Effects of a Third Year of Exercise Training Preceded by Two Years of Exercise Training with Isoflavone Supplementation on Bone in Postmenopausal Women

A Thesis Submitted to the College of Graduate and Postdoctoral Studies in Partial Fulfillment of the Requirements for the Degree of Master of Science in the Department of Kinesiology
University of Saskatchewan

by

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Abstract

Osteoporosis is a chronic disease characterized by low bone mineral density (BMD) and micro-architectural deterioration and is especially prevalent in postmenopausal women. Soy isoflavones (i.e. “phytoestrogens”) have been proposed as a safe alternative to hormone replacement therapy for preventing loss of BMD. Exercise is also recommended for slowing the loss of BMD. The present research is a one year extension of a previous randomized controlled trial which measured bone loss in women who did or did not take isoflavone or exercised for two years. The present study consisted of 86 participants from the same previous groups with: n=28 exercised and did not take isoflavone; n=26 stopped isoflavone supplementation but continued to exercise; n=18 stopped isoflavone supplementation and did not exercise; and n=14 controls who did not exercise or take isoflavone. Exercise training included two one-hour resistance training sessions and four 20-30 min brisk walking sessions per week. Areal BMD (aBMD) of the hip and lumbar spine, and fat and lean tissue mass were measured at baseline, and at one, two, and three years. There were exercise group x time interactions for total hip and lumbar spine BMD with it decreasing in the exercise groups compared to non-exercise groups (p<0.05). There were no changes in lean tissue mass but fat mass was reduced from a baseline of 25 ± 6.6 kg in the exercise group after year one and this was maintained into year three (24 ± 7.1 kg) (p < 0.01). The fat mass in the non-exercise groups increased from baseline (28.9 ± 6.7 kg) to year three (30.3 ± 7.1 kg) (p < 0.01). There were also exercise group x time interactions for trunk and percent fat with more decreases in the exercise compared to non-exercise groups. There were exercise x time interactions for muscular strength, with chest
press (upper body) and hack squat (lower body) strength 33% and 88% higher in exercise groups than non-exercise groups by the end of three years (p<0.01). We conclude that exercise and removal of isoflavone supplements did not stimulate or maintain aBMD beyond the first two years of training, but exercise did reduce whole body fat mass, trunk and percent fat, and led to increased muscular strength.
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My thanks and appreciation also to Dr. Jonathan Farthing, and to all of the faculty members in the College of Kinesiology, University of Saskatchewan. Lastly, I would like to thank the external reviewer whose comments and suggestions helped to further refine this thesis.
Dedication

I dedicate this thesis to my mother, father, and my brother Munir, without whose endless support, care, love, optimism, generosity and patience, I would not be the person I am.
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<td>1RM</td>
<td>One Repetition Maximum</td>
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<tr>
<td>ANOVA</td>
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<td>aBMD</td>
<td>Areal Bone Mineral Density</td>
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<td>CSMI</td>
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<td>CVD</td>
<td>Cardiovascular Disease</td>
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<td>DXA</td>
<td>Dual-Energy X-Ray Absorptiometry</td>
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<tr>
<td>HRT</td>
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<td>pQCT</td>
<td>Peripheral Quantitative Computed Tomography</td>
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<td>SD</td>
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<td>SPSS</td>
<td>Statistical Package for the Social Sciences</td>
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<td>Z</td>
<td>Section Modulus</td>
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<td>WHO</td>
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1. Introduction

The skeleton, comprised of bone, is the scaffold on top of which the rest of the human body forms. Bone is a complex nanocomposite structure composed of both organic and inorganic entities (Wang et al., 2014). The organic portion is largely type I collagen arranged into nanofibers ranging from 50 to 500 nm in diameter (Kadler, Holmes, Trotter, & Chapman, 1996) while the primary inorganic portion consists of hydroxyapatite crystals which are embedded between the collagen fibers (Wang et al., 2014). This interweaving of the organic and inorganic components provides architectural strength. Formation of bone, which is the deposition and resorption of the organic and inorganic components noted above, begins during fetal growth and continues into the second and third decade of life (Weaver et al., 2016). During childhood, bone mass develops slowly but accelerates prior and during pubertal growth spurt (Weaver et al., 2016). Peak bone mineral accretion rate occurs at approximately 12 years of age in girls and 14 in boys while approximately 95% of the total adult bone mass will have been acquired approximately four years after the peaks (Weaver et al., 2016). One of the most important factors in bone development is that it is sensitive to loading so that physical activity promotes bone modeling while a lack of activity slows bone accretion rates while also compromising its strength and flexibility (Rubin & Lanyon, 1984; Weaver et al., 2016).

During aging, especially after the age of 50 (postmenopausal age), besides a decrease in muscle mass, there is also deterioration in bone mass and a weakened bone tissue microarchitecture which leads to bone fragility and a greater susceptibility to fractures (Figure 1) (Consensus Development Conference, 1993). This loss of bone tissue is termed osteoporosis, or “porous bone”, with the World Health Organization (WHO)
defining it as when areal bone mineral density (aBMD) is less than 2.5 standard deviations (S.D.) below that of the sex-specific mean of young adults (World Health Organisation, 1994). In Canada, there is a high incidence of hip and other bone fractures as a result of osteoporosis. In 1981, it was estimated that 20,000 people had hip fractures and this had increased to 27,342 in 1995, with 73% of the hip fractures occurring in women (Lorrain et al., 2003). However, between 1996 and 2005, fracture rates have declined annually by 2.4% in both men and women (Leslie et al., 2009). Although this may be encouraging, it should be noted that currently seniors comprise 16.9 percent of the Canadian population and this is projected to increase to 23 percent by 2031 (Sinha, 2013). This means that fracture rates due to osteoporosis may increase in the future. Moreover, these statistics mask the fact that there are minority populations within Canada who have significantly higher rates of fractures as compared to their average Canadian cohorts (Leslie et al., 2004). In First Nations Canadians, the standardized incidence ratios for each fracture type, as compared to average Canadians, were 1.93 for spine, 3.01 for wrist, 1.88 for hip, and 2.23 for all types of fracture (Leslie et al., 2004).
Osteoporosis is a chronic illness that affects a large proportion of adults over the age of 50 with incidence rates being higher in women than men. In Canada in 2011, there were approximately 164,763 people who had a fracture with 69% of them being women while the rest, or 31%, were men (Hopkins et al., 2016). Of these, the number of fractures directly attributable to osteoporosis was estimated to be 131,443 (81%) with women twice as likely to have fractures as compared to men (Hopkins et al., 2016). Moreover, the fracture rates are positively correlated with age so that the elderly are more likely to have any types of fractures as compared to their younger same sex cohorts. Osteoporosis and the resultant fractures place a substantial economic burden on Canadian society. Acute care costs were estimated to be $1.5 billion (18% increase since 2008) in 2011 and when outpatient care, prescription drugs, secondary admissions, and indirect costs are added, the overall yearly cost of osteoporosis was estimated to be $4.63 billion (83% increase since 2008) (Hopkins et al., 2016).
As one approaches the age of 50, there is a decline in bone formation, increased bone resorption, and loss of bone mass (Figure 2). This loss of bone is especially marked in women as compared to men. Women begin losing bone around the age of 35 at a rate of 0.5% to 1% annually and this accelerates to 3% to 5% per year in the decade after menopause (Josse et al., 1996). One of the main reasons ascribed to why women lose more bone mass as compared to men, especially after menopause, is due to a decrease in estrogen levels. It is known and generally accepted that estrogen plays a significant role in regulating bone homeostasis, especially given the fact that there are estrogen receptors on osteoblasts as well as osteoclasts (Lerner, 2006; Lindsay, 1995). Estrogen inhibits osteoclasts from dissolving and resorbing bone, and therefore increases or maintains bone structure and mass (Lerner, 2006). It is thought that estrogen causes apoptosis in osteoclasts and indirectly promotes osteoblast proliferation which then allows the human body, especially in females, to maintain or increase bone formation (Wang et al., 2015). In fact, one of the therapies used to stabilise osteoporosis is estrogen therapy (Klein-Nulend, van Oers, Bakker, & Bacabac, 2015).
Figure 2: Bone mass across the male and female lifespan. Figure from (Weaver et al., 2016).

Osteoporosis is also common in postmenopausal women because concomitant with hormonal changes, such as a decrease in estrogen, there is reduced mobility. As noted above, bone formation is stimulated by mechanical load and stress and because this becomes reduced with age, the result is a decrease in bone formation (Klein-Nulend, Bacabac, & Bakker, 2012). It is known that stress from exercise subjects bone to strain and this results in a biochemical signal to stimulate osteocytes and initiate bone formation and remove damaged bone (Frost, 1992). However, postmenopausal estrogen deficiency causes bone to be less sensitive to mechanical force, resulting in decreased BMD even with a constant load. Therefore, a combined therapy of exercise and estrogen replacement could be the optimal intervention to maintain bone mass and offset bone loss in postmenopausal women.
One promising treatment for osteoporosis, or to at least maintain adequate BMD, is the use of phytoestrogens (Wang, Sathyamoorthy, & Phang, 1996). Phytoestrogens are naturally occurring, plant produced bioactive compounds which have antioxidant and antibiotic properties, as well as the ability to decrease male fertility in herbivores as a defense against herbivore overpopulation, and therefore overgrazing of plants (Aresta, Cotugno, Massari, & Zambonin, 2017). Depending on the structure of the phytoestrogen, they are classified into a number of different groups and include lignans, found in flaxseed and wheat, and isoflavones which are found in high concentrations in soybeans (Poluzzi et al., 2014; Upmalis et al., 2000). Some phytohormones are known to interact with the α and β estrogen receptors in the human body (Sirotkin & Harrath, 2014) and trigger a biological response similar to that caused by natural estrogen (Rafii, 2015). The reason for this is due to the remarkably structural similarity between phytoestrogens and estrogen. Isoflavones act as natural selective estrogen receptor modulators by acting as agonists for estrogen receptors on bone and antagonists for estrogen receptors on breast tissue (Wang et al., 1996). These properties have made isoflavone supplementation an attractive alternative to estrogen therapy for post-menopausal women. However, isoflavones are, at best, currently considered only as a second-line therapy after HRT (Brown, Josse, & Canada, 2002).

