The Effect of β-blockers on Bone Mineral Density and Fractures in the Canadian Multicentre Osteoporosis Study (CaMos)

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Statement of Originality

This work presented in this thesis, including the design of the study, literature review, methodology, compilation of medication lists, analysis of all data, and writing of the thesis, was conducted by Dr. Vautour. Data acquisition, clean-up and entry into the CaMos database were completed by the various staff of CaMos throughout Canada.

Abstract

Objectives: β-blockers can alter bone turnover and increase bone formation in animals. It is unknown whether β-blockers have similar bone protective effects in humans. We aimed to estimate the effects of β-blockers on bone mineral density (BMD) and fractures using data from the Canadian Multicentre Osteoporosis Study, a large prospective cohort study.

Methods: All medications, including β-blockers, taken at baseline and after five years of follow-up were recorded. BMD was measured at baseline. During the five years of follow-up, incident minimal trauma fractures were documented by yearly questionnaires. To compare users of β-blockers to non-users while controlling for possible confounders, multiple linear regression was utilized to estimate between group differences in BMD and multivariate logistic regression was employed to estimate differences in fracture risk.

Results: Of the 9423 participants, 236 of 2884 males (8.2%) and 600 of 6539 females (9.2%) used β-blockers at some point during the study. In men, β-blocker users had differences of +1.1% (95% confidence interval [CI] -0.9%, 3.0%) and +1.2% (95% CI -0.5%, 4.0%) in baseline BMD at the total hip and at the lumbar spine, respectively, compared to non-users. In women, β-blocker users had differences of +0.05% (95% CI -1.2%, 1.3%) and +0.2% (95% CI -1.3%, 1.7%) for the BMD of the total hip and the lumbar spine, respectively, compared to non-users. For users of β-blockers at baseline, the adjusted odds ratio (OR) for any minimal trauma fracture was 1.23 (95% CI 0.67-2.25) in men and 1.02 (95% CI 0.76-1.35) in women. Chronic use (user at baseline and year 5) in men had an OR for any minimal trauma fracture of 2.1 (95% CI 1.0-4.3). In women who used β-blockers at baseline but not at year 5, the OR for hip fracture was 6.3 (95% CI 2.0-19.3). The risk of fractures for other sites was inconclusive owing to wide confidence intervals.

Conclusion: Despite relatively large numbers of subjects, wide confidence intervals do not permit strong conclusions with regards to the effect of β-blockers on BMD in men. Using a 2% limit of clinical importance for BMD, there appears to be no effect of β-blockers on BMD in women. There is some evidence from our study that β-blockers may be associated with an increased risk of fractures in certain subsets of users.

Résumé

Objectifs : Les β-bloquants peuvent augmenter la formation osseuse chez les animaux. On ignore si ceux-ci ont des effets préventifs au niveau osseux chez les humains. La présente étude visait à déterminer les effets des β-bloquants sur la densité minérale osseuse (DMO) et les fractures à partir de données provenant de l'Étude canadienne multicentrique sur l'ostéoporose, une étude de cohorte longitudinale.

Méthodes : Les médicaments, incluant les β-bloquants, que prenaient les participants à la visite initiale et celle de la cinquième année ont été notés. La DMO a été mesurée à la visite initiale. Durant les 5 années de suivi, les fractures reliées à un traumatisme mineur ont été documentées à l'aide de questionnaires annuels. Afin de comparer les participants prenant des β-bloquants ou non tout en contrôlant les éventuels facteurs confondants, une régression linéaire multiple a été utilisée pour évaluer les différences de DMO et une régression logistique multivariable pour déterminer les différences au niveau du risque fracturaire.

Résultats : Sur un total de 9 423 participants, 236 des 2 884 hommes (8,2%) et 600 des 6 539 femmes (9,2%) prenaient des β-bloquants. Chez les hommes recevant des β-bloquants, on notait une différence de +1,1% (intervalle de confiance [IC] de 95%: -0,9%; 3,0%) au niveau de la DMO de la hanche totale et de +1,2% (IC de 95%: -0,5%; 4,0%) à la colonne lombaire par rapport aux hommes n'en recevant pas. Chez les consommatrices, la différence au niveau de la DMO était de +0,05% (IC de 95%: -1,2 %; 1,3%) à la hanche totale et de +0,2% (IC de 95%: -1,3%; 1,7%) à la colonne lombaire par rapport aux femmes n'en recevant pas. Chez les consommateurs à la visite initiale, le rapport de cotes (RC) comparatif pour les fractures à traumatisme mineur était de 1,23 (IC de 95%: 0,67-2,25) chez les hommes et de 1,02 (IC de 95%: 0,76-1,35) chez les femmes. La prise prolongée de β-bloquants chez les hommes (visite initiale et 5 ans) se traduisait par un RC de 2,1 (IC de 95%: 1,0-4,3) pour les fractures reliées à un traumatisme mineur. Chez les femmes prenant des β-bloquants à la visite initiale seulement, le RC pour les fractures de la hanche était de 6,3 (IC de 95% : 2,0-19,3). Le risque fracturaire aux autres sites n'était pas concluant en raison de l'étendue des intervalles de confiance.

Conclusion : Malgré le nombre élevé de participants, l'étendue des intervalles de confiance ne permet pas de conclure de façon définitive sur les effets des β-bloquants au niveau de la DMO chez les hommes. Si on utilise une limite de valeur clinique de 2 % pour la DMO, les β-bloquants ne semblent avoir aucun effet sur la DMO des femmes. Certaines données de cette étude tendent à prouver que les β-bloquants seraient associés à un risque fracturaire plus élevé chez certains sous-ensembles de participants prenant ce type de médication.

Chapter 1 – Introduction

Osteoporosis is a serious health problem in Canada and around the world. Despite the great strides made in the last 2 decades in our understanding of its epidemiology, pathogenesis and treatment, this disease is projected to have an even greater impact on our health care system as the population ages and the number and proportion of people at risk increases substantially.

Osteoporosis was defined by a United States National Institutes of Health consensus development conference as "a skeletal disorder characterized by compromised bone strength predisposing a person to an increased risk of fracture. Bone strength reflects the integration of 2 main features: bone quantity and bone quality".[1] The diagnosis of osteoporosis is usually made by the finding of a low bone mineral density (BMD) measurement, which acts as a surrogate for bone quantity. Our ability to clinically assess bone quality, however, is limited. Efforts to identify additional risk factors for low BMD and fractures are imperative as fracture rates are expected to rise considerably in the decades ahead. The goals of prevention and treatment are meant to ultimately reduce the incidence of fractures which can have devastating health consequences and impose a huge economic burden on our society.

1.1 The Burden of Osteoporosis

Fractures can cause substantial morbidity and mortality. It is well recognized that hip fractures are particularly devastating. By one year after a hip fracture, mortality is 20% for women.[2] Mortality is even higher for men after a hip fracture.[2-4] The highest risk of death occurs immediately following the hip fracture and declines after about 6 months,

but still remains higher than in the general population.[5-7] This increased risk of death post-fracture is associated in large part with underlying co-morbidities, which are related to both low-bone density and death, but up to 25% of the increase in death rate may be directly attributable to the fracture itself.[8] In 2000, there was an estimated total of 9.0 million osteoporotic fractures, including 1.5 million hip fractures, that occurred worldwide.[9] It is projected that by 2050, there will be 6.26 million or more hip fractures occurring annually worldwide.[10, 11] By 2041 in Canada, there will be an estimated 88,124 hip fractures and 7,000 deaths associated with the acute care of these fractures.[12]

In addition to the increased mortality associated with hip fractures, there is also a great deal of morbidity. In the year following a hip fracture, 40% of women are unable to walk without aid and 80% have difficulty performing at least one task of daily living.[2] It is estimated that 20-25% of those suffering hip fractures become partially dependant or need assisted living or nursing home care. Similarly, incident vertebral fractures are associated with acute back pain, disability and many days of limited activity and bed rest.[13] Vertebral fractures may also lead to chronic back pain, deformity, loss of respiratory function and a reduced quality of life. At the age of 50, it is estimated that a woman has a 40% chance of having an osteoporosis-related fracture in her remaining lifetime and a 20% chance of having a hip fracture.[14, 15] At age 50, 1 out of 5 men will have an osteoporosis-related fracture in their remaining lifetime.[15] Hence, the health impact and the social burden associated with osteoporotic fractures are large.

From an economic perspective, the annual cost of treating osteoporosis-related fractures is great. In a study of hospitalizations in Sweden, osteoporosis ranked between that of stroke and ischemic heart disease in the annual number of bed days.[16] In women, osteoporosis was the most common diagnosis for prolonged hospitalization.[17] In Canada,

the burden of care associated with hip fractures was estimated to be around $650 million annually by 1995.[18]

1.2 β-Blockers: Current roles in the practice of medicine

β-blockers are compounds that block the receptors for the endogenous catecholamines, adrenaline and noradrenaline, which are neurotransmitters released by axons belonging to the sympathetic nervous system (SNS). The SNS is part of the autonomic nervous system, which is composed of control centres in the central nervous system and a peripheral network of nerves which innervate and control normal homeostasis of most organ systems, including the cardiovascular system. For this reason, β-blockers are established medications in the treatment of hypertension and angina. They are also recommended after myocardial infarction or in heart failure where they have been shown to reduce mortality. Aside from these more common uses, β-blockers are also employed for cardiovascular protection during high-risk surgery and for the management of arrhythmias, social anxiety disorders, tremors and esophageal varices as well as other conditions.

The frequent use of β-blockers in hypertension is likely due to the fact that they are effective and importantly, relatively inexpensive. Hypertension is a common medical condition, affecting about 25% of adult Canadians, 36% of whom take anti-hypertensive medications.[19] β-blockers are considered first-line therapy in the management of hypertension for many patients[20] and have been shown to reduce the cardiovascular outcomes of hypertension in adults less than 60.[20] In Saskatchewan, β-blockers were prescribed initially to 26% of patients diagnosed with uncomplicated hypertension.[21] The National Health and Nutrition Examination Survey (NHANES) is a large scale national

health survey which periodically provides information on the health of the US population. In the 1999-2000 NHANES, 19.7% of subjects with hypertension were taking a β-blocker.[22] If we were to extrapolate these anti-hypertension prescribing patterns to the whole of Canada, we can clearly imagine that β-blockers account for a large number of prescriptions annually.

1.3 β-Blockers and their potential impact on osteoporosis and fractures

While the effectiveness and advantages of β-blocker use in various conditions are well known, evidence for a possible effect of these drugs on bone homeostasis emerged only in 2002. Takeda et al. showed that treatment of normal mice with the β-blocker propranolol could increase bone mineral density and prevent the bone loss associated with ovariectomy.[23] The premise for the effects of β-blockers on the skeleton rests on the novel discovery that there is a central regulation of bone mass mediated through the sympathetic nervous system. Axonal fibers, part of the SNS, were demonstrated in bone and it was then shown that either enhancing the release of neurotransmitters from these axons or antagonizing them with a β-blocker had a profound effect on the bone turnover of these mice. This report generated major interest in the osteoporosis field as it provided a novel mechanism of control of bone turnover and also implied that commonly used drugs such as β-blockers could have a positive effect on bone density. This work was quickly followed by further animal studies which confirmed some of these novel findings and by several observational studies in humans of the effect of β-blockers on BMD and fractures. The results of the human studies have been divergent and hence it is still not clear whether this class of drugs truly has an effect on skeletal homeostasis. The issue is important as β-blockers are so commonly used in the population, and often for extended periods of time, so that even small effects on bone strength could be

clinically significant and have an impact on the overall burden of osteoporotic fractures in the population. Studies are therefore clearly needed to further elucidate their potential effect, if any, on human bone.

1.4 The Canadian Multicentre Osteoporosis Study

The analyses conducted in this thesis employ data from the Canadian Multicentre Osteoporosis Study (CaMos).[24] Started in 1995, CaMos was first established as a 5-year prospective study of the skeletal health of Canadian adults aged ≥25 and randomly selected from 9 regional centres across the country. The original study aims were both descriptive and analytical. CaMos provided normative data for peak bone mass and described the prevalence of osteoporosis and its risk factors in Canada.[24] The prospective component of the study allowed for estimates of fracture incidence and BMD changes with aging. As many sociodemographic and health characteristics were collected at baseline, CaMos data have been used to study various measures of health and their relationships with BMD and fractures in the Canadian population.[25-27] Some of the strengths of this study are its random selection of the study subjects, its inclusion of males in addition to females (who are more often studied), and the very high retention rates in the prospective follow-up period. Further details about CaMos will be provided in chapter 3.

1.5 Objectives

This thesis has two main objectives: Our first objective is to estimate differences in BMD in users of β-blockers compared to non-users, using data from the CaMos study. A second objective is to estimate differences in fracture risk in users versus non-users of β-blockers using the same data source.

1.6 Outline

Chapter 2 will provide a review of the literature. This will include a review of the animal and in-vitro studies that have demonstrated an effect of β-blockers on bone homeostasis, as well as a summary of the previously published studies of β-blocker effects on bone in human subjects. Chapter 3 will provide details about CaMos and the specific methods used in this work. Chapter 4 will provide results of all analyses and we will finish with our conclusions and a discussion in Chapter 5.

Chapter 2 - Literature Review

Of the many factors known to influence fracture risk, obesity ranks as one of the most important predictors, along with age. The adipocyte-derived hormone leptin has emerged as a potential mediator of the fat mass-bone relationship. In section 2.1 of this chapter, we provide an overview of the associations between obesity, leptin levels, and bone mass. Understanding the possible mechanism of action of leptin on bone turnover is crucial if we wish to consider any potential actions of β-blockers on the skeleton, in view of the fact that these agents have been implicated in the skeletal actions of leptin. Therefore, we will discuss the possible physiological mechanisms of how leptin might regulate bone metabolism in section 2.2, and in this context, review the possible role of β-blockers in causing increased bone formation in section 2.3. The effects of β-blockers on markers of bone turnover in humans will be summarized in section 2.4. A review of the studies examining the effects of β-blockers on BMD and fractures in humans will be presented in sections 2.5 and 2.6, respectively. Finally, in section 2.7, we will provide an overview of the important potential confounders to consider when studying the relationship between β-blockers and outcomes, such as BMD and fractures, in humans.

2.1 Obesity, leptin and the skeleton

Osteoporotic fractures are often regarded as a disease of frail, thin older women. Epidemiological studies have confirmed that increasing age and frailty are risk factors for fractures. In addition, a substantial number of observational studies have also demonstrated that obesity is one of the strongest predictors of bone density and fractures, conveying a protective effect on the skeleton.[28-32] This raises the question of how fat mass, or adipocytes, might control bone remodeling and strength. Several

mechanisms have been postulated to explain how increasing body weight could positively influence bone density and reduce fractures. These include the direct effect of load bearing on the skeleton, the increased secretion of insulin with increasing fat mass, the increased aromatization of androgens to estrogens by adipocytes, the protection of the skeleton during falls, and the increased secretion of other, potentially important, bone regulating hormones from the adipocytes. Leptin is one such hormone that has emerged as having potentially important effects on the skeleton.

The study of mouse models of obesity led to the discovery of the *obese* (ob) gene, and its protein product leptin, in 1994.[33, 34] The best understood physiological role of leptin is to signal energy sufficiency by binding to its receptors in the hypothalamus, where it produces an anorexigenic or satiety effect.[35, 36] Periods of starvation lead to leptin reductions, which in turn lowers metabolic rate, lowers energy expenditure, increases appetite, suppresses growth and reduces fertility. Increased food intake increases leptin production thereby causing reduced appetite, an increase in energy expenditure, restoration of fertility and an increase in growth hormone secretion. Leptin deficient, *ob/ob* mice, when injected with subcutaneous leptin, will reduce their food consumption, start to lose weight, and have correction of their other endocrine defects.[37]

In humans, congenital leptin deficiency is exceptionally rare. Moreover, contrary to mice, leptin levels are highly correlated with increasing fat mass.[38, 39] The paradoxical increase of leptin levels in most forms of human obesity has been difficult to understand but is thought to be related to central leptin resistance to its anorexigenic effects, due to an alteration of leptin transport across the blood-brain barrier.[40] Nonetheless, since the recognition that leptin levels increase proportionally with increasing fat mass in humans,

there has been a great deal of interest in leptin as the potential mediator of the relationship between fat and bone density.

Evidence suggests that leptin might control bone metabolism through two different pathways: one involving a direct, stimulatory effect on bone growth, and another that is indirect, involving a hypothalamic circuit that suppresses bone formation. Thus, in the direct pathway, leptin has anabolic or osteogenic effects while in the indirect pathway, it acts as an antiosteogenic factor. Which of these two alternative pathways predominates in the human skeleton is still a matter of debate.

There have been a number of clinical and epidemiological studies of the effect of leptin excess or deficiency on BMD in humans. From observational studies, the association between leptin levels and BMD are not clear. Adjusting for potential confounders, different studies have shown a positive,[41-45] a negative,[46-50] or no association[43, 50-52] with BMD. Associations, if any are found, often vary between the sexes and between premenopausal and postmenopausal women within the same study suggesting an effect modification of sex steroids on leptin and potential BMD effects.

There is limited data concerning the effects of leptin deficiency or leptin treatment on BMD in humans. In a report of a family with congenital leptin deficiency due to a leptin gene mutation, four affected members had abnormalities of PTH and calcium metabolism. One of these four had osteopenia despite the severe obesity, while the other three had normal bone density.[53] In another report, subcutaneous leptin administration to two patients, aged 31 and 33, with generalized lipodystrophy and hypoleptinemia failed to produce a clinically meaningful difference in BMD after 18 months of therapy.[54] Patients with anorexia nervosa have relative leptin deficiency and

hypothalamic amenorrhea due to their low weight. Leptin administration for up to 3 months to 8 such patients led to clinically important increases of bone specific alkaline phosphatase and osteocalcin, markers of bone formation, but there were no changes in markers of bone resorption or total BMD.[55]

Thus, the effects of leptin on human bone metabolism are certainly not obvious from these observations and are probably quite complex. This relationship is likely confounded and modified by many other factors, such as age, stage of skeletal maturity and various hormonal influences.

2.2 The effects of leptin on bone metabolism: lessons from animals

One approach to understanding the effects of leptin on bone homeostasis has been to ascertain the bone phenotype of animals with leptin deficiency. The *ob/ob* and *db/db* mice, deficient in leptin and its receptor, respectively, are two such animal models. Although they eventually develop severe obesity, these leptin-signaling deficient animals are born with a normal appearance. They start to become distinguishable from their wild-type littermates usually by 4-6 weeks of age, when they appear to have a slightly shorter body, are rather square and have wide hind quarters.[56] The *ob/ob* and *db/db* mice also have hypogonadism and hypercortisolism, a hormonal milieu that would predict a low bone mass phenotype.

However, in 2000, Ducy et al., from the Karsenty laboratory, showed that despite expectations, these two mice strains actually have a high bone mass phenotype, preceding the onset of obesity.[57] These leptin signaling deficient animals have an increase in trabecular bone volume and a 2-3 fold increase in the trabecular number with

little change in the cortical bone volume. Using histomorphometry techniques, they showed that the high bone mass was due to an increased osteoblast activity yet normal osteoblast numbers. These animals had a bone formation rate almost 70% higher then that of a normal wild-type littermate. Their osteoclasts have normal function but are increased in number leading to increased bone resorption. However, this increased resorption corrects when the hypogonadism is treated with estrogen replacement, leading to an even higher bone density. Hence, the high bone mass develops in these animals despite the increased bone resorption. Other mice models of obesity have not been found to have a high bone mass suggesting that it is the leptin deficiency and not the obesity that leads to this increased bone mass.

Ducy et al. also showed that the intracerebroventricular (ICV) infusion of leptin in the *ob/ob* mice, who are leptin deficient, could cause bone loss and reverse the high bone mass phenotype. An ICV infusion of leptin could even cause bone loss in normal mice.[57] This bone loss occurred through the inhibition of bone formation. By chemically lesioning different areas of the hypothalamus, they were able to show that the ICV mediated effects of leptin occurred through hypothalamic nuclei distinct from those responsible for the anorexigenic effects of this hormone. The idea that there is a common, central endocrine control of energy metabolism, bone remodeling and reproduction through leptin was certainly novel and very surprising. In addition, these findings introduced the concept that leptin might have antiosteogenic effects on bone, contrary to conventional thinking.

This same group later reported that these antiosteogenic effects of leptin on bone metabolism are mediated through the sympathetic nervous system.[23] Thus, the postulated regulatory loop involves leptin binding to its receptors at the antiosteogenic

nuclei in the hypothalamus, which in turn sends signals through sympathetic neurons to alter bone homeostasis. Consistent with this, axons of the sympathetic nervous system were found to be present in bone. Furthermore, β_2-adrenergic receptors, which bind noradrenaline, have been found to be expressed in osteoblasts.[23] In keeping with this mechanism, they also showed that mice with mutations in the enzyme necessary to synthesize noradrenaline and adrenaline in sympathetic neurons also had a high bone mass phenotype. Thus, from this study, leptin was shown to produce its effect on bone through an indirect, central neuroendocrine regulatory pathway.

Despite the elegant and convincing work by Karsenty's group, there have been some contradictory findings by other investigators. Thus, Steppan et al. found that *ob/ob* mice had a lower total body and lower femur bone mineral content, and a lower femur BMD compared to wild-type mice.[58] Lorentzon et al. found that the tibia and femurs of db/db mice had reduced trabecular and cortical bone volume with impaired mineralization.[59] Hamrick et al. confirmed the finding of a high bone mass phenotype in the *ob/ob* mice at the spine but at the femur, they found a lower bone mineral content, BMD, cortical thickness, and trabecular bone volume compared to wild-type mice.[60] The reasons for these discrepancies are not clear, but suggest that perhaps there are regional skeletal differences of leptin effects on bone mass regulation. Indeed, most of the studies of the central regulation of bone mass by leptin performed by Ducy et al.[57] and Takeda et al.[23] focused on findings in the vertebrae. It is possible that differences in sympathetic innervation throughout the skeleton could explain these disparate findings of the effects of leptin deficiency on the skeleton.

There is also considerable evidence strongly supporting the notion that leptin has direct, local effects in bone tissue. Although leptin is primarily produced in adipocytes, it is also

expressed in many other tissues, including bone.[61] Similarly, the leptin receptor is expressed in many areas of the central nervous system and other tissues, including bone cells.[62, 63] Leptin receptors, capable of active signaling, are expressed in rat osteoblasts.[64] Furthermore, *in vitro* studies have provided evidence for the direct effects of leptin on bone formation. Marrow stromal cells in culture can be induced to differentiate into osteoblasts under the influence of leptin.[65] Furthermore, leptin has been shown to promote osteoblast proliferation, new collagen synthesis and mineralization in primary cultures of human osteoblasts[61, 66] and to inhibit osteoclast development.[66, 67]

In addition, to these *in vitro* findings, peripherally administered leptin reduced bone fragility in adult male mice,[63] reduced ovariectomy-induced bone loss in rats,[68] and led to an increase in femoral length, total body bone area, bone mineral content, and bone density in leptin-deficient (*ob/ob*) mice.[58]

These findings of a direct positive or anabolic effect of leptin on bone homeostasis are in keeping with the concept that humans with more fat mass have a higher BMD. While it is difficult to reconcile the indirect, central versus the direct, peripheral effects of leptin, it does appear that when administered systemically, the direct actions of leptin outweigh its centrally mediated effects on bone. Which effect predominates under normal physiological conditions in humans is entirely conjectural at this time. It is apparent that further clinical investigations are necessary to clarify this issue

2.3 β-blockers and bone metabolism in animals

Once the sympathetic nervous system was suspected to play a role in bone homeostasis, drugs that mimicked or antagonized its effects were tested in animals to further define their roles. Hence, Tekeda et al. showed that the sympathomimetic drug, isoproterenol, caused marked bone loss in the vertebrae and long bones of *ob/ob* mice and wild-type mice by reducing bone formation, yet without causing weight changes.[23] Furthermore, bone mass was shown to increase even when wild-type mice were treated with the β-adrenergic antagonist (β-blocker) propranolol. The increase in bone mass was due to an increase of bone formation and osteoblast number. Propranolol was also shown to mitigate against bone loss in ovariectomized wild-type mice.[23] Hence, findings from these animal studies suggested a role for β-blockers in the regulation of bone formation and the prevention of bone loss. If these results were applicable to humans, β-blockers could promote bone formation, attenuate the bone loss due to menopause and increase BMD. This could potentially then lead to a reduction in fractures in those who use these medications.

2.4 β-blockers and bone turnover in humans

The assessment of bone turnover, using serum and urine markers of osteoblast and osteoclast function, is often used as a surrogate for bone mineral density measurements. Bone turnover markers can estimate the relative amount of bone formation versus resorption in the skeleton. Their levels can also change rapidly with certain drugs that have effects on bone metabolism. Hence, their measurement can provide earlier evidence of response to a drug compared to BMD changes, which take longer to detect.

In order to evaluate the effect of β-blockers on bone turnover, Reid et al.[69] conducted a randomized controlled trial examining the effect of treatment with the non-selective β-blocker propranolol, at 160 mg/day, versus placebo, in 41 post-menopausal women over 3 months. At the end of the study, there was a clinically important reduction in serum osteocalcin, a marker of bone formation, in the group receiving the β-blocker propranolol (-21%; 95% confidence interval [CI] -17%, -25%) compared to placebo (+3%; 95% CI -6%, +11%). (Note: the above confidence intervals were approximated by the author as they were not included in the original article.) For the other markers of bone turnover examined at 3 months, the confidence intervals were wide and included values representing no clinically important differences between the treatment groups. It was inconclusive as to whether there were differences in the BMD at the lumbar spine and total proximal femur between the two groups over the 3-month period, however, this study was not designed to detect a difference in this measurement. While preliminary, this randomized controlled trial examining the β-blocker effects on bone markers demonstrated that the β-blocker propranolol, at the dosage given in this trial, may be potentially detrimental to bone formation.

