V. RESULTS

A. Overview of Participants and Non-participants in the STORM-EBT examination

1. Baseline characteristics of men who participate and did not participate in STORM-EBT examination

Baseline characteristics of participants and non-participants in STORM-EBT examination are shown in Table 23. Men who participated in EBT examination were younger, and taller than men who did not participate in the examination. The mean age of participants at baseline examination were 64.1 years (SD: 6.25) compared with 68.1 years (SD: 7.63) among non-participants (p-value < 0.001). Participants were taller than non-participants in measured baseline height and reported height at age 25 years.

Systolic blood pressure was lower among participants than that of non-participants. Mean systolic blood pressure of participants was 138.3 ± 13.44 mmHg compared with 141.8 ± 15.53 mmHg among non-participants (p=0.010). The frequency of current smoker and ever-smoker were similar between two groups. However, mean pack-years for smokers were significantly less among participants than non-participants (31.6 ± 24.1 years; 38.7 ± 27.3 years, respectively). The medical history of two groups did not differ significantly except the history of MI and angina. Of interest, the frequency of myocardial infarction (heart attack) and angina were greater (p< 0.001) among non-participants. Men who participated in STORM EBT examination are less likely to have a history of heart attack than people who did not participate, 3.5 % for participants and 16.2% for non-participants, respectively. In addition, the frequency of angina shows a similar pattern, and approximately 22.4% of non-participants experienced more previous chest pain (Angina) compared with 9.9% of participants.

However, there was no significant difference in history of fracture, family history of osteoporosis, use of oral glucocorticoids or thiazide diuretics. Furthermore, there was no significant difference in bone mineral density (BMD) of total hip among participants

and non-participants (Table 24). There was no difference in lipid measurement at baseline among participants and non-participants.

Table 23. Baseline Characteristics of participants and non-participants in the STORM-EBT examination

	Participants (n=144)	Non-participants (n= 397)	p-value
Age (yrs)	**64.14 (6.25)**	**68.12 (7.63)**	**< 0.001**
Height (cm)			
Baseline	**174.55 (5.88)**	**173.11 (6.61)**	**0.021**
At Age 25	**177.46 (5.71)**	**175.98 (6.70)**	**0.012**
Weight (Kg)			
Baseline	83.97 (12.39)	83.16 (13.57)	0.530
Age 25 years	73.49 (10.85)	73.79 (11.02)	0.782
BMI (kg/m2)	27.54 (3.63)	27.70 (3.94)	0.658
Waist to Hip ratio	0.93 (0.04)	0.93 (0.05)	0.139
Systolic BP (mmHg)	**138.30 (13.44)**	**141.84 (15.53)**	**0.010**
Diastolic BP (mmHg)	84.40 (6.73)	84.53 (7.27)	0.844
Education (years)[+]	12.63 (2.39)	12.27 (2.55)	0.181
Smoking			
Current smokers, n (%)	15 (10.42)	37 (9.32)	0.702
Ever smokers, n (%)	110 (76.39)	280 (70.53)	0.179
Pack-years for smokers [+]	**31.62 (24.10)**	**38.73 (27.32)**	**0.022**
Alcohol intake (drinks/wk) [+]	6.44 (14.11)	5.63 (10.50)	0.131
Dietary calcium intake (mg/day)[+]	625.90 (503.17)	608.86 (393.53)	0.706
Calcium supplement use, n(%)	11 (7.64)	31 (7.81)	0.948
Vit D supplement use, n(%)	4 (2.78)	10 (2.53)	0.874
Physical activity [+]			
Baseline (METS/day)	2.93 (2.96)	2.82 (2.48)	0.711
Historical (METS/day)	4.07 (4.76)	4.84 (5.81)	0.330
Back related disability score [+]	1.10 (2.05)	1.18 (1.92)	0.230

Values are presented as mean (SD) or n (%)

[+] : P-value from Wilcoxon Two-sample T-test

Table 23 (continued)

	Participants (n=144)	Non-participants (n= 397)	p-value
Health Status (fair/poor), n (%)	26 (18.06)	89 (22.42)	0.273
Any Fracture since age 50, n (%)	31 (21.53)	72 (18.32)	0.403
Osteoporosis, n (%)[#]	1 (0.70)	6 (1.52)	0.461
Fall last year, n (%)	26 (18.06)	65 (16.37)	0.644
Arthritis, n (%)	55 (39.86)	147 (37.69)	0.653
Hypogonadism, n (%)[#]	1 (0.70)	2 (0.52)	0.805
Infertility, n (%)[#]	1 (0.69)	4 (1.02)	0.791
Hyperthyroidism, n (%)[#]	1 (0.70)	8 (2.05)	0.288
Diabetes, n (%)	11 (7.75)	46 (11.70)	0.190
Hypertension, n (%)	31 (21.53)	113 (28.46)	0.106
Myocardial infarction, n (%)	**5 (3.47)**	**64 (16.24)**	**< 0.001**
Angina, n (%)	**14 (9.86)**	**89 (22.42)**	**0.001**
Stroke, n (%)	3 (2.10)	21 (5.32)	0.110
COPD, n (%)	8 (5.63)	28 (7.11)	0.548
Cancer, n (%)	12 (8.33)	45 (11.42)	0.303
Oral glucocorticoid, n (%)	4 (2.78)	14 (3.53)	0.792
Thiazide diuretics, n (%)			
Past	13 (9.03)	46 (11.62)	
Current	12 (8.33)	49 (12.37)	0.250
Thyroid hormone use, n (%)[#]	**0**	**10 (2.52)**	**0.070**
HMG-CoA reductase, n (%) [#]	**0 (0.0)**	**11 (2.77)**	**0.042**
Calcium channel blocker, n (%)	**19 (13.19)**	**83 (20.91)**	**0.043**
Family history of osteoporosis, n(%)	52 (36.11)	116 (29.22)	0.126

[#] : Fisher Exact Test

Table 24. Baseline bone density and serum levels of lipid of participants and non-participants in STORM-EBT examination

	Participants	Non-participants	p-value
Bone mineral Density (g/cm^2)	(n=143)	(n= 397)	
Total hip	0.961 (0.14)	0.949 (0.14)	0.368
Femoral Neck	0.776 (0.12)	0.771 (0.13)	0.654
Trochanter	0.740 (0.12)	0.725 (0.12)	0.203
Intertrochanter	1.134 (0.16)	1.113 (0.17)	0.187
Lipid (mg/dL)	(n=144)	(n=395)	
HDL-c	47.72 (10.56)	47.27 (11.02)	0.668
HDL2-c [&]	5.18 (2.57)	4.90 (2.82)	0.588
HDL3-c	40.68 (8.00)	40.25 (7.88)	0.581
LDL-c	138.53 (35.48)	138.62 (34.18)	0.978
Triglycerides [&]	133.09(1.65)	128.68 (1.68)	0.545
Total Cholesterol	217.52 (36.38)	216.13 (36.68)	0.696

Values are mean (SD)

[&] : Analyses were performed on log-transformed variables

2. Follow-up characteristics of men who participate and did not participate in STORM-EBT examination

Men who participated in STORM-EBT examination were recruited from the participants of the follow-up examination. Participants of STORM-EBT examination were compared with men who only joined the study during follow-up examination. Characteristics of participants and non-participants based on follow-up examination are described in Table 25 through 27. The average age of STORM-EBT participants was 70.9 $\pm$ 6.3 years compared with 74.7 $\pm$ 7.4 years among all non-participants ($p < 0.001$). In general, men who participated in the STORM-EBT examination were: younger, taller, more active, and drink more alcohol containing beverages. They were less likely to experience heart attack (myocardial infarction) (p=<0.001), and angina (p=0.009). In addition, they were less likely to use selected medications (thiazide diuretics, HMG-CoA reductase, and calcium channel blocker).

Although BMD at follow-up examination did not differ by participation in STORM-EBT examination, the rate of change in BMD among participants was less than the rate of change in BMD among non-participants (Table 26). This pattern only remained significant at the femur neck (p=0.040) after adjusting for age. Further adjustment for weight changes since baseline yielded no significant difference between participants and non-participants. Broadband ultrasound attenuation (BUA) and speed of sound (SOS) was assessed through the calcaneus quantitative ultrasound (QUS) measurement. QUS parameters were not significantly different among men who participate and those who did not participate in EBT examination. Two biochemical bone turnover markers, osteocalcin as a bone formation marker and N-telopeptide (NTx) as a bone resorption marker was measured at follow up examination. There was no significant difference among participants and non-participants in the levels of bone turnover markers (Table 26). However, the serum osteocalcin concentration in our study sample is approximately 57% higher than those of reported values at similar age groups of men (Gallagher et al., 1998). The mean (SD) value of urinary N-telopeptide level (mean: 35.32 nmolBCE/mmol Cr) was similar to those of reported values of younger men (mean: 35.7) and older men (mean: 36.3). The range of NTx was, however, quite large ranged from 14 to 378 nmol BCE/mmol Cr in our sample.

Serum levels of sex steroid hormones or C-reactive protein did not differ by participation in EBT examination. However, the serum levels of sex hormone binding globulin were significantly different between participants and non-participants. The significant difference was not persistent after adjustment for age (Table 27).

Table 25. Follow-up characteristics of participants and non-participants in the STORM-EBT examination

	Participants (n=144)	Non-participants (n=183)	p-value
Age (yrs)	**70.93 (6.27)**	**74.66 (7.43)**	**<0.001**
Weight (kg)	84.70 (13.21)	83.12 (14.12)	0.308
Weight changes since baseline	**0.69 (5.71)**	**-0.73 (4.90)**	**0.017**
Height (cm)	**173.73 (5.91)**	**172.33 (6.19)**	**0.040**
BMI (kg/m^2)	28.03 (3.87)	27.96 (4.29)	0.886
Waist circumference (cm)	99.81 (9.36)	98.88 (9.81)	0.398
Waist to Hip Ratio (WHR)	0.97 (0.05)	0.96 (0.05)	0.321
Diastolic blood pressure, mmHg	77.08 (8.74)	75.82 (9.75)	0.238
Systolic blood pressure, mmHg	136.79 (17.19)	139.01 (20.37)	0.301
Dietary calcium intake (mg/day) [+]	789.76 (399.57)	765.91 (380.14)	0.524
Total calcium intake (mg/day) [+]	1022.44 (567.99)	941.93 (491.57)	0.291
Total vitamin D intake (IU/day) [+]	350.67 (223.62)	320.29 (210.91)	0.358
Calcium supplement use, n (%)	19 (13.29)	19 (10.80)	0.494
Vit D supplement use, n (%)	3 (2.10)	7 (3.98)	0.521
Physical activity (Kcal/day) [+]	**353.84 (655.93)**	**185.37 (119.37)**	**0.016**
Current Cigarette use, n (%)	9 (6.29)	6 (3.41)	0.226
Alcohol Intake (drinks/wk) [+]	**5.29 (10.28)**	**4.61 (9.86)**	**0.078**
Caffeine consumption (mg/day) [+]	232.64 (201.79)	213.25 (206.27)	0.378
Back related disability score [+]	1.11 (1.95)	1.15 (1.95)	0.698
Health Status (fair/poor), n(%)	**29 (20.28)**	**51 (28.98)**	**0.075**

Values are mean (standard deviation) or n (%)

[+]: P-value from Wilcoxon two-sample test

Table 25 (continued)

	Participants (n=144)	Non-participants (n=183)	p-value
Any Fracture since baseline, n(%)	13 (9.03)	19 (8.74)	0.928
Osteoporosis, n(%)[#]	2 (1.43)	1 (0.58)	0.589
Fall in past year, n (%)	29 (20.57)	35 (20.35)	0.962
Vertebral fracture, n (%)	19 (13.38)	25 (14.45)	0.785
Osteoarthritis, n (%)	20 (14.18)	23 (13.37)	0.835
Rheumatoid arthritis, n (%)	5 (3.50)	6 (3.41)	0.966
Hyperthyroidism, n (%) [#]	0	3 (1.70)	0.256
Diabetes, n (%)	13 (9.09)	20 (11.36)	0.507
Hypertension, n (%)	66 (46.81)	80 (46.51)	0.958
Myocardial infarction, n (%)	**12 (8.39)**	**45 (25.57)**	**<0.001**
Angina, n (%)	**10 (7.09)**	**29 (16.86)**	**0.009**
Stroke, n (%)	9 (6.29)	15 (8.52)	0.453
COPD, n (%)	14 (9.29)	18 (10.23)	0.897
Cancer, n(%)	28 (19.58)	35 (19.89)	0.946
Oral glucocorticoid use, n (%)[#]	3 (2.14)	5 (2.91)	0.735
Thiazide diuretics, n (%)	**9 (6.43)**	**24 (13.95)**	**0.032**
HMG-CoA reductase use, n (%)	**15 (10.42)**	**32 (17.49)**	**0.070**
Calcium channel blocker, n (%)	**14 (9.72)**	**34 (18.58)**	**0.025**
Family history of osteoporosis, n (%)	54 (38.30)	52 (29.55)	0.101

[#] : Fisher exact test

Table 26. Follow up bone mineral density (BMD), rate of changes in BMD, QUS parameters and bone turnover markers of participants and non-participants in STORM-EBT examination

	Participants	Non-participants	p-value
BMD at follow-up (g/cm^2)	(n=143)	(n=183)	
Total hip	0.965 (0.14)	0.947 (0.16)	0.295
Femoral neck	0.776 (0.13)	0.765 (0.14)	0.481
Trochanter	0.744 (0.12)	0.735 (0.13)	0.507
Intertrochanter	1.134 (0.17)	1.106 (0.19)	0.178
Percent rate of change in BMD(%/yr)	(n=142)	(n=173)	
Total hip	-0.034 (0.70)	-0.257 (0.64)	**0.003**
Femoral neck	-0.076 (0.83)	-0.308 (0.78)	**0.012**
Trochanter	0.019 (0.85)	-0.152 (0.77)	**0.061**
Intertrochanter	-0.069 (0.75)	-0.296 (0.70)	**0.006**
Absolute rate of change in BMD ($mg/cm^2/yr$) (n=142)		(n=173)	
Total hip	-0.271 (6.73)	-2.422 (6.29)	**0.004**
Femoral neck	-0.573 (6.56)	-2.377 (6.22)	**0.013**
Trochanter	0.112 (6.55)	-1.118 (5.63)	**0.074**
Intertrochanter	-0.672 (8.43)	-3.314 (8.00)	**0.005**
Calcaneal QUS	(n=120)	(n=146)	
BUA (dB/MHz)	77.87 (16.42)	79.73 (16.43)	0.360
Speed of Sound (SOS) (M/S)	1554.7 (31.41)	1557.9 (31.47)	0.410
Bone turnover markers [&]	(n=140)	(n=174)	
Osteocalcin (ng/ml)	8.58 (1.48)	9.08 (1.57)	0.242
N-telopeptide (nM BCE/mM Cr)	35.26 (1.59)	35.69 (1.60)	0.823

Values are mean (SD)

[&] : Analysis was performed on log-transformed variable.

