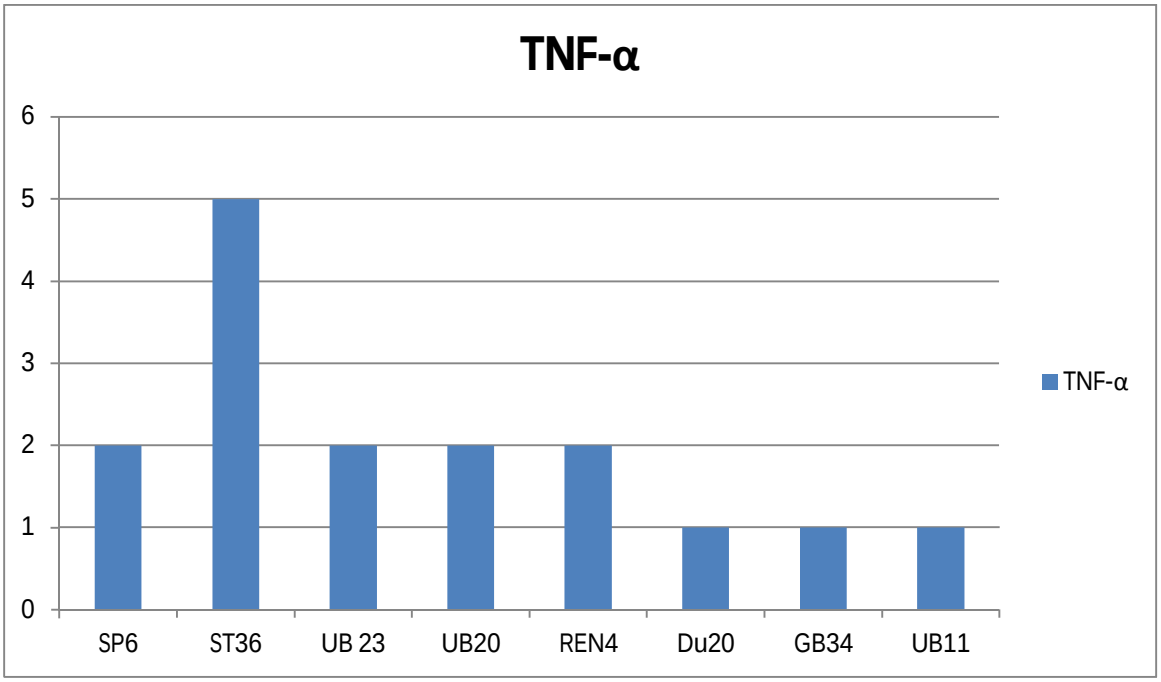
Study #23 measured the difference between single points on TNF-α levels using regular acupuncture. ST36 group increased TNF-α level by 2.06 pg/ml. ACU group LI11 had the greatest decrease at -2.57 pg/ml. ACU group SP6 decreased by a -2.43. ACU group DU14 decreased by -1.74, and the Sham ACU point, which was an off channel point, increase by 1.73 pg/ml.

Table 8 – Measurement outcomes for TNF-α

Study #	Blood EA	Blood ACU	Synoviol EA	Synovial ACU	
8 – Before	146.32 ng/L	147.02	149.76	148.81	
After	139.25	141.26	142.75	142.19	
Difference	-7.53	-5.89	-6.87	-6.39	
	Normal	Control	ACU	EA 1	EA2
12	750pg/ml	1380	1250	525	450
	ACU – D14	ACU –LI11	ACU – ST36	ACU– SP6	ACU- Sham pt.
23 Change after treatments	-1.74pg/ml	-2.57	2.06	-2.43	1.73
25	EA ↓ over model P<.01				

Points for TNF-α. Graph 5 below shows the most common used points for studies on TNF-α. ST36 is used the most followed by the favorites SP6, UB23, UB20 and REN4.

Graph 5 -TNF-alpha Points



Deoxypyridinoline

Deoxypyridinoline (Dpd) is a biomarker for bone resorption. High levels of Dpd indicates higher breakdown of bone. A 24 hour urine sample can tell the amount of bone degraded in a day (Bilezikian et al. 1996).

Table 9 displays the measurement outcomes for Dpd shown below and will be discussed in this section. Study results for Dpd showed acupuncture decreased levels of Dpd over OVX model groups but did not lower it to the level of the normal control groups.

In study #2, Zhang et al. (2010), showed that Dpd levels in the OVX treatment groups decreased significantly, the HRT group was the lowest at 50 nmol/mmol, and the herb and EA

groups were the same at 60nmol/mmol, and OVX model was at 95nmol/mmol and normal control group was 40 nmol/mmol.

Study #3 done by Zhang et al. in 2004, showed that the ACU-A a group which needled ST36 and SP6 lowered Dpd level to 650 compared to OVX model and OVX-ACU-B groups level of 780n mol, OVX-ACU-B group needled UB20 an UB23. None of the OVX groups were as low as the normal control group at a level of 450n mol at week 11 of treatments. The normal control, OVX-ACU-A and OVX-ACU-B groups continued to drop and at four weeks laterOVX- ACU-B had a significant decrease to 500n mol, while OVX-ACU-A dropped to 520n mol and the normal control dropped to 300n mol, the OVX-model group increased to 850 n mol.

Study #11 showed significant decrease in OVX-ACU-A to 35nμ/mm compared to the OVX model of 45 nμ/mm but not as low as normal control at 20nμ/mm. OVX-ACU-A group was normal needling of ST36 and SP6. OVX-ACU-B was needled off the channel and opposite of ST36 and SP6, lowered slightly to 40nμ/mm, while OVX-ACU-C group, which needled ST36 and SP6 but deep enough to touching the bone, increased to 50nμ/mm. (Zhang et al., 2005)

Study #25 which compared different point combination had a decrease of $p<.01$ in Dpd in EA compared to model. (Bao et al., 2012)

Table 9 - Deoxypyridinoline Measurements Outcome Results

Study #	Normal Control	OVX Model	OVX – ACU-A	OVX – ACU-B	OVX – ACU-C
3* – At week 11	450n mol	780	650	780	
3* At week 15	300	850	520	500	
11*	20nμ/mm	45	35	40	50
	Control normal	Model OVX	HRT – estrogen	Herb	ACU
2	40nmol/mmol	95	50	60	60
25	EA ↓ compared to model P<.01				

*estimate from graph

Bone Specific Alkaline-phosphate (ALP) measures the level of bone formation, it is a byproduct of osteoblast; higher level shows increase in bone building. The following is a description of the Table 10; ALP measurement results.

Study #4 compared no treatment to EA at ST36 and SP6 and Zhou et al. (2012) study showed an increase in APL in EA group over both OVX model and normal Control, EA was 120 U/I, the model OVX was 100 U/I and the normal control was 90U/I.

Study #6: (The effect of acupoint cat-embedding on the quality of life, reproductive endocrine and bone metabolism of postmenopausal women, Chen et al., 2010). This study showed catgut implantation had a greater decrease than the normal control supplement group

but both groups had a decrease after starting treatment. Catgut 13.13 KU before and 9.73KU after with a 3.40KU decrease. Supplement 12.07 before and 9.66 after with a 2.40 decrease.

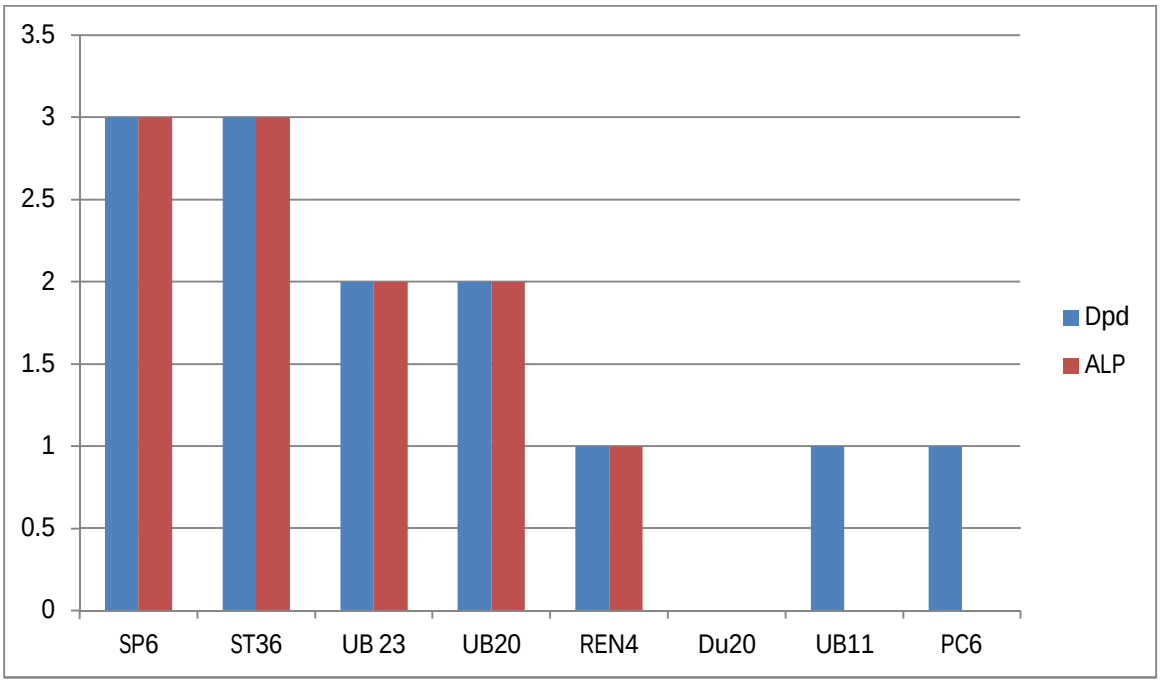
In study #25, Bao et al. (2012), showed that ALP had a significant decrease over model p<.01 and in study 30,Maet al., 2008, showed that the ACU group had the same decreasing effect as HRT group over the OVX-model.

Table 10 - Alkaline-Phosphate Measurement Results

Study #	Control Normal	OVX Model	OVX - EA	
4	90 U/l	100	120	
	Control Normal	Model OVX	OVX – Acu A	OVX – Acu B
6 Before	12.06 KU	13.13		
After	9.66	9.73		
25	EA↓ over model P<.01			
30	Acu and HRT both ↓ over model no significant diff between two.			

Points for Dpd and ALP. Points used that affected Dpd and ALP are among the usual points SP6 and ST36 being most studied followed by UB23 and UB20. These data are shown in the following Graph 6. REN 4 was studied as well, a point commonly used in Estrogen studies. The following graph shows these results.

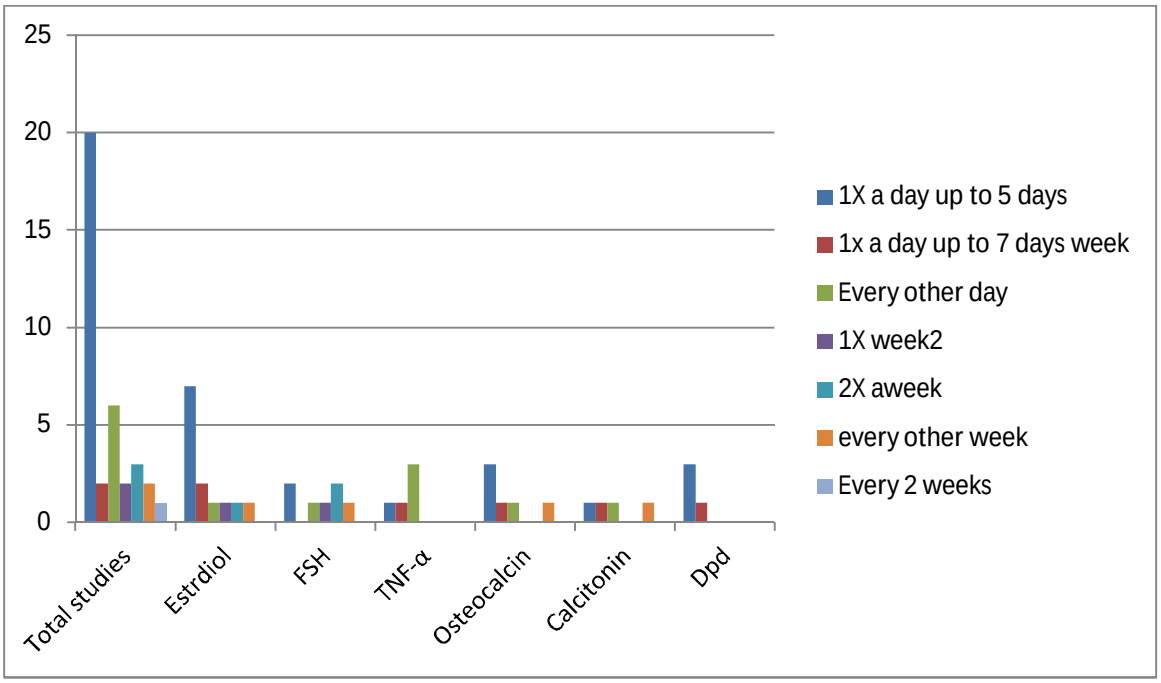
Graph 6 Dpd and ALP points used



Treatment Detail Results for Biochemicals

Treatment frequency for Biochemicals. The following graph shows the treatment frequency for Bio chemical studies. The treatment of one time a week for 5 days with two days off is the most commonly used method for the studies reviewed. TNF-α did every other day treatments. This observation follows the trend for all studies reviewed where one time a day for 5 day was the most used, followed by every other day. The length of the studies was average of 8 weeks. The following Graph 7 displays these results.

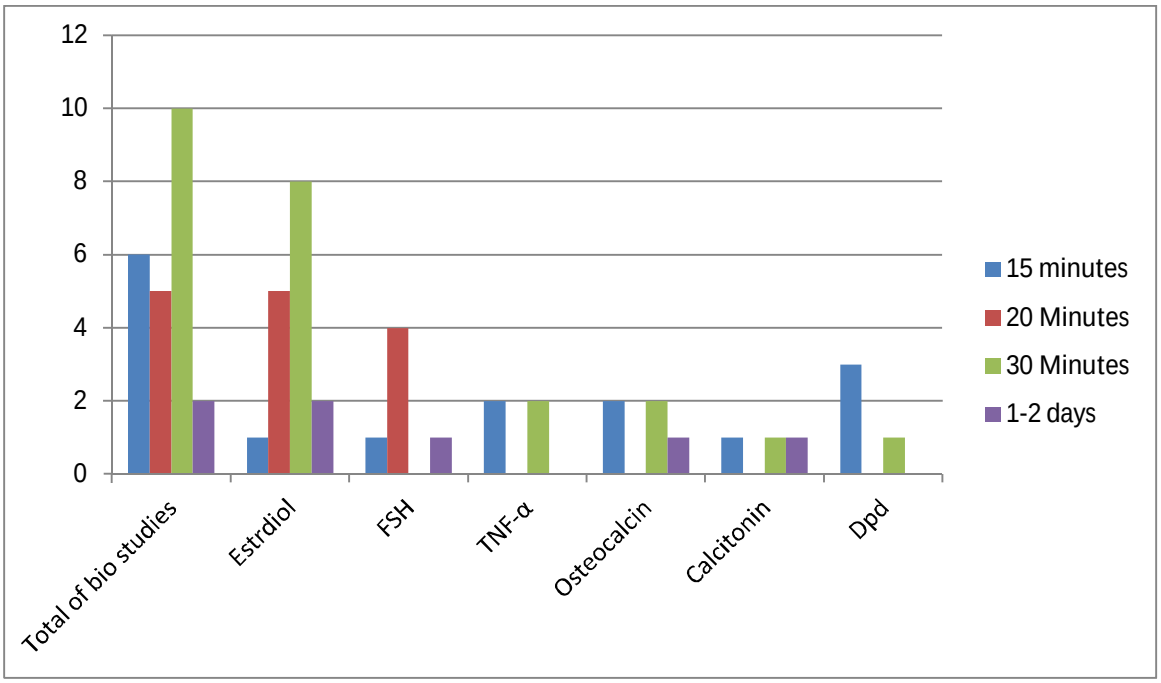
Graph 7 - Treatment Frequency Biochemicals



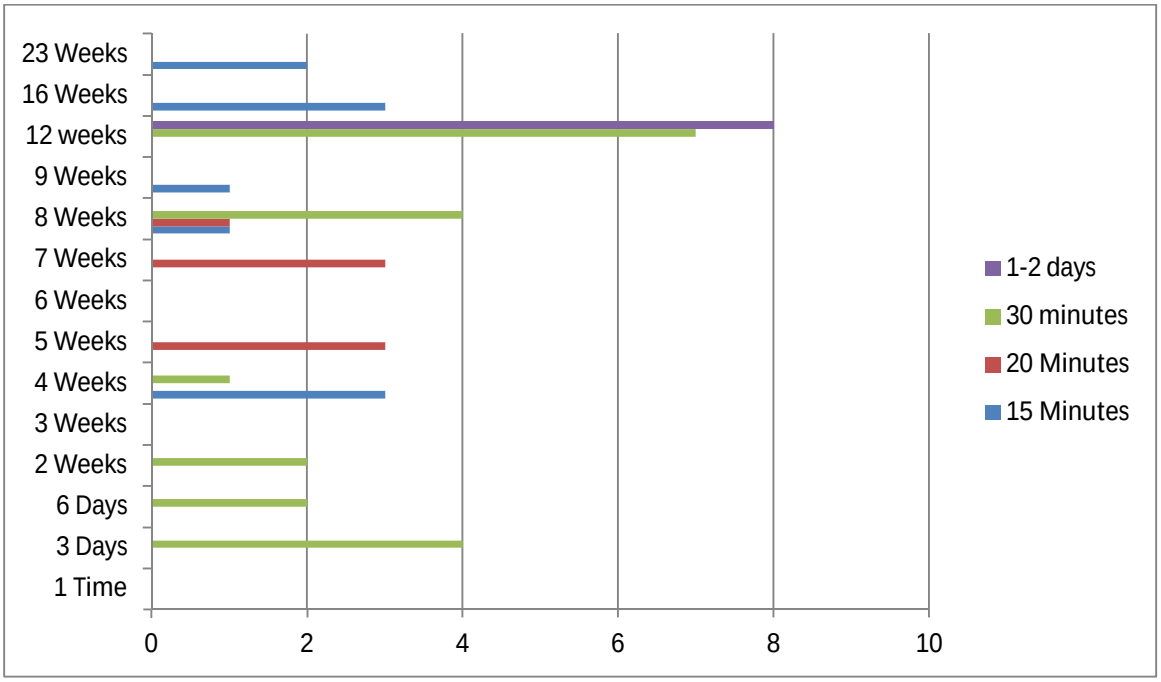
Treatment duration of Biochemicals

Biochemical needle retention time and study length. Needle retention time for bio chemical studies is shown in the following Graph 8. Of the studies that reported retention times 43% had a retention time of 30 minutes; 26% had a retention time of 15 minutes; and 21% had a retention time of 20 minutes. Estradiol favored 30 minute retention times at 50%, followed by 20 minutes at 31% of reported studies. Dpd favored a 15 minute retention time. Graph 9 shows bio-chemical treatment length and needle retention time. Needle retention time was 30 minutes for 66% of the studies. The studies in bio-chemicals used a 12 week study length for 50% of the studies, followed by 20% using an 8 week study length.