The present study is an extension of a previous study (Chilibeck et al., 2013) on the effects of combining an isoflavone supplement with exercise, and with isoflavone and exercise alone on bone mineral properties. In the Chilibeck et al., (2013) study, women were randomly placed into four different groups: exercise only, isoflavone only, combined exercise and isoflavone, and control (no exercise or isoflavone). It was hypothesized that the combined isoflavone and exercise training would have a positive and additive effect on
BMD of the proximal femur and a smaller effect on the lumbar spine. Chilibeck et al., (2013) reported, however, a negative outcome on bone mineral properties when isoflavone was combined with exercise training for two years (i.e. there was a negative interaction when isoflavone supplementation was combined with exercise), while the isoflavone or exercise alone improved bone mineral properties. The main aim of the present study is to assess the effect of an additional year of exercise training with cessation of isoflavone therapy. To the author’s knowledge, this is the first ever research study which evaluates, over three years, the effects of exercise in women who are confirmed to be postmenopausal and are not undergoing any medicinal therapy. Eighty-six out of 298 original participants volunteered for the additional year of exercise training, or be assessed for another year if they were not originally assigned to the exercise groups. We hypothesized that there would be an improvement in bone mineral properties, bone geometry, body composition, and muscle strength among those who exercised over an additional year as well as in the combined therapy group after cessation of isoflavone supplementation.

2. Literature Review

2.1. Post-menopausal Bone Loss

The onset of bone loss in healthy women occurs at about two years before the last menses and accelerates to a significantly higher rate four to five years after menopause (Recker, 2011) (Figure2). In early menopause, the main bone loss occurs in the trabecular bone and is thought to be caused by estrogen deficiency (Lerner, 2006). As previously noted, bone loss is thought to be due to increased activity by osteoclasts whose primary function is to dissolve and resorb bone. When young, the osteoblasts and osteoclasts work synchronously with osteoblasts dominating thus resulting in a net bone gain. However, due
to aging and reduction in estrogen, concomitant with reduced mobility, the proliferation of osteoblasts becomes reduced thus resulting in bone resorption dominating (Klein-Nulend et al., 2012; Klein-Nulend et al., 2015; Lerner, 2006). This phase of post-menopausal bone loss continues for four to eight years and is followed by the second phase with continuation of trabecular bone and cortical bone loss. Menopause by itself is not a partition so that prior to it there is no bone loss. In fact, women begin losing bone around the age of 35 but this accelerates after menopause (Josse et al., 1996). This is likely due to a decrease in estrogen levels, which is considered to be the leading cause of bone loss and osteoporosis in postmenopausal women. With the decline in estrogen, the bone remodelling process reconfigures and results in a high rate of bone loss (Riggs & Melton, 1992). Although hormone replacement therapy (HRT) was at one time considered to be the first line of management to lessen bone loss and therefore prevent bone fractures (Torgerson & Bell-Syer, 2001), it was later found that HRT increased the risks for invasive breast cancer and cardiovascular diseases (CVD), and the risks outweighed the benefits (Poluzzi et al., 2014; Rossouw et al., 2002). Given these associated risks, it was recommended that HRT should not be undertaken (Writing Group for the Women's Health Initiative Investigators, 2002). Due to these side effects, many women are hesitant to start HRT in order to alleviate the symptoms of osteoporosis and many women have turned away from prescription drugs and towards alternative remedies (Poluzzi et al., 2014).

2.2. Isoflavones.

Phytoestrogens, including isoflavones, are naturally produced plant compounds which are non-steroidal, structurally similar to estradiol and have been shown to compete
with estradiol for estrogen receptor sites in the human body (Shutt & Cox, 1972; Verdeal, Brown, Richardson, & Ryan, 1980). They may act as agonists with respect to bone and antagonists with respect to uterine and breast tissue (Mueller & Korach, 2001). Furthermore, they have a higher affinity to the estrogen receptors specific to osteoblasts and a lower affinity for the estrogen receptors that are found predominantly in uterine tissue (Picherit et al., 2001). Given these characteristics of isoflavones, and the negative side effects of estrogen HRT, phytoestrogens have been considered as a therapeutic option for osteoporosis (Komm & Chines, 2012). In contrast to estrogen, isoflavones do not increase the incidence of breast cancer and in fact have been associated with a decreased risk for breast cancer (Ingram, Sanders, Kolybaba, & Lopez, 1997; Murkies et al., 2000; Torres-Sánchez, López--Carrillo, Ló--Cervantes, Rueda-Neria, & Wolff, 2000). Isoflavones, therefore, may act as natural selective estrogen receptor modulators by acting as agonists for estrogen receptors on bone and thereby stimulate osteoblast activities (Wang et al., 1996). Although the benefits of isoflavone supplementation may be attractive and promising, a number of recent publications and meta-analyses have critically examined if soy isoflavones are effective in treating or stabilising postmenopausal bone loss (Lanou, 2011; Taku et al., 2010). The authors of the aforementioned studies have concluded that there is insufficient evidence to recommend the use of isoflavones for bone health. In fact, there are many factors, such as menopausal status, supplement type, isoflavone dose and intervention duration, which still need to be studied in order to fully understand if isoflavones are efficacious when it comes to bone health in postmenopausal women (Wei, Liu, Chen, & Chen, 2012).
2.3. Exercise and BMD

As noted previously, osteocytes are sensitive to and stimulated by mechanical stress on bones which is exerted during movement and physical exercise and these can, by stimulating bone formation, help to reduce the risk for fractures in postmenopausal women (Kemmler, Bebenek, Kohl, & von Stengel, 2015). Exercise programs, through increasing dynamic balance, reflexes and coordination, can help to reduce falls and all fracture risks (Gillespie et al., 2012), fall impacts (Groen, Smulders, De Kam, Duysens, & Weerdesteyn, 2010), and increase bone strength (Marques, Mota, & Carvalho, 2012). Exercise training has been known to improve BMD and neuromuscular function as well as reduces the number and severity of fractures in older adults. Furthermore, exercise contributes to a decrease in age-related bone loss as well as in promoting faster recovery if there has been a fracture (Kemmler & von Stengel, 2014). Indeed, exercise is a highly effective prescription for improving or stabilizing BMD, reducing falls and fracture risk, and in the recovery of osteoporotic fracture in postmenopausal women (Kemmler et al., 2015).

The most recent systematic review of exercise and BMD in postmenopausal women showed that bones' adaptive response to exercise is significantly more favorable when exercising >2–4 times a week as compared to exercising <1.5–2 times per week (Kemmler & von Stengel, 2014). In addition, it has also been noted that exercising six hours per week is needed to maintained BMD (Karlsson, Magnusson, Karlsson, & Seeman, 2001). Moderate-impact aerobic activity (e.g., walking, jogging, skipping, hopping) has long-term benefits in that it helps to prevent osteoporosis in postmenopausal women (Cussler et al., 2005). Moreover, mechanical vibration has also shown to be beneficial on bone microarchitecture, bone density and strength, as well as in elevating physical function and
mobility (Moreira et al., 2014). As one gets older, bone turnover (e.g. bone formation and resorption) slows but with exercise, the BMD increases by about 1-2% per year (Bonaïuti et al., 2002). However, a 5% increase in BMD is necessary for a clinically significant reduction in the risk of a fracture (Guyatt et al., 2002).

While Chilibeck et al., (2013) studied the effects of isoflavone and exercise on BMD, no study has investigated the effects of a resistance-training program greater than two years in duration on BMD in postmenopausal women (Howe et al., 2011). The present study is especially relevant as a recent meta-analysis reported favorable outcomes of exercise on nearly almost all fracture risks (Kemmler, Haberle, & von Stengel, 2013). Furthermore, an exercise regime is currently considered to be a first line of treatment in stabilizing and preventing osteoporosis in postmenopausal women (Papaioannou et al., 2010).

2.4. Exercise and Bone Geometry

Most studies which measure the effects of exercise on bone density in postmenopausal women utilize aBMD as their primary indicator. Some studies have noted that in some exercise studies, aBMD does not vary and that there were no changes in bone mass. This is somewhat misleading as a recent review of aBMD and exercise noted that aBMD may not fully capture the benefits of exercise on bone (Harding & Beck, 2017). The primary reason for this is that bone is three dimensional while DXA scans, which are the primary mode of measurements for bone density, provide only planar data. Furthermore, two dimensional data can lead to further inaccuracies because the relationship between bone area and volume is non-linear thereby leading to underestimation as to the effects of
exercise. This means that studies in postmenopausal women and exercise are overlooking bone morphology and geometry, which requires a volumetric measurement of BMD in g/cm$^3$ and would be more representative and accurate a criteria as compared to areal BMD (g/cm$^2$) (Harding & Beck, 2017). This means that exercise is having an effect on bone morphology and geometry but the DXA is unable to capture it.