The effect of β-blockers on bone turnover markers was also examined using a cross-sectional design within the Danish Osteoporosis Prevention Study, a population–based, prospective study of 2016 perimenopausal women.[70] In multiple linear regression, treatment with a β-blocker was an independent predictor of serum osteocalcin level with lower values for the subjects on β-blockers (β coefficient -2.8, 95% CI not available). Thus, the adjusted difference in serum osteocalcin for subjects treated with a β-blocker versus those not treated was -17.5% (95% CI not provided) in this cohort. In their analysis, they adjusted for many potential confounders including age, years since menopause, number of previous fractures, calcium and vitamin D intake, alcohol,

smoking, activity level and other medication use including thiazides and loop diuretics. Consequently, this study supported the study of Reid et al.[69]

2.5 β-blockers and bone mineral density in humans

In addition to effects on bone turnover, since 2004, there have been several publications that have examined the effect of β-blockers on BMD; all of these are observational

Table 1: Published studies on the effect of β-blockers on BMD

Reference	Design	Population	N	Adjusted % difference of BMD at the spine‡ (95% CI)	Adjusted % difference of BMD at the hip‡* (95% CI)
Pasco et al. 2004	Case-control	Postmenopausal women	1,344	Cases 2.4 (-0.8 to 5.7) Controls 2.3 (-0.5 to 5.2)	Cases 2.9 (0.3 to 5.5) Controls 2.8 (0.6 to 4.9)
Rejnmark et al. 2004	Case-control	Postmenopausal women	2,016	"No difference"#	"No difference" for femur neck#
Reid et al. 2005	Prospective cohort	Postmenopausal women	8,142	Not measured	0.0 (-1.1 to 1.2)
Levasseur et al. 2005	Prospective cohort	Postmenopausal women	7,598	Not measured	"No difference" for femur neck#
Lynn et al. 2006	Cross-sectional	Men and women age ≥ 65	3887	Men 1.2 (-1.1 to 3.5) Women 1.3 (-0.8 to 3.6)	Men -0.6 (-2.2 to 1.0) Women -0.1 (-1.8 to 1.5)
Bonnet et al. 2007	Cross-sectional	Postmenopausal women	944	Figures not provided**	Figures not provided**

‡Percent difference of BMD in β-blockers users versus non-users
*Hip refers to the total hip unless stated otherwise in the column
**Data provided allowed for the calculation of an unadjusted difference, which suggested an inconclusive effect owing to wide confidence intervals. Text reports that adjustments did not change results.
Conclusion of the publication authors, figures were not provided

studies. Shown in table 1, are those published studies, including only those that attempted to control for potential confounders either through design or statistical methods.

An important consideration in the interpretation of these studies is the value that represents a clinically meaningful BMD difference, a parameter which not universally considered. From observational studies of population-based cohorts, a BMD that is 1% lower than the mean value for young adult is associated with an increased fracture risk of 3-4%.[71, 72] For a BMD that is 2% lower from the young adult mean, there is a 6-8% increase in fracture risk. The latter clearly represents a more clinically meaningful risk. Thus, for the remainder of this thesis, a BMD difference of 2% is used as a clinically meaningful difference.

In the first report published on this subject in 2004, Pasco et al.[73] used data from the Geelong Osteoporosis Study, a case-control study of fractures in women, ≥50 years of age, conducted over 2 years from February 1994-1996. Fracture cases were identified prospectively from the radiology department while controls were an age-stratified sample of women without incident fracture who were randomly selected from the same geographic region. An interviewer assisted questionnaire and BMD were completed for all 569 fracture cases and 775 controls. Within the fracture cases, β-blockers users had a +2.9% (95% CI, 0.3% to 5.5%) difference in BMD at the total hip and a +2.4% (95% CI, -0.8% to 5.7%) difference in BMD at the lumbar spine compared to non-users. Within the controls, β-blockers users had a +2.8% (95% CI, 0.6% to 4.9%) difference in BMD at the total hip and a +2.3% (95% CI, -0.5% to 5.2%) difference in BMD at the lumbar spine compared to non-users. BMD means were adjusted for age and weight at the total hip and for age, weight, and height at the spine. Thus, the effects of β-blockers on BMD are

inconclusive in this study despite the increased estimates of the percent differences in BMD, owing to the wide confidence intervals crossing the null yet including clinically meaningful changes. There are several limitations to this study. First, there was a small number of β-blockers users (N=117) limiting the power to precisely estimate the BMD effects of this drug. Also, recall bias may have been a problem as not all subjects had their medications verified. In the analysis, the BMD mean for the users and non-users of β-blockers were not adjusted for several other important potential confounders, such as hormonal replacement therapy (HRT) and thiazides. Finally, patients identified from a hospital department may differ in important ways from controls in the general populations. Patients with many chronic diseases, who are frequently in the hospital, may be sent more readily for spinal radiographs while controls from the general population may be healthier, more active, and less likely to have spinal radiographs. Consequently, a selection bias may have occurred in choosing fracture cases from within hospital radiology departments and controls from the general population.

Data from the Danish Osteoporosis Prevention Study (discussed earlier in section 2.4) was also used to estimate the effects of β-blockers on BMD in perimenopausal women.[70] Only 2% of the 2019 women participating were on β-blockers. Adjusting for potential covariates using multiple linear regression, "no association" could be found between β-blocker users and non-users with respect to BMD measurements at the lumbar spine or femoral neck (data was not available to compute the mean differences in BMD or confidence intervals). Their study had a limited power to detect a BMD difference in view of the fact that they had so few users of β-blockers. The authors do state that their data "do not exclude" the possibility that β-blockers may affect BMD. This appears synonymous to saying that their results were inconclusive.

Reid *et al.*[74] used data from the Study of Osteoporotic Fractures (SOF) to evaluate the effect of β-blockers on total hip BMD and fractures. SOF is a multicentre study prospectively following a large cohort of white women, aged ≥ 65 years old, who were recruited from the population. Recruitment of subjects was done through mailings to women on population-based listings and by advertisements targeting volunteers. In this cohort, there were 1099 users of β-blockers and 7028 non-users at the time of the analysis. In general, the women who used β-blockers weighed more, smoked less, were less active, frailer and reported worse health. Users of β-blockers were also taking more estrogen, thiazides, nitrates, and statins but less glucocorticoids. After adjusting for potential confounders in a cross-sectional analysis, there was no difference in the total hip BMD between users of β-blockers and non-users (difference 0.0% (95% CI, -1.2% to 1.2%). A major limitation of this study is the method of subject recruitment which contrasts to the random selection of CaMos subjects from the population. Furthermore, in contrast to the CaMos cohort, there were no men and no representation from age groups under 65.

These findings are similar to those of Levasseur et al. who used data from the Epidémiologie de l'ostéoporose study (EPIDOS study) to look at the effect of β-blockers on BMD and fracture risk.[75] This prospective cohort study recruited a total of 7,598 women (mean age ± standard deviation (SD) of 80.5 ± 3.8) from voter registration lists in five French cities and followed them for a mean of 3.6 ± 1.2 years. At baseline, information on lifestyle, medical history, medication use and duration of medication use was collected by questionnaires. Measurements, including BMD, were also done. β-blockers were used by 3.9% of subjects for an average of 13.9 ± 10.1 years. Unadjusted for potential confounders, β-blocker users had a "higher" mean BMD than non-users but the authors report that "no association" could be found between β-blocker use and BMD

of the femur neck or whole body, after adjustment for potential confounders in a multiple linear regression model (data provided do not permit computations of estimates of BMD differences or confidence intervals).

Lynn et al. examined the effects of various anti-hypertensive medications, including β-blockers, on BMD in a cross-sectional study of community-dwelling Chinese men and women who were ≥ 65 years of age.[76] Overall, 16.8% of the 1929 women and 15.8% of the 1958 men were on β-blockers. Adjusting for potential confounders, male users of β-blockers had a -0.6% (95% CI, -2.2% to 1.0%) difference in BMD at the total hip and a +1.2% (95% CI, -1.1% to 3.5%) difference in BMD at the lumbar spine compared to non-users. In women, β-blockers users had a -0.1% (95% CI, -1.8% to 1.5%) difference in BMD at the total hip and a +1.3% (95% CI, -0.8% to 3.6%) difference in BMD at the lumbar spine compared to non-users (these percent differences and confidence intervals were approximated the author). Therefore, for men, the effect of β-blockers on BMD of the spine and total hip was inconclusive. For women, those who used β-blockers had no difference in the BMD at the total hip compared to non-users, while the effect of β-blockers on the spine BMD were inconclusive. A major limitation of this study was the non-random selection of the subjects whose participation was solicited through a media campaign.

More recently, Bonnet et al. also examined the effect of β-blockers on BMD and fractures in a cross-sectional study design.[77] They recruited 944 postmenopausal women (mean age ±SD = 65.2±9.3) from clinical referrals to a bone densitometry center. All participants provided information on medications use, diet and lifestyle through interviewer-administered questionnaires. Physical measurements, including BMD and spine radiographs, were done. In this cohort, 158 women were on β-blockers. From the

remaining group, 341 age and weight-matched women were selected to be controls. In unadjusted comparisons, β-blocker users had a +3.3% (95% CI, 0.0% to 6.7%) difference in BMD at the lumbar spine compared to non-users, a 4.3% (95% CI, 0.7% to 8.0%) difference in BMD at the femoral neck of the hip compared to non-users, and a +3.3% (95% CI, -1.3% to 7.9%) difference in BMD at the total hip compared to non-users. Adjusting for potential confounders using multiple linear regression was said to not alter these percent differences or their "statistical significance". Unfortunately, the estimates for the percent differences and the confidence intervals of these latter regressions were not reported and could not be computed with the data provided. Thus it is unclear if there truly was a clinically meaningful difference in BMD between users of β-blocker and non-users in this study. In addition to the poor reporting, this study has other limitations, the most important being the recruitment of subjects from a hospital-based department. Subjects selected from a BMD testing center may be very different from the general population, including different access to health care, difference in severity of metabolic bone diseases, differences in risk factors for low bone mineral density or differences in potential confounding illnesses. These limitations would affect the ability of the study's authors to generalize any of their findings to other β-blocker users.

In summary, six studies investigating the effects of β-blockers on BMD were discussed in this section. In the studies by Lynn et al.[76] and Reid et al.,[74] sufficient data in the publications allow us to see that there was clearly no difference in the BMD at the total hip between β-blockers users and non-users in women. In two other studies looking at BMD of the femur neck and spine in women, there was "no difference" between users of β-blockers and non-users as reported by the study authors, but sufficient data was not provided to firmly conclude if these results were truly negative, or inconclusive owing to wide confidence intervals[70, 75] Finally, for the remaining studies and BMD sites examined,

the results were inconclusive owing to the wide confidence intervals for the percent BMD differences[73, 76, 77] In the only study that included men, the results were inconclusive for both the total hip and spine BMD differences.[76]

2.6 β-blockers and risk of fractures in humans

There are several observational studies in humans that have examined the effect of β-blockers on fractures, and all have been published since 2004. These are shown in table 2. The only studies that are included are those that controlled for confounders either through design or analysis, and those that used the incidence of fractures as the outcome. This latter criterion excludes only one cross-sectional study which correlated β-blockers use with a past history of fractures.[77] Past fractures are difficult to verify and their timing, in relation to drug use, cannot be reliably ascertained, hence this latter study did not meet our criteria for inclusion in this review.

The effects of β-blockers on fractures were examined in the Geelong Osteoporosis Study, described in section 2.5.[73] In this case-control study, β-blocker users had an unadjusted OR for all fractures of 0.68 (95% CI, 0.48-0.96). Using multivariate logistic regression and adjusting for potential confounders, β-blocker users had an OR for fractures of 0.71 (95% CI, 0.55-0.99) for all fractures. Thus it, appears from this study that β-blockers reduced fracture risk. However, in addition to the limitations of this study already discussed in section 2.5, another is in the selection of cases and controls. While the incident fracture cases were identified prospectively through two hospital radiology departments, the controls were selected randomly from the population in the same geographic region. It is not clear if cases and controls are sufficiently comparable, with equal opportunity to be exposed to β-blockers and with equal opportunity to be correctly

Table 2: Published studies on the effect of β-blockers on fracture risk

Reference	Design	Population	Number	OR for any fractures* (95% CI)	OR for hip fractures* (95% CI)
Pasco et al. 2004	Case-control	Postmenopausal women	1,344	0.71 (0.55-0.99)	0.56 (0.24-1.33)**
Rejnmark et al. 2004	Nested Case-control	Postmenopausal women	2,016	3.3 (1.1-9.4)	Not examined
Schlienger et al. 2004	Case-control	Men and women in general practice clinics	150,420	0.77 (0.72-0.83)	0.68 (0.52-0.89)
Reid et al. 2005	Prospective cohort	Postmenopausal women	8,142	0.87 (0.75-1.00)	0.66 (0.49-0.90)
Levasseur et al. 2005	Prospective cohort	Postmenopausal women	7,598	1.2 (0.9-1.5)	Not examined
Rejnmark et al. 2006	Case-control	General population	498,617	0.91 (0.88-0.93)	0.91 (0.85-0.98)
De Vries et al. 2007	Case-control	General population	46,2000	Not examined	GPRD 0.82 (0.74-0.93) RLS 0.87 (0.80-0.95)

*OR shown are unadjusted unless indicated otherwise
**Figures for the adjusted OR not available but reported to be similar to unadjusted OR

opportunity to be exposed to β-blockers and with equal opportunity to be correctly classified as cases. From the routine use of spinal radiographs, it is recognized that 50% or more of vertebral fractures are asymptomatic.[78] In this study by Pasco et al., control subjects did not routinely receive spinal radiographs, which could have caused a misclassification of cases and controls for those with vertebral fractures, a form of measurement bias. [73] If there was non-differential misclassification of the outcome, this could lead to a bias away from the null and a greater apparent protective effect of β-blockers. Furthermore, inasmuch as cases may be receiving more radiographs because of frequent hospital care and greater access, these subjects may be different from controls in other ways that cannot be recognized or adjusted for. Another limitation is the

potential for recall bias as the subjects were interviewed a median of 59 days after the fracture episode. Finally, it is not entirely clear that all patients who suffer a fracture are later available to participate in a study. For example, as hip fractures are often associated with prolonged hospital stays and mortality, these subjects may be under-represented in a case-cohort study. Subjects who take β-blockers may have more comorbid diseases, such as cardiovascular diseases, and be under-represented after fracture. Failure to include them in the study would bias the results away from the null.

Fracture risk associated with β-blocker use was assessed in the Danish Osteoporosis Prevention Study discussed in Section 2.4 and 2.5.[70] This prospective study of 2,016 perimenopausal women (mean age 50) looked at the effects of HRT on fractures in an open-labeled trial without placebo control. Only 38 subjects were taking β-blockers at the study inception. The authors used a nested case-control study design to look at the effect of β-blocker on fractures. There were 163 women who sustained an incident fracture over the 5 years of the cohort follow-up. These cases were matched to 6 subjects from the study population who had not sustained a fracture during that same period. These controls were randomly selected but matched on HRT use. Spinal x-rays were done on all subjects at baseline and at 5 years. Adjusting for a long list of potential confounders, the risk of all low-trauma fractures with the use of β-blockers was increased (multivariate OR 3.3; 95% CI, 1.1-9.4). There was a fracture risk increase for those who reported the use of β-blockers for over 8 years (OR 5.3; 95% CI, 1.1-26.3). For those women who had been treated for less than 8 years, the fracture risk was inconclusive but the point estimate for the OR was at least consistent with what was found for those with a longer duration of use (OR 2.4; 0.6-9.5). This report does not specify if there was an increased risk for any specific fracture site, such as the hip. The analysis was repeated, restricting it to those who had not received HRT and the same increased risk for

fractures with β-blockers was found. The strengths to this study include the prospective design, the spinal radiographs which were done on all subjects, and adjustments for a long list of potential confounders. This study had some limitations including the relatively small number of β-blockers users, and also fractures that occurred over the 5 years of follow-up which limited specific fracture site analysis.

In a large case-control study, Schlienger et al. also examined the potential association between β-blocker use and fractures.[79] Employing data from the United Kingdom General Practice Research Database (GPRD), they identified all patients with an incident fractures occurring during the 7 year period from January 1993 to December 1999. The data in GPRD is entered by general practitioners and includes past medical history, new diagnoses, and contains all prescription information, which is computerized. All cases were between the ages of 30 and 79 at the time of the fracture. From the same database, up to 4 controls were randomly selected and matched for age, sex, center, calendar period (of the index date) and years of prior data entry in the database. They excluded any patients who had metabolic bone diseases, including osteoporosis, or who were taking bisphosphonates prior to the index date. They had 30,601 cases and 120,819 matched controls. Of the cases, 57.1% had fractures of the hand/lower arm or feet. In determining exposure to β-blockers, they categorized patients as current users, recent users and past user. The current users were also categorized by the total number of prescriptions given as a surrogate of duration of use. Pooling all patients who were current users of β-blockers with 3 or more prescriptions before the index date, the adjusted OR for fracture associated with β-blockers was 0.77 (95% CI, 0.72-0.83) compared to non-users. Compared to non-users, current users with 3-19 prescriptions and >20 prescriptions prior to the index date had adjusted ORs of 0.63 (95% CI, 0.55-0.73) and 0.83 (95% CI, 0.76-0.91), respectively. Thus, the estimate of the fracture risk

was lower for those with more prescriptions. A sex difference seemed to account for this as the adjusted ORs for men and women with ≥20 prescriptions were 0.69 (95% CI, 0.59-0.81) and 0.92 (95% CI, 0.83-1.02), respectively. The effect of β-blockers on fracture risk for current users with only 1 or 2 prescriptions and recent users was inconclusive. Adjustments were made for smoking, BMI, number of practice visits prior to the fracture and use of several other potentially confounding medications.

This study has several strengths, including a most impressive sample size, and analyses that were adjusted by matching and statistical techniques. However, information on BMI and smoking was missing for 30% and 15% of patients, respectively. A random review of 200 charts suggested that less than 1% of fractures were due to polytrauma but the exact cause of fractures is not known from the GPRD. It could be postulated that patients with less cardiovascular diseases, who don't take β-blockers, are more active and have more activity related, high-trauma fractures, which would be included in this database. In keeping with this, 57.1% had fractures of distal extremities, fracture sites not typically related to osteoporosis. Furthermore, the GPRD has no information on falls, physical activity, diet, calcium and Vitamin D supplements. Additionally, since cases and controls were identified through their general practitioner's office, it is possible that a certain number of patients who suffered fractures never returned for an office visit, as might be the case when transferred to a nursing home or in the event of mortality after a hip fracture. Patients who are β-blocker users and have more co-morbities may be more likely to suffer this course compared to non-users. Finally, not all patients had radiographs to confirm or exclude vertebral fractures, which is problematic considering so many such fractures are not clinically detected, as was discussed earlier.

Data from SOF, discussed in section 2.5, was also used to examine the effects of β-blockers on fractures. In this prospective cohort study of postmenopausal women, there were 2167 incident fractures, including 585 hip fractures, occurring over a mean 7 years of follow-up. Only the first fracture of any type per subject was included in this analysis. After adjusting for potential confounders, β-blockers reduced the hip fracture risk (OR 0.66; 95% CI, 0.49-0.90) while the effect of β-blockers on total fracture risk was inconclusive (OR 0.87; 95% CI, 0.75-1.00). Separating the β-blockers into selective and non-selective β-blockers, a reduction in hip fracture risk was seen with selective β-blockers. The hip fracture risk with non-selective β-blockers was inconclusive. No effects of β-blocker dose was found for fracture outcomes (not enough data was provided to compute confidence intervals).

Levasseur et al. also looked at the effects of β-blocker use on fracture risk using the EPIDOS data. During a mean follow-up of 3.6 years, 1,311 women had a nonvertebral fracture. After adjusting for multiple potential confounders, the effect of β-blockers on fracture risk was inconclusive (OR 1.2; 95% CI, 0.9-1.5).

Rejnmark et al. published another study on this subject. These authors, who previously examined the effect of β-blockers on BMD and fractures using data from DOPS (Danish Osteoporosis Prevention Study), now used a population-based case-control study design to explore the same subject. Using the Danish National Hospital Discharge Register, 124,655 fracture cases and 373,962 age and gender-matched controls were identified in the year 2000. Electronic linkage with other national health and statistics databases provided information on prescription medication use, including β-blockers, over the preceding 5 years, other medical diagnoses, number of general practitioner visits, and socioeconomic status. Adjusting for potential confounders, β-blockers were associated

with a reduced risk for any fractures (OR 0.91; 95% CI, 0.88-0.93) and hip fractures (OR 0.91; 95% CI, 0.85-0.98). In an analysis examining the effect of increasing daily doses of β-blockers, the OR for any fractures and hip fractures do have point estimates consistent with a dose effect but the confidence intervals are wide and overlap for these different dosages. Stratification by age and gender did not materially change the interpretation of the results. The investigators also examined the effect of other, non-diuretic, anti-hypertensives on the fracture risk. Notably, they found that calcium-channel blockers and angiotensin converting enzyme inhibitors were also associated with a clinically important fracture risk reduction of similar magnitude as that found with β-blockers.

Despite the very large sample size and the extensive confounder adjustment in this study, there are nonetheless some limitations. The investigators could not adjust for weight, a very important potential confounder, falls, dietary intake of calcium and vitamin D, lifestyle, smoking and alcohol intake. The cause of fracture is not known from these databases. The mean age of the fracture cases was 43 years, suggesting that many fractures are occurring at a relatively young age. This may be an indication that many fractures are the result of severe trauma (e.g. motor vehicle accident, fall off a ladder, down the stairs) or recreational injuries (e.g. skating, running, skiing). These latter fracture types are more likely to occur in healthier subjects who are more active rather than in those with cardiovascular diseases who may be taking β-blockers. In other population-based studies where records could be individually reviewed to ascertain the cause of fractures, severe trauma fractures accounted for 43% of all fractures for a cohort with a mean age of 37.4, while they accounted for only 7% of fractures for a cohort with a mean age of 57.8 years.[80, 81] They could not adjust for level of physical activity of the cases and controls which may have been helpful. By combining both fragility fractures and severe trauma or recreational fractures, the relationship between β-

blockers and fractures is likely confounded by other aspects of health that cannot be adjusted for by the Charlson co-morbidity index alone. This may also explain how 3 different classes of anti-hypertensives are associated with a fracture risk reduction in this study.

Finally, the most recent publication on this subject is from de Vries et al.[82] To examine the effects of β-blockers on hip and femur fractures, they conducted two case-control studies using data from population-based databases, the UK General Practitioner Research Database (GRPD) and the Dutch PHARMO Record Linkage System (RLS). From the GPRD, there were 22,247 cases and 22,247 controls (matched on year of birth, sex, medical practice and calendar time). From the RLS, there were 6,763 cases and 26,341 controls identified (matched by year of birth, gender, region, and calendar time). For each patient, medication histories, including β-blocker use, was obtained through the prescription and dispensing records or the GPRD and RLS, respectively. Analyses, adjusted for confounders, were conducted separately for the two databases. From 46 to 54% of patients were ≥ 80 years old in the GPRD and RLS, respectively. Current use of β-blockers (prescription given or dispensed in the 3 months prior to the index date) was associated with an OR for hip/femur fracture of 0.82 (95% CI, 0.74-0.93) in the GPRD and 0.87 (95% CI, 0.80-0.95) in the RLS. The data was not consistent with a dose-effect among current users of β-blockers on hip/femur fracture risk. There were inconsistent effects with respect to the effect of β-blockers receptor selectivity and lipophilicity on hip/femur fracture risk. When stratified by the use of other antihypertensive medications, β-blockers had a protective effect in those who were current or past users other anti-hypertensive agents in both the GPRD (OR 0.73, 95% CI 0.64-0.83) and the RLS (OR 0.76, 95% CI 0.67-0.86). The effect of β-blockers used on hip/femur fracture risk in patients who used them alone, without other antihypertensive

drugs, was inconclusive as the confidence intervals were wide in both the GPRD and the RLS. However, the point estimates of the ORs were very close to 1.0 in both cases.

These last two large case-control studies have the advantage of power to detect an effect of β-blockers on fracture risk. On the other hand, these two studies have limitations similar to those discussed earlier with Schlienger et al.[79] While the authors did adjust for a list of specific co-morbid illnesses and medications as potential confounders, the databases do not provide information on dietary factors, physical activity level, smoking and alcohol use. There was also very limited information on the history of falls. Data on body mass index (BMI) was missing for 58% of patients in the GPRD and unavailable in the RLS. The cause of fracture could also not be ascertained in the cases. This was perhaps less problematic for the study by de Vries et al.[82] than that discussed for the case-control study by Schlienger et al.[79] This is because the subjects in de Vries et al.[82] were older and hip/femur fractures, the only outcome examined, are strongly associated with low bone strength in older age groups. Nonetheless, the fact that analyses stratified by use of other antihypertensive agents showed very different point estimates for the ORs for the effect of β-blockers on hip/femur fracture risk does suggest that there are other unmeasured confounding variables associated with advanced cardiovascular diseases in these large case-control studies. Of course, the other possibility is that all of these anti-hypertensive agents actually do have bone protective effect, a seemingly unlikely possibility.

In summary, seven publications reporting on the effect of β-blockers on fracture risk were discussed. In three of these, β-blockers were associated with a decreased risk for any fractures.[73, 79, 83] One study with a nested case-control design, that only included women, did find an increased risk for any fracture associated with β-blocker use.[70] Looking at hip

fracture risk associated with β-blockers use, four studies found a reduced risk for fracture[74, 79, 82, 83] while one study was inconclusive[73] In the three studies that included men, stratification according to gender did not reveal major differences in the results.