Graph 8 - Needle Retention Time for Biochemical Studies



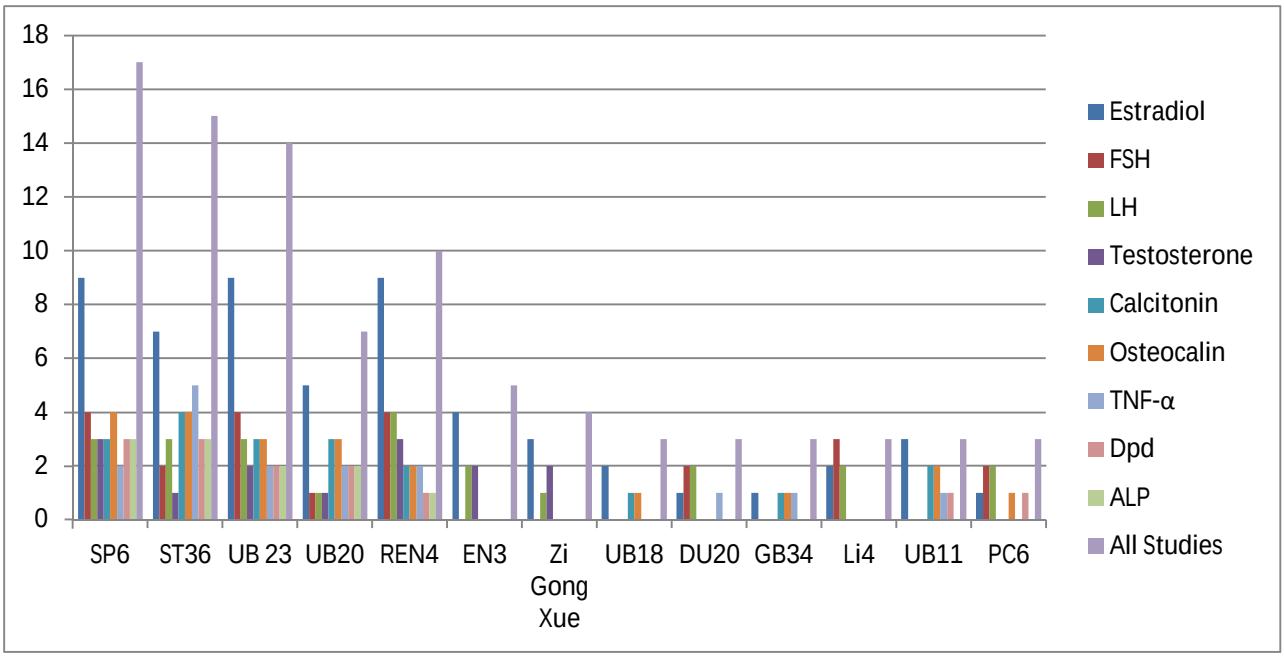
Graph 9 - Bio-Chemical needle retention time and length of treatment.



Points used for Biochemicals

Points used for all biochemicals are shown in the following Graph 10. The trend is SP6, ST36, UB23, UB20, REN4, REN3 and Zigongxue. These points are the most common used for biochemical studies as well as all studies. The graph shows the number of studies that used a certain point and then broken down by biochemicals.

Graph 10 - Points Used Biochemical



Bone Mass Density, Formation and Strength

This section will look at the strength and structure of the bones. This is important to determine fracture risk and assess the building structure and health of the bones. First we will look at bone mass density (BMD). Bone mass density is a measurement of the density of bones at a specific site and is used to determine the strength of bones and predict fracture risk. BMD measurements are taken at the lumbar region, the calcaneous, tibia, and femur. Then we will investigate the structure of the bones by researching the mineralizing apposition, formation rate, energy to failure rate, trabecular thickness, separation and number.

Bone Mass Density – Lumbar. This section will explain the results also shown in Table 11 BMD – Lumbar.

Study #1 by Feng et al. (2008), on ovariectomized osteoporosis rats measured the BMD with DEXA scan for small animals on Lumbar 1-6 and right thigh. The BMD in the OVX-model, Estrogen (OVX-E) and OVX-EA groups were all lower than the normal intact group, but compared to the OVX-model, the OVX-E group increased BMD on right thigh slightly more than OVX-EA and OVX-EA increased BMD of Lumbar 1-6 slightly more than OVX-E group. When compared to the normal intact and sham groups' numbers, the treatment groups; OVX-E and OVX-EA were very close in levels, but the OVX-model group was significantly lower than the normal intact and OVX - treatment groups.

In study #2, Zhang et al. (2007), showed an increase in BMD in all treatment groups over the OVX model group, with the OVX-ACU group having the greatest increase of 17 mg/cm^2over the course of the study, but the Herb Fructus Citri Sarcodactylis OVX group wasn't

far behind the OVX-ACU group with an increase of 12mg/cm^2, and the OVX propranol group only had a 3 mg/cm^2 increase. None of the groups had as good of an increase as the normal control group, showing an increase of 32mg/cm^2 over the course of the study trial.

Study #3, by Zhang et al. (2004), showed an increase in BMD in both treatment groups over the model but not over the control. The OVX-ACU –A group, needled ST36 and SP6, did not increase BMD as much as the OVX-ACU-B, with a needling of UB23 and UB20, after the first 11 weeks of treatment but OVX-ACU-A did kept the BMD from declining sharply by the fifteenth week as the OVX-ACU-B group did. OVX-ACU-A group increased 31 points by week 11 and only dropped 3 points by week 15, whereas OVX-ACU-B group increase 34.34 points by week 11 only to drop 12.37 points by week 15. OVX-ACU-A and the OVX-model group were the same at week 11 with BMD of 155, but then OVX-ACU- A maintained its increase, whereas as the OVX-model and OVX-ACU-B group both dropped to similar level of 144.88 for OVX-model and 148.13 for the OVX-ACU-B group.

Study #11 had three OVX needling groups, ACU-A true needling of ST36 and SP6, ACU-B off the channel opposite side of ST36 and SP6, and ACU-C group which needling ST36 and SP6 but touched the bone. Zhang Et al. (2005), showed in this study that the normal control group increased to 180 mg/cm^2 at the midpoint of study and remained the same through to the end of the study. The ACU-A and ACU-C groups both had increases at week 28, with ACU-A significantly higher than ACU-C, at 150 mg/cm^2 and 140 mg/cm^2 for ACU-C, but then both dropped to 130 mg/cm^2 at week 35, whereas ACU-B declined steadily to 125 mg/cm^2 by week 35, but was still higher than the model OVX group, which declined to 120 mg/cm^2. This study showed the ACU-A, with needling of ST36 and SP6 had the greatest effect on the BMD.

In study #4, Zhou et al. (2012) showed that the OVX-EA group, with a needling of ST 36 and SP6, had a great effect on BMD compared to the model OVX group. The control normal group had a BMD of .179 g/cm^2, and OVX-EA group BMD was .155g/cm^2 and the OVX- odel group was .123 g/cm^2 at the end of the study.

Table 11 – Measurement results for BMD - Lumbar

Study # Lumbar	Control Normal	Model OVX	ACU	Herb	HRT Estrogen
1	.25 g/cm^2	.12	.125 - EA		.13
2 before	138 mg/cm^2	138	138	138	138
2 after	170 mg/cm^2	138	155	150	141 - propranolol
	Control Normal	OVX Model	OVX – ACU-A	OVX – ACU-B	OVX – ACU- C
3 – start	124.84	123.75	124.88	126.16	
3 – Week 11 TX	178.57	155.3	155.78	160.5	
3 – week 15 TX	184.2	144.88	152.59	148.13	
11 – At week 10 start	140 mg/cm^2	140	140	140	140
11 – At week 28	180	130	150	130	140
11 – At week 35	180	120	130	125	130
4	.179 g/cm^2	.123	.155 - EA		

BMD – Tibia. This section will explain the results in Table 12 BMD – Tibia.

Study #2 (The effects of B-blocker and acupuncture on BMD and sympathetic nervous system of ovariectomized rats, by Zhang et al.,2007). This study showed an increase in tibia BMD that continued from week 7, at the beginning, to week 36, the end of the study in all groups. The OVX-ACU group had the greatest increase of the OVX groups with a 19 mg/cm^2 increase by the 36th week but not significantly higher than the OVX model group at week 24. All OVX groups had about the same BMD of tibia at week 24 with the OVX-propranolol group at the lowest level at 138 mg/cm^2, the OVX-ACU and OVX-herb group were only slightly higher at 141 and 142 mg/cm^2, respectively compared with the OVX-model group at 140 mg/cm^2. All OVX groups were significantly lower than the normal control group which increased to 158 mg/cm^2. This study by Zhang et al,. 2007 showed that acupuncture the acupuncture had the greatest effect on the BMD of the tibia over herb and medication groups.

In study #3, Zhang et al. (2004) compared Acupuncture needling at ST36 and SP6, OVX-ACU-A group to needling of UB20 and UB23 of OVX-ACU-B., and showed all groups had significant increase in BMD of the tibia but OVX-ACU-A group had the greatest increase over the treatment groups with 49.25 increase from start to finish and was closest to the normal control groups' increase of 59.9. The BMD of the OVX-ACU-A group and the normal control group were close at a 166.94 for control and 160.6 for the OVX-ACU-A group, whereas the OVX-model group and OVX-ACU-B group were close with 152.12 for the OVX-model and 155.13 for the OVX-AUC-B group.

Table 12 – Measurement results for BMD-Tibia

Study # Tibia	Control Normal	Model OVX	ACU	Herb	HRT Estrogen
2 At 7 weeks	133 mg/cm^2	133	133	133	133
2 At 24 weeks	153 mg/cm^2	140	141	142	138
2 At 36 weeks	158 mg/cm^2	141	152	149	148
	Control Normal	Model OVX	OVX – ACU-A	OVX – ACU-B	OVX – ACU-C
3 – At the start	107.04	109.03	111.35	110.73	
3 – At week 11	153.47	141	146.58	144.38	
3 – At week 15	166.94	152.12	160.6	155.13	

BMD – Femur. This section will explain the results shown in Table 13 BMD- Femur.

In study #1, Feng et al. (2008), compared the effects EA to HRT on IGF-1 on BMD in ovariectomized rats and as stated earlier the OVX-model, estrogen (OVX-E) and OVX-EA groups BMD were all lower than the normal control intact, but compared to the OVX-model group, the OVX-E group increased BMD on right thigh slightly more than OVX-EA, though not as high as normal control group which was at .249 g/cm^2, the OVX-E and OVX-EA groups were very similar

in numbers at .137 g/cm^2 and .13 g/cm^2 respectively, but they were both higher than the OVX model group which was at .28g/cm^2.

Study #2, Zhang et al. (2007), looked at the effect of B-blockers and acupuncture on BMD and found that the OVX groups were lower than the normal control groups significantly by 20 to 25 mg/cm^2. The OVX-model, propranolol, and herbal groups all had about the same BMD of the femur of a 180 mg/cm^2 by the end of the study, at week 36. The acupuncture group's BMD was higher than all the OVX groups at week 36 with a 185 mg/cm^2.

In study #3, Zhang et al., 2004, showed that OVX-ACU-A groups needling of SP6 and ST36 had the greatest effect on the femur BMD with a 193.85 g/cm^2 at the end of the 15 week treatments. OVX-ACU-B group increased the BMD of the femur to 189.02 g/cm^2 which was higher than the OVX-model group at 186.33 g/cm^2, but none of the OVX groups were as high as the normal control group at 220.23 g/cm^2. The increase from each groups base line to end of the 15 week study showed that the OVX-ACU-A and OVX-ACU-B groups were very close with OVS-ACU-A having a 44.85 g/cm^2 increase and OVS-ACU-B having a 44.48 g.cm^2 increase, the OVX- model increased to 43.23 g/cm^2, and the normal control groups increase was 75.36 g/cm^2.

Study #4, Zhou et al. (2012), compared OVX-EA, needling of ST36 and SP6, to the OVX-model group and normal control group. The OVX-EA group increased the BMD of the femur to .229 g/cm^2 over OVX-model group at .224 g/cm^2 but under the control group which was .232 g/cm^2.

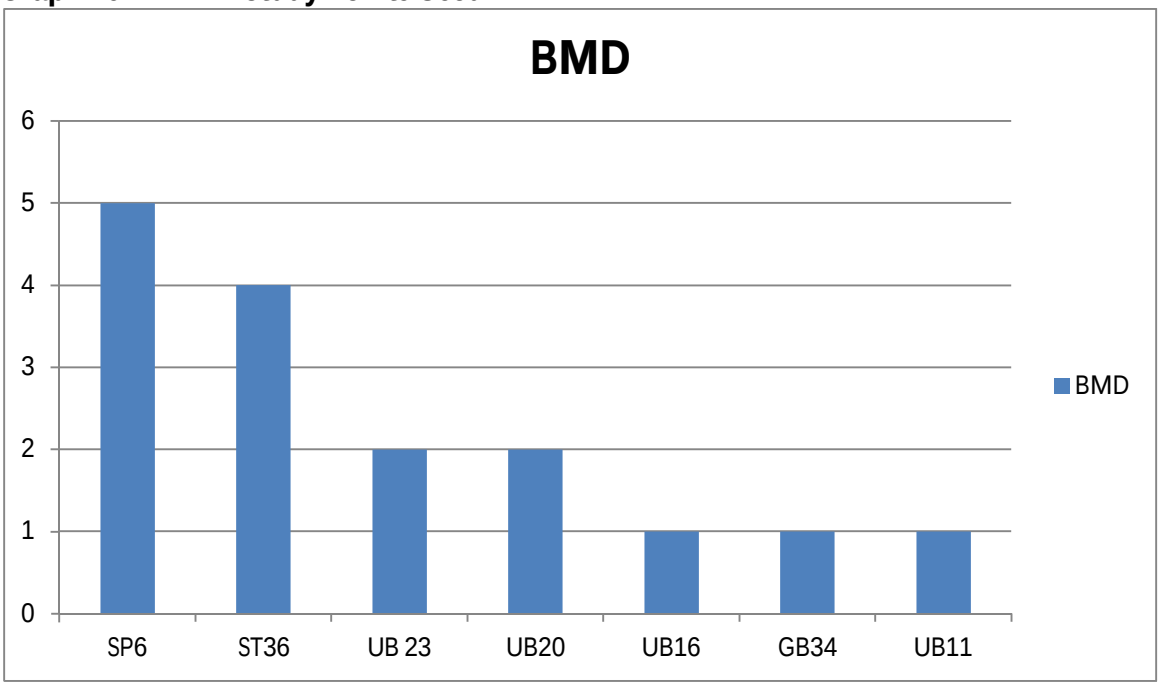
Table 13 – Measurement results for BMD Femur

Study # Femur	Control Normal	OVX Model	ACU	Herb	HRT Estrogen
1	.249 g/cm^2	.128	.13 - EA		.137
2 – Start at 7 weeks	168 mg/cm^2	168	168	168	168
2 At 24 weeks	190 mg/cm^2	165	174	174	165
2 At 36 weeks	200 mg/cm^2	180	185	180	180
	Control Normal	OVX Model	OVX – ACU-A	OVX – ACU-B	OVX – EA
3 At start	144.87	143.1	149	144.54	
3 At week 11	204.57	185.04	188.25	183.47	
3 At week 15	220.23	186.33	193.85	189.02	
4	.232 g/cm^2	.224			.229

BMD Study Points

The points most commonly used for BMD studies SP6, ST36 followed by UB23 and UB20 shown in Graph 10.1. Frequency of treatment as shown in Graph 12 was once a day five times a week for at least 8 weeks or more.

Graph 10.1 - BMD Study Points Used



Mineralization and Bone Formation. Bone mineralization is the process of osteoblast adding to the bone matrix by adding minerals and can be used to estimate bone mass. Mineral apposition rate (MAR) is the rate at which the new minerals are added to the trabecular surface. Bone formation rate (BFR) is how much of the bone surface is being rebuilt (Bilezikian et al., 1996). This lets us know how active the osteoblasts are and how many are active. All MAR results measurements numbers are in μm/d and BFR results measurement numbers are in $\mu m^3/\mu m^2/d$. The following narrative in this section will explain the results shown in Table 14 Mineral Apposition rate and Table 15 Bone formation rate. This section will discuss the measurement outcomes shown is Table 14 mineral apposition rate and Table 15 bone formation rate.

Study #2: (B-Blockers and other analogous treatments that affect bone mass and sympathetic nerve activity in ovariectomized rats, by Zhang et al., 2007). This study showed an increase in both the MAR and BFR in the treatment groups over both the OVX-model and the normal control. The acupuncture group had the highest BFR at 3.7 but had lowest MAR at 3.1 of the treatments groups. Whereas the Propranolol groups had the highest MAR at 5.3 and only slightly lower BFR than the acupuncture group at 3.4 and same as the herb group which was at 3.3 BFR. The OVX-model group was the lowest of the OVX groups at 3.2 MAR and 23. BFR but still higher than the normal control at 1.9 Mar and 1.1 BFR. This study appears to show that OVX causes an increase in MAR and BFR as OVX-model and OVX-0ACu group had the same numbers, the herbal group was slightly better and the propranolol had a significant increase.

In study #3, Zhang et al. (2004) compared OVX-ACU-A group, needling of Sp6 and St 36 to OVX-ACU-B group, needling of UB20 and UB23. OVX-ACU-B group had the highest MAR and BFR over all groups with MAR of 10 and BFR of 7. OVX-ACU-A group was higher than the OVX-model but lower than OVX-ACU-B with a MAR of 7 and a BFR of 3.7. The OVX-model group had a MAR of 3.9 and BFR of 1, and normal control group had lowest of all groups with MAR of 2 and BFR <1. This study by Zhang et al. (2004) on the effects of acupuncture on BMD showed that acupuncture had a greater effect over BMD than no treatment OVX model group and the normal control group.

Study #11, by Zhang et al. (2005) compared types of treatment true ST36 and Sp6 ACU-A group, sham off channel ACU -B group and hitting the bone at ST36 and SP6 ACU -C groups. ACU -A and ACU -B had increase in MAR and BFR over control and model. ACU -A had the greatest results with a 6 Mar and 4.5 BFR. ACU -C had the lowest numbers with a 3 MAR and

1.5 BFR which was lower than the normal control group with 3.5 MAR and 2 BFR. ACU -C group was better than model and control but only slightly with MAR of 3.9 and BFR 2.5. The model had MAR of 3 and BFR of 2.1.

Study #29: (The changes in histomorphometric and mechanical properties of femurs induced by acupuncture at Shenshu point in the SAMP6 mouse model of senile osteoporosis, by Zhang et al., 2009). This study showed that regular consistent daily needling of UB23 in the OVX-ACU group had a 14.7% BFR and 9.9% MAR increase over the OVX-model.

Table 14 - Mineralizing Apposition Rate Study Results

Study # Mineral Apposition rate	Control Normal	OVX Model	ACU	Herb	HRT Propranolol
2	1.9 µm/d	3.2	3.1	3.9	5.3
	Control Normal	OVX Model	OVX – ACU-A	OVX – ACU-B	OVX – EA
3	2 µm/d	3.9	7	10	
11*	3.5 µm/d	3	6	3.9	3
29			Increase 9.9% over model		

*Estimate from graph

Table 15 - Formation Rate Study Results

Study # Formation rate	Control Normal	OVX Model	ACU	Herb	HRT Propranolol
2	1.1 $\mu m^3/\mu m^2/d$	2.3	3.7	3.3	3.4
	Control Normal	OVX Model	OVX – ACU-A	OVX – ACU-B	OVX – EA
3	>1 $\mu m^3/\mu m^2/d$	1	3.2	3.9	
11*	2 µm/d	2.1	4.5 Acu-A	2.5 –Acu-B	1.5 –Acu-C
29			14.7% increase over model		

*Estimate from graph

Energy to Failure

This section will report the results on studies that recorded the amount of energy it takes for the bone to fail or break as displayed in Table 16.