Table 27. Follow-up serum levels of sex steroid hormones and C-reactive protein of participants and non-participants in STORM-EBT examination

	Participants	Non-participants	p-value
Estradiol (pg/ml)			
Total	22.02 (7.18)	23.52 (8.92)	0.107
Bioavailable	13.59 (4.43)	14.10 (5.55)	0.382
Testosterone (ng/dL)			
Total	413 (148.05)	436.86 (155.82)	0.168
Bioavailable	129.42 (42.93)	126.98 (47.82)	0.638
Sex hormone binding globulin (μg/dL) [&]	**1.00 (1.67)**	**1.14 (1.50)**	**0.012**
C-reactive protein (mg/L) [&]	1.44 (2.64)	1.55 (2.46)	0.259

Values are mean (SD)

[&] : Analysis was performed using log-transformed variable (SHBG) or inversed variable (C-reactive protein)

3. Prevalence of clinical cardiovascular disease and coronary artery calcification (CAC) score of men who participated in STORM-EBT examination

a. Prevalence of clinical cardiovascular disease and Rose questionnaire

Prevalence of clinical cardiovascular disease was assessed at the time of EBT measurement (Table 28). 12% of men reported history of myocardial infarction, and 9% of men had stroke, angina, transient ischemic attack, or carotid surgery. Approximately 6% of men reported having chronic heart failure. Family history of cardiovascular disease was also obtained. A family history of cardiovascular disease defined as either a maternal or paternal history of CVD was reported from 60% of men. Among them, 4% of men reported having paternal history of CVD before age 50 years, and same number of men reported having maternal history of CVD before age 50. However, history for both of maternal and paternal CVD before age 50 was not reported.

Rose questionnaire for angina or intermittent claudication was addressed at the time of EBT measurement (Table 29). Three men had Rose positive angina but two of them

had a history of angina. One was categorized as Rose positive claudication and had no history of intermittent caludication.

Table 28. Updated clinical cardiovascular diseases and family history of cardiovascular diseases of men who participated in STORM-EBT examination

Clinical disease (n=144)	n (%)
History of myocardial infarction	17 (12.14)
History of angina	13 (9.29)
History of stroke, transient ischemic attack, or carotid surgery	12 (8.57)
History of CHF	8 (5.71)
History of intermittent claudication or peripheral vascular surgery	5 (3.57)
History of coronary artery bypass surgery, percutaneous transluminal angioplasty	7 (5.00)
Pacemaker	3 (2.14)
Family history of cardiovascular disease, n (%)	84 (60.0)
Paternal: Before age 50 years	6 (4.3)
Maternal: Before age 50 years	6 (4.3)

Table 29. Rose questionnaire for angina and intermittent claudication (IC) of participants in STORM-EBT examination

	No pain	Exertional pain	Rose positive
Rose questionnaire for angina pectoris (n=139)	103 (74.10)	33 (23.74)	3 (2.16)
Rose questionnaire for claudication (n=136)	107 (78.68)	28 (20.59)	1 (0.07)

b. Distribution of coronary artery calcification (CAC) score

Coronary artery calcification (CAC) score ranged from 0 to 10705.93 with median of 406.44 (Table 30). Seven men (5 %) had a zero score and more than 50% of participants had a CAC score grater than 400 (Figure 7). The median CAC score in our participants was comparable with other populations (Newman et al., 2001). The median CAC score increased with age and it was similar to Cardiovascular Health Study except for the individuals in the age group of 65 to 74 years, which had higher median score (Table 31 & Figure 8).

Table 30. Coronary artery calcification score of total participants in STORM-EBT examination

Sample (n = 144)	Median	Range
Calcium score		
Left main	0.00	0 – 352.89
LAD	208.37	0 -- 4264.88
LCX	36.90	0 – 2506.20
RCA	57.32	0 – 3934.85
Total	406.44	0 – 10705.93
Calcium volume score		
Left main	0.00	0 – 279.57
LAD	153.25	0 – 3424.19
LCX	28.67	0 – 1956.21
RCA	51.77	0 – 3064.58
Total	336.70	0 – 8444.98

Table 31. Median CAC scores by age group (N=144)

Age Group	n	%	Median CAC scores (Range)
< 65	30	20.4	151.72 (0 – 3211.72)
65 -69	32	21.8	401.14 (0 – 5494.85)
70-74	39	27.5	534.83 (0 -- 10705.93)
75-79	32	22.5	543.41 (1.03 – 4049.32)
80 >	11	7.7	788.17 (286.29 – 3933.98)

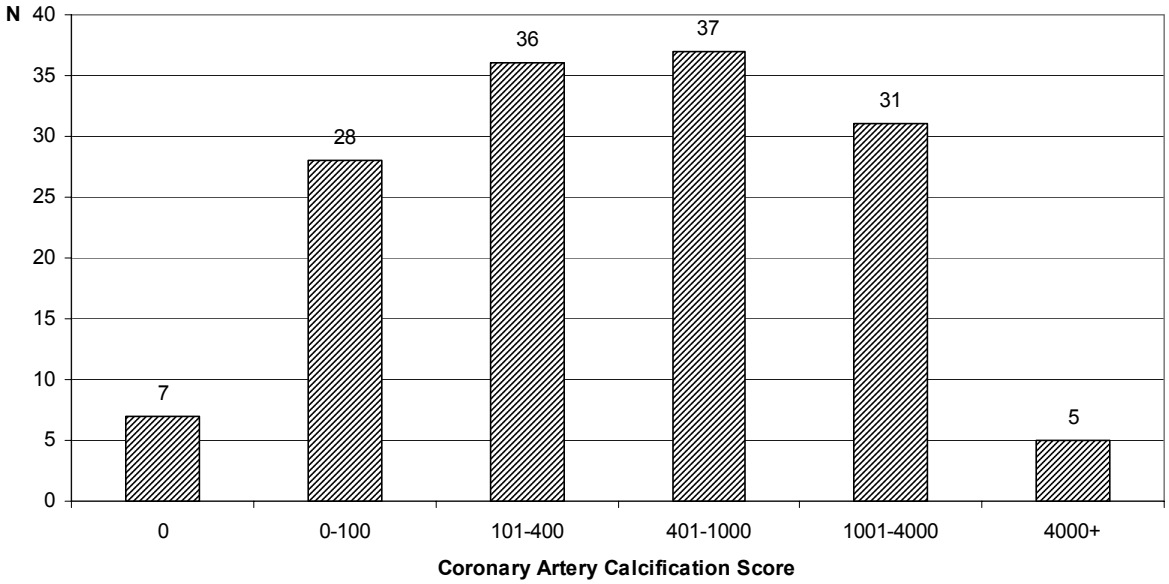

Figure 7. Distribution of CAC scores in STORM men

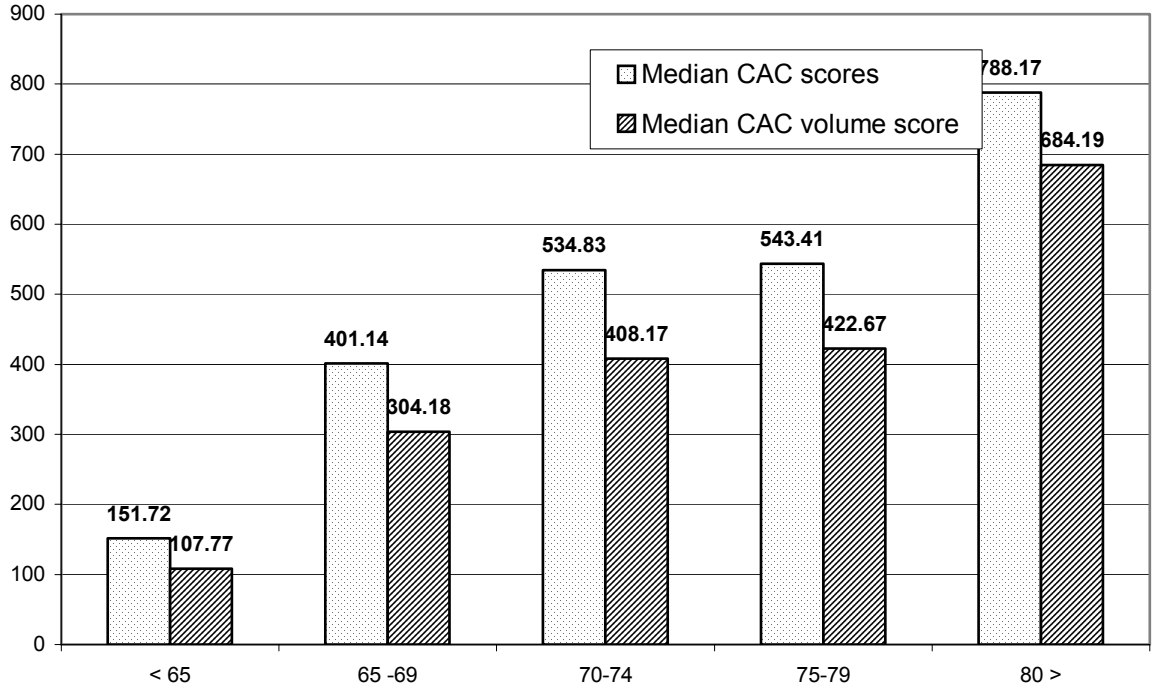

Figure 8 . Median CAC scores in men by age group (n=144)

B. Determinants of BMD, rate of change in BMD of study participants

1.Characteristics of the participants

Two of the 144 men who completed the EBT examination were excluded from further statistical analysis because they did not have a bone density measurement either at baseline or follow-up examination. Due to differences in BMD levels and small sample size, we excluded non-Caucasian men n=3). Three non-Caucasian men had higher BMD (1.06 $\pm$ 0.10 g/cm^2) of the total hip than mean values (0.96 $\pm$ 0.13 g/cm^2) of Caucasian men. Non-Caucasian men had 0.8 SD greater BMD of the total hip compared with white men. To analyze the sex steroid hormonal effects on BMD, or rate of change in BMD, we further excluded one man who was taking medications that influenced sex steroid hormones (anti-androgen). Thus, a total of 138 men were included in statistical analyses for evaluating risk factors that affect rate of change in BMD, CAC score, specific aim I (correlation between bone density, bone loss and CAC score), and specific aim 2-1 (mediating effect of estrogen, and C-reactive on the relationship between BMD and Calcification). In the analyses of osteoprotegerin (OPG) polymorphisms and BMD or CAC scores, we were able to complete genotyping on 134 men for OPG T-950C single nucleotide polymorphism (SNP), and 133 men for OPG G-1181C SNP.

The mean age of participants was 64.1 $\pm$ 6.3 years at the baseline examination and 72.9 $\pm$ 6.42 years at the STORM-EBT examination. The mean number of years between the baseline and EBT examination was 8.8 $\pm$ 0.3 years ranged from 8.3 to 9.6 years. The mean weight of the men in the sample was 84. 2 $\pm$ 12.4 kg and mean weight change from baseline to follow-up examination was 0.58 kg. The mean BMI was 27.6 and standard deviation was 3.6. 10% of the men were current smokers but men who smoked in the past and current comprised 76.1% of the sample. The median dietary calcium intake was 542 mg/day, and only 8% of men reported that they took additional calcium supplements. Averaged estimates of dietary calcium intake in our sample were

less than that of NHANES III in older men (Alaimo et al.,1994). The participants consumed an averaged six drinks of any alcoholic beverages per week. The median baseline physical activity presented as metabolic equivalent (MET value) was 2.25 and the median value of total energy expenditure for leisure time activity measured at follow up examination was 158.2 kcal/day. Men who were taking oral glucocorticoid, thiazide diuretics in past and currently, calcium channel blocking agents were 4 (3%), 12(9%) and 12 (9%), and 18 (13%), respectively, of the study participants. None of participants did not take HMG CoA reductase inhibitor (STATIN) at the baseline examination, but thirteen men (9%) reported the use of statin at the time of follow up examination.
Thirty men (22%) reported any fracture since age 50 years, and 53 men (40%) had arthritis. Men who had history of diabetes, hypertension, heart attack, and cancer were 11(8%), 29(21%), 5(4%), and 11(8%), respectively.

Mean BMD of the total hip (0.97 $\pm$ 0.13 g/cm^2), femoral neck (0.78 $\pm$ 0.12 g/cm^2) and trochanter (0.74$\pm$0.12 g/cm^2) in our study population were comparable with the mean values for older U.S men in the NHANES III estimates (Looker et al., 1998). The mean values of percentage rate of change in the total hip were –0.03 $\pm$ 0.68 %/yr at the total hip, -0.07 $\pm$0.82 %/yr at the femoral neck, 0.01 $\pm$ 0.78 %/yr at the trochanter, and –0.07 $\pm$ 0.73 %/yr at the intertrochanter, respectively. Annualized rate of change in the total hip and any hip subregion by age groups is shown in Figure 9. In men at age from 65 to 74 years, they gained bone while men over age 75 years lost bone at all sites. There was significant difference in the rate of change in the total hip across age groups (p-value 0.027). Using WHO definitions based on mean value of 20-29 year old white men, 5% (n=7) of our study participants were osteoporotic and 51% of men were osteopenic at the femur neck, respectively. The prevalence of osteoporosis (1%) and osteopenia (31%) was less likely when using the cutoff value based on total hip BMD.

The mean values of QUS parameters at the calcaneus among study participants (n=115) were 78.1 $\pm$ 15.9 dB/MHz of BUA, and 1555.3 $\pm$ 30.6 m/s of SOS, respectively. There were significant correlations between BMD and QUS parameters (BUA) ranged from 0.39 to 0.42 (Table 32), but we could not observe any significant relationship

between age and QUS parameters (Table 32). Neither did osteocalcin (r_s= -0.02, p=0.82) nor NTx (r_s= -0.05, p=0.54) correlated with age at follow-up examination. The correlation coefficients between BMD at each site and both osteocalcin and NTx were statistically significant ranged from –0.20 to -0.31 (Table 32). Retrospective analysis of bone loss and bone turnover markers also yielded similar results. Bone resorption marker, NTx seemed to show more strong correlation with total hip BMD and any other sites.