2.7 Factors that predict BMD and fractures – potential confounders

In addition to BMI and fat mass, which have been found to be strong independent predictors of BMD and fracture risk as discussed in section 2.1, there are many other independent risk factors for BMD and fractures. Any risk factor for a low BMD will also be a risk factor for fracture but some clinical variables are a fracture risk over and above that for BMD alone.[84] These clinical risk factors for low BMD and fractures have usually been identified in large prospective, population-based cohort, studies such as CaMos. In addition, many of these risk factors for fractures have been identified through meta-analyses using the individual source data of several of these large cohort studies.[31, 85-92]

BMD is probably the single best predictor of fracture.[92, 93] The clinical variables that are considered major predictors of both low BMD and fracture risk (associated with a relative risk greater than 2) include: age, premature menopause, hypogonadism, previous fragility fracture, glucocorticoid therapy, family history of fragility fracture, malabsorption syndromes, propensity to fall, long-term immobilization, low body weight, anorexia nervosa, primary hyperparathyroidism, and chronic renal failure.[84, 94] In addition, several other variables are considered predictors of low BMD but considered moderate risk factors for fracture (relative risk for fracture between 1 and 2) including rheumatoid arthritis, past history of hyperthyroidism, calcium intake, smoking, excessive alcohol intake, excessive caffeine, and other medication such as chronic heparin and anticonvulsant therapy.[94] Other less prevalent, potential predictors for BMD and fracture

that have been reported and possibly confound the relationship between β-blockers, BMD and fractures include medications such statins,[95] nitrates,[96, 97] thiazide diuretics,[98, 99] and loop diuretics.[100, 101]

In investigating the effects of β-blockers on BMD and fractures, it is important to consider adjusting for the underlying conditions for which these drugs are prescribed, as these may be potential confounders. A recent study found an increased risk of hip fracture after a cardiovascular event (OR 2.38; 95% CI 1.92-2.94) after adjustment for potential confounders.[102] Furthermore, a diagnosis of hypertension, heart failure, or cerebrovascular were independent predictors of hip fracture, with a 2 to 3-fold increase in hip fracture risk in this study. Other studies have found that aortic calcification, a marker for cardiovascular diseases, is associated with an increased relative risk for low BMD and fractures.[103, 104] Thus, cardiovascular disease, cerebrovascular diseases and hypertension, which are frequent indications for β-blockers, need to be controlled for in an observational study such as ours.

2.8 Summary of the literature review

Leptin has been identified as an important regulator of bone metabolism in the last decade. It likely has both direct, positive effects on bone homeostasis as well as indirect, antiosteogenic effects. While the debate continues over with which regulatory pathway predominates *in vivo* in animals and in humans, there has nonetheless emerged from these recent findings the possibility that β-adrenergic receptor antagonists could have protective effects on the skeleton. Given the wide prevalence of use of these medications, clarifying their skeletal effects, if any, could have a major impact on their use in the population. Observational studies published so far have been contradictory as

to the effects of β-blockers on BMD and fractures in humans. Some of the case-control studies, despite the advantage of power to detect differences, have several limitations which might explain the disparate findings. Use of the CaMos population database has several advantages. As it is a prospective cohort study, it collects the information on potential risk factors before the occurrence of any events, thereby limiting recall bias. Medications were verified by an interviewer at the time of questionnaire, limiting errors. CaMos also collected information on a host of other anthropometric and lifestyle factors that may impact BMD and fractures. As the timing and cause of fractures could be determined in CaMos, analyses can include only incident minimal trauma fractures. Finally, CaMos enrolled both men and women of different ages and there were many users of β-blockers within this population. Thus, we believe the CaMos population offers advantages over previous studies in studying the effects of β-blockers on BMD and fractures.

Chapter 3 - Methods

This chapter describes the methods used in this thesis. A description of the data collection methods is given in section 3.1. This is followed by section 3.2 which describes the statistical analyses done to estimate the effect of β-blockers on BMD, and section 3.3, which presents the details of the analyses carried out to examine the effects of β-blockers on fractures.

3.1 Data Collection

CaMos Study Design and Population

CaMos is a population-based, prospective cohort study of non-institutionalized subjects 25 years of age and older who reside within 50 kilometers from one of 9 CaM*os* Research Centers across Canada (Vancouver, Calgary, Saskatoon, Hamilton, Toronto, Kingston, Quebec, Halifax and St-John's). This catchment area comprises 37% of the Canadian population. Initiated in 1995 with funding from the National Health Research Development Program (NHRDP), CaM*os* was designed to obtain basic epidemiologic information on osteoporosis and fractures in Canada. Among its objectives, the cross-sectional component of the study was intended to describe the normal range of bone mineral density for community dwelling Canadian men and women across all ages, to estimate the peak bone mass for men and women, and to estimate the prevalences of low bone density and fractures in the population. The follow-up cohort component of the study was designed to estimate the patterns of bone density changes over time and the incidence of fractures in the Canadian population. An analytical objective of the study was to estimate the relationship between bone density, fractures and potential risk or

protective factors, information on which was collected in a detailed questionnaire, described below.

The sample population was stratified by age, sex and center. This was necessary to ensure sufficient numbers of subjects to accurately estimate the prevalence of fractures, mean bone mineral density and the magnitude of bone loss across ages and regions in men and women. Subjects were randomly selected from household telephone listings. Approximately 1,000 subjects were recruited from each CaMos Research Center. Postal codes in the regions of interest were enumerated and a random sample of listed telephone numbers for each postal code obtained. Initial contact was made by sending all potential households an introductory letter which was then followed by a telephone call to formally request participation. Once enumeration of eligible household members age 25 or older was made, a single subject was selected randomly from each household. If the selected individual refused to participate, an agreeable member of the household was not selected in lieu. Selected household members who refused to participate were nonetheless asked to complete a one-page questionnaire requesting information about the major risk factors for osteoporosis, including age, sex, race, previous fractures, smoking and family history of osteoporosis. This questionnaire is termed the "non-responder questionnaire". There were few exclusion criteria in CaMos, but potential subjects were excluded if they were institutionalized or could not understand and speak English, French or for the Toronto and Vancouver centres, Mandarin.

The centres recruited subjects over an 18 month period beginning February 1996. Sampling by calendar period was done to ensure a representative number of subjects across ages were obtained in each season. This was determined to be necessary after a pilot study found that younger subjects were being recruited faster which could have a

potential impact on the ability to compare seasonal exposures and habits in the young versus the older age groups. In particular, vitamin D intakes can vary greatly by season, potentially affecting some items measured by the CaMos questionnaire. Written informed consent was obtained from each participating subject and the Institutional Ethics Review Boards from each of the 9 participating centers approved the study.

All participants received an in-person, interviewer-administered questionnaire (IAQ or "long" questionnaire) at entry. The interviewers were trained and usually administered the questionnaires at the CaMos research centres. Those few participants who refused to or couldn't undergo the physical assessment had the IAQ conducted elsewhere, usually at their home. This questionnaire obtained sociodemographic information, medical and fracture history, medication use in the past year, diet and physical activity, fall history, smoking status, reproductive history, and family history of osteoporosis and fractures. The subjects also completed the Rand SF-36 Questionnaire and Health Utilities Index. Subjects were requested to bring the content of their medicine cabinets to the study centre for interviewer verification of all currently used prescription and non-prescription medications or supplements. When there was a discrepancy, the interviewer noted the participant's reported usage of the medication. Subjects were made aware of the discrepancies. When subjects failed to bring these medications and supplements to the study centre, the interviewer contacted them at home later to review this. The cohort also underwent a number of physical measurements at entry including weight, height, bone densitometry testing by dual energy x-ray absorptiometry (DXA) of the lumbar spine (L1-4) and proximal femur and for those subjects 50 years and older, a radiograph of the lateral thoracic and lumbar spine. A small number of participating individuals refused the physical assessment including the BMD measurement or could not perform the tests because of severe deformity or previous surgery.

A complete reassessment, including the IAQ and all the physical and densitometry measurements, was repeated at year 3 for those subjects who were between ages 40-60 or who were pregnant at baseline and at year 5 for all participants.

All completed questionnaires and measurement results were sent for coding and entry at a central data centre. Data entry error rates were found to be substantially less than 1% at this centre in a quality control assessment. The appendix to this thesis contains a copy of all questionnaires used in CaMos that relate to variables used in this study

Assessment of β-blocker use

As with all other medications, the IAQ collected information on the type, route of delivery, dose and frequency of use of β-blockers by study subjects. As β-blockers were the drugs of interest, stringent efforts were made to ensure that no patients taking this drug would systemically be missed. CaMos created a large database compiling all known medications and supplements from multiple pharmaceutical registries. Medications and supplements in their various doses and combinations, and from different manufacturers, all are given a unique identification number within this database. The CaMos database had 10,238 medication entries (file version April 25, 2005). Two search strategies were employed to find all entries containing a β-blocker ingredient within this CaMos medication database. The first was a manual search with a process of elimination of all non-pertinent medications and retention of relevant drugs. Medications which possibly included a β-blocker or other confounding medication as an ingredient were kept. The list was pared down to include only β-blockers using a careful elimination process done by the author. The second search strategy utilized a compilation of β-blockers found

listed in the Compendium of Pharmaceuticals and Specialties (CPS, 2005 edition), other journal articles,[79] and through drug classification system such as the Anatomical Therapeutic Chemical Classification System[105] and the "Banque de Données Automatisée sur les Médicaments".[106] Indeed the number of listed β-blockers from these different sources varied from 10 in the CPS to 26 in BIAM. From a combination of such lists, we included the β-blockers listed in table 3 in our search of the CaMos database. There were 232 entries found in the CaMos drug database using these search strategies. Only oral β-blockers taken daily were included in this study. This list of 232 possible β-blockers was used in a search to identify those subjects who used any of these. Subjects taking β-blockers at both the baseline and year 5 visits were classified as chronic users. Those who were taking β-blockers at only one of these visits were classified separately as users at year 0 or year 5 only.

Table 3

β-blockers included in the CaMos database search		
Acebutolol hydrochloride	Celiprolol chlorhydrate	Oxprenolol chlorhydrate
Alprenolol chlorhydrate	Esmolol hydrochloride	Penbutolol sulfate
Atenolol	Labetolol	Pindolol
Befunolol chlorhydrate	Labetolol chlorhydrate	Practolol
Bxolol	Levobunolol chlorhydrate	Propranolol
Bxolol chlorhydrate	Metipranolol	Propranolol hydrochloride
Bisoprolol	Metoprolol	Sotalol
Bisoprolol fumarate	Metoprolol succinate	Sotalol chlorhydrate
Bupranolol chlorhydrate	Metoprolol tartrate	Tertatolol chlorhydrate
Butofilolol	Nadolol	Timolol
Carteolol chlorhydrate	Nebivolol	Timolol maleate
Carvedilol	Oxprenolol	
Celiprolol	Oxprenolol hydrochloride	

Bone Mineral Density Assessment

BMD of the lumbar spine (L1-L4) and the proximal hip were measured by DXA using Hologic QDR 1000 or 2000 or Lunar DPX densitometers. All Lunar density measurements were converted to a Hologic base according to the method by Genant et al.[107] Calibration of the machine was done daily. Quality assurance tests were done at least weekly at each centre according to the manufacturers' instructions. Cross-calibration across centres was done at the start of the study and then annually with the same European spine phantom. The BMD measurements reported in this thesis are those technically adequate results of the spine or hip taken at baseline.

Fracture Assessment

Every year, a "short" fracture questionnaire (see copy in Appendix) was mailed to the study participants which collected information on incident fractures, cause of fractures, other medical disorders and hospitalizations. In the event of a new fracture, consent was obtained to allow contact with the treating physician or hospital to verify diagnoses and details. For 73% of subjects in CaMos, original radiology reports were obtained to confirm the details of incident fragility fractures. All reported clinical fragility fractures were included in this analysis. Although spine radiographs were done at baseline and at 5 years to determine the radiographic vertebral fracture incidence, the results of these are not included in this analysis since for technical reasons these data are not yet available. Fractures were classified according to the circumstances of the injury. Falls from standing height or less were considered minimal trauma. Fractures occurring through motor vehicle accidents and falls from greater than standing height are deemed severe trauma.

In CaMos, fractures were reported at a variety of sites, but to obtain adequate numbers for analysis, they are grouped in 3 ways as shown in table 24. The grouping "any minimal trauma fractures" includes fractures at any site occurring without significant trauma. The grouping "main fragility fractures" includes only minimal trauma fractures of the vertebrae, hip, distal radius, pelvis and ribs fractures. This grouping excludes fractures of the hands, fingers and feet, for example, which are not always associated with a low BMD. Finally, we looked at the incidence of minimal trauma hip fractures as these are particularly devastating and any detection of differences between β-blocker users and non-users would be particularly important. Furthermore, this outcome would be more reliably diagnosed in all subjects as hip fractures are almost never missed in practice so the chance of detection bias is reduced for this fracture. All fractures discussed in our results refer to minimal trauma fractures. In all analyses, only the first incident fracture sustained per subject, during the 5 years of follow-up, was used, since multiple fractures per subjects are not independent events. Hence, for any minimal trauma fractures, an incident fracture is the first low-trauma fracture at any site in a given subject; for main fractures, any first fracture of the vertebrae, hip, distal radius, pelvis and ribs was used; for hip fractures, the first hip fracture per subject was used.

Other Measurements

The use of β-blockers may be associated with other determinants of BMD and fractures. Information on such confounding variables was obtained from the baseline questionnaire and physical measurements. These included the categorical variables age, sex, current cigarette smoking (referred in the rest of this thesis as "smoker"), previous daily cigarette smoking for at least 6 months (referred to as "ever smoked cigarettes" in the rest of this thesis), menopausal status for women, current participation in any regular activity or

program (referred to as "physical program"), any previous minimal trauma fractures and history of falls in the past month. The categorical variables also included certain comorbities such as physician-diagnosed, patient-reported history of hypertension, heart attack, stroke and transient ischemic attack. Exposure to potentially confounding, currently used medications, including oral contraceptives, hormonal replacement therapy, thiazide and loop diuretics, nitrates, statins, bisphosphonates, fluoride, selective estrogen receptor modulators (SERMs), calcitonin and systemic glucocorticoids was reported as categoricals. The continuous variables included weight, height, body mass index (BMI), average alcohol intake (continuous variable reported as number of beverages/week), energy expenditure from strenuous, vigorous, or moderate activity (reported as number of kilocalories expended/week performing these activities; referred to in the rest of the thesis as "energy expenditure"), and the number of falls in the month prior to the IAQ. Continuous variables also included the average daily calcium intake combining the average daily quantity in the diet, as assessed by a food frequency questionnaire, and supplements (reported in mg/day) and approximate vitamin D intake obtained by combining the average quantities from any supplements and fortified milk (reported in I.U./day).

Of the two variables for physical activity examined, only the continuous variable energy expenditure was selected to represent physical activity in the multiple linear regression and multiple logistic regressions.

3.2 Statistical methods for the effects of β-blockers on BMD

Descriptive statistics (means, standard deviations, medians with inter-quartile ranges and percentages, as appropriate) were compiled to assess any differences in baseline

characteristics between the users and nonusers of β-blockers for both continuous and dichotomous variables. A Pearson correlation matrix was created across all continuous variables to also aid in assessing the potential for confounding, and similarly, cross-tables were created for categorical variables for the same purposes. Simple linear regressions with each independent variable regressed against the dependant variables were first run for all the variables, followed by multiple linear regression. This procedure again allowed us to assess the effects of any confounding, leading to a robust estimate of the effect of β-blocker use on bone mineral density. Finally, the Bayesian Information Criterion (BIC) as implemented in the BMA package in R was used to search for the best multivariate predictive models which included β-blockers, to again ensure that no confounding was missed.[108]

The independent variables for men and women included in the models were: age, BMI, smoking, alcohol, energy expenditure, falling in the past month (categorical), thiazides, loop diuretics, statins, nitrates, glucocorticoids, myocardial infarction, cerebrovascular diseases and prior minimal trauma fractures. In men, we also included calcium intake. In women, we also included menopause, HRT, SERMs, and vitamin D intake. The analyses for men and women were conducted separately.

The percent BMD difference between users and non-users of β-blockers was calculated using the formula [(β parameter)/BMD in the non-users] x 100%.

3.3 Statistical methods for the effects of β-blockers on fractures

To examine the effect of β-blocker use on fracture incidence, we conducted two different analyses. In the first, all CaMos subjects were classified at baseline as users or non-

users of β-blockers. The fracture incidence for these groups and the adjusted relative risk of fracture for users of β-blockers were then calculated via logistic regression. In the second analysis, CaMos subjects were classified into 4 groups: non-users of β-blockers, those who used β-blockers at baseline only, those who used β-blockers at year 5 only and those who were chronic users (users at both baseline and year 5). This analysis allowed us to see if duration of β-blockers use had an effect on the risk for fracture.

Descriptive statistics were redone within each of the two sets of β-blocker user groups described above, in order to examine any differences in baseline characteristics between the various categories of users and nonusers of β-blockers. Similarly, correlation matrix and cross-tables were redone for the same purposes as described in section 3.2. Univariate logistic regression models across all independent variables were first run, followed by multiple logistic regression. This procedure again allowed us to assess the effects of any confounding, leading to a robust estimate of the effect of β-blocker use on fracture risk. Finally, the Bayesian Information Criterion (BIC) as implemented in the BMA package in R was used to search for the best predictive models including β-blockers, to further check that no confounding was missed.[108] All of the potential confounding medications with a prevalent use of 1% in at least one of the groups were included in the multivariable logistic regression model.

The independent variables included in the models were: age, BMI, smoking, alcohol, calcium intake, vitamin D intake, energy expenditure, falls in the past month (categorical), thiazides, loop diuretics, statins, nitrates, glucocorticoids, hypertension, myocardial infarction, cerebrovascular diseases and prior minimal trauma fractures. Menopause, HRT and SERMs were added for the women. The analyses for men and women were conducted separately.

Chapter 4 – Results

In this chapter, we present the results based on the methods of the previous chapter. In particular, in section 4.1 we will present descriptive statistics based on the CaMos dataset and the subjects exposed to β-blockers. Next, in section 4.2, the results of the analysis of β-blocker effects on BMD will be presented, followed in sections 4.3 and 4.4 by the results of two separate analyses of the effects of β-blockers on fractures. In the first analysis, presented in section 4.3, all CaMos subjects were classified according to their use of β-blockers at baseline only. In the second analysis, presented in section 4.4, CaMos subjects were classified by their duration of use of β-blockers to determine if there are varying effects on fracture risk with differences in exposure duration.

4.1 CaMos recruitment and subjects exposed to β-blockers

During the enrollment period from February 1996 to September 1997, there were 9,423 subjects enrolled in CaMos. Of these, 6,539 were women and 2,884 were men. Approximately equal numbers were recruited from each of the nine centers. The recruitment rate for full participation was 42% of all potential subjects contacted. Another 30% of individuals sampled completed only the "refusal" questionnaire for a total response rate of 72%. At the end of the year 5 follow-up, 87% of women and 84% of men remained as full participants in the cohort.

Of all the subjects recruited in CaMos, 836 (8.9%) were using oral β-blockers daily at baseline. Of these, 8.2% (236) of the men and 9.2% (600) of the women were using these drugs. Most of the β-blockers users were older, reflecting the pattern of onset of the disorders for which they are indicated. In men, 2.0% of those less than age 50 took

β-blockers while 10.2% of those age 50 or older were taking them. The figures were similar in women (2.4% and 10.4%, respectively). Indeed, CaMos had very few younger subjects taking β-blockers, since 96% of women and 94% of men taking them were aged 50 years and older (see tables 4 and 5).

Table 4: Number (%) of β-blockers users and non-users in women by age groups

Age Groups	Total number	β-blockers Users	β-blockers Non-Users
25-29	89	1 (0.2)	88 (1.5)
30-39	262	6 (1.0)	256 (4.3)
40-49	622	16 (2.7)	606 (10.2)
50-59	1351	71 (11.8)	1280 (21.6)
60-69	2045	192 (32.0)	1853 (31.2)
70-79	1647	237 (39.5)	1410 (23.7)
80-89	480	74 (12.3)	406 (6.8)
90 and older	43	3 (0.5)	40 (0.7)
Total	**6539**	**600**	**5939**

Table 5: Number (%) of β-blockers users and non-users in men by age groups

Age Groups	Total number	β-blockers Users	β-blockers Non-Users
25-29	86	0 (0)	86 (3.2)
30-39	238	1 (0.4)	237 (9.0)
40-49	373	13 (5.5)	360 (13.6)
50-59	602	29 (12.3)	573 (21.6)
60-69	759	76 (32.2)	683 (25.8)
70-79	629	84 (35.5)	545 (20.6)
80-89	188	33 (14.0)	155 (5.9)
90 and older	9	0 (0)	9 (0.3)
Total	**2884**	**236**	**2648**

Apart from being of older age, subjects who took β-blockers were different in other ways from non-users as shown in table 6. In general, they also had a slightly a greater BMI

Table 6: Characteristics of patients who used β-blockers and those who did not at baseline. Continuous variables presented as means (SD) and categorical variables as number (%).

	β-blockers Users n = 836	Non-Users n = 8587
Sex Female	600 (71.8)	5939 (69.2)
Male	236 (28.2)	2648 (30.8)
Age (years)	68.7 (9.9)	61.4 (13.5)
Weight (kg)	73.7 (15.2)	72.5 (15.17)
Height (meters)	1.6 (0.1)	1.6 (0.1)
BMI	27.8 (4.9)	26.9 (4.8)
Ever smoked cigarettes	428 (51.2)	4579 (53.3)
Smoker	1372 (16.0)	95 (11.4)
Alcohol (No. beverages/wk)	2.8 (6.0)	3.0 (5.9)
Calcium (mg/day)	960.8 (587.8)	1000.0 (608.2)
Vitamin D (IU/day)	125.0 (531.8)	149.2 (594.7)
Physical program (yes/no)	4744 (55.3)	451 (53.4)
Energy expenditure (kcal/wk)	3974 (3869)	4671 (3973)
Falls in the past month (yes/no)	50 (6.0)	571 (6.7)
Thiazide diuretics	206 (24.6)	758 (8.8)
Loop diuretics	61 (7.3)	230 (2.7)
Statins	114 (13.6)	329 (3.8)
Nitrates	129 (15.4)	235 (2.7)
Glucocorticoids	12 (1.4)	124 (1.4)
Hormone replacement therapy	122 (14.6)	1363 (15.9)
SERMs	7 (0.8)	49 (0.6)
Bisphosphonates	17 (2.0)	143 (1.7)
Calcitonin	0 (0.0)	1 (0.01)
Fluoride	0 (0.0)	3 (0.03)
Hypertension	627 (75.2)	2041 (23.9)
Myocardial infarction	169 (20.4)	417 (4.9)
Cerebrovascular disease	55 (6.6)	305 (3.6)
Prior minimal trauma fracture	237 (28.4)	2252 (26.3)

and utilized more thiazide diuretics, loop diuretics, statins, and nitrates. Further details on the baseline characteristics broken down by sex will be presented in the next two sections.

4.2 The effect of β-blockers on BMD

Of all the subjects who agreed to participate in CaMos, 12.9% did not have a BMD measurement taken at baseline because of severe physical deformity, bilateral hip replacements or refusal. In order to investigate whether there might be a selection bias associated with the presence or absence of this measurement, the characteristics of those men and women who did versus those who did not have a baseline BMD are presented in table 7. Subjects who did not have a BMD done at baseline were older, participated less in physical activity programs, used more anti-hypertensives, diuretics, nitrates, and had more hypertension and cardiovascular diseases. Hence, these subjects appeared to have worse health and increased comorbities. Since our main objective is to estimate the effects of β-blockers on BMD and fractures, a selection bias would imply not only a difference in baseline characteristics, but that those without a BMD measurement somehow had a different effect of this medication on their bones, which seems unlikely. For the remainder of this section, all descriptive statistics and analyses are limited to those 8,209 CaMos subjects who had a BMD measurement.

4.2.1 Descriptive statistics for the effect of β-blockers on BMD

Of the 8209 subjects from CaMos who had a BMD at entry into the study, 5,648 were women and 2,555 were men. Men (table 8 below) who used β-blockers were older, heavier, smoked less, and used more thiazide diuretics, loop diuretics, statins and

Table 7: Characteristics of subjects who had a BMD measurement and those who did not at baseline. Continuous variables presented as means (SD) and categorical variables as number (%).

	BMD measured n=8203	BMD not measured n=1220
Age (years)	61.0 (13.0)	69.5 (13.5)
Female Sex	5648 (68.9)	891 (73.0)
Weight (kg)	72.9 (14.7)	70.1 (18.3)
Height (meters)	164.0 (9.2)	162.0 (9.5)
BMI (kg/m^2)	27.0 (4.7)	26.7 (6.2)
Ever smoked cigarettes	4347 (53.0)	660(54.1)
Smoking	1260 (15.4)	207 (17.0)
Alcohol (No. beverages/wk)	3.1 (5.8)	2.4 (6.3)
Calcium (mg/day)	1004.3 (606.1)	943.6 (606.6)
Vitamin D (IU/day)	143.5 (559.0)	171.2 (762.8)
Physical program (yes/no)	4641 (56.6)	554 (45.4)
Energy expenditure (kcal/wk)	4792 (3999)	3076 (3333)
Falls in the past month (yes/no)	534 (6.5)	87 (7.1)
β-blocker use	691 (8.4)	145 (11.9)
Thiazide diuretics	786 (9.6)	178 (14.6)
Loop diuretics	188 (2.3)	103 (8.4)
Statins	384 (4.7)	59 (4.8)
Nitrates	260 (3.2)	104 (8.5)
Glucocorticoids	96 (1.2)	40 (3.3)
Hormone replacement therapy	1358 (16.6)	127 (10.4)
SERMs	44 (0.5)	12 (1.0)
Bisphosphonates	117 (1.4)	43 (3.5)
Calcitonin	0 (0)	1 (0.1)
Fluoride	3 (0.04)	0 (0)
Hypertension	2203 (26.9)	465 (38.3)
Myocardial infarction	457 (5.6)	129 (10.6)
Cerebrovascular disease	284 (3.5)	76 (6.3)
Prior minimal trauma fracture	2134 (26.1)	355 (29.2)

nitrates. They also had higher rates of hypertension and history of myocardial infarction or stroke. Similar to the men, women (table 9 below) who used β-blockers were also older, heavier, smoked less, and used more thiazide diuretics, loop diuretics, statins, and nitroglycerin. They also had more hypertension and a greater history of myocardial infarction and stroke.

Pearson correlation matrixes were obtained for all continuous variables and are presented in tables 10 and 11 for men and women, respectively. For men, there were strong positive correlations between weight and height and between weight and BMI. Total hip BMD and lumbar spine BMD were also strongly correlated with each other. There were also moderate positive correlations between weight and BMD at either the total hip or the lumbar spine, and between BMI and BMD measured at either the total hip or the lumbar spine. There were small negative correlations between age and BMD at the hip, between age and energy expended on moderate or strenuous physical activity, and between age and height. For women, there were strong positive correlations between weight and BMI and between the BMD at the two sites with each other. There were small to moderate correlations between weight and the variables height, energy expenditure, total hip BMD, and lumbar spine BMD. There were small to moderate correlation between BMD and height and between BMD and BMI. Age was negatively correlated with BMD and with height. As BMI and weight are both correlated with BMD and are probably collinear to some degree, only BMI was used in the multivariate regressions throughout this thesis.