Study #4: (Electroacupuncture prevents ovariectomy-induced osteoporosis in rats: a randomized controlled trial, by Zhou, J., Chen, S., Gou, H., Xia, L., Lui, H., Qin, Y. and He, C. in 2007) This study used 30 female rats, these were divided into 3 groups; a sham operation control group (sham), an ovariectomy group with no other treatment (OVX-Model), and an ovariectomy group treated with 3Hz and 1mA of intensity of electroacupuncture bilaterally on

ST36 and SP6 for 30 minutes a day, 5 days a week for 12 weeks (OVX-EA). This study used .25mm diameter and .25mm length needles inserted 3-5 mm for ST36 and 2-3mm for SP6.

Zhou et al. (2007) then tested both the Lumbar 5 and femur for their energy to failure number. The OVX-EA group had a greater effect on the lumbar with a .039N compared to the femur .03N, OVX-AE was stronger than the model for both the lumbar and the femur but significantly lower than the normal control, the OVX-model lumbar and femur was .028N and the femur was .035N, the normal control failure number on the lumbar was .05N and .035N on the femur. This showed that acupuncture increase the failure number over OVX-model but still wasn't as good as the normal group.

Study #10: (Effect of electroacupuncture on the healing process of tibia fracture in a rat model: a randomized control trial done by Nakajima, M., Inoue, Motohiro, M., Hojo, T., Inoue, N., Tanaka, K., Takatori, R., Itoi, M., 2009) This study used 30 male rats split into three groups. All rats had osteotomies causing fractures to the tibia. The first group was the control group and had no treatment. The second group received EA of 50 Hz at 20uA daily for three weeks, and the third group received needling but no electro stimulation daily for three weeks. The needles were placed above the fracture site and 15mm proximal to the site for both needling groups using .24mm-10mm needles. All rats were then observed for an additional three weeks after treatment.

In study #10, the EA group had significant change in total area of the callus and bone of the tibia from the x-rays. The Control and sham groups increased, but were not different from each other in amount. At 4 weeks the EA group had significant improvement over the other

two groups. At six week the three groups were different by three points, but the EA group had the best improvement in growth at 35.66 compared to the ACU group at 32.6 and control group was the lowest at 29.72. The ACU group had the most improvement from week 4 to week 6 whereas the EA group had less but the EA group had a greater increase week 0 to 4 then leveled off and still had the greatest improvement over all of the three groups. The longitudinal and transverse diameter increased in all groups but EA had a higher increase, and the control and ACU groups were about the same with EA only slightly better.

Study #10 EA had the significant increase in energy it took to make the bone fail with a 16.54N compared to the acupuncture group at 7.13N and both treatment groups were higher than the normal control group at 6.67 N.

Study #11: (The more efficacious acupoints of zusanli and sanyinjiao than that of non-acupoints on bone mass in osteopenic ovariectomized rats done by Zhang et al.,2005). This study showed a higher energy to failure in the OVX-N-A group that needled ST36 andSP6 at the correct depth than the other treatment groups.

Study #29: (Changes in histomorphometric and mechanical properties of femurs induced by acupuncture at Shenshu point in the SAMP6 mouse model of senile osteoporosis were both higher that control and sham groups by Zhang, X., Peng, Y., Yu, C., Cheng, H., Liu, L.and Han, J.,2009) This study used male mice divided into three groups. A control group received no treatment. A sham group received two needles on the hypochondria, and ACU group that received needling at UB23. The treatment groups were treated daily for eight

weeks. This study showed that the ACU group improved the energy to failure over the other two groups.

Table 16 – Measurement results for Energy to Failure Studies

Energy to failure Study #	Control Normal	OVX Model	OVX – ACU A	OVX – ACU B	OVX – EA
4* – The Femur	.035 N	.028	.03		
4 *– The Lumbar 5	.05 N	.028	.039		
10	6.67 N		7.13		16.54
11			Higher than OVX		
29			Higher than Control and sham groups		

*Estimate form graph

Trabecular Separation, Number and Thickness.

Trabecular number, thickness and separation help to determine the strength and stability of the bone. Increase in thickness and trabesular number indicates a stronger bone while an increase in trabecular separation means there are larger gaps between the trabeculae, causing weakness in the bones. This section will look at the results of studies that reported on Trabecular micro architecture.

Trabecular Separation. The following section explains Table 17 – Trabecular separation. All number measurements are μm.

Study #2, Zhang et al. (2007), compared OVX- model, OVX-ACU, OVX-Herbal and OVX-Propranolol. The study showed that the treatment groups all had a decrease in Tb.Sp over model OVX group which was at 520.2, with the Propranolol group the lowest at 441 followed by herb group at 481.9 and the acupuncture group at 490.5. The normal control groups was at 331.5

Study #4: (Electroacupuncture prevents ovariectomy-induced osteoporosis in rats: a randomized controlled trial, by Zhang et al.,2012) This study showed the EA group lowered Tb.Sp. to 229.70 which was lower than the OVX-model group at 337.27. Both of the OVX groups where higher than the normal control group which was 157.84.

Study #11 compared different needling points on OVX rats, OVX-ACU-A needled ST36 and SP6, OVX-ACU-B needled off channel on the opposite side of ST36 andSP6 and OVX-ACU-C that needled ST36 and SP6 but was inserted seep enough to hit the bone. The results showed that the Tb.Sp. of all the OVX groups where higher than the normal control group which was at 350 μm. The OVX-ACU-A and OVX-AUC-B were similar at 410 μm and 420 μm respectively which were lower than the OVX-model group which was at 550μm, and OVX-ACU-C was higher than all the groups at 900μm.

In study #3, Zhou et al. (2004), compared the needling of St36 and SP6, OVX-ACU-A, to UB20 and UB23, OVX-ACU-Band found that the Tb.Sp of OVX-ACU-A was the lowest at 140μm

and OVX-ACU-B was highest at 160μm, which was even higher than the OVX-model which was at 152μm., But all the OVX groups were higher than the normal control which was 100μm.

Table 17 - Trabecular Separation

Tb. Sp. Study #	Control Normal	OVX Model	ACU	Herb	HRT propranolol
2	331.5 μm	520.2	490.5	481.9	441
	Control Normal	OVX Model	OVX – ACU-A	OVX – ACU-B	OVX – ACU-C
4	157.84 μm	337.27	229.70		
11 *	350 μm	550	410	420	900
3 *	100 μm	152	140 -EA	160	

*Estimate form graph

Trabecular Thickness and Number. This section will report results from Table 18 – Trabecular thickness (Tb.Th.) and Table 19 Trabecualr number (Tb.N.). All measurements for Tb.Th are in μm and Fro Tb.N. are in No/mm

Study #2 showed an increase in the Zhang et al. (2007), treatment groups for Tb.Th. and Tb.N. over the OVX-model abut all were less than the control. The Tb.Th. for OVX-EA group was 34.5 with a Tb.N. of 3.1, the highest of the treatment groups. OVX-Herb group was the next best with a Tb.Th. number of 37 and Tb. N. of 2.9, the propranolol group had the lowest numbers of the three treatment groups with a Tb.Th. of 31.6 and Tb.N. of 2.7, followed by the OVX- model group at a Tb. Th. of 23.2 and Tb. N. of only 1.1. Showing EA helped build a stronger bone.

In study #4, Zhou et al. (2012), it was found that OVX-EA had a significant increasing Tb.Th over both OVX-model and control group with a 77.39μm compared to OVX-model group with a 59.88μm and the control normal group of 62.29μm, these results were not carried to Tb.N. wear the OVX-EA group was better than the OVX-model at 3.31μm over 2.54μm but less than the control group which was at 4.57μm.

Study #11, Zhang et al. (2005), found that all the Tb.Th in the treatment groups were higher than the OVX-model which was at 31μm. The Tb.Th of the OVX-ACU-A group was less that of the control group at 34μm and OVX-ACU-B was the same as the control group at 36μm, OVX-ACU-C was highest of all groups at 52μm but it was the lowest in Tb.N at 1μm. Tb.N of OVX-ACU-A and OVX-ACU-B was higher than the OVX-model but less than the control with OVX-ACU-A at 2.48μm, OVX-ACU-B at 2μm, the OVX-model at 1.59μm and the control at 3μm.

Study #3, Zhang et al. (2004), found that OVX-ACU-B group with back points UB20 and UB23 had the highest Tb.Th of the OVX groups at 75 μm. OVX-ACU-A had Tb.Th of 65μm which was higher than the model at 50μm. But none of the OVX groups were higher than the control at 130μm. But for Tb.N the OVX-ACU-A group at 4.2μm was highest of all groups. OVX-ACU-B was very close to the control group at 3.5μm and control was 3.55μm. The OVX-model was close to the OVX-ACU-A group at 4μm.

Table 18 - Trabecular Thickness

Study # Tb. Th	Control Normal	OVX Model	ACU	Herb	HRT Estrogen
2	36.8 µm	23.2	34.5	37	31.6
	Control Normal	OVX Model	OVX – ACU A	OVX – ACU B	OVX – ACU C
4	62.29 µm	59.88	77.39 - EA		
11*	36 µm	31	34	36	52
3 *	130 µm	50	65	75	

*Estimate from graph

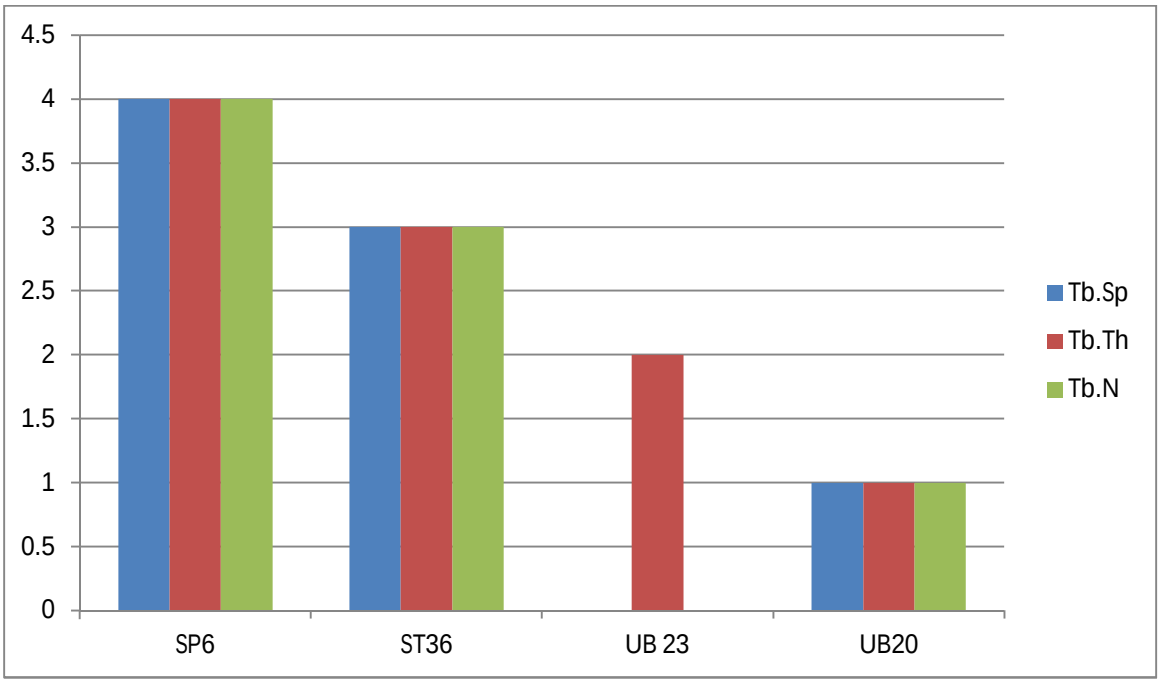
Table 19 - Trabecular Number

Study # Tb.N.	Control Normal	OVX Model	ACU	Herb	HRT Estrogen
2	3.7 No./mm	1.1	3.1	2.9	2.7
	Control Normal	OVX Model	OVX – ACU-A	OVX – ACU-B	OVX – ACU-C
4	4.57 No./mm	2.54	3.31- EA		
11*	3 No./mm	1.59	2.48	2	1.2
3*	3.55 No./mm	4	4.2	3.5	

*Estimate from graph

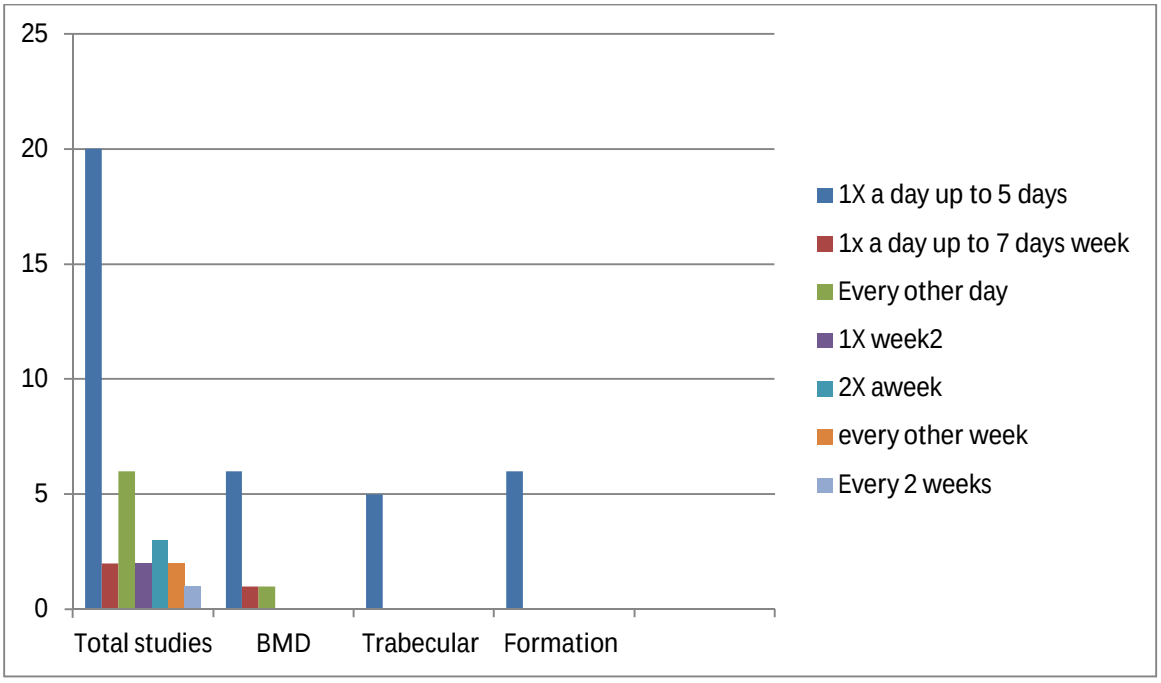
Points for Trabecular studies. The primary points studied for trabecular measurements were SP6, ST36 and UB23. (Graph 11)

Graph 11 -Points Used for Trabecular Measurements Studies

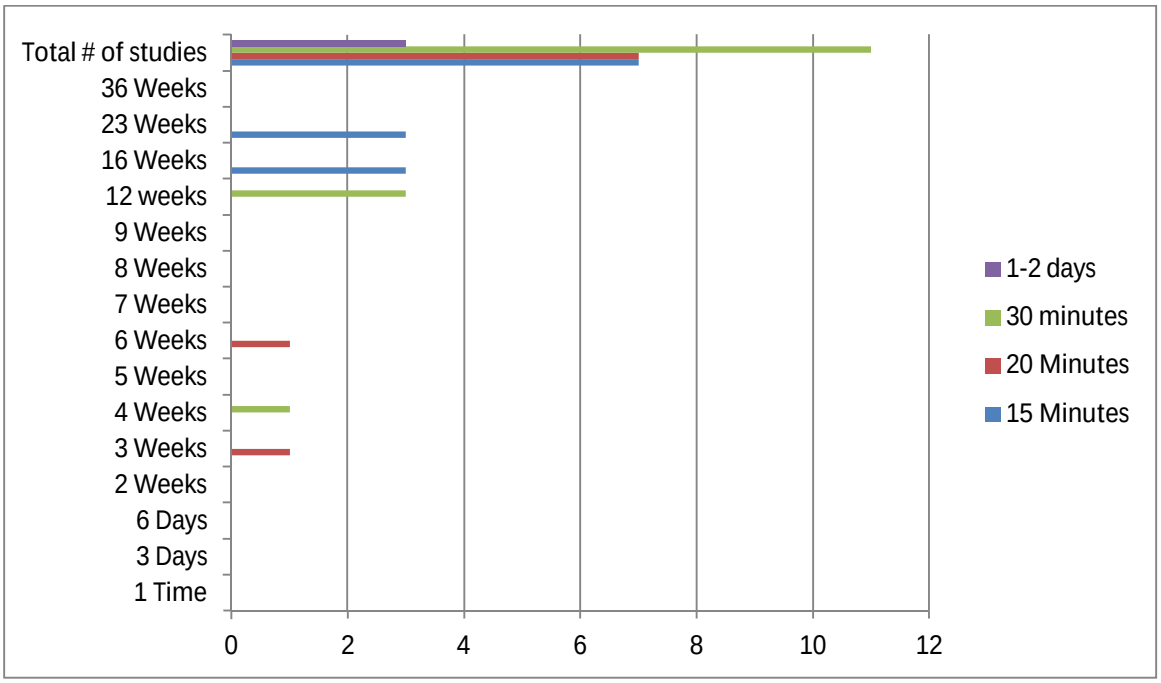


Treatments for Bone Formation and Density. The frequency of treatment for BMD and bone formation studies follows the same patter as biochemical with frequent, once a day treatment, five times a week followed (see Graph 12). The next common course was every other day treatments. Most of the study lengths were 8 weeks or 12 weeks (see Graph 13). And duration of needle retention was mostly 30 minutes. Points used most were ST36, SP6, UB20, UB23 and Ren4 as shown in Graph 14. These graphs are followed by chart 26 treatment details for studies on BMD, bone formation and trabecular studies.