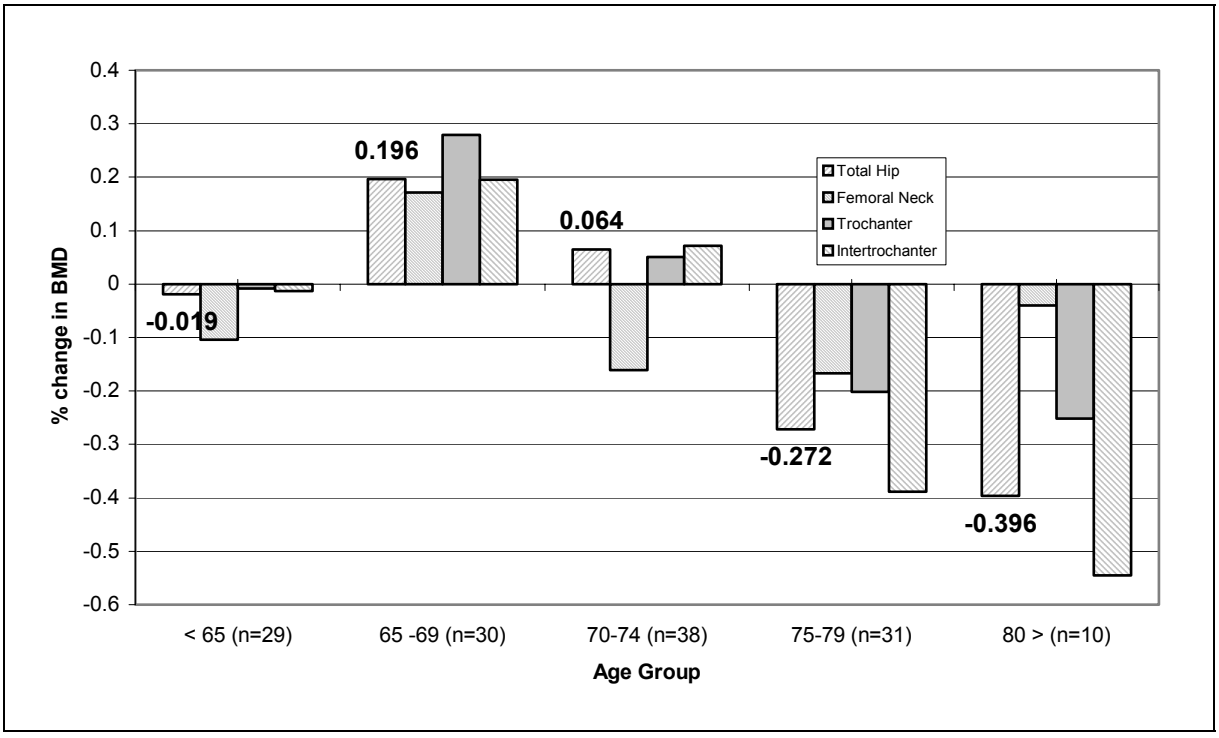

Figure 9. Percentage rate of change in BMD by age group

Table 32. Correlation coefficients for selected characteristics and QUS parameters, and bone turnover markers

r	BUA (p-value)	SOS (p-value)	Osteocalcin+ (p-value)	N-telopeptide+ (p-value)
Age (yrs)	-0.05 (0.554)	-0.08 (0.385)	0.04 (0.656)	-0.01 (0.877)
Height (cm)	0.18 (0.058)	0.09 (0.336)	-0.02 (0.843)	-0.14 (0.10)
Weight (kg)	0.14 (0.141)	-0.03 (0.742)	-0.03 (0.751)	-0.03 (0.71)
BMD at follow-up (g/cm^2)				
Total hip	0.42 (<0.001)	0.44 (<0.001)	-0.23 (0.007)	-0.30 (<0.001)
Femoral neck	0.39 (<0.001)	0.41 (<0.001)	-0.22 (0.010)	-0.24 (0.004)
Trochanter	0.42 (<0.001)	0.45 (<0.001)	-0.20 (0.020)	-0.31 (<0.001)
Intertrochanter	0.40 (<0.001)	0.42 (<0.001)	-0.23 (0.007)	-0.29 (<0.001)
Percentage rate of change in BMD (%/yr)				
Total hip	-0.01 (0.946)	0.003 (0.977)	-0.22 (0.011)	-0.28 (<0.001)
Femoral neck	0.11 (0.226)	0.12 (0.195)	-0.20 (0.018)	-0.30 (<0.001)
Trochanter	-0.01 (0.944)	0.01 (0.913)	-0.16 (0.053)	-0.25 (0.003)
Intertrochanter	0.01 (0.902)	0.03 (0.765)	-0.16 (0.067)	-0.22 (0.008)

+: Spearman Rank Correlation (r_s)

2. Determinants associated with percent rate of changes in BMD

The mean (SD) percent rate of change of BMD in the total hip and femoral neck were –0.03 (0.68) and –0.07 (0.82) % per year, respectively. Absolute rate of change of BMD per year were –0.29 (6.47) and –0.50(6.46) mg/cm^2/year. Table 33 describes the significant and non-significant correlations between determinants of BMD and percent rate of change of BMD of the total hip, femoral neck, trochanter, and intertrochanter.

Age was inversely related to the percent rate of change of total hip BMD (r=-0.16, p=0.055) and intertrochanter BMD (r=-0.21, p=0.011). Weight change since baseline were significantly correlated with total hip and all sub-regions, and correlation coefficients ranged from r=0.16 to 0.33. Systolic blood pressure was inversely correlated with the changes of BMD in total hip, trochanter, and intertrochanter. Current

smoking was inversely correlated with femoral BMD. Unexpectedly, a history of MI and angina were associated with positive rate of changes of total hip BMD and at the other measurement sites. Percent rate of change of BMD of the total hip was significantly and positively correlated with a history of heart attack (myocardial infarction) (r=0.17, p=0.051), and angina (r=0.20, p=0.017). There were inverse correlations between percent changes of total hip BMD and fracture history since age 50 (r=-0.27, p=0.001) and use of oral glucocorticoid (r=-0.17, r=0.051). Physical activity was inversely correlated with percent changes of total hip (r_s= -0.14), but was not statistically significant (p=0.102). We could not find any significant association between percent changes of total hip BMD and dietary calcium intake, and use thiazide diuretics.

In a stepwise regression analysis including age, weight change since baseline, history of heart attack, angina, fracture, use of oral glucocorticoids, current smoking and systolic blood pressure, weight changes (p<0.001), history of fracture (p<0.001), glucocorticoid (p=0.012), and current smoking (p=0.048) were identified as significant predictors of the variation in percent change of total hip BMD. 23.5% of variance in percent change of total hip was explained by these four variables. Weight change since baseline explained 8.6% of total variance in percent changes of total hip BMD and history of fractures explained another 8.7% of the variability. Glucocorticoid use, history of fracture, and current smoking was associated with bone loss of total hip BMD. Age, history of heart attack, angina and systolic blood pressure in the model did not show a significant effect on the prediction of variability in percent change in total hip BMD.
Likewise, weight change, diabetes, current smoking, and history of fracture explained 17.6% of the variance in percent change of femoral neck BMD in multivariate regression model. Weight change and diabetes were positively related to changes in BMD, and explained the 3.9% and 4.4% of the variability, respectively. History of fracture and current smoking were associated to negative changes of BMD, and accounted for 4.4% and 4.9% of variance, respectively (Table 34).

Table 33. Correlation coefficients for baseline characteristics and bone loss of men in STORM-EBT examination (n=138)

Percentage rate of change in BMD (%/yr)	Total hip (p-value)	Femoral Neck (p-value)	Trochanter (p-value)	Intertrochanter (p-value)
Age at baseline (yrs)	**-0.16 (0.055)**	-0.03 (0.715)	-0.11 (0.211)	**-0.21 (0.011)**
Height (cm)				
Baseline	0.12 (0.141)	0.05 (0.550)	0.08 (0.363)	**0.17 (0.048)**
At Age 25	0.06 (0.464)	-0.003(0.97)	0.06 (0.499)	0.10 (0.234)
Weight (Kg)				
Baseline	0.10 (0.226)	-0.02 (0.767)	0.08 (0.349)	**0.17 (0.049)**
At Age 25	0.04 (0.636)	-0.02 (0.782)	0.05 (0.571)	0.09 (0.293)
Weight change since baseline	**0.29 (<0.001)**	**0.16 (0.05)**	**0.17 (0.039)**	**0.33 (<0.001)**
BMI (kg/m2)	0.05 (0.530)	-0.05 (0.549)	0.05 (0.552)	0.10 (0.227)
Waist Girth (cm)	-0.02 (0.773)	-0.04 (0.611)	-0.01 (0.926)	0.04 (0.639)
Waist to Hip ratio	-0.12 (0.155)	-0.09 (0.309)	-0.08 (0.336)	-0.06 (0.477)
Systolic BP (mmHg)	**-0.18 (0.030)**	-0.09 (0.315)	**-0.19 (0.027)**	**-0.22 (0.011)**
Diastolic BP (mmHg)	-0.09 (0.280)	-0.09 (0.279)	-0.10 (0.251)	-0.10 (0.256)
Education (years) [+]	0.11 (0.180)	0.149 (0.081)	0.11 (0.186)	0.05 (0.526)
Smoking				
Current smoking (yes/no)	-0.11 (0.181)	**-0.22 (0.008)**	-0.14 (0.110)	-0.02 (0.772)
Ever smokers (yes/no)	-0.05 (0.519)	0.05 (0.567)	-0.04 (0.666)	-0.04 (0.593)
Pack-years for smokers [+]	0.07 (0.457)	-0.01 (0.873)	0.08 (0.428)	0.07 (0.484)
Alcohol intake (drinks/wk) [+]	0.03 (0.719)	0.11 (0.203)	0.02 (0.804)	0.02 (0.829)
Dietary calcium intake (mg/day) [+]	-0.02 (0.807)	0.07 (0.442)	0.04 (0.658)	-0.03 (0.720)
Calcium supplement use (yes/no)	-0.03 (0.744)	0.08 (0.338)	0.01 (0.866) +	-0.04 (0.668)
Vit D supplement use (yes/no)	-0.08 (0.363) [+]	-0.02 (0.843)	-0.02 (0.49)	-0.05 (0.544) [+]
Physical activity [+]				
Baseline (METS/day)	-0.14 (0.102)	-0.12 (0.17)	-0.03 (0.745)	-0.13 (0.133)
Historical (METS/day)	0.06 (0.445)	0.08 (0.373)	0.09 (0.314)	0.07 (0.421)
Back related disability score [+]	-0.09 (0.265)	-0.08 (0.358)	**-0.14 (0.098)**	-0.05 (0.528)

[+]: Spearman Rank Correlation (r_s)

Values are correlation coefficients (p-value)

Table 33 (continued)

Percentage rate of change in BMD (%/yr)	Total hip	Femoral Neck	Trochanter	Intertrochanter
Health Status (fair/poor) (fair/poor vs. excellent/good)	-0.02 (0.708)	0.01 (0.897)+	0.02 (0.792)	-0.05 (0.544)
Fall in past year (yes/no)	-0.04 (0.645)	0.03 (0.736)	-0.06 (0.513)	-0.02 (0.808)
Any Fracture after age 50 years (yes/no)	**-0.27 (0.001)**	**-0.20 (0.019)**	**-0.23 (0.006)**	**-0.24 (0.004)**
Diabetes (yes/no)	0.12 (0.177)	**0.21 (0.012)**	0.08 (0.324)	0.11 (0.215)
Hypertension (yes/no)	0.02 (0.797)	0.02 (0.789)	0.09 (0.279)	-0.05 (0.529)
Myocardial infarction (yes/no)	**0.17 (0.051)**	**0.14 (0.096)**	0.11 (0.200)	**0.16 (0.053)**
Angina (yes/no)	**0.20 (0.017)**	0.10 (0.232)	**0.14 (0.101)**	**0.20 (0.022)**
Stroke (yes/no)	-0.04 (0.605)	0.04 (0.618)	0.004 (0.961)	-0.07 (0.425)
COPD (yes/no)	0.07 (0.438)	**0.19 (0.025)**	0.01 (0.862)	0.03 (0.696)
Cancer (yes/no)	0.04 (0.645)	0.06 (0.442)	0.07 (0.438)	0.004 (0.960)
Arthritis (yes/no)	-0.08 (0.344)	-0.09 (0.282)	-0.01 (0.860)	-0.07 (0.434)
Oral glucocorticoid (yes/no)	**-0.17 (0.051)**	-0.05 (0.578)	-0.13 (0.115)	**-0.17 (0.051)**
Thiazide diuretics (yes/no)	-0.05 (0.528)	-0.01 (0.913)	0.03 (0.677)	**-0.14 (0.096)**
Family history of osteoporosis (yes/no)	0.02 (0.779)	0.05 (0.562)	-0.01 (0.853)	0.01 (0.874)

+: Spearman Rank Correlation (r_s)

Values are correlation coefficients (p-value)

Table 34. Summary of multivariate association with rate of change in BMD of the total hip and its sub-region

Measurement Site	Predictor Variables	Partial R^2	P-value
	Weight Change (+)	0.086	<0.001
Total Hip	Any Fracture (-)	0.087	<0.001
(Total R^2 0.235)	Current Smoking (-)	0.039	0.012
	Glucocorticoid use (-)	0.023	0.048
	Weight Change (+)	0.039	0.008
Femoral Neck	Any Fracture (-)	0.044	0.010
(Total R^2 0.176)	Current Smoking (-)	0.049	0.002
	Diabetes (+)	0.044	0.012
Trochanter	Weight Change (+)	0.038	0.008
(Total R^2 0.123)	Any Fracture (-)	0.056	0.002
	Current Smoking (-)	0.030	0.036
Intertrochanter	Weight Change (+)	0.108	<0.001
(Total R^2 0.220)	Any Fracture (-)	0.074	<0.001
	Glucocorticoid use (-)	0.038	0.012

C. Determinants of Coronary calcification of study participants in STORM-EBT examination

Risk factors related to coronary artery calcification (CAC) score were also determined in this analysis. Spearman rank correlation coefficients between CAC and several risk factors are shown in Table 35. Age at the time of EBT scan or age at baseline was only a significant predictor of CAC (r_s= 0.25, p=0.003). Waist circumference was positively correlated with CAC but it was not statistically significant (r_s = 0.14, p=0.098). History of heart attack was also positively, but not significantly, related to CAC (r_s= 0.14, p=0.10). We could not find any relationship between baseline lipid levels, especially LDL-c, HDL-c, triglycerides and CAC. Use of calcium channel blocker or other medications including diuretics was not related to CAC. There was no association of LDL-c. When regression analysis was performed, a small fraction of

variability in CAC was explained by age (7.8%, p=0.001). 1.9% of CAC was explained by waist circumference, but this was not statistically significant (p=0.09).