Table 8: Descriptive baseline statistics for the men who underwent BMD testing at baseline: all men, men who did not use β-blockers, and men who did use β-blockers at baseline.
Continuous variables reported as mean (SD), median (IQR), categorical variables reported as number (%).

	All Men (n=2555)	Men who are non-users (n = 2353)	Men who are β-blockers users (n = 202)
Age (years)	58.8 (14.1) 60.0 (49.0-70.0)	58.1 (14.2) 59.0 (49.0-69.0)	67.4 (9.8) 69.0 (61.0-74.0)
Weight (kg)	81.7 (13.4) 80.7 (72.5-90.0)	81.6 (13.3) 80.7 (72.5-90.0)	83.7 (14.1) 82.0 (74.0-93.0)
Height (centimeters)	173.7 (7.1) 173.5 (169.0-178.0)	173.7 (7.1) 174.0 (169.0-178.0)	172.5 (6.9) 172.0 (168.0-177.8)
BMI	27.1 (3.8) 26.7 (24.4-29.2)	27.0 (3.8) 26.6 (24.4-29.1)	28.1 (3.9) 27.8 (25.2-30.1)
Ever smoked cigarettes	1701 (66.6)	1564 (66.5)	137 (67.8)
Current Smoker	466 (18.3)	445 (18.9)	21 (10.4)
Current Alcohol Use (No. beverages/week)	5.2 (8.0) 2.0 (0.0-7.0)	5.2 (7.9) 2.0 (0.2-7.0)	5.7 (9.1) 2.0 (0.0-7.0)
Calcium/day (mg)	912 (580) 781 (518-1158)	915 (579) 785 (522-1165)	878 (594) 752 (500-1082)
Vitamin D/day (IU)	92 (501) 0.0 (0.0-0.0)	93 (519) 0.0 (0.0-0.0)	75 (168) 0.0 (0.0-0.0)
Currently physically active	1440 (56.4)	1319 (56.1)	121 (59.9)
Energ Exp from stren, vig, mod activity (#kcal/wk)	5706 (5196) 4353 (2037-7724)	5576 (5149) 4407 (2087-7849)	4891 (5667) 3329 (1764-5956)
Falls in the past week	55 (2.2)	53 (2.3)	11 (5.5)
Falls in the past month	165 (6.5)	154 (6.5)	36 (17.8)
β-blocker use	202 (7.9)	0 (0.0)	202 (100.0)
Thiazide diuretic use	137 (5.4)	101 (4.3)	36 (17.8)
Loop diuretic use	56 (2.2)	47 (2.0)	9 (4.5)

Statins	128 (5.0)	93 (4.0)	35 (17.3)
Nitrate Use	98 (3.8)	53 (2.3)	45 (22.3)
Glucocorticoid use	26 (1.0)	24 (1.0)	2 (1.0)
Bisphosphonate use	3 (0.1)	3 (0.1)	0 (0.0)
Total hip BMD (g/cm^2)	1.0099 (0.1492) 1.0065 (0.9120-1.1108)	1.0097 (0.1489) 1.0058 (0.9123-1.1110)	1.0116 (0.1536) 1.0191 (0.9120-1.1048)
L1-4 BMD (g/cm^2)	1.0467 (0.1689) 1.0340 (0.9360-1.1455)	1.0441 (0.1686) 1.0325 (0.9333-1.1418)	1.0768 (0.1698) 1.0505 (0.9597-1.1860)
Hypertension	612 (24.0)	472 (20.1)	140 (69.3)
Heart attack	219 (8.6)	149 (6.4)	70 (35.4)
Stroke/TIA	99 (3.9)	85 (3.6)	14 (6.9)
Prior Min. Trauma Fracture	609 (23.9)	566 (24.1)	43 (21.4)

Table 9: Descriptive baseline statistics for the women who underwent BMD testing at baseline: all women, women who did not use β-blockers, and women who did use β-blockers at baseline.
Continuous variables reported as mean (SD), median (IQR), categorical variables reported as number (%)

	All women (n=5648)	Women who are non-users (n =5159)	Women who are β-blockers users (n =489)
Age (years)	61.9 (12.4) 64.0 (54-71)	61.4 (12.4) 63.0 (53-70)	68.0 (9.8) 69.0 (62-75)
Weight (kg)	68.8 (13.5) 67.0 (59.3-77.0)	68.7 (13.5) 67.0 (59.0-76.5)	70.1 (13.2) 68.0 (60.7-78.0)
Height (centimeters)	159.8 (6.4) 160.0 (155.0-164.0)	159.9 (6.5) 160.0 (155.0-164.0)	158.6 (6.1) 159.0 (154.9-162.6)
BMI	26.9 (5.0) 26.3 (23.3-29.8)	26.9 (5.0) 26.3 (23.3-29.7)	27.9 (5.1) 27.1 (23.3-30.8)
Ever smoked cigarettes	2646 (46.9)	2427 (47.0)	219 (44.8)
Current Smoker	794 (14.1)	739 (14.3)	56 (11.5)
Current Alcohol Use (No. beverages/week)	2.1 (4.2) 0.2 (0-2.0)	2.1 (4.2) 0.2 (0-2.0)	1.9 (4.0) 0.1 (0-2.0)
Calcium/day (mg)	1046 (613) 937 (598-1366)	1048 (615) 937 (601-1367)	1026 (593) 957 (564-1362)
Vitamin D/day (IU)	167 (582) 0.0 (0-250)	170 (588) 0.0 (0-250)	137 (512) 0.0 (0-114)
Currently physically active	3201 (56.7)	2939 (57.0)	262 (53.6)
Energ Exp from stren, vig, mod activity (#kcal/wk)	4378 (3235) 3625 (1880-6456)	4423 (3253) 3682 (1897-6490)	3906 (2998) 3060 (1566-5565)
Falls in the past month	369 (6.5)	339 (6.6)	30 (6.1)
Menopause (Perstop)	4651 (82.4)	4186 (81.2)	465 (95.1)
HRT use	1358 (24.0)	1246 (24.2)	112 (22.9)
SERM use	44 (0.8)	39 (0.8)	5 (1.0)
Thiazide diuretic use	649 (11.5)	518 (10.0)	131 (26.8)

Loop diuretic use	132 (2.3)	103 (2.0)	29 (5.9)
Statins	256 (4.5)	192 (3.7)	64 (13.1)
Nitrate Use	162 (2.9)	104 (2.0)	58 (11.9)
Glucocorticoid use	70 (2.0)	65 (1.3)	5 (1.0)
Bisphosphonate use	114 (2.0)	101 (2.0)	13 (2.7)
Total hip BMD (g/cm^2)	0.8544 (0.1445) 0.8518 (0.7565-0.9479)	0.8563 (0.1444) 0.8546 (0.7592-0.9498)	0.8353 (0.1447) 0.8375 (0.7331-0.9288)
L1-4 BMD (g/cm^2)	0.9369 (0.1720) 0.9301 (0.8149-1.0477)	0.9380 (0.1721) 0.9308 (0.8168-1.0503)	0.9257 (0.1698) 0.9250 (0.7997-1.0216)
Hypertension	1591 (28.3)	1218 (23.7)	373 (76.6)
Heart attack	238 (4.2)	166 (3.2)	72 (14.8)
Stroke/TIA	185 (3.3)	154 (20)	31 (6.4)
Prior Minimal Trauma Fracture	1525 (27.0)	1369 (26.6)	156 (31.9)

Table 10: Correlation matrix for the men with a BMD

	Weight (kg)	Height (cm)	BMI (kg/m^2)	Alcohol*	Calcium (mg per day)	Vitamin D (IU per day)	Energy expenditure*	Total hip BMD*	Lumbar spine BMD*
Age (years)	-0.09	-0.24	0.03	-0.02	-0.02	0.02	-0.22	-0.22	0.02
Weight (kg)		0.50	0.87	0.05	0.08	0.01	0.18	0.43	0.30
Height (cm)			0.00	0.05	0.10	0.03	0.18	0.22	0.16
BMI (kg/m^2)				0.03	0.03	-0.01	0.10	0.37	0.26
Alcohol*					-0.01	0.02	0.01	0.05	0.07
Calcium (mg per day)						0.05	0.08	0.07	0.07
Vitamin D (IU per day)							-0.02	-0.01	0.00
Ergergy expenditure*								0.16	0.08
Total hip BMD*									0.66

*Alcohol reported as number of beverages per week; energy expenditure as kcal/week; BMD as g/cm^2.

Table 11: Correlation matrix for the women with a BMD

	Weight (kg)	Height (cm)	BMI (kg/m^2)	Alcohol*	Calcium (mg per day)	Vitamin D (IU per day)	Energy expenditure*	Total hip BMD*	Lumbar spine BMD*
Age (years)	-0.09	-0.28	0.03	-0.04	0.06	0.06	-0.14	-0.43	-0.32
Weight (kg)		0.34	0.91	-0.07	-0.01	-0.01	0.21	0.48	0.36
Height (cm)			-0.07	0.07	0.05	0.00	0.19	0.26	0.25
BMI (kg/m^2)				-0.10	-0.04	-0.01	0.14	0.40	0.27
Alcohol*					-0.02	-0.00	-0.02	0.02	0.04
Calcium (mg per day)						0.20	0.06	-0.01	-0.01
Vitamin D (IU per day)							-0.03	-0.06	-0.05
Ergergy expenditure*								0.18	0.08
Total hip BMD*									0.72

*Alcohol reported as number of beverages per week; energy expenditure as kcal/week; BMD as g/cm^2.

4.2.2 Simple Linear Regression

Simple linear regression (SLR) analyses were conducted and residual plots obtained for each independent variable against the dependent variables, BMD of the total hip and BMD of the lumbar spine. The SLR models, with their point estimates, corresponding confidence intervals, and R^2 values are shown for the hip in tables 12 and 13 for men and women, respectively and for the spine in tables 14 and 15 for men and women, respectively. The scatter and residual plots reasonably supported the assumptions of linearity, normality and homoscedasticity for any univariate regressions of the dependant on the independent variables (plots too numerous to include here).

Covariates from the univariate analyses which seemed to have any chance of an association with the total hip and lumbar spine BMD in men and women were included for further evaluation in the multivariate analyses, except the reciprocal BMD site. For men at the total hip, these were age, weight, height, BMI, smoking, alcohol use, calcium intake, energy expenditure in moderate, strenuous or vigorous activities, participation in a physical activity program, loop diuretics, nitrates, glucocorticoids, bisphosphonates, myocardial infarction, and prior minimal trauma fracture. For men, at the lumbar spine, these were: weight, height, BMI, smoking, alcohol use, calcium intake, energy expenditure in moderate, strenuous or vigorous activities, participation in a physical activity program, β-blockers, thiazides, glucocorticoids, bisphosphonates, hypertension, cerebrovascular disease and prior minimal trauma fracture.

For women, at the total hip, variables included age, weight, height, BMI, vitamin D intake, energy expenditure in moderate, strenuous or vigorous activities, falls in the past month, menopause, HRT, β-blockers, loopdiuretics, nitrates, glucocorticoids, bisphosphonates,

hypertension, myocardial infarction, cerebrovascular disease and prior minimal trauma fracture. For women, at the lumbar spine, included in further analyses were age, weight, height, BMI, alcohol use, vitamin D intake, participation in a physical activity program, energy expenditure in moderate, strenuous or vigorous activities, falls in the past month, menopause, HRT, thiazides, statins, nitrates, glucocorticoids, bisphosphonates, myocardial infarction, cerebrovascular disease and prior minimal trauma fracture.

In addition, some variables were added based on the a priori knowledge of being suspected or potential confounders. For example, statins, nitrates, and loop diuretics have all been previously shown to have potential effects on mineral metabolism or bone density and are clearly associated with β-blocker use. The variables representing falls and cerebovascular disease were also chosen for further modeling as they represent markers of co-morbidities or frailty which could be confounding the association between exposure and outcome. Conversely, the variable bisphosphonate was not selected for further modeling, as we considered this would represent overadjustment, since their use is so closely associated with the outcomes. Bisphosphonates are nearly always used by patients with low BMD. Similarly, lumbar spine BMD and total hip BMD are so strongly correlated that measurement of one closely predicts the other. Hypertension is a diagnosis so strongly associated with β-blocker use that the effects are difficult to separate in an observational study, so this variable was not included in the multivariate linear regressions (MLRs) even when it seemed associated with the outcome. Finally, only one variable was chosen to represent the degree of routine physical activity, energy expenditure in moderate, strenuous or vigorous activities. All chosen variables for the MLR analyses are shown in bold in these tables.

Table 12: Univariate regression results for total hip BMD against independent variables at baseline in men

Variable	β coefficient	95% CI	R^2
Age	**-0.0024**	**-0.0028, -0.0020**	**0.05**
Weight (kg)	0.0048	0.0044, 0.0051	0.18
Height (centimeters)	0.0045	0.0038, 0.0053	0.05
BMI (kg/m^2)	**0.0143**	**0.0129, 0.0157**	**0.14**
Smoking (yes/no)	**-0.0303**	**-0.0452, -0.0154**	**0.01**
Alcohol (No. beverages/week)	**0.0010**	**0.0003, 0.0017**	**0.03**
Calcium (mg/day)	**0.00002**	**0.000007, 0.00003**	0.004
Vitamin D (IU/day)	-0.000004	-0.00001, 0.000007	0.002
Physical program (yes/no)	0.0341	0.0225, 0.0456	0.01
Energy expenditure (kcal/wk)	**0.000005**	**0.000004, 0.000006**	**0.03**
Falls in the past month (yes/no)	**0.0179**	**-0.0057, 0.0414**	**0.001**
β-blockers	**0.0018**	**-0.0196, 0.0233**	**0.0000**
Thiazides	**-0.0031**	**-0.0288, 0.0226**	**0.0000**
Loop diuretics	**-0.0602**	**-0.0996, -0.0207**	**0.004**
Statins	**-0.0107**	**-0.0372, 0.0159**	**0.0002**
Nitrates	**-0.0502**	**-0.0803, -0.0202**	**0.004**
Glucocorticoids	**-0.1167**	**-0.1742, -0.0592**	**0.006**
Bisphosphonates	-0.2647	-0.4334, -0.0960	0.004
BMD lumbar spine (g/cm^2)	0.5794	0.5536, 0.6053	0.43
Hypertension	0.0014	-0.0121, 0.0150	0.0000
Myocardial infarction	**-0.0268**	**-0.0475, -0.0062**	**0.003**
Cerebrovascular disease	**-0.0273**	**-0.0573, 0.0027**	**0.001**
Prior minimal trauma fracture	**-0.0220**	**-0.0355, -0.0084**	**0.004**

Table 13: Univariate regressions for total hip BMD against independent variables at baseline in women

Variable	β coefficient	95% CI	R^2
Age	**– 0.0050**	**-0.0053, -0.0047**	**0.1822**
Weight (kg)	0.0051	0.0049, 0.0054	0.2288
Height (centimeters)	0.0058	0.0052, 0.0063	0.0657
BMI (kg/m^2)	**0.0115**	**0.0108, 0.0122**	**0.1589**
Smoking (yes/no)	**-0.00266**	**-0.01351, 0.00818**	**0.0000**
Alcohol (No. beverages/week)	**0.0005**	**-0.0004, 0.0014**	**0.0002**
Calcium (mg/day)	-0.000002	-0.000008, 0.000004	0.0001
Vitamin D (IU/day)	**-0.00001**	**-0.00002, -0.000008**	**0.0035**
Physical program (yes/no)	0.0016	-0.0060, 0.0092	0.0000
Energy expenditure (kcal/wk)	**0.000008**	**0.000007, 0.000009**	**0.03**
Falls in the past month (yes/no)	**0.0180**	**0.0027, 0.0332**	**0.0009**
Menopause	**-0.1074**	**-0.1169, -0.0979**	**0.08**
HRT	**0.0366**	**0.0278, 0.0453**	**0.01**
SERMs	**0.0000**	**-0.0429, 0.0430**	**0.0000**
β-blockers	**-0.0205**	**-0.0339, -0.0071**	**0.0016**
Thiazides	**0.0074**	**-0.0044, 0.0192**	**0.0003**
Loop diuretics	**-0.0477**	**-0.0725, -0.0229**	**0.003**
Statins	**-0.0104**	**-0.0284, 0.0077**	**0.0002**
Nitrates	**-0.0648**	**-0.0873, -0.0423**	**0.006**
Glucocorticoids	**-0.0827**	**-0.1167, -0.0486**	**0.004**
Bisphosphonates	-0.1333	-0.1598, -0.1068	0.02
BMD lumbar spine at baseline (g/cm^2)	0.6079	0.5927, 0.6230	0.5227
Hypertension	-0.0111	-0.0195, -0.0027	0.001
Myocardial infarction	**-0.0571**	**-0.0758, -0.0384**	**0.01**
Cerebrovascular disease	**-0.0571**	**-0.0782, -0.0360**	**0.01**
Prior minimal trauma fracture	**-0.0478**	**-0.0563, -0.0394**	**0.02**

Table 14: Univariate regression results for lumbar spine BMD against independent variables at baseline in men

Variable	β coefficient	95% CI	R^2
Age	**0.0003**	**-0.0002, 0.0008**	**0.001**
Weight (kg)	0.0038	0.0033, 0.0042	0.09
Height (centimeters)	0.0038	0.0029, 0.0047	0.03
BMI (kg/m^2)	**0.0112**	**0.0096, 0.0128**	**0.06**
Smoking (yes/no)	**-0.0407**	**-0.0576, -0.0239**	**0.01**
Alcohol (No. beverages/week)	**0.0014**	**0.0006, 0.0022**	**0.004**
Calcium (mg/day)	**0.00002**	**0.000008, 0.00003**	**0.004**
Vitamin D (IU/day)	-0.00000006	-0.00001, 0.00001	0.0000
Physical program (yes/no)	0.0218	0.0087, 0.0350	0.004
Energy expenditure (kcal/wk)	**0.000003**	**0.000001, 0.000004**	**0.0062**
Falls in the past month (yes/no)	**0.0125**	**-0.0141, 0.0390**	**0.0003**
β-blockers	**0.0323**	**0.0081, 0.0564**	**0.003**
Thiazides	**0.0446**	**0.0158, 0.0733**	**0.004**
Loop diuretics	**-0.0184**	**-0.0632, 0.0264**	**0.003**
Statins	**0.0016**	**-0.0284, 0.0315**	**0.0000**
Nitrates	**0.0102**	**-0.0238, 0.0442**	**0.0001**
Glucocorticoids	**-0.0665**	**-0.1306, -0.0023**	**0.0016**
Bisphosphonates	-0.1978	-0.3893, -0.0063	0.0016
Total hip BMD (g/cm^2)	0.7422	0.7091, 0.7754	0.43
Hypertension	0.0326	0.0174, 0.0478	0.07
Myocardial infarction	**0.0186**	**-0.0047, 0.0419**	**0.07**
Cerebrovascular disease	**0.0501**	**0.0166, 0.0835**	**0.007**
Prior minimal trauma fracture	**-0.0209**	**-0.0362, -0.0056**	**0.003**

Table 15: Univariate regression results for lumbar spine BMD against independent variables at baseline in women

Variable	β coefficient	95% CI	R^2
Age	**-0.0045**	**-0.0048, -0.0041**	**0.1025**
Weight (kg)	0.0046	0.0043, 0.0049	0.13
Height (centimeters)	0.0066	0.0059, 0.0073	0.06
BMI (kg/m^2)	**0.0095**	**0.0086, 0.0104**	**0.0759**
Smoking (yes/no)	**-0.00179**	**-0.01103, 0.01461**	**0.0000**
Alcohol (No. beverages/week)	**0.0017**	**0.0006, 0.0028**	**0.002**
Calcium (mg/day)	0.000003	-0.00001, 0.000005	0.0001
Vitamin D (IU/day)	**-0.00002**	**-0.00002, -0.000006**	**0.002**
Physical program (yes/no)	-0.0125	-0.0215, -0.0036	0.001
Energy expenditure (kcal/wk)	**0.000004**	**0.000003, 0.000006**	**0.007**
Falls in the past month (yes/no)	**0.0190**	**0.0009, 0.0371**	**0.001**
Menopause	**-0.1274**	**-0.1386, -0.1162**	**0.08**
HRT	**0.0530**	**0.0426, 0.0633**	**0.02**
SERMs	**-0.0100**	**-0.0610, 0.0411**	**0.0000**
β-blockers	**-0.0126**	**-0.0284, 0.0033**	**0.0004**
Thiazides	**0.0367**	**0.0228, 0.0506**	**0.005**
Loop diuretics	**-0.0067**	**-0.0361, 0.0227**	**0.0000**
Statins	**-0.0345**	**-0.0559, -0.0130**	**0.002**
Nitrates	**-0.0283**	**-0.0550, -0.0015**	**0.0007**
Glucocorticoids	**-0.0628**	**-0.1028, -0.0228**	**0.002**
Bisphosphonates	-0.1336	-0.1650, -0.1021	0.01
Total hip BMD (g/cm^2)	0.8599	0.8385, 0.8813	0.52
Hypertension	0.0040	-0.0059, 0.0139	0.0001
Myocardial infarction	**-0.0444**	**-0.0664, -0.0224**	**0.003**
Cerebrovascular disease	**-0.0496**	**-0.0744, -0.0249**	**0.003**
Prior minimal trauma fracture	**-0.0477**	**-0.0576, -0.0377**	**0.02**

4.2.3 Multivariate linear regression of the effect of β-blockers on BMD

For both men and women, β-blockers had to be forced into all the final regression models in order to report their effects while adjusting for potential confounders, since the BIC criterion always eliminated this variable from all models. For the men, the variables selected, once β-blockers were forced in, for the total hip and lumbar spine BMD are shown in tables 16 and 17, respectively. For the women, the variables selected for our final models, with β-blockers forced in, for the total hip BMD and lumbar spine BMD are shown in tables 18 and 19, respectively.

For men, the results of the MLR are inconclusive with respect to an effect of β-blockers on BMD and preclude any strong conclusions. Converting the coefficients to percent differences in BMD is somewhat easier to interpret. In men, β-blockers users had a +1.1% (95% CI, -0.9% to 3.0%) difference in BMD at the total hip and a +1.2% (95% CI, -0.5% to 4.0%) difference in BMD at the lumbar spine compared to non-users.

Table 16: Multiple linear regression for the total hip using the variables of the final model in men.

Variable	Point Estimate	95% CI
Intercept	0.771	0.727, 0.817
Age (years)	-2.47e-3	-2.86e-3, -2.08e-3
BMI (kg/m^2)	0.014	0.012, 0.015
Smoking	-0.032	-0.046, -0.018
Calcium (mg/day)	1.31e-5	4.08e-6, 2.21e-5
Energy expenditure from moderate, strenuous activity (kcal/week)	1.87e-6	8.25e-7, 2.91e-6
β-blockers	0.011	-0.009, 0.030
Glucocorticoids	-0.078	-0.130, -0.026
Prior minimal trauma fracture	-0.025	-0.037, -0.012

Table 17: Multiple linear regression for the lumbar spine using the variable of the final model in men.

Variable	Point Estimate	95% CI
Intercept	0.739	0.692, 0.785
BMI (kg/m^2)	0.011	9.11e-3, 1.24e-2
Smoking	-0.032	-0.049, -0.016
Alcohol intake (no. beverages/week)	1.56e-3	7.57e-4, 2.36e-3
Calcium (mg/day)	1.88e-5	7.87e-6, 2.98e-5
β-blockers	0.018	-0.006, 0.042
Cerebrovascular disease	0.049	0.016, 0.819
Prior minimal trauma fracture	-0.0248	-0.040, -9.81e-3

Displayed in figures 1 and 2 are these percent differences in BMD for the total hip and lumbar spine, respectively, in users versus non-users of β-blockers in men. In each figure, the unadjusted percent difference is shown, followed by the percent difference which was adjusted for age only and finally the percent difference which was multiply-adjusted with the variables chosen in our final model. At the total hip, when unadjusted, there appears to be little difference between the 2 groups. When adjusted for age only, the β-blocker users have a potentially clinically important increase in the BMD compared to the non-users. This is because age is a confounder of the relationship between β-blockers and BMD in view of the fact that β-blocker users are older and older people have generally lower bone densities. However, once adjusted for the final variables in our model, we can see that the point estimates for the differences in BMD are positive but small at the total hip and at the lumbar spine. However, using a 2% limit of clinical importance, these differences are inconclusive. In particular, the wide confidence intervals at both the hip and the spine sites both contain the null value of zero, as well as potentially important clinical effects on BMD.

Table 18: Multiple linear regression for the total hip using the variable of the final model in women.

Variable	Point Estimate	95% CI
Intercept	0.800	0.776, 0.824
Age (years)	-4.23e-3	-4.57e-3, -3.88e-3
BMI (kg/m^2)	0.012	0.011, 0.013
Alcohol intake (No. beverages/week)	1.46e-3	0.001, 0.002
Energy expenditure from moderate, strenuous activity (kcal/week)	3.17e-6	2.2e-6, 4. 1e-6
β-blockers	4.02e-4	-0.010, 0.011
Thiazides	0.018	8.89e-3, 2.8e-2
Hrt	0.040	0.033, 0.048
Menopausal status	-0.031	-0.042, -0.020
Prior minimal trauma fracture	-0.037	-0.044, -0.031

Table 19: Multiple linear regression for the lumbar spine using the variable of the final model in women.

Variable	Point Estimate	95% CI
Intercept	0.875	0.843, 0.906
Age (years)	-2.55e-3	-0.003, -0.002
BMI (kg/m^2)	0.010	0.010, 0.011
Alcohol intake (No. beverages/week)	2.44e-3	1.5e-3, 3.5e-3
β-blockers	0.00156	-0.01261, 0.01573
Thiazides	0.048	0.035, 0.060
Hrt	0.066	0.056, 0.075
Menopausal status	-0.094	-0.109, -0.079
Prior minimal trauma fracture	-0.038	-0.047, -0.029

For women, β-blockers appeared to have little impact on the BMD of either the spine or the hip. In terms of percent differences, β-blockers users had a +0.05% (95% CI, -1.2% to 1.3%) difference in BMD at the total hip and a +0.2% (95% CI, -1.3% to 1.7%) difference in BMD at the lumbar spine compared to non-users.