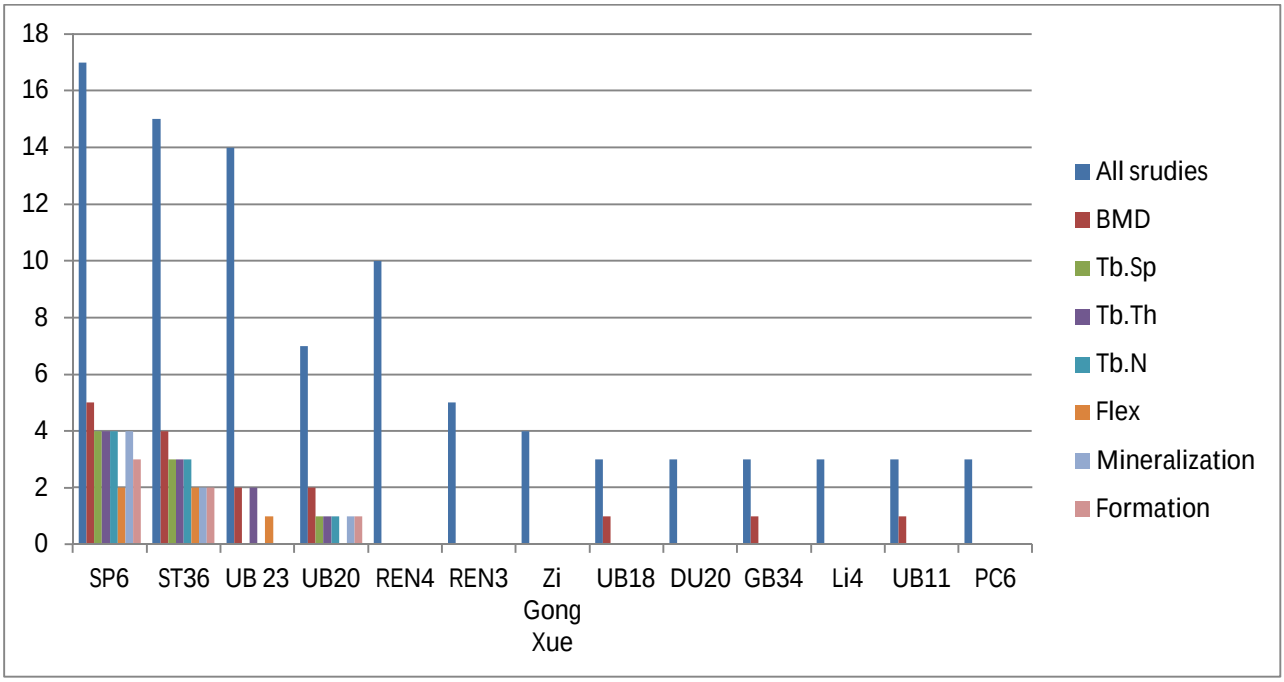
Graph 12 - Frequency of Treatments for Bone Formation and Density



Graph 13 Needle retention time and treatment lengths for all bone formation and BMD studies



Graph 14 - Points Used Bone formation, BMD

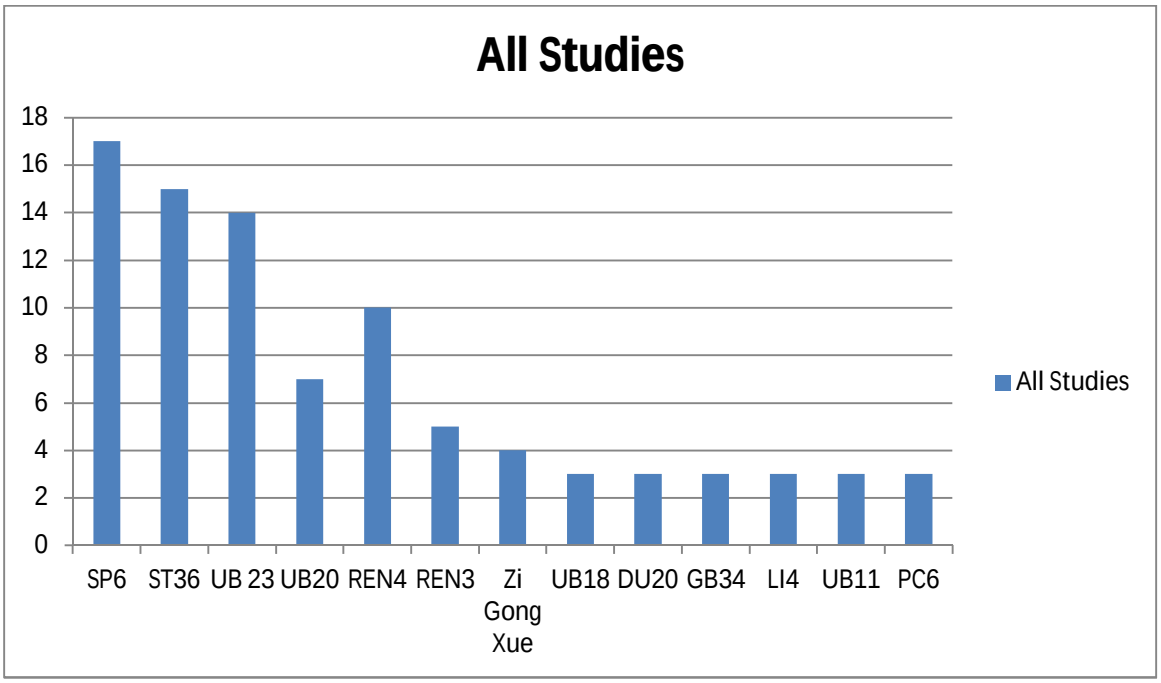


Treatment Results for All Studies

This section will look at treatment results for all studies. Points used to determine which points are being studied. Then the frequency of treatments, how long the needles were retained and how long the treatment study were. This analysis will provide data regarding how often and how effective treatments need to be, as well as what type of needling was done.

Points for all studies. The points that were studied the most shown in Graph 15 all studies points. Most of the studies used SP6 and ST36 at 56% and 50% of the studies using them. With 46% of the studied used UB23 and at 33% usage are UB20 and REN4. REN3 and Zigongxue were also used frequently. Graph 15 also shows that UB18, DU20, GB34, LI4 UB11 and PC6 were noted used a few times as well.

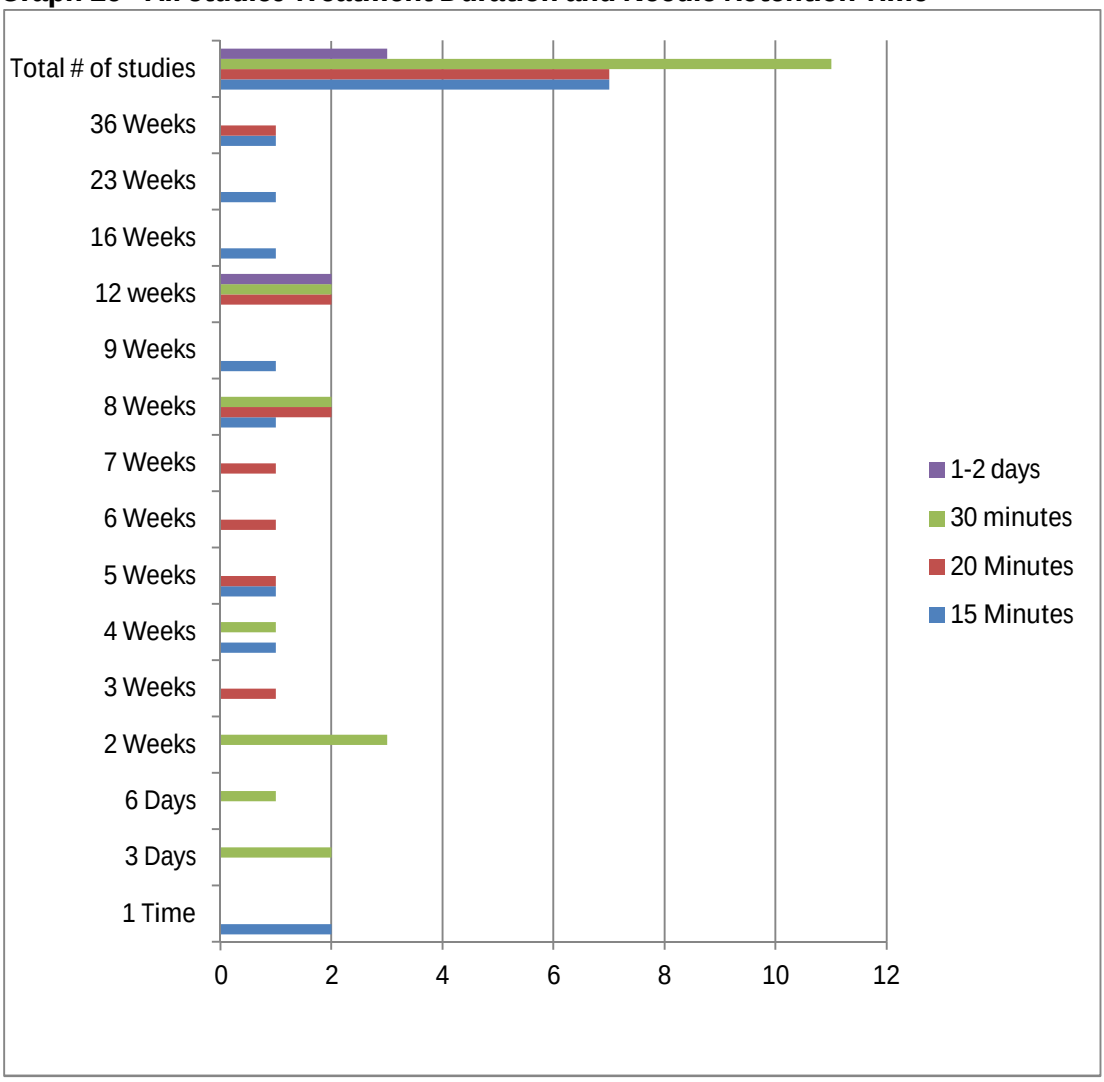
Graph 15 - Points for All Studies



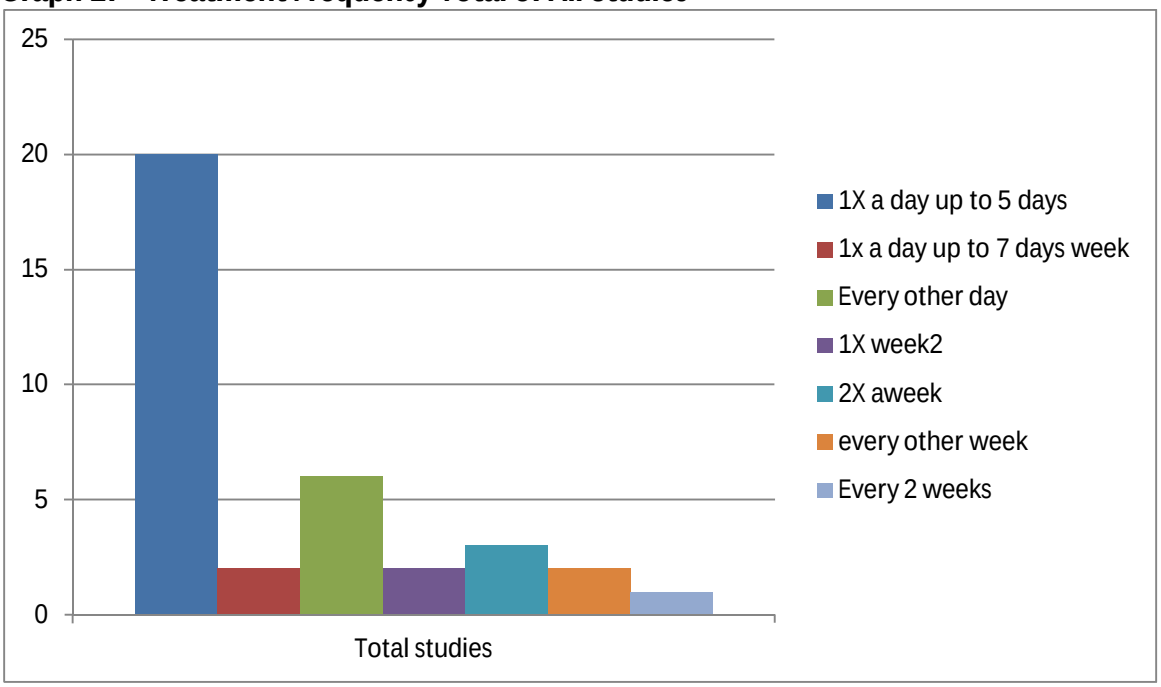
Treatment frequency. In the following Graph 16, for all of the studies, treatment duration and needle retention time are displayed to show how long the studies were, and how long they retained the needles. The studies are noted in from 1 time to 36 weeks. Colored bars note the amount of time the needles were retained per study length. 36% of studies had a needle retention time of 30 minutes, followed by 15 and 20 minutes (23% of the studies). The most common study length was 12 weeks (20% of the studies). Of the 12 weeks studies, they were evenly divided between 1-2 day retention and 20 and 30 minute retention. The next most popular study length was 8 weeks, with 16% of the studies using needle retention from 15 -30 minutes.

Graph 17 shows that most studies did once a day treatments, 5 days a week by an over whelming 66%. This is followed by every other day at 20% of the studies.

Graph 16 - All Studies Treatment Duration and Needle Retention Time

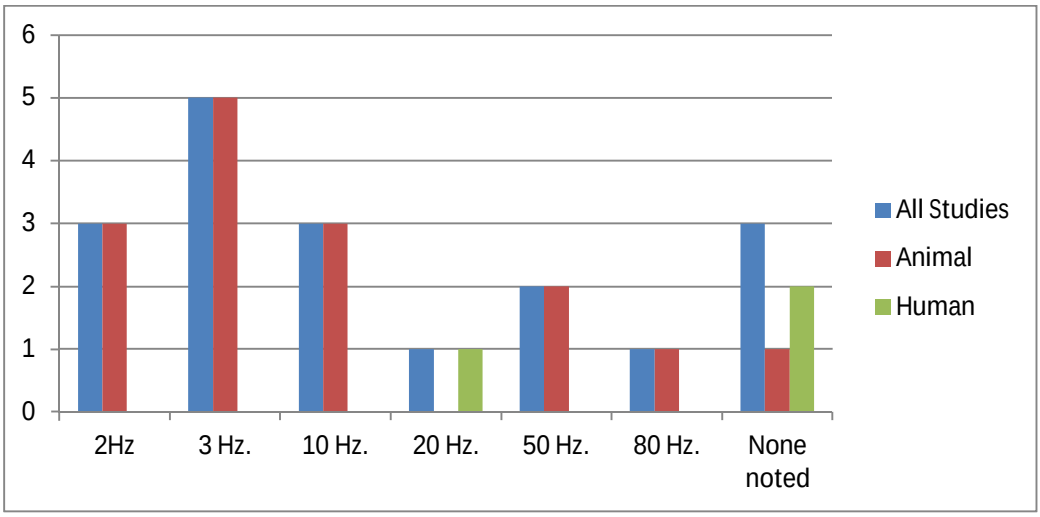


Graph 17 - Treatment Frequency Total of All Studies

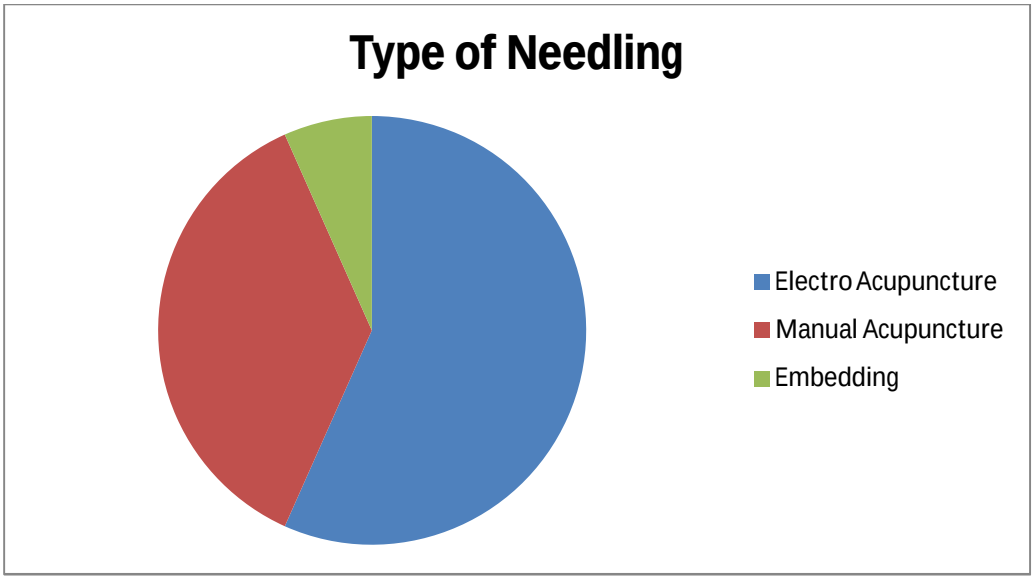


Type of needling. There were two main types of needling: simple needling with manual stimulation, and electroacupuncture. The data revealed 56% being electroacupuncture and 36% manual acupuncture (see Graph 19). In the studies 83% of EA treatments were on animal subjects, and 57% of manual acupuncture was done on human subjects. Most of the EA studiesused 3-4 Hz. stimulation. Graph 18 shows the usage of EA stimulation.

Graph 18 - Amount of Hz Used in EA studies



Graph 19 Type of needling



Chapter 5 – Discussion

Summary of Findings

In this research synthesis stuty, 30 articles were compiled and reviewed regarding the effects of acupuncture and electroacupuncture on the biochemicals that impact bone health and bone maintenance. Through qualitative analysis, a number of factors were examined including which chemicals were studied. Data were gathered regarding whether the biochemicals increased or decreased, and their impact on bone, as well as what treatment methods were used. The current study was accomplished to investigate the scientific effectiveness of electroacupuncture on the biochemicals involved in osteoporosis. The hypothesis of this study was that electroacupuncture could be a viable alternative treatment for osteoporosis based on the scientific validity of the biochemicals it influenced.

The information complied shows that electroacupuncture has a positive effect on the bones, showing an increase in BMD, trabecular thickness and trabecular number and a decrease in trabcular separation. EA treatments revealed that levels of estradiol and ALP increased, showing more bone formation. FSH, Dpd, TNF-α and urinary calcium were decreased showing less bone resorption. These factors all balanced each other out, showing a good balance of biochemicals for bone maintenance.

Implications of Theory

As we have seen in chapter four, acupuncture can have a significantgreat effect on bone maintenance and health by having an impact on bone formation, bone resorption and micro architecture. Investigating some of the pathways that acupuncture is affecting the body's

hormones and chemical pathways, we begin to see how on a theoretical scientific level acupuncture may be able to help with osteoporosis.

Acupuncture and electroacupuncture have been shown to increase estradiol, bone mass density, trabecular thickness and number, alkaline phosphate and osteocalcin showing an improvement in bone formation and structure. This impct is balanced with a decrease in deoxypyridinoline (Dpd), TNF-α, urinary calcium and follicle stimulating hormone (FSH) which shows how acupuncture provides a slowdown in bone resorption. Through these biochemical markers, we can see how acupuncture may have a cascading affect on the body as a whole, and not just on these specific markers. By investigating these markers it was theorized that different pathways have been activated to create a positive effect on bones, the formation and resorption seem to be balanced with acupuncture treatments and create a homeostasis in bone maintenance.

Estradiol is often looked in cases of osteoporosis, for it tends to be the hormone for which postmenopausal women manifest deficiency. Acupuncture has been shown to have an effect of the hypothalamic-pituitary-ovarian axis (HPOA), which influences hormones like estradiol, estrone, estriol. These hormones are depleted as we age. Estradiol, which is the most potent of the estrogen hormones, is all but diminished by age 50. This depletion is like Yin deficiency in TCM, the classic stage by age 40 when Yin is cut in half. When this happens we have symptoms of hot flashes, irritability, dryness of the skin, mouth, eyes and vaginal secretions. Yin controls the fire, and when it is depleted, we start to "bake our bone" causing them to become brittle and weak. This cycle mirrors what happens when we become estrogen deficient. Estrone, which is ten times less potent than estradiol, is what is most predominant in

menopausal women. With a deficiency of estradiol there is less osteoblast activity and therefore less bone formation. Estrogen and estradiol have an effect on BMD by stimulating osteoclast apoptosis and preventing osteoblast and osteocytic apoptosis (Takeda, 2005).