In another review of the literature, but with a focus on studies which measured the effects of exercise on both bone mass and volume using DXA and peripheral quantitative computed tomography (pQCT), the authors reported that in nearly all of the studies analyzed there were positive changes in bone mass and geometry as measured by the latter even though DXA data showed little to no change (Hamilton, Swan, & Jamal, 2010). Although pQCT has its limitations, one being the inability to measure sites which are more clinically relevant to fractures such as the hips and the lumbar spine, it has its benefits in that it can measure bone size and shape, and volume. The authors also pointed out that in postmenopausal women, exercise effects were largely seen in cortical rather than the trabecular portions of the bones. This suggests that the instruments used to scan and collect data may be limited in some way and the full scope of changes may have been underestimated. Furthermore, it was noted that bone mass and geometric changes were highly dependent on the type of exercise the participants engaged in. Generally, the more vigorous the physical activity, the greater the benefits of exercise so that exercises which required running, bounding and jumping were much more beneficial than simple weight bearing exercises or dancing (Hamilton et al., 2010). Therefore, it has been shown that exercise does have a positive effect on bone mass and geometry but that DXA scans may be unable to measure the changes.
2.5. Exercise, Dynamic Balance and Fracture Prevention

As noted previously, as people age there is a commensurate decrease in their muscle mass and mobility, and this negatively affects BMD due to a lack of mechanical stress. Furthermore, there is a positive correlation between fracture rates and age so that the elderly are more likely to have all-type fractures as compared to people who are younger (Hopkins et al., 2016). Given these scenarios, research has indicated that one of the best interventions to prevent falls, and injuries and fractures resulting from falls, is exercise. The latter strengthens the muscles, increases balance and coordination, and the speed and effectiveness of reflexes to mitigate the injurious effects of falls, such as extending an arm to grab an object or to soften an impact. A recent meta-analysis examined 17 exercise trials which involved 4305 participants with a mean age of 76.7, and with 77 percent of the participants being women (El-Khoury, Cassou, Charles, & Dargent-Molina, 2013). The authors found that in the exercise group there was a 37 percent reduction in all injurious falls, 43 percent reduction in falls which led to severe injuries, and a decrease of 61% for falls which resulted in fractures.

Similarly, another meta-analysis consisting of a comparison of 88 trials comprised of 19,478 participants who were 65 years of age or older found that there was a 21% decrease in falls in people who exercised more than three hours per week as compared to those who did not exercise (Sherrington et al., 2016). Moreover, these reductions extended to people who had Parkinson’s disease as well as those who were cognitively impaired. In another recent study, the authors examined the effects of exercise on 70 to 80 years old women who had fallen previously (Patil et al., 2015). The authors found that although there was no decrease in the number of falls between the exercise and non-exercise group, the
women who exercised were half as likely to need medical attention as those who did not exercise. These studies show that exercise can significantly help to prevent falls and also reduce the severity of injuries and fractures if one falls.

However, the above data should be treated with caution as there have been reports that the correlation between BMD and fracture risk is not simple and that the primary reasons for fractures may be other than osteoporosis. In a population-based random sample study on postmenopausal women over the age of 60, the authors conducted aBMD scans over a number of years (Pasco et al., 2006). The authors reported that in scans conducted, with a median time of 5.6 years between scans, 37.6%, 48.0%, and 14.5% of the women showed normal, osteopenic (e.g. less than one standard deviation below peak bone mass), and osteoporotic total hip aBMD, respectively. At the study’s end, 73.1% (56.5% osteopenic and 16.6% with normal aBMD) of all low trauma fractures were reported by women without osteoporosis. Moreover, the authors noted that of the women with osteopenia who reported a low level fracture, the chance of them not having another fracture over the next five years was lower than women with osteoporosis and no fractures (Pasco et al., 2006). This suggests that the loss of coordination and mobility may play a greater risk for fractures, or that women who know they have osteoporosis are more likely to be more cautious in their activities as compared to women who are not diagnosed as having osteoporosis.

2.6. Previous Study Results.

The hypothesis of the Chilibeck et al., (2013) study, of which the present study is a continuation, was that isoflavone supplementation (165 mg total isoflavone d\(^{-1}\) or 105 mg aglycone equivalent d\(^{-1}\)) and exercise training would be additive and increase lumbar spine
and proximal femur BMD as compared to the controls (no isoflavone and no exercise), those who only exercised (no isoflavone), and those who only took isoflavone (no exercise). Contrary to the hypothesis, it was found that in the exercise plus isoflavone group, similar to that of the control group, there was a decrease in total hip BMD. Furthermore, there was a deterioration in BMD at the narrow neck and femoral shaft regions of the proximal femur in the combined exercise plus isoflavone group compared to the exercise-only group that approached statistical significance (p = 0.055). In the exercise alone and the isoflavone by itself groups, on the other hand, total hip BMD was maintained. These findings suggested that there was a negative interaction between combining exercise and isoflavone supplements. The authors hypothesized that the reason why the therapy combination led to a decrease in total hip BMD was due to the higher affinity of isoflavone to interact with the β-estrogen receptor rather than the α-estrogen receptor on bone. The α receptor is known to activate osteoblast activity (Damien, Price, & Lanyon, 2000) thereby promoting bone formation, whereas activation of the β receptor dampens or blocks the positive effects of mechanical stress on bone (Saxon & Turner, 2005). However, the reason as to why the combination therapy resulted in greater total hip BMD loss is likely to be more complex than receptor affinity. This is because if it was simply due to isoflavone blocking the stimulating mechanical stress on bone, the isoflavone without exercise group should have lost more BMD than both the control and the combination therapy group.

Nonetheless, besides the above, Chilibeck et al., (2013) also found, as could be expected, that there was a positive effect on dynamic balance and leg strength in the exercise groups compared to non-exercise groups, with a greater increase in leg strength also apparent in isoflavone groups compared to non-isoflavone groups. The stimulating
The effect of isoflavone on leg strength was thought to be due to the fact that there are estrogen receptors in muscle and the isoflavone may have led to increased muscle mass which then translated into greater leg strength.

3. Research Aim and Hypothesis

3.1 Primary Aim

The primary objective of this study was to investigate the BMD at lumbar spine and proximal femur in postmenopausal women after cessation of isoflavone therapy, but with continuation of exercise training.

3.2 Primary Hypothesis

It was hypothesized that an additional year of exercise training after cessation of isoflavone therapy, or continued exercise training for an additional year in postmenopausal women who never received isoflavone in the first study, would maintain lumbar spine and proximal femur BMD as compared to loss in the control groups who did not exercise. Furthermore, it was also hypothesized that the group that previously received the exercise + isoflavones treatment would have the greatest increase in bone measures since the inhibitory effect of isoflavones was removed for the third year.

3.3 Secondary Aim

The secondary objective was to assess the effects of exercise on bone geometry at the proximal femur.
3.4 Secondary Hypothesis

An additional year of exercise training after cessation of isoflavone therapy, or continued exercise training for an additional year in postmenopausal women who never received isoflavone in the first study, would be effective in improving bone geometry as compared to the control group who did not exercise. Moreover, it was further hypothesized that the group that previously received the exercise + isoflavone treatment would have the greatest increase in bone geometry as compared to the other groups since the inhibitory effect of isoflavones was removed for the third year.

3.5 Tertiary Aim

The tertiary objective was to assess the effects of exercise on body composition, anthropometry, balance, walking speed, and strength (bench press and hack squat strength).

3.6 Tertiary Hypothesis

An additional year of exercise training after cessation of isoflavone therapy, or continued exercise training for an additional year in postmenopausal women who never received isoflavone in the first study, would be effective in improving body composition, anthropometry, balance, walking speed, and strength (bench press and hack squat strength).
4. Methods

4.1. Participants

4.1.1. Number of Participants and Group Allocation

A total of 86 participants completed the additional year of exercise training or were part of the control group which did no training, after complete cessation of soy isoflavone supplementation. Participants were divided into four groups (Figure 3) as in the previous study: exercise, isoflavone, exercise + isoflavone, and control group. The isoflavone and control groups did stretching exercise at home as an exercise placebo. The other two groups attended the exercise training for an additional year with supervision. All participants were postmenopausal, as verified by determining levels of follicle stimulating hormone and luteinizing hormone at the start of the original study (Chilibeck et al., 2013).

4.1.2. Inclusion Criteria

Postmenopausal women

4.1.3. Exclusion Criteria for the Chilibeck et al., (2013) study were the following:

Previous fragility fractures (defined as fractures resulting from minimal trauma); having taken bisphosphonates, HRT, selective estrogen receptor modulators such as Roloxofene or calcitonin within the previous 12 months before the original study; currently taking corticosteroids; Crohn’s disease; Cushing’s disease; kidney disease, allergy to soy; severe osteoarthritis; currently involved in vigorous exercise training as defined by jogging or resistance training for more than 20 minutes per session, more than twice per week; planning to travel outside of Saskatoon for an extended period during the study; osteoporosis (lumbar spine or proximal femur aBMD 2.5 SD below the young adult mean
(i.e. T-score of –2.5 or lower); current or previous breast cancer; and current or previous endometrial cancer.