Table 36 shows determinants at follow up examination, which significantly related to CAC score. Characteristics of participants who completed the follow up examination were assessed for the relationships to coronary calcification measured two years later. Age at follow up was significantly correlated with CAC (r_s=0.24, p=0.005). Conversely, we found an inverse relationship between diastolic blood pressure and CAC. Interestingly, total calcium intake and vitamin D intake was correlated with CAC score, and showed the borderline significance. A moving average plot demonstrated the positive relationship between total calcium intake and coronary calcification (Figure 9). In multivariate regression analysis, age at follow up, total calcium intake, and history of MI were emerged as significant explanatory variables for CAC. A total of 13.4% of variance in CAC was explained by these three factors, taken together. Age alone explained 6.4% of variability in CAC (p=0.003), and calcium intake explained another 4% of the variation in CAC (p=0.018). History of diabetes was an independent factor (partial r^2= 0.02, p=0.068) but was not significant at the level of alpha equal to 0.05. Height, diastolic blood pressure, total vitamin D intake, and angina did not show any significance in explaining the variability of CAC in multivariate models (Table 37).

27% of men reported clinical cardiovascular diseases at the time of EBT measurement, and they had higher CAC score compared with those without cardiovascular disease (Table 38). Men with MI, angina, chronic heart failure, stroke, or peripheral vascular diseases (clinical CVD) had a median CAC score of 300.88 (range, 0 to 10706) whereas men without any clinical CVD had a median value of 714.53(range, 11 to 5495) (p=0.001). In age adjusted transformed CAC model, this association was attenuated but it remained significant (p=0.015).

Table 35. Spearman rank correlation coefficients (r_s) for baseline characteristics with coronary calcification

(n=138)	r_s	p-value
Age at scan (yrs)	**0.25**	**0.003**
Height (cm)		
Baseline	**-0.14**	**0.101**
Age 25 years	**-0.13**	**0.105**
Weight (Kg)		
Baseline	0.01	0.852
Age 25 years	-0.01	0.877
BMI (kg/m2)	0.10	0.249
Waist Girth (cm)	**0.14**	**0.098**
Waist to Hip ratio	0.01	0.912
Systolic BP (mmHg)	0.12	0.154
Diastolic BP (mmHg)	0.03	0.716
Education (years)	-0.04	0.640
Smoking		
Current smoking (yes/no)	-0.01	0.866
Ever smokers (yes/no)	0.05	0.570
Pack-years for smokers	0.03	0.774
Alcohol intake (drinks/wk)	-0.05	0.559
Dietary calcium intake (mg/day)	-0.04	0.621
Calcium supplement use (yes/no)	0.04	0.579
Vit D supplement use (yes/no)	-0.08	0.318
Physical activity		
Baseline (METS/day)	-0.07	0.395
Historical (METS/day)	0.10	0.248
Back related disability score	0.02	0.820

Table 35 (continued)

	r_s	p-value
Health Status (fair/poor)		
(fair/poor vs. excellent/good)	-0.06	0.450
Diabetes (yes/no)	0.09	0.270
Hypertension (yes/no)	0.11	0.216
Myocardial infarction (yes/no)	**0.14**	**0.100**
Angina (yes/no)	0.13	0.125
Stroke (yes/no)	0.01	0.883
COPD (yes/no)	0.04	0.621
Cancer, n(%)	0.05	0.579
Oral glucocorticoid (yes/no)	0.002	0.980
Thiazide diuretics (yes/no)	0.06	0.466
Calcium channel blocker (yes/no)	0.03	0.704
Lipid (mg/dL)		
HDL-c	0.04	0.614
HDL2-c	-0.02	0.849
HDL3-c	0.06	0.493
LDL-c	0.09	0.301
Triglycerides	0.07	0.401
Total Cholesterol	0.08	0.333

Table 36. Spearman rank correlation coefficients for follow-up characteristics significantly related to coronary artery calcification (CAC) score in men

n=138	r_s	p-value
Age at follow-up (yrs)	0.24	0.005
Height (cm)	-0.15	0.087
Diastolic blood pressure (mmHg)	-0.20	0.019
Total calcium intake (mg/day)	0.15	0.073
Total Vit D intake (mg/day)	0.17	0.053
Diabetes (yes/no)	0.17	0.051
Myocardial infarction (yes/no)	0.19	0.028
Angina (yes/no)	0.18	0.040

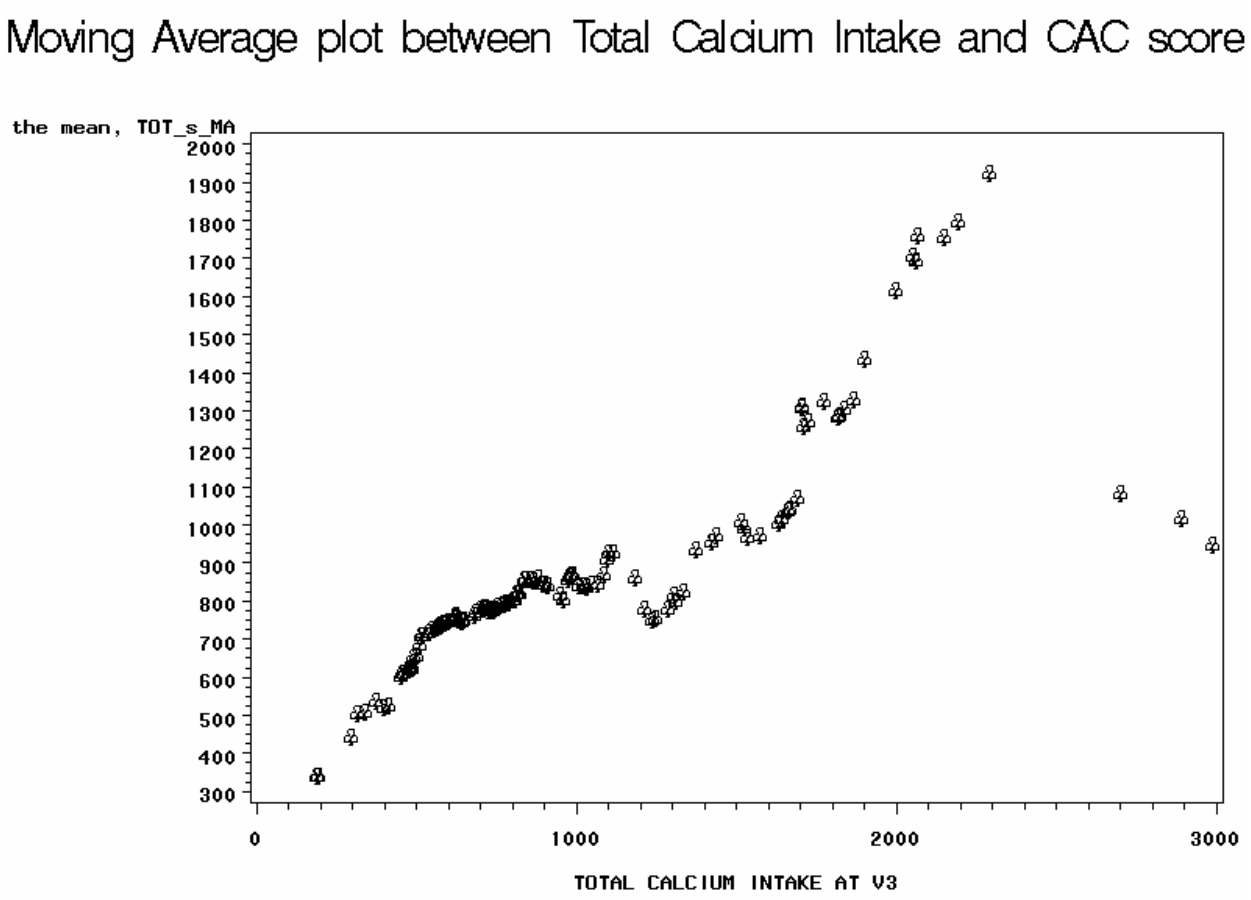

Figure 10. Moving average plot of total calcium intake at follow up examination and CAC score

Table 37. Summary of multivariate association with coronary artery calcification (CAC) score

Visit	Variables	β-coefficients	Partial R2	P-value
Baseline (Total R^2 0.078)	Age	0.278	0.078	0.001
Follow-up (Total R^2 0.134)	Age	0.270	0.064	0.001
	Total Calcium intake (Log)	0.195	0.040	0.019
	MI (Yes/No)	0.174	0.030	0.036

Table 38. Spearman rank correlation coefficients for updated clinical disease and coronary calcification in the STORM-EBT examination

n=138	r_s	p-value
Myocardial infarction (yes/no)	**0.25**	**0.003**
Angina (yes/no)	**0.18**	**0.036**
Chronic heart failure (yes/no)	**0.20**	**0.021**
Stroke (yes/no)	0.09	0.306
Peripheral vascular disease (yes/no)	0.05	0.539
Clinical cardiovascular disease (total) (yes/no)	**0.28**	**0.001**

D. Assessment of Specific Aim I: Relationship between BMD, rate of change in BMD, bone turnover markers, QUS parameters and CAC score

1. Correlation between bone mineral density and CAC score

The associations between coronary artery calcification (CAC) and BMD, rates of changes in BMD and coronary artery calcifications (CAC) score were estimated using Spearman Rank Correlation coefficients (Table 39). Baseline BMD of the total hip, femoral neck, trochanter, and intertrochanter were negatively but not related to CAC score (r_s = - 0.05; r_s= -0.11; r_s=-0.06; r_s= -0.11, respectively). The regression model checking the assumption of linear regression (linearity, normality, or homogeneity of variance) showed similar results. The ranges of variation in CAC score explained by BMD were only from 0.01% at the intertrochanter to 0.5% at the femoral neck (p-value = 0.93; p-value = 0.397). We could not find any significant correlation between percent change in BMD, and rate of changes in BMD of the hip.

Neither QUS parameters (BUA, and SOS) nor biochemical bone turnover markers (N-telopeptide and osteocalcin) were associated with CAC score.

In conclusion, our data could not demonstrate significant inverse associations between coronary artery calcification and BMD of the total hip, rate of changes in BMD, QUS parameters, and biochemical bone turnover markers.

Therefore, we limited the analysis of further specific aims (mediator effects by estradiol, CRP, or osteoprotegerin T-950C or G-1181C polymorphisms) as of independent association of each variable on BMD, rate of changes in bone, QUS parameters, bone turnover markers, and coronary artery calcification.

Table 39. Spearman Rank Correlation between BMD, rates of change in BMD, QUS parameters, and bone turnover markers and coronary calcification in STORM men

Correlation (rs) (n=138)	CAC Score	p-value
BMD (g/cm^2)		
Total hip	-0.05	0.517
Femoral neck	-0.11	0.204
Trochanter	-0.06	0.477
Intertrochanter	-0.11	0.192
Percentage rate of change in BMD(%/yr)		
Total hip	0.02	0.841
Femoral neck	0.10	0.258
Trochanter	0.001	0.992
Intertrochanter	-0.03	0.749
Absolute rate of change in BMD($mg/cm^2/yr$)		
Total hip	0.03	0.712
Femoral neck	0.11	0.206
Trochanter	0.01	0.906
Intertrochanter	-0.01	0.868
Calcaneal QUS (n=115)		
BUA (dB/MHz)	0.10	0.280
Speed of Sound (SOS) (m/s)	0.08	0.401
Bone turnover marker (n=138)		
Osteocalcin (ng/ml)	-0.06	0.506
N-telopeptide (nM BCE/mM Cr)	-0.02	0.805

E. Sex steroid hormones and C-reactive protein with BMD and CAC score

We could not observe any relationship between CAC score and bone mineral density in the STORM men so that we examined independent relationships of sex steroid hormone and CRP with BMD, or with coronary calcification.

1. Correlation coefficients between sex steroid hormone and CRP in STORM men

The analyses were conducted using pearson and spearman rank correlation coefficients analysis. Serum levels of total estradiol (22.0 $\pm$ 7.2 pg/ml) and bioavailable estradiol (13.6 $\pm$ 4.4 pg/ml) were similar to the values from Rancho Bernardo Study (Greendale et al., 1997). Total testosterone and bioavailable testosterone concentrations were a little higher in our study sample compared to the Rancho Bernardo Study, but similar to the values from a reported study (Khosla et al, 1998). Table 40 shows the correlation coefficients between age, body weight, sex steroid hormones, and C-reactive protein. Age was highly correlated with total and bioavailable estradiol, total and bioavailable testosterone and sex hormone binding globulin (Table 40). Age was related to lower bioavailable estradiol (r=-0.18, p=0.033) and lower bioavailable testosterone (r=-0.32, p=0.001). We could not find any significant relationship between weight and serum levels of estradiol.

Among sex steroid hormone, there were significant correlations between hormones for each other. Bioavailable estradiol was positively correlated with bioavailable testosterone (r=0.40, p<0.001), total testosterone (r=0.15, p=0.081), and negatively correlated with SHBG (r=-0.15, p=0.072).

The average CRP levels (mg/l) in our study individuals was 2.22 $\pm$ 2.35 (median 1.43 and ranges 23.24) and this levels was comparable to a normal range of < 2 mg/l in population without episode of acute illness (Macy et al., 1997). CRP was positively correlated with bioavailable estradiol (r_s=0.19, p=0.03) and sex hormone binding globulin (r_s= -0.18, p=0.03). Adjusting for age and body weight, there was of borderline significance in the relationship between bioavailable estradiol and CRP (r_s=0.16, p-value =0.064).

Table 40. Unadjusted correlations between sex steroid hormone and CRP in STORM men (n=138)

	Total E2	Bio E2	Total T	Bio T	SHBG+	CRP+
Age	**0.17**	**-0.18**	**0.24**	**-0.32**	**0.54**	-0.10
	(0.047)	**(0.033)**	**(0.004)**	**(<0.001)**	**(<0.001)**	(0.213)
Weight	-0.02	0.11	**-0.24**	-0.03	**- 0.27**	0.13
	(0.846)	(0.198)	**(0.005)**	(0.740)	**(0.001)**	(0.124)
Total E2	---	**0.80**	**0.49**	**0.18**	**0.38**	0.09
		(<0.001)	**(<0.001)**	**(0.035)**	**(<0.001)**	(0.27)
Bio E2		---	**0.15**	**0.40**	**- 0.15**	**0.19**
			(0.081)	**(<0.001)**	**(0.072)**	**(0.028)***
Total T			---	**0.50**	**0.65**	-0.10
				(<0.001)	**(<0.001)**	(0.249)
Bio T				---	**- 0.24**	0.03
					(0.005)	(0.681)
SHBG					---	**-0.18**
						(0.033)

E2, 17 β estradiol ; T, Testosterone; SHBG, Sex hormone binding globulin; CRP, C-reactive protein

+ : Spearman Rank correlation coefficients

* : Age and body weight adjusted partial correlation between Bio E2 and CRP was r_s = 0.16 (0.064).

