THE EFFECT OF WATER FLUORIDATION ON THE BONE MINERAL DENSITY OF YOUNG WOMEN

A Thesis Submitted to the College of Graduate Studies and Research in Partial Fulfilment of the Requirements for the Degree of Masters of Science in Physical Education in the Department of Physical Education
University of Saskatchewan
Saskatoon

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Spring 1996

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Abstract

The effect of long term exposure to low level water fluoridation on BMD (bone mineral density) was studied in a sample of female university students (18 to 25 years). Subjects were 24 women from Regina, Saskatchewan (natural level of fluoride in the drinking water is 0.1 mg./litre) and 33 women from Saskatoon, Saskatchewan (supplementation of fluoride in the drinking water is 1.0 mg./litre). These two cities are very similar in population, climate and demographic data. The subjects had not moved outside of their resident city for more than four years, had no bone affecting medical disorders and were not using any bone affecting medications. All subjects completed a lifestyle and dietary history evaluation and a food frequency questionnaire. There were no differences between the two groups for age, weight, height, bone mineral free lean mass, fat mass, estrogen status, calcium and vitamin D intake, and past or present physical activity level.

Areal bone mineral density of the proximal femur, lumbar spine (AP and lateral companion scan) and total body were measured using dual energy x-ray absorptiometry (Hologic 2000, array mode). Volumetric BMD was also determined for the lumbar spine from the AP and lateral companion spine scans (VLS). The hypotheses predicted greater BMD in the fluoridated community as compared to the non-fluoridated community, with the greatest difference occuring at the axial skeleton. To determine an overall difference in BMD between Regina and Saskatoon groups, MANCOVA was performed with place of residence as the independent variable and AP Spine, Total Body and Total Proximal Femur BMD as the dependent variables. Weight was used as the covariate. There was a significant difference between Regina and Saskatoon adjusted mean BMD (F (52,3) = 2.44, $p < 0.05$, one-tailed). Post-hoc univariate F-tests found the differences in BMD were due to differences at

the AP Spine (Regina = 0.975 g/cm^2, Saskatoon = 1.039 g./cm^2, $\underline{F}$ (54,1) = 7.33, $\underline{p} < 0.05$, one-tailed), and Total Body (Regina = 1.044 g/cm^2, Saskatoon = 1.073 g/cm^2, $\underline{F}$ (54,1) = 3.16, $\underline{p} < 0.05$, one-tailed), but not at the proximal femur. Based on the significant finding at the axial spine, ANCOVA was performed for the lateral companion spine (L3) and VLS (L3). There was a significant difference between the Regina and Saskatoon groups at VLS ((L3), Regina = 0.216 g/cm^3, Saskatoon = 0.227 g/cm^3, $\underline{F}$ (53,1) = 4.37, $\underline{p} < 0.05$, one-tailed). This study supports that low level water fluoridation may have a positive effect on the bone mineral density of young women, the greatest effect occurring at the axial skeleton.

ACKNOWLEDGEMENTS

I would like to thank Dr. Don Bailey for being my advisor; I respect and admire the contributions he has made in the field of osteoporosis prevention and I am honoured to have had the opportunity to be one of his graduate students. I would also like to thank my committee members for their support and advice, Dr. R. Mirwald, Dr. R. Faulkner, Dr. L. Kikulis and my external examiner, Dr. E. Harrison. I would also like to recognize the contributions of Dr. D. Drinkwater and Dr. P. Crocker for their willingness to provide advice and assistance.

This study would not have been successful without the assistance and support from Dr. R. McCulloch who recruited and transported the volunteers necessary for this research to happen. Thank-you also to Dr. H. McKay, who not only contributed hours of technical assistance, but also provided continued support and advice throughout this project. Finally, I would like to thank my family: my husband, Jim, my children, Zoe and Lauren, and my parents, Bill and Joan McCusker for their love, support and guidance throughout these past two years.

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OPERATIONAL DEFINITIONS

1. Bone mineral: Primarily composed of hydroxyapatite (a micro-crystalline compound containing primarily calcium, with phosphate, sodium, potassium, zinc, magnesium, fluoride and other trace elements) and is the basic structural element of bone.

2. Bone Mineral Content (BMC): The total amount of bone mineral as measured by the Hologic 2000 dual x-ray absorptiometer, expressed in grams.

3. Bone Mineral Density (BMD): The ratio of bone mineral content to the unit area of skeletal material measured. For the purpose of this investigation, will be expressed as areal bone mineral density in g/cm^2 as measured by the Hologic 2000.

4. Volumetric Bone Mineral Density: Lumbar Spine (VLS): This is a width adjusted estimate of a volumetric bone density calculated by basic mathematical laws from the two projections of the same region and is expressed as g/cm^3. It is calculated by the Hologic 2000 software by dividing the bone mineral content obtained from the lateral view of the vertebrae by the estimated lumbar spine volume. The estimated lumbar spine volume is the average width (cm) of the vertebral body determined from the Anterior-Posterior (AP) projection and the skeletal area scanned (cm^2) from the companion lateral projection. VLS for the third lumbar vertebrae (L3) was calculated in this study.

5. Peak Bone Density: In order to avoid confusion over the terminology peak bone mass and peak bone density, I will refer to peak bone density as the highest level of bone mineral density in the skeleton that is achieved by young adulthood, expressed by g/cm^2. As there is no direct measure of bone mass, I will refer to peak bone mass as synonymous with peak bone density.

GLOSSARY OF TERMS

1. Trabecular Bone: A porous, honeycomb configuration where there is a network of small, interconnected plates and rods of bone surrounding marrow-fat spaces. This type of bone is usually found in the metaphysis of long bones and in the interior of irregular bones (ie. vertebrae or os calcis).

2. Cortical Bone: Bone with less porosity than trabecular bone, a solid dense bone that is found in the outer layer of all bones and is most evident in the diaphysis of long bones.

3. parts per million (ppm): Number of grams of solute per million grams of sample. Used in expressing amount of fluoride present and can be considered synonymous with mg/l.

Chapter 1

Scientific Framework

1.1 Introduction

Osteoporosis, low bone mass and microarchitectural deterioration of bone tissue leading to enhanced bone fragility and an increased fracture risk, is a major health concern for postmenopausal women (Consensus Development Conference, 1991). One in two women will sustain an osteoporotic fracture in their lifetime (Hillard & Stevenson, 1991). The lifetime risk for a hip fracture for white females is 15%, equivalent to the combined lifetime risk of developing breast, uterine or ovarian cancer (Cummings, Kelsey, Nevitt & O'Dowd, 1985). The lifetime risk of vertebral fracture for a white female has been reported to be as high as 27% (Melton III, 1990; Kanis & McCloskey, 1992). Fracture frequency increases as bone mineral density decreases; thus, lower bone mineral density is strongly associated with an increased risk for osteoporotic fracture (Mazess, 1989; Stevenson, 1990).

Recent international evidence reveals that the prevalence of osteoporosis and the incidence of vertebral and hip fractures is rising, primarily due to an increasing number of people in the elderly population (Avioli, 1991; Kanis & McCloskey, 1992). According to the 1991 Census Report (Statistics Canada [1], 1991) the total number of people in Canada over the age of 65 has increased by approximately 30% from 1981 to 1991. The total number of people over the age of 85 has increased by approximately 50% in this same time span. This increase in life expectancy means the prevalence

and health care costs of osteoporosis will continue to rise.

While osteoporosis is primarily a health problem in the older population, optimizing bone density in young adulthood may be the best preventative measure for osteoporotic fractures later in life (Stevenson, 1990). For example, a woman with high peak bone density as a young adult, who loses the average 20% of bone later in life, does not lose enough bone to reach her fracture threshold. However, a woman with low peak bone density who loses only 10% of bone, subsequently is at a greater risk of fracture (Stevenson, 1990). Thus, determining the factors that affect bone mineral density in the growing years could be extremely important in determining preventative measures for a costly and prevalent disease in the elderly.

Research to date examining lifestyle and environmental factors that affect bone mineral density in the young is limited. There is some empirical support that weight bearing physical activity during the growing years is associated with higher bone mineral density. However, there are also equivocal results that there is no association of past physical activity to bone mineral density in the young (Bailey & Martin, 1994; Gutin & Kasper, 1992). Similarly, research is inconclusive on the influence of dietary habits on peak bone density. There is some limited evidence that increased calcium intake during the growing years has a positive association with higher peak bone density values (Lloyd et al, 1993; Johnston et al, 1992).

In addition, there is limited knowledge on the influence of other environmental factors such as exposure to fluoride during the growing years on BMD. Fluoride is known to stimulate bone formation, making it an attractive substance to consider for the prevention of osteoporosis (Riggs, 1991). However, little is known about the optimal level of fluoride or the length of time required for it to have a positive effect on bone density. As well, despite fluoride's bone stimulating effect, there continues to be debate

about fluoride's ability to decrease the risk of fracture. There are reports of a detrimental effect of fluoride in high dosages on the quality of bone, that may result in a higher fracture risk (Melton III, 1990; Riggs, 1991; Inkovaara, 1991). Exposure to low doses of fluoride in the water supply is unlikely to have any detrimental effects on bone; however, there is no conclusive evidence to support a positive effect on bone mineral density or fracture risk (Melton III, 1990).

Supplemental water fluoridation at 1.0 mg./l. has been implemented in many Canadian communities as there is strong empirical support that it is an effective method of reducing dental caries especially during the growing years (Health and Welfare Canada, 1990). It is unclear whether water fluoridation at this low level provides any benefit to the rest of the skeleton during accrual of bone. To date, there is no empirical evidence to support a beneficial effect of drinking fluoridated water on bone mineral density in the young.

Because this study will focus on the impact of an environmental factor on peak bone density in the young, the following two areas will be reviewed in more detail in the Introduction: First, other genetic, lifestyle and environmental factors associated with peak bone density and second, a description of water fluoridation and the impact of fluoride on the young skeleton.

1.1.1 Factors Associated with the Attainment of Peak Bone Density

Two major factors determine the extent of adult bone mineral density and the risk of developing osteoporosis. These factors are peak bone density (the highest bone density established in young adulthood) and subsequent bone loss later in life. Matkovic, Fontana, Tominac, Goel & Chestnut III (1990) report that by age 16 daughters have obtained 90 to 97% of the bone mineral content and 80 to 95% of the bone density of their premenopausal mothers. Geusens et al (1991) found the highest values for bone mineral density and

bone mineral content were found between the ages of 21 to 25 years for both sexes and all locations, with the exceptions of bone mineral content at the distal radius and bone mineral density at the lumbar spine for females which peaked between 16 to 20 years.

Factors associated with the attainment of peak bone density include: heredity, body composition, endocrine factors, physical activity, calcium intake and other factors such as alcohol, smoking, caffeine intake and other medical conditions. The empirical evidence of the influence of these factors on the attainment of peak bone density will be summarized briefly.

Heredity. The extent of the genetic contribution to bone mineral density has been reported by only a few sources. Familial resemblance of bone mineral density is supported by Seeman et al (1989) where lower bone mineral content has been found in the lumbar spine, femoral neck and midshaft of the femur in premenopausal daughters of osteoporotic mothers as compared to normals. McKay, Bailey, Wilkinson & Houston (1993) found a strong familial resemblance in bone mineral density at the lumbar spine and proximal femur for both mother-daughter and mother-grandmother pairs. Krall & Dawson-Hughes (1993) report ranges of 0.46 to 0.62 of the total variance due to heredity for five skeletal sites (total body, femoral neck, lumbar spine, radius and os calcis). From this evidence, heredity does appear to play a substantial role in the attainment of peak bone density, however approximately 50% of the variance in bone mineral density may be explained by other factors.

Body Composition. Several sources report a positive association between body weight and bone mineral density (Dawson-Hughes, Shipp, C., Sadowski, L. & Dallal,G., 1987; Katzman, Bachrach, Carter & Marcus, 1991; Geusens et al, 1991). Low body weight has been identified as one of the risk factors for developing osteoporosis (Cooper, 1989). Much less is known

about the relative associations of bone free lean mass and fat mass on BMD. Faulkner et al (1993) found bone free lean tissue to be an important predictor of total body BMD for both boys and girls aged 8 to 16 years. Others have reported positive associations of fat mass to BMD in premenopausal females but not in males (Reid, I., Plank, L. & Evans, M., 1992).

Endocrine Factors. Endocrine changes associated with menopause are related to a rapid loss of bone mineral density in older women (Stevenson, 1990). Endocrine changes can be a factor in some young women in the attainment of peak bone mass. Lower estrogen levels associated with anorexia nervosa or amenorrhea associated with intense physical training has been associated with lower bone mineral density in young women (Davies, M., Hall, M. & Jacobs, H., 1990; Young, Formica, C. & Szmukler, 1994). Women with lower estrogen exposure, including later age of menarche and irregular menses during adolescence, had lower spine and wrist bone mineral density values (Dhuper, Warren, Brooks-Gunn & Fox, 1989). Women with normal bone mineral density have their first menstrual period an average of 14 months earlier than women with low bone mineral density (Armamento-Villareal, Villareal, Avioli & Civitelli, 1992).