All exclusion criteria were determined by questionnaire except for osteoporosis, breast cancer, and endometrial cancer, which were evaluated by bone densitometry, mammography, and ultrasound, respectively. Participants who developed osteoporosis or started taking medications for low aBMD during the study were excluded from the study. This was required for ethical reasons as women who developed osteoporosis were referred to their family physicians for treatment. Participants were recruited into the extension study by asking them to participate in a one year extension of the previous study, but with cessation of the isoflavone supplement. A new consent form was signed for the extension study.

86 participants

Group1 - Ex
n = 28

Group2 - past Iso
n = 18

Group3 (past Iso+Ex)
n = 26

Group4 - Control
n = 14

Figure 3: Extension study participant groups

Group1-Ex= Exercise training (combined weight training and walking) plus previously received isoflavone placebo;
Group2-past Iso= exercise training placebo (flexibility program) plus previously received isoflavone therapy;
Group3-past Iso+Ex= exercise training; and previously received isoflavone;
Group4-control= exercise training placebo plus isoflavone placebo.
4.1.4 Subject Withdrawal Criteria

A participant who developed any of the conditions outlined in the exclusion criteria, or who had a serious adverse event likely related to the intervention, was removed from the study.

An attempt was made to collect all follow-up data on the participants after they were removed so data could be analyzed as “intent to treat”.

4.2. Exercise Protocol

The exercise training intervention was adopted from a previous study and consisted of weight training combined with walking (Chilibeck, 2005). The exercise training placebo consisted of a home-based flexibility program, which was found to have no effect on aBMD. Exercise involved strength (resistance, weight) training twice a week and brisk walking four times per week. The strength training and walking were combined two days per week, at which time exercise sessions were fully supervised. On two other days of the week, subjects were required to perform walking exercise on their own. Compliance to the exercise program was monitored by activity logs.

Exercises done during strength training included:

- hack squat,
- hip abduction - on a multi-hip machine,
- hip adduction - on a multi-hip machine,
- hip flexion - on a multi-hip machine,
- hip extension - on a multi-hip machine,
- hamstring curl,
- quadriceps (knee) extension,
- back extension,
- abdomen flexion,
- bench press,
- lat-pull down,
- shoulder press
- Biceps curl, and triceps extension (presses).

Two sets of 8 repetitions for each exercise were done at intensities corresponding to approximately 80% of 1 repetition maximum (1RM). The walking program involved 20-30 minutes of brisk walking per session at an intensity corresponding to 70% of age-predicted maximal heart rate (220-age). Intensity and duration of exercise was increased progressively on an individual basis. Pedometers were given to the women to track walking distance and to encourage compliance.

The exercise placebo participants were given a home-based flexibility program that involved stretching exercises for all of the major muscle groups, requiring 20-30 minutes of stretching four days per week. The exercise placebo participants were telephoned periodically to assess compliance to the flexibility program.

The duration of the extension study was one year.

4.3. Measurements

All of the measurements and protocols were adapted from Chilibeck (2005).

4.3.1. Primary Outcomes

The primary outcome was to measure the change in proximal femur aBMD at 12, 24, and 36 months.
4.3.2. Secondary Outcomes

The secondary outcomes included:

- Lumbar spine aBMD
- Whole body aBMD
- Bone geometry at the proximal femur (bone cross-sectional area (CSA), cross-sectional moment of inertia (CSMI), and section modulus (Z), at the narrow part of the femoral neck, the intertrochanteric region of the hip, and the shaft of the femur.
- Body composition by densitometry (i.e. collected at the same time as whole-body aBMD)
- Anthropometry (waist circumference, body mass, height, and body mass index (BMI))
- Balance
- Walking speed
- Bench press and hack squat strength

Changes in all secondary measures were assessed at 36 months, or one year after cessation of the two years of the Chilibeck et al., (2013) study.

4.4. Methods and Measurements

All of the methods and measurement protocols were adapted from Chilibeck et al., (2013).
Body composition was assessed with dual-energy X-ray absorptiometry (DXA) (Figure 4). aBMD of the whole body, lumbar spine (L₁–L₄ vertebrae), and proximal femur (including the femoral neck, trochanter, Ward’s, and total hip) were measured by DXA in array mode (QDR Discovery Wi; Hologic, Inc., Bedford, MD, USA) using QDR software for Windows XP (QDR Discovery, Hologic, Inc. Marlborough, MA, USA). The
coefficients of variation for these measures were 0.5% for the whole body, 0.7% for the lumbar spine, and 1.0% for the proximal femur. Lean tissue and fat mass were assessed from the whole-body scans and the coefficients of variation for these measurements were 0.5%, and 3%, respectively. Hip structural analysis (HSA), adapted from Beck et al., (2011), was used to assess structural characteristics of three regions of the proximal femur from the DXA scans: the narrow neck region, which is located across the narrowest segment of the femoral neck; the intertrochanteric region along the bisector of the neck-shaft angle; and the femoral shaft, which is located 2 cm distal to the midpoint of the lesser trochanter (Beck et al., 2011). For each region, the distribution of the bone mass across the bone is extracted, then CSA, which is equivalent to cortical area of CSMI, and aBMD (grams of bone divided by bone area) were measured. Like BMC, CSA measures the amount of bone within the cross-section but expresses the quantity in terms of cortical equivalent surface area (important for axial bone strength) rather than mineral mass. The coefficients of variation for narrow neck, intertrochanteric, and femoral shaft regions, respectively, were as follows: aBMD (1.7%, 1.3%, and 1.3%); subperiosteal width SPW (5.3%, 1.8%, and 1.2%); CSA (2.6%, 2.2%, and 1.8%); CSMI (7.2%, 4.3%, and 3.7%); Z (3.5%, 3.4%, and 2.1%). aBMD and geometric measurements of multiple areas of the proximal femur were included as outcomes because each has been associated with fracture risk (LaCroix et al., 2010; Wu, Wang, & Shyu, 2011).
Strength in the lower body was assessed by determining the 1-RM during the hack squat whereas upper body strength was determined by the 1-RM during the bench press (Chrusch, Chilibeck, Chad, Davison, & Burke, 2001) (Figure 5). Dynamic balance was measured as time taken to perform backward tandem walking (i.e., toe to heel) over a distance of 6 m on a board that was 10 cm in width and raised about 4 cm off of the ground. Number of errors (i.e., number of times the participant stepped off the walking board) during the test was also recorded as a measure of dynamic balance (Figure 5). This test is sensitive to the effects of exercise training (Nelson et al., 1994). Walking speed was assessed by timing walking over an 80 m course at a fast pace (Himann, Cunningham, Rechnitzer, & Paterson, 1988). The coefficients of variation for squat strength, bench press strength, dynamic balance (backward tandem walking time), and walking time over 80 m, were 31.3%, 8.2%, 19.3%, and 4.5%, respectively.
4.5. Participant numbers

A total sample size of 86 participants was based on volunteers from a previous study agreeing to complete an additional year of the research trial. Participants continued in the 4 groups to which they were originally randomized, except that the groups assigned to take isoflavone supplement stopped taking the supplement:

- **Group 1 (n = 28)** - exercise training (combined weight training and walking)
- **Group 2 (n = 18)** - exercise training placebo (flexibility program) plus previously received isoflavone (94 mg aglycone equivalent d⁻¹ (157 mg total isoflavone d⁻¹));
- **Group 3 (n = 26)** - exercise training; and previously received isoflavone.
- **Group 4 (n = 14)** - exercise training placebo plus isoflavone placebo.

4.6. Data Analysis

Statistical analysis of change in aBMD, bone geometry, body compensation, anthropometry and strength between the groups over a 3-year period were assessed using IBM’s Statistical Package for the Social Science (SPSS). The statistical analysis utilized a 3-factor ANOVA with a between-groups factor for exercise, a between-groups factor for isoflavone (i.e. removal of isoflavone) and a within-groups factor for time (baseline, 1 year, 2 years, 3 years). A p < 0.05 was accepted as significant. If there was statistically significant group × time interaction, Tukey's honestly significant difference (HSD) post hoc test was run on the data.
5. Results

Baseline data for the participants who took part in the extension study are presented below in Table 1. The total number of participants who agreed to be a part of the extended third year study was 86. However, the bone geometry data consisted of 68 participants as some scans were unable to be analyzed due to poor positioning during the scans.

Table 1. Baseline Data

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<th>Chest Press (kg)</th>
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<th>Balance Time (s)</th>
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<td>27</td>
<td>37</td>
<td>16.22</td>
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</table>
5.1 Bone Outcomes

For total hip and lumbar spine aBMD there was an exercise group x time interaction (p < 0.05). In the exercise groups, total hip aBMD decreased from baseline to year 2 and year 3, and lumbar spine aBMD decreased from baseline to year 3 (Table 2) (Figures 6 and 7). There were time main effects for aBMD at the femoral neck, trochanter, and whole body, with aBMD decreasing over time (p<0.01) (Table2). For most geometric measures (i.e. CSMI, section modulus, CSA, superiosteal width) there were time main effects (p<0.05) with measures increasing over time (Table2). The exceptions were an exercise group x time interaction for CSMI of the femoral shaft (p<0.05) (Figure 8) and an isoflavone group x time interaction for superiosteal width of the femoral shaft (p<0.05) (Figure 9). Post-hoc analysis showed that both the exercise groups and non-exercise groups increased femoral shaft CSMI from baseline to year 3; the exercise group x time interaction
was due to a greater increase in the non-exercise groups. Post-hoc analyses showed no difference over time for any groups for femoral shaft superiosteal width; the isoflavone group x time interaction is therefore difficult to interpret.