2. Correlation coefficients between sex steroid hormones, CRP and BMD, rate of change in BMD, QUS parameters, and bone turnover markers

Table 41 shows the results of the univariate analyses between sex steroid hormone and CRP to the BMD of the total hip and its sub-regions in our population.

Serum bioavailable estradiol levels, not total estradiol levels, were significantly correlated with BMD of total hip, trochanter, and intertrochanter region (r=0.25, p=0.03; r=0.20, p=0.017; r=0.24, p=0.005, respectively). There was of borderline significance observed at the femoral neck (p=0.063). Total and bioavailable estradiol did not correlate with yearly percent rate of change in BMD or absolute rate of change in BMD, except weakly at the trochanter (Table 41). After adjusting age and body weight, there

was no significant correlation between serum estradiol levels and changes of BMD at the total hip, or any sub-region (Table 42). Total estradiol alone was positively associated with unadjusted BUA (r=0.17, p=0.074) or age and body weight adjusted BUA and SOS parameters (r=0.18, p=0.053, r=0.17, p=0.063, respectively), but it was not statistically significant (Table 43 and 44). There was no association between biochemical markers (NTX and osteocalcin) and any of the hormones (Table 43 and 44).

Serum bioavailable testosterone concentrations were significantly correlated with BMD (for total hip, r=0.20, p=0.018; for femur neck, r=0.16, p=0.056; for intertrochanter, r=0.20, p=0.017, respectively), except at the trochanter (Table 41). In contrast, serum total testosterone levels were not correlated with BMD at all. There were the weak and inverse correlations between total testosterone and the percent rate of changes in BMD of the total hip and intertrochanter (r=-0.16, p=0.067 and r=-0.16, p=0.051, respectively). Adjustment for age and body weight, however, attenuated the relationships between testosterone and the rate of changes in BMD (Table 42). There was no association between total or bioavailable testosterone, and QUS parameters or biochemical bone turnover markers.

Higher serum levels of SHBG were correlated with lower BMD of the total hip and intertrochanter (r=-0.19, p=0.026 and r= -0.22, p=0.008). Percent changes or absolute changes in BMD of the total hip, trochanter, and intertrochanter were significantly correlated to SHBG levels. After adjusting for age and body weight, however, the statistical significance did not remain in the relationship between serum level SHBG and BMD or rate of changes. Conversely, SHBG levels were not correlated to QUS parameters in univariate analyses. After age and body weight adjustment, however, SHBG concentrations were significantly correlated with BUA (r=0.19, p=0.042). There was no association between SHBG and biochemical bone turnover markers.

Overall, no significant relationship was found between CRP and BMD, rates of change in BMD, QUS parameters, and bone turnover markers. To examine the relative

effects of estradiol, testosterone, and SHBG in determining percent rate of changes in BMD of the total hip, multivariate analyses were performed. Only 2.6% of the variability of the rate of change in BMD of the total hip was explained by SHBG levels (p=0.056).

Table 41. Simple correlation of sex steroid hormones, CRP with bone density and rate of change in BMD in STORM men (n=138)

	Total E2	Bio E2	Total T	Bio T	SHBG[+]	CRP [+]
BMD (g/cm^2)						
Total Hip	0.14	**0.25**	-0.00	**0.20**	**-0.19**	0.03
	(0.101)	**(0.003)**	(0.975)	**(0.018)**	**(0.026)**	(0.697)
Femoral neck	0.10	**0.16**	0.04	**0.16**	-0.08	0.01
	(0.235)	**(0.063)**	(0.629)	**(0.056)**	(0.378)	(0.894)
Trochanter	0.12	**0.20**	0.00	0.13	-0.10	0.02
	(0.144)	**(0.017)**	(0.976)	(0.111)	(0.233)	(0.848)
Intertrochanter	0.12	**0.24**	-0.02	**0.20**	**-0.22**	-0.00
	(0.162)	**(0.005)**	(0.825)	**(0.017)**	**(0.008)**	(0.952)
Percentage rate of change in BMD(%/yr)						
Total hip	-0.09	0.01	-0.16	-0.02	**-0.19**	0.04
	(0.284)	(0.872)	(0.067)	(0.843)	**(0.021)**	(0.631)
Femoral neck	-0.04	0.01	-0.03	0.02	-0.08	0.01
	(0.617)	(0.908)	(0.756)	(0.823)	(0.307)	(0.915)
Trochanter	-0.13	-0.07	-0.11	-0.00	**-0.16**	0.01
	(0.113)	(0.412)	(0.208)	(0.774)	**(0.050)**	(0.917)
Intertrochanter	-0.04	0.09	**-0.16**	0.02	**-0.24**	0.06
	(0.616)	(0.309)	**(0.051)**	(0.766)	**(0.004)**	(0.495)
Absolute rate of change in BMD(g/cm^2/yr)						
Total hip	-0.13	-0.005	**-0.19**	-0.03	**-0.20**	0.05
	(0.126)	(0.951)	**(0.024)**	(0.700)	**(0.014)**	(0.548)
Femoral neck	-0.07	-0.002	-0.05	0.01	-0.10	0.01
	(0.434)	(0.935)	(0.571)	(0.877)	(0.261)	(0.848)
Trochanter	**-0.17**	-0.09	-0.13	-0.04	**-0.18**	0.01
	(0.046)	(0.269)	(0.132)	(0.631)	**(0.037)**	(0.866)
Intertrochanter	-0.08	0.07	**-0.20**	0.01	**-0.25**	0.07
	(0.358)	(0.409)	**(0.016)**	(0.924)	**(0.003)**	(0.443)

Table 42. Age, and body weight adjusted partial correlation of sex steroid hormone, CRP with BMD, and bone loss

	Total E2	Bio E2	Total T	Bio T	SHBG [+]	CRP [+]
BMD (g/cm^2)						
Total Hip	**0.21**	**0.19**	0.16	**0.17**	0.04	-0.02
	(0.015)	**(0.022)**	(0.064)	**(0.045)**	(0.672)	(0.800)
Femoral neck	0.15	0.10	**0.19**	0.13	0.16	-0.03
	(0.072)	(0.255)	**(0.023)**	(0.119)	(0.066)	(0.689)
Trochanter	0.16	0.16	0.11	0.13	0.048	-0.04
	(0.068)	(0.054)	(0.217)	(0.137)	(0.581)	(0.578)
Intertrochanter	**0.19**	**0.18**	0.15	0.16	-0.001	-0.03
	(0.026)	**(0.036)**	(0.086)	(0.057)	(0.988)	(0.680)
Percentage rate of change in BMD(%/yr)						
Total hip	-0.07	-0.02	-0.11	-0.02	-0.12	0.01
	(0.434)	(0.801)	(0.202)	(0.843)	(0.180)	(0.856)
Femoral neck	-0.04	0.01	-0.03	0.02	-0.08	0.01
	(0.664)	(0.938)	(0.758)	(0.823)	(0.353)	(0.945)
Trochanter	-0.12	-0.10	-0.07	-0.02	-0.11	-0.01
	(0.160)	(0.263)	(0.407)	(0.774)	(0.200)	(0.896)
Intertrochanter	-0.01	0.04	-0.10	0.02	-0.15	0.02
	(0.909)	(0.634)	(0.253)	(0.766)	(0.089)	(0.784)
Absolute rate of change in BMD(g/cm^2/yr)						
Total hip	-0.11	-0.04	-0.15	-0.09	-0.13	0.03
	(0.211)	(0.638)	(0.081)	(0.319)	(0.135)	(0.757)
Femoral neck	-0.06	-0.001	-0.06	0.003	-0.10	0.02
	(0.455)	(0.988)	(0.505)	(0.968)	(0.233)	(0.848)
Trochanter	-0.15	-0.12	-0.09	-0.082	-0.12	-0.01
	(0.074)	(0.154)	(0.274)	(0.342)	(0.179)	(0.848)
Intertrochanter	-0.05	0.02	-0.14	-0.05	-0.16	0.03
	(0.583)	(0.773)	(0.099)	(0.538)	(0.069)	(0.712)

[+] : Spearman Rank Correlation

Table 43. Simple correlations between QUS parameters and bone turnover markers and sex steroid hormone, and CRP

	Total E2	Bio E2	Total T	Bio T	SHBG [+]	CRP [+]
Calcaneal QUS (n=115)						
BUA (dB/MHz)	0.17	0.11	0.06	-0.02	0.11	-0.01
	(0.074)	(0.250)	(0.519)	(0.848)	(0.241)	(0.925)
SOS (m/s)	0.15	0.10	0.10	0.01	0.10	-0.05
	(0.113)	(0.266)	(0.293)	(0.877)	(0.298)	(0.600)
Bone turnover markers (n=138)						
Osteocalcin	0.05	-0.01	0.01	-0.05	0.02	-0.03
(ng/mL) [+]	(0.578)	(0.908)	(0.899)	(0.558)	(0.783)	(0.722)
NTX (nM	-0.05	-0.11	-0.04	-0.10	-0.02	0.04
BCE/mM Cr) [+]	(0.582)	(0.191)	(0.659)	(0.233)	(0.798)	(0.660)

[+] : Spearman Rank Correlation

Table 44. Age, and body weight adjusted partial correlation of sex steroid hormone, CRP with QUS parameters and bone biochemical markers.

	Total E2	Bio E2	Total T	Bio T	SHBG [+]	CRP [+]
Calcaneal QUS						
BUA (dB/MHz)	**0.18**	0.09	0.12	-0.03	**0.19**	-0.02
	(0.053)	(0.323)	(0.200)	(0.756)	**(0.042)**	(0.824)
SOS (m/s)	0.17	0.10	0.13	-0.01	0.17	-0.06
	(0.063)	(0.281)	(0.178)	(0.945)	(0.065)	(0.489)
Bone turnover markers						
Osteocalcin	0.04	-0.01	-0.002	-0.05	-0.001	-0.02
(ng/mL) [+]	(0.676)	(0.918)	(0.980)	(0.590)	(0.986)	(0.778)
NTX (nM	-0.04	-0.11	-0.04	-0.11	-0.03	0.04
BCE/mM Cr) [+]	(0.618)	(0.219)	(0.598)	(0.219)	(0.739)	(0.633)

[+] : Spearman Rank Correlation

3. Spearman rank correlation coefficients of sex steroid hormones and CRP with coronary artery calcification (CAC) score

The relationship between CAC scores and sex steroid hormone and CRP were assessed (Table 45). Coronary calcification was inversely correlated with serum levels of bioavailable testosterone (r_s= - 0.17, p=0.04), and positively related to the levels of SHBG (r_s= 0.18, 0.036). However, the relationship did not remained after adjusting for age and body weight (Table 45).

To assess the relative contributions of sex steroid hormones and CRP in explaining CAC score, stepwise regression was performed in which CAC score was the dependent variable, and sex steroid, and CRP were the predictor variables. SHBG level was the only significant independent variable explaining a small fraction (r^2=0.068, p=0.002) of the variance of CAC score. After age and body weight was introduced in the model, SHBG was no longer a significant predictor of CAC.

Table 45. Unadjusted and adjusted Spearman Rank correlation coefficients of sex steroid hormone with CRP and coronary calcification (n=138)

r_s	Total E2	Bio E2	Total T	Bio T	SHBG	CRP
Coronary CAC	0.11	-0.04	0.08	**-0.17**	**0.18**	0.002
score	(0.171)	(0.627)	(0.322)	**(0.040)**	**(0.036)**	(0.978)
Adj. CAC	0.08	0.002	0.04	-0.10	0.07	0.02
score *	(0.348)	(0.980)	(0.620)	(0.262)	(0.416)	(0.764)

Values are correlation coefficients (p-value)

* : Adjusted for age and body weight

F. Assessment of Specific Aim 2-2: BMD, rate of change in BMD and CAC score by Osteoprotegerin (OPG) genotype

1. Single nucleotide polymorphisms (SNPs) and allele frequencies in OPG

The frequency distribution of two osteoprotegerin single nucleotide polymorphisms in STORM men is shown in Table 46. Both of polymorphisms are in agreement with Hardy-Weinberg equilibrium in the total study sample. The first allelic frequency was found at the –950 promoter site of osteoprotegerin gene (50% for the T allele and 50% for the C allele) and the distribution of the genotypes were similar to those reported in Caucasian European men and women (47.5%) (Brandstrom et al., 2002). The most common genotype in our study participants was T/C (n=64, 47.8%). A G to C polymorphism is located in the first exon of OPG gene and causes an amino acid change from lysine to asparagines. The frequency of G and C allele was 0.45 and 0.55, respectively. The distribution of the OPG G-1181C genotypes was similar to the result from the recent study on Danish population (Langdahl et al., 2002). For instance, the frequency of G allele of our sample is same as the frequency of G allele in men from previous study G =0.45, C=0.55). The frequency of men with G/G homozygous and C/C homozygous was 22.6 % and 32.3%, respectively.

Table 46. Distribution of osteoprotegerin (OPG) genotypes

OPG/ SNPs		Sample, n (%)	Frequency of allele	P value (HWE)
T-950C (HINC II)	-/- (T/T)	35 (26.1)	T = 0.500	> 0.05
(Promoter region)	-/+ (T/C)	64 (47.8)	C = 0.500	(χ^2 =0.268)
N=134	+/+ (C/C)	35 (26.1)		
G-1181C	G/G	30 (22.6)	G = 0.451	> 0.05
(Exon 1)	G/C	60 (45.1)	C = 0.549	(χ^2 = 1.054)
N=133	C/C	43 (32.3)		

Table 47. Contingency table of the distribution of T-950C and G-1181C genotype

	T-950 C SNP		
G-1181C SNP	T/T	T/C	C/C
G/G	21 (7.53)	8 (13.72)	0 (7.75)
G/C	12 (15.57)	40 (28.40)	8 (16.03)
C/C	1 (10.9)	14 (19.88)	27 (11.22)

: Values are presented as the number of observed subjects and the number of expected (in parentheses) under the assumption of independence. χ^2 =76.755, d.f =4, p<0.0001

In our study population of 134 individuals, T-950C and G-1181C polymorphisms were in highly linkage disequilibrium (Table 47). Lewontin's Coefficient D' was 0.70 (Devlin and Risch, 1995; Terwilliger and Ott, 1994; Hartl, 1999). In combination of these genotypes, the frequencies of two polymorphisms were: T/TG/G 21 (16.0%), T/TG/C 12 (9.2%), T/TC/C 1 (0.8%), T/CG/G 8(6.1%), T/CG/C 40 (30.5%), T/CC/C 14 (10.7%), C/CG/C 8 (6.1%), C/CC/C 27 (20.6%). One haplotype, C/CG/G, was not found in present study population.