There are reports of a positive relationship between bone mineral density and the use of oral contraceptives (Lindsey, Tohme, Kanders, 1986). However, others have found no relationship between bone mineral density and long term contraceptive use (Lloyd, Buchanan, Ursilo, Wood & Halbert, 1989; Hreshchyshyn, Hopkins, Zylstra & Anbar, 1987). It is possible that exogenous estrogen may suppress endogenous production so that circulating levels may not be excessively high (Armamento-Villareal, Villareal, Avioli & Civitelli, 1992). Thus, the long term effect of oral contraceptives on bone mineral density is still not established, but the current literature would suggest there is no relationship.

Physical Activity. There is empirical support that weight bearing physical activity and mechanical loading is associated with increased bone mineral density. This support has been documented in animal studies, controlled trials on humans, cross sectional observational studies and unilateral studies using one limb as a control (Bailey & Martin, 1994; Smith & Gilligan, 1991). Both weight bearing exercise and load produced on the skeleton by the pull of muscles (ie. weight lifting) may be beneficial (Marcus et al, 1992). Although, the research is promising, data on the effect of physical activity on the growing skeleton in children is still limited. One of the most encouraging studies examining the influence of past physical activity on BMD was a study by Slemenda et al (1991) of 118 healthy children, 5-14 years. They reported children who were more physically active had a five to ten percent greater bone mass than less active children once they reached adolescence.

Calcium Intake. In a quantitative review, Cummings (1990) concluded that there is a consistent positive effect of calcium supplements in increasing bone mineral density in postmenopausal women, however, well designed studies are lacking in the premenopausal age group. Recent studies are emerging in this area, supporting that dietary calcium may play a role on the attainment of peak bone density during the growing years. Calcium intake is often reduced during the adolescent years. Chan (1991) found only 15% of children older than 11 years met the recommended daily allowance for calcium. There is some empirical support that higher calcium intakes result in increased bone mineral density in children and adolescents (Johnston et al, 1992; Chan, 1991; Lloyd et al, 1993). However, various methodological problems including small sample sizes and inconsistent methods of measuring calcium intake have made it difficult to make any definite conclusions on the role of calcium in optimizing peak bone mass.

Vitamin D is essential for the absorption of calcium. Early studies found that children deficient in Vitamin D are not able to absorb calcium, in some situations the amount of calcium in the faeces was greater than the dietary intake (Fourman & Royer, 1968). Sunlight exposure and supplemental vitamin D in milk or milk based foods are the primary sources of Vitamin D.

Other Factors. Women who smoke have an earlier menopause, lower body weight and lower serum estrogen concentrations than women who do not smoke (Lindquist & Bengtsson, 1979). This may put smokers at a greater risk for developing osteoporosis than non-smokers. A recent study of bone density in female twins found a 2.0% reduction in bone mineral density of the lumbar spine in the twin who was the heavier smoker. The authors concluded that women who smoke one pack of cigarettes everyday throughout adulthood would have a deficit of 5 to 10% in bone density, enough to increase the risk of fracture (Hopper & Seeman, 1994).

Alcohol can reduce bone formation by decreasing osteoblast activity (Cooper, 1989). Long term heavy alcohol intake is associated with losses in bone density; but there is no evidence that moderate alcohol intake affects bone mineral density (Heaney, 1993). A moderate intake of caffeine, one to four cups per day, likely has little impact on bone mineral density. However, there are reports of decreased bone mineral density in women with higher caffeine intakes, possibly due to caffeine promoting an increased loss of calcium in the urine or a heavy caffeine intake related to a lower intake of dairy products (Cooper, 1989).

Other medical conditions associated with increased risk of osteoporosis are: corticosteroid use, hyperthyroidism, hyperparathyroidism, biliary cirrhosis, kidney disease, anticonvulscent medication and intestinal malabsorption (Cooper, 1989).

1.1.2 Water Fluoridation and Fluoride's Effect on the Skeleton

Fluoridation of drinking water was first introduced in Canada in 1945 in Brantford, Ontario. By 1977 approximately 8.6 million Canadians were supplied with fluoridated water at a level considered optimal for dental health (Health and Welfare Canada, 1977). Since 1968, Health and Welfare Canada has endorsed fluoridation of drinking water, recommending the optimal fluoridation level be established at 1.2 mg/l. (Health and Welfare Canada, 1990; Canadian Public Health Association, 1979). At this concentration the incidence of dental caries in children has been reported to be reduced by as much as 50% (Health and Welfare Canada, 1990). The decreased prevalence of dental caries in communities with fluoridated water has been consistently reported throughout the world. The most pronounced beneficial effect being on children age 3 to 7 and children age 11 to 14 (Royal College of Physicians, 1976).

Drinking water is the main source of fluoride for most Canadians. The average daily intake of all tap water derived beverages including coffee and tea for females age 18 to 34 in Canada is 1.33 litres per day. There is little difference in reported daily tap water intake in different seasons during the year (Health and Welfare Canada, 1981). Food is a secondary source of fluoride, with fish and tea being the only major dietary sources. In provinces such as Newfoundland where there is a higher intake of foods such as fish and tea, there is a higher fluoride intake from food. Greater amounts of fluoride have been found in the teeth of children from these areas (Elliot & Smith, 1960). In heavy tea drinking areas such as Britain, with an average adult tea intake of 6 to 8 cups per day, higher daily intakes of fluoride are common (Priest & Van De Vyver, 1990). Fluoride is frequently added to products such as toothpastes (0.1 %), mouthwashes (0.02 to 0.1%) and vitamin supplements (0.1 %, Canadian Public Health Association, 1979). The fluoride

from dentifrices and mouthwash is primarily absorbed in teeth enamel, ingested only if swallowed. In adults, the daily fluoride intake from dentifrices is minimal, 0.018 to 0.145 mg./day (Department of Health and Human Services, 1991). Air-borne fluoride is another source of exposure, with fluoride concentrations higher in areas where there is high industrial emission of dusts and gases containing fluoride and in active volcanic regions. The ambient air in Canadian cities contains very low fluoride concentrations (Canadian Public Health Association, 1979).

It is estimated that in Canadian communities with fluoridated water (1.0 mg./l.) the average daily intake of fluoride from food, water, air and fluoride dentifrices such as toothpaste, mouthwashes and vitamins, is approximately 2.7 mg./ day for an adult and less than 2.0 mg/ day for a child (Health and Welfare Canada, 1990). Approximately 80% of daily fluoride intake comes directly from drinking water (Priest & Van De Vyver, 1990). In communities without artificial fluoridation (0.1 mg./l.) daily fluoride intake is 0.3 to 0.5 mg./day for adults and children over 12 years (Canadian Public Health Association, 1979). Food cooked in fluoridated water has three to five time more fluoride than foods cooked in non fluoridated water (Canadian Public Health Association, 1979). This likely is the main contributor to the difference in amount of fluoride ingested in fluoridated communities compared to non fluoridated communities.

Some homes may have filtration systems that remove fluoride from the water. The amount of fluoride removed with water filter systems used in the home may vary depending on the type of system being used (personal communication with Public Health Inspection, Saskatoon, April, 1995). Bottled, demineralized water and water filtered with reverse osmosis have only trace amounts of fluoride. Carbon filter systems that can be attached to the tap, carbon filtered jugs and water softening systems do not remove any

fluoride (Saskatchewan Consumer and Commercial Affairs [1,2], 1989; personal communication with Culligan Water Suppliers, Saskatoon, July, 1995). The percentage of Canadian households that use purification systems is low, approximately 3.7%, but somewhat higher in the Prairie provinces, where approximately 6.4% use water purifiers (Health and Welfare Canada, 1981).

Fluoride has two major effects on bone. First, fluoride substitutes for hydroxyl ions in the mineralization phase forming fluorohydroxyapatite. This substitution increases bone crystallinity and decreases the ability of osteoclasts to resorb bone. Second, fluoride will increase osteoblast number and activity. At long term, low exposure rates, the new bone formed is normal. At higher concentrations as used for therepeutic treatment, the formation of this new bone may have structural defects. The mechanism of these structural changes is not clear (Riggs, 1991). One explanation suggests that if bone mineral is deficient in calcium and phosphate, the mineralization process of the newly formed bone stimulated by fluoride is impaired (Priest & Van De Vyver, 1990). Deficiency in calcium may result in poorly mineralized bone despite the stimulation of bone accretion by fluoride. There is empirical support that calcium supplementation in humans can negate the tendency of higher fluoride concentrations to reduce bone mineral (Likimani, Whitford & Kunkel, 1992). Nonetheless, this does not completely explain structural changes that may occur with higher doses of fluoride as there are reports of no improvement in bone quality or fracture rates in controlled trials of fluoride therapy even with supplemental calcium (Riggs, 1991; Sogaard, Mosekilde, Richards & Mosekilde, 1994).

Approximately ninety-six percent of the fluoride ingested by food and water is absorbed and is taken up by the bones and teeth (Royal College of Physicians, 1976). There is strong experimental data from animal studies that there is a higher uptake of fluoride in the young, growing skeleton as

compared to the mature, adult skeleton. It appears that similar accumulation of fluoride occurs in humans, however comparable data is lacking. The most rapid absorption of fluoride in human bone likely occurs between 10 to 30 years of age, reaching a maximum plateau at approximately age 55 (Canadian Public Health Association, 1979).

The amount of fluoride in bone has been consistently found to correlate positively and exhibit a linear relationship with the amount of fluoride ingested in the water and diet (Grynpas & Rey, 1992; Turner, Akhter & Heaney, 1992). There are also regional differences in incorporation of fluoride in bone. Within individual bones, the distribution of fluoride corresponds closely to the biological activity of bone, where cancellous bone has higher concentrations of fluoride than cortical bone (Weidmann & Weatherell, 1970). Gedalia & Zipkin (1974) report trabecular bone as having 1.5 to 3 times more fluoride than adjacent cortical bone. There are also reports that vertebral fluoride contents are consistently higher than other more peripheral bone sites (Canadian Public Health Association, 1979). This is likely due to the higher trabecular content of bone in the vertebrae. It is widely accepted that the higher accumulation of fluoride in trabecular bone is due to the higher turnover of cancellous bone (Likimini, Whitford & Kunkel, 1992).

1.2 Review of Related Literature

Because of fluoride's ability to stimulate new bone formation, it has been considered as a possible preventative measure to decrease the risk of osteoporotic fracture. There are two ways to consider its use: 1) as a secondary preventative measure by using high therapeutic doses of fluoride to increase bone mass in individuals with low bone mass and 2) as a primary prevention in the form of low level fluoridation in the water supply to help

prevent age-related bone loss. The evidence for both methods will be reviewed, the latter in more detail as it is more directly related to this study.

Sodium fluoride's ability to stimulate new bone formation has made it an attractive treatment for osteoporosis. For example, fluoride therapy has been found to dramatically increase trabecular bone mass in dosages of 20 to 80 mg. / day (Riggs, 1991; Dure-Smith, Kraenzlin, Farley, Libinati, Schultz & Bayink, 1991). In a randomized clinical trial of 202 osteoporotic women (Riggs et al, 1990), the fluoride therapy group (75 mg./day) increased their mean bone mineral density in the lumbar spine by 35%, 12% increase in the femoral neck and a 10% increase in the trochanter of the proximal femur as compared to a placebo control group over a four year period. However, cortical bone mass as measured in the shaft of the radius actually decreased by 4%. Fluoride therapy has been found to consistently increase trabecular bone mass but it appears to have no effect or it may have a negative effect on cortical bone (Melton III, 1990; Riggs, 1991). As well, a recent study suggests that there is no improvement in trabecular bone quality or strength in other areas such as the iliac crest after five years of fluoride therapy (Sogaard, Mosekilde, Richards & Mosekilde, 1994).

The mechanism for these divergent effects of fluoride in different areas in the skeleton is not clear. It is possible that there is a redistribution of mineralized bone from cortical to cancellous areas with fluoride therapy as there might not be enough bioavailable calcium in the cancellous bone to keep up with mineralizing the newly formed bone stimulated by large doses of fluoride (Riggs, 1990). For example, at the proximal femur where there are relatively equal amounts of cancellous and cortical bone, there are moderate increases in BMD in response to fluoride therapy, however, there is no evidence that these increases in BMD result in any decrease in fracture risk (Riggs, 1991). Bone formed in response to large doses of fluoride has

increased crystallinity, decreased elasticity, and decreased tensile strength, thus the increase in bone density does not necessarily equate with stronger bone.

In the Riggs et al randomized trial (1990) there was no significant decrease in vertebral fractures in the treatment group despite a significant increase in BMD. The number of non vertebral fractures was actually significantly higher in the treatment group. Another retrospective study of 389 patients treated with sodium fluoride (Farley et al, 1992) found that the spinal fracture rate decreased significantly as a function of time with fluoride therapy. There was a subgroup of patients who had a 48% decrease in spinal fracture rate as compared with nonresponders. Nonresponders tended to be older, had more fractures prior to therapy and had a much slower rate of increase in spinal bone density. Others have reported this nonresponse to fluoride therapy, which sheds further questions on its clinical usefulness and reinforces the individual responses to fluoride. Inkovaara (1991) reported approximately fifteen to twenty per cent of fluoride therapy patients do not respond to therapy.

It appears from the current research that there is a substantial increase in axial BMD in response to fluoride in therapeutic doses in the range of 20 to 80 mg./day. There is some support that this results in a slight decrease in vertebral fracture rate for osteoporotic women. However, there is no evidence that fluoride affects hip fracture rate and there is some evidence that it may actually increase hip fracture rates (Melton III, 1990). Thus, the use of fluoride to decrease the incidence of osteoporotic fracture cannot be currently supported until further research is done (Melton III, 1990; Riggs, 1991; Inkovaara, 1991; Dure-Smith et al, 1991).