5.2. Balance, Exercise, Anthropometric tests, and Body Composition

There were no changes in lean tissue mass between the groups. There were exercise group x time interactions for total body fat mass, percent fat, and trunk fat mass (p<0.05) (Figures 10, 11, 12) (Table 3), with all decreasing over time in the exercise groups but with no change in the non-exercise groups.

There were exercise group x isoflavone group x time interactions for body weight and waist girth (p<0.05) (Figures 13, 14). Body weight decreased in the exercise + past isoflavone group from baseline to years 2 and 3 (p<0.01) with no changes in other groups. The post-hoc of the 3-way interaction for waist girth showed no differences over time within groups, but again it appeared this interaction was due to a decrease in the exercise + past isoflavone group with no change in the other groups.

As could be expected, the exercise groups improved significantly (p < 0.01) than the non-exercise groups in squat strength and chest press (Figures 15 and 16). There were time main effects (p<0.01) for walking time and balance time with both decreasing over time (indicating an improvement in performance across groups) (Table4). There were no significant changes in balance errors.
Figure 6: Decreased total hip bone mineral density (aBMD) in the exercise groups ($p < 0.05$).

* = ($p < 0.05$) Versus Baseline.

Figure 7: Decreased lumbar spine bone mineral density (aBMD) in exercise groups ($p < 0.05$).

* = ($p < 0.05$) Versus Baseline.
**Figure 8:** Exercise group x time interaction for cross-sectional moment of inertia (CSMI) at the femoral shaft \((p < 0.05)\)

**Figure 9:** Isoflavone group x time interaction for superiosteal width of the femoral shaft \((p < 0.05)\)
Figure 10: Decreased whole body fat mass in exercise groups.

*= (p < 0.05) Versus Baseline.

Figure 11: Decreased percent fat in exercise groups.

*= (p < 0.05) Versus Baseline.
Figure 12: Decreased trunk fat mass in exercise groups (*p < 0.05*).

* = (p < 0.05) Versus Baseline.

Figure 13: Body weight decrease in the exercise + past isoflavone groups (*p < 0.05*).

* = (p < 0.05) Versus Baseline.
Figure 14: Exercise group x isoflavone group x time interaction ($p < 0.05$).

Figure 15: Chest press improvement in exercise groups ($p < 0.05$).

(*) = ($p < 0.05$) Versus Baseline, (**) = Between-group differences ($p < 0.05$)
Figure 16: Squat strength improvement in exercise groups (exercise group x time interaction) ($p < 0.05$).

(*) = ($p < 0.05$) Versus Baseline, (**) = Between-group differences ($p < 0.05$)
Table 2. Bone Outcomes.

<table>
<thead>
<tr>
<th>Variable</th>
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<th>Control</th>
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<td>Baseline Mean (SD)</td>
<td>Baseline Mean (SD)</td>
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<td>24 mo Mean (SD)</td>
<td>24 mo Mean (SD)</td>
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<td>36 mo Mean (SD)</td>
<td>36 mo Mean (SD)</td>
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<tr>
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<tr>
<td>Lumbar Spine aBMD**</td>
<td>0.94 (0.11)</td>
<td>0.95 (0.11)</td>
<td>0.96 (0.11)</td>
<td>0.97 (0.11)</td>
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<tr>
<td>Total Hip aBMD**</td>
<td>0.88 (0.09)</td>
<td>0.91 (0.09)</td>
<td>0.89 (0.09)</td>
<td>0.89 (0.09)</td>
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<tr>
<td>Femoral Neck aBMD*</td>
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<td>0.77 (0.08)</td>
<td>0.74 (0.08)</td>
<td>0.74 (0.08)</td>
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<td>Trochanter aBMD</td>
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<td>Whole Body aBMD*</td>
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<td>1.13 (0.09)</td>
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<tr>
<td>Intertrochanter Cross-sectional area*</td>
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<td>4.91 (0.79)</td>
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<td>Intertrochanter CSMI*</td>
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<td>Shaft Cross-sectional Area*</td>
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<td>(0.67)</td>
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<td>(0.68)</td>
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<td>(0.41)</td>
<td>(0.35)</td>
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<td>(0.40)</td>
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<td>2.43</td>
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aBMD = Areal bone mineral density  
CSMI = cross-sectional moment of inertia  

*Notes, Rows demarcated with ** indicate group x time interaction (see figures).  
*Rows demarcated with * indicate a time main effect (p<0.05).
Table 3. Body Composition and Anthropometry.

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<td>Baseline Mean (SD)</td>
<td>24 mo Mean (SD)</td>
<td>36 mo Mean (SD)</td>
<td>Baseline Mean (SD)</td>
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<tr>
<td>Whole Body Fat Mass (kg) **</td>
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<td>Whole Body Lean Mass (kg)</td>
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<td>42 (5)</td>
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<td>Trunk Fat Mass (kg)**</td>
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<td>11 (4)</td>
<td>14 (4)</td>
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<td>73 (11)</td>
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<td>82 (10)</td>
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<td>%Fat **</td>
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<td>38 (5)</td>
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Notes, Rows demarcated with ** indicate exercise group x time interaction (see figures).

Rows demarcated with *** indicate exercise group x isoflavone group x time interaction (see figures)
Table 4. Exercise Tests Outcomes.

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<td>36 mon Mean (SD)</td>
<td>Baseline Mean (SD)</td>
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<tr>
<td>Chest Press (kg)* *</td>
<td>66 (23.54)</td>
<td>92 (23.86)</td>
<td>92* (25.06)</td>
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</tr>
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<td>Squat Strength (kg)* *</td>
<td>59.35 (36.32)</td>
<td>159.6 (54.32)</td>
<td>178.5* (59.04)</td>
<td>65 (36.32)</td>
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<td>Balance Time Average (seconds) *</td>
<td>41.89 (14.41)</td>
<td>30.52 (12.35)</td>
<td>27.27 (14.30)</td>
<td>42.06 (14.41)</td>
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<td>Balance errors</td>
<td>2.30 (2.23)</td>
<td>1.78 (2.20)</td>
<td>1.81 (2.54)</td>
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<td>Walking Test (seconds) *</td>
<td>52.3 (6.37)</td>
<td>50 (5.92)</td>
<td>52.3 (6.18)</td>
<td>51.88 (6.37)</td>
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</tbody>
</table>

Notes,

Rows demarcated with ** indicate exercise group x time interaction (see figures).

Rows demarcated with * time main effect (p<0.05).

5.3. Adverse Events and Symptoms.

There were no serious adverse events recorded during the extension study. The frequency of non-serious adverse effects from baseline until the second year was reported in a previous study (Chilibeck et al., 2013).
6. Discussion

The present study is the first to examine the effect of exercise training among postmenopausal women over a three-year period. Although there have been previous studies conducted to demonstrate the benefits of exercise training, this is the first to show the effect of persistent exercise training over a three-year duration following the cessation of isoflavone. The specific aim of this study was to investigate the BMD at lumbar spine and proximal femur in postmenopausal women after cessation of isoflavone therapy, but with continuation of exercise training. We hypothesized that an additional year of exercise training after cessation of isoflavone therapy, or continued exercise training for an additional year in women who never received isoflavone in the first study (Chilibeck et al., 2013), would be effective in increasing lumbar spine and proximal femur aBMD as compared to participants who did not exercise and did not take isoflavone. Moreover, we also hypothesized that an additional year of exercise training after cessation of isoflavone therapy, or continued exercise training for an additional year in women who never received isoflavone in the first study (Chilibeck et al., 2013), would be effective in improving bone geometry as compared to the control groups who did not exercise.

The results of the present study indicated that there were significant differences in the aBMD, and geometry between the exercise and non-exercise groups. Unexpectedly, the exercise groups lost more aBMD at the lumbar spine and total hip in comparison to the non-exercise groups. Although the majority of data supports the idea that exercise can delay or stabilize osteoporosis, and in fact exercise is used as one of the first line of treatment for osteoporosis (Papaioannou et al., 2010), the results of the present study were at odds with this guideline. Our findings, however, are not unusual.
A study similar to ours, but without isoflavone supplements or cessation, researched the effects of exercise (home-based daily exercise; 20 min d⁻¹), over a 30-month period in post-menopausal women (Korpelainen, Keinänen-Kiukaanniemi, Heikkinen, Väänänen, & Korpelainen, 2006). The authors of this study measured the aBMD in the femoral neck and trochanter and found that there was a slight decrease in the control group while there were no changes in the exercise group. In both groups, as they aged, there was significant bone loss at the radius and calcaneus. The authors concluded that exercise, besides improving physical conditioning, had little effect on the aBMD and the other bone variables measured. There are a number of reasons as to why the author of this study did not find differences between the exercise and non-exercise group in terms of aBMD, and in bone quality and architecture. First, bone expansion is dependent on factors such as age, gender, hormonal variables, stress and weight changes (Korpelainen et al., 2006). Furthermore, it has been found that obesity has a protective effect against osteoporosis in post-menopausal women (Albala et al., 1996). The explanation for this is that excess fat leads to an increase in free sex steroids within the body, and this in turn stimulates osteoblasts to form bone mass. Moreover, although it may seem counter intuitive, the excess weight in obese people means that they are constantly exerting mechanical loading on their bones and this may lead to further proliferation of osteoblasts. This means that as people lose weight, the mechanical stress they exert on the bones decreases. Secondly, as people age there is an aBMD decline of 3% to 5% per year in the decade after menopause so that over the length of the study, even though there may have been bone mass which formed due to the stimulating effects of exercise, it was insufficient to offset the declines (Josse et al., 1996).
In the present study, any benefits or negative effects of isoflavone in the third year can be discounted as the intestinal half-life of soy isoflavones is very short, varying from 3.3 to 7.5 h (Xu, Harris, Wang, Murphy, & Hendrich, 1995). This is because the bacteria in the gut break down the isoflavone. Therefore, any residual effects of isoflavone over the one year period of the present study can be discounted and the only effect measured was that of exercise on bone. Overall, these results indicate that exercise had no benefit on aBMD beyond the first two years of training (as indicated in the Chilibeck et al. (2013) study) and the removal of isoflavone supplements did not affect this result.