2. Characteristics of participants by T-950C promoter genotype

At baseline, there were no significant differences noted in age, height, weight, weight changes since baseline among genotypes (Table 48). Osteoprotegrin promoter T-950C polymorphism was not significantly different in variables of body mass, height, or body mass index (BMI).

The characteristics of other osteoporosis-related lifestyle factors including dietary calcium intake, smoking, history of fracture, and history of fall were similar across genotypes. No significant differences were observed among genotypes related to current smoking, past smoking, and pack-years for smokers.

There were weak but significant differences across genotypes were observed in the variables including historical physical activity, the prevalence of cancer, and use of thiazide diuretics. Men with T/T genotypes reported higher historical physical activity

calculated as a multiple of resting metabolic rate (MET score) compared to men with C/C genotype ($p = 0.057$). Overall, ten men reported the history of cancer. Interestingly, men with T/T homozygous had higher prevalence of cancer than percentage of cancer among heterozygous or C/C homozygous (22.9% vs 1.6 or 2.6%, $p < 0.001$). In C/C and T/C genotypes group, men were more likely to use thiazide diuretics currently and there was statistically significant difference among the use of thiazide diuretics across genotypes.

Lipid levels measured at baseline did not differ across genotypes (Table 49). Also, serum levels of total estradiol (17β-estradiol) and bioavailable estradiol did not significantly differ across genotypes (Table 50). However, men with C/C genotype had 20% or 0.5SD higher levels of bioavailable testosterone compared to T/T genotypes (131.91 ± 47.36 vs 116.88 ± 42.53). The significance level of association did not reach the significance level of 0.05, but we found significant p-value for trend of bioavailable testosterone levels across genotype ($p=0.10$, trend=0.038). Total testosterone levels were also higher in men with C/C homozygous but it did not reach the statistical significance level.

Table 48. Characteristics of STORM-EBT men by OPG T-950C genotypes

	OPG T-950C genotypes			
	T/T (n=35)	T/C (n=64)	C/C (n=35)	p-value
Age (yrs)	65.48 (6.14)	62.94 (6.13)	64.07 (6.51)	0.155
Height (cm)				
Baseline	175.32 (5.51)	174.54 (5.75)	174.05 (6.07)	0.651
At Age 25	178.42 (4.68)	177.20 (5.81)	176.80 (5.67)	0.431
Weight (Kg)				
Baseline	83.85 (11.18)	85.12 (13.45)	83.94 (11.48)	0.849
At Age 25	74.64 (9.15)	73.80 (11.02)	72.06 (12.15)	0.593
Weight change since baseline	0.52 (4.06)	0.75 (6.94)	0.24 (4.86)	0.914
BMI (kg/m2)	27.31 (3.68)	27.90 (3.86)	27.67 (3.13)	0.739
Waist Girth (cm)	95.23 (9.25)	97.81(11.04)	96.56 (8.28)	0.468
Waist to Hip ratio	0.93 (0.04)	0.94 (0.04)	0.93 (0.04)	0.278
Systolic BP (mmHg)	137.71 (13.99)	138.26 (14.13)	137.71 (9.51)	0.971
Diastolic BP (mmHg)	85.01 (6.41)	83.94 (6.33)	85.26 (6.02)	0.542
Education (years)[+]	12.43 (2.45)	12.86 (2.80)	12.51 (1.54)	0.875
Smoking				
Current smoking, n (%) [#]	4 (11.43)	7 (10.94)	3 (8.57)	1.000
Ever smokers, n (%)	28 (80.00)	47 (73.44)	27 (77.14)	0.754
Pack-years for smokers [+]	28.70 (26.65)	34.10 (23.04)	30.53 (22.48)	0.365
Alcohol intake (drinks/wk) [+]	3.77 (5.41)	5.33 (8.40)	9.98 (23.32)	0.594
Dietary calcium intake (mg/day) [+]	642.49 (335.46)	644.91 (591.66)	586.26 (405.57)	0.398
Total calcium intake (mg/day)[+]	1051.3 (490.8)	1032.2 (625.8)	1055.3 (574.9)	0.449
Calcium supplement use, n(%)[#]	2 (5.71)	8 (12.50)	1 (2.86)	0.280
Vit D supplement use, n(%)	12 (34.29)	20 (31.25)	9 (25.71)	0.730
Physical activity [+]				
Baseline (METS/day)	3.23 (2.96)	2.70 (3.08)	2.82 (2.62)	0.648
Historical (METS/day)	**5.34 (4.66)**	**3.77 (4.81)**	**3.19 (3.53)**	**0.057**
Back related disability score [+]	1.14 (2.20)	0.98 (1.95)	1.26 (2.13)	0.762

Values are presented as mean (SD) or n (%)

[+] : Kruskal-Wallis test

: Fisher Exact test

Table 48 (continued)

	OPG T-950C genotypes			
	T/T (n=35)	T/C (n=64)	C/C (n=35)	p-value
Health Status(fair/poor), n(%)	6 (15.79)	13 (20.31)	6 (17.14)	0.895
Any Fracture since age 50, n(%)	7 (20.00)	14 (21.88)	8 (22.86)	0.956
Fall last year, n(%)	9 (25.71)	12 (18.75)	4 (11.43)	0.308
Arthritis, n(%)	16 (44.12)	23 (36.51)	13 (40.63)	0.757
Diabetes, n(%) [#]	2 (5.88)	7 (11.11)	2 (5.71)	0.609
Hypertension, n(%)	6 (17.14)	13 (20.31)	9 (25.71)	0.669
Myocardial infarction, n(%)[#]	0	4 (6.25)	0	0.186
Stroke, n(%)[#]	0	1 (1.56)	2 (5.71)	0.330
COPD, n(%)[#]	2 (8.82)	3 (4.76)	1 (2.86)	0.540
Cancer, n(%)[#]	**8 (22.86)**	**1 (1.56)**	**1 (2.56)**	**< 0.001**
Oral glucocorticoid, n(%)[#]	2 (5.71)	1 (1.56)	1 (2.86)	0.580
Thiazide diuretics, n(%)				
Past	**5 (3.73)**	**6 (4.48)**	**1 (0.75)**	
Current	**0 (0.0)**	**6 (4.48)**	**6 (4.48)**	**0.055**
Calcium channel blockers, n (%)	5 (14.29)	9 (14.06)	3 (8.57)	0.696
Family history of osteoporosis, n(%)	16 (45.71)	24 (37.50)	10 (28.57)	0.333
Family history of CVD, n(%)	21 (60.00)	41 (64.06)	28 (80.00)	0.157

[#] : Fisher Exact Test

Table 49. Serum levels of lipid by OPG T-950 C genotypes

	OPG T-950C genotypes			p-value	
Lipid (mg/dL)	T/T (n=35)	T/C (n=64)	C/C (n=35)	ANOVA	Trend
HDL-c	46.96 (10.56)	48.18 (10.69)	47.47 (11.31)	0.860	0.844
HDL2-c [&]	4.56 (2.98)	5.59 (2.39)	5.28 (2.64)	0.596	0.520
HDL3-c	40.34 (7.07)	41.21 (9.02)	40.03 (7.70)	0.766	0.877
LDL-c	137.67 (37.02)	134.25 (34.76)	143.34 (33.00)	0.467	0.498
Triglycerides [&]	128.27 (1.65)	136.09 (1.86)	141.99 (1.71)	0.755	0.457
Total Cholesterol	214.51 (34.95)	216.05 (37.96)	221.83 (32.16)	0.654	0.393

[&]: Analysis was performed on log-transformed value

Table 50. Sex steroid hormones and C-reactive protein by OPG T-950 C genotypes

	OPG T-950C genotypes			p-value	
	T/T (n=35)	T/C (n=64)	C/C (n=35)	ANOVA	Trend
Estradiol (pg/ml)					
Total	21.97 (8.28)	21.62 (7.33)	22.91 (6.20)	0.703	0.591
Bioavailable	13.28 (4.65)	13.84 (4.95)	13.71 (3.37)	0.839	0.692
Testosterone (ng/dl)					
Total	386.51 (129.39)	401.45 (131.70)	445.34 (169.25)	0.190	**0.085**
Bioavailable	116.88 (42.53)	131.55 (35.54)	137.91 (47.36)	**0.099**	**0.038**
SHBG (µg/dL) [&]	1.03 (1.68)	0.94 (1.66)	1.01 (1.70)	0.669	0.852
C-reactive protein (mg/L)[&]	1.96 (2.10)	1.94 (1.77)	1.60 (1.76)	0.295	0.183

[&]: Analysis performed on log-transformed value.

3. BMD, rate of changes in BMD, QUS parameters, bone turnover markers and coronary artery calcification by T-950 C promoter genotype

There was no significant difference in BMD of the total hip, and its sub-regions among T-950C genotypes. Contrary to our hypotheses, men with C/C genotype were more likely to lose BMD at all sites compared to T/T genotype although it was not statistically significant except of borderline significance at the intertrochanteric region (for percent rate of change, p=0.067; for absolute change, p=0.071). We found no significant association between OPG T-950C genotype and QUS parameters or bone turnover makers (Table 51). Adjusting for possible confounding factors across T-950C genotype did not change the results from unadjusted analyses (Table 52). Men with the C/C genotype showed significantly higher bone loss at the intertrochanter (p=0.03), when compared with those of the T/T or T/C genotypes (Table 53). There were similar trend of bone loss in the total hip and femoral neck, but we could not find statistical significance.

We could not find any significant difference in CAC score across genotype (Table 54).

Table 51. BMD, rate of change in BMD, QUS parameters, bone turnover markers by OPG T-950 C genotypes

	OPG T-950C genotypes			p-value	
	T/T (n=35)	T/C (n=64)	C/C (n=35)	ANOVA	Trend
BMD at follow-up (g/cm^2)					
Total hip	0.94 (0.14)	0.98 (0.12)	0.97 (0.15)	0.241	0.239
Femoral neck	0.76 (0.13)	0.79 (0.11)	0.79 (0.13)	0.447	0.363
Trochanter	0.73 (0.12)	0.75 (0.11)	0.75 (0.12)	0.765	0.560
Intertrochanter	1.09 (0.16)	1.16 (0.15)	1.15 (0.18)	0.125	0.129
Percent rate of change in BMD(%/yr)					
Total hip	0.03 (0.69)	0.05 (0.68)	-0.18 (0.65)	0.228	0.184
Femoral neck	0.07 (0.89)	-0.02 (0.84)	-0.26 (0.72)	0.206	**0.090**
Trochanter	0.13 (0.78)	0.04 (0.79)	-0.10 (0.79)	0.476	0.227
Intertrochanter	-0.04 (0.71)	0.07 (0.75)	-0.28 (0.62)	**0.067**	0.170
Absolute rate of change in BMD ($mg/cm^2/yr$)					
Total hip	0.01 (6.21)	0.56 (6.65)	-1.62 (6.28)	0.274	0.291
Femoral neck	0.52 (7.05)	-0.12 (6.76)	-1.94 (5.40)	0.253	0.116
Trochanter	0.67 (5.75)	0.29 (6.47)	-0.78 (6.02)	0.584	0.325
Intertrochanter	-0.72 (7.41)	0.95 (8.73)	-2.91 (6.89)	**0.071**	0.252
Calcaneal QUS	(n=30)	(n=53)	(n=28)		
BUA (dB/MHz)	77.30 (16.43)	76.59 (17.04)	81.22 (13.12)	0.451	0.353
SOS (M/S)	1552.78 (28.91)	1553.15 (31.80)	1560.31 (30.67)	0.556	0.354
Bone turnover markers	(n=35)	(n=64)	(n=35)		
Osteocalcin (ng/ml)[&]	8.32 (1.41)	8.63 (1.55)	8.81 (1.47)	0.831	0.550
N-telopeptide (nM BCE/mM Cr)[&]	32.94 (1.48)	38.27 (1.71)	34.52 (1.38)	0.148	0.463

Values are mean (SD).

[&] : Analysis was performed on log transformed variable.

Table 52. Adjusted BMD, rate of change in BMD, QUS parameters, bone turnover markers by OPG T-950 C genotypes [a]

	OPG T-950C genotypes			p-value	
	T/T (n=35)	T/C (n=64)	C/C (n=35)	ANCOVA	Trend
BMD (g/cm^2)					
Total hip	0.93 (0.02)	0.98 (0.02)	0.97 (0.02)	0.211	0.214
Femoral neck	0.75 (0.02)	0.79 (0.01)	0.79 (0.02)	0.357	0.280
Trochanter	0.73 (0.02)	0.75 (0.01)	0.75 (0.02)	0.751	0.541
Intertrochanter	1.08 (0.03)	1.16 (0.02)	1.15 (0.03)	0.109	0.124
Percent rate of change in BMD(%/yr)					
Total hip	0.002 (0.12)	0.06 (0.09)	-0.17 (0.12)	0.256	0.315
Femoral neck	0.01 (0.15)	0.002 (0.10)	-0.24 (0.14)	0.330	0.241
Trochanter	0.11 (0.15)	0.05 (0.10)	-0.09 (0.13)	0.565	0.322
Intertrochanter	-0.06 (0.13)	0.08 (0.09)	-0.26 (0.12)	0.079	0.283
Absolute rate of change in BMD ($mg/cm^2/yr$)					
Total hip	-0.25 (1.20)	0.67 (0.83)	-1.51 (1.11)	0.283	0.451
Femoral neck	0.002 (1.21)	0.005 (0.84)	-1.82 (1.12)	0.381	0.282
Trochanter	0.47 (1.14)	0.44 (0.79)	-0.72 (1.05)	0.637	0.452
Intertrochanter	-0.89 (1.46)	1.02 (1.01)	-2.71 (1.36)	0.082	0.375
Calcaneal QUS	(n=30)	(n=53)	(n=28)		
BUA (dB/MHz)	78.47 (3.20)	76.29 (2.24)	80.80 (3.05)	0.483	0.607
SOS (M/S)	1555.02 (6.15)	1552.14 (4.32)	1559.94 (5.87)	0.558	0.573
Bone turnover markers	(n=35)	(n=64)	(n=35)		
Osteocalcin (ng/ml)[&]	8.34 (1.08)	8.64 (1.64)	8.84 (1.07)	0.854	0.576
N-telopeptide (nM BCE/mM Cr)[&]	32.00 (1.08)	38.35 (1.06)	34.87 (1.08)	0.197	0.456

Values are mean (SD).