Displayed in figures 3 and 4 are these percent differences in BMD for the total hip and lumbar spine in users versus non-users of β-blockers in women. Just as shown for the men, the unadjusted percent difference is shown, followed by the percent difference which was adjusted for age only and finally the percent difference which was multiply-adjusted with the variables chosen in our final model. Unadjusted, β-blocker users have a lower BMD at both the hip and the spine. However when adjusted for age only, the β-blocker users have a potentially clinically significant increase in the BMD compared to the non-users. This complete reversal when adjusting for age alone is explained by the fact that the β-blocker users are older. However, once adjusted for all the final variables in our model, the point estimates for the differences in BMD are near zero for the hip and for the spine. There is some controversy as to whether 1% or 2% constitutes a clinically important percent difference in BMD. Using a 2% limit of clinical importance, the confidence intervals fall within these limits. Therefore, there is no difference in the BMD at these sites between the β-blockers users and non-users in women.

Figure 1

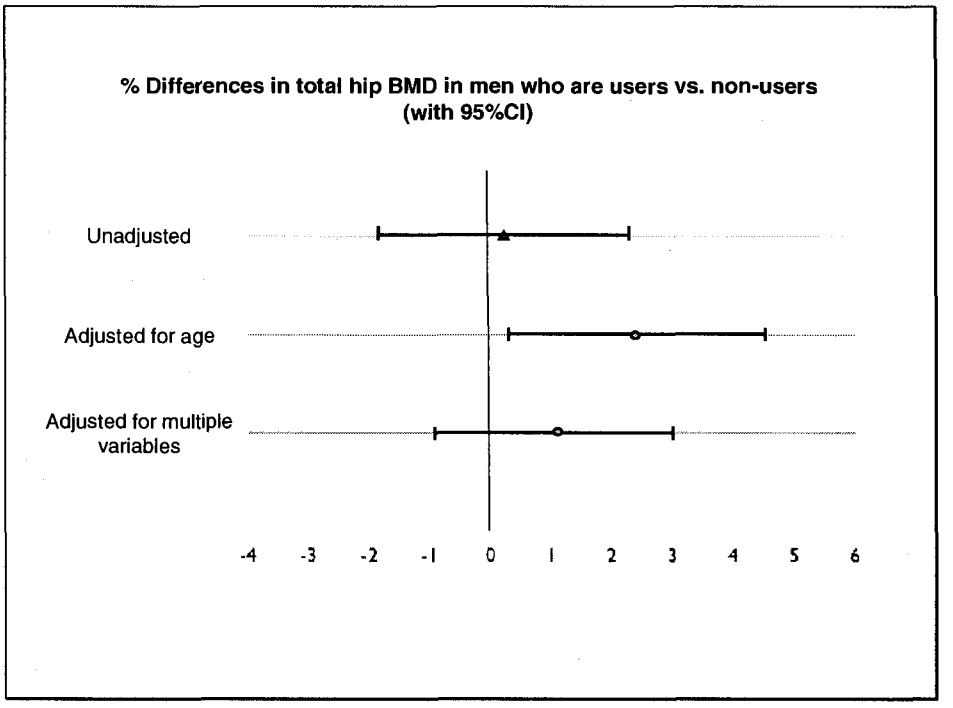


Figure 2

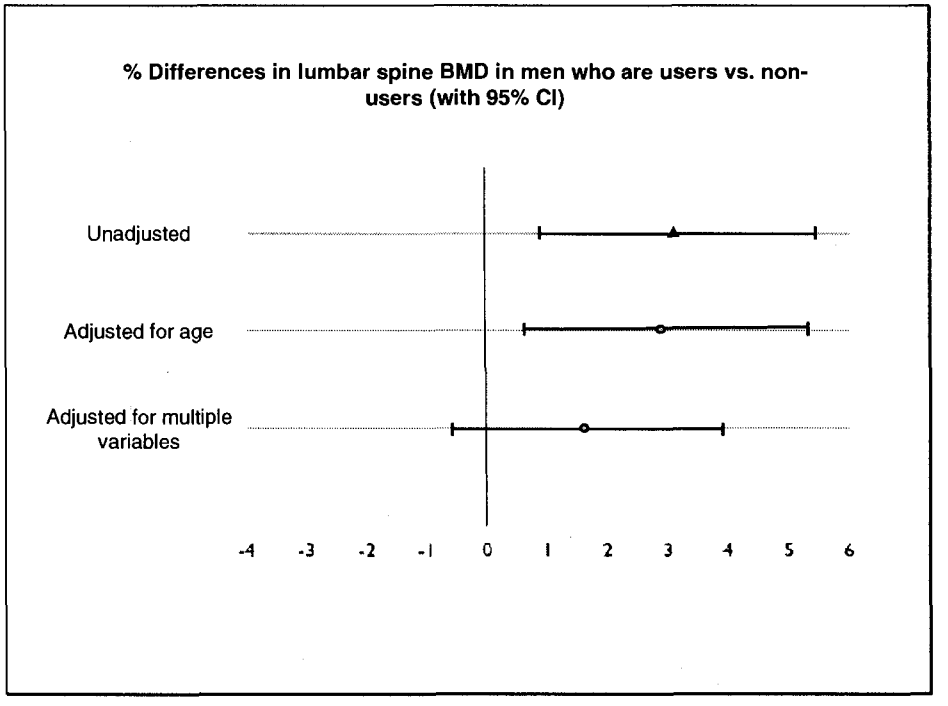


Figure 3

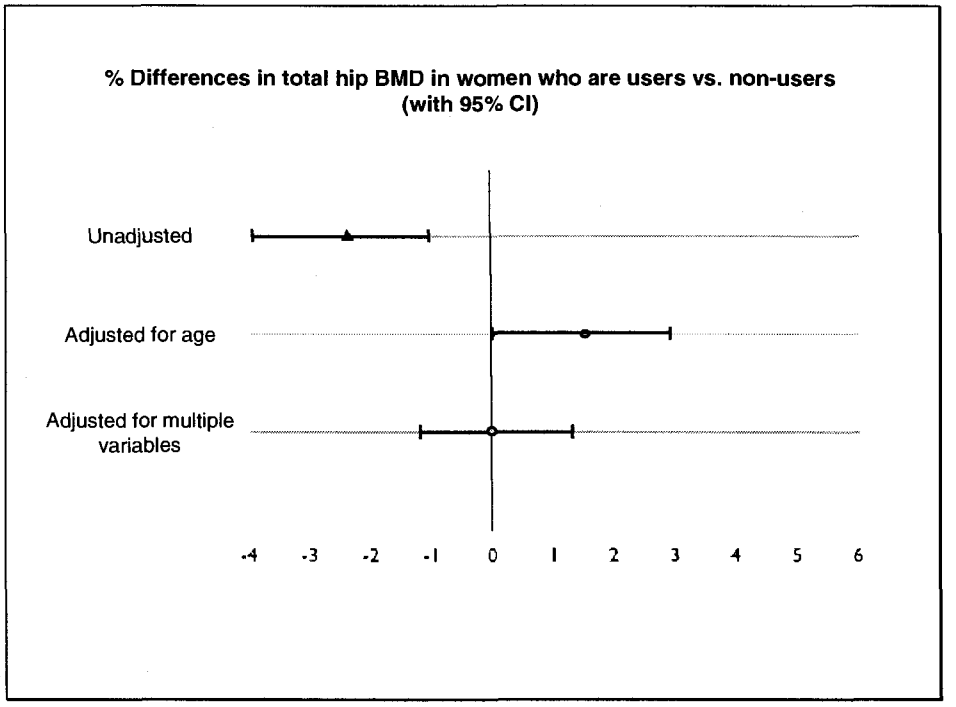
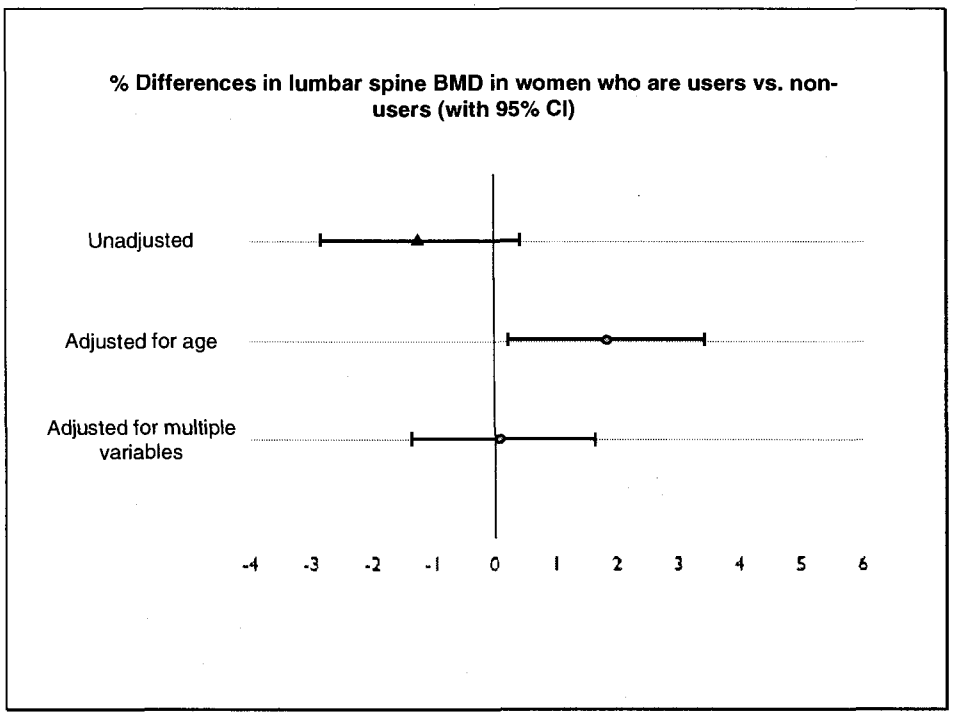


Figure 4



4.3 The effect of baseline β-blocker use on fractures

For this section and the following section 4.4, we will present the results of our analysis of the effect of β-blockers on fractures in all CaMos subjects. As this analysis does not depend on BMD measurements, all the subjects enrolled at baseline in CaMos are included. However, as the cohort has then changed somewhat compared to that in section 4.2, we have redone the descriptive statistics for the entire cohort separately, and these results will be presented in section 4.3.1.

To examine the effect of β-blocker use on fracture risk, we conducted 2 analyses as shown in figures 5 and 6. In the first analysis, all CaMos subjects were classified as users or non-users at baseline. The fracture incidences for these groups and the odds ratios of fracture for users of β-blockers compared to non-users were then calculated. For this first analysis, the composite of "all minimal trauma fractures" was employed for men and women.

In the second analysis (see figure 6), CaMos subjects were classified into non-users of β-blockers, those who used β-blockers only at baseline, those who used β-blockers only at year 5 and those who were chronic users (using β-blockers at both baseline and year 5). Fracture incidence and the odds ratios for fracture were calculated comparing users to the non-users. The non-users served as the reference group in all cases. In contrast to our first analysis, this second analysis allowed us to estimate whether duration of β-blockers use had an effect on the risk for fracture.

By year 5, there were 1064 (13.9%) subjects who were using β-blockers. Of these, 366 (6.7%) females and 120 (5.4%) males were chronic users. In this more detailed analysis,

we also looked at more detailed fracture types. These included "any minimal trauma fractures", "main" fragility fractures and hip fractures. These will be described in more detail later.

Figure 5: Fracture Analysis 1

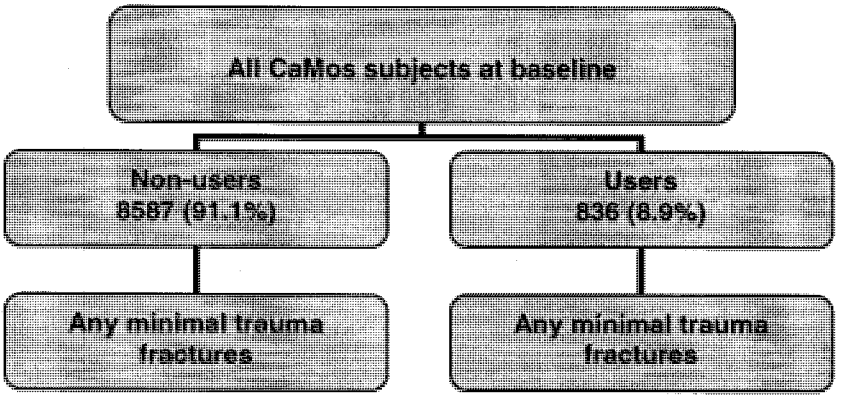
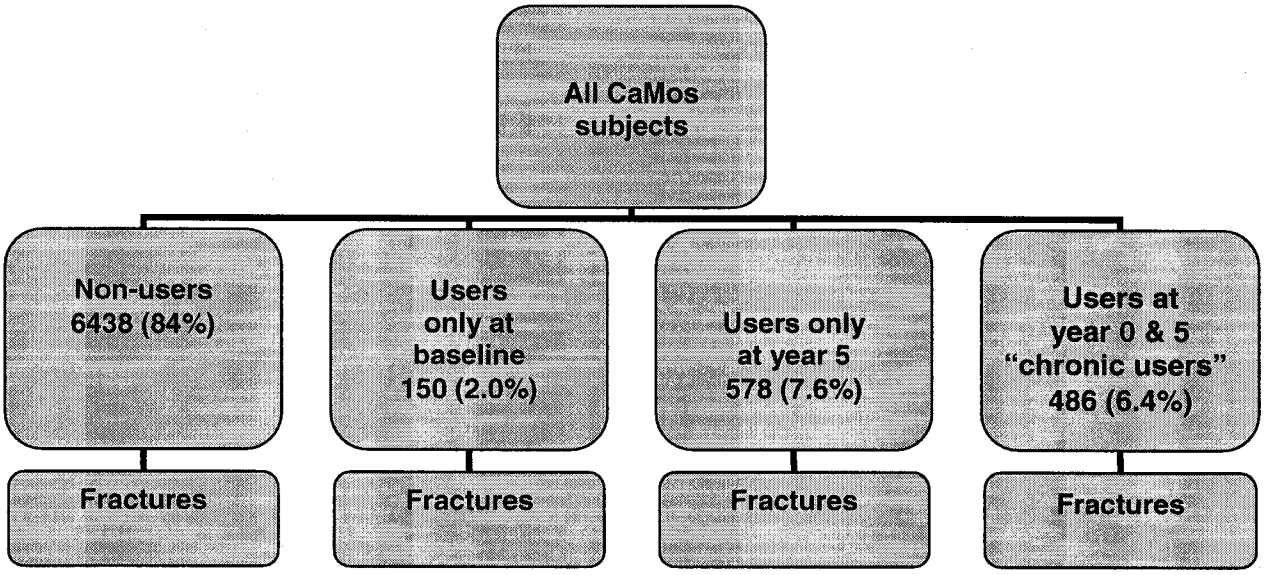


Figure 6: Fracture Analysis 2



4.3.1 Descriptive Statistics of the effect of β-blocker use at year 0 on fractures

The descriptive characteristics across all CaMos subjects enrolled at baseline, broken down by β-blocker use or not are presented in tables 20 and 21, respectively, for men and women. Similar to the results of the BMD analysis discussed in section 4.2, men and women who used β-blockers were older, had a higher BMI, smoked less, and used more thiazide diuretics, loop diuretics, statins and nitrates. They also had more hypertension and a greater history of myocardial infarction or stroke.

The correlation matrices for all the continuous variables considered in this section are presented in tables 22 and 23. In men there were more important positive correlations between weight, height and BMI with BMD perhaps more so for the total hip. There were strong correlations between weight and BMI and between weight and height. There was a small negative correlation between age and height, energy expended on moderate or strenuous physical activity and BMD at the hip but not at the spine. As expected, total hip BMD and lumbar spine BMD were strongly correlated. For women, the findings were similar. There were small to moderate positive correlations between BMD with weight, BWD with height, BMD with BMI. Weight was also positively correlated with height and strongly correlated with BMI. There were small to moderate negative correlations between age with height, energy expended on moderate or strenuous physical activity and BMD at both the hip and spine. As the calculation of the BMI incorporates both weight and height, the strong positive correlation between weight and BMI is therefore expected and presents potential collinearity problems in the analysis. For these reasons, BMI was the only one of these 3 variables used in the regressions.

Table 20: Descriptive statistics of all men, men who did, and men who did not use β-blockers at baseline. Continuous variables reported as mean (SD), median (IQR), categorical variables reported as number (%).

	All Men (n=2884)	Men who are non-users (n =2648)	Men who are β-blockers users (n = 236)
Age (years)	59.9 (14.48) 62.0 (50-71)	59.1 (14.6) 61.0 (49-70)	68.1 (10.1) 69.0 (61.5-75.0)
Weight (kg)	81.5 (14.09) 80.0 (72.0-89.9)	81.3 (14.0) 80.0 (72.0-89.5)	83.6 (14.9) 81.0 (73.5-92.5)
Height (centimeters)	173.5 (7.2) 173.0 (168.3-178.0)	173.6 (7.3) 173.4 (168.8-178.0)	172.6 (6.9) 172.6 (168.0-177.8)
BMI	27.0 (4.09) 26.6 (24.2-29.3)	26.9 (4.1) 26.5 (24.3-29.1)	28.0 (4.1) 27.7 (25.0-30.1)
Ever smoked cigarettes	1935 (67.1)	1773 (67.0)	162 (68.6)
Current Smoker	532 (18.5)	506 (19.1)	26 (11.0)
Current Alcohol Use (No. beverages/week)	5.3 (8.3) 2.0 (0-7.0)	5.22 (8.3) 2.0 (0-7.0)	5.68 (9.0) 2.0 (0-7.0)
Calcium/day (mg)	903 (582) 773 (507-1153)	908 (582) 778 (509-1162)	846 (575) 737 (476-1070)
Vitamin D/day (IU)	88 (476) 0.0 (0.0-0.0)	90 (494) 0.0 (0.0-0.0)	69 (161) 0.0 (0.0-0.0)
Currently physically active	1603 (55.6)	1461 (55.2)	142 (60.2)
Energ Exp from stren, vig, mod activity (#kcal/wk)	5502 (5146) 4141 (1901-7491)	5581 (5106) 4248 (1935-7626)	4615 (5512) 3075 (1608-5917)
Falls in the past month	191 (6.6)	177 (6.7)	14 (5.9)
Thiazide diuretic use	169 (5.9)	126 (4.8)	43 (18.2)
Loop diuretic use	80 (2.8)	66 (2.5)	14 (5.9)
Statins	145 (5.0)	104 (3.9)	41 (17.4)
Nitrate Use	128 (4.4)	75 (2.8)	53 (22.5)
Glucocorticoid use	36 (1.3)	33 (1.3)	3 (1.3)

Bisphosphonate use	5 (0.2)	5 (0.2)	0 (0)
Total hip BMD (g/cm^2)	1.0099 (0.1492) 1.0066 (0.9125-1.1108)	1.0098 (0.1488) 1.0058 (0.9126-1.1111)	1.0116 (0.1536) 1.0191 (0.9120-1.105)
L1-4 BMD (g/cm^2)	1.0472 (0.1691) 1.0355 (0.9362-1.1466)	1.0447 (0.1689) 1.0333 (0.9339-1.1426)	1.0769 (0.1701) 1.0505 (0.9582-1.1863)
Hypertension	712 (24.7)	549 (20.8)	163 (69.1)
Heart attack	267 (9.3)	185 (7.0)	82 (35.5)
Stroke/TIA	121 (4.2)	103 (3.9)	18 (7.6)
Prior Min. Trauma Fracture	697 (24.2)	644 (24.4)	53 (22.6)

Table 21: Descriptive statistics of all women, women who did, and women who did not use β-blockers at baseline. Continuous variables reported as mean (SD), median (IQR), categorical variables reported as number (%).

	All women (n=6539)	Women who are non-users (n =5939)	Women who are β-blockers users (n =600)
Age (years)	63.0 (12.8) 65.0 (55-72)	62.5 (12.9) 64.0 (54-72)	68.9 (9.8) 70 (63.5-75.0)
Weight (kg)	68.6 (13.9) 67.0 (59.0-76.4)	68.5 (14.0) 66.7 (59.0-76.2)	69.7 (13.3) 68.0 (60.0-78.0)
Height (centimeters)	159.6 (6.5) 160.0 (155.0-164.0)	159.7 (6.5) 160.0 (155.0-164.0)	158.4 (6.2) 158.0 (154.9-162.6)
BMI	26.9 (5.2) 26.3 (23.2-29.7)	26.8 (5.1) 26.2 (23.2-29.6)	27.8 (5.2) 27.0 (23.8-30.8)
Ever smoked cigarettes	3072 (47.0)	2806 (47.3)	266 (44.3)
Current Smoker	935 (14.3)	866 (14.6)	69 (11.5)
Current Alcohol Use (No. beverages/week)	2.0 (4.0) 0.2 (0-2.0)	2.0 (4.0) 0.2 (0-2.0)	1.7 (3.8) 0.0 (0-1.0)
Calcium/day (mg)	1037 (613) 930 (589-1360)	1041 (615) 931 (593-1363)	1006 (587) 921 (548-1328)
Vitamin D/day (IU)	173 (631) 0 (0-229)	176 (633) 0 (0-250)	147 (618) 0 (0-100)
Currently physically active	3592 (54.9)	3283 (55.3)	309 (51.5)
Energ Exp from stren, vig, mod activity (#kcal/wk)	4215 (3242) 3398 (1716-6206)	4265 (3267) 3486 (1769-6297)	3719 (2939) 2703 (1437-5359)
Falls in the past month	430 (6.6)	394 (6.6)	36 (6.0)
Menopause (Perstop)	5471 (83.7)	4897 (82.5)	574 (95.7)
HRT use	1484 (22.7)	1362 (22.9)	122 (20.3)
SERM use	56 (0.9)	49 (0.83)	7 (1.2)
Thiazide diuretic use	795 (12.2)	632 (10.6)	163 (27.2)

Loop diuretic use	211 (3.2)	164 (2.8)	47 (7.8)
Statins	298 (4.6)	225 (3.8)	73 (12.2)
Nitrate Use	236 (3.6)	160 (2.7)	76 (12.7)
Glucocorticoid use	100 (1.5)	91 (1.5)	9 (1.5)
Bisphosphonate use	155 (2.4)	138 (2.3)	17 (2.8)
Total hip BMD (g/cm^2)	0.8545 (0.1447) 0.8518 (0.7567-0.9479)	0.8563 (0.1445) 0.8545 (0.7594-0.9497)	0.8358 (0.1447) 0.8376 (0.7332-0.9294)
L1-4 BMD (g/cm^2)	0.9369 (0.1721) 0.9302 (0.8146-1.0483)	0.9380 (0.1723) 0.9308 (0.8167-1.0507)	0.9254 (0.1696) 0.9250 (0.7987-1.0236)
Hypertension	1956 (30.0)	1492 (25.2)	464 (77.6)
Heart attack	319 (4.9)	232 (3.9)	87 (14.6)
Stroke/TIA	239 (3.7)	202 (3.4)	37 (6.2)
Prior minimal trauma fracture	1792 (27.5)	1608 (27.1)	184 (30.7)

Table 22: Correlation matrix for all men of CaMos

	Weight (kg)	Height (cm)	BMI (kg/m^2)	Alcohol*	Calcium (mg per day)	Vitamin D (IU per day)	Energy expenditure*	Total hip BMD*	Lumbar spine BMD*
Age (years)	-0.14	-0.24	-0.02	-0.03	-0.04	0.01	-0.26	-0.22	0.03
Weight (kg)		0.49	0.87	0.05	0.08	0.01	0.21	0.43	0.30
Height (cm)			0.01	0.04	0.11	0.04	0.19	0.22	0.16
BMI (kg/m^2)				0.03	0.03	-0.01	0.12	0.37	0.26
Alcohol*					0.00	0.02	0.01	0.05	0.07
Calcium (mg per day)						0.06	0.09	0.07	0.07
Vitamin D (IU per day)							-0.02	-0.01	0.00
Ergergy expenditure*								0.16	0.08
Total hip BMD*									0.66

*Alcohol reported as number of beverages per week; energy expenditure as kcal/week; BMD as g/cm^2.

Table 23: Correlation matrix for all women of CaMos

	Weight (kg)	Height (cm)	BMI (kg/m^2)	Alcohol*	Calcium (mg per day)	Vitamin D (IU per day)	Energy expenditure*	Total hip BMD*	Lumbar spine BMD*
Age (years)	-0.12	-0.29	-0.01	-0.06	0.02	0.06	-0.18	-0.43	-0.32
Weight (kg)		0.33	0.91	-0.05	-0.01	-0.01	0.22	0.48	0.36
Height (cm)			-0.07	0.08	0.06	0.01	0.20	0.26	0.25
BMI (kg/m^2)				-0.09	-0.03	-0.02	0.15	0.40	0.28
Alcohol*					-0.01	-0.01	-0.00	0.02	0.04
Calcium (mg per day)						0.18	0.08	-0.01	-0.01
Vitamin D (IU per day)							-0.02	-0.06	-0.05
Ergergy expenditure*								0.18	0.08
Total hip BMD*									0.72

*Alcohol reported as number of beverages per week; energy expenditure as kcal/week; BMD as g/cm^2.

During the 5 years of follow-up after enrollment, there were 117 men and 600 women who reported a fragility fracture. Therefore, a minimal trauma fracture had occurred in 9.2% of all the female subjects and 4.1% of all the male subjects of CaMos.

The numbers of fractures and the percentages of men and women in each group of users versus non-users of β-blockers who experienced this type of fracture are shown in table 24. Obviously these are unadjusted for potential confounders but provide an idea of the numbers of fractures upon which the analytical components are based. In order to obtain adequate numbers for the first analysis, we used the composite of "any minimal trauma fracture".

Table 24: Counts and percentages of subjects experiencing a first incident fragility fracture of each type in the 5 years of follow-up in men and women using β-blockers at baseline compared to non-users

Types of fractures	Men		Women	
	Non-users	**Users**	**Non-users**	**Users**
Any minimal trauma fracture	104 (3.9)	13 (5.5)	533 (9.0)	67 (11.2)
Main fragility fracture*	57 (2.2)	6 (2.5)	307 (5.2)	46 (7.7)
Hip fracture	8 (0.3)	2 (0.85)	47 (0.8)	11 (1.8)

*Fractures of vertebrae, hip, distal radius, pelvis and ribs combined

4.3.2 Univariate logistic regression of the effect of β-blocker use at baseline on fractures

The odds ratios and corresponding 95% confidence intervals for the univariate logistic regression models looking at the dependant variable, any minimal trauma fractures, with each of the independent variables, are shown in tables 25 and 26, for men and women,

respectively. In men, the more interesting associations were with the variables age, alcohol intake, smoking, loop diuretics and myocardial infarction, all of which appeared associated with an increased risk of fracture. In the univariate model, it was inconclusive at to whether or not β-blockers were associated with the outcome of any minimal trauma fractures in men.