In the present study it was found that there were time main effects for aBMD (decreasing) and for geometric properties (increasing). This finding can be explained by the fact that although there is a net loss of aBMD in both men and women after maturity, there have been reports of increased bone thickness with aging due to bone growth on the periosteal, or exterior of the bone surface (Weaver et al., 2016). The majority of studies have examined bone modelling and bone loss at the endocortical, or the interior of the bone, and very few at the periosteal (Szulc, Seeman, Duboeuf, Sornay-Rendu, & Delmas, 2006). It is known that bone formation occurs on both the periosteal and endocortical surfaces with bone deposition on the former resulting in an increase in the external diameter. During the growth phase, periosteal apposition surpasses endocortical resorption so that there is a net thickening and elongation of the cortex. During aging, however, it may be that periosteal development continues and supersedes endocortical bone loss so that there is either no net loss of bone and strength or that there is an increase in bone development on the exterior surface thereby leading to an increase in bone width. A number of studies have reported increases in pelvic and L4 lumbar vertebra growth (Berger, May, Renner, Viradia,
& Dahners, 2011), femoral neck (Johnson, Renner, & Dahners, 2012), and the hand bones (Kalichman, Malkin, Seibel, Kobyliansky, & Livshits, 2008).

In one of the few studies on periosteal development, the authors studied bone mass changes and bone geometry and strength in 821 women divided into four groups: 151 premenopausal, 33 perimenopausal, 279 postmenopausal women, and 72 postmenopausal women receiving HRT (either equine estrogens or transdermal 17β-estradiol) (Szulc et al., 2006). The authors found that while in premenopausal women there was an increase in the radius width, it only partly offset cortical bone loss. In all of the other groups, the authors found that there was a decrease in both periosteal and endocortical bone resorption leading to reduced aBMD and strength as the women aged. However, the HRT therapy was effective in slowing bone loss and strength. In another study, the authors measured, besides other factors such as aBMD, subperiosteal and endocortical width (cm) at specific locations of the proximal femur in white (non-Hispanic) group of 2719 men and 2904 women ranging in age from 20 to 99 (Beck, Looker, Ruff, Sievanen, & Wahner, 2000). The authors found that in females the width, or Z, at the narrow neck and the shaft regions was constant until the age of 50 after which there was a slight decline but at a rate lower than aBMD. In males there was a marginal decline in the narrow neck Z until the fifth decade after which it remained constant but this was followed by an increase as the age increased. In both sexes, although there was a decline in aBMD there was a concomitant expansion in subperiosteal diameter in both sexes and in both regions. The authors concluded that although there was a loss in aBMD, the increases in width may have offset the reduction in mechanical strength caused by aBMD loss. The aforementioned reports suggest that
further work needs to be conducted in order to fully elucidate bone loss and modelling as one ages so that fracture risk decreases as one ages.

Another possible explanation for bone geometric increase in the non-exercise group is the inaccuracies in the DXA bone measurements based on the changes in soft tissue and its distribution within the scanned region. In an in vivo study by Bolotin et al., (2001), it was found that DXA scans in regions where there were two absorbtiometrically distinguishable components, the BMD error was as high as 20% (Bolotin, Sievänen, Grashuis, Kuiper, & Järvinen, 2001). The authors noted that the errors were especially high in subjects with osteoporotic bones, such as in postmenopausal women and the elderly. This error, in the authors’ opinion, was attributable to the body fat in the region. In a follow up study to the preceding one, the authors measured BMD in regions where the body fat and the lean muscle tissue was unevenly distributed and reported BMD errors ranging from 20 to 50 percent (Bolotin, Sievänen, & Grashuis, 2003). In a clinical setting, this means that the errors which can occur when testing individuals for BMD can be highly variable and are dependent on the fat and lean muscle distribution within the scanned region. In the present study, there were no statistically significant changes in lean tissue mass, body weight and waist girth in the non-exercise groups but there was a decrease in total body fat mass, percent fat, and trunk fat mass in the exercise group. Given this data, the BMD measurement errors within the non-exercise group should have offset (e.g. since the fat and lean mass did not change from the baseline to the third year, any measurement errors should have been offset).
6.1. Body Weight and aBMD.

The results in the present study showed a statistically significant decrease in body weight in the exercise + Past Iso group (Figure 13) and a significant loss of whole body fat mass (Figure 10). This finding is in line with other reports in the literature where the loss of body weight correlated to a greater loss in aBMD. In a recent study of 165 post-menopausal women, the researchers measured body weight, BMI and aBMD of the spine and proximal femur and found that both body weight and BMI positively correlated with aBMD (Mir et al., 2017). In fact, the authors concluded that increased body weight and a greater BMI protected the women against osteoporosis. The rationale of the authors for this conclusion was that the increased mass in obese and overweight people led to mechanical loading, or inadvertent weight or load bearing exercise, which stimulated the osteoblasts. Moreover, the authors noted that adipose tissue is a source of estrogens and that this too may have had a positive effect on aBMD in postmenopausal women.

Similarly, several other studies have indicated that BMI can be used to identify the risk of osteoporosis in postmenopausal women (Hendrijantini, Alie, Setiawati, Astuti, & Wardhana, 2016; Ravn et al., 1999). As with the study above, it was noted that thinner women are at a greater risk for postmenopausal bone loss as compared to heavier women. These findings support our results that the women in the exercise group lost significantly more body fat from baseline to the third year and showed greater aBMD loss as compared to women in the non-exercise group. Other studies too have come to the same conclusion that weight loss is one of the leading causes of bone loss in older populations (Albala et al., 1996; Khosla, 2013; Villareal et al., 2011).
Although there seems to be strong evidence suggesting that a higher weight can mitigate the risks of osteoporosis, the diseases linked with a high BMI or fat mass may offset the benefits. A number of serious diseases and increased mortality have been attributed to being overweight/obese and include many cardiovascular diseases (CVDs) such as high blood pressure, coronary heart disease, congestive heart failure, stroke, high total cholesterol and triglycerides in blood serum, and others such as diabetes mellitus 2, and cancer (Hensrud & Klein, 2006). A higher trunk fat mass, often measured by waist circumference, correlates with lipid measures that are associated with increased CVD so that the loss of fat mass, especially in the trunk region, could reduce CVD risk (Haslam, Sattar, & Lean, 2006; Vatanparast et al., 2009). Furthermore, with increased obesity, a body’s routine functional capacity such as sitting/standing, walking, and balance control are affected so that there are greater risks for falling (Pataky, 2014; Wee et al., 2011). Economically, too, there are benefits to losing fat mass as overweight adults have health care costs that are 10-20% higher as compared to people with normal weights (Hammond & Levine, 2010).

6.2. Exercise and Fitness Parameters.

As was expected, the exercise groups improved in strength as compared to the non-exercise groups (Figures 15, 16, and Table 4). The participants in the exercise groups improved their fitness parameter fitness parameter (body composition, chest press and leg strength) from the baseline and this continued into the third year of the study. These results were to be expected as, with exercise, the muscles adapt and become stronger (Morgan, Cobb, Short, Ross, & Gunn, 1971). A recent study examined the incidences of falls and the
effects of exercise on leg strength, walking speed, and backward walking (dynamic balance) in post-menopausal women aged between 70 to 80 (Patil et al., 2015). The authors found that, besides exercise reducing falls by 50 percent, there were statistically significant (P < 0.05), improvements in all of the aforementioned parameters as compared to the non-exercise group. Another study similar to the present one found that over a 30 months period, postmenopausal women who exercised had statistically significantly greater leg strength, walking speed, and in the distance they were able to walk in two minutes as compared to those who did not (Korpelainen et al., 2006). While the authors did not find any positive effects on aBMD, or bone geometry, they did note that exercise helped the women to be more able to maintain their balance and this may lead to a lower fracture incidence rate as compared to those who did not exercise.