Evidence that long term exposure to low level of fluoride in the water supply can reduce bone loss later in life, is also sparse and inconclusive. There are no controlled, clinical trials of exposure to low level fluoride on

bone density or fracture rate. The majority of the studies in this area are population based studies examining hospital admission data and hip fracture rate in communities with differing water fluoridation levels. There are numerous problems with this type of research: difficulty determining other predisposing factors for fracture, migration, comparison of urban verses rural communities, and difficulty determining actual daily intake of fluoride. Because of these difficulties, the evidence is mainly circumstantial; the fracture rate may differ for a number of other reasons.

The results of these studies are mixed. There are reports of no effect of water fluoridation on fracture risk (Suarez-Almazor et al, 1993; Madans, Kleinman & Cornoni-Huntley, 1983), a positive effect of low level fluoride on BMD and reducing fracture risk (Kroger, Alhava, Honkanen, Tuppurainen & Saarikoski, 1994, Simonen & Laitenan, 1985), and a negative effect on hip fracture risk (Danielson, Lyon, Egger & Goodenough, 1992). Comparing these studies is difficult as the results may vary due to varying fluoridation levels, different skeletal sites measured, different measurement methods, varied exposure time, inconsistent evaluation of confounding factors and variability of communities being studied. The related research in this area will be reviewed.

During the 1950s and 60s there were reports of a decreased prevalence of osteoporosis in geographical areas with water fluoridation greater than 4.0 mg./l. (Bernstein, Sadowsky, Hegsted, Guri & Stare, 1966). More recent cross-sectional and prospective studies have found no protective effect at these levels and in some cases a negative effect on cortical bone. Sowers, Wallace & Lemke (1986) investigated bone density and fracture history in women from three communities in Iowa. Fluoride levels were 4.0 mg./l in one community and 1.0 mg./l in the two other communities. Mid radius bone density was measured via photon absorptiometry in women aged 20 to 80 years in the high fluoride community and 20 to 35 years and 55 to 80 years in the low fluoride

communities (n = 417). There was no significant difference between the three communities in confounding factors such as socioeconomic background, sunlight exposure, exercise history, height, weight and body fat. For all women there was no significant difference in mid-radius bone mineral density, but there was an increased fracture frequency in the postmenopausal women in the high fluoridated community. An analysis of covariance including calcium and vitamin D intake as covariates revealed that premenopausal women in the lower fluoride group (1.0 mg./l.) had greater mean bone density values than premenopausal women in the high fluoride community (4.0 mg./l.). In a subsequent prospective study of the same three communities (Sowers, Clark, Jannausch & Wallace, 1991) radial bone density was significantly lower in both premenopausal and postmenopausal women. As well, there was an increased rate of radial bone mass loss in premenopausal women and significantly more fractures among postmenopausal women in the high fluoridated community.

Another study in the United States with similar water fluoridation levels, examined 151 women 39 to 87 years, lifelong residents of two communities, one with optimal water fluoridation of 1.0 ppm, another community with a higher fluoridation level of 3.7 ppm (Phipps & Burt, 1989). Mid radius bone density was measured via single photon absorptiometry. Using multiple regression analysis, significant predictors of bone density were body weight, years since menopause, estrogen supplementation, diabetes and fluoride exposure. Exposure to the higher fluoride level was a negative predictor of bone density. In support of the Sowers study, there may be a negative effect on cortical bone density at fluoride levels of 3.5 ppm or greater. These authors suggest water fluoridation at 3.5 ppm may be a threshold limit where fluoride becomes detrimental to cortical bone.

It is not clearly established at what level of fluoride exposure impaired mineralization or osteofluorosis develops, as there are individual reactions to fluoride. Findings from Arnala, Alhava, Kivivuori & Kauranen (1986) suggest 4000 ppm of fluoride in trabecular bone ash is the upper limit for developing osteofluorosis. This level of fluoride has not been found in the bones of individuals exposed to less than 1.5 mg./l. of fluoride in their water supply. However, long term exposure above 1.5 mg./l. does result in a greater number of individuals with osteofluorosis (Arnala, Alhava, Kivivuori, Kauranen, 1986; Arnala, Alhava & Kauranen, 1985).

There is discrepancy in the literature on the effect of low level fluoridation on animal bone strength. An early study reported no increase in rat's bone strength up to fluoridation levels of 20 ppm with bone strength diminishing at greater than 45 ppm (Saville, 1967). More recently, Turner, Akhter & Heaney (1992) reported that rats exposed to fluoridation levels of 16 ppm for 16 weeks had greater femoral bone strength as measured by a three point bending test as compared to no fluoridation and fluoridation at 64 ppm and 128 ppm. Fluoride levels in bone were determined by ash weight measurements in the lumbar vertebrae. The vertebral fluoride content at which femoral bone strength was maximum was between 1100 and 1500 ppm. This is approximately the equivalent bone fluoride level in axial spine areas observed in humans exposed to 1.0 ppm fluoride for greater than 10 years. These results suggest that fluoridated water levels of 1.0 ppm may lead to increased bone strength whereas water fluoridation levels greater than 4.0 ppm may lead to decreased bone strength.

Kuipo, Finland and surrounding communities have been the location of several studies due to differing water fluoridation practises in close proximity. The first was by Alhava, Olkkonen, Kauranen & Kari (1980), where 158 autopsies were performed using the iliac crest. They measured

trabecular bone compressive strength with a strain transducer and BMD with gamma ray attenuation. Histomorphic evaluation of the bone was also performed using bone ash to establish the amount of fluoride present. The mean age of the subjects was 68.6 years. The women from Kuipio, where there is water fluoridation of 1.0 mg./l. had a much more rapid accumulation of fluoride in their bones. Women in Kuipio had a mean of 2070 ppm of fluoride in cancellous bone compared to 622 ppm in the non fluoridated communities outside Kuipio. The fluoride content in cortical bone was much less, 1720 ppm in Kuipio women compared to 443 ppm in women from the other communities. There was no significant difference between the BMD values between the two groups and even though there was a significant difference between the compressive strength, the authors concluded that there appeared to be no beneficial effect of low level water fluoridation on BMD or bone strength.

In the second study in this region, three different water fluoridated communities were studied: Kuipio, with moderate (1.0 mg./l.) fluoridation, communities with low (0.3 mg./l) fluoridation and a high fluoridated community (>1.5 mg./l., Arnala, Alhava, Kauranen, 1985). One hundred and eighty-five bone autopsy samples were used from both men and women from the three areas. As before, there was a significantly higher fluoride concentration in trabecular bone in the moderate (1.0 mg./l.) and high fluoride regions (>1.5 mg./l.) compared to the low fluoridated region (< 0.3 mg./l.) They also reported a linear relationship between fluoridation and fluoride content of the bone ($r= 0.761$, $p<0.0001$). There was no significant difference between histomorphic changes between the low and moderate fluoridated regions; although there was a significant increase in the osteoid component in the high fluoridated region. The osteoid (non mineral) component of bone increases with fluoridation higher than 1.5 ppm. They surmise that there may

be an upper limit of water fluoridation where there is no change in bone structure up to 1.5 mg./l., beyond this structural changes can occur in the bone that may increase risk of fracture. Thus, there may be no protection of water fluoridation to prevention of bone loss up to 1.5 mg./l. but it does not appear to be harmful to bone quality.

In a third study in this region of Finland, hip fracture incidence was also evaluated in the same 185 individuals in the previous study by Arnala, Alhava, Kivivuori & Kauranen (1986). The incidence of hip fractures did not differ between these three communities based on hospital data taken between 1972 to 1981. However, an earlier study by Simonen & Laitenen (1985), which examined the same regions in Finland during the period 1967 to 1978, found a significant difference in hip fracture rate. The difference between men in the two communities, Kuipio, fluoridated at 1.0 mg./l. and Jyvaskyla, at 0.3 mg./l. was significant ($p < .001$) in all age groups over 50 for men, but only over 70 years of age for women. They suggest fluoride in the water supply at 1 ppm is an essential mineral and may assist in the strengthening of bone tissue as prevention against osteoporosis. This certainly raises the question of how two investigations of the same communities could produce such different results. This emphasizes the difficulty in drawing conclusions from these population based studies where hip fracture incidence may vary over time for unknown reasons.

Danielson, Lyon, Egger & Goodenough (1992) compared hip fracture rates in three communities in Utah, one with fluoridation to 1.0 ppm and two without fluoridation, < 0.3 ppm. There was a small but significant increase in hip fracture rates for both men and women in the fluoridated community. This is one of the first reports of an increased fracture risk in an optimal fluoridated community. However, despite the communities being similar in population and migration status, there was no method used to determine the

role of other confounding factors such as estrogen status, dietary habits and physical activity status. Another study in the United States (Jacobsen et al, 1990) reported an increased hip fracture risk in communities with water fluoridation. Based on a weighted least squares regression model, they found that soft and fluoridated water, poverty, reduced sunlight exposure and rural location all increase the risk of hip fracture. However, the amount of fluoride in the water may have differed significantly in the different communities examined, with no specific definition of the level of fluoridated water used in the analysis.

The majority of studies examining hospital recorded hip fracture data or BMD, suggest there is either no effect or a small, positive effect of low level water fluoridation on bone. Madans, Kleinman & Cornoni-Huntley (1983) found no protective effect of water fluoridated to 0.7 ppm in examining the NHIS (National Health Interview Surveys from 1973 to 1977). However, preliminary investigation of communities in the NHIS with natural fluoride in their water greater than 0.7 ppm suggests there may be a decreased risk of hip fracture in women only with higher levels of water fluoridation.

Cooper, Wickham, Lacey & Barker (1990) examined the relationship of hip fracture hospital discharge rate and water fluoridation levels in nine counties in England. They found no relationship between discharge rates and water fluoridation ranging from 0.005 mg./l. and 0.93 mg./l. The only known study in Canada to date was by Suarez-Almazor and colleagues (1993). Two similar communities (Edmonton and Calgary), were compared, with water fluoridation of 1.0 mg./l. and the other with no supplemental fluoridation. Hip fracture hospital admission rates were examined for the two communities from 1981 to 1987. There was a small but significant difference for men over age 65. However, the authors point out that these small differences in a large population based study may be significant, but likely not clinically relevant.

They concluded there was no effect of low level water fluoridation on hip fracture hospitalization rate.

The most recent study and most relevant to this study's design was again in Finland. This is the only other study, to my knowledge, that has specifically investigated bone mineral density in a female population in communities with low level fluoridation and no fluoridation. Kroger, Alhava, Honkanen, Tuppurainen & Saarikoski (1994) compared BMD in two communities , one with fluoridated drinking water at levels of 1.0 - 1.2 mg./l., another with no supplemental fluoridation, < 0.3 mg./l. The age range of the women participating were 47 to 59. A total of 969 women drinking the fluoridated water for greater than 10 years and 2253 women drinking the nonfluoridated water were tested on a Lunar DPX, examining BMD of the AP spine and the femoral neck. BMD was significantly higher in the fluoride community as compared to the non fluoridated community for the spine only. When BMD values were adjusted for confounding factors (age, weight, menopausal status, calcium intake, physical activity, deliveries, alcohol consumption and estrogen use) the mean spinal BMD remained significantly higher in the fluoridated community (1.151 vs. 1.121 g/cm^2, $p < .001$) and also was significantly different for the femoral neck (0.940 vs. 0.930, $p = 0.004$). There was no significant difference between the groups in prevalence of reported fractures sustained during a nine year period just prior to the study.

To date, there is limited evidence that fluoridated water at the level recommended for dental caries, approximately 1.0 mg./l., protects against bone loss associated with osteoporosis. Only one other study to my knowledge (Kroger et al, 1994) has investigated BMD in people exposed to low level water fluoridation compared to no supplemental fluoridation and they found an increase in axial BMD in women in the fluoridated community. There are substantial reports of increased axial BMD in older women exposed to

therapeutic doses of fluoride. However, there is no evidence that low level fluoridation in the water supply provides a protective effect to fracture risk.

There may be a threshold level where water fluoridation has a beneficial effect on bone within the optimal range of 1.0 mg./l. up to 2.5 mg./l. as suggested by Simonen & Laitinen (1985) with possibly a negative impact occurring beyond 4.0 mg./l as suggested by Sowers (1986). It appears from the research that the quality of bone is not compromised at long term exposure to water fluoridation at a low level (1.0 mg./l.) as there are no reports of skeletal fluorosis in communities fluoridated at these levels (Canadian Public Health Association, 1979). The amount of fluoride found in the skelton of women exposed to low level fluoride does not reach toxic levels (Arnal, Alhava, Kauranen, 1985). However, due to the mixed results of the available research, there is no recommended level of fluoride for bone health as there is for dental health. It is possible that research examining BMD in the young adult skeleton will shed further light on the impact of fluoride during the growing years. Fluoride has its greatest positive impact on teeth during the growing years and is accumulated more rapidly in the growing skeleton. In spite of this, there is no research to date examining the effect of long term, low level exposure to fluoride on the establishment of peak bone density in the young adult skeleton.