In women, age, vitamin D intake, falls in the past month, menopause, glucocorticoids, hypertension, history of cerebrovascular disease and prior minimal trauma fracture were associated with an increased risk of any minimal trauma fractures. On the other hand, HRT seemed protective. Similar to the analysis for men, it was inconclusive whether or not β-blockers were associated with the outcome of any minimal trauma fractures in women.

As was the case with our multiple linear regression models, variables were selected for further investigation in multivariate logistic regression (MLR) models based on the univariate results, that is, if they appeared to have a potential association with the dependant variable or were potential confounders of the relationship between β-blockers and fractures. Additional variables were selected for multivariate modeling based on *a priori* knowledge of being a potential confounder. The variables representing myocardial infarction and cerebrovascular disease were chosen for modeling as they represent markers of co-morbidities or frailty which could be confounding the association between exposure and outcome. The variable representing falls was also chosen for modeling as we postulated that patients on anti-hypertensive agents could have more falls, falls can cause fractures, and we were interested to see if β-blockers would increase fractures through another mechanism, i.e. independent of falls. BMD was not selected for modeling, despite its apparent strong and known association with fractures, as we

postulated BMD may be in the causal pathway between β-blockers and fractures so it should not be included as a potential confounder. As bisphosphonates are usually prescribed for osteoporosis with or without fractures, the use of these drugs could be considered as a surrogate for the diagnosis of low BMD, which we postulate is in the causal pathway. If this were the case we would not want to adjust for this. On the other hand, individuals on bisphosphonates may fracture less at a given BMD than those not on these drugs. Therefore, the variable bisphosphonate was added to the final model to check whether it was a confounder in this analysis if it had a prevalent use of 1% or greater.

In both men and women, variables selected for modeling included age, BMI, calcium and vitamin D intake, alcohol intake, smoking, falls, energy expenditure, thiazides, loop diuretics, nitrates, statins, glucocorticoids, history of hypertension, myocardial infarction and cerebrovascular disease, prior minimal trauma fractures and a quadratic term for BMI. In women, the variables for menopause, hormone replacement therapy and SERMs were also included.

Table 25: Fracture Analysis 1, Univariate regression of "Any minimal trauma fractures" versus independent variable in men

Variable	Odds Ratio	95% CI
Age (years)	1.03	1.01-1.04
BMI (kg/m^2)	1.01	0.97-1.06
Calcium intake per SD = 582 mg/day	0.96	0.79-1.16
Vitamin D intake per SD = 476 IU/day	1.13	1.00-1.29
Energy Expenditure per SD = 5146 kcal/week	1.12	0.95-1.32
Alcohol intake (No. beverages/week)	1.02	1.01-1.04
Alcohol intake per SD (= 8.3 beverages/week)	1.20	1.04-1.38
Smoking (yes/no)	1.63	1.07-2.48
Falls past month (yes/no)	1.65	0.86-2.06
β-blockers	1.43	0.79-2.58
Thiazides	1.72	0.90-3.25
Loop diuretics	2.74	1.29-5.85
Statins	1.21	0.55-2.65
Nitrates	1.17	0.51-2.72
Glucocorticoids	0.67	0.09-4.96
Hypertension	1.49	1.00-2.21
Myocardial infarction	2.23	1.36-3.63
Cerebrovascular diseases	1.24	0.54-2.89
Prior minimal trauma fracture	1.30	0.86-1.95

Table 26: Fracture Analysis 1, Univariate regression of "Any minimal trauma fractures" versus independent variable in women

Variable	Odds Ratio	95% CI
Age (years)	1.04	1.03-1.05
BMI (kg/m^2)	0.99	0.97-1.01
Calcium intake per SD = 613 mg/day	1.07	0.98-1.16
Vitamin D intake per SD = 631 IU/day	1.08	1.02-1.15
Energy expenditure per SD = 3242 kcal/week	0.89	0.81-0.98
Alcohol intake (No. beverages/week)	1.00	0.98-1.02
Smoking (yes/no)	0.96	0.75-1.22
Falls past month (yes/no)	1.43	1.058-1.93
β-blockers	1.28	0.97-1.67
Thiazides	1.02	0.79-1.32
Loop diuretics	1.47	0.98-2.23
Statins	1.11	0.76-1.64
Nitrates	1.18	0.77-1.80
Glucocorticoids	2.86	1.77-4.63
Bisphosphonates	2.67	1.79-3.97
Menopause	2.84	2.05-3.93
HRT	0.74	0.59-0.92
SERMs	1.66	0.78-3.52
Hypertension	1.29	1.08-1.09
Myocardial Infarction	1.36	0.96-1.94
Cerebrovascular disease	2.40	1.72-3.36
Prior minimal trauma fracture	2.31	1.95-2.74

4.3.3 Multivariate logistic regression of the effect of β-blockers used at year 0 on fractures

Table 27 shows the multivariate adjusted OR for fragility fracture of all subjects who were β-blocker users at baseline versus the non-users, our first analysis. For comparison, the univariate ORs for β-blockers are repeated in this table. Again, the fracture outcome shown is "any minimal trauma fractures". For both men and women, the estimates of the odds ratios for fractures are relatively close to 1.0, but the confidence intervals are wide. Therefore, our results are inconclusive for an effect of β-blockers on "any minimal trauma fracture" risk in this analysis.

Table 27: β-blockers at year 0 and risk of any fragility fracture

	OR (95%) for any minimal trauma fracture:	
	Univariate	**Multivariate Adjusted**
MEN	1.4 (0.8-2.6)	1.2 (0.7-2.3)
WOMEN	1.3 (1.0-1.7)	1.0 (0.8-1.4)

In men, our final model included age, β-blockers and smoking. In women, our final model included age, β-blockers, prior minimal trauma fractures and cerebrovascular diseases.

It is not clear whether an analysis focusing on the effects of β-blockers should adjust for bisphosphonates, as they may be on the causal pathway. Therefore, we ran our analyses both with and without this variable. While we did find that bisphosphonates have an effect on any minimal trauma fractures in this analysis, we did not uncover any confounding with the effect of β-blockers as adding it did not substantially change the point estimate or CI of the OR for β-blockers. In particular, in men, we found that

bisphosphonates, added to the final model, had an OR of 5.2 (0.6-49.6) for any minimal trauma fracture. That there were few men on bisphosphonates, and that there were few events in men, likely explains the wide confidence intervals. Similarly in women, bisphosphonates, when added to the final model, had an OR of 1.8 (1.2-2.7) for any minimal trauma fractures. The OR's for fractures with bisphosphonates suggest an elevated risk yet this drug reduces fractures in randomized controlled trials. As it is usually taken in individuals with low BMD and high fracture risk, this variable is in effect a marker for low BMD. To find out the true risk of fracture while on these drugs compared with non-users, we would need to adjust for BMD. In our case, since BMD changes may be in our causal pathway, we would not want to adjust for this.

4.4 The effect of β-blocker duration of use on fractures

In this section, we present descriptive statistics, univariate logistic regressions and multivariate logistic regression of our second analysis, as described at the beginning of section 4.3 and represented in figure 6 earlier. Subjects were classified into 4 groups: those using β-blockers only at the baseline visit, those who were using β-blockers at only the year 5 visit, those who were using β-blockers at both the baseline and year 5 visit (chronic users) and the non-users, who were our reference group in all analyses. The fracture outcomes, previously described in section 4.3.1 were "any minimal trauma fractures", main fragility fractures and hip fractures. The absolute numbers of hip fractures occurring in men was particularly low. For this reason, regression analyses were not performed for hip fractures in men as there are insufficient numbers of events for accurate estimation. For this second fracture analysis, all descriptive statistics and linear regressions were limited to those 7,652 subjects who participated at both the baseline and the five year follow-up visit.

4.4.1 Descriptive statistics of the effect of β-blockers duration of use on fractures

The descriptive statistics, including all the continuous and categorical variables, and correlation matrices were done separately for this analysis but the full tables are not included here due to their size. The subjects, categorized in 4 groups according to duration of β-blocker use, were different in many ways. Briefly, men and women who were in any of the 3 categories of users were older, somewhat heavier, used more thiazides, nitrates, statins, loop diuretics and had much more cardiovascular disease (hypertension, myocardial infarction, cerebrovascular disease). More of the women users had reached menopause compared to the non-users. Differences were also apparent within the three classes of users of β-blockers. Chronic users and baseline users had more cardiovascular disease and used more thiazides, nitrates, statins, and loop diuretics.

The correlation matrices revealed similar associations to what was seen in the previous two sections. In men, there were strong positive correlations between weight and height, weight and BMI, and between total hip BMD and lumbar spine BMD. There were small to medium positive correlations between weight and energy expenditure, weight and BMD at both sites, height and energy expenditure, height and BMD at both sites, BMI and BMD at both sites, energy expenditure and total hip BMD. There were small negative correlations between age and energy expenditure, age and total hip BMD, age and height. In women, there were strong positive correlations between weight and BMI, lumbar spine BMD and total hip BMD. There were small to medium positive correlations between weight and height, weight and energy expenditure, weight and BMD at both sites, height and energy expenditure, height and BMD at both sites, BMI and energy expenditure, lumbar spine BMD and total hip BMD, calcium intake and vitamin D intake,

energy expenditure and total hip BMD. There were small negative correlations between age and height, age and energy expenditure, age and BMD at reciprocal sites, menopause and BMI, menopause and lumbar spine BMD.

4.4.2 Univariate logistic regression of the effect of duration of β-blockers use on fractures

Univariate logistic regression analyses were conducted for each independent variable against the dependent variables, "any minimal trauma fractures", "main" fragility fractures and hip fractures. Again, only the first two fracture types were analyzed in men. The results are shown in tables 28 and 29.

For the men, there were associations between any minimal trauma fractures and the variables: β-blockers chronic use [OR 2.4 (1.2, 4.8)], age, alcohol, smoking, loop diuretics, bisphosphonates and myocardial infarctions. Similarly, there were interesting associations between main fragility fractures and the variables age, smoking, falls in the past month, loop diuretics, bisphosphonates and myocardial infarctions.

For women, the univariate models revealed associations between any minimal trauma fractures and age, vitamin D, loop diuretics, glucocorticoids, bisphosphonates, menopause, HRT, hypertension, cerebrovascular disease and prior minimal trauma fracture. There were interesting associations between main fragility fractures and chronic use of β-blockers [OR 1.7 (1.1-2.5)], age, energy expenditure, menopause, loop diuretics, glucocorticoids, bisphosphonates, hypertension, myocardial infarction, cerebrovascular disease and prior minimal trauma fracture. There were associations between hip fractures and all durations of use of β-blockers [chronic use OR 3.0 (1.1-8.0)], use at year 5 only [OR 3.3 (1.3-8.1)] and use at baseline only [OR 8.7 (3.0-25.9)],

age, vitamin D, menopause, loop diuretics, bisphosphonates, hypertension, myocardial infarction and cerebrovascular disease.

For men, the variables selected for the MLR were: β-blockers in the 3 categories of duration, age, BMI, calcium, vitamin D, energy expenditure, smoking, alcohol, falls in the past months, thiazides, loopdiuretics, statins, nitrates, hypertension, myocardial infarction, cerebrovascular disease and prior minimal trauma fractures. After checking for linearity, some continuous variables were transformed if deemed appropriate. This included energy expenditure which was modeled as a categorical variable, divided into quartiles. Alcohol was actually treated in 2 ways: as a continuous variable, but also as a categorical variable, divided into quartiles.

In men, there were too few subjects on glucocorticoids to include this variable in our multivariate logistic regression models. Of subjects who used β-blockers at year 5 only, only 1.16% were taking glucocorticoids. The other groups of users and non-users had a prevalence of glucocorticoid use of less than 1%. The highest prevalence of bisphosphonate use was 0.2% in one group so this variable was not included in this analysis. In any event, the same argument for not including it in the MLR as in section 4.3 would apply here.

In women, the variables selected for the MLR were: β-blockers in the 3 categories of duration, age, BMI, calcium, vitamin D, energy expenditure, alcohol, smoking, falls in the past months, thiazides, loop diuretics, statins, nitrates glucocorticoids, menopause, hormone replacement therapy, SERM's, hypertension, myocardial infarction, cerebrovascular disease and prior minimal trauma fractures. After checking for linearity, some continuous variables were transformed as deemed appropriate. Age was run in

Table 28: Fracture Analysis 2, Univariate logistic regression of "Any minimal trauma fractures" and "main" fragility fractures against independent variables in men by duration of β-blockers use

	OR (95% CI)	
	Any minimal trauma fracture	**Main fragility fracture**
β-blocker chronic use	2.42 (1.22-4.84)	2.17 (0.84-5.62)
β-blocker year 5 only	1.46 (0.72-2.99)	1.18 (0.47-3.35)
β-blocker baseline only	0.58 (0.08-4.27)	NA*
Age (years)	1.03 (1.01-1.05)	1.03 (1.00-1.05)
BMI (kg/m^2)	1.01 (0.96-1.06)	1.00 (0.93-1.08)
Calcium pre SD (=585 mg/day)	0.96 (0.77-1.20)	1.08 (0.82-1.42)
Vitamin D per SD (=513 IU/day)	1.16 (0.98-1.37)	1.19 (0.97-1.45)
Energy expenditure per SD (=5306 kcal/week)	1.17 (0.98-1.41)	1.30 (1.05-1.61)
Alcohol intake per SD (=8.3 beverages/week)	1.19 (1.01-1.40)	1.17 (0.93-1.46)
Smoking	1.87 (1.16-3.03)	2.16 (1.14-4.09)
Falls in the past month	1.47 (0.70-3.10)	2.67 (1.17-6.08)
Thiazides	1.59 (0.72-3.52)	1.26 (0.39-4.13)
Loop diuretics	4.57 (1.54-13.56)	1.91 (0.25-14.42)
Statins	1.33 (0.57-3.12)	1.26 (0.39-4.13)
Nitrates	1.18 (0.36-3.83)	0.73 (0.10-5.40)
Glucocorticoids	NA*	NA*
Bisphosphonates	8.17 (0.84-79.29)	16.10 (1.64-157.76)
Hypertension	1.54 (0.97-2.44)	1.43 (0.76-2.70)
Myocardial Infarction	2.82 (1.58-5.05)	2.38 (1.05-5.40)
Cerebrovascular disease	1.12 (0.35-3.64)	0.70 (0.10-5.14)
Prior minimal trauma fractures	1.24 (0.77-1.99)	1.67 (0.90-3.09)

*NA, not available as could not be computed

Table 29: Fracture Analysis 2, Univariate logistic regression of "any minimal trauma fractures", "main" fragility fractures and hip fractures against independent variable in women by duration of β-blockers use.

	OR (95% CI)		
	Any minimal trauma fracture	**Main fragility fracture**	**Hip fractures**
β-blocker chronic use	1.28 (0.90-1.81)	1.66 (1.09-2.51)	2.99 (1.12-7.98)
β-blocker year 5 only	1.08 (0.76-1.53)	1.20 (0.76-1.88)	3.25 (1.30-8.09)
β-blocker baseline only	1.41 (0.77-2.60)	1.75 (0.84-3.65)	8.73 (2.95-25.89)
Age (years)	1.04 (1.03-1.05)	1.06 (1.05-1.07)	1.15 (1.10-1.20)
BMI (kg/m^2)	1.00 (0.98-1.02)	0.99 (0.97-1.02)	0.97 (0.90-1.04)
Calcium pre SD (=617 mg/day)	1.07 (0.98-1.17)	1.01 (0.89-1.14)	1.26 (0.95-1.67)
Vitamin D per SD (=628 IU/day)	1.08 (1.01-1.16)	1.08 (0.99-1.17)	1.18 (1.05-1.32)
Energy expenditure per SD (=3254 kcal/week)	0.93 (0.84-1.02)	0.86 (0.75-0.99)	0.70 (0.47-1.06)
Alcohol intake per SD (=4.1 beverages/week)	1.00 (0.91-1.10)	1.02 (0.90-1.14)	0.80 (0.51-1.28)
Smoking	0.91 (0.68-1.20)	0.96 (0.67-1.38)	1.54 (0.67-3.52)
Falls in the past month	1.24 (0.87-1.78)	1.30 (0.82-2.06)	0.88 (0.21-3.67)
Menopause	2.70 (1.92-3.78)	5.90 (3.12-11.14)	7.73 (1.06-56.52)
Thiazides	0.85 (0.62-1.16)	1.22 (0.85-1.75)	1.24 (0.48-3.21)
Loop diuretics	1.77 (1.02-3.09)	2.61 (1.41-4.82)	6.62 (2.30-19.06)
Statins	1.23 (0.80-1.87)	1.32 (0.77-2.26)	1.99 (0.61-6.53)
Nitrates	1.34 (0.79-2.28)	1.50 (0.78-2.89)	1.07 (0.15-7.88)
Glucocorticoids	2.43 (1.35-4.37)	3.06 (1.56-6.03)	2.06 (0.28-15.19)
Bisphosphonates	2.77 (1.77-4.35)	3.78 (2.28-6.27)	7.28 (2.78-19.04)
HRT	0.76 (0.60-0.95)	0.75 (0.55-1.02)	0.50 (0.19-1.29)
SERMS	1.30 (0.46-3.69)	0.55 (0.08-4.05)	NA*
Hypertension	1.29 (1.06-1.58)	1.67 (1.30-2.16)	2.34 (1.21-4.52)
Myocardial Infarction	1.37 (0.87-2.15)	1.83 (1.08-3.09)	3.46 (1.21-9.87)
Cerebrovascular disease	2.27 (1.51-3.43)	2.79 (1.72-4.53)	4.03 (1.41-11.53)
Prior minimal trauma fractures	2.31 (1.91-2.80)	2.05 (1.59-2.64)	1.84 (0.93-3.62)

*NA, not available as could not be computed

two separate models, in one as a continuous variable and the other categorized by quartiles. BMI was categorized into 4 groups for analyses: underweight (BMI <18.5), normal weight (BMI 18.5-24.9), overweight (BMI 25.0-29.9) and obesity (BMI 30.0+). Alcohol was transformed to a squared variable.

4.4.3 Multivariate logistic regression modeling of the effect of β-blocker duration of use on fractures.

Tables 30 and 31 show the results of our second fracture analysis. The multivariate adjusted OR for fragility fractures are shown. Again, the three categories of subjects using β-blockers are compared to the non-users and the fracture outcomes are as previously explained.

In men, the multivariate logistic regressions were done twice for each fracture outcome, once with alcohol as a continuous linear variable and a second time with alcohol broken down into quartiles. The OR for our main variable of interest did not change in either case so here we report the results only using alcohol as a continuous variable. In men, the final model for the outcome "any minimal trauma fracture" included the variables age, β-blocker by duration of use and smoking. The final model for the outcome main fragility fractures included the variables: β-blocker by duration of use and vitamin D.

For women, the multivariate logistic regression was done twice for each of the 3 fracture outcomes, once with age as a continuous linear variable and a second time with age broken down into quartiles. For all outcomes, age entered the final models both as a continuous variable or in quartiles and the results were almost the same for the exposure of interest in all cases so we report here the results for the models only with age as a continuous variable model.

In women for the outcome any minimal trauma fracture, the final model included the variables: age, β-blocker by duration of use and prior minimal trauma fractures. For the outcome main fragility fracture, the final model included the variables: age, β-blocker by duration of use and prior minimal trauma fractures. For the outcome hip fracture, the final model included the only 2 variables: age and β-blocker by duration of use.

As discussed in section 4.3.2, it is not clear whether an analysis focusing on the effects of β-blockers should adjust for bisphosphonates, as they may be on the causal pathway. Therefore, we ran our analyses both with and without this variable. While we did generally find that bisphosphonates have an effect on fractures, we did not uncover any confounding with the effect of β-blockers. In particular, in men, we found that if bisphosphonates were added to the final model, these had an OR of 9.4 (0.9-95.1) for any minimal trauma fractures and an OR of 18.5 (1.8-191.4) for main fragility fractures. The wide confidence intervals in men likely reflect the rare prevalence of bisphosphonate use and the fact that there were so few events in these subjects. Similarly in women, bisphosphonates, added to the final model, had an OR of 1.8 (1.1-2.9) for any minimal trauma fractures, 2.3 (1.4-4.0) for main fragility fractures and 4.1 (1.5-11.3) for hip fractures. The OR's for fractures with bisphosphonates suggest an elevated risk yet this drug reduces fractures in randomized controlled trials. As it is usually taken in individuals with low BMD and high fracture risk, this variable is in effect a marker for low BMD. To find out the true risk of fracture while on these drugs, we would need to adjust for BMD. In our case, since BMD changes may be in our causal pathway, we would not want to adjust for this.

In the men, there were actually too few or no fractures of certain types occurring in some subgroups so that we could not compute any OR's for these. This was the case for the

outcome "main fragility fractures" in the users of β-blockers at year 0 only. For men, as can be see in table 30, there was evidence of an effect for chronic users of β-blockers on the composite "any minimal trauma fractures" as the OR was 2.1 (1.0-4.3). A similar OR was found for the main fragility fractures but here, the CI crossed unity.

For women, the results are shown in table 31. Among women using β-blockers at baseline only, there was a 6-fold increased hip fracture risk [OR 6.3 (2.0-19.3)] and the CIs indicate that the true odds ratio may be as low as 2.0 or as high as 19.3. When we looked at chronic use in women, we did not find any evidence of an effect of β-blockers on increased fracture risk as the confidence intervals are wide and cross 1. However, the OR for hip fracture in the chronic users did have a point estimate consistent with that found for users at baseline only.

Table 30: Risk of fragility fracture associated with increased duration of β-blocker use in men

			OR (95% CI)	
			Univariate	**Adjusted**
Duration of β-blocker use	**Year 0**	Any fracture	0.6 (0.1-4.3)	0.6 (0.1-4.3)
		Main fragility*	ND	ND
	Year 5 only	Any fracture	1.5 (0.7-3.0)	1.1 (0.5-2.4)
		Main fragility*	1.2 (0.4-3.4)	1.0 (0.3-3.1)
	Chronic User	Any fracture	2.4 (1.2-4.8)	2.1 (1.0-4.3)
		Main fragility*	2.2 (0.8-5.6)	2.3 (0.9-6.1)

*Includes vertebrae, hip, distal radius, pelvis and ribs

Table 31: Risk of fragility fracture associated with increased duration of β-blocker use in women

			OR (95% CI)	
			Univariate	**Adjusted**
Duration of β-blocker use	**Year 0**	Any fracture	1.4 (0.8-2.6)	1.2 (0.6-2.2)
		Main fragility*	1.8 (0.8-3.7)	1.4 (0.7-3.0)
		Hip fracture	8.7 (3.0-25.9)	6.3 (2.0-19.3)
	Year 5 only	Any fracture	1.1 (0.8-1.5)	0.9 (0.6-1.3)
		Main fragility*	1.2 (0.8-1.9)	1.0 (0.6-1.5)
		Hip fracture	3.2 (1.3-8.1)	2.3 (0.9-5.9)
	Chronic User	Any fracture	1.3 (0.9-1.8)	1.0 (0.7-1.4)
		Main fragility*	1.7 (1.1-2.5)	1.2 (0.8-1.8)
		Hip fracture	3.0 (1.1-8.0)	1.5 (0.5-4.5)

* Includes vertebrae, hip, distal radius, pelvis and ribs

Chapter 5 – Conclusions and Discussion

As discussed in section 2.5, there is no consensus about whether 1% or 2% should be considered a clinically meaningful BMD difference. Depending on the value chosen, it can change the interpretation of our results for the women. Using data from observational studies, a 2% decrease in BMD compared to the young adult mean is associated with an increased risk of fracture of 6-8%, which is a clinically important risk.[71, 72] The situation is different in clinical trials where a 1% increase in BMD due to anti-resorptive treatment is associated with an 8% or more reduction in vertebral and non-vertebral fracture risk.[109-111] It is well recognized that the risk reduction seen with these treatments is in part due to improvements in BMD, but also due to other mechanisms such as improvements of bone quality. Thus, choosing a 2% limit of clinical importance for BMD makes sense in the context of evaluating BMD differences in observational studies. In this population-based cohort study therefore, using a 2% value as a minimum clinically important difference in BMD, there appears to be no effect of β-blockers on BMD at either the total hip or lumbar spine in women. However, we cannot make strong conclusions with regards to the effect of β-blockers on BMD at the total hip or lumbar spine in men, owing to the wide confidence intervals.

There were differences between the β-blocker users and the non-users in two important osteoporosis risk factors, namely age and body weight. β-blocker users were on average older and heavier. Age and body weight are major predictors of BMD in women and men. When we adjusted for age only, the β-blocker users had increased BMD compared to the non-users. This is because age is a confounder of the relationship between β-blockers and BMD, since β-blocker users are older and older people have generally lower bone densities. However, once adjusted for other potential confounders, including

BMI, the results become inconclusive for men, and for women, there is no difference in BMD.

CaMos has relatively large numbers of subjects, and yet despite this, our results for the effect of β-blockers on BMD in men were inconclusive, as were some of our results for the fracture sites examined in both men and women. CaMos was designed to assess the prevalence of low bone density and to estimate the incidence of fractures in the Canadian population.[112] It was also designed to evaluate the relationship between low BMD, fractures and major clinical risk factors. CaMos is perhaps underpowered to investigate the relationship between risk factors that have a more complex and weaker relationship with the outcome, particularly in men. Hence, larger studies are needed to evaluate the β-blocker effects on BMD in men and on fractures at all sites in different subgroups of users.