Of the seventeen studies that measured estradiol, all but one showed an increase. In study one, Feng et al. 2008, a decrease in estradiol was observed. The estrogen levels in the EA group did not increase as much as the estrogen group, but the BMD was still increased. It is theorized that this increase occured though an enhancement of IGF-1. The OVX-model and EA groups, estrogen levels were markedly lowerm but showed an increase in IGF-1 levels, IGF-1 has an effect on bone remodeling. Feng et al. (2008), theorized that IGF-I was enhanced in two ways. The first theory is that the enhancement resulted through EA stimulating enkephalins, which had an effect on the neuroendocrine process, subsequently stimulating the secretion of growth hormone. The growth hormoe was found to increase after EA. The increase in plasma growth hormone stimulated the pathway of IGF-I causing IGF-I transcription to the bone. Then it recruits pre-osteoblast cells to remodel the surface of the bones, and bone rebuilding begins (Takeda, 2005). The second theory is that IGF-I was increased through the enhanced binding to IGF-BP. When IGFI-I binds to IGF-BP, it increases the half life of IGF-I, and allows IGF-I to leave the capillaries and travel to its target tissue, the bones. Thereby bone remolding is enhanced (Feng et al., 2008). As we age, both estrogen and IGF-I decrease, especially in post menopausal women. This observation explains why post menopausal women tend to be more prone to osteoporosis. Study #1 showed how this can be reversed and increase BMD.

This review found that electroacupuncture helped balance estradiol with FSH and TNF-α. This balance is important for bone maintenance. As we age, estradiol tends to decrease and

FSH increases. According to Gilbert, He, Farmer, Boden, Kozlowski, Rubin & Nanes (2000), TNF-α is produced in excess postmenopause. Estradiol lowers the number and activity of osteoclast and stimulates the activity of osteoblast. Whereas, FSH stimulates osteoclast through the modulation of TNF-α. TNF-α synergistically activates existing osteoclast with RANKL. This activation is important because estrogen slows down RANKL induced osteoclast differentiation (Shevde, Bendixen, Dienger, Pike. 2000). Osteoclast differentiation in turn helps mediate the TNF-α osteoclast increase and balances the bone loss. Therefore, acupuncture is able to restore balance by decreasing FSH, which slows down the stimulation of TNF-α osteoclast resorption. At the same time acupuncture increases estradiol, which increases osteoblast and slows down RANKL, which in turn slows down TNF-α osteoclast resportion. This cycle puts back into balance the bone remodeling process thrown off by menopausal hormone level changes.

Colaianni, Cuscito, Colucci (2013), noted that women with normal levels of etradiol were still prone to osteoporosis. The authors theorized that this was due to higher levels of FSH. Studies show that if FSH is higher in pre menopause women, bones become fragile due to trabecular perforations. The performations do not affect the mass, but do impact the micro architecture making\e the bones fragile and putting women at risk for fractures even with good BMD numbers (Colaianni, Cuscito, Colucci. 2013).

Studies have shown that maintaining a lower level of FSH keeps the expression of cytokines that influence osteoclast production in check. The current study has shown that electroacupuncture has a positive effect on FSH, estradiol, BMD, trabecular thickness and numbers. Not only is the BMD increased, but so is the overall sturdiness of the bone structure. In conclusion higher levels of estradiol keep FSH and TNF-α in check and balance pre and

postmenopausal osteoporosis. Therefore, this researcher suggests that EA be done *preventatively* on premenopausal women to help restore the balance of hormones whether it is FSH or estradiol or both.

In study #5, the effects of electro-acupuncture on estrogen levels, body weight, articular cartilage histology and MMP-13 expression in ovariectomised rabbits, by Qin et al. (2013), showed an increase in estradiol but also measured MMp-13. MMp are from the matrix metalloproteinase family that are expressed in articulating cartilage and help degrade the extracellular matrix, making them part of the clean up process. MMp-13 digests type II collagen, and along with MMp-9, MMp-13 is part of the bone remodeling. MMp-13 cleave collagens from the spicules of the trabecular bone and MMp-9 cleaved collagen from the last transverse septa of the tibiae. It iss thought that MMp-13 is the dominate collagenase in cartilage, and together with other MMP's contribute to the collagen turnover in bone (Li, Liao, Dai, Wei, Luo. 2004). MMp-13 cleaves the collagen and MMp-9 clears the fragments. This combination promotes bone maintenance and health (Li, Liao, Dai, Wei, Luo. 2004). In the Qin et al. (2013) study, the cartilage samples were assessed, and the OVX groups had more damage than the control group. However, the treatment groups had much less damage to the cartilage. The estrogen group had less damage to the cartilage than the EA group. The estrogen group seemed to help with lowering MMp-13 and cartilage damage better than EA group. However, the numbers were only a few points different, and when compared to the OVX - no treatment group, both Ea and estrogen are very impressive in helping lower MMp-13 levels and regulate damage. With EA being about as good as estrogen therapy, it might be a better alternative because it has less side effects than estrogen therapy.

Study #7 and study #13, done by Zhao et al. in 2004 and 2005, showed an increase in aromatase activity, which may account for the increase in estrogen levels. Aromatase is the key enzyme responsible for estrogen biosynthesis. The Zhao et al. (2005) study on ovariectomized rats showed that SA adipose and liver contribute to the extragonadal aromatization to promote the increase in estradiol levels post ovariectomies. The study suggested that the adrenals might be helping with the compensation. An increase in the OVX and OVX-EA aromatase expression shows a compensation for a decrease in estrogen production from the ovaries. The body weights of the OVX rats increased as well, perhaps due to the need for other extragonadal aromatization in menopause and ovariectomized females. EA can improve and speed up this process to help restore homeostasis. In estrogen deficiency the liver steps up and converts more testosterone to estradiol to help with restoring balance to the system. This restoration is shown in these two studies by an increase in tissue aromatase in SA adipose and liver in the OVX-EA group and the increased circulation of estrogen in the OVX rats. This observation suggests that the SA adipose and liver is contributing to the circulating estrogen.

In studies, #7 and #13, EA showed an increase in the weight and volume of the adrenals, as well as an increase in the adrenal cortex cell activation, in the OVX-EA group but not in the INT-EA group. This observation suggests that the activation of the adrenal function may be another compensatory mechanism in estrogen deficiency by EA in OVX. It is interesting that the INT-EA did not always affect the hormone and steroid levels when the OVX-EA did. This effect was seen in study #23 as well, The effects of some acupoints (Du-14, li-11, St-36 and Sp-6) on serum TNF-α and hsCRP levels in healthy young subjects, by Karatay et al. (2011). This

effect of the balance of hormones occurs perhaps because they are already in balance and acupuncture is a regulatory system, only restoring balance when out of balance.

Changes in blood levels of steroids may suggest that EA is having an effect on the GnRH network and may help balance the hypothamus pituitary ovarian axis (HPOA) in OVX rats. Studies #7. by Zhao et al. (2005), study #18 by Wang et al. (2010), and study #19, by Chen (1997) all had an effect on GnRH, with each showing an increase. Wang et al. (2010), looked at specific points for their effect on GnRH neurons. His results help show that REN3, REN4, SP6, ST36 and Zigongxue have an excitatory effect on the GnRH neuron. This excitatory effect maybe the reason for the changes in hormone levels to a balanced state, showing that acupuncture can activate GnRh neurons in hypothalamus. The GnRH is secreted by the hypothalamus which stimulates luteinizing hormone to be released. That release in turn stimulates the secretions of estradiol. These secretions are regulated by the negative feedback loop of sex hormones and CNS (Wang et al. 2010). Wang et al. (2010) also measured the number of hypothalamic neurons discharged when acupuncture points were stimulated. The results showed that points on the same neural segments as reproductive organs, had a greater effect on GnRH neurons, such as REN4, SP6, Zigongxue, Yao Yan, which are all innervated by the 3rd and 4th segments of the lumbar spinal cord. This study showed that acupuncture creates a chain reaction of hormone secretions and stimulations. This observation may constitute another way acupuncture affects hormone secretion levels and why there is the increase in estradiol that we have seen in many studies that have used SP6 and REN4.

Acupuncture can have a systemic effect on the body. Therefore, needling one point can improve overall function of the body. As we age, and our hormones decline, and so does the

function of our digestive system. By improving our digestive system we improve the nutrient content in our bodies. This improvement has a systemic effect on our overall health and consequently our bones. Zhang, et al. (2004) postulated that homone deficiency decreased the intake of calcium and motility and secretion of gastric juices. The lack of calcium results in over secretion of parathyroid hormone which results in bone resportion.

In study #3, the preventative and therapeutic effects of acupuncture on bone mass density in osteopenic ovariectomized rats, by Zhang et al. (2004), showed a decrease in urinary excretion of calcium and phosphorus with the needle group that used ST36 and SP6, showing an improved calcium and phosphorus absorption, the two biomarkers for healthy bones. ST36 and SP6 have effects on the middle jiao, which in western medicine is related to the digestion and absorption of nutrients. Therefore, ST36 and SP6 improve the digestive track function and improve the overall body function and hence the bones.

Stress can have detrimental consequences on the body. Study two, (Zhang et al., 2007), explored this theory by seeing if EA could have the same effects as beta blockers. Studies have shown that B-agonist caused a decrease in bone formations and that B-Blockers had the opposite effects. The theory is that the sympathetic nervous system has a catabolic effect on the bones (Zhang et al., 2007). The imiplicaitdon is that when we are in that fight or flight state, the bone formations mechanisms is shut down so the body can concentrate on producing more nor-epinephrine, catecholamine, adrenalin, and increase blood calcium levels for muscle and cell activity. In this excited state osteoblast production is shut down, heart rate increased and gastrointestinal tract shuts down, causing a lack of nutrients to be absorbed. However, osteoclast resorption continues to supply, the added need of calcium to stimulate the longer

action potentials needed for ”fight or flight.” PC6 and SP6 are parasympathetic switches that puts the system back into that rest and restore state and turns the bone forming mechanisms back on. This study by Zheng et al. (2007) showed a decrease of noepinephrine and lowered heart rates in the treatment groups over the normal control group. The EA group and herb group both increased BMD of the vertebrae, tibia and femur. However, the beta-blocker group only increased the BMD of the vertebrae. This findin illustrates the fact that herbs and EA have a sympathetic regulating effect by lowering the level of stress hormone noepinephrine and may be part of the action in increasing BMD.

This research synthesis has shown that BMD is increased with electroacupuncture. Howver, it also showed an improvement in bone formation through trabecular thickness and numbers, and lower trabecular separation and increased BMD, creating a stronger architecture of the bones. Zhang et al. (2007) notes that this strengthening may be accomplished through re-establishing bone connectivity by bridging the marrow space between disconnected trabeculae and advancing the cortical bone area.

In study #9, Inoue et al. (2013) did needling distal to a bone fracture gap to measure for the gap width healing process. The results showed EA caused a greater healing of the gap compared to the control group. The control group grew, but then receded again over the course of the study. The study noted that EA increased the number of osteoblasts at the fracture site causing a remarkable increase in bone formation and healing in the fibula. EA stimulates the bone matrix healing system as a whole. This stimulation is also shown in the energy to failure results. The energy to failure rates were much improved, showing an increase in structure stability not just mass.

This review found that osteocalcin levels were shown to be lower after treatments, which means less osteoblast activity. This may seem like a negative finding for bone mass density. However, this finding is normal with postmenopause and ovariectomy patients. When looking at the study results, acupuncture slowed the normal decline of osteoblast formation. When combined with the Dpd results, which were markedly decreased in electroacupuncture treatments, there was less resorption. As a result, the the ratio between the Dpd and osteocalcin balance out. Postmenopause or ovariectomy increases bone loss due to increase osteoclast activity, which is not put in check by osteoblast activity. This bone loss still happens, but the rate is slowed down by acupuncture which helps restores this balance by suppressing bone resorption rather than increasing bone formation.

ALP is a biochemical marker for osteoblast. The current study found that EA slowed the decline of ALP compared to the OVX-model groups. This decline is normal in OVX and menopause. However, in study four, electroacupuncture prevents ovariectomy-induced osteoporosis in rats. In a randomized controlled trial done by Zhou et al. (2012), the researchers observed a significant increase of ALP over OVX-model and the normal control groups. The observed increase of ALP helps to keep the balance between resorption and formation. This observation may be the reason why acupuncture can increase BMD. It maintains the ratio balanced between biochemical markers, that western medications don't seem to have.

Implications of Practice

The current study has shown that EA improved the levels of hormones better than supplements. Even though EA is not as numerically effective as hormone replacement therapies, it comes close, and has fewer side effects. EA is a regulatory system that adjusts levels back to normal. It doesn't just do the adjusting to one hormone. but to all, as shown in the balancing between osteoblast biomarkers and osteoclast biomarkers.

The points used in these studies are those that have system wide effects. SP6 and ST36 were the most studied points, followed by UB20, UB23, REN3, REN4 and Zigongxue. SP6, ST36 and REN4 as noted earlier normalize gastric function. However, these points do much more. SP6 is the meeting place of the Yin and is closely related to estrogen production. SP6 is considered most Yin in TCM. Helping to harmonize the liver and tonify the kidneys, SP6 is helpful in two ways. The kidneys dominate bones govern aging and growth. Therefore, by tonifying the kidneys, the progression of decline of hormones, and aging is slowed. Harmonizing the liver creates a smooth flow of Qi and blood, allowing for Qi and blood to circulate and nourish the body, while also keeping liver Qi from stagnating and causing a fire condition. As mentioned before, fire will bake the bones and make them fragile. ST36 helps to fortify pre-heaven Qi. The pre-heaven Qi comes from the kidneys, but it also needs post-heaven Qi, which is created from the spleen and stomach, to keep it supplemented and nourished. This means that ST36 can help slow the decline of kidney Yin and Qi thus keeping the aging process and bone loss to a minimum. The use of SP6 and ST36 in the studies maybe due to the fact that they affect multiple systems and help to maintain health and allow for healthy aging.

Bladder points UB20 and UB23 were used often in the studies as well. Some of the studies noted that they did not have the same effectiveness on BMD as SP6 and ST36 did, even though they have similar functions as ST36 and SP6. UB23 and UB20 were also not studied as much and may affect different hormones and biochemical pathways. Since UB23 is the back shu of the kidneys, one would think it would have a great effect on essence, marrow, and bones and hence BMD. UB23 being the back shu of the kidneys, means it has direct action on the kidneys and its functions. This point nourishes kidney Yin and Yang and benefits essence, some. Some of the main components that effect aging. Once Yin, Yang and essence decline, symptoms of grey hair, memory problems, hot flashes, blurry vision, dry eyes, skin and vaginal secretions, hearing loss, urinary incontinence and osteoporosis can occur. UB20 is the back shu of the spleen. which we have noted is involved in the making of nutrients for the body. Deficiency of spleen causes digestive issues, fatigue, mental cloudiness, and emaciation. When the Spleen is functioning properly. it allows for blood and Qi to be formed so that it can be spread through the body and nourish it. While TCM suggests that UB20 and UB23 directly affect the pre and post heaven Qi, it is unclear why they did not have a greater effect on bone mass and health. More studies would need to be done.

The study of Karatay et al. (2011) regarding TNF-α have shown the effectiveness of DU14 and LI11. DU14 functions include expel wind, firm the exterior, clear heat, tonifies deficiency, pacifies wind and treats malaria. The impact is perhaps through clearing heat and fire, which dries up the bones and makes them brittle. LI11 functions include clearing heat, cooling the blood, eliminating wind and draining damp, regulating Qi and blood plus activation

of the channel and alleviating pain; clearing heat might be the action though which it is benefiting the bones.

REN4 on the abdomen is local to uterus, ovaries and reproduction organs, which decline as we age and causes a depletion of estrogen and increase in FSH. This review has shown that EA had a direct effect on the deficiency caused by the removal of ovaries or their decline as seen in menopause. REN4 tonifies and nourishes the kidneys, fortifies the pre-heaven Qi and benefits essence, which as we have noted, has an effect on aging and bone health. The use of REN4 can help balance these core deficiencies. REN4 also warms and fortifies the spleen, which has a direct effect on digestion, nutrient assimilation and blood formation, helping to maintain health through post heaven Qi. REN3 has similar action to REN4 it also benefits the uterus, regulates menstruation and fortifies the kidneys. Zigongxue is on the same level as REN3 and it also regulates menstruation. These points help with the main female causes of disease that can then relate to menopause and osteoporosis. Wang et al. (2010) study showed that REN4 and SP6 activated GnRH neurons in the hypothalamus showing they are important points for the reproductive endocrine function.

Point selection is important as we have seen in Zhang et al.'s (2004) study. Zhang showed that SP6 and ST36 had a better effect than UB20 and UB23 on BMD, the ST36 and SP6 group increased BMD in all areas much more than UB20 and UB23. Point location is important as well. This research synthesis noted that many of the studies that compared true acupuncture to sham, showed that true had better effect. Howver, in many cases the sham acupuncture still had an effect, just not as much. Sham acupuncture included off channel points, a sham device or many studies used Hautuojaiji points with SJ5.

Studies that used the sham device had results close to the true results. This observation is likely because in sham procedures you are still touching the point. Therefore,m you are effecting a change. This observation can also be seen in studies that compared EA to manual needling with no stimulation. Both had an effect, but the point with the most stimulation had the greater effect. Study 22, (Ovarian blood flow responses to electroacupuncture stimulation depend on estrous cycle and on site and frequency of stimulation in anesthetized rats, by Stener-Victorin, E., Fujisawa, S. and Kurosawa, M. 2006), looked at the impact of different frequencies, 2 Hz, 10 Hz, and 80 Hz on ovarian blood flow. The abdominal stimulation of 10 Hz showed a decrease, followed by significant increase in blood flow to the ovaries. 80 Hz had a great decrease with only a slight increase in blood flow to the ovaries. Increased blood flow increases function of the ovaries, and this study showed that 10 Hz stimulation would be good for increasing blood flow to the ovaries. However, stimulation of 2 Hz wasn't very effective. This finding is interesting, since most of the studies use 2-3 Hz stimulation. However, most of those studies were on animals, and the few studies on human subjects did use 10-20 Hz of stimulation. This finding may be due to a body mass differentiation. With more mass, more stimulation is needed. The amount of hertz needed to effect the greatest change is something that needs to be studied further, especially in human subjects.

A review of the studies on the depth of the needles and whether touching the bone was better, reveals unclear results. Study #11, (Zhang et al., 2005) compared true manual needling of ST36 and SP6, to sham of the channel points, to SP6 and ST36 touching the bone. The group that had ST36 and SP6 touching the bone. was better than the sham group in BMD, and about the same as true group. When considering the mineralizing apposition rate, the results of the

needle touching the bone group were as low as the OVX - no treatment group. The touching the bone group's bone formation rate was lower than all the other groups significantly. The touching the bone group's Tb.Th. and Tb.Sp numbers were significantly higher than all the groups. The Tb.N. was significantly lower than the true group and slightly lower than the OVX-no treatment group. These results show that touching the bone group had thicker bones but a weaker structure. On the other hand. studies 9, Inoue et al. (2013) and 10, Nakajima et al. (2009), regarding the healing process of tibia fractures, showed that EA touching the bone had faster healing than the no treatment and manual needling groups at the same location and depth. These studies also noted that there were more osteoblasts at the site of bone fracture. In study 11 the researchers used specific points and assessed the whole bone. In studies 9 and 10 the researchers needled proximal to the fracture and did not asses the quality of the bone. More studies are needed to assess which technique is better. However, this reviewer's opinions is that point location, depth and angle with EA may be factors that are responsible for creating the healthiest bones.