1.3 Rationale for Study

The purpose of this study was to examine the relationship of drinking fluoridated water during the growing years on the attainment of peak bone density in young adult females. The communities selected for this study, Saskatoon and Regina, are similar in sunlight exposure, economic structure, population and climate (see Appendix A). Thus, they are ideal communities for a comparative study. Saskatoon has had fluoridated water, 0.9 mg./l. to

1.2 mg./l. for the past 35 years. Regina's water fluoride content is 0.12 mg./l. and has never been supplemented. The objectives of this study are to: 1) determine if there are any differences in BMD between two samples of young women who have lived most of their growing years in communities with different water fluoride levels and 2) determine if long term fluoride exposure has differing effects on the axial skeleton as compared to more peripheral skeletal sites.

1.4 Hypothesis

The major hypothesis is that the young women drinking from the fluoridated water supply in Saskatoon will have higher bone density values as compared to the young women drinking from a non-supplemented water supply in Regina. The second hypothesis is that the differences in BMD values will be site specific, with a greater difference in BMD at the axial spine sites (AP Spine, Lateral Spine (L3) and VLS) as compared to the proximal hip and total body.

1.5 Limitations and Delimitations

The sample populations will be restricted to female University students from Regina and Saskatoon. The results of this study can only be applied to females age 18 to 25 years in similar communities.

Chapter 2

Method

2.1 Subjects

The total sample of 57 females between the ages of 18 and 25 years of age volunteered from the University of Regina in Regina, Saskatchewan and the University of Saskatchewan in Saskatoon, Saskatchewan. All females selected were long term residents, not travelling outside of their resident city for longer than four years. The mean number of years in their respective cities were 20.1 years for Regina subjects and 21.3 years for Saskatoon subjects. Eighty-three percent of the subjects in Regina and 95% of the subjects in Saskatoon had lived in their respective cities for 100% of the time during their growing years (age 1 to 18).

A screening questionnaire was administered to all potential participants. Individuals with bone affecting disorders, use of potential bone affecting medications, long term use of fluoride supplements, a history of amenorrhea (less than 3 menses per year, as defined by Armamento-Villareal, Villareal, Avioli & Citivelli, 1992), and those who were currently pregnant were excluded (see Appendix B). All subjects signed consent forms (see Appendix C). This study was approved by the University of Saskatchewan Advisory Committee on Ethics in Human Experimentation.

2.2 Measures

DXA Hologic 2000. DXA is a dual energy x-ray source used for the purpose of measuring bone mineral density. The Hologic 2000, array mode, was used in this study. The radiation entrance dose ranges from 1 mrem for the whole body to 12 mrem for the lumbar spine. When these surface doses are corrected for body attenuation, as well as type and volume of tissue being irradiated, and the reproductive capacity of the subject based on the protocols established by the International Commission on Radiological Protection (ICRP 60, 1990), the effective dose equivalent for the entire protocol is 5.61 mrem. This is assuming that the ovaries are exposed fully or partially for all scans.

Quality control phantom scans were performed daily on the Hologic 2000. The short term precision values are 0.9% and 1.1% at the femoral neck and total proximal femur respectively. Short term precision values for the total AP lumbar spine are 0.7 % and 2.1 % for the lateral spine using L3 alone. The short term precision value for the total body is 0.5 %. These values are in agreement with other sources (Mazess, Collick, Trempe, Barden, & Hanson, 1989). The validity of the DXA is confirmed by testing the accuracy of the instrument to detect a known amount of hydroxyapatite in phantom cadavers. There is a linear increase in measured bone mineral content as the level of hydroxyapatite increases (SEE= 0.8% Mazess, Collick, Trempe, Barden & Hanson, 1989).

In this study, BMD of the AP Spine, Total Body and Proximal Femur were measured. A companion lateral spine scan of the lumbar spine was also done to further evaluate the trabecular bone in the vertebral body. Width adjusted BMD measure of the lumbar spine (VLS) was calculated by the Hologic 2000 software (refer to Operational Definitions, page viii). Only L3 was used for VLS due to the increased error at L2 and L4 from overlying ribs close to L2 and the close proximity of the pelvis at L4. Because, the posterior

elements of the spine (high in cortical content) are eliminated from the analysis, the VLS estimate has been recommended as a more accurate measure of trabecular bone in the spine (Peel & Eastell, 1994; Uebelhart, Duboeuf, Meunier & Delams, 1990).

Screening Questionnaire A questionnaire to screen for any bone disorders, use of medication that could affect bone, menstrual dysfunction or pregnancy, was administered to all participants prior to testing (see Appendix B). This questionnaire was based on the MEDOS (Mediterranean Osteoporosis Study Questionnaire, Dequeker, Ranstam, Valsson, Sigurgevisson & Allander, 1991). Questions detailing place of birth and current residence were asked and individuals who had lived outside of their birth city for greater than four years were excluded. Four years was chosen as a conservative length of time that would not result in any differences in fluoride exposure. Other sources have recommended participants be exposed to fluoride for at least five years (Sowers, 1991). The subjects in this study were all exposed to the water supply for over 80 % of their growing years.

Lifestyle and Dietary History Questionnaire A comprehensive questionnaire evaluating various lifestyle factors was filled out by all participants at the time of testing. This questionnaire was based on the MEDOS questionnaire (Dequeker, Ranstam, Valsson, Sigurgevisson & Allander, 1991, Appendix D). Other detailed questions were included on physical activity, calcium intake, food and water intake and estrogen history.

The physical activity questions used in this questionnaire included rating current physical activity level and past physical activity during childhood and youth on a five category rating scale. This scale was developed from the activity rating scale used by Sallis, Buono, Roby, Micale & Nelson (1993). This scale has been correlated with cardiovascular risk factors, and although not a sensitive measure for change; it has been reported as an

appropriate screening questionnaire. A two week recall of current physical activity was also administered, individuals checking off from a comprehensive list of physical activities spent in the last two weeks. For the two week recall, the total number of minutes spent in weight bearing activity/week was calculated by adding the highest level of minutes indicated in each category marked by the participant and then dividing the total number of minutes by two. Housework, yardwork and swimming were not included (see Appendix D, question 6.0). The two week recall has been used in previous and ongoing bone density research at the University of Saskatchewan (McCulloch, 1989). The two week recall was moderately correlated with the activity rating scale, $\underline{r}$ = 0.67, in this study. Test-retest reliability of physical activity questionnaires over three months has demonstrated respectable correlations ($\underline{r}$ = 0.74 to 0.88, Baecke, Burema & Frijters, 1982).

Calcium history was assessed based on a milk history questionnaire. This form was developed in conjunction with the National Health and Nutrition Evaluation Survey III (NHANES III, Woteki, Briefel & Kuczmarski, 1988). Estrogen exposure was evaluated based on the scoring criteria proposed by Armamento-Villareal, Villareal, Avioli & Civitelli (1992). This is a modification of the method described by Dhuper (1989). In the modified method, the estrogen score is based on age of menarche, length and frequency of menstrual cycles and use of oral contraceptives. Weighted scores are assigned, with a possible range of scores from 2 to 24 (see Appendix E).

Food Frequency Questionnaire. A food frequency questionnaire (FFQ) developed by the Department of Nutrition and Dietetics at the University of Saskatchewan was administered to all participants at the time of testing (see Appendix F). Individuals provided their usual intake of foods in a typical day or week, using food models as guides. The daily intake of calcium and vitamin D was determined by a nutritional assessment software package with

1988 Canadian Nutrition File information. The validity of this FFQ was tested by correlating results with three day estimated food records in a sample of forty undergraduate university students ($\underline{r}$ = 0.45 for calcium, $\underline{r}$ = 0.65 for Vit. D). The reliability coefficient for the FFQ over three weeks was r= 0.91 for both calcium and vitamin D (Whiting, unpublished data).

Fluoride Level Saskatoon's tap water has been supplemented with fluoride for greater than 25 years. The level of fluoride in Saskatoon's tap water has been consistent at 0.9 mg./l. to 1.0 mg./l. The fluoride level is tested four times per day by pulling a sample of water and testing with a fluoroelectrode. A safety control ensures that the fluoride level does not exceed 1.25 mg./l. The average calcium ion concentration in the water is 31 mg./l. (Personal communication, Water Treatment Plant, Saskatoon).

Regina's water supply has not been supplemented with fluoride for the past 25 years. For the past five years Regina has received their tap water supply from the Buffalo Pound Water Supply. The average natural fluoride in Regina's tap water is 0.12 mg./l to 0.15 mg./l. The average calcium ion concentration in the water is 42 mg./l. (Personal communication, Buffalo Pound Water Treatment Plant).

2.3 Procedure

All testing was performed from October, 1994 to January, 1995. Regina and Saskatoon subjects were dispersed randomly over this time frame. Subjects from Regina were transported to Saskatoon by van or car, with all testing being completed in one day. Three qualified technicians were used to perform the Hologic 2000 scans. A standard protocol was used for all scans as outlined in the HOLOGIC QDR Operators Manual and User Guide (1991). Subjects wore loose fitting shorts and a T-shirt for measurement. All metal objects (jewellery, glasses, etc) and shoes were removed prior to the bone

density scans.

The total body scan was performed with the subject positioned in supine. The body was centered and straightened along the midline within the longitudinal total body lines as outlined on the scan mat. The shoulders were depressed below the level of the jaw and the arms were pronated. The great toes were inverted and taped together to maximize femoral neck display and immobilize this region for consistency of measurement. For the lumbar spine, subjects were positioned supine and to the right edge of the scanning pad, ensuring their body was parallel to the longitudinal midline. Legs were positioned on a firm block cushion, with the angle of the hip at approximately 45 degrees. Subjects were cautioned not to move their position prior to the lateral spine scan (see Figure 1). For the proximal hip, the limb was positioned in internal rotation using the positioning fixture and strapping the foot and leg to immobilize it in this position (see Figure 2).

The machine was calibrated using a standard phantom spine prior to each testing session. Height and weight measures and administration of the food frequency questionnaire and lifestyle questionnaire were performed by the same individual using a standard protocol.