Our finding that there is no difference in the BMD of the hip between β-blockers users and non-users in women is consistent with results from four other studies[70, 74-76] though only two of these[74, 76] provided enough data to verify the CIs. Our finding that there is no difference in the BMD at the lumbar spine between β-blocker users and non-users in women is consistent with results from one other study but their CIs could not be verified.[70] Other investigators reported on the effects of β-blockers on BMD. Of these, Pasco et al. reported that BMD was increased in β-blocker users compared to non-users.[73] However, a closer examination of their data reveals that, in fact, their results were inconclusive as to the effects of β-blockers on BMD. The confidence intervals indicate that the true percent BMD difference may be as low as 0.3% or as high as 5.5%. The other study results reporting on BMD effects of β-blockers were inconclusive, including the only two that included men. Thus, including our results, there are now five

observational studies that have found no difference in the BMD between β-blocker users and non-users for women, and 2 such findings of no difference in BMD for the spine. The wealth of evidence combined suggests that, in fact, β-blockers have no effect on BMD in women.

There is some evidence from our study that β-blockers may be associated with an increased risk of fractures in certain subsets of users. In men, chronic use of β-blockers (user at both baseline and year 5) appeared to increase the risk for any minimal trauma fracture. There were too few hip fractures in men to conduct the analysis for this site. For women who used β-blockers at baseline only (intermittent use), there was an increased risk of hip fracture. Among women who were chronic users of β-blockers, the odds ratio for any fracture was 1.5, which is consistent with what was found in users at baseline only, but the confidence intervals indicate that the true odds ratio may be as low as 0.5 or as high as 4.5. The risk of fractures for other sites in women was inconclusive, again owing to wide confidence intervals.

The finding of an increased hip fracture risk in women who were users of β-blockers at baseline only is hard to explain. Consistent with the users at baseline only, women who were chronic users of β-blockers had an increased point estimate for the OR for hip fracture, but the wide CIs rendered this inconclusive. It is possible that confounding by severity or a channeling bias could explain these findings. This situation could arise if patients perceived to be at highest risk for subsequent falls and repeated fractures are taken off the β-blocker because of their association with occasional dizziness and orthostatic hypotension. Thus, these high risk fracture patients would no longer be using at the year 5 visit. This could cause an apparent increased risk of hip fractures for the

subjects using β-blockers only at baseline and at the same time, reduce the apparent effect of chronic use of β-blockers on causing an increased fractures.

Our findings of an increased risk of any fracture in men who were chronic users of β-blockers and of hip fractures in women who were users of β-blockers at baseline only is not consistent with the majority of previous studies. Nonetheless, our results are in accord with those of Rejnmark et al.[70] Using data from the Danish Osteoporosis Prevention Study (DOPS) and a nested case-control design, they found an increased risk of any fracture for women who were users of β-blockers. Evidence for a dose effect was also found in this study. There are similarities between CaMos and DOPS. Both are prospective cohort studies that collected extensive information on potential confounders at baseline. Recall bias is not a concern in either study as information was collected before outcomes occurred and medications taken were verified. As in our study, statistical analyses of the data from DOPS were adjusted for all available confounder information, including lifestyle variables, something not possible in some other studies.

In total, five studies reported that β-blocker use was associated with a reduced risk of any fracture or of hip fracture in either women alone, or in both men and women. Three of these were large case-control studies that used administrative databases or computerized record systems. Though the confidence intervals are narrow due to the large sample sizes, they have missing or limited information on potential confounder information such as BMI, smoking, alcohol, physical activity, falls, and dietary factors and as a consequence may be biased. Also, for many of these studies, it was not possible to ascertain the cause of fractures and therefore all fractures were included regardless of cause. Subjects who frequently engage in exercise or have active lifestyles and suffer more trauma or recreational fractures may differ in many ways which may be linked to

their use of β-blockers. Specifically, non-users of β-blockers, may be more active and suffer more of these "non-fragility" fractures. Thus, activity and exercise confound the relationship between β-blockers and fractures. Thus, the inclusion of all fractures, without adjustment for activity level, could produce biased results.

One of these studies using administrative databases made a particularly interesting and relevant finding.[83] A protective effect on fracture risk could be demonstrated with the use of anti-hypertensive agents from two other classes in addition to the β-blockers. The protective effect found with calcium-channel blockers and angiotensin-converting enzyme inhibitors was of similar magnitude to that found for β-blockers in the same population. As these other drugs are not currently considered to have bone-modulating effect, this finding is consistent with a systematic form of bias in the use of these drugs. Specifically, this could be due to a form of selection bias in the prescribing of these drugs. Thus, owing to concerns about the adverse effects, such as dizziness and orthostatic hypotension with β-blockers and other anti-hypertensive drugs, these may not be prescribed to patients perceived by physicians to be frail and at higher risk for falls. This could produce an exposed group at lower risk for fracture.

Another explanation for the reduction in fracture seen in some of these studies could be the so called "healthy user effect", whereby patients who use a drug consistently and for a long duration differ in health characteristics from others in the population. Studies that can adjust for lifestyle variables, such as diet, smoking, alcohol, and physical activity may correct for this, at least in part. This is not possible with the use of administrative databases.

In many studies, there was limited adjustment for the potential confounding by indication.[70, 73, 75, 82, 96] In our fracture analyses, we did adjust for hypertension and cardiovascular diseases, which have been associated with low BMD and fractures and are probably the two most frequent reasons subjects are prescribed long term β-blockers.[102-104]

In the end, these inconsistent findings as to the effects of β-blockers on fracture risk in observational studies are disconcerting. The suggestion that β-blockers influence bone density and fractures in humans is mechanistically plausible. According to findings in animal studies, β-blockers would block the effects of an increased sympathetic tone generated by leptin. However, the effects of leptin on bone certainly appear quite complex and are probably mediated by age, other hormonal influences and specific skeletal site. The contrasting findings of high bone density at the spine and low bone density at the hip in mice with leptin deficiency could be explained by differences in innervations of the various skeletal sites. This could also result in variable responses to pharmacological intervention. Thus, our finding of an increased risk of fractures in subsets of our patients treated with β-blockers is not necessarily inconsistent with the current understanding of the physiological effects of leptin on bone.

Our findings of an increased risk of fractures in certain subsets of users are also in keeping with other evidence. Data from *in vitro* studies suggest that different β-blockers may exert divergent effects on skeletal tissue, i.e. catabolic as well as anabolic.[113] Our findings also agree with the reduction of bone formation markers seen in a 3 month randomized controlled trial of β-blockers[69] Finally, there has been a pooled analysis of 9 randomized clinical trials investigating the non-selective β-blocker carvedilol for the treatment of heart failure.[74] Unadjusted for falls, the relative risk for fracture was 1.15

(95% CI, 0.81-1.64). Although these pooled results are inconclusive, the relative risk estimate is at least consistent with some of our findings.

The main strengths of our study are the prospective cohort study design, the random selection of subjects from the population, the high retention rate, the long-follow-up of subjects, the inclusion of men, data on numerous confounding factors, and the ability to assess associations with bone mineral density and fracture in the same study group. There were few exclusion criteria in CaMos so that our findings should be generalizable to the Canadian population.

Our study does have some limitations. The small number of incident fractures limited site-specific fracture risk analysis, especially for the men. The duration of use of β-blockers before the initial questionnaire is unknown as was the persistence of use of β-blockers between baseline and year 5. A wrong assumption of persistent use between the baseline and year 5 visits may weaken any association between fractures and those labeled as chronic users. Of course, as with any observational study examining possible associations among drug use, bone density, and fractures, our study is subject to residual or unmeasured confounding, which may also explain some of our findings. Finally, subjects of CaMos may have agreed to participate because they were more health conscious. Thus, if they were healthier than average, they could have a higher BMD than the general population. Conversely, participants may have chosen to take part because they believed they were at higher risk of osteoporosis because of family history or background of osteoporosis, and therefore had a lower BMD. In either of these situations, the choice of participating is probably unrelated to their use of β-blockers and should not bias our findings. Of course, this may not be the case if β-blockers were strongly related to BMD.

There are likely to be complex interactions between cardiovascular disease and fracture risk, operating through falls, BMD, genetic risks, lifestyle, or other as yet unknown factors. These complex relationships may be difficult to evaluate in observational studies given their potential for bias and confounding. Future research with larger numbers of β-blocker users will be important to determine whether β-blocker use is prospectively associated with changes of bone density or fractures.

Our findings suggest that, in the cohort we have studied, use of β-blockers in women is not associated with a difference in BMD compared to non-users. However, our findings also suggest that use of β-blockers is associated with increased rates of hip fractures for subsets of users in women, and increased rates of any minimal trauma fractures in the men who were chronic users. Although some of these associations are possibly due to residual confounding, further investigation of β-blocker use and fractures in other populations with longer follow-up or ideally, by randomized clinical trial, is warranted given the presence of β-adrenergic receptors in bone and the wide use of these drugs in the population. While conducting a randomized controlled trial of β-blockers to establish their effect on fractures may not be ethical given the possibility of an increased risk for fracture from ours and other studies, future randomized controlled trials of β-blockers given with intent to prevent or treat cardiovascular diseases should include fractures as a secondary or safety outcome. At present, the epidemiologic data relating β-blocker use to fractures are equivocal. Certainly the cumulative evidence does not warrant the use of β-blockers as agents to prevent or treat osteoporosis.

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Appendices

CaMos baseline questionnaires

Human ethics approval forms

Respondent I.D. # _____

CaMos

Canadian Multicentre Osteoporosis Study

Étude canadienne multicentrique sur l'ostéoporose

QUESTIONNAIRE

Respondent I.D. #_____

CaMos
Canadian Multicentre Osteoporosis Study

RESPONDENT

*PROVINCIAL HEALTH # _____

NAME _____ (Last (Maiden in Quebec)) _____ (First)

_____ (* ETHNIC NAME (Last)) (First)

ADDRESS _____ (No.) _____ (Street) _____ (Apt. #)

_____ (City) _____ (Province) |_|_|_|_| |_|_|_|_| (Postal Code)

TELEPHONE # ( ) _____ (Area Code)

DO YOU PLAN TO MOVE IN THE NEXT YEAR? ☐ YES ☐ NO

→ When? _____

CONTACT PERSON *

NAME _____ (Last (Maiden in Quebec)) _____ (First)

ADDRESS _____ _____ _____

_____ _____

TELEPHONE #

( ) _____ (Home) ( ) _____ (Work)

RELATION TO RESPONDENT: _____

*See notes in manual

Respondent ID # _____

CaMos
Canadian Multicentre Osteoporosis Study

CENTRE NUMBER ⌊_⌋⌊_⌋

INTERVIEWER ID # _____ NAME _____

LOCATION OF INTERVIEW ☐ HOSPITAL ☐ HOME ☐ OTHER _____

DATE OF INTERVIEW _____ / _____ / _____ (Day / Month / Year)

TIME BEGAN ⌊_⌋⌊_⌋ HRS ⌊_⌋⌊_⌋ MIN.

TIME ENDED ⌊_⌋⌊_⌋ HRS ⌊_⌋⌊_⌋ MIN.

RESPONDENT

NUMBER OF RESIDENTIAL TELEPHONE # IN HOME ⌊_⌋⌊_⌋

IF RESPONDENT ASSISTED, BY WHOM? _____

LANGUAGE OF INTERVIEW ☐ FRENCH ☐ ENGLISH ☐ OTHER _____

HEARING IMPAIRMENT ☐ YES ☐ NO VISUAL IMPAIRMENT ☐ YES ☐ NO

FIRST INTERVIEW (PHASE I) SCHEDULED _____ D/M/Y ☐ INCOMPLETE ☐ COMPLETED _____ D/M/Y

CLINICAL ASSESSMENT

DEXA	ULTRASOUND	BLOOD	URINE	X-RAY
☐ Yes	☐ Yes	☐ Yes	☐ Yes	☐ Yes
☐ No	☐ No	☐ No	☐ No	☐ No
		☐ N/A	☐ N/A	☐ N/A

RESULTS TO BE SENT TO PHYSICIAN ☐ YES ☐ NO FOLLOW UP ☐ YES ☐ NO

CAMOS DATA ENTRY DATE _____ / _____ / _____ (Day / Month / Year)

COMMENTS _____

* See note manual

To begin the questionnaire I would like to ask you general questions about yourself.

1. SOCIO-DEMOGRAPHIC INFORMATION

1.1 Sex: ☐ Male ☐ Female

1.2 Date of Birth: _____/ _____/ _____ (Present age _____)
Day Month Year

1.3 In what country were you born? _____

1.4 a) * How many years have you lived in Canada? _____ years

b) If less than 5 years, Country where respondant has lived for the most number of years _____

1.5 * To which ethnic or cultural group(s) did your ancestors belong?
(*For example: French, British, Chinese, etc.*)

(*Do not read list. Mark all that apply*)

- ☐ Black
- ☐ Canadian
- ☐ Chinese
- ☐ Dutch (Netherlands)
- ☐ English
- ☐ French
- ☐ German
- ☐ Inuit/Eskimo
- ☐ Irish
- ☐ Italian
- ☐ Jewish
- ☐ Métis
- ☐ North American Indian
- ☐ Polish
- ☐ Portuguese
- ☐ Scottish
- ☐ South Asian
- ☐ Ukrainian
- ☐ Other ethnic or cultural group(s) (Specify _____)

1.6 * What is the language that you first learned at home in childhood and can still understand?

(*If can no longer understand the first language learned, choose the second language learned*).

(*Do not read list. Mark all that apply*)

- ☐ English
- ☐ French
- ☐ Arabic
- ☐ Chinese
- ☐ Cree
- ☐ German
- ☐ Greek
- ☐ Hungarian
- ☐ Italian
- ☐ Korean
- ☐ Persian (Farsi)
- ☐ Polish
- ☐ Portuguese
- ☐ Punjabi
- ☐ Spanish
- ☐ Tagalog (Filipino)
- ☐ Ukrainian
- ☐ Vietnamese
- ☐ Other (Specify _____)

* See notes in manual

1.7 * How would you best describe your race or colour?
(*Do not read list. Mark all that apply*)

- ☐ White
- ☐ Chinese
- ☐ South Asian (e.g. East Indian, Pakistani, Punjabi, Sri Lankan)
- ☐ Black (e.g. African, Haitian, Jamaican, Somali)
- ☐ Native/Aboriginal Peoples of North America (North American Indian, Métis, Inuit/Eskimo)
- ☐ Arab/West Asian (e.g. Armenian, Egyptian, Iranian, Lebanese, Moroccan)
- ☐ Filipino
- ☐ South East Asian (e.g. Cambodian, Indonesian, Laotian, Vietnamese)
- ☐ Latin American
- ☐ Japanese
- ☐ Korean
- ☐ Other (Specify _____)

1.8 How many years of school have you finished? (*Mark the highest grade completed*)

- ☐ less than grade 9
- ☐ grades 9-13, without certificate or diploma
- ☐ high school certificate or diploma
- ☐ trades or professional certificate or diploma (CEGEP in Quebec)
- ☐ some university without certificate or diploma
- ☐ university certificate or diploma
- ☐ university degree

1.9 * What is your current employment status?

- ☐ employed full time
- ☐ homemaker (full time)
- ☐ employed part time
- ☐ unemployed
- ☐ disability
- ☐ retired ⟶ How old were you? ____ years
- ☐ other (specify _____)

1.10 Do you live alone ? ☐ Yes ☐ No

↳ Do you live with another adult?
☐ Yes ☐ No

1.11 Do you have a particular doctor or clinic that you would call your regular doctor or clinic? ☐ Yes ☐ No

* See notes in manual

Now we'll review your past health.

2. MEDICAL HISTORY

2.1 * Has a doctor ever told you that you have any of the following conditions?

	DIAGNOSIS			TREATMENT			
	Yes	No	DK	Yes	No	DK	N/A
Osteoporosis							
Rheumatoid arthritis							
Osteoarthritis							
Thyroid disease: 1 = Hyperthyroidism 2 = Hypothyroidism							
Liver disease							
Scoliosis							
Eating disorder							
Breast cancer * *(for all)*							
Uterine cancer *(for females)*							
Inflammatory bowel disease							
Kidney stones							
Hypertension							
Heart attack							
Stroke TIA (*Transient Ischemic attack*)							
Neuromuscular disease: 1 = Parkinson's 2 = Multiple Sclerosis 3 = Other							
Diabetes: Age 1 = Insulin Dependent							
2 = Non Insulin Dependent							
Kidney disease							
Phlebitis, thrombophlebitis							
Prostate cancer *(for males)*							
Paget's Disease of Bone							

* See notes in manual

2.2 * Have you ever been confined to a bed, a wheelchair or by a cast for more than one month at a time

☐ Yes ☐ No

↓

How many episodes? _____

(1st episode) At what age? _____ years

For how long? _____ months

(most recent episode) At what age? _____ years

For how long? _____ months

2.3 * Which of the following surgeries have you had in the past? How old were you?

	YES	NO	AGE
Parathyroid			
Thyroid			
Stomach			
Intestine			
Gall Bladder			

How many times? _____

2.5 * Have you fallen in the past month? ☐ Yes ☐ No

↓

How many times? _____

* See notes in manual

Now I will ask you about any medicines you may have taken.

3. DRUGS AND MEDICATIONS

3.1 * Have you ever taken any of the following medications daily for more than one month?

If YES: For approximately how many months total have you taken it?

	YES	NO	TOTAL # OF MONTHS TAKEN
Thyroid pills (Synthroid®)			
Dilantin (Seizure Pills) / Phenobarbital			
Tamoxifen (Nolvadex)			
Calcitonin (Calcimar)			
Didronel® / Etidronate			
Fluoride (Fluatic)			
Diuretics - Thiazide / Other			
Laxatives			
Cortisone / Prednisone			
1 = Oral			
2 = Inhaled *			
			FREQUENCY OF INJECTION
3 = Injection a) Intravenous			
b) Intramuscular, Subcutaneous			

* See notes in manual

3.2 * Current medications and or self administered supplements taken on a regular basis.

Medications: From contents of medicine cabinet		
NAME	DOSE	FREQUENCY

* See notes in manual

Now I would like to know about any broken bone you may have had.

4. FRACTURES

4.1 * Have you ever fractured any bones? ☐ Yes ☐ No → Go to 5.1 If female
→ Go to 6.1 If male

Complete the table below

(Refer to picture of body skeleton if necessary)

Use the following trauma codes to indicate how it happened.

1 = severe trauma
2 = minimal trauma
3 = other disease

(See manual for definitions)

INCIDENT(S)	TRAUMA CODE	AGE (years)	BONE SITE										OTHER					
			BACK		RIBS		PELVIS		FOREARM /WRIST		HIP		BONE SITE		BONE SITE		BONE SITE	
			#	X	#	X	#	X	#	X	#	X	#	X	#	X	#	X
1																		
2																		
3																		
4																		
5																		
6																		

# = fracture
x = x-ray

* See notes in manual

Respondent I.D. # _____

In this section I would like to ask you questions that will help us understand how women's hormones relate to bone structure. We ask everyone these questions.

5. Reproductive History (Females)

5.1 * Before menopause, have you ever gone 3 months or more without a menstrual period?
(not including pregnancy or during breastfeeding)

☐ Yes ☐ No → Go to 5.2

What was the longest single period of time without a menstrual flow? _____ months

If you count all the periods you have missed throughout your menstruating years, how many months would that be? _____ months
(this question asks for the cumulative time)

5.2 * Have your menstrual periods stopped for more than one year?
(No period one year or more after last menstruation)

☐ Yes ☐ No

→ At what age? _____ years

5.3 Have you had your uterus removed (*hysterectomy*)?

☐ Yes ☐ No

→ At what age? _____ years

5.4* Have you ever had one or both ovaries removed?

☐ Yes, one ovary removed at what age? _____
☐ Yes, both ovaries removed at what age? _____
(if ovaries were removed on separate occasions, write the age at which the second ovary was removed)
☐ Yes, do not know how many at what age? _____
☐ No

* See notes in manual

5.5* Do you or did you ever take **estrogen** for menopause or for any other reason ?

☐ Yes, currently
☐ Yes, but not now

☐ No
↳ Go to 5.6

What type(s)?

(Interviewers to show Ogen®, Premarin® pills, colors and doses and Estraderm®, Estracomb®, patches, sizes and doses)

☐ Pill

Pill N°	Number of days/month	Age started	Age stopped	Total number of months taken

☐ Patch

Patch N°	Number of days/month	Age started	Age stopped	Total number of months taken

☐ Injection — How many times/year? _____
How many years? _____

☐ Vaginal cream — How frequently? _____

* See notes in manual

5.6 * Do you or did you ever take Provera[R], for menopause or for any other reason?

☐ Yes, currently
☐ Yes, but not now
↓

☐ No
↳ Go to 5.7

What type(s)? (*Interviewers to show Provera[R] pills, colors and doses*)

☐ Pill

Pill N°	Number of days/month	Age started	Age stopped	Total number of months taken

☐ Injection

How many times/year? _____
How many years? _____

5.7 * Have you ever used birth control pills or oral contraceptives?

☐ Yes
↓

☐ No → Go to 5.8
→ Go to 5.9 (*if periods have stopped permanently through natural/surgical menopause)*

At what age did you start? _____ years (*approximately*)

* For approximately how long did you use birth control pills? _____ years _____ months

Are you still using birth control pills?

☐ Yes
↓
Go to 5.9

☐ No
↳ At what age did you stop using birth control pills?
_____ years

* See notes in manual

5.8 * *(If not using birth control pills, not menopausal, have not had both ovaries removed)*

Can you tell by the way you feel that your period is coming?

- ☐ Yes, every month
- ☐ Yes, most months
- ☐ Yes, less than half the time
- ☐ Yes, one or twice a year
- ☐ Never

If YES, to any of the above:

What signs or symptoms indicate to you that your period is coming?

- ☐ menstrual cramps or aching back or legs
- ☐ bloating, fluid retention
- ☐ increased appetite (*in general or for sweet, salty or spicy foods*)
- ☐ moodiness (*frustration, irritability, sadness*)
- ☐ breast tenderness in the front or the nipple
- ☐ breast tenderness up under the arm or on the outer sides of the breast
- ☐ breast swelling
- ☐ headaches (*migraine or tension*)
- ☐ acne / pimples / blemishes
- ☐ other _____

5.9 * How many times have you been pregnant? _____ → If 0 : Go to 5.12
(Pregnancy confirmed by a physician or pregnancy test)

5.10 * How many of these pregnancies resulted in at least one live birth? _____ → If 0 : Go to 5.12
(Count twins and triplets as 1)
↓
Age at 1st birth? _____ years

5.11 Did you breast feed any of your children? ☐ Yes ☐ No
↳ For how many months total ____ months
(i.e. adding up the months with each child)

5.12 How old were you when you had your first menstrual period? _____ years

* See notes in manual

5.13* a) Did you have regular periods once they began?

☐ Yes ☐ No
→ Go to 5.14

b) If you had irregular periods, did they become regular?

☐ Yes ☐ No → Go to 5.14
→ At what age _____ years

c) Have your periods been made regular by medication?

☐ Yes ☐ No
→ At what age _____ years

5.14 On average, how often did you have menstrual periods when you were in your 20's and 30's?

☐ 20 days or less
☐ 21-25 days
☐ 26-30 days
☐ 31-36 days
☐ 37 or more days
☐ do not know

In this section, I would like to ask you questions that will help us understand how men's hormones relate to bone structure. We ask everyone these questions.

6. Reproductive History (Males)

6.1 Have you fathered any children?

☐ Yes
↓
How many ? ____

☐ No
→ Have you ever been diagnosed with a fertility problem?
☐ Yes ☐ No ☐ Don't know

6.2* Which of the following is your usual experience regarding spontaneous erections not related to sex?

☐ one or more times a day (*for example, first thing when I wake up*)
☐ most days
☐ some days
☐ occasionally
☐ rarely
☐ never

* See notes in manual

Now I will ask about your family history.

7. FAMILY HISTORY

7.1 * How many brothers and/or sisters do/did you have? (*not adopted*)

_____ siblings ☐ do not know

7.2 * I would like to ask about the following family members and their medical history.

DIAGNOSIS	PARENTS			SIBLINGS				CHILDREN			
	Yes	No	DK	Yes	No	DK	NA	Yes	No	DK	NA
Fracture											
Osteoporosis											
Osteoarthritis											
Scoliosis											
CVD, stroke, aneurysm, hypertension											
Breast cancer											
Ovarian cancer											
Uterine cancer											
Prostate cancer											

* See notes in manual

In this section I will ask you about diet, exercise programs and eating habits.

8. PHYSICAL CHARACTERISTICS

8.1 What was your **greatest** adult height? ___ feet ___ inches - or - ___ cm ☐ do not know

Go to 8.3 if subject to undergo DEXA measurement

8.2 * *If not scheduled for DEXA, measure height with carpenter's ruler)*

What is your current height? ___ feet ___ inches - or - ___ cm *(to be measured in the home)*

8.3 What was your **greatest** adult weight? ____ lbs - or - ___ kg ☐ do not know
(when over 25 yrs old and not pregnant)

8.4 What was your **lowest** adult weight? ___ lbs - or - ___ kg ☐ do not know
(over age 25)

Go to 8.6 if subject to undergo DEXA measurement

8.5 * *If not scheduled for DEXA, weigh with portable scale*

What is your current weight? ___ lbs - or - ___ kg *(to be measured in the home)*

8.6 * Have you ever lost more than 10 pounds: *(other than after childbirth, re: one year post-partum)*

☐ Yes ☐ No → Go to 8.7

↳ Did you regain the lost weight?

☐ Yes ☐ No

↳ How much did you lose? ____ lbs -or- _____ kg

(In lifetime)

How many times have you lost and regained 10-20 pounds (6-10 kg)? _____

How many times have you lost and regained over 20 pounds (over 10 kg)? _____

* See notes in manual

I'm going to ask you a few questions on your eating habits.

8.7 a) I am going to read two sentences for you. Please answer True (T) or False (F) for each statement as it pertains to you.

I enjoy eating too much to spoil it by counting calories or watching my weight. T ☐ F ☐

I consciously hold back at meals in order not to gain weight. T ☐ F ☐

b) Which of these best describes you?

On a scale of 0 to 5, where 0 means no restraint in eating (*eating whatever you want, whenever you want it*) and 5 means total restraint (*constantly limiting food intake and never "giving in"*), what number would you give yourself?

0 Eat whatever you want, whenever you want it
1 Usually eat whatever you want, whenever you want it
2 Often eat whatever you want, whenever you want it
3 Often limit food intake, but often "give in"
4 Usually limit food intake, rarely "give in"
5 Constantly limiting food intake, never "giving in"

Now the questions I will ask will relate to the use of tobacco.