Through the investigation it has been found that acupuncture and electroacupuncture have a systemic effect on the body and not just the bones. The use of ST36 and SP improve estrdiol levels, through activation of the GnRH, adrenals, aromatase, and also through improved digestion and reducing stress. Stress as we know has a very detrimental effect on the whole body effecting digestion, heart rate, sleep, and mental clarity. Even though the current study focuses on bone health and maintenance, it is clear to the researcher that acupuncture has an advantage over western drug treatment in that it balances the whole system not just on biochemical or hormone. In TCM treating the whole body is the approach. TCM looks for the

root cause of the problems and doesn't just treat the symptoms. By using this approach better overall health is created. if a patient has digestive issue, by treating that area, the entire body is treated. Better nutrients means better functioning of the systems and may clear up problems that have not been identified like osteoporosis. Most people don't realize they have osteoporosis until they have a fracture. However, wth regular acupuncture treatments of other issues, sicj as stress, digestiive issues or insomnia, one is helping to prevent future problems like osteoporosis. Even though the focus of this review was on biochemical factors and how EA affects them, the goal was to show the science behind it and introduce how systemic EA could be.

In terms of the practice implications of this research study, it is suggested that regular, consistent treatment of electroacupuncture of 10-20 Hz, of ST36 and SP6, once a week for at least 12 weeks or daily for 8 weeks, will have the greatest effect on the biochemicals of osteoporosis and the body as a whole.

Limitations of Study

The research of this topic was limited by the article sources available; this is particularly true for articles available in English. This limitation of sources resulted in the use of five abstracts to compile enough data. Articles which had actual measurements for biochemical factors were limited to the few reported on in this study. There were 17 studies that dealt with Estradiol. Howedver, for other factors there were only three or four studies. Most of the articles were animal studies, and those studies were detailed. Human studies lacked diagnosis and point prescriptions. Human studies used sham devices which were ineffective or were only

case studies. Nonetheless, levels of biochemicals were able to be extracted. A further limitation was actual level numbers in the text, a few of the results had to be pulled from graphs within the studies, making accuracy subjective, and these instances have been noted in the tables.

Comparing levels of each biochemical was difficult because the comparison groups were not all the same. Some were using different points, some were using different types of needling, and some compared to hormones therapies or supplements or herbs. On the other hand, most studies did have a normal control group and an OVX-model group in their comparisons.

Suggestions for Future Research

The point of this research was to show that acupuncture has an effect on the body. Future research needs to be done on human subjects and under STRICTA guidelines. These guidelines will help clear up the problems with sham protocols. Acupuncture is not practiced like western medicine, and should not be studied under those guidelines but under the STRICTA rules. When transitioning to human subjects doing one and two point studies will be helpful in narrowing down impact. However, as we have seen with SP6 and ST36 there is a systemic effect. Perhaps setting up studies with inclusion and exclusion guidelines on a TCM diagnosis and treatment protocols will help. Studies on hertz frequencies would be beneficial to see what level has the best affect.

It is suggested thast a long one to two year study be conducted on human subjects to see what the effect acupuncture can have on bone maintenance and ultimately on

osteoporosis. Biochemicals should be tested at the beginning and every three months. Treatments should be given in at least three groups. One group should be treated every day for 5 day a week for 8 weeks. Another group should be treated once a week for two years. A third group should receive treatemtns twice a week for two years. Ideally, there should be four treatment groups, wht the fourth group receiving no treatment. The treatment groups might be further differentiated with group one ST36 and SP6, group two UB20 and UB23, group three GB34 and UB11. Group four should be points picked per each patient's diagnosis. Each of the above treatments groups should be separated into a manual needling group and a 10 Hz. electroacupuncture group.

Animal studies are great for establishing a foundation. However, we need to move to human subjects now. This study did not look at herbs. However, herbs should be included in future research because they can be as effective, or more effective than current western medications.

Conclusion

It has been shown that electroacupuncture can affect many of the biochemicals associated with bone formation and remodeling, and ultimately help with osteoporosis. This review also found the pathways that acupuncture maybe activating to do influence biochmicals and open the door to better understand and future research. Perhaps with this information Western and Eeastern practitioners can collaborate to help patients slow down the progress of bone loss. More studies need to be accomplished. However, the ground work has been laid and needs to be further developed.

Osteoporosis affects the majority of the population. With proper education of both patients and doctors this researcher believes that we can find a happy meeting ground to help prevent, manage and reverse this degenerative disease.

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Zhao, H., Chen, B. (2005). Evaluation of electroacupuncture on ovariectomized rats: Implications of modern scientific mechanisms on acupuncture curing woman perimenopausal syndrome. *Acupuncture & Electro-Therapeutics Res. Int. J., 30*, 275-288.

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Zhang, W., Kanehara, M., Zhang, Y., Wang, X. & Ishida, T. (2007) B-Blockers and other analogous Treatments that affect bone mass and sympathetic nerve activity in ovariectomized rats. *American Journal of Chinese Medicine*, 35 (1) 89-101.

Zhang, W., Kanehara, M., Zhang, Y., Yu, Z., Zhang, G., Yang, Y., Sun, Y., Zhang, J., Ishida, T. (2005). The more efficacious acupoints of zusanli and sanyinjiao than that of non-acupoints on bone mass in osteopenic ovariectomized rats. *Chinese Journal of Integrated Traditional and Western Medicine. 11*(3), 209-216.

Appendix A: IRB Approval Letter

YO SAN UNIVERSITY OF TRADITIONAL CHINESE MEDICINE

April 4, 2013

Rochan Olson, L.Ac
1012 Justin Ave, Apt C
Glendale, CA 91201

Dear Rochan,

Your Claim for Exemption from the Institutional Review Board (IRB) has been reviewed. Your research proposal has been approved, with no recommendations effective April 1, 2013 through March 31, 2014.

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,

Shelley Cerny, L.Ac
IRB Coordinator

Appendix B: Articles Used for Research Synthesis, Listed in Order of Study number

Articles Used for Research Synthesis, Listed in Order of Study Number.

Study 1

Feng, Y., Lin, H., Zhang, Y., Wu, X., Wang, T., Liu, Y. & Tan, Y. (2008) Electroacupuncture promotes insulin-like growth factors system in ovariectomized osteoporosis rats. *American Journal of Chinese Medicine*, 36 (5) 889-897.

Study 2

Zhang, W., Kanehara, M., Zhang, Y., Wang, X. & Ishida, T. (2007) B-Blockers and other analogous Treatments that affect bone mass and sympathetic nerve activity in ovariectomized rats. *American Journal of Chinese Medicine*, 35 (1) 89-101.

Study 3

Zhang, W., Kanehara, M., Ishida, T., Guo, Y., Wang, X., Li, G., Zhang, B., Kondo, H., Tachi, S. (2004). Preventative and therapeutic effects of acupuncture on bone mass density in osteopenic ovariectomized rats. *American Journal of Chinese Medicine, 32*(3), 427-443

Study 4

Zhou, J., Chen, S., Gou, H., Xia, L., Lui, H., Qin, Y., He, C. (2012). Electroacupuncture prevents ovariectomy-induced osteoporosis in rats: a randomized controlled trial. *Acupuncture Medicine, 30*, 37-43. Doi: 10.1136/acupmed-2011-010114

Study 5

Qin, Y., He, J., Xia, L., Guo, H., He, C. (2013). Effects of electro-acupuncture on oestrogen levels, body weight, articular cartilage histology and MMP-13 expression in ovariectomised rabbits. *Acupuncture Med, 31*, 214-221. Doi: 10.1136/acupmed-2012-010289

Study 6

Chen, G., Xu, T., Zhang, J., Liu, S., Guo, Z. (2010). Effect of acupoint cat-embedding on the quality of life, reproductive endocrine and bone metabolism of postmenopausal women. *Chinese Journal of Integrated Medicine, 16*(6), 489-503.

Study 7

Zhao, H., Chen, B. (2005). Evaluation of electroacupuncture on ovariectomized rats: Implications of modern scientific mechanisms on acupuncture curing woman perimenopausal syndrome. *Acupuncture & Electro-Therapeutics Res. Int. J., 30*, 275-288.

Study 8

Ouyang, B., Gao, J., Che, J., Zhang, Y., Li, J., Yang, H., Hu, T., Yang, M., Wu, Y., Ji L. (2011). Effect of Electro-acupuncture on tumor necroses factor-α and vascular endothelial growth factor in peripheral blood and joint synovial of patients with rheumatoid arthritis. *Chinese Journal of Integrated Traditional and Western Medicine., 17*(7), 505-509.

Study 9

Inoue, M., Nakajima, M., Hojo, T., Itoi, M., Hiroshi, K. (2013). The effect of electroacupuncture on osteotomy gap healing in a rat fibula model. *Acupuncture Medicine, 31*, 222-227. Doi: 10.1136/acupmed-2012-010294

Study 10

Nakajima, M., Inoue, Motohiro, M., Hojo, T., Inoue, N., Tanaka, K., Takatori, R., Itoi, M. (2009). Effect of electroacupuncture on the healing process of tibia fracture in a rat model: a randomized control trial. *Acupuncture Medicine, 28*, 140-143. Doi : 10.1136/aim.2009.001800

Study 11

Zhang, W., Kanehara, M., Zhang, Y., Yu, Z., Zhang, G., Yang, Y., Sun, Y., Zhang, J., Ishida, T. (2005). The more efficacious acupoints of zusanli and sanyinjiao than that of non-acupoints on bone mass in osteopenic ovariectomized rats. *Chinese Journal of Integrated Traditional and Western Medicine. 11*(3), 209-216.

Study 12

Yim, Y., Lee, H., Hong, K., Kim, Y., Lee, B., Son, C., Kim, J. (2006) Electro-acupuncture at acupoint ST36 reduces inflammation and regulated immune activity in collagen-induced arthritic mice. *eCam. 4*(1). 51-57. Doi: 10.1093/ecam.nel1054.

Study 13

Zhao, h., Tain, Z., Cheng, L., Chen, B. (2004). Electro acupuncture enhances extragonadal aromatization in ovariectomized rats. *Reproductive Biology and Endocrinology. 2*(18). Retrieved from WWW.rebj.com/content/2/1/18.

Study 14

Sunay, D., Ozdiken, M., Arslan, H., Seven, A., Aral, Y. (2011). The effect of acupuncture on postmenopausal and reproductive hormones: a sham controlled clinic trial. *Acupuncture Med. 11*(29), 27-29. Doi: 10.1136/aim/2010.003285

Study 15

Xiaoming, S., Yuanhoa, D., Li, Y., Yang, X., Hong, Y., Guiru,H., Yongtie, G., Xuemin, S. (2005). Acupuncture for treatment of climacteric syndrome – a repot of 35 cases. *Journal of Traditional Chinese Medicine. 25*(1), 3-6.

Study 16

Zhou, K., Jiang, J., Wu, J., Liu, Z. (2013). Electroacupuncture modulated reproductive hormone levels in patients with primary ovarian insufficiency: Results from a prospective observational study. *Evidence Based Complementary and Alternative Medicine. 2013*, 657234. Retrieved from http://dx.doi.org/10.1155/2013/657234.

Study 17

Kim, D., Jeong, J., Kim, K., Rho, J., Choi, M., Yoon, S., Choi, S., Kang, K., Ahn, H., Lee, M. (2011). Acupuncture for hot flushes in perimenopausal and postmenopausal women: a randomized, sham controlled trial. *Acupuncture Medicine. 2011*(29), 249-256. Doi: 10.1136/aim.2011/004085.

Study 18

Wang, S., Zhu, B., Ren, X., Tan, L. (2010). Experimental study on acupuncture activating the gondotropin-releasing hormone neurons in hyponthalmus. *Journal of Traditional Chinese Medicine. 30* (1). 30-39.

Study 19

Chen, B. (1997). Acupuncture normalizes dysfunction of hypothalamic-pituitary-ovarian axis. *Acupuncture and Electro-Therapeutics Res. 22*, 97-108.

Study 20

Azizi, H., Liu, Y., Du, L., Wang, C., Bahrami-Taghanaki, H., Esmaily, H., Azizi, H., Xue, X. (2011). Menopause-related symptoms: traditional Chinese medicine vs hormone therapy. *Alternative Therapies. 17*(4). 48-53.

Study 21

Pastore, L., Williams, C., Jenkins, J., Patrie, J. (2011). True and sham acupuncture produced similar frequency of ovulation and improved LH to FSH in women with polycystic ovary syndrome. *Journal of Endocrinology Metab. 96*(10). 3143-3150. Doi: 10.1210/jc.2011-1126

Study 22

Stener-Victorin, E., Fujisawa, S., Kurosawa, M. (2006). Ovarian blood flow responses to electroacupuncture stimulation depend on estrous cycle and on site and frequency of stimulation in anesthetized rats. *Journal of Applied Physiology. 101*. 84-91. Doi: 10.1152/japplphysiol.01593.205

Study 23

Karatay, S., Akcay, F., Yildirim, K., Erdem, F., Alp, F. (2011). Effects of some acupoints (Du-14, li-11, St-36 and Sp-6) on serum TNF-α and hs CRP levels in healthy young subjects. *The Journal of Alternative and Complementary Medicine. 17*(4). 347-350. Doi: 10.1089/acm.2009.0461

Study 24

Chen, GZ., Xu, YX., Zhang, JW., Liu, SH., Gou, ZY. (2010). Effect and safety evaluation of catgut implantation at acupoint for levels of bone metabolism and free radicals in postmenopausal women. [Abstract]. *Zhongguo Zhen Jiu, 30*(3), 177-181.

Study 25

Bao, SY., Zhang, SJ., Lin, WJ., Chen, JF. (2012). Effect of electroacupuncture on the biochemical indices of bone and bone collagen metabolism and TNF-alpha in osteoporosis model rats without ovaries [Abstract}. *Zhongguo Zhen Jiu, 32*(12), 1108-1112.

Study 26

Zhao LH., Nong ZN., Zhong, X., Pang, Y., Liang, JS. Li, XD., Ye, FW. (2008). Effects of warm needle moxibustion on bone mass density and biochemical indexes of bone metabolism in patients of postmenopausal osteoporosis [Abstract]. *Zhongguo Zhen Jiu, 28*(12), 897-900.

Study 27

Wei, YF., Liu, YL., Zhang, SH., Wang, ZO., Liu, Y., Wang, HC., Yao, JF., Li, F., Wang, CH. (2007) Effect of electroacupuncture on plasma estrin and bone mineral density in ovariectomized rats [Abstract]. *Zhen Ci Yan Jiu, 32*(1), 38-41.

Study 28

He, J., Yang, L., Qing, Y., He, C. (2013). Effect of electroacupuncture on bone mineral density, oestradiol level and osteoprotegerin ligand expression in ovariectomized rabbits [Abstract]. *Acupuncture Medicine*. Doi: 10.1136/acupmed-2012-010271.

Study 29

Zhang, X., Peng, Y., Yu, C., Cheng, H., Liu, L., Han, J. (2009). Changes in histomorphometric and mechanical properties of femurs induced by acupuncture at Shenshu point in the SAMP6 mouse model of senile osteoporosis [Abstract]. *Gerontology, 55*(3), 322-332. Doi: 10.1159/000214845

Study 30

Ma, J., Yun-guang, H., Zhang, DH. (2008). Effect of acupuncture on bone metabolism and serum estradiol level in ovariectomy-induced osteoporosis rats [Abstract]. Zhen Ci Yan Jiu, 33(4), 235-239.

Appendix C: Article Abstraction Form

Article Abstraction form – Rochan Olson – Research Synthesis.

Article Title: _____
Article number: _____ Date of Article: _____
Authors: _____
Publisher: _____

Human _____ Animal: _____ Male ____ Female_____ Age_____
Number of Subjects: _____ Length of Study_____
Acupuncture:_____Electro Acup: _____ Herbs: _____
TMC or Western Study # of Patient treated:
Organ system studied:

Bio marker	**Model**	**Control**	**other**
E2			
FSH			
T			
Osteocalcin			
Calcitonin			
PTH			
AL Phospatase			
BMD			
BMD Femur			
BMD Vertabra			
BMD Tibia			
Tra bone volume			
Tra Thickness			
Tra Number			
Bone Formation rate			
# node			
Node terminus struts			
Marrow Cavity surface			
TRa Separation			
Cortial Area			
Mineral Apposit			
Mineral Surface			
Load Max			
Flexibility energy to failure			
Noepinphrin			
DPd			
Calcium			
Phosphorus			
BP			
HT Rate			

Body Weight			
MMP-13			
IGF-I			
IGF-II			

Points Used:

Needle type:

Manipulation:

Length TX:

Conclusions

Notes

Appendix D: Systems Studies Table

1. Systems studied Table

Organ system studied	**Studies that used this system**	**Total Number of studies that used this system**
Bone	1, 2, 3, 4, 6, 9,10, 11, 24, 25, 26, 27, 28, 29, 30	14
Gynecological & Menopause	2, 6, 7, 13, 14,15, 16, 17, 18, 19, 20, 21, 22, 24	14
Neurological	18	1
Immune	12, 23	2
Sympathic Nervous system	2, 22	2
Cartilage	5, 8	2

Appendix E: Type of Subjects Used Table

Table 2: Type of subjects Used

Type of subjects used	Studies which used these subjects	Total number of subjects	Total number of Studied
Animal	1	50	19
	2	43	
	3	35	
	4	30	
	5	24	
	7	--	
	9	40	
	10	30	
	11	40	
	12	58	
	13	60	
	18	60	
	19	18	
	22	23	
	25	60	
	27	32	
	28	21	
	29	--	
	30	40	
Rodents	1	40	17
	2	43	
	3	35	
	4	30	
	7	--	
	9	40	
	10	30	
	11	40	
	12	58	

	13 18 19 22 25 27 29 30	60 60 18 23 60 32 -- 40	
Rabbits	5 28	32 21	2
Human	6 8 14 15 16 17 20 21 23 24 26	93 63 53 65 11 54 57 84 90 65 40	11
Human Female	6 14 15 16 17 20 21 24 26	93 53 65 11 54 57 84 65 40	9
Human Mixed	8 23	63 90	2

Appendix F: Biomarkers Screened For

Table 3: Bio-Markers screened for

Biochemical marker	Study #s that screened for this marker	Did it increase or decrease with treatment	System studied	Total number of studies
Osteocalcin	2 6 11 25 26 29	Lowered Lowered Increased Decreased Decreased Decreased	Bone Repro & Bone Bone Bone Bone Bone	
Calcitonin	6	Lowered	Repo & Bone	
IGF-1	1	increased	bone	
BP	2	lowered	Bone	
Heart rate	2	lowered	bone	
Noepinephrine	2	lowered	bone	
Cartilage damage	5	Lowered	Cartilage	
Deoxypyridinoline	2 3 11 25	Lowered Lowered Lowered ovx Increased sham Decreased	 Bone Bone	
Carboxyterminal Propetided	25	Decreased	Bone	
Creatinine/calcium	25	Decreased	Bone	

	26	Decreased	Bone	
Calcium	3 26	Lowered Lowered	Bone Bone	
Bone specific Alkaline-Phosphate	4 6 25 30	Increased Lowered Lowered Lowered	Bone Repo & bone Bone Bone	
Tartrate-resistant acid phosphatase	4 30	Decreased Decreased	Bone Bone	
Ovarian blood flow	22	Increased	Gyno/nerve	
IL-6	12	Decreased	Immune	
Bone weight -ash	3	Increased		
Energy to failure	4 10	L5 increased increased		
Bone Flex	4 29	L5 increased Femur increased Increased- Femur	 Bone	
CRP	23	Same	Immune	