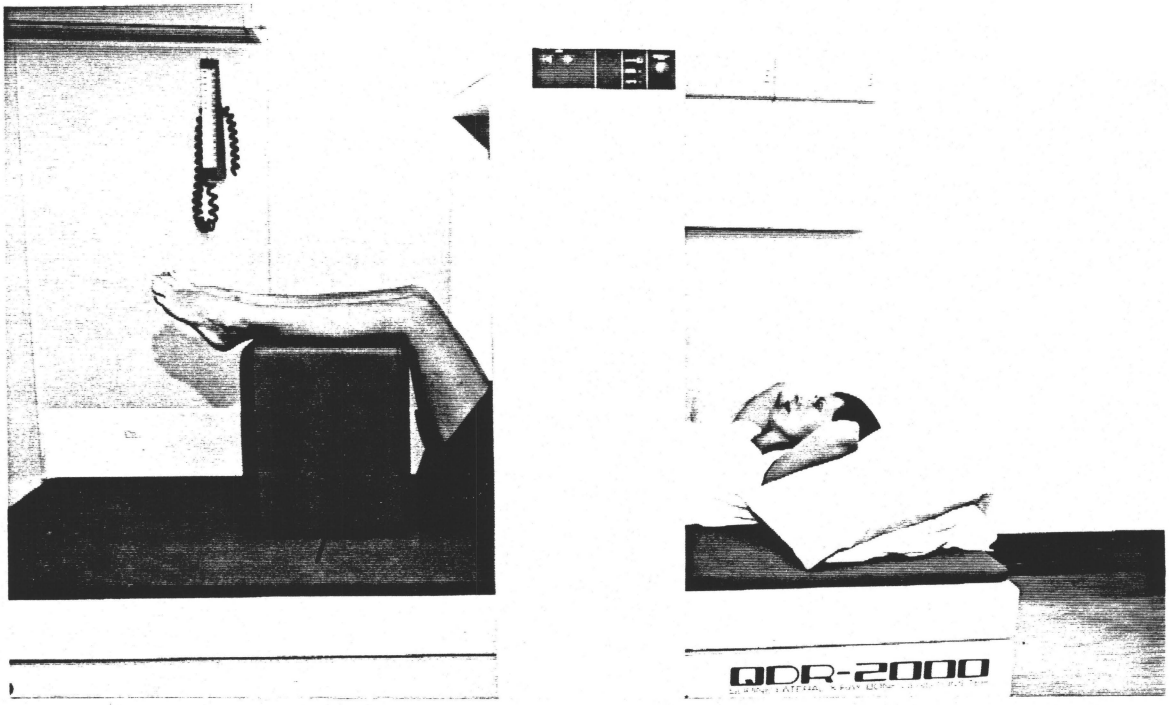

Figure 1. Subject on Scan Table for AP/Lateral Lumbar Spine Scan

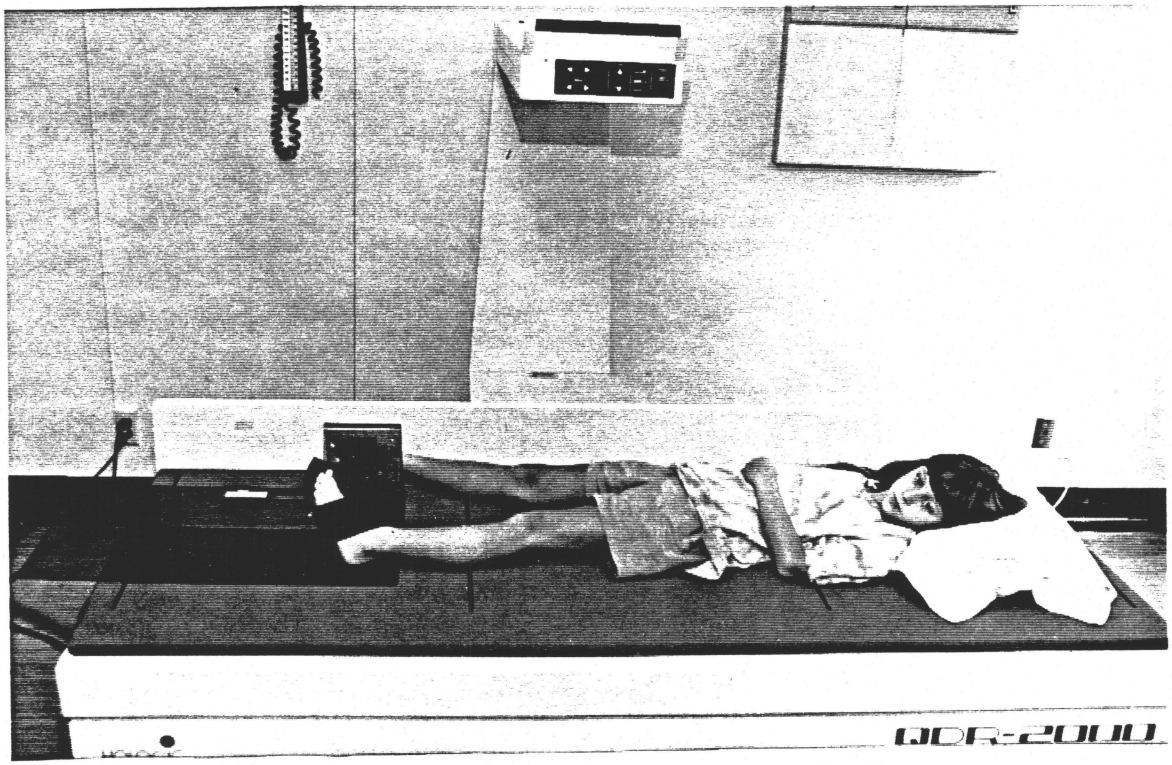

Figure 2. Subject on Scan Table for Left Femur Scan

3.4 Statistical Design.

Descriptive statistics of the following variables were generated to evaluate various factors that may affect BMD: height, weight, lean body mass, fat mass, estrogen status, past and present physical activity level, calcium history, current calcium and vitamin D intake, alcohol and caffeine intake, smoking history, oral contraceptive use, family history of osteoporosis and water intake. The rating scales used for physical activity, milk history and estrogen score were analyzed as interval data. The assumption that ordinal scales are interval is often made as the conclusions drawn are similar (Diekhoff, 1992).

To determine if there was an overall difference in BMD between Saskatoon and Regina samples, MANCOVA , with weight as the covariate, was calculated. Weight was chosen as a covariate based on its significant, positive relationship with BMD. This has been supported in previous literature (Dawson-Hughes, Shipp, Sadowski & Dallal, 1987). The three primary bone sites used as dependent variables in the MANCOVA were: AP Spine, Total Body and Proximal Femur. Post-hoc univariate tests were conducted for these three primary sites. Based on the significant finding at the AP Spine, further analysis was conducted for the axial spine (lateral spine scan and VLS for L3) using ANCOVA.

An alpha level of 0.05 using a one-tailed test of significance was selected for all analyses. A one-tailed test was chosen based on the related research supporting either a small positive effect of water fluoridation on BMD or no effect (Kroger et al, 1994; Arnala, Alhava & Kaurenen, 1985). There is no evidence to support a detrimental effect of low level fluoridation on BMD (Melton III, 1993). However, the one-tailed test does increase the chance of making a Type I error.

Chapter 3

Results and Discussion

3.1 Results

The Results section will be divided into three subsections: First, descriptive data of various factors that may affect BMD (body composition parameters, dietary history and lifestyle factors) will be reported. Second, results of MANCOVA for differences between BMD for the two groups and univariate analysis for AP Spine, total body and proximal femur BMD will be presented and third, results of univariate ANCOVA for the axial spine measures: lateral spine (L3) and VLS (L3), will be reported.

3.1.1 Descriptive Data: Body Composition, Lifestyle and Diet

Several factors were assessed to determine if there were any differences between the two groups that might contribute to differences in BMD other than exposure to water fluoridation. The primary variables chosen as potential confounding variables were: age, weight, height (measured and recorded at time of testing), bone mineral free lean mass and fat mass (measured by DXA Hologic 2000) , estrogen score (modified Dhuper scoring system), past physical activity level (rating scale), present physical activity (rating scale and two week recall of weight bearing activities), calcium history (milk history) and current calcium and Vitamin D intake (food frequency questionnaire). Independent t-tests were performed and there were no significant differences between any of these variables for the two groups (see Tables 3.1 to 3.3).

Table 3.1. Descriptive Data and Independent t-tests for Age, Height, Weight, Fat Mass and Bone-free Lean Mass

Variables	mean	range	SD	t	p*
Age					
Regina	20.8	18-25	1.72	-1.21	0.23
Saskatoon	21.3	18-25	1.63		
Height (cm)					
Regina	166.6	155.1-175.8	4.71	-0.66	0.51
Saskatoon	167.6	153.5-179.5	6.24		
Weight (kg)					
Regina	63.60	49.20-75.85	7.36	1.37	0.18
Saskatoon	60.83	43.85-78.45	7.66		
Bone-free lean (kg)					
Regina	40.05	30.50-48.68	4.42	0.07	0.95
Saskatoon	39.97	31.29-52.67	5.29		
Fat Mass (kg.)					
Regina	20.29	5.69-30.74	4.78	-1.85	0.07
Saskatoon	17.69	6.87-26.60	5.80		

*two-tailed significance

Table 3.2. Descriptive Data and Independent t-tests for Estrogen Score, Milk History, Current Calcium Intake, Vitamin D Intake, Past and Present Physical Activity.

Variables	mean	range	SD	t	p†
Estrogen Score (2-24)					
Regina	18.17	12-22	2.46	0.27	0.79
Saskatoon	18.33	15-23	2.15		
Milk History (rating scale 1-7)					
Regina	5.54 (child)	1-7	1.44	-0.44	0.66
	5.30 (teen)	1-7	1.55	-0.55	0.58
Saskatoon	5.70 (child)	2-7	1.24		
	5.48 (teen)	2-7	1.09		
Daily Calcium (mg)					
Regina	1016.3	314-2519	552.9	-0.94	0.35
Saskatoon	904.6	337-1743	346.2		
Daily Vitamin D (RDA- ug)					
Regina	6.42	1.12-19.41	4.22	0.95	0.31
Saskatoon	5.48	1.11-13.91	2.73		
Physical Activity Rating (1 to 5)					
Past					
Regina	3.96	2-5	0.86	0.05	0.96
Saskatoon*	3.97	2-5	0.92		
Present					
Regina*	3.57	2-5	0.99	-0.31	0.75
Saskatoon	3.48	2-5	0.91		
Weight Bearing Activity (min./week)					
Regina	291.04	15-872.5	219.5	-0.69	0.50
Saskatoon*	256.30	52-626.0	158.9		

Note. n= 24 for Regina, 33 for Saskatoon except where noted * n=33,23,31
† = two-tailed significance

Table 3.3. Mean Differences and Confidence Intervals for Differences in Body Composition and Lifestyle Variables Between Saskatoon and Regina

Variables	Mean Difference	95% Confidence Intervals
Age	0.54	(-0.36, 1.44)
Height (cm.)	1.01	(-2.03, 4.05)
Weight (kg.)	-2.76	(-6.81, 1.29)
Bone-free lean (kg.)	-0.87	(-2.74, 2.57)
Fat Mass (kg.)	-2.60	(-5.41, 0.21)
Estrogen Score	0.17	(-1.06, 1.39)
Milk History		
Child	0.16	(-0.56, 0.87)
Teen	0.19	(-0.51, 0.89)
Daily Calcium (mg.)	-111.67	(-350.65, 127.30)
Daily Vitamin D (RDA)	-0.94	(-2.78, 0.91)
Physical Activity Rating		
Past	0.01	(-0.47, 0.49)
Present	-0.08	(-0.59, 0.43)
Current Weight Bearing Activity (minutes/week)	-34.74	(-136.05, 66.56)

These primary confounding variables were correlated to the BMD values used in the analysis: AP Spine, total body and proximal femur. The Pearson r correlation table is reported in Appendix G. Weight and lean body mass were the highest correlation coefficients for the three BMD sites. Other correlation coefficients that reached statistical significance were height and past physical activity rating.

Secondary variables examined were: family history of osteoporosis, estrogen status, alcohol and caffeine intake, smoking history and water consumption. Family history of osteoporosis was defined as a report of the disease in paternal and maternal parents, grandparents, aunts, uncles or cousins. The percentage of participants with a family history of osteoporosis was similar in both groups, slightly higher in Saskatoon, 36.4% reporting family history in Saskatoon, 20.8% in Regina. A Mann-Whitney U test for independent samples was non significant. Estrogen status was measured by use of oral contraceptives, number of years since menarche and scores on the modified Dhuper estrogen score (see Appendix H). In Regina, 54.2%, and in Saskatoon, 45.5%, reported current or past use of oral contraceptives. The mean number of years since menarche was 7.90 for Regina, 8.56 for Saskatoon. The difference in estrogen scores can be found in Table 2.

Results for alcohol, caffeine, water consumption and smoking patterns can be found in Appendices I and J. In Saskatoon, 54% and in Regina, 41.7% reported never drinking coffee. Over 80% of the respondents in Saskatoon and Regina were alcohol abstainers or reported only occasional use of alcohol (once or twice a month). Smoking history was also very low in both groups. The mean number of pack years for the 20.8% in Regina who had smoked at sometime in their life was 1.23. In Saskatoon only 9.1% had ever smoked with a mean number of pack-years of 2.70. The highest frequency of tap water consumption in both groups was between 3 to 5 cups

per day, including powdered and concentrated beverages mixed with water, but excluding coffee and tea (see Appendix J). The frequency of bottled water consumption was similar in both groups but slightly higher in Regina, with over 90% in both groups reporting never or occasional use of bottled water.

3.1.2 Multivariate Analysis of Differences between BMD Values for the Regina and Saskatoon Samples

Bone mineral content and density mean values are summarized in Appendix K for the whole sample and Regina and Saskatoon groups. One subject's lateral spine scan was eliminated from the final analysis because of an unknown anomaly resulting in an abnormally extreme value at lateral L3.

The first hypothesis was that the young women from a water fluoridated community (Saskatoon) would have greater BMD values as compared to a non fluoridated community (Regina). This hypothesis was supported as a MANCOVA revealed a significant main effect of city on BMD, using Wilks Lambda test of significance (F (52,3) = 2.44, $p < 0.05$, one-tailed test, see Table 4). Because the MANCOVA was significant, post-hoc univariate ANCOVA was conducted on the three measurement sites used: AP Spine, total body and proximal femur. Univariate ANCOVA revealed that the main effect could be contributed to differences in BMD at the AP spine (F (54,1) = 7.33, $p < 0.05$, one-tailed) and total body (F (54,1) = 3.16, $p < 0.05$, one-tailed), but not at the proximal femur (see Table 5).

3.1.3 Univariate Analysis for the Axial Spine

The second hypothesis stated that the greatest difference in BMD between the two groups would be at the axial spine as compared to the total body and proximal femur regions. This was supported as the greatest

difference in BMD was observed at the AP spine. The effect size at the AP spine, as measured by eta^2, was 11.9% as compared to 5.5% at the total body (see Table 3.7). In order evaluate the BMD differences at the axial spine further, ANCOVA was also performed for the lateral companion scan for L3 and VLS (L3). VLS has been reported as a more accurate estimate of the trabecular BMD at the axial spine (Peel & Eastell, 1994). There was a significant difference (F (53,1) = 4.37, $p < 0.05$, one-tailed) between VLS (L3) for the two groups; but no difference at the lateral (L3) scan. The eta^2 for VLS was 7.6% (see Tables 3.6 and 3.7).