It was found that at the end of the third year, the exercise groups were 65% stronger than the non-exercise groups. This is a substantial difference and if extended into older age, is highly likely to have benefits for increasing functional abilities. A 20% difference in strength is predictive of differences in functional impairments; therefore, the 65% difference in strength could be considered clinically significant (Brill, Macera, Davis, Blair, & Gordon, 2000). As noted above, besides increasing dynamic balance and strength, exercise also helps to reduce falls and the severity of injuries. Besides these benefits, exercise is also known to prevent and decrease the risks of CVD and death in postmenopausal women (Kushi et al., 1997). In fact, regular physical activity is known to reduce the risk, by 20% to 30%, of at least 25 chronic medical conditions (Warburton & Bredin, 2018), including type 2 diabetes, depression, and cancer of the breast and colon (Thompson et al., 2003).
Our study found that waist girth and body mass decreased in the exercise + past isoflavone group with no change in the other groups. These results are similar to a six month long clinical trial where the effects of exercise training combined with isoflavones supplement were measured on 34 postmenopausal women (Lebon, Riesco, Tessier, & Dionne, 2014). The authors found that in the combined therapy group there was a decrease in total fat mass with decreases in hip, leg and trunk circumferences. This suggests that isoflavones may have synergistic effects with exercise for improving some components of body composition.

6.3. Strengths, Limitations and Future Considerations.

One of the limitations of the present study was the small number of participants. Regardless, the present study is the only study, to the author’s knowledge, which investigated the effect of exercise training in postmenopausal women on aBMD over a three-year period using DXA scan as a primary tool of investigation. No other study has investigated the effects of a resistance-training program greater than 2 years in duration on aBMD (Howe et al., 2011). Another limitation may be that using DXA for monitoring the aBMD in the elderly may not be the best way to monitor and measure aBMD (Engelke, Libanati, Fuerst, Zysset, & Genant, 2013), especially given the fact that there are now more sensitive and accurate instruments, such as those utilizing computed tomography, which may be more useful and are able to detect minute differences which were imperceptible with DXA.

Future studies could research if the high dose of isoflavones supplements had a greater affinity towards the β estrogen receptor and if this blocked the stimulating effects
of exercise on bone by downregulating bone synthesis (Saxon, Robling, Castillo, Mohan, & Turner, 2007). This is further supported by the fact that it has been reported that a low dose soy isoflavone supplement resulted in aBMD increases in early postmenopauseal women as compared to high dose isoflavone (Huang et al., 2006). A study researching aBMD in postmenopausal women, with one control group and two other groups who receive a low-level supplement and the second receiving a high dose, may be able to unravel if varying dosages of isoflavone has any effect on aBMD.

6.4. Conclusion.

The results of the present study showed that exercise had no benefit on aBMD in the third year of the study as the bone loss in the exercise group was greater than the non-exercise group. The exercise program, however, improved body composition (i.e. reduced fat mass, trunk fat, and percent fat) and increased strength of the participants from the baseline to year 3.
7. References


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8. Appendices

8.1. Consent Form

**Title of the study:** Effect of exercise training beyond 2 years on prevention of osteoporosis

**Researchers:** Philip D. Chilibeck, Ph.D., Associate Professor, College of Kinesiology, University of Saskatchewan (966-1072).
Muhedeen Abdulmula, Master Student, College of Kinesiology, University of Saskatchewan

You are being asked to participate in an extension to a research study. Currently you are performing resistance training exercise and walking OR flexibility exercises and taking a soy-based nutritional supplement or placebo. We are inviting you to continue taking part in the exercise portion of this study for one more year. You will stop taking the isoflavone or placebo during this study extension.

Before you decide, it is important for you to understand what the research involves. This consent form will tell you about the study, why the research is being done, what will happen to you during the study and the possible benefits, risks and discomforts. If you wish to participate, you will be asked to sign this form. Your participation is entirely voluntary, so it is up to you to decide whether or not to take part in this study. If you do decide to take part in this study, you are free to withdraw at any time without giving any reasons for your decision nor will this affect the medical care you are currently receiving or your access to services at the university. Please take time to read the following information carefully and to discuss it with your family, friends, and doctor before you decide.
**Purpose of the study**

Exercise training has small benefits for increasing bone mineral density and therefore increasing bone strength. Very few studies have evaluated the effects of exercise training for longer than 2 years on bone mineral density. The purpose of this study is to determine the effects of 3 years of exercise training on bone mineral density.

**Possible benefits of the study**

Possible benefits include an increase in bone mineral density, an improvement in bone quality, a loss of body fat, an increase in muscle mass, or an increase in flexibility. These benefits are not guaranteed.

**Procedures to be followed**

You will discontinue the soy-based nutritional supplement or placebo you are currently taking, but will continue with the exercise portion of the study. This involves either strength training twice per week (45 minutes to an hour each session) and brisk walking four times per week (about 30 minutes each time) OR flexibility exercises (20-30 minutes per day) four days per week.

**12 Month follow-up measurements**

Your bone mineral density and body composition will be measured by dual-energy x-ray absorptiometry. The bone in your lower leg and wrist will be measured by ultrasound (to measure bone quality). Exercise testing will be performed. This will involve determining the maximal amount of weight you can lift on several exercises, a walking test over 80 meters, a flexibility test, and a test of balance. You will be required to fill out
questionnaires to assess your food intake and physical activity levels. Testing is done in one session and will require about 2 hours of your time.

With your permission, your family physician will be informed of your enrolment and of your test results.

**Foreseeable risks, side effects and discomfort**

There is some radiation exposure with the dual energy x-ray absorptiometry. This is equivalent to the amount of radiation you would receive during a return airplane flight to Toronto.

There is a risk of injury during exercise testing or training. This will be minimized by proper warm-up procedures and supervision by qualified exercise trainers. There may be unforeseen risks during the study or after the study is completed.

You are free to withdraw from the study at any time and this withdrawal will not affect access to health care or other services.

**Alternatives to this study**

You do not have to participate in this study to improve your bone mineral density.

You can join any gym and receive instructions from staff on a proper exercise training program. You can also take various medications such as HRT or bisphosphonates to improve your bone mineral density.

**Research-Related Injury**

There will be no cost to you for participation in this study. In the event you become ill or injured as a result of participating in this study, necessary medical treatment will be
made available at no additional cost to you. By signing this document you do not waive any of your legal rights.

**Confidentiality**

Precautions will be taken to protect your anonymity during the study. All data collected will be stored in a locked office in the College of Kinesiology. While absolute confidentiality cannot be guaranteed, every effort will be made to ensure that the information you provide for this study is kept entirely confidential. Your name will not be attached to any information, nor mentioned in any study report, nor be made available to anyone except the research team. It is the intention of the research team to publish results of this research in scientific journals and to present the findings at related conferences and workshops, but your identity will not be revealed.

If you have questions concerning the study you can contact Dr. Philip Chilibeck at 966-1072, 343-6577, or 230-3849 (24 hour number).

If you have any questions about your rights as a research subject or concerns about the study, you should contact the Chair of the Biomedical Research Ethics Board, c/o the Office of Research Services, University of Saskatchewan at (306) 966-4053.

If, during the course of this study, new information becomes available that may be related to your willingness to continue to participate, this information will be provided to you by the investigator.
Your participation in the research is entirely voluntary. You are free to withdraw from this study at any time and this withdrawal will not affect your relationship with the researchers or your standing at the university. If you choose to enter the study and then decide to withdraw at a later time, all data collected about you during your enrolment in the study will be retained for analysis.

Please read the following before signing this consent form:

- I have read or have had this read to me and understood the research subject information and consent form.
- I have had sufficient time to consider the information provided and to ask for advice if necessary.
- I have had the opportunity to ask questions and have had satisfactory responses to my questions.
- I understand that all of the information collected will be kept confidential and that the result will only be used for scientific objectives.
- I understand that my participation in this study is voluntary and that I am completely free to refuse to participate or to withdraw from this study at any time without changing in any way the quality of care that I receive.
- I understand that I am not waiving any of my legal rights as a result of signing this consent form.
- I understand that there is no guarantee that this study will provide any benefits to me (if applicable).
• I have read this form and I freely consent to participate in this study.

• I have been told that I will receive a dated and signed copy of this form.

Please notify my primary care physician of my participation in this study:

Yes_____  
No______

Research Subject’s Signature:___________________

Signature of the Individual responsible for conducting the consent process:

______________________

Date: ______________________
PROJECT TITLE: Effect of exercise training beyond 2 years on prevention of osteoporosis

PRINCIPAL INVESTIGATOR: Philip D. Chilibeck, Ph.D., Associate Professor
Muhedeen Abdulmula, Master Student

DEPARTMENT: College of Kinesiology

SUB-INVESTIGATOR(S):

DEPARTMENT: College of Kinesiology

RESEARCH WILL BE CONDUCTED AT: College of Kinesiology

1. Hypothesis (State briefly the nature and purpose of the research proposal, and the proposition the research is seeking to uphold. What potentially useful knowledge or clarification about therapeutic options will be advanced to justify the participation of human subjects in this research project?)

We hypothesize that:

Post-menopausal women participating in an extended resistance training and walking exercise program will achieve a greater increase in bone mineral density than women participating in an extended flexibility program.