9. TOBACCO

9.1 Have you ever used any of the following tobacco products daily for at least 6 months?

Cigarettes	☐ Yes	☐ No	
Pipes	☐ Yes	☐ No	
Cigars	☐ Yes	☐ No	
Chewing tobacco	☐ Yes	☐ No	→ If NO to all: go to 9.3

9.2 Complete the following table for each product used.

→ At what age did you begin to ..... daily? (*for at least 6 months*)
→ Are you currently smoking?
→ At what age did you stop?
→ Approximately how many every day? (*number of cigarettes, bowls of pipe tobacco, number of cigars, number of chews*)
→ Have you temporarely stopped ..... and started again? (*total up all periods and covert to years*)

	AGE STARTED	CURRENTLY SMOKING		AGE STOPPED	AMOUNT PER DAY	TEMPORARELY STOPPED (YEARS)
		YES	NO			
Cigarettes						
Pipe						
Cigar						
Chewing tobacco						

9.3 a) On average, over the last month, have you been exposed to the tobacco smoke of others (*i.e. environmental tobacco smoke (ETS)*)?

☐ Not at all
☐ < 3 hours/day
☐ 3-8 hours/day
☐ 9 or more hours per day

b) Have you ever been exposed to ETS for more than 6 months?

☐ Yes ☐ No

↳ ☐ < 3 hours/day
☐ 3-8 hours/day
☐ 9 or more hours per day

Number of years _____

* See notes in manual

Now I will ask you in detail about the foods you eat

10. FOOD INTAKE

10.1 * How often (on the average) have you eaten the following items?

	During the last 12 months?					In your 30's (If subject 40 years or over)				In your teens?				As a child?			
Food	Never	servings per month	servings per week	servings per day	Serving Size	Never	Less	Same	More	Never	Less	Same	More	Never	Less	Same	More
Milk to drink incl. choc. milk & hot cocoa w/milk					☐ 125 ml (0.5 cup) ☐ 250 ml (1.0 cup) ☐ 375 ml (1.5 cup)												
Milk on cereal					☐ 60 ml (.25 cup) ☐ 125 ml (0.5 cup) ☐ 250 ml (1.0 cup)												
Milk/cream in tea/coffee					☐ 15 ml (1 tbsp) ☐ 30 ml (2 tbsp) ☐ 60 ml (4 tbsp)												
Milk desserts (tapioca, rice pudding)					☐ 125 ml (0.5 cup) ☐ 250 ml (1.0 cup)												
Hard cheese (to eat, in sandwich or mixed dish)					☐ 15 g (0.5 oz) ☐ 30 g (1 oz) ☐ 60 g (2 oz)												
Yogurt					☐ 125 ml (0.5 cup) ☐ 175 ml (single) ☐ 250 ml (1 cup)												
Ice-cream, ice milk or frozen yogurt					☐ 125 ml (0.5 cup) ☐ 250 ml (1.0 cup) ☐ 375 ml (1.5 cup)												
Cream soups made with milk					☐ 125 ml (0.5 cup) ☐ 160 ml (.67 cup) ☐ 250 ml (1.0 cup)												

* See notes in manual

Respondent I.D. # _____

	During the last 12 months?					In your 30's (If subject 40 years or over)				In your teens?				As a child?			
Food	Never	servings per month	servings per week	servings per day	Serving Size	Never	Less	Same	More	Never	Less	Same	More	Never	Less	Same	More
Canned salmon or sardines with bones					☐ 30 g (1 oz) ☐ 60 g (2 oz) ☐ 90 g (3 oz)												
Broccoli					☐ 60 ml (.25 cup) ☐ 125 ml (0.5 cup) ☐ 250 ml (1 cup)												
Dark leafy greens (bok choy, kale, gailan (Chinese broccoli), collards, dandelion greens)					☐ 60 ml (.25 cup) ☐ 125 ml (0.5 cup) ☐ 250 ml (1 cup)												
Dried peas or beans (navy, pinto, kidney)					☐ 60 ml (.25 cup) ☐ 125 ml (0.5 cup) ☐ 250 ml (1 cup)												
Whole wheat buns, bread, rolls, bagels					☐ 1 serving = 1 slice ½ bagel ½ pita												
White bread, buns, rolls, bagels, etc.					☐ 1 serving = 1 slice ½ bagel ½ pita												
Tofu					☐ 60 ml (.25 cup) ☐ 125 ml (0.5 cup) ☐ 250 ml (1 cup)												
Multivitamin, Vit. D or cod liver oil					☐ 1 supplement												
Calcium suppl. or "TUMS"					☐ 200 mg ☐ 300 mg ☐ 500 mg												

Respondent I.D. # _____

Now some questions about the liquids/fluids you might choose to drink.

BEVERAGES *

10.2 How many of the following drinks did you consume?

In these questions, one serving of alcoholic beverage is:
- *1 bottle or can of beer or a glass of draft (12 oz):*
- *1 glass of wine or a wine cooler (4-5 oz)*
- *1 straight or mixed drink with (1-1½ oz) hard liquor*
- *1 serving of tea or coffee is 6 oz*
- *1 serving of cola is 12 oz - 1 can (355 ml)*

Beverages		During the past 12 months?				In your 30's (If subject is 40 years or over)				When in your teens?			
		None	Serving /month	Serving /week	Serving /day	None	Less	Same	More	None	Less	Same	More
Coffee	caffeinated												
	decaffeinated												
Tea	caffeinated												
	decaffeinated												
Colas	caffeinated												
	decaffeinated												
Alcoholic beverages													

* See notes in manual

Respondent I.D. # _____

In this section I will ask you about your physical activities and exercise.

11. Physical Activity

11.1 During a typical week in the past 6 months, how much time did you usually spend walking to work or school or while doing errands?

- ☐ None
- ☐ Less than 1 hour
- ☐ Between 1-5 hour
- ☐ Between 6-10 hours
- ☐ Between 11-20 hours
- ☐ More than 20 hours

11.2 Which of the following describes the paid work you usually do or what you consider your job? Or if retired or unemployed, which best describes your (*past or longest*) job?

- ☐ I am usually sitting during the day and do not walk around very much
- ☐ I stand or walk quite a lot during the day but I do not have to lift or carry heavy things
- ☐ I usually lift or carry light loads or I often have to climb stairs or hills
- ☐ I do heavy work or have to carry loads

11.3 Do you currently participate in any regular activity or programme (*either on your own or in a formal class*)?

☐ Yes ☐ No

→ How many times a week? _____

→ How long per session ? _____ minutes

11.4* On the average, during the last year, how many hours <u>in a week</u> did you spend in the following activities?

	Never	1/2-1 hr	2-3 hrs	4-6 hrs	7-10 hrs	11-20 hrs	21-30 hrs	31 hrs +
STRENUOUS SPORTS (such as jogging, bicycling on hills, tennis, racquetball, swimming laps, aerobics)								
VIGOROUS WORK (such as moving heavy furniture, loading or unloading trucks, shovelling, weight lifting, or equivalent manual labour)								
MODERATE ACTIVITY (such as housework, brisk walking, golfing, bowling, bicycling on level ground, gardening)								

11.5 * On the average, during the last year, how many hours <u>in a day</u> did you spend in the following sitting activities?

	Never	Less than 1 hr	1 to 2 hrs	3 to 4 hrs	5 to 6 hrs	7 to 10 hrs	11 hrs or more
Sitting in car or bus							
Sitting at work							
Watching TV							
Sitting at meals							
Other sitting activities (such as reading, playing cards, sewing)							

11.6 On the average, during the last year, how many hours <u>in a day</u> did you sleep (*include naps*)?

☐ 5 hours or less ☐ 7 hours ☐ 9 hours
☐ 6 hours ☐ 8 hours ☐ 10 hours or more

* See notes in manual

11.7 * Rate your overall level of physical activity compared to your peers during certain times in your past life.

	When you were about 50 ~~if subject 60 y. and over~~	When you were about 30 ~~if subject 40 y. and over~~	Teenager	Child
A lot less active				
Somewhat less active				
About the same				
Somewhat more active				
A lot more active				

Now I want to ask you questions about being in the sunlight

12. Sunlight Exposure

12.1 * Did you ever expose a considerable part of your body to direct sunlight?

A. During the past 12 months?
- ☐ never
- ☐ seldom
- ☐ regularly
- ☐ often

If 60 years old or more.

B. When you were about 50 years old?
- ☐ never
- ☐ seldom
- ☐ regularly
- ☐ often

If 40 years old or more.

C. When you were about 30 years old?
- ☐ never
- ☐ seldom
- ☐ regularly
- ☐ often

For all.

D. When you were a child or teenager?
- ☐ never
- ☐ seldom
- ☐ regularly
- ☐ often

* See notes in manual

PLEASE ADMINISTER THE MMSE
HERE IF RESPONDENT
MEETS CRITERIA

Now I would like to ask you how your health has been on the average, over the past week. I will ask you about different areas of general health. For some of the questions, I want you to tell me which statement most closely describes how you felt.

13. HEALTH STATUS QUESTIONNAIRE : * TORRANCE QUESTIONNAIRE

INTERVIEWER ADMINISTERED VERSION

NOTE to interviewer: For each question that lists a number of choices, circle the letter for the one choice that the respondent feels best describes the usual level of ability over the past week.

1.1 Are you able to see well enough without glasses or contact lenses to read ordinary newsprint?

- ☐ Yes - Go to 2.1
- ☐ No

1.2 If not, which of the following describes your *usual* ability to see well enough to read ordinary newsprint? Are you:

a. Able to see well enough but with glasses or contact lenses.
b. Unable to see well enough even with glasses or contact lenses.
c. Unable to see at all.

2.1 Are you able to see well enough without glasses or contact lenses to recognize a friend on the other side of street?

- ☐ Yes - Go to 3.1
- ☐ No

2.2 If not, which one of the following best describes your *usual* ability to see well enough to recognize a friend on the other side of the street? Are you:

a. Able to see well enough but with glasses or contact lenses.
b. Unable to see well enough even with glasses or contact lenses.
c. Unable to see at all.

---

* GW Torrance and DH Feeny, McMaster University
Questionnaire development supported through research grants funded by the
Ontario Ministry of Health and US Agency for Health Care Policy and Research.

3.1 Are you able to hear what is said in a group conversation with at least three other people *without* a hearing aid?

☐ Yes → Go to 4.1
☐ No

3.2 If not, which statement describes your *usual* ability to hear in a group conversation with at least three other people? Are you:

a. Able to hear what is said with a hearing aid.
b. Unable to hear what is said even with a hearing aid.
c. Unable to hear what is said, but don't wear a hearing aid.
d. Unable to hear.

4.1 Are you able to hear what is said in a conversation with one other person in a quiet room without a hearing aid?

☐ Yes → Go to 5.1
☐ No

4.2 If not, which one of the following best describes your usual ability to hear what is said in a conversation with one other person in a quiet room? Are you:

a. Able to hear what is said with a hearing aid.
b. Unable to hear what is said even with a hearing aid.
c. Unable to hear what is said, but don't wear a hearing aid.
d. Unable to hear.

5.1 Are you able to be understood when speaking the same language with strangers?

☐ Yes → Go to 6.1
☐ No

5.2 If not, which of the following best describes your *usual* ability to be understood when speaking the same language with strangers? Are you:

a. Able to be understood partially.
b. Unable to be understood.
c. Unable to speak at all.

6.1 Are you able to be understood when speaking the same language with people who know you well?

☐ Yes → Go to 7.1
☐ No

6.2 If not, which of the following best describes your *usual* ability to be understood when speaking the same language with people who know you well? Are you:

a. Able to be understood partially.
b. Unable to be understood.
c. Unable to speak at all.

7.1 Which one of the following best describes how you usually feel? Are you:

a. Happy and interested in life.
b. Somewhat happy.
c. Somewhat unhappy.
d. Very unhappy.
e. So unhappy that life is not worthwhile?

8.1 Are you free of pain and discomfort?

☐ Yes → Go to 9.1
☐ No

8.2 If not, which one of the following best describes your level of pain? Do you have:

a. Mild to moderate pain that prevents no activities.
b. Moderate pain that prevents a few activities.
c. Moderate to severe pain that prevents some activities.
d. Severe pain that prevents most activities.

9.1 Are you able to walk around the neighbourhood **without** difficulty and **without** walking equipment, and have no health limitation in vigourous activities such as running and strenuous sports?

NOTE: Walking equipment refers to mechanical supports such as braces, a cane, crutches or a walker.

☐ Yes → Go to 10.1
☐ No

9.2 If not, which of the following best describes your *usual* ability to walk. Are you:

a. Able to walk around the neighbourhood without difficulty and without walking equipment, and have some health limitation in vigourous activities such as running and strenuous sports.

b. Able to walk around the neighbourhood with difficulty, but without walking equipment or a helper.

c. Able to walk around the neighbourhood with walking equipment, but without a helper.

d. Able to walk only short distances with walking equipment. Able to walk short distances with a helper, and require a wheelchair to get around the neighbourhood.

e. Unable to walk alone, even with walking equipment. Able to walk short distances with a helper, and require a wheelchair to get around the neighbourhood.

f. Cannot walk at all.

10.1 Do you have full use of two hands and ten fingers?

☐ Yes → Go to 11.1
☐ No

10.2 If not, which of the following best describes your usual ability to use your hands and fingers? Do you have:

a. Limited use of hands or fingers, but do not require special tools or help from others.

b. Limited use of hands or fingers, require special tools but do not require help from others.

c. Limited use of hands or fingers, require the help of another person for some tasks.

d. Limited use of hands or fingers, require the help of another person for most tasks.

e. Limited use of hands or fingers, require the help of another person for all tasks.

11.1 Are you able to remember most things?

☐ Yes → Go to 12.1
☐ No

11.2 If not, which of the following best describes your *usual* ability to remember things?

a. Somewhat forgetful.

b. Very forgetful.

c. Unable to remember anything at all.

12.1 Are you able to think clearly and solve day to day problems?

☐ Yes → Go to 13.1
☐ No

12.2 If not, which of the following best describes your usual ability to think and solve day to day problems?

Do you:

a. Have a little difficulty when trying to think and solve day to day problems.
b. Have some difficulty when trying to think and solve day to day problems.
c. Have great difficulty when trying to think and solve day to day problems.

or are you:

d. Unable to think or solve day to day problems.

JUST A FEW MORE QUESTIONS:

13.1 Do you eat, bathe, dress and use the toilet normally?

☐ Yes → Go to 14.1
☐ No

13.2 If not, which of the following best describes your usual ability to perform these basic activities?

a. Eat, bathe, dress and use the toilet **independently, with difficulty.**
b. Requires mechanical equipment to eat, bathe, dress or use the toilet independently.
c. Requires the help of another person to eat, bathe, dress or use the toilet.

14.1 Are you generally happy and free from worry?

☐ Yes → Go to 15.1
☐ No

14.2 If not, which of the following best describes how you usually feel?

a. Occasionally fretful, angry, irritable, anxious or depressed.
b. Often fretful, angry, irritable, anxious or depressed.
c. Almost always fretful, angry, irritable, anxious or depressed.
d. Extremely fretful, angry, irritable, anxious or depressed, usually requiring hospitalization or psychiatric institutional care.

This is the last question. It is a different question about pain. Just to remind me:

15.1 Are you free of pain and discomfort?

☐ Yes → *That ends the questionnaire. Thank you for your help.*
☐ No

15.2 If not, which one of the following best describes your usual level of pain?

a. Occasional pain. Discomfort relieved by non-prescription drugs or self-control activity without disruption of normal activities.

b. Frequent pain. Discomfort relieved by oral medicines with occasion disruption of normal activities.

c. Frequent pain. Frequent disruption of normal activities. Discomfort requires prescription narcotics for relief.

d. Severe pain. Pain not relieved by drugs and constantly disrupts normal activities.

In this section, I will give you a small questionnaire for you to complete by yourself. For each question, you are asked to read the question, and then circle the number you choose as closest to your experience.

14. RAND HEALTH SCIENCE PROGRAM (SF-36)

1. In general, would you say your health is:

(Circle One Number)

Excellent 1
Very good 2
Good 3
Fair 4
Poor 5

2. Compared to one year ago, how would you rate your health in general now?

(Circle One Number)

Much better than one year ago 1
Somewhat better now than one year ago 2
About the same 3
Somewhat worse now than one year ago 4
Much worse now than one year ago 5

3. The following items are about activities you might do during a typical day. Does your health now limit you in these activities? If so, how much?

(Circle One Number on Each Line)

ACTIVITIES	Yes, limited a lot	Yes, limited a little	No, not limited at all
a. Vigorous activities, such as running, lifting heavy objects, participating in strenuous sports...	1	2	3
b. Moderate activities, such as moving a table, pushing a vacuum cleaner, bowling or playing golf...	1	2	3
c. Lifting or carrying groceries...	1	2	3
d. Climbing several flights of stairs...	1	2	3
e. Climbing one flight of stairs...	1	2	3
f. Bending, kneeling or stooping...	1	2	3
g. Walking more than one mile...	1	2	3
h. Walking several blocks...	1	2	3
i. Walking one block...	1	2	3
j. Bathing or dressing yourself...	1	2	3

4. During the past 4 weeks, have you had any of the following problems with your work or regular daily activities as a result of your physical health?

(Circle One Number on Each Line)

	Yes	No
a. Cut down the amount of time you spent on work or other activities...	1	2
b. Accomplished less than you would like...	1	2
c. Were limited in the kind of work or other activities...	1	2
d. Had difficulty performing the work or other activities (for example, it took extra effort)...	1	2

5. During the past 4 weeks, have you had any of the following problems with your work or other regular daily activities as a result of any emotional problems (such as feeling depressed or anxious)?

(Circle One Number on Each Line)

	Yes	No
a. Cut down the amount of time you spent on work or other activities...	1	2
b. Accomplished less than you would liked...	1	2
c. Didn't do work or other activities as carefully as usual...	1	2

6. During the past 4 weeks, to what extent has your physical health or emotional problems interfered with your normal social activities with family, friends, neighbours, or groups?

(Circle One Number)

Not at all 1
Slightly 2
Moderately 3
Quite a bit 4
Extremely 5

7. How much bodily pain have you had during the past 4 weeks?

(Circle One Number)

None 1
Very mild 2
Mild 3
Moderate 4
Severe 5
Very severe 6

8. During the past 4 weeks, how much did pain interfere with your normal work (including both work outside the home and housework)?

(Circle One Number)

Not a bit 1
A little bit 2
Moderately 3
Quite a bit 4
Extremely 5

9. These questions are about how you feel and how things have been with you during the past 4 weeks. For each question, please give the one answer that comes closest to the way you have been feeling.

How much of the time during the past 4 weeks.....

(Circle One Number on Each Line)

	All of the time	**Most of the time**	**A good bit of the Time**	**Some of the time**	**A little of the time**	**None of the time**
a. Did you feel full of pep?	1	2	3	4	5	6
b. Have you been a very nervous person?	1	2	3	4	5	6
c. Have you felt so down in the dumps that nothing could cheer you up?	1	2	3	4	5	6
d. Have you felt calm and peaceful?	1	2	3	4	5	6
e. Do you have a lot of energy?	1	2	3	4	5	6
f. Have you felt downhearted and blue?	1	2	3	4	5	6
g. Did you feel worn out?	1	2	3	4	5	6
h. Have you been a happy person?	1	2	3	4	5	6
i. Did you feel tired?	1	2	3	4	5	6

10. During the past 4 weeks, how much of the time has your physical health or emotional problems interfered with your social activities (like visiting with friends, relatives, etc.)?

(Circle One Number)

All of the time 1
Most of the time 2
Some of the time 3
A little of the time 4
None of the time 5

11. How TRUE or FALSE is <u>each</u> of the following statements for you?

(Circle One Number on Each Line)

	Definitely True	Mostly True	Don't Know	Mostly False	Definitely False
a. I seem to get sick a little easier than other people...	1	2	3	4	5
b. I am as healthy as anybody I know...	1	2	3	4	5
c. I expect my health to get worse...	1	2	3	4	5
d. My health is excellent...	1	2	3	4	5

THAT ENDS THE QUESTIONNAIRE. THANK YOU VERY MUCH FOR YOUR HELP.

Respondent I.D. # _____

INTERVIEWER'S ASSESSMENT

As an interviewer my assessment of the process and the respondent was:

(Circle One Number on Each Line)

	Not at all	Not much	Moderate	Somewhat	A great deal
a. The respondent appeared or seemed interested in the research	1	2	3	4	5
b. The respondent seemed to cooperate with me	1	2	3	4	5
c. I believe that the respondent understood the questions	1	2	3	4	5
d. I believe that the respondent listened well	1	2	3	4	5
e. I perceived that the respondent was restless or wanted to hurry the process	1	2	3	4	5
f. The respondent expressed feelings of tiredness during the interview	1	2	3	4	5

The respondent required assistance with the Rand SF-36 ☐ Yes ☐ No

Comments :

_____

_____

_____

_____

_____

_____

_____

_____

Time finished _____ hrs _____ min.

Follow-up Questionnaire

This questionnaire was designed to find out how you have been since (date of last questionnaire).

Please indicate your answer by putting a check in the appropriate box.

1. a) Have you broken one or more bones in the past year?

   ☐ Yes ☐ No → *Go to question # 3*

   ↓

   b) How many times have you fractured a bone, in the last year? _____

2. a) Which bone(s) were broken the last time that you had a fracture, in the last year?

   ☐ Back
   ☐ Hip
   ☐ Ribs
   ☐ Forearm / Wrist
   ☐ Pelvis
   ☐ Other _____

   b) For the most recent incident, how did it happen?

   ☐ Fell out of bed or off a chair
   ☐ Fell climbing a chair or ladder
   ☐ Fell on stairs
   ☐ Motor vehicle accident
   ☐ Sporting injury *(i.e. skiing, playing hockey, cycling, running or jogging, etc.)*
   ☐ Slipped or tripped in home *(on carpet, wet floor, getting in/out of bath, etc)*
   ☐ Slipped or tripped and fell outside the home other than sporting *(on ice, on the curb, etc)*
   ☐ Heavy object fell or struck body causing the fracture
   ☐ Bone(s) broke with no fall or injury
   ☐ Other → Specify: _____

(Please turn over)

RESPONDENT I.D. #

3. a) Have you had any hospital admissions in the past year?

❑ Yes ❑ No

↓

b) For what reason? *(Check all that apply)*

- ❑ Heart disease
- ❑ Pregnancy
- ❑ Breast cancer
- ❑ Cancer of the uterus
- ❑ Other cancer *(specify)*: _____
- ❑ Removal of the uterus
- ❑ Removal of ovaries
- ❑ Other surgery *(specify)*: _____
- ❑ Other hospital admission *(specify)* _____

4. Have you taken any of these medications in the past year? *(Circle all that apply)*

❑ Yes ❑ No

↓

Alendronate	Clodronate	Estrogen	Pamidronate
Aredia	Deca-Durabolin	Etidronate	Premarin
Bonefos	Didrocal	Fluotic	Progesterone
Calcimar	Didronel	Fosamax	Prometrium
Calcitonin	Durabolin	Nandrolone	Provera
Calcitriol	Estracomb	Ogen	Rocaltrol
C.E.S.	Estraderm	Ostac	Tamoxifen
Climacteron	Estrace	Ostoforte	Vivelle

ADDITIONAL INFORMATION

5. Date of birth: ____ / ____ / 19____ (Day / Month / Year) Sex: ❑ Male ❑ Female

Comments:

DATE ____/____/____ (Day / Month / Year)

PLEASE RETURN THE QUESTIONNAIRES IN THE POSTAGE PAID ENVELOPE PROVIDED.

THANK YOU!

Fracture Questionnaire

1. *Incident #* _____

2. Which bone was broken?
   - ☐ Back
   - ☐ Hip
   - ☐ Ribs
   - ☐ Forearm / Wrist
   - ☐ Pelvis
   - ☐ Other _____

3. How did it happen?
   - ☐ Fell out of bed or off a chair
   - ☐ Fell climbing a chair or ladder
   - ☐ Fell on stairs
   - ☐ Motor vehicle accident
   - ☐ Sporting injury *(i.e. skiing, playing hockey, cycling, running or jogging, etc.)*
   - ☐ Slipped or tripped in home *(on carpet, wet floor, getting in/out of bath, etc)*
   - ☐ Slipped or tripped and fell outside the home other than sporting *(on ice, on the curb, etc)*
   - ☐ Heavy object fell or struck body causing the fracture
   - ☐ Bone(s) broke with no fall or injury
   - ☐ Other → Specify: _____

4. What was the date of the fracture? _____ Month _____ Year ☐ don't know

5. a) Were X-rays of the fracture taken?
   - ☐ Yes
   - ☐ No → *Go to question 6.a)*

   b) What was the date of the X-rays? _____ Month _____ Year ☐ don't know

   c) At what clinic/hospital were the X-rays done? _____ ☐ don't know

6. a) Was the fracture treated?
   - ☐ Yes → *Go to question 6.b)*
   - ☐ No → *Go to question 8*

   b) Where was the fracture treated?
   - ☐ in hospital
   - ☐ in physician's office → *Go to question 6.d)*
   - ☐ in home → *Go to question 7*

RESPONDENT I.D. # _____

6. c) IN HOSPITAL Date: _____ (Month) _____ (Year)

❑ in emergency clinic - OR - ❑ in-patient

Hospital name: _____ Length of stay: _____ days

Treating doctor: _____

Treatment received: ❑ Surgery ❑ Cast ❑ Other → specify _____

Where did you go when you left the hospital? *(Pick one from the list)*

❑ Home

❑ Rehabilitation centre ┐→ What was the name? _____

❑ Convalescent home ┘ How long did you stay? _____ days

❑ Other : specify _____

d) IN PHYSICIAN'S OFFICE

Physician's name? _____

Date of the first visit? _____ (Month) _____ (Year) Total number of visits? _____

Treatment received: ❑ Surgery ❑ Cast ❑ Other → specify _____

7. Were you treated with physiotherapy?

❑ Yes ❑ No → *Go to question 8*

↓

How many visits per week? _____ How many weeks? _____

8. Were you visited by an occupational therapist? ❑ Yes ❑ No

9. Since the fracture:

Have you given up any of your usual daily activities? ❑ Yes ❑ No
(i.e. household chores, personal hygiene)

Do you go out? ❑ less often ❑ the same ❑ more often

THANK YOU!

DATE _____/_____/_____ (Day / Month / Year)