Table 3.4. Results of MANCOVA Comparing BMD Between Regina and Saskatoon Groups, Using Weight as the Covariate

Main Effects	Value	DF	Exact F
Wilks lambda:			
Within + Residual	0.54	52,3	9.43*
City	0.14	52,3	2.44*

* $p < 0.05$, one-tailed test

Table 3.5. Univariate Post-Hoc F tests, Using Weight as the Covariate, for AP Spine, Total Body and Proximal Femur Comparing Regina and Saskatoon Groups

Variable	SS	DF	MS	F
AP Spine				
Within+Residual	0.40	54	0.01	
City	0.05	1	0.05	7.33†
Total Body				
Within+Residual	0.20	54	0.00	
City	0.01	1	0.01	3.16*
Proximal Femur				
Within+Residual	0.64	54	0.01	
City	0.02	1	0.02	1.30

† $p < 0.025$, one-tailed test, * $p < 0.05$, one-tailed test

Table 3.6. ANCOVA for Axial Spine Sites: Lateral Spine (L3) and VLS (L3) Comparing BMD for Regina and Saskatoon Groups

	SS	DF	MS	F
Lat. Spine (L3)				
Within+Residual	0.34	53	0.01	
City	0.01	1	0.01	1.92
VLS (L3)				
Within+Residual	0.02	53	0.00	
City	0.00	1	0.00	4.37*

* $p < 0.05$, one-tailed test

Table 3.7. Adjusted and Unadjusted Mean BMD Values and eta^2 for the Effect of City on AP Spine, Lateral Spine (L3), VLS (L3), Total Body and Proximal Femur

Variable	Regina	Saskatoon	Effect Size (eta ^{2})
	Adjusted mean values *(unadjusted values ± SD)*		
AP Spine (Total) (g/cm^2)	**0.975** (0.986 ± .07)	**1.039** (1.028 ± .12)	0.119
Lateral Spine (L3) (g/cm^2)	**0.746** (0.752 ± .07)	**0.777** (0.771 ± .09)	0.035
VLS (g/cm^3)	**0.216** (0.218 ± .02)	**0.227** (0.225± .02)	0.076
Total Body (g/cm^2)	**1.044** (1.051 ± .06)	**1.073** (1.065 ± .08)	0.055
Proximal Femur (g/cm^2)	**0.927** (0.936 ± .14)	**0.961** (0.951 ± .09)	0.024

3.2 Discussion

The purpose of this study was to determine if long term exposure to water fluoridation would have a positive effect on BMD. The major hypothesis in this study, that the women from Saskatoon would have greater BMD values than women from Regina was supported from the significant effect of city on BMD in the MANCOVA , using weight as a covariate. Results of the univariate post-hoc tests, showing that the difference in BMD was primarily a result of differences at the AP spine, supported the second hypothesis that the greatest difference in BMD would be at the axial skeleton. This has been supported in previous literature, where there has been a positive effect of water fluoridation on the axial skeleton (Kroger et al, 1994), with little impact on hip fracture rates (Suarez-Almazor et al, 1994; Arnala, Alhava, Kivivuori & Kauranen, 1986).

There has been great debate in the literature regarding the role of both high doses of fluoride and exposure to low levels of fluoride on BMD, bone strength and fracture rates. It is widely agreed upon that high doses of fluoride does increase bone density in the axial skeleton (Likimini, Whitford & Kunkel, 1992; Gedalia & Zipkin, 1974; Riggs, 1991; Dure-Smith, Kraenzlin, Farley, Libinati, Schultz & Bayink, 1991). However, it is unknown if low level fluoride in the water supply would have a similar effect on the axial skeleton following long term exposure.

There are very few studies examining communities with low level fluoridation verses no fluoridation and even fewer examining BMD in the axial skeleton. This study supported the findings by Kroger et al (1994) examining BMD by DXA in women age 47 to 59 years in two communities, one with water fluoridation at 1.0 to 1.2 mg./l. and another with less than 0.3

mg./l. They also found a significant difference in axial BMD between the two communities suggesting that low level fluoridation has a slight increasing effect on axial bone mass with long term exposure.

In the Kroger et al study (1994) a much larger sample was used: 3222 perimenopausal women were measured. In their study, the adjusted mean difference in AP Spine BMD, taking into account age, weight, menopausal status, calcium intake, physical activity level, deliveries, alcohol consumption and estrogen use, was 0.03 g/cm^2. In this study examining young women in Regina and Saskatoon, a greater difference in AP Spine BMD was found in a smaller sample. The difference in the adjusted mean AP Spine BMD, using weight as the covariate, was 0.06 g/cm^2 or a 6.5 % difference in BMD. The effect size as measured by eta^2 was 11.9% which is fairly substantial considering a large percentage of BMD variance, 46% to 62%, has been reported as the contribution of heredity factors (Krall & Dawson-Hughes, 1993).

It is puzzling that no significant difference could be found at the lateral spine site. The lateral spine projection decreases the amount of cortical bone from the spinous processes exposed during the scan, providing evaluation of the mainly trabecular bone in the vertebral body (Uebelhart, Duboeuf, Meunier & Delmas, 1990). Thus, if fluoride does have a greater impact on trabecular bone, it would be expected that BMD at the lateral spine site would be significantly higher in the Saskatoon sample. Even though it was close to reaching significance, the difference was less than at the AP spine. One possible explanation for this could be due to the greater measurement error in the lateral spine projection as compared to the AP spine. Even though the short term precision of the Hologic 2000 for lateral scans is improved with the ability to perform a lateral scan in the supine position, it is still substantially higher than the precision values at the AP spine and total body (2.1%

coefficient of variation compared to 0.7% at the AP spine and 0.5% at the total body). The combination of increased measurement error and a relatively small sample size may have made it difficult to find a significant finding at the lateral spine site.

The volumetric estimate of BMD at L3 (VLS) was significantly greater in the Saskatoon group as compared to the Regina group with an effect size of 7.6%. This finding does support a positive association of exposure to water fluoridation on the BMD of the highly trabecular bone of the axial skeleton. The VLS may provide a more sensitive measure of the impact of low level fluoridation on trabecular bone; however precision is jeopardized by improving the sensitivity. Similar to lateral L3, the precision value of the volumetric estimate is greater than the AP spine. The clinical and empirical use of the lateral spine and volumetric estimate at the lumbar spine is a fairly recent advancement (Peel & Eastell, 1994; Compston, 1995). It has been proposed that the volumetric estimate (VLS) may provide a more sensitive assessment of the more central trabecular bone of the vertebral body as it corrects for the effect of body size (Uebelhart, Duboeuf, Meunier & Delmas, 1990). However, the diagnostic sensitivity of the VLS as compared to areal density measures is still unclear (Compston,1995). Further research is needed evaluating the precision and sensitivity of the VLS estimate and the lateral spine scan.

The significantly higher total body BMD values in the Saskatoon group may reflect a general effect of low level fluoride on the skeleton. Fluoride is known to have a bone stimulating effect resulting in increased osteoblast activity (Gedalia & Zipkin, 1973). Thus, an overall increase in BMD as observed in the total body scan would be expected if there was a positive effect of fluoride on the skeleton. The difference in BMD between the two samples for the total body was certainly smaller than the AP spine, an effect

size of 5.5% as compared to 11.9%. This would be expected as the total body scan represents primarily cortical bone.

The finding of no significant difference in BMD at the proximal femur supports other research that there is likely no beneficial effect of long term exposure to low levels of fluoride on the BMD of the femur or hip fracture rates. (Arnala, Alhava, Kivivuori & Kauranen, 1986; Madans, Kleinman & Cornoni-Huntley, 1983; Suarez-Almazor et al, 1993). Even though the Kroger et al study (1994) did find a significant difference in BMD at the femoral neck when the effect of various extraneous factors were removed, the difference was small and likely not clinically relevant. The only other study to our knowledge in Canada found no difference in hip fracture rates in a large sample comparison between Edmonton and Calgary (Suarez-Almazor et al, 1993).

Limitations in this study are similar to other studies in this field. Because subjects are residents of a community and not randomly assigned, there could be already established differences in BMD from a number of other factors related to genetic, lifestyle or environmental influences. As well, even though fluoride levels are higher in one city compared to another, this does not necessarily mean all residents benefit the same. There are various dietary and drinking habits that are difficult to evaluate. In this study, these limitations were addressed by a detailed evaluation of lifestyle and dietary habits to determine if there were any differences between the two groups that would explain differences in BMD other than water fluoridation. This evaluation found that the two samples were very similar in a number of parameters.

Regina and Saskatoon are very similar in demographic data. Direct sunlight exposure is similar, general climate varies little. Population, ethnic diversity, and professional backgrounds are almost identical (see Appendix A).

The two samples in this study from Saskatoon and Regina were recruited from a University population. They were very similar in various lifestyle and dietary factors. Caffeine intake, smoking and alcohol consumption were low. Previous research supports long term smoking, high alcohol consumption and high caffeine intake may affect BMD (Hopper & Seeman, 1994; Heaney, 1993). These would be unlikely contributing factors in this study as consumption of these products was low in both groups.

Tap water consumption, the greatest contributor to fluoride in the diet (Priest and Van De Vyver, 1990) was very similar in both groups. The use of bottled water, although slightly higher in the Regina group, was generally low. If the Regina group tended to drink more bottled water, this would not increase their fluoride intake as there are only trace amounts of fluoride in bottled water. It is likely that the daily fluoride intake between individuals in the same community was similar with the majority of fluoride ingested from tap water and a relatively low intake of tea, the other major source of fluoride. Nonetheless, there is no well established method to determine the exact amount of fluoride intake for each individual; there may be some differences that have not been accounted for. For example, the use of water filter systems in the home was not evaluated. Because of the relatively low use of such systems reported (Health and Welfare Canada, 1981), the various methods used with differing amounts of fluoride removed (Saskatchewan Consumer Affairs, 1989), and exposure to other water systems at work or at school it would be difficult to determine the effect of these systems on the daily fluoride intake.

Genetic factors such as height, weight, bone mineral-free lean mass and fat mass play a significant role in determining BMD (Dawson-Hughes, Shipp, Sadowski & Dallal, 1987). Calcium intake and physical activity have also been associated with BMD (Bailey & Martin, 1994, Johnston et al, 1992).

Even though this study was not designed to measure the impact of other lifestyle factors on BMD, it is interesting that the past physical activity rating scale was significantly correlated with BMD. This finding has been reported in previous research (McCulloch, 1989). In order to delineate the effect of water fluoridation on BMD, it was important to determine as much as possible, any differing genetic and lifestyle factors between the two communities. In this study, there were no significant differences between the two groups for height, weight, bone-free lean mass, fat mass, physical activity patterns or calcium intake. There appeared to be no differences between the two groups that would explain differences in BMD other than exposure to different water supplies.

This study was unique in evaluating the effect of long term exposure to low level fluoridation in young women (aged 18 - 25). Because fluoride has its most beneficial effect on teeth during the growing years (Canadian Public Health Association, 1979) and it is absorbed more rapidly in the young skeleton (Weidmann & Weatherell, 1970), fluoride may have its greatest impact on the skeleton during bone mineral acquisition in childhood and adolescence. It is at this time that maximizing one's potential level of bone density may be crucial in the prevention of osteoporosis later in life (Stevenson, 1990).

From this study, it appears that low level fluoride can have a positive impact on the skeleton at peak bone density, if exposed to fluoride throughout the growing years. Previous research found similar differences in BMD for older perimenopausal women exposed to water fluoridation. However, the length of exposure time varied, the majority exposed for greater than 10 years (Kroger et al, 1994). Fluoride does continue to be absorbed at a fairly steady rate in the skeleton until the age of 55 (Health and Welfare Canada, 1977). Therefore, one would expect there to be a greater difference in BMD in an

older group of women exposed to water fluoridation throughout their lifespan. Further research evaluating BMD in older premenopausal women who have been exposed to water fluoridation throughout their growing and adult years would assist in determining if fluoride continues to increase BMD prior to the rapid bone loss associated with menopause. If this is so, exposure to water fluoridation may be an important preventative measure to osteoporotic fracture later in life.

Chapter 4

Summary and Conclusions

This study was unique in examining a young female population with long term exposure to differing water fluoridation practises. The first hypothesis was supported as the women from the fluoridated community had significantly greater BMD than women from the non fluoridated community. The conclusions that can be drawn from this are:

1. Fluoride absorbed from food and water over an extended period of time (15 to 25 years) may have a small, positive influence on BMD.
2. Low level fluoridation exposure may have a positive impact on the BMD in the young skeleton at the time when peak bone density is determined.
3. It is unknown what effect, if any, this would have on the likelihood to develop osteoporosis or the risk of fracture later in life.

The second hypothesis was also supported as the difference in BMD was greater at the axial skeleton as compared to the total body and proximal femur. Conclusions that can been drawn from this are:

1. In support of other research on older, perimenopausal women, the greatest impact of exposure to low level fluoride is at the AP Spine.
2. This continues to support the theory of site specific absorption of fluoride in the highly trabecular bone of the lumbar vertebrae.
3. There appears to be no beneficial impact of exposure to water fluoridation on the proximal femur. This supports previous research finding no difference in hip fracture rates between communities with and without water fluoridation.

Future studies in this area should consider the following:

1. Because the effect of water fluoridation is site specific, subsequent research should concentrate on evaluating the difference in BMD at the axial skeleton between communities with and without water fluoridation.
2. Longitudinal studies are needed to determine differences in BMD over time in the same individuals exposed to water fluoridation. Ideally, following a group of children during the growing years would increase our knowledge of the impact of low level fluoridation on the growing skeleton.
3. Studies are also needed in an older premenopausal group of women to determine if fluoride continues to improve BMD with exposure over a lifetime.
4. Communities such as Saskatoon and Regina are ideal for subsequent research in this area and should continue to collaborate efforts to evaluate the impact of water fluoridation on the skeleton.