2. Academic Validity (Provide evidence that the scientific reasoning and design of the project are sufficiently sound to meet the objectives of this project. Provide your own comments and if possible those resulting from peer review. Indicate if any other committee or agency has assessed the project’s scientific validity):
Currently we are running a study (Bio#03-1077) in which 351 post-menopausal women are taking part in either a resistance training and walking exercise program or a flexibility exercise program. They have also been randomized to receive a soy-based nutritional supplement or a placebo. Bio#03-1077 involves 2 years of the intervention, with the primary outcome of bone mineral density. The first subjects in this study were enrolled in December 2004 and therefore are close to completing the initial study. No study has investigated the effects of a resistance training program greater than 2 years duration on bone mineral density (Bonaiuti et al., Cochrane Database Systematic Review, 2002). Bone turns over slowly; therefore, the increases observed with exercise training are usually only about 1-2% per year (Bonaiuti et al., 2002). A 5% increase in bone mineral density is necessary for a clinically significant reduction in chance of fracture (Guyatt et al. Endocrinology and Metabolism Clinics of North America, 2002). We want to extend the exercise portion of the current study to investigate the effects of 3 years of resistance training on bone mineral density. Women completing Bio#03-1077 will stop taking their soy-supplement and placebo, but continue with the exercise portion of the study. While stopping the soy-supplement or placebo may have an effect on their bone mineral density, there should be an approximate equal number of women originally randomized to the resistance training and flexibility groups who are also randomized to supplement and placebo; therefore, the effect of stopping isoflavone or placebo should be equal across the two different exercise groups.

If applicable, please indicate whether TPD approval has been obtained:

Yes _____  No _____  Pending _____  N/A __x____

3. **Funding** (indicate the source of funds supporting the research. If externally funded, state whether the grant or contract is still in application, or has already been awarded):

Residual CIHR funds from Bio #03-1077 will be used for the extension study

4. **Disclosure of Potential Conflicts of Interest** (indicate any motivation or incentives for conducting this study that arise external to the objectives of the study, e.g., will the investigator or institution be paid to conduct this research project? Note: The consent form should also include an introductory disclosure of potential conflict of interest statement, where applicable, indicating that this is a medical research study for which the study doctor, the institution, or both are being paid):

None

5. **Subjects**
   a) **Target Population** (e.g., age, gender, medical condition, target enrollment, significant inclusion/exclusion criteria):
Post-menopausal women currently participating in Bio 03-1077 will be invited to continue with the exercise portion of the study. The main exclusion criteria are that they have to refrain from taking any medications (i.e. bisphosphonates or hormone replacement therapy) that affects bone mineral density.

b) Proposed Strategies for Recruitment (e.g., use of advertisements, brochures, physician patient records):

Subjects currently participating in Bio 03-1077 will be invited to participate in the extension.

6. Procedures (clearly identify treatment allocation design, and describe the medical and other procedures to be followed in obtaining research data, including questionnaires):

Subjects will continue to perform the exercise programs they are currently doing in Bio 03-1077. This involves either strength training twice per week (45 minutes to an hour each session) and brisk walking four times per week (about 30 minutes each time) OR flexibility exercises (20-30 minutes per day) four days per week.

Subjects will be measured for bone mineral density at their lumbar spine, hip, and whole body by dual energy X-ray absorptiometry, bone quality at the tibia and radius by ultrasound, strength for squat and bench press exercise, flexibility (sit and reach test), walking speed, and balance after 12 months. Subjects will be asked to fill out a food frequency questionnaire and physical activity questionnaire at 6 and 12 months. All these procedures are identical to those approved for Bio 2003-1077.

7. Time Period (indicate the dates when the research project is expected to begin and to be completed. A final status report must be filed with the Ethics Office once data collection from the last subject is complete. The Ethics Office should be notified once the study site is closed.):

The extension study will start in December 2006. The latest the study would run is April 2009 because the last women to enter Bio 2003-1077 entered in April 2006 (and therefore will complete Bio 2003-1077 in April 2008).

8. Data Storage (In accordance with recommended guidelines provide a statement outlining the procedures you will use to store securely the research data. State how long and where the data will be stored and identify the person who will be assuming responsibility for data storage):

Data will be stored in a locked office in the college of Kinesiology for a period of 25 years.
9. **Consent Form** (include a copy of the consent form and/or any study information that will be used. If not using a consent form give reasons why).

Attached

9. **Signatures**

________________________________________________________________________
Principal Investigator                                          Phone
________________________________________________________________________
Fax                                                e-mail

________________________________________________________________________
Department Head, Dean, Director, or Administrative Head

10. Contact Person and Mailing Address for Correspondence:
8.3. Study Renewal, Extension Study (06-234).  

**Biomedical Research Ethics Board Study Renewal Form**

*Note, if your study is complete please fill out the study closure form available at: [http://www.usask.ca/research/files/index.php?id=22](http://www.usask.ca/research/files/index.php?id=22)*

Please type in your responses, print, and then send the original signed copy to our office. Do not fax.

Double click on boxes to check.

1. **Title:** Effect Of Exercise Training Beyond 2 Years On Prevention Of Osteoporosis

2. **Bio #:** 06-234  
3. **Protocol #:**

4. **Expiry Date:** November 28, 2008  
5. **Clinical Trial Registration Number (If applicable):**

6. **Contact Information:** Philip Chilibeck

<table>
<thead>
<tr>
<th>Name:</th>
<th>Department:</th>
<th>Phone Number, Email, Fax Number (Provide only if different from previously submitted information):</th>
</tr>
</thead>
<tbody>
<tr>
<td>Philip Chilibeck</td>
<td>Kinesiology</td>
<td>966-1072, <a href="mailto:phil.chilibeck@usask.ca">phil.chilibeck@usask.ca</a>, 966-6464</td>
</tr>
</tbody>
</table>

7. **Sponsor/Funding Agency:** CIHR

8. Indicate whether delegated or full board review is required for this renewal.

- [ ] Delegated Review (Please indicate which of the following apply)
  - [ ] Research involves no more than minimal risk
  - [ ] No research subjects have been enrolled in the study
  - [ ] Research remains open only for the long term follow up of participants
  - [ ] Remaining research is limited to data analysis
- [ ] Full Board Review:
  - Regulatory/Sponsor requirement [e.g. US Federal Agency (e.g. NIH) or some sponsoring organization (e.g. NCIC, COG)]
  - Other (please indicate):

9. Did the initial protocol require a “No Objection Letter” (NOL) from Health Canada?  
- [ ] Yes  
- [X] No

If Yes, please submit a copy of the original “NOL” if not already submitted.

- [ ] Already submitted  
- [ ] Attached to renewal

10. **Location where research will be conducted (if different from previously submitted information):**

College of Kinesiology, Williams Building

11. **Does this research involve another institution?**  
- [ ] Yes  
- [X] No

12. Is there interim analysis by a data safety monitoring board (DMSB) or some monitoring committee?  
- [ ] Yes  
- [X] No

Has there been a report from a DMSB or safety monitoring committee in the last year?  
- [ ] Yes  
- [X] No

If Yes, what was the outcome?  
- [ ] Continue study as planned  
- [ ] Modifications were suggested  
- [ ] Close study temporarily  
- [ ] Close study permanently

13. Have there been any changes to the study (study design, changes in recruitment material, procedures, consent process,) that have not already been reviewed and approved by the Bio-REB?  
- [ ] Yes  
- [X] No

If Yes, please submit an amendment.

14. Please indicate which version of the consent form(s) is(are) currently being used (date and/or version number).

November 8, 2006
15. Have there been any changes in research personnel, such as principal investigator, sub-investigators, Clinical Research Assistants, residents or students?  
   ☐ Yes  ☒ No  
   If Yes, please list the former/new personnel and position.

16. What is the current status of the study? (Please mark all that apply)  
   ☐ Recruitment has not yet started.  
   ☐ Research participants are currently being recruited.  
   ☒ Recruitment is closed.  
   ☐ Recruitment is closed and data collection involving participants is on-going.  
   What was the original number of participants to be recruited? __268____
   How many participants have been screened? __268____
   How many participants were enrolled? __107____
   How many research participants are currently in the study and receiving treatment? __54____
   Is there a significant change in anticipated enrollment? Is yes, please explain.  
   ☐ Yes  ☒ No

   ☐ The data collection is complete except for long-term follow-up of participants.  
   ☐ How many participants are currently not receiving treatment but still in the follow-up stage of the study? __________
   ☐ The data collection is complete, remaining research activities are limited to data analysis only.  
   ☐ The study is closed (Please complete the Biomedical REB Study Closure Form)

17. How many research participants have been withdrawn from or discontinued the study? __11____
   Please provide a reason for each withdrawal (if known):  
   ☐ Need for Other Treatment, number _____  ☒ Withdrawn Consent/Dropped Out, number: __11____  
   ☐ Serious Adverse Event, number: _______  ☐ Other, number _____ (Specify reason, if known)

18. Since receiving original ethics approval, have any ethical concerns arisen?  
   ☐ Yes  ☒ No  
   If Yes, please describe concerns in detail.

19. Have there been any serious adverse or unexpected events at this site?  
   ☐ No serious adverse events  ☐ Expected serious adverse events only  ☒ Unexpected serious adverse events  
   (Please attach a Serious Adverse Event Report for any unreported unexpected serious adverse events.)

20. Were there major protocol deviations:  
   ☐ Yes  ☒ No  
   Please attach a Protocol Deviation Report form for any unreported major protocol deviations.

21. Have any findings, new information or study modifications changed the risk level of this study for current and future participants?  
   ☐ Yes  ☒ No  
   If Yes, explain the changes made, how participants will be notified and whether or not participants will be re-consented or refer to approved or submitted protocol amendment.

23. Provide a brief summary of study progress.  
   Of the 268 subjects who completed bio#03-1077, 107 volunteered to continue with the exercise training program. Fifty-four of these participants remain in the study.

24. Indicate the expected closure date of this study. November 30, 2009

______________________________________________________________________________
Signature of Principal Investigator            Date
8.4. Completed Course Work

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