[a] : Covariates are historical physical activity, use of thiazide diuretics, and cancer.

[&] : Analysis was performed on log transformed variable

Table 53. Comparison of bone loss between OPG T-950C genotype groups

	T/T + T/C n= 99	C/C n= 35	p-value
Age (yrs)	63.84 (6.22)	64.07 (6.51)	0.851
Height (cm)	174.81 (5.65)	174.05 (6.07)	0.502
Weight (Kg)	84.67 (12.65)	83.94 (11.48)	0.763
BMD (g/cm^2)			
Total hip	0.968 (0.13)	0.975 (0.15)	0.780
Femoral neck	0.781 (0.12)	0.787 (0.13)	0.805
Trochanter	0.745 (0.12)	0.751 (0.12)	0.819
Intertrochanter	1.137 (0.15)	1.152 (0.18)	0.639
Percent rate of change in BMD(%/yr)			
Total hip	0.043 (0.68)	-0.186 (0.65)	0.086
Femoral neck	0.015 (0.86)	-0.262 (0.72)	0.089
Trochanter	0.070 (0.79)	-0.097 (0.79)	0.282
Intertrochanter	0.031 (0.74)	-0.279 (0.625)	0.028

Table 54. Coronary calcification (CAC) score by OPG T-950C genotypes [+]

N=134	OPG T-950C genotypes			p-value	
	T/T (n=35)	T/C (n=64)	C/C (n=35)	Unadj. & Adj.	Trend
Total calcium score	276.21 (14.71)	380.30 (22.27)	460.70 (6.51)	0.496	0.242
Age adjusted CAC score	240.93 (0.01)	409.73 (0.003)	454.78 (0.01)	0.277	0.142

Values are mean (SD).

[&] : Analysis was performed using transformed total CAC score (power of 0.25).

4. Characteristics of participants by OPG G-1181C genotypes.

Age and education level were of borderline significance by G-1181C genotype (Table 55). Men with G/C genotype were more likely to have history of cancer (20.0%) than either men with heterozygous G/C (3.3%) or those with C/C genotype (7.0%) (p=0.035). In contrast, men with C/C genotype were more likely to use thiazide diurectics (5.26%) currently compared with those with G/C or G/G genotype (p=0.067). There was no association between height, weight, weight change, or waist girth and genotype. Likewise, dietary calcium intake, total calcium intake at the follow up, calcium and vitamin D supplement use, physical activity or prevalence of medical history did not differ by G-1181C genotype. Interestingly, the distribution of family history of cardiovascular disease in the study population was significantly different across genotype (p=0.041). Men with C/C genotype reported higher frequency of family history of cardiovascular disease (79.5%) compared with men with heterozygous, G/C and those with homozygous, G/G (50.0% and 69.8%, respectively). There was a significant linear trend among genotypes (trend=0.015). Serum levels of lipid, sex steroid hormones and CRP were compared across OPG G-1181C genotype (Table 56 and Table 57). However, there was no significant association between genotype and these measurements.

Table 55. Characteristics of STORM-EBT men by OPG G-1181C genotypes

	OPG G-1181 C genotypes			
	G/G (n=30)	G/C (n=60)	C/C (n=43)	p-value
Age (yrs)	**66.84 (5.27)**	**62.65 (6.41)**	**64.23 (6.43)**	**0.067**
Height (cm)				
Baseline	174.65 (4.78)	175.47 (6.65)	173.78 (4.94)	0.339
Age 25 years	177.59 (4.39)	178.33 (6.37)	176.33 (4.75)	0.193
Weight (Kg)				
Baseline	84.36 (12.09)	85.72 (13.58)	82.83 (10.37)	0.520
Age 25 years	74.29 (10.41)	73.39 (10.84)	73.43 (11.85)	0.926
Weight Change since baseline	0.32 (4.03)	1.30 (7.00)	-0.41 (4.88)	0.330
BMI (kg/m2)	25.67 (3.82)	27.80 (3.83)	27.43 (3.27)	0.892
Waist Girth (cm)	96.48 (9.45)	96.88 (11.61)	96.60 (7.96)	0.981
Waist to Hip ratio	0.93 (0.04)	0.93 (0.05)	0.94 (0.04)	0.715
Systolic BP (mmHg)	141.38 (13.98)	136.72 (13.13)	137.19 (11.67)	0.402
Diastolic BP (mmHg)	86.22 (7.89)	83.88 (5.54)	84.45 (6.07)	0.597
Education (years)[+]	**11.83 (2.33)**	**13.15 (2.52)**	**12.63 (2.22)**	**0.056**
Smoking				
Current smoking, n (%)	1 (3.33)	10 (16.67)	3 (6.98)	0.121
Ever smokers, n (%)	22 (73.33)	46 (76.67)	32 (74.42)	0.933
Pack-years for smokers [+]	29.57 (27.20)	31.98 (21.71)	31.94 (23.97)	0.747
Alcohol intake (drinks/wk) [+]	4.47 (5.98)	6.73 (12.89)	6.57 (18.01)	0.274
Dietary calcium intake (mg/day)[+]	626.83 (382.54)	625.67 (572.59)	677.83 (525.11)	0.789
Total calcium intake (mg/day)[+]	971.32 (527.1)	993.55 (599.8)	114.71(577.5)	0.223
Calcium supplement use, n(%)[#]	2 (2.67)	6 (10.00)	3 (6.98)	0.849
Vit D supplement use, n(%)	10 (33.33)	15 (25.00)	15 (34.88)	0.507
Physical activity [+]				
Baseline (METS/day)	3.12 (3.37)	3.03 (3.04)	2.64 (2.62)	0.703
Historical (METS/day)	4.29 (3.81)	4.11 (5.21)	3.74 (4.04)	0.680
Back related disability score [+]	0.93 (2.20)	0.85 (1.57)	1.56 (2.50)	0.638

Values are presented as mean (SD) or n (%)

[+] : Kruskal-Wallis Test

[#] : Fisher Exact test

Table 55 (continued)

	OPG G-1181 C Genotypes			
	G/G (n=30)	G/C (n=60)	C/C (n=43)	p-value
Health Status(fair/poor), n(%)	6 (20.00)	12 (20.00)	8 (18.60)	0.982
Any Fracture since age 50, n(%)	6 (20.00)	15 (25.00)	7 (16.28)	0.556
Fall last year, n(%)	7 (23.33)	10 (16.67)	7 (16.28)	0.692
Arthritis, n(%)	15 (51.72)	22 (37.93)	15 (36.59)	0.380
Diabetes, n(%)	1 (3.45)	5 (8.47)	4 (9.30)	0.763
Hypertension, n(%)	9 (30.00)	10 (16.67)	9 (20.93)	0.343
Myocardial infarction, n(%)	0	4 (6.67)	0	0.146
Stroke, n(%)	0	1 (1.67)	2 (4.65)	0.597
COPD, n(%)	2 (6.90)	3 (5.08)	2 (4.65)	1.000
Cancer, n(%)	**6 (20.00)**	**2 (3.33)**	**3 (6.98)**	**0.035**
Oral glucocorticoid, n(%)	0	2 (3.33)	2 (4.65)	0.686
Thiazide diuretics, n(%)				
Past	5 (3.76)	5 (3.76)	2 (1.50)	
Current	0	4 (3.01)	7 (5.26)	**0.067**
Calcium channel Blockers, n (%)	5 (16.67)	7 (11.67)	5 (11.63)	0.769
Family history of osteoporosis, n(%)	11 (36.67)	26 (43.33)	12 (27.91)	0.278
Family history of CVD, n(%)	**13 (50.00)**	**37 (69.81)**	**31 (79.49)**	**0.041**

[#] : Fisher Exact Test

Table 56. Serum levels of lipid by OPG G-1181C genotypes

	OPG G-1181C genotypes			p-value	
Lipid (mg/dL)	G/G (n=30)	G/C (n=60)	C/C (n=43)	ANOVA	Trend
HDL-c	47.34 (10.89)	47.94 (11.18)	47.18 (10.09)	0.933	0.950
HDL2-c [&]	4.82 (2.48)	5.26 (2.82)	5.47 (2.42)	0.854	0.578
HDL3-c	40.77 (7.46)	40.91 (8.90)	39.95 (7.48)	0.832	0.674
LDL-c	136.91 (43.01)	135.99 (32.02)	138.41 (32.46)	0.942	0.857
Triglycerides [&]	128.48 (1.77)	130.739 (1.72)	145.54 (1.18)	0.564	0.362
Total Cholesterol	215.03 (44.60)	215.32 (32.89)	218.98 (31.82)	0.850	0.641

[&] : Analysis was performed on log transformed variable.

Table 57. Sex steroid hormone and C-reactive protein by OPG G-1181C genotype

	OPG G-1181 C genotypes			p-value	
	G/G (n=30)	G/C (n=60)	C/C (n=43)	ANOVA	Trend
Estradiol (pg/ml)					
Total	23.13 (9.33)	20.67 (6.20)	22.63 (6.48)	0.210	0.765
Bioavailable	14.20 (5.07)	13.38 (4.54)	13.90 (3.82)	0.714	0.757
Testosterone (ng/dL)					
Total	396.50 (118.73)	389.67 (124.31)	438.42 (170.28)	0.200	0.209
Bioavailable	121.33 (32.51)	132.40 (42.14)	131.60 (48.50)	0.476	0.311
SHBG (μg/dL)[&]	1.01 (1.68)	0.89 (1.66)	1.08 (1.60)	0.162	0.557
C-reactive protein (mg/L)[&]	2.05 (2.12)	1.88 (1.83)	1.67 (1.72)	0.383	0.174

[&]: Analysis was performed on log transformed variable.

5. BMD, rate of changes in BMD, QUS parameters, bone turnover markers and coronary artery calcification by G-1181C genotype

We found no significant difference between the genotype groups with respect to BMD, percent rate of change in BMD, or absolute rate of change in BMD in the study population. However, men with C/C homozygous exhibited a significantly higher BUA and SOS parameters compared with men with heterozygous (Table 57). Furthermore, the statistical significance remained across genotypes after adjusting for significant confounding factors (Table 58). There was a less significant linear trend in BUA or SOS parameters across genotypes (p=0.068, p=0.045). The unadjusted CAC score was significantly different among the polymorphisms in the first exon G-1181C in participants (p- value 0.055; trend = 0.032) (Table 59). We hypothesized that subjects with C/C genotype would have lower coronary calcification compared with subjects with G/G or G/C genotypes. However, we found that men with C/C genotype showed higher CAC score than men with G/G genotype. Adjusting for age demonstrated a more significant

result (p-value 0.037; trend = 0.013). However, there was no interaction effect between age and G-1181C genotypes (p = 0.554). Additional adjustments for potential confounding factors (education, use of thiazide diurectics, and cancer), which differed by G-1181C genotype, did not change the significant effect of G-1181C polymorphism on coronary calcification (CAC) score in participants (p = 0.029; trend 0.010). Adding family history of cardiovascular diseases yielded more significant difference in CAC across genotype (p=0.017, trend =0.006).

When we looked at the interaction effect on coronary calcification between total calcium intake tertiles (the lowest =< 710 mg/day vs. the highest > 1087 mg/day) and G-1181C genotypes, we could not observed any interaction effect on coronary calcification score (p for interaction = 0.61) (Figure 11).

Table 58. Bone mineral density (BMD), rate of change in BMD, QUS parameters, and bone turnover markers by OPG G-1181C genotype

	OPG G-1181 C genotypes			p-value	
	G/G (n=30)	G/C (n=60)	C/C (n=43)	ANOVA	Trend
BMD (g/cm2)					
Total hip	0.96 (0.12)	0.97 (0.14)	0.97 (0.12)	0.869	0.754
Femoral neck	0.78 (0.12)	0.78 (0.12)	0.77 (0.12)	0.920	0.780
Trochanter	0.75 (0.11)	0.75 (0.13)	0.74 (0.10)	0.998	0.964
Intertrochanter	1.11 (0.14)	1.15 (0.17)	1.14 (0.16)	0.676	0.490
Percent rate of change in BMD(%/yr)					
Total hip	0.12 (0.68)	-0.07 (0.73)	-0.04 (0.60)	0.455	0.334
Femoral neck	0.19 (0.99)	-0.12 (0.79)	-0.18 (0.74)	0.140	**0.062**
Trochanter	0.26 (0.83)	-0.08 (0.79)	0.005 (0.75)	0.145	0.165
Intertrochanter	0.07 (0.66)	-0.06 (0.82)	-0.10 (0.61)	0.602	0.331
Absolute rate of change in BMD ($mg/cm^2/yr$)					
Total hip	0.91 (6.20)	-0.46 (6.99)	-0.42 (5.94)	0.604	0.388
Femoral neck	1.37 (7.76)	-0.78 (6.35)	-1.47 (5.70)	0.173	**0.068**
Trochanter	1.77 (6.11)	-0.56 (6.46)	-0.09 (5.71)	0.233	0.205
Intertrochanter	0.61 (7.00)	-0.38 (9.25)	-1.17 (6.96)	0.652	0.356
Calcaneal QUS	(n=27)	(n=51)	(n=32)		
BUA (dB/MHz)	**76.67 (16.10)**	**74.97 (15.75)**	**84.15 (14.69)**	**0.031**	**0.068**
SOS (M/S)	**1551.67 (28.71)**	**1549.41 (29.01)**	**1567.65 (32.94)**	**0.024**	**0.045**
Bone Turnover Markers	(n=30)	(n=60)	(n=43)		
Osteocalcin (ng/ml)	7.88 (1.39)	9.37 (1.51)	8.28 (1.50)	0.101	0.605
N-telopeptide (nM BCE/mM Cr)	**31.93 (1.38)**	**39.01 (1.76)**	**33.30 (1.41)**	**0.083**	0.699

Table 59. Adjusted Bone mineral density (BMD), rate of change in BMD, QUS parameters, and bone turnover markers by OPG G-1181C genotype