Appendix G: Biomarkers screened for Table 3.1

Table 3.1: Bio-Markers screened for

Biochemical marker	Studies that screened for this marker	Did it increase or decrease with treatment	System studied	Total number of studies
Testosterone	6 7 13 29	Increased Decreased No change Decreased	Repo & Bone Repo Menopause Bone	4
BMD	1 2 3 4 11 26 27 28	increase increase - vertebrae, femora, tibia increased tibia L5 increased Femur increased L4-6 Increased Femur Increased Increased Increased Increased	Bone Bone, Sympathetic Nervous system Bone Bone Bone Bone Bone Bone Bone Bone	8
BMC	1	Increase	Bone	1
IGF-1	1	increase	Bone	1
Serum Estradiol	1 5 6	Increase Increased Increased	Bone Cartilage Repo & Bone	17

	7 13 14 15 16 17 19 20 24 25 26 27 28 30	Increased Increased Increased Increased Increased Increased Increased Increased Increased Increased Increased Increased Increased Increased	Repo Menopause Menopause Menopause Gynecology Menopause Gynecology Menopause Bone & menopause Bone Bone Bone Bone Bone	
Uterine weight	2 11 30	Increase Increased Increased	Bone Bone Bone	3
Bone volume	2 11 29	Increased Increased Increased	Bone Bone Bone	3
Trabecular number	2 3 4 11	Increased Increased Increased Increased	Bone Bone Bone Bone	5

	29	Increased	Bone	
Trabecular separation	2 3 4	Decreased Decreased Decreased	Bone Bone Bone	3
Trabecular thickness	2 3 4 11	Same Increased Increased Increased	Bone Bone Bone	4
Mineralizing and bone formation	2 3 9 10 11 29	Not significant change Increased Increased Increased Increased Increased	Bone Bone Bone Bone Bone Bone	6
LRP5	4	Increased	Bone	1
MMP-13	5	Decreased	Cartilage	1
Serum bone GLA protein	30	decreased	Bone	1
Tartate-resistant acid phosphatse	30	Decreased	Bone	1
Parathyroid hormone	6	Lowered	Repo & Bone	1
Adrenal weight	19	Increased	Gynecology	1
Luteinizing hormone	6 14	Lowered Decreased	Repo & bone Menopause	7

	15 16 17 19 21	Decreased Decreased Decreased Decreased Decreased	Menopause Gynecology Menopause Gynecology Gynecology	
Follicular stimulating hormone	6 14 15 16 17 20 21	Lowered Decreased Decreased Decreased Decreased Decreased Decreased	Repo & Bone Menopause Menopause Gynecology Menopause Menopause Gynecology	7
B-catenin	4	increased		1
Mature Vaginal epithelia	7 13 19	Increased Increased Increased	Repo Menopause Gynecology	3
Corticosterone	7 13 19	Increased Increase increase	Repo Menopause Gynecology	3
Gonadotropin releasing hormone	7 18 19 -hypothamic	Increased Increased Decreased	Repo Neuro gynecology	3
Corticotrophin releasing hormone	7	Increased	Repo	1

Aromatase activity	7 13	Increased Increased	Repo	2
Aromatase –SA adipose	13	Increased	Menopause	1
Aromatase - liver	13	Increased	Menopause	1
Aromatase - mRNA	13	Increased	Menopause	1
TNF-α	8 12 23 25	Decreased Decreased Decrease Decreased	RA Immune Immune Bone	4
Vascular endothelial growth factor	8	Decreased	RA	1
Bone Morphogenic Protein 2 (BMP-2)	9	Increased	Bone	1
Osteoblast	9	Increased	Bone	1
Callus accretion	10	Increased	Bone	1
AgNOR	19	Increased	Gynecology	1
B-endorphin	19	Increased	Gynecology	1
ER mRNA Cell inner adrenal cortex	19	Increased	Gynecology	1

Appendix H: Method of Treatment Table

Table 4: Methods of Treatments

Method of treatment used	Studies that Used This Type of Treatment	Bone system study	Other organ system study	Total number of studies
Acupuncture	2	Y	Sympathic nervous system, hormones	14
	3	Y	Bone	
	8	N	RA	
	11	Y	Bone	
	14	N	Menopause	
	15	N	Menopause	
	17	N	Menopause	
	18	N	Nuero	
	20 +CHM	N	Menopause	
	21	N	Gynecology	
	23	N	Immune	
	26 – moxa	Y	Bone	
	29	Y	Bone	
	30	Y	Bone	
Electro-acupuncture	1	Y	Bone	15
	4	Y	Bone	
	5	N	Cartilage	
	7	N	Menopause	
	8	N	RA	
	9	Y	Bone	
	10	Y	Bone	

	12 13 16 19 22 25 27 28	N N N N N Y Y Y	Immune Menopause Gynecology Gynecology Gyno/Nerve Bone Bone Bone	
Sham	13 17 19 21 22 29	N N N N N Y	Menopause Menopause Gynecology Gynecology Immune Bone	6
Moxa	26 - needle	Y	Bone	1
Catgut Embedding	6 24	Y Y	Repo & Bone Bone & Repo	2

Appendix I: Treatment Details of TCM

Table 5: Treatment details of TCM

Study that used points	Type of treatment	Points used	Needle/ instruments used	Length of needle retention	Number of treatments	Length of study
1	EA -2hz 1mA	GB39 Du4	.22mm-10mm needles	30 minutes daily	28	4 weeks
2	Acu – manual 2hz 120 turns/min	Pc6, Sp6	.22mm – 10mm	15 min 5 days week two days off	180	36 weeks
3	Acu –maunual 2 Hz 120 turns/min for 1 min at beginning and end of treatment	St36, SP6 UB20, Ub23	.22mm - .8 mm Inserted .3cm	15 Min 5 days a week	80	16 weeks
4	EA – 3Hz, 1mA	SP6, St36	.25mm-.25mm	30Min. 5 Days a week	60	12 weeks
5	EA -10 Hz, 1-5mA Acu	UB23, St36 UB18, UB20, Sp6	0.3mm-25mm .3mm-25mm	30 min. 5X a week	10	2 weeks
6	Cat gut embedding Retained 1-2days Ethinyl, provera, vit A, D2, E and calglucon capsule	UB23, Sp6 R4 every tx every person Gan shen Yin Xu UB18 Pi shen yang xu ST36, UB20	3/0 Catgut 1.5 cm length. Lifting and thrusting till Da Qi then embedded cat gut.	Retain 1-2 days Once every two weeks	6	3 months

7	EA 3Hz 1-2mA	True - R4, R3, on side Sp6, lateral Zi Gong Xue Sham - Huotuojiaji T3-5, One side SJ5	Didn't say	30 min	3	3 days
8	EA Continuous wave Needling	Both Support yang D20, LI11, SJ6, R4, St36, GB34, Sp6, UB23, UB20, ect. Plus local ashi points With Thrust lift to da qi stimulation. EA Local ashi points15 min Flip and UB20, 23 15 min	.25x25mm or .30x30mm	30 total 15 minutes front treatment 15 minute back treatment	30	About 2 months
9	EA – 50hz. 20 min	Local point	.30mmx24mm	20min 5 days a week	30	6 weeks
10	EA -50 Hz. Acu	Local points	.30mmx24mm	20min Dailey	15-21	3 weeks
11	Acupuncture with reinforcing/reducing method	ST36, SP6	.22mmx10mm	15min	115	23 weeks

	1 – normal needling 2- sham needling 3- normal needling but hit bone					
12	EA 2 hz. 6-7mA. Acupuncture	St36	.25X30mm	15 min	15 45	5 week 9 weeks
13	EA 3 Hz. 1-2mA	R3, R4, Sp6, Zigongxue		30 min.	3	3days
14	Acupuncture with da qi sensation	St36, K3, R3, Liv3, Li4, Yin Tang	.25x25mm	20 min.	10 total 2x week	5 weeks
15	Acupuncture Reinforcing / reducing method. 2 manual stimulation per treatment	D16, D20, R6, UB23 Plus to condition Ub20, UB2\18, K3, Sp6, ST36, P6, LIv3, sishencong	None noted	15-20	24 6X week for	4 weeks
16	Electro-acupuncture 20 Hz, 1-4mA	P1- UB23 P2- R4, St25, ST29 Alternating between two	.45mmX125mm .30mmX75mm	20 Min	Daily 5X week for 4 weeks Then every other day 3X week for 2 months 3 months of follow-up	6 months
17	Acupuncture	Acu - P6, Ht7,	.30mmX30mm	20 min	2X week	7

	Sham acupuncture	Ht8, Li4, St36, Sp6, R4 Sham: Below Sp9 Above LI11 8 points around umbilicus 2 cm up 2 cm down and 3 and 6 cm lateral			for 4 weeks 1Xweek for three weeks	weeks 8 week of follow up
18	Acupuncture	D9, 3 St21, 36 Gb26, 34 Ub23,40 Sp14, 6 Liv14, Sp11 K10, 12 Tia yang Qian Zheng Zigongxue yaoyan Dingchuan jing Bi Erbai Dan nan baichongwu	None noted	3 min	1	1time
19	Electro-acupuncture 4-5 Hz, 7-8mA	R3, R4, Sp6, Zigongxue Sham	None noted	30 min	1 x day 3 days	3 days

		Houtuojiaji SJ5				
20	Acupuncture and Herbal medicine HRT	UB23, UB15, K3, Sp6, Liv3,Lu7, K6, R4, Ht6, K7, LI4 Kun Bao Wan Estrogen and progesterone	None noted	20 min	10	2 months
21	Electro-acupuncture plus manual acupuncture Sham device acupuncture	EA UB23, UB28, Sp6, Sp9 ACU P6, SJ5, D20 Sham off channels and points	Not noted	Not noted	12 session 2x week 4 weeks then 1x week for 4 weeks	8 weeks
22	EA	Abdomen KD16/ST36 area Leg ST36 area	.3mm diameter needles	30sec	Once	once
23	Electro-acupuncture 2Hz. Acupuncture	Du14 ST36 Li11 SP6 Sham 1.5 cun lateral UB57	.25X.25 steel needles	30 min.	6 session	2 weeks
24	Catgut imbedded	SP6 UB23 REN4 Plus Syndrome	catgut	2 weeks	6 Once Every other week	3 months

		differentiation points				
25	Electro-acupuncture Not note	R4 St36 Ub23 UB17 UB11 UB24 UB20 UB21	None noted	30 Minutes	1X a day for 6 days a week 72 sessions	12 weeks
26	Moxa needle	UB11 UB18 UB23 ST36 GB34 ECT.	None Noted	None noted	1X a day every other day	3 months
27	Electro-acupuncture 1-3Hz., .7 -1mA	ST36 SP6	None Noted	20 min	1X a day 56 sessions	8 weeks
28	Acupuncture plus Electro-acupuncture 10 Hz, 2mA	Acu ST35, UB20, UB23 EA UB20 UB23	None noted	30 min	1X a day 14 sessions	14 days
29	Acupuncture Non-point	UB23 2pts on hypochondria	None noted	None noted	1X day 56 sessions	8 weeks
30	Acupuncture Diethylstibestrol	UB 11, 23, 20	None noted	30 min	1X day 60 sessions	60 days

Appendix J: Studies Outcomes

6. Studies Outcomes

Study #	# of Subjects	Type of subjects	System studied	Bio chemical markers measured	Treatment intervention(s) $X_1, X_2, X_3...$	Outcome results	Results related to BMD
1	50	Rats	BMD	BMD BMC IGF-I IGF-BP Estradial	X_1 Intact – nothing done to them X_2 2- Sham – removed fat from ovaries then nothing done to them X_3 Model – Removed ovaries then nothing done to them X_4 -estrogen-removed ovaries – given .9mg/week/100 g rat weight X_5 - EA - ovaries removed – Elector acu daily for 4 weeks, 30 min day, GB39 and Du4, 2 Hz, 1mA intensity	EA increase BMD L1-6, increase in IGF_I and BP E- increase BMD right thigh and estradial	Through IGF-I Ea can increase BMD.
2	43	rats	Bone Sympathetic nervous system	BMD Vertebrae Femora Tibia BP – Ht rate	X_1 – Sham operation X_2 – Model ovaries removed no treatment X_3 –	Increase BMD but not in bone mineralization , Increase in uterine	Needling increased BMD through suppressing bone resorption and also effected the

				Noepinephrine Deocypyridinoline Osteocalcin Bone Histomorhpolgy Uterine weight Body weight	Propranolol – ovaries removed- given 2.8mg/d X_4 – FCS – ovaries removed, given ethanol extract Fructus citri Sarcodactylis .9 g/kg Dailey X_5 – Needling – ovaries removed needled p6, sp6, 5 days week 15 min	weights of treatment groups Lower Ht rate, and Noepinephrine levels Lower deoxypyridinoline and osteocalcin	sympathetic nervous system causing calmer body to encourage bone formation.
3	35	Rats	BMD	Urinary calcium Phosphorus Deoxypridinoline BMD Bone Ash Weight Trabecular thickness Number Separation Trabecular node number Node-node struts Trabecular termini	X_1 – Sham – sham operation control group X_2 – Model – ovaries removed not treatment X_3 – Acu A – ovaries removed bilateral SP6, ST 36 – 15 min, 5 days a week. X_4 - Acu B – Ovaries removed, bilateral needles UB20, UB23, 15 min 5 days a week.	Body weight slightly higher in ovariectomy groups acu groups higher than model BMD – Lumbar – Acu A had greater increase then Acu B and both acu groups higher than model but less than sham. Tibia – Acu A group had higher BMD than B or model Acu B	Acu a group had greater systemic effect on bone Acu B had greater effect locally. Acu increased BMD and Structure and bone formation. Acu A had effect on Gastric function increase absorption.

				Node-termini struts Cortical bone measurement Mineral apposition rate	All treatments for 16 weeks	and Model about same at end experiment. Acu A less than sham but a lot closer than other OVX groups. Femora – Acu A group higher than Model and B but still lower then sham. Ash weight – Acu A group had increase over model but still less then sham. Dpd – Higher in ovx groups but and same between Acu A and B. Phosphorus – Acu A And B were much lower than model and sham. Calcium – Acu A and B were lower than model and sham Cortical area same in ovx	

						groups and ovx much lower than sham Marrow area – higher in ovx than sham same in ovx. Mineral apposition rate Acu B higher than A and much higher than model and sham. Acu A Higher than Model and sham Model sl. higher than sham Mineral Surface – Acu A highest. Acu B higher than Sham and model. Sham and model same Bone Formation Rate – Acu much higher than sham and model. Model and sham same Bone connectivity – ovx less but	

						Acu A increased over ovx	
4	30	Rats	Bone	BMD – L5 Femur Bone Histomphomolgy Bone strength Serum Bone-specific alkaline phosphates Tartrate-resistant acid phosphatase Gene Expression LRP5, B-catenin	X_1- Sham operation, no treatment control X_2 – OVX with no treatment X_3 – OVX with EA 3Hz, 1mA at SP6, ST36, 30 min, 5 times a week for 12 weeks	Increase in BALP over sham and model Decrease of TRACP5b over model and increase over sham BMD increase femoral metaphyseal and L5 over model but lower than sham mid-shaft femur same on 3 groups Load strength of femur increase in EA over model Load and Energy to failure significant increase over model EA increased Trabecular area, width, and number over model and	

						decreased Trabcular separation over model EA increased LRP5 and b-catenin sign over model. model and EA sig increase over sham	
5	32	Rabbit	Cartilage	MMp-13 Weight Estradial 17B	X_1 – Cotrol – nothing done to them X_2- Model - Ovariectomy with no tx X_3 – Est – Ovariectomized and given .625 mg/day 5 x/week for two weeks X_4 – EA – Ovariectomized and needle UB18, UB20, SP 6 with Electro-acupuncture at UB23 and ST36, 10Hz, 1-5mA. 5x/week for two weeks.	17B estradial levels were highest in model group. EA and Est levels were sl. Higher than control group. EA group was the highest level Cartilage damage levels were higher in OVX groups but EA and EST lower than model. MMP-13 OVX groups were higher than Control EA and EST were lower than model. Est was lower than EA. Body weights EA was lower	Higher 17B estradial helps maintain bone health so EA had a great effect on this Lower body weight of EA rats helps maintain systemic heath. MMP-13 being higher helps in bone turn over for MMP-13 is part of cleavage process in repairing and building bones. It was higher than control but lower than model helping maintain a healthier

						than all other groups OVX was the highest and Est and Control were the same	level.
6	93	Human femal e	Reproduction and Bone	FSH LH Testostrone Osteocalcin PTH Calcitonin Estradial Alkaline phosphates	X_1-Normal- no treatment childbearing women with normal cycle X_2 – ControL Given daily capsule of Ethinyh, provera, vitamin A, D2, E and Calglucan. X_3 – Catgut embedding once every two weeks. UB23, SP6, R4 every patient every time then SX of Gan Shen Yin Xu received UB18 SX of Pi She Yang Xu Received ST36, UB20 Treament lasted for 3month on all groups	All levels were higher than Normal group. Comparison is from before treatment to after treatment. LH Was lowered Acu was lowest. E2 was higher Control Was Higher. FSH Was Lower and Control was Lower. Testosterone was Higher and Acu was highest Osteocalcin was lower similar in both Calcitonin was lower similar in both groups PTH was Lower with	Bone turnover rate was reduced. Bone resorption was more than Formation. Bone loss was prevented

						Acu be much lower than control Alkaline Phospatase was lower with Acu lowest.	
7	58	Rats	Menopause	Estradiol Testosterone Corticosterone Gonadotrophin Vaginal epithelia number Corticotrophin releasing hormone Aromatase	X_1 – Int – intact no treatment X_2 – Int-EA – specific point Tx – REN3, REN4, Sp6, ZiGongxue, 3Hz 1-2 mA X_3 – OVX – Ovaries removed and no treatment X_4 – OVX-EA – Ovaries removed specific point tx REN3, REN4, SP6 one side, ZiGongXue, 3Hz, 1-2mA X_5 – OVX EA sham – Ovaries. removed, sham points, HauTuoJiaJi T-3-5, Sj5 one side 3Hz, 1-2mA	E2 increased Significantly over ovx but less than INT Testosterone Decreased compared to Int and OVX. Corticosterone Increased over all other groups GnRH was lower than OVX but higher than INT Aromatase Increased significantly over ovx and Int for both SA adipose and Liver	Increase in estradiol helps to slow the osteoclasts. Aromatase increase helps with estrogen biosynthesis of estrogen. With increased adrenal suggest activation of adrenal function. Corticosterone increased in ovx-EA suggests EA stimulates inner adrenal cortex cells then transformed to estrogen. CEH increase influence female repo system and

							hence estrogen.
8	63	Human	RA	TNF-α Vascular endothelial growth factor	Both groups received support the Yang points selected form D20, GB20, Li11, SJ6, R4, ST36, GB34, GB39, SP6, UB23, UB20 ect. Plus local ashi points for 30 minutes every other day for 10 times per course for round of 3 courses. Needles were thruster, lifted and twisted to achieve Da Qi. X_1 – EA- Received support the Yang needling. The first 15 minute EA was applied to Local ashi points, then patient was flipped and received 15 minute of treatment on UB20 and	Both TNF-α and VEGF blood and synovial levels were lower in both groups after treatment. EA Was lower in TNF-α in blood level than SN and lowering value in both for TNF-α was same in Synovial fluids VEGF blood level was significantly lower than SN groups for both blood and synovial but considerably more in synovial.	TNF-α stimulates osteoclast so lower levels of this means less bone resorption. VEGF cause vascular hyperplasia causing cartilage destruction

					UB23 for 15 minutes. X_2- Received simple needling same as EA but with no EA		
9	40	Rats	Bone	Osteoblast Bone Morphogenic Protein 2 (BMP-2) Fracture healing distance	Both groups had their Fibula osteotomy to create 2 mm gap. X_1 – EA Received local EA to fracture site needle touching periosteum. 5 days a week for 6 weeks. 50 Hz for 20 minutes X_2- Control – received no treatment	EA Increased number of osteoblast Increased double over control number of BMP-2. Increased the healing by decreasing the fracture gap for 2mm to .48 mm. Interestingly the control group gap increased .34 mm.	EA increased the number of osteoblast which increase the bone building and reduced the gap. EA increased the BMP-1 protein which is needed to promote bone formation.
10	30	Rats	Bone	Energy to failure Callus of tibia Longitudinal tibia length.	All groups had fracture of the tibia preformed just below the knee X_1 – Control had no treatment done. X_2 – EA group had needle inserted at	EA group showed increased healing of fracture over the other two groups almost double. EA showed increased longitudinal length and callus of the tibia over the	Increased bone formation from EA could be very beneficial to BMD on OP. Sham did not show any real increase over the control maybe needed more manual

					fracture and 15 mm above fracture site touching the periosteum. 50 Hz EA at 20 Ua was applied for 20 minute daily for 3 weeks. X_3 – Sham – had same needling as EA group but not electricity was applied.	other two groups. The sham and Control groups had similar numbers.	manipulation.
11	40	Rats	Bone	BMD Cancullous volume Tra. Thicknes Tra. Number Surface mineralizati on Mineral apposite Osteocalcin Dpd Bone Volume Body weight Uterine Weight	X_1 – Sham operation and no treatment X_2 – OVX model – OVX and no treatment X_3 – OVX NA – OVX with needling of St 36 and Sp6 for 15 minutes 5x a week for 23 weeks X_4- OVX NB – OVX with needling opposite side of St36 and SP6 for 15 min. 5 x a week for 23 weeks X_5 – OVX NC –	OVX NA increased osteocalcin over all other groups. OVX NA had lower levels of Dpd than all the groups and OVX NC had the highest and only slightly higher than OVX model group. EA BMD was higher than other ovx group but lower than Sham group. BMD of Femur was	OVX causes decrease in BMD OVX NA has increasing effect on the deficiency of ovx cause op.