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Appendix A

Sunlight Exposure and Demographic Data for Saskatoon and Regina

	Saskatoon	Regina
Sunlight Exposure (yearly)	2380 hrs.	2365 hrs.
Total population	186,060	179,180
Females	96,395	92,170
Females 20-24 years	8885	7590
Total born in province	137,920	136,225
Immigrant population	16,225	15,435
Ethnic Origin (%)		
British	14.7	15.2
German	10.1	12.3
Aboriginal	4.1	4.2
Ukraine	6.4	4.4
French	2.7	2.1
Canadian	4.1	2.8
Norwegian	1.4	0.8
Other	10.4	11.4
Multiple origin	45.4	45.6
Highest level of Education for Age 15 and Over		
Less than grade 9	9.2	10.1
Grade 9 to 13	37.3	40.4
Trade certificate	2.7	3.1
Non university education	19.0	15.9
University education		
no degree	15.9	16.5
degree	15.9	14.0
Labor Force (%)		
Females in labor force	63.3	65.4
Unemployment rate	9.0	7.6
for females	9.2	7.7
Income		
Family average income	48,927	52,466
Average income for females	16,859	18,304

References: Crop Science and Plant Ecology, University of Saskatchewan

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Appendix B

Screening Questionaire

The questions on this survey provide information on factors that may influence your bone mineral density. The answers to these questions help to determine your eligibility for this study. Read the questions carefully and mark the appropriate response with a (✓). If you are unable to respond or the question is irrelevant to you mark the answer space with an N/A. All answers on this questionaire remain strictly confidential. Please complete this questionaire and return to:

In Regina:
R. McCulloch
Physical Activity Studies
University of Regina
Regina, Sask.

In Saskatoon:
C. Arnold
College of Physical Education
University of Saskatchewan
Saskatoon, Sask., S7N 0W0

---

1. Identification

1.1 Surname_____ Given Name(s)_____
1.2 Address_____
1.3 City or Town_____Postal Code_____
1.4 Telephone (Home)_____ (Other)_____
1.5 Date of Birth: Day_____ Month_____ Year_____
1.6 Sask Health Services #_____
1.7 Family Doctor_____

2. Demographic Data

2.1 What city/town were you born in?_____

2.2 Have you ever moved from the location noted above? ( )yes ( )no
(If you answered "no" go to question 3)

2.3 Where else have you lived?_____

2.4 How long have you lived outside of your birth city?
( ) less than two years
( ) greater than two years
( ) greater than five years

2.5 During what time span did you live outside of your birth city?
( ) age 1 to 10
( ) age 10 to 16
( ) over age 16

3. Reproductive History

3.1 Are you currently pregnant? ( )yes ( )no

3.2 Do you menstruate regularly? ( )yes ( )no

3.3 How many periods do you usually have in a year?
over 13 periods _____
9 to 13 periods _____
3 to 8 periods _____
less than 3 periods _____

3.4 Have you ever had an absence or loss of periods? ( )yes ( )no

(pregnancy and lactation not included)

If Yes, at what age(s) did you miss a period(s)

1.____years old

2.____years old

For how long did your period stop?

1._____mos. _____yrs.

2._____mos. _____yrs.

4. Medical History and Status

4.1 In the last six months have you seen a doctor? ( )yes ( )no

If Yes, what was the reason for you visit?_____

_____

_____

4.2 Has there been any change in your general health in the past six months? ( )yes ( )no

If Yes, please specify_____

_____

_____

4.3 Have you ever been hospitalized, confined in bed or had a limb immobilized for a period of 21 days or longer? ( )yes ( )no

If Yes, list condition, approximate date and time involved

(Example	wrist fracture	summer 1990	10 weeks)
	Reason	Date	Time involved
	_____	_____	_____
	_____	_____	_____

4.4 Have you had any surgery in the past two years? ( ) yes ( )no

If Yes, list procedure and approximate date

(Example:	gall bladder removed	summer,1990)
	Surgery	Date
	_____	_____
	_____	_____

5. Medication

5.1 Are you currently taking any medications? ( )yes ( )no

If Yes, What medications are you taking? What are they for?

_____

_____

5.2 Have you ever taken any of the following medications? Please specify at what age you began to use them and for how long you used them.

Medication	Current use(✓)	Age at Start	Duration
calcium preparations	____	____	_____
antacids	____	____	_____
anabolic steroids	____	____	_____
fluoride	____	____	_____
vitamin D compounds	____	____	_____
calcitonin	____	____	_____
diuretics	____	____	_____
heparin	____	____	_____
cortisone (oral)	____	____	_____
corticosteroids (other)	____	____	_____
anti-inflammmatories	____	____	_____
thyroid preparations	____	____	_____

Appendix C

General Information and Consent Form for Participants

Dear Participant:

For the past few years our research team at the University of Saskatchewan has been conducting a series of studies which investigate the effects of various lifestyle habits on current levels of bone mineral density. Bone mineral density achieved in the young adult years is closely related to the potential risk of osteoporosis later in life. The results of these studies on physical activity level and calcium intake have been helpful in establishing guidelines for optimizing bone mineral density in young adults. This study will examine another environmental factor, exposure to fluoridated drinking water, and its effect on bone mineral density in young women. This study is unique in examining the long term effects of low level fluoride exposure in a young female population.

Your involvement in this study involves evaluation of your bone mineral density: total body status, lumbar (lower) spine and proximal femur (hip). This procedure involves minimal exposure to x-radiation and is painless. Each participant in this study will be provided with a written summary of the results once analysis has been completed. You will also be asked to fill out two questionaires: one on lifestyle habits and another on dietary intake.

If you are interested in participating in this study and wish to have your bone mineral density evaluated, please read the attached material carefully, sign the consent form and fill out the Bone Density Study Questionaire. Return the consent form and completed questionaire to one of the following addresses:

C. Arnold (Graduate Student)
College of Physical Education
University of Saskatchewan
Saskatoon, SK S7N 0W0

Dr. R. McCulloch
Faculty of Physical Activity Studies
Regina, SK S4S 0A2

Should you have any questions regarding the study, any one of the persons listed below would be pleased to assist you.

Cathy Arnold	966-6500 or 931-0966
Dr. R McCulloch	585-4854 (Regina)
Dr. Don Bailey	966-6524
Dr. Bob Faulkner	966-6469

Consent Form: Bone Density Study

The Effect of Fluoridated Drinking Water on the Bone Mineral Density of Young College-Aged Women

Investigators:

Cathy Arnold, College of Physical Education, U. of S.
Dr. Don Bailey, College of Physical Education, U of S.
Dr. Bob McCulloch, Physical Activity Studies, U. of R.
Dr. Bob Faulkner, College of Physical Education, U. of S.
Heather McKay, College of Physical Education, U. of S.

Purpose and Benefits of Study:

Higher values of bone mineral density in young adulthood may decrease the risk of osteoporosis later in life. Therefore, investigating the impact of various lifestyle factors on bone density in young adults is of considerable importance. The specific purpose of this study is to investigate the effect of drinking fluoridated water on the bone mineral density in young women. (Saskatoon has had a fluoridated water supply since 1954 whereas Regina has no fluoride supplementation).

Procedures:

Your participation in this project will involve one testing session using an established test protocol as follows:

Your total body, lumbar spine (lower back) and proximal femur (hip) bone mineral density will be evaluated with a bone densitometer. This procedure is painless, is routinely used in the practise of modern medicine and represents minimal exposure to x-radiation. The exposure from the bone density test represents approximately 1% of the yearly permissable exposure, similar to the exposure one would receive from radiation during a return trip by air across Canada. All bone density measurements will be conducted in the Department of Medical Imaging at the Royal University Hospital in Saskatoon and will be administered by qualified technologists.

In addition to the bone density measurements you will be asked to complete a food frequency questionaire and a physical activity and health information questionaire. The entire procedure will take approximately one hour. Participants from Regina wil be transported to and from the testing site on the same day in a rented van or bus.

Rights and Welfare of the Participant:

It is understood that you will be free to withdraw from any or all parts of the study at any time without penalty. Your identity will remain confidential and only those directly involved in the study (namely the investigators, project assistants and Medical Imaging Staff) will have access to your records and results. In any publication or presentation arising from this investigation only aggregate data will be reported. All individual results will remain strictly confidential.

Please be assured that you may ask questions at any time. We will be glad to discuss your results with you when they become available and we welcome your comments and suggestions. Should you have any questions contact any of the investigators listed on the previous page.

Bone Density Study Consent

Participant's Statement:

I,_____, understand the purpose
(please print name)
and procedures of this study as I have read or have had described to me, and I voluntarily agree to participate. I understand that at any time during the study I will be free to withdraw without any penalty. I understand the contents of the consent form, the proposed procedures and any possible risks.

I have had the opportunity to ask questions and have received satisfactory answers to all inquiries regarding this study.

_____

Signature of the participant Date

_____

Saskatchewan Health #

_____

Signature of Investigator

Skeletal fragility in older adults appears to be a function of peak bone density attained in young adulthood. Nutritional factors and physical activity may have an impact on the attainment of an optimal level of bone mineral density. Exposure to a fluoridated water supply throughout life may also have an impact on peak bone density. This provides the rationale for the current bone density
research.

Appendix D

Lifestyle and Dietary History Questionnaire

Name_____ **Today's Date**_____

Height: _____cm. _____cm. **Weight: _____kg. _____kg.**

The following questions are directed towards events in childhood, adolescence and current life that may have some influence on bone mineral density. Read the questions carefully and mark the appropriate response with a (✔). Mark those questions which are not relevant to you or to which you are unable to respond with **N/A**. All information on this questionnaire remains strictly confidential.

1. Smoking History

1.1 Have you ever smoked? ( )yes ( )no
If no, go to question 2

1.2 Have you ever smoked for 6 months or more? ( )yes ( )no
If no, go to question 2
If yes, how many years did you smoke? _____
At what age did you start smoking?_____
How many cigarettes per day did you usually smoke?_____

1.3 Do you still smoke? ( )yes ( )no
If yes, how many cigarettes do you usually smoke per day?
_____

2. Alcohol Intake

How often do you drink some form of alcoholic beverage?

Daily or almost every day	( )
3 or 4 times per week	( )
Once or twice a month	( )
Less than once a month	( )
Never	( )
Don't know	( )

3. Current and Past Dietary Habits

The following questions concern your dietary habits during three different time spans in your life: **current** , **teenage** years (between the ages of 13 to 17) and **childhood** years (between the ages of 5 to 12). When trying to recall past dietary habits thinking of the school you attended or the home you lived in at the time may assist you.

	Current	**Teenage (13-17 yrs)**	**Childhood (5-12 yrs.)**
3.1 How often do you/ did you drink coffee?			
never	( )	( )	( )
sometimes	( )	( )	( )
1 to 2 cups per day	( )	( )	( )
3 cups or more per day	( )	( )	( )
don't know	( )	( )	( )
3.2 How often do you/did you drink tea?			
never	( )	( )	( )
sometimes	( )	( )	( )
1 to 2 cups per day	( )	( )	( )
3 cups or more per day	( )	( )	( )
don't know	( )	( )	( )

	Current	Teenage (13-17 yrs)	Childhood (5-12 yrs.)
3.3 How often do you/did you drink cola (cans/ bottles)?			
never	( )	( )	( )
sometimes	( )	( )	( )
1 to 2 cans per day	( )	( )	( )
3 cans or more per day	( )	( )	( )
don't know	( )	( )	( )
3.4 How often do you/did you drink water? (tap water)			
never	( )	( )	( )
sometimes	( )	( )	( )
1 to 2 cups per day	( )	( )	( )
3 cups or more per day	( )	( )	( )
don't know	( )	( )	( )
3.5 How often do you/did you drink bottled water (not tap water)?			
never	( )	( )	( )
sometimes	( )	( )	( )
1 to 2 cups per day	( )	( )	( )
3 cups or more per day	( )	( )	( )
don't know	( )	( )	( )

	Current	Teenage (13-17 yrs)	Childhood (5-12 yrs.)

3.6 How often do you/did you drink tap water mixed with powdered mixtures such as juice powder, iced tea mix or kool-aid or with frozen concentrates such as fruit juice or lemonade?

never	( )	( )	( )
sometimes	( )	( )	( )
1 to 2 cups per day	( )	( )	( )
3 cups or more per day	( )	( )	( )
don't know	( )	( )	( )

3.7 How often do you/did you drink any type of milk (including milk on cereal)? Do not include milk added to coffee or tea.

a. never	( )	( )	( )
b. less than once per week	( )	( )	( )
c. once per week	( )	( )	( )
d. less than once per day but more than once per week	( )	( )	( )
e. once per day	( )	( )	( )
f. more than once per day	( )	( )	( )
g. more than three times per day	( )	( )	( )
h. don't know	( )	( )	( )

	Current	Teenage (13-17 yrs)	Childhood (5-12 yrs)
3.8 How often do you/did you eat any type of cheese?			
a. never	( )	( )	( )
b. less than once per week	( )	( )	( )
c. once per week	( )	( )	( )
d. less than once per day but more than once per week	( )	( )	( )
e. once per day	( )	( )	( )
f. more than once per day	( )	( )	( )
g. more than three times per day	( )	( )	( )
h. don't know	( )	( )	( )
3.9 How often do you/did you eat yoghurt, ice cream or pudding?			
a. never	( )	( )	( )
b. less than once per week	( )	( )	( )
c. once per week	( )	( )	( )
d. less than once per day but more than once per week	( )	( )	( )
e. once per day	( )	( )	( )
f. more than once per day	( )	( )	( )
g. more than three times per day	( )	( )	( )
h. don't know	( )	( )	( )

4. Dietary Supplements

4.1 Do you currently or have you ever taken a calcium supplement?

( )yes ( )no

If yes, how many times per day do you/did you take it?____

For how long did you take it?_____

What is the name of the supplement?_____

How many milligrams of calcium does it contain?____

4.2 Do you currently or have you ever taken a multivitamin?

( )yes ( )no

If yes, how often do you/did you take it?_____

For how long did you take it?_____

What is the name of the multivitamin?_____

4.3 Do you currently or have you ever taken any of the following antacids on a daily basis?

Rolaids, TUMS ( )yes ( )no

If yes, how many times per day do you/did you take it?_____

For how long did you take it?_____

4.4 Do you currently or have you ever taken a bran or fibre supplement?

( )yes ( )no

If yes, how often do you/did you take it?_____

For how long did you take it?_____

What is the name of the supplement?_____

4.5 Do you currently or have you ever taken a fluoride supplement?

( )yes ( )no

If yes, how often do you take it?_____

For how long did you take it?_____

What is the name of the supplement?_____

5.0 Reproductive History

5.1 When did you start to have menstrual cycles?

_____years _____months

5.2 Do you now or have you ever used oral contraceptives ?

( ) yes ( ) no

If yes, for how long? ____years ____months

5.3 Do you now or have you ever taken estrogen supplements? (Other than oral contraceptives) ( )yes ( )no

if Yes, what medication did you or are you taking?

(please give brand name)_____

When did you begin taking this medication_____

When did you stop taking this medication_____

6.0 Family and Medical History

6.1 Have you ever been treated for any of the following conditions?

food allergies	__yes __no	asthma	__yes __no
other allergies	__yes __no	kidney disease	__yes __no
back pain	__yes __no	chronic liver problems	__yes __no
scoliosis	__yes __no	gastrointestinal disease	__yes __no
epilepsy	__yes __no	muscular dystrophy	__yes __no
osteoporosis	__yes __no	osteoarthritis	__yes __no
rheumatoid arthritis	__yes __no	anemia	__yes __no
diabetes	__yes __no	malabsorption	__yes __no
excess urinary calcium	__yes __no	excess blood calcium	__yes __no
hyperparathyroidism*	__yes __no	hypoparathyroidism▫	__yes __no
hyperthyroidism*	__yes __no	hypothyroidism▫	__yes __no

*hyper = excess
▫hypo = deficiency

other conditions (please list)
_____
_____

6.2 Have you ever had any problems with your bone such as fractures? ( )yes ( )no

If Yes, how many fractures have you had?_____

Please list type of fracture and approximate date of occurrence

Type of fracture	Date
_____	_____
_____	_____

6.3 Is there a history of osteoporosis in your family? ( )yes ( )no

If yes, indicate who was affected_____

6.4 Is there a history of wrist, hip or spine fractures in your family?

( )yes ( )no

If yes, indicate who was affected_____

7.0 Physical Activity

7.1 Rate your **current** overall activity level. Compared to others of your age and sex, how much activity do you get? (circle a number)

1	2	3	4	5
much less than others		same as others		much more than others

7.2 Rate your activity level as a **child and youth** (during school years, age 6-18). Compared to others of your age and sex, how much activity did you get as a child and youth?

1	2	3	4	5
much less than others		same as others		much more than others

7.3 Did you participate in organized sport as a child or youth?

( )yes ( )no If yes, list the sports you participated in and the approximate number of years of participation.

Activity	Year(s)
_____	_____
_____	_____
_____	_____

7.4 During the last two weeks how many times did you do any of the activities listed below:	# of times	About how much time did did you spend on each occasion: minutes each time			
		1-15	16-30	31-59	60+
Walking	_____	( )	( )	( )	( )
Skating	_____	( )	( )	( )	( )
X-country skiing	_____	( )	( )	( )	( )
Aerobics	_____	( )	( )	( )	( )
Weight training	_____	( )	( )	( )	( )
Bicycling	_____	( )	( )	( )	( )
Jogging or running	_____	( )	( )	( )	( )
Bowling	_____	( )	( )	( )	( )
Social Dancing	_____	( )	( )	( )	( )
Jazz, ballet or modern dancing	_____	( )	( )	( )	( )
Racquet sports	_____	( )	( )	( )	( )
Golf	_____	( )	( )	( )	( )
Swimming	_____	( )	( )	( )	( )
Gardening/Yardwork	_____	( )	( )	( )	( )
Housework	_____	( )	( )	( )	( )
Other (please specify)					
_____	_____	( )	( )	( )	( )
_____	_____	( )	( )	( )	( )
_____	_____	( )	( )	( )	( )

OR ( ) I did nothing like this in the last two weeks
Thank-you for completing this questionnaire.

Appendix E

Estrogen Score

Parameter	Score
Age at menarche (yr)	
≤ 10	10
11	9
12	8
13	7
14	6
15	5
16	4
17	3
18	2
19	1
≥ 20	0
Menstrual Cycle	
Eumenorrheic	10
Oligomenorrheic	
(Total months)	
< 6	8
6-12	6
>12	4
Amenorrheic (total mo)	
< 6	6
6-12	4
>12	2
Use of birth control pills (yrs)	
<1	1
1-3	2
>3-5	3
>5	4

Appendix F

Food Frequency Questionnaire

Name_____ Today's Date_____

Instructions:

1. We want to know how often you eat or drink certain foods.

2. Tell us if you eat or drink the food for each question, then give the number of serving sizes.

3. The first question may ask about a food you might have every day; if you do not eat it every day go to the next question, which asks if you eat it once or more in a typical week.

4. Think about a typical day or week, not what you ate yesterday or today.

5. Medium portion sizes are given, but they do not represent the right or correct size. They are there to help you determine the usual size of food or drink.

6. For each food the medium size is described; we will show you what each food looks like on the portion board.

> If you eat less than the medium portion size, then give a fraction. For example, a small glass of milk is "1/2" the medium so "1/2" describes your usual intake. If you drink 2 small glasses of milk per day this is the same as drinking 1 medium portion.
>
> If you eat more than a medium portion, then indicate this by giving the number of portions your size is equal to. for example, a very large plate of spaghetti would be 2 or 3 medium portions.

7. Fill out the form similar to this example:

MILK

Do you drink milk every day? How many times a medium portion?

A. YES **2**

The medium portion for milk is 1 cup (250 ml.) and you drank a large

chocolate milk (500 ml.). Choose 2 medium portions.

Food Type	**Medium Portion**	**How many times a medium portion**
1. MILK		
A. Do you drink milk every day?		
If YES:		
white or chocolate (treat as same)	glass or drink carton (8 oz. or 250 ml.)	
If NO:		
B. Do you drink milk weekly?		
If yes, white or chocolate (treat as same)	glass or carton (8 oz. or 250 ml.)	
If NO:		
C. Do you ever drink milk? (use in coffee or tea?)		
If yes, how often?_____		
If no, Explain_____		
2. MILK ON CEREAL		
A. Do you eat cereal with milk every day?		
If YES:		
How much milk do you use?	1/2 cup per bowl	
If NO:		
B. Do you eat cereal with milk weekly?		
If yes, how much milk do you use and how much per week?	1/2 cup per bowl	
3. CHEESE		
A. Do you eat cheese every day?		
If YES:		
cheese single slice (in sandwich or as snack)	single slice	
hard cheese (such as cheddar)	1 oz. piece	

soft cheese	1 tbsp.
cottage cheese	1 cup

If NO:

B. Do you eat cheese weekly?

If yes: cheese single slice (in sandwich or as snack)	single slice
hard cheese (such as cheddar)	1 oz. piece
soft cheese	1 tbsp.
cottage cheese	1 cup

---

4. BREAD

A. Do you eat bread or buns every day?

(Remember sandwiches)

If YES:

bread	one slice
bun or roll	one dinner roll

If NO:

B. Do you eat bread or buns weekly?

If yes:

bread	one slice
bun or roll	one dinner roll

---

5. DESSERT

A. Do you eat dessert every day?

If YES:

ice cream, pudding, frozen yoghurt	1/2 cup, one scoop or pudding cup
Donut	one cake donut
Cookies	one regular

If NO:

B. Do you eat dessert weekly?

If yes: ice cream, pudding, frozen yoghurt	1/2 cup, one scoop or pudding cup

Donut	one cake donut
Cookies	one regular

6. BUTTER/MARGARINE

A. Do you use butter or margarine every day?

IF YES:

butter	one square (1 tsp.)
margarine	one square (1 tsp.)

IF NO:

B. Do you use butter or margarine weekly?

If yes,

butter	one square (1 tsp.)
margarine	one square (1 tsp.)

---

7. LUNCHES OR DINNER

Do you have any of the following once a week or more?

___Spaghetti with tomato sauce or noodles and sauce	1 plate (1 cup)
___Macaroni and cheese	1 plate (1 cup)
___Canned salmon (in sandwich or casserole)	1 serving (1 oz.)
___Tuna: in sandwich or casserole:	1 serving (1 oz.)
___Seafood: shrimp, lobster, salmon steak	3 oz.
___Lasagne	1 square
___Perogies	Give usual number eaten
___Do you have sour cream?	Yes or No
___Tacos	1 regular
___Pizza	
take-out	1 slice
frozen mini	1 round
___Cheeseburgers/hamburgers (circle)	
Where is it made_____	1 regular

___baked beans or other beans	1/2 cup

8. OTHER FOODS

Do you eat any of these foods weekly?

____Yoghurt	one container (175 ml.)
____Potatoes: mashed	one scoop (1/2 cup)
____eggs: any type	one whole (with yolk)
____Cream soups (made with milk)	one bowl (1 cup)
____Orange	one medium
____Orange juice	one juicepack (1 cup)
____Chocolate bar usual brand_____	1 regular
____Hot chocolate (in addition to other milk)	one cup (250 ml.)
____Milkshake (If not already included as dessert)	homemade (10 oz.) or purchased at _____
____Eggo-type waffle	one
____Pancake, waffles, french toast	one
____Broccoli, spinach or beet greens	1/2 cup
____taco chips, nacho chips	28 g. (1/2 small bag)

Appendix G

Pearson r Correlation Coefficients for Body Composition and Lifestyle Parameters to AP Spine, Total Body and Total Proximal Hip BMD

	AP Spine	Total Body	Total Proximal Femur
Height	.39*	.36*	.25
Weight	.52*	.54*	.43*
Lean Mass	.52*	.59*	.47*
Fat Mass	.20	.16	.12
Past Phys. Activity	.15	.30*	.26*
Current Phys. Activity	.13	.17	.12
Calcium Intake	-.13	-.13	-.14
Estrogen Score	-.12	-.11	-.17

* $p < .05$, two-tailed significance

Appendix H

Estrogen Status: Menstrual Status, Oral Contraceptive Use, Number of years since menarche and Modified Dhuper Estrogen Score

	Regina	Saskatoon	t-value
Eumenorrheic 9 - 13 cycles	24	32	
Oligomenorrheic 3 - 8 cycles	0	1	
Missed menstrual cycle on one occassion < 4 mo.	2	1	
Currently taking oral contraceptive (%)	45.8	54.5	
Have never used oral contraceptive (%)	54.2	45.5	
mean # of years since menarche	7.90	8.56	-1.17,n.s.
SD	1.82	2.26	
range	5 - 12	4 - 14	
Mean modified Dhuper estrogen score	18.17	18.33	-0.27,n.s.
SD	2.46	2.15	
range	12 - 22	15 - 23	

Appendix I

Lifestyle Habits: Smoking history, Caffeine and Alcohol Intake for Regina and Saskatoon Samples

	Regina	Saskatoon
Nonsmokers (%)	79.2	90.9
Smokers (past / current %)	20.8	9.1
mean # of pack years	1.23	2.70
Alcohol use (%)		
never	4.2	12.1
1 - 2 /mo.	83.3	78.8
3 - 4 /wk.	12.5	6.1
daily	0	0
don't know	0	3
Coffee intake (%)		
never	41.7	54.0
sometimes	25	42.4
1-2 cups/day	25	3
3-more cups/day	8.3	0
Cola intake (%)		
never	0	12.1
sometimes	70.8	69.7
1 - 2 cans/day	29.2	18.2
Tea intake (%)		
never	25	18.2
sometimes	75	66.7
1 - 2 cups/day	0	15.2

Appendix J

Tap Water and Bottled Water Consumption.for Regina and Saskatoon Samples

	Regina	Saskatoon
Current tap water consumption (%)		
< 3 cups per day	20.8	18.2
< 5 cups per day	58.3	51.5
5 or more cups / day	20.8	30.3
Current bottled water consumption (%)		
never	33.3	63.3
sometimes	58.3	33.3
1 to 2 cups per day	4.2	3

Appendix K

Descriptive Data for Bone Mineral Content and Density Measures.

Variable	mean(total)	SD(total)	mean (Regina)	mean (S'toon)
Total Body				
BMC (g.)	2179.58	300.72	2165.98	2189.46
BMD (g/cm^2)	1.06	.07	1.05	1.07
AP Spine				
BMC	59.64	8.65	58.31	60.61
BMD	1.01	0.10	0.99	1.03
Lat. Sp. (L3)				
BMC	7.99*	1.50	7.92	8.05
BMD	0.76*	0.09	0.75	0.77
VLS (L3)				
BMD (g/cm^3)	0.22*	0.02	0.22	0.23
Proximal Femur				
BMC	31.34	5.53	31.06	31.55
BMD	0.94	0.12	0.94	0.95

Note. *n = 56, n = 57 for all other total sample values