				Single and double perimeter	Needling ST36 and Sp6 hitting the bone for 15 min 5 days a week for 23 weeks. Needles were manipulated with reinforcing/reducing method	higher in OVX NA groups over other OVX groups but lower than Sham. BMD of L4-6 was higher than other OVX groups and significantly higher than OVX NC. Also significantly lower than sham group. OVX NA was higher in mineralizing surface, bone formation rate, mineral apposite than all groups and significantly higher than OVX NC groups. Trabcular thickness and number were all higher in OVX NA group compared to other ovx groups. Body weight of OVX NA Was lower than the	

						control but higher than all groups and significantly higher than sham. OVX Na had less uterine atrophy than other acu groups.	
12	50	Rats	Immune	TNF-α IL-6 TNF-γ IgG IgM	X_1 – Normal – No injection or treatments X_2 – Control – Injection of collagen to induce arthritis X_3 – NR – Injection of collagen. Needle retention – Needle St36 3 times a week for 15 minutes. For 9 weeks X_4 - EAI – Injection of collagen. Then Needle St36 and E-stime applied to needle with a probe attached to ST41 for 15 minutes, 3 times a week for 5 weeks.	EA lowered IL-6, TNF-α significantly over control and NR groups. But not as low as normal group. EAII was lower than EAI but not statically significant.	Lowering of TNF-α and IL-6 lowers the proliferation of osteoclast and hence slow bone resorption.

					X_5 – EAII - Injection of collagen. Then Needle St36 and E-stime applied to needle with a probe attached to ST41 for 15 minutes, 3 times a week for 5 weeks.		
13	60	Rats	Menopause	Estradiol Corticotrophin Testosterone Carticosterone SA adipose aromatase Liver aromatase Vaginal epithelial cells GnRH	X_1- INT - Intact no treatment X_2 – INT-EA - intact with EA to R4, R3, Sp6 unilateral, zigongxue bilateral. 3Hz wit 1-2 mA for 30 min a day for 3 days. X_3 – OVX - Ovariectomized with no treatment X_4 – OVX-EA – ovariectomized with EA to R4, R3, Sp6 unilateral, zigongxue bilateral. 3Hz wit 1-2 mA for 30 min a day for 3 days. X_5 – OVX-Sham	E2 was increased in OVX-EA over OVX groups but lower than INT. T was decrease in OVX-EA over all groups Cortictrophin was higher in OVX-Ea over all groups. Corticosterone was increased in OVX-Ea over all groups. Mature epithelial cells were increased in OVX-EA group but still lower than INT	Increase in estrogen will help maintain bone health. Increase in SA- Adipose and Liver Aromatase increase the amount of serum estradiol this allows the balance to be restored in the loss of estrogen fro ovaries. Estrogen helps slow osteoclast

					– Ovariectomized with EA 3 Hz 1-2 Ma on Hautoujiaji T3-T5 unilateral SJ5 for 30 min a day for 3 days.	groups. GnRH was increased over OVX groups but lower than INT groups. SA Adipose and Liver Aromatase activity was significantly increased of all groups	
14	53	Human female	Menopause	Estradiol FSH LH	X_1- ACU - Acupuncture group received acupuncture on ST36, KD3, Liv3, Li4, Yin tang for 20 minute 2x a week for 10 sessions. Needled to acquire Da Qi. X_2- SHAM – Sham acupuncture group received sham needle acupuncture on ST36, K3, Liv3, Li4, Yin tang for 20 minute 2x a week for 10 sessions. Needled to acquire Da Qi.	ACU had increased in E2 after first treatment and still increased thru to the last treatment. Sham had a drop in E2 after 1st treatment and the slight increase by end of treatments. FSH was significantly lowered in ACU but not sham. LH was lowered in Acu after 1st treatment but	This showed that Estradiol was increase with acupuncture which helps slow osteoclast production. Also shows that true needling is more effective than sham.

						then stayed the same to end of tx. Sham increased after 1st tx and then stayed the same.	
15	65	Human Female	Menopause	Estradiol FSH LH	X_1 – ACU – received acupuncture on do20, d16, R6 and UB23 for all in group and then points selected to patient condition from the following. UB20, 18, K3, Sp6, ST36, PC6, Liv3, sishencong. All receive 15-20 minutes with 2x manipulation per session with 6 session's week for 4 weeks. X_2- MED – Received oral oryzanol .01-.02g, vit. B1 .01-.02g, bit E .5-1g daily for 4 weeks.	Acu group had significantly increased in E2 and significantly decrease in FSH and LH. MED group did not have as good of results in lowered E2 and LH and increased FSH. ACU had effective rate of 97.14 15 cured 16 markedly improved 6 improved 1 failure MED group had 83.33 effective rate	ACU improve E2 levels which slow bone resorption and help to maintain bone balance. ACU seems to be better than oral medication for adjusting hormone levels and alleviating menopause symptoms.

						3 cured 8 markedly improved 14 improved 5 failure	
16	11	Human female	Gyno	Estradiol FSH LH	Received daily 5X week for 4 weeks then, every other day 3Xa week for 2 month and then follow-ups for 3 months. All received the following two treatments alternating every other TX. EA 20Hz 1-4mA. Pt.1- UB33 Pt.2 – REN4, ST25, ST29.	Estradiol increased significantly and stayed high through TX and follow-ups FSh deceased significantly and stay low thru tx and follow-ups LH deceased and stayed low thru tx and follow-ups	Higher Estradiol keeps osteoclast in check so there is less break down of bone Lower FSH again keeps osteoclast activation down and improves bone strength.
17	54	Human female	Menopause	Estradiol FSH LH	X_1- ACU- Received true acupuncture at p6, HT7, HT8, LI4, ST36, SP6, REN4. X_2- Sham- received acu off channels below SP9, above LI11, 8 points 2cm above and	Estradiol increased in acu group and decreased in the sham group. FSH decreased in the acu group and increased in the sham group.	Higher estradiol and lower FSH helps to maintain healthy bone turn over. Very interesting that Estradiol decreased significantly in sham group

					2cmm below umbilicus and 3 and 6 cm lateral to umbilicus All TX were 20minute, Da qi achieved, 2X week for 4 weeks then 1Xweek for 3 Weeks. And 8 week follow ups	LH decreased in acu group and unchanged in sham group.	and LH increased this is not normal results for acu show that point location and selection is important for good results.
18	60	Rats	Nuero	GnRH Paraventricular nucleus	Sacrificed and brain wired with micro electrodes to record hypothalamus neuron activity of D9, 3 ST21, 36 GB26, 34 UB23,40 SP14, 6 Liv14, Sp11 KD10, 12 Tia yang Qian Zheng Zigongxue yaoyan Dingchuan jing Bi Erbai	Points on level with reproductive organ had most stimulatory effect. Points on lower abdomen 183.56% and lower limbs 180.31% Thoracolumbar only had 83.31% stimulation of GnRh neuron Points that had best effect R4 262.91% Sp6 221.79% Zigongxue 266.67%	Helps to show that specific acu points effect the hormone regulation from paraventricualr zone of the hypothalamus this has an effect on balance the hormone that effect bone maintenance and health.

					Dan nan Baichongwu	St36 200% Sp11 185.86% GB34 190.53% Yaoyan 184.07% Dannan 191.56%	
19	18	Rats	Gyno	Estradiol LH Corticosterone GnRh E2 receptor AgNOr # Adrenal Weight Epithelium cells B-endorphin ER mRNA	X_1- OVX – Ovariectomized no treatment X_2- OVX-EA Ovariectomy with EA 1x day for 3 days REN3, REN4, SP6, Zigongxue 30 min of 3Hz EA. Then another 3 days control points houtoujiaji, Sj5 EA 3Hz daily for 3 days X_3- Int – Nothing done to them X4- INT-EA – intact with EA 1x day for 3 days REN3, REN4, SP6, Zigongxue 30 min of 3Hz EA. Then	Estradiol increase 6.13 points. Corticosterone increased 1.12 points and OVX increased 1.28pts. OVX-EA had increase in vaginal epithelium. OVX-Ea had increase in adrenal weight. OVX had significant increase in AgNor number and ER MRNA 199.25 compared to 129.75 ovx and 87.25 INT OVX-Ea increase in ER receptor over	Compensatory mechanism of the adrenal to produce more estrogen in the deficiency after ovariecotomy Show that the EA helps the body to restore balance in hormones and hence maintenance of bone health.

					another 3 days control points houtoujiaji, Sj5 ea 3Hz daily for 3 days	OVX	
20	57	Human Female	Menopause	Estradiol FSH	X_1- CHM – received 5g 2x day of Kun Bao Wan formula for 2 months X_2 – CHM+ACU received 5g 2x day of Kun Bao Wan formula for 2 months plus 10 sessions of acupuncture for 20 minutes at UB23, UB15, KD3, SP6, Liv3, Lu7, KD6, REN4, HT6, KD7, LI4 X_3- HRT received hormones Conjugated estrogen and medroxy progesterone acetate for 2 months.	All tx increased estaradiol with HRT have the largest increase at 27.83Pg/mL CHM+ACU had the lowest at 20.42Pg/mL and CHM similar to HRT at 26.9Pg/mL FSH was decreased in all groups with greatest at 33.4IU/L for HRT then CHM+ACU at 14.18IU/L and CHM having the least effect only decreased 8.74IU/L	This shows that CHM+Acu had similar effect on hormone replacement as HRT with les side effects. The ratio was similar to HRT but in some ways better for bone health. Having higher estradiol keeps number of osteoclast down and having higher level of FSH keeps the new osteoclast formation in check making for less bone resorption.
21	84	Human female	Gynecology	FSH LH	Both groups received 2 tx a week for 4 weeks and	True had increase of FSH and sham remained the	Lowering of FSH causes there to be less

					then 1 tx a week for 4 weeks. For a total of 12 sessions over 8 weeks X_1 – True – received EA on UB23, UB28, Sp6, Sp9 and manual manipulation on P6, SJ5, D20 X_2 – Sham had park sham device on point of the extremities off meridians and away from know points.	same True had a decrease in LH But sham had a higher decrease	osteoclast production and hence less bone breakdown.
22	23	Rats	Gynoc	Ovarian blood flow Mean artial blood flow	X_1 – Intact2 - needle during estrus and diestrus cycle S25 and ST36 areas 30 sec of 2hz EA X_2 – Intact10 - needle during estrus and diestrus cycle S25 and ST36 areas 30 sec of 10 Hz EA X_3 – Intact80 - needle during estrus and diestrus phases cycle	No real difference between the Estrus and Diestrus phases. Frequency did effect change 10 and 80 htz had greatest effect on OBF causing a decrease and increase MAP with stimulation. 10 and 80 Hz in Hind leg same.	Shows that Sympathetic nerve is efferent pathway EA stimulate of increase blood flow to the ovaries and higher frequencies had a greater effect than 2 hzs. Stimulation of ovaries may influence the hormone levels of estradiol and hence bone

					ST25 and ST36 areas 30 sec of 80 Hz EA X_4 - – Severed2 -needle during estrus and diestrus cycle ST25 and ST36 areas 30 sec of 2hz EA after sympathetic nerve was severed X_5 – Severed10 - needle during estrus and diestrus cycle ST25 and ST36 areas 30 sec of 10 Hz EA after sympathetic nerve was severed X_6 – Severed80 - needle during estrus and diestrus phases cycle S25 and ST36 areas 30 sec of 80 Hz EA after sympathetic nerve was severed	80 in abdomen had great decrease than significant increase over 10hz but both more than 2 Hz. Once nerve was severed OBF was effected but very little compared to before. MAF stayed about the same after nerve severed	formation.
23	90	Human	Immune	TNF-α CRP	All groups were needled 3x week for 30 minutes for 2	No real change in levels of TNF-	Acu is a regulatory system if there is no

					weeks for total of 6 sessions. No manipulation X_1- DU14 – X_2- LI11 X_3 – ST36 X_4 –SP6 X_5 – Sham – 1.5 cun lateral UB57	α or CRP Except Sham group showed significant decrease in CRP over other groups.	problem then there is nothing to regulate. But most groups did show a slight decrease in TNF-α This is good for bone for TNF-α causing increase in OC
24	65	Human female	Bone	Bone gla protein Calcitonin PTH Alkaline phonsphatase Estradiol	X_1 – Catgut implantation at SP6, UB23, REN4 plus syndrome differentiation points once every 2 weeks. X_2 – Med – oral admin of Fu fuchun capsule once a day. Both groups were treated for 3 months	Catgut effective rate was 93.9% and Med group was 96.9% Bone gla protein, calcitonin, PTH, and alkaline phaspatase had significant changes but abstract did not specify how much or which way. Estradiol increased in both groups with medication group better than catgut	Lowering Estradiol lowers number and activity of osteoclast thereby slowing bone resorption. Catgut having lower adverse reactions makes it safer course of treatment.

						group. But adverse reaction was lower in catgut group over medication group	
25	60	Rats	Bone	Estradiol TNF-α Osteocalcin Alkaline phonshatase Deoxyridinoline Creatinine Carboxyterminal propeptide Calcium	X_1- Model – no TX All needle treatments are 1X a day for 6 days a week for 12 weeks. X_2- EA – electro-acupuncture at UB 20, 21, 23, 24. X_3 – EAT – Tonification points. Electro acupuncture at REN4, ST36 X_4 – EAD – Blood stagnation points. EA at UB23, 17, 11 X_5 – EATD – Blood stagnation and tonification points. EA at	All EA decreased compared to the model – Deoxypyrdinoline Creatinine Alkaline phosphatase osteocalcin TNF-α Calcium All EA increased over model Eatradiol and Carboxyterminal propeptide EADT was significantly better than other EA groups in decreasing Alkaline phosphatase	All of these markers show an improvement of bone health by lowering resorption and increasing building.

					REN4, ST36, UB23, 17, 11	osteocalin TNF-α Calcium And increasing Estradiol and Carboxytermi nal propeptide	
26	40	Huma n femal e	Bone	BMD Estradiol Osteocalcin Calcium/ creatinine	X_1 – Warm needle moxibustion every other day for 3 months. Points UB11, 18, 23, ST36, GB34, ect. X_2 – Medicaiton Oral admin of caltrate with Vit D2.	BMD increased in acu group P<0.05, P<0.01 but not in the medication group. Estradiol increased in both groups P,0.01 Osteocalcin decreased in both groups P<0.01 Calcium/creat inine levels decreased in both groups P<0.05 Between the two groups osteocalcin and calcium/creati nine were	By increasing estradiol and decreasing urine calcium shows a slowdown of bone resorption and increase in osteocalcin shows and increase in bone formation. Effectively increasing bone health and slowing down the progression to osteoporosis

						significantly different P<0.05 or 0.01. Acu group was better than medication group P<0.01	
27	32	Rats	Bone	Estrin BMD	X_1 – Normal control – no tx X_2 – OVX – model ovaries removed no tx X_3 – EA – Ovaries removed and EA ST36 and SP6 every day, 20 minutes 1-3 Hz at .7-1mA for 8 weeks X_4 – Med – Ovaries removed and given 5% nilestriol 5 mL/week for 8 weeks	BMD and Estrin increased in both Med and EA groups compared to the OVX model group. P<0.01, 0.05	EA can have similar effect to BMD and estrin as medication with less adverse reactions.
28	21	Rabbits	Bone	Estradiol BMD Osteoprogerin ligand	X_1- Control – normal no tx X_2- OVX – model ovaries removed and given no tx	EA increased BMD and Estradiol over OVX group P<0.49, 0.012 and decreased osteoprogerin ligand express	This shows that EA can bring BMD to a more normal level. Increase in estradiol and decrease in osteoprogerin

					X_3 – EA – Ovaries removed and given Acupuncture ST35, UB20, 23 And Electro-acupuncture to UB23 and UB20, 10 Hz, 2mA, for 20 a day for 14 days	P<0.22	ligand shows a slowdown in bone resorption.
29	None noted	Mice – male	Bone	Testosterone Osteocalcin Bone Flexion Trabecular thickness Trabecular bone valume Osteoid volume Mineral apposition rate Bone formation rate	X_1- Control – no treatment X_2 – NP – received acupuncture on 2 points on hypochondria 1X a day for 8 weeks. X_3 – Acu – received acupuncture to UB23 1X a day for 8 weeks.	Acu group Increased testosterone and decreased osteocalcin. All the following had a increase compared to the control Trabecular Thickness 20.4% Trabecular bone volume 18.1% Osteoid volume 14.1% Mineral apposition rate 9.9% Bone formation	Shows that bone formation and architecture improved creating stronger bones. Increasing testosterone helps maintain bone formation and slows bone resorption

						rate 14.7% Bone flex of acu group improved over control and non-point 99groups	
30	40	Rats	Bone	Alkaline phosphates Tartrate-resistant acid phosphates Estradiol Bone gls protein Uterine weights	X_1 – Sham – sham operation and no tx X_2- OVX - ovaries removed and no tx X_3- Acu - Ovaries removed and Needle UB 11, 23, 20 for 30 minutes a day for 60 days with manual stimulation $X4_4$ – DES – ovaries removed and given 22.5 micro g/ml of diethylstibestrol, 1x day for 60 days	Both Acu and Des groups had similar results. Both increased estradiol and uterus weights over model. Both decreased Alkaline phosphatase, tartate-resistant acid phosphast and bone gla protein over model group.	Shows that Acu is as effective as meds in effecting bone maintenance and health by having positive effects on biochemical that effect bone turn over with less adverse reaction then medications.