	OPG G-1181 C genotypes			p-value	
	G/G (n=30)	G/C (n=60)	C/C (n=43)	ANCOVA	Trend
BMD (g/cm2)					
Total hip	0.96 (0.12)	0.97 (0.14)	0.97 (0.12)	0.988	0.881
Femoral neck	0.78 (0.12)	0.78 (0.12)	0.77 (0.12)	0.824	0.546
Trochanter	0.74 (0.11)	0.74 (0.13)	0.74 (0.10)	0.944	0.790
Intertrochanter	1.11 (0.14)	1.14 (0.17)	1.14 (0.16)	0.992	0.921
Percent rate of change in BMD(%/yr)					
Total hip	0.14 (0.13)	-0.08 (0.09)	-0.03 (0.10)	0.361	0.306
Femoral neck	**0.19 (0.16)**	**-0.12 (0.11)**	**-0.17 (0.13)**	0.170	**0.076**
Trochanter	0.29 (0.15)	-0.09 (0.10)	0.003 (0.12)	0.121	0.141
Intertrochanter	0.10 (0.13)	-0.09 (0.09)	-0.08 (0.11)	0.494	0.318
Absolute rate of change in BMD ($mg/cm^2/yr$)					
Total hip	1.12 (1.22)	-0.61 (0.85)	-0.35 (0.10)	0.503	0.354
Femoral neck	**1.35 (1.23)**	**-0.79 (0.86)**	**-1.43 (1.01)**	0.210	**0.085**
Trochanter	1.97 (1.15)	-0.66 (0.80)	-0.10 (0.94)	0.183	0.169
Intertrochanter	0.92 (1.50)	-0.71 (1.05)	-0.93 (1.23)	0.601	0.345
Calcaneal QUS					
BUA (dB/MHz)	**77.34 (3.05)**	**74.69 (2.21)**	**84.04 (2.79)**	**0.036**	**0.109**
SOS (M/S)	**1553.14 (5.91)**	**1548.35 (4.28)**	**1568.10 (5.40)**	**0.019**	**0.065**
Bone Turnover Markers [&]					
Osteocalcin (ng/ml)	7.84 (1.08)	9.39 (1.05)	8.28 (1.06)	0.105	0.574
N-telopeptide (nM BCE/mM Cr)	32.36 (1.09)	38.54 (1.06)	33.55 (1.07)	0.163	0.743

&: Analysis was performed on log transformed variable

Table 60. CAC score by OPG G-1181C genotypes[&]

N=137	OPG G-1181 C genotypes			p-value	
	G/G (n=30)	G/C (n=60)	C/C (n=43)	Unadj. & adj.	Trend
Total calcium score (Transformed score)	238.13 (11.53)	293.79 (11.85)	584.27 (17.48)	**0.055**	**0.032**
Age adjusted CAC score	201.70 (0.01)	323.30 (0.003)	570.83 (0.01)	**0.037**	**0.013**

[&] : Statistical analysis was performed using transformed total CAC score (power of 0.25)

[+] : Values are mean (SD) and adjusted for age

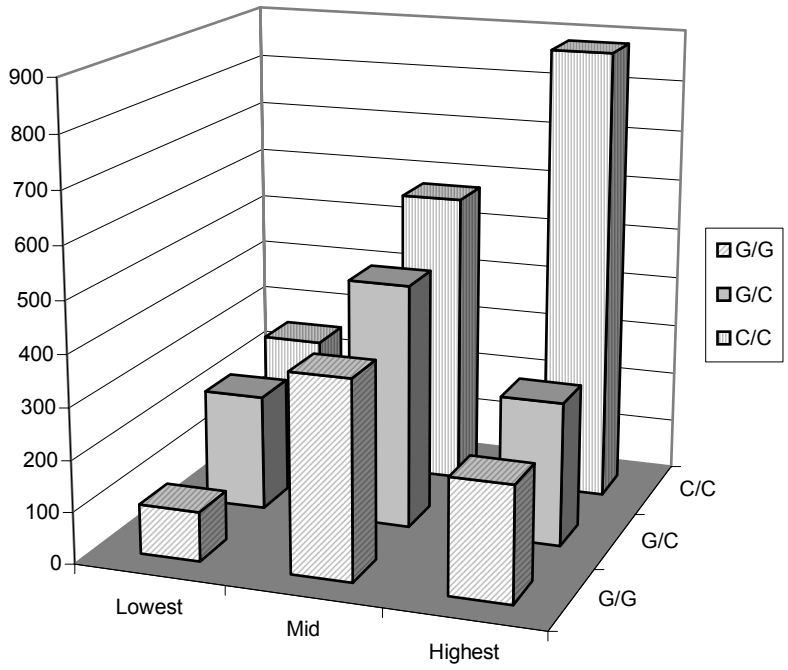

Figure 11. CAC score by total calcium intake and OPG G-1181C genotype.

VI. DISCUSSION

A. Summary

The primary aim of the present study was to examine the association between coronary artery calcification and measures of bone strength, including BMD, bone loss, quantitative ultrasound (QUS) measurements, and biochemical markers of bone turnover. In addition, we aimed to evaluate potential mechanisms underlying the associations between coronary calcification and osteoporosis. Specifically, sex steroid hormones, markers of inflammatory response and polymorphisms at the osteoprotegerin (OPG) gene were examined as potential mechanisms. We studied 138 older Caucasian American men, aged 51 to 78 years at baseline (60-87 at the time of EBT scan), who were recruited from a longitudinal cohort study, Study of Osteoporotic Risk in Men or STORM. All participants underwent coronary artery calcification measurement using electron beam computed tomography (EBT).

We found that the median score of coronary artery calcification (CAC) was 414 ranging from 0 to over 10,000. 8% of the variability in CAC score was explained by age. There were no significant associations between BMD, bone loss and coronary calcification. Neither quantitative ultrasound measurements at the heel nor bone turnover markers were significantly related to coronary calcification.

Total and bioavailable estrogen levels of participants were significantly related to cross- sectional measurements of BMD but not with the rate of change of BMD. Calcaneal BUA was related to estrogen levels, but this was only of borderline significance. There was a borderline significance between calcaneal BUA and unadjusted and age adjusted mean value of total estrogen levels. There was no significant relationship between bone formation marker (osteocalcin), bone resorption marker (urinary N-telopeptide) and total bioavailable estrogen levels. We could not find any significant correlation of serum levels of C-reactive protein (CRP) with any of bone

related measurements. Coronary calcification was not correlated to either estrogen or C-reactive protein.

There was no significant difference in unadjusted value of BMD, rate of change in BMD, calcaneal QUS measurements and bone turnover markers across OPG T-950C genotypes. However, men with C/C genotypes are more likely to lose bone compared to men with T allele at the intertrochanter and the significance was persistent after controlling for factors associated with polymorphisms. OPG T-950C genotypes (T/T, T/C vs. C/C) significantly add to the prediction of rate of change of intertrochanteric BMD (partial R^2= 0.03, p=0.02). There was no significant difference in coronary calcification across OPG T-950C genotypes.

Finally, the OPG G-1181C polymorphism in the exon 1 did not have a significant effect on BMD or bone loss. However, unadjusted and adjusted mean values of calcaneal BUA and SOS differ across genotypes. Men with C/C genotype tend to have higher values in QUS parameters. Men with C/C genotypes also had significantly 0.5 SD higher coronary calcification score (584.3) compared with men with G/G (238.1) or G/C genotypes (293.8). There was a statistically significant trend in increasing coronary calcification with the number of C alleles (p for trend=0.03).

B. Determinants of coronary calcification

Age at the time of EBT scan or baseline was most significant predictor of coronary artery calcification. The prevalence of coronary artery calcification increased from the median value of 152 at age less than 65 years, to 788 at age 80 years and older, which is consistent with previous studies (Newman et al, 2001). Age at the time of EBT scan or baseline was most significant predictor of coronary artery calcification in the present study. Waist girth measured at baseline was weakly but not statistically significant related to coronary artery calcification (r_s=0.14).

However, we found no significant associations between quantity of CAC and conventional cardiovascular risk factors including lipid, diabetes, and hypertension. Unlike previous studies, which have reported a strong association between risk factors and CAC (Wong et al., 1994; Maher et al., 1996; Valdes et al., 2001), our study is more consistent with studies pointing out diminished associations between CAC and cardiovascular risk factors with aging (Newman et al., 2001; Frost et al., 1996). Recent studies in older people suggest that the characteristics and progression rate of CAC may differ from those of younger adults (Newman et al., 2001).

Total calcium intake from food or dietary supplement measured in follow-up examination showed a significant association with the extent of coronary calcification in the multivariate model. Interestingly, we found significant correlation between total calcium or vitamin D intake, but not dietary intake and coronary calcification. In multivariate analysis model, total calcium intake was remained to be significantly associated with coronary calcification. Because vitamin D and calcium supplementation along with dietary intake are widely used for the prevention of osteoporosis, especially, in the elderly, it is important to investigate whether large intake of calcium or vitamin D for osteoporosis is related to vascular calcification.

For instance, one in vitro study of vitamin D_3 on vascular smooth muscle cells demonstrated that the $1,25(OH)_2D_3$ (10^{-7} mol/L) affected the vascular smooth muscle cells and there was a dose dependent effect of $1,25(OH)_2D_3$ on the stimulation of vascular calcification (Jono et al., 1998). Furthermore, our study finding is similar to the result from a study, which showed the total calcium intakes had weakly associated with the presence of aortic calcification in postmenopausal women (Aoyagi et al., 2001). The study investigators found that one standard increase in calcium intake (386mg/day) was related to a 20% increased risk of having aortic calcification in Japanese-American women. Our study demonstrated that 4 % of the variability in CAC was attributable to calcium intake.

C. Determinants of Bone loss

Few longitudinal studies of bone loss at the hip and lumbar spine in men have been reported for various populations of Australian (Jones et al., 1994), European (Burger et al., 1998; Dennison et al., 1999), and US cohorts (Hannan et al., 2000). Body mass index (BMI), dietary calcium intake, physical activity, smoking, and alcohol intake all have been shown to be determinants of BMD or bone loss in elderly men and women.

Weight change since baseline, history of fractures, the use of glucocortioid, and current smoking were significantly associated with the rate of changes in BMD of total hip or femoral neck. Weight change since baseline was consistently associated with rate of change in total hip and all its sub-regions. These results are consistent with findings from cross sectional and longitudinal studies that reported that weight gain appeared to protect against bone loss in older men and women while weight loss are associated with significant bone loss (Hannan et al., 2000: Dennison et al., 1999).

Our previous cross sectional study in the STORM population did not find any significant effect of current cigarette smoking on low BMD (Glynn et al., 1995). However, we found that men who were current smokers lost more BMD than men who were non-smokers during 7-year follow-up. A detrimental effect of smoking on total hip and femoral neck BMD in the present study is similar to the reports from male twins (Slemenda et al., 1992), cross sectional studies (Kiel et al., 1996; Nguyen et al., 1994) or longitudinal studies (Burger et al., 1998; Dennison et al., 1999; Hannan et al., 2000). The higher bone loss in elderly men who smoked cigarettes has been linked to increased bone loss at the radius (Krall and Dawson-Hughes, 1991), lumbar spine (Dennison et al., 1999) and femoral neck (Burger et al., 1998).

A previous history of fracture after age 50 was inversely related to the bone loss rate at the hip. The history of fracture accounted for bone loss varied from 4% at the femoral neck to 9% at the total hip. History of any fracture predicted the increased risk

of subsequent new incident fractures (Cauley and Zmuda, 1999). Use of corticosteroid was significantly related to bone loss in our study sample. Chronic corticosteroid users showed a great extent of bone loss, especially in trabecular bones (Reid et al., 1999). For instance, glucocorticoid use was significantly related to two fold increased risk of vertebral fracture (Cauley and Zmuda, 1999).

Interestingly, bone loss in our study was not affected by physical activity, and thiazide diuretic use, which previously predicted BMD at baseline (Glynn et al., 1995)

D. Relationship between coronary calcification and bone density, and bone loss

The relationship of coronary calcification to bone mineral density (BMD) is controversial. There are a number of studies linking biological or pathophysiological pathways between bone and vascular calcification (coronary or aortic calcification). The molecular components of calcium deposit (hydroxyapatite: calcium and phosphate product) detected in atherosclerotic plaques have similar composition to bone (Anderson et al., 1983). Embryonic bone formation and bone remodeling has been detected in the atherosclerotic lesions and calcified heart valves. Clinical studies have found an association between low bone mass and increased osteoporotic fracture risks with atherosclerotic calcification (Fujita et al., 1984; Ouchi et al., 1993; Byers et al., 2001) or cardiovascular mortality (Browner et al., 1993;Browner et al., 1991; von der Recke et al., 1999; Barengolts et al., 1998). In some studies, investigators have suggested the observed association merely as age-related process (Frye 1992; Anderson, 1964; Barengolts 1998; Vogt, 1997), whereas others have supported a causal relationship (von der Recke 1999; Barengolts, 1993) between aortic or coronary calcification and BMD. Moreover, Women with low BMD and greatest bone loss had higher frequency of presence of calcification or severe aortic calcification (Kiel et al., 2001; Hak et al., 2000) However, there is only few study examined whether the calcification is associated with BMD and whether progression of vascular calcification is related to bone loss in men.

1. BMD, bone loss and coronary calcification

In this study, we were unable to link BMD at either the hip or any hip subregion to the extent of coronary artery calcification (CAC) measured with EBT in older men. Moreover, there was no relationship between bone loss and coronary calcification. Few studies in men have demonstrated a significant relationship between BMD, cardiovascular disease and cardiovascular mortality (Laroche et al., 1994; Trivedi et al., 2001). In Framingham study, however, the relationship between BMD and aortic calcification in men was negative while one percent decline in metacarpal cortical area was related to 7.3% increased in aortic calcification index in women (p=0.01). Men with age adjusted highest quartiles of bone loss to have no differences in abdominal aortic calcification index by X-ray than men with the lowest quartiles of bone loss in Framingham Study (Kiel et al., 2001).

It is possible that the lack of association between BMD and coronary calcification in our study participants is due to the gender differences in prevalence of cardiovascular disease and osteoporosis. Men had higher BMD compared to women at the same age levels. The BMD values of the present study were similar to the values of NHANES III (Looker et al. 1997) but the rate of change in BMD of the total hip and other sites in our study sample was remarkably lower that other bone loss studies (Hannan et al., 2000; Burger et al., 1998). For instance, the bone loss rates at the femoral neck in our study are smaller than those reported by Jones et al. (1994) and by Hannan et al. (2000) in longitudinal studies. The annualized femoral neck bone loss for our study sample was –0.07% per year compared with –0.4% per year in men from the Framingham Study (Hannan et al., 2001). In the Dubbo study, men mean age 70 years were reported to have mean annual percent loss in the femoral neck BMD of 0.82% compared with the mean value of bone loss in the femoral neck of 0.07% in our study (Jones et al., 1994). The difference in bone loss between our study sample and other studies may due to healthy volunteer effect and the exclusion criteria of study. However, men in the current study gained weight significantly compared with men who did not participate. In addition, we excluded men, who underwent coronary bybass surgery, so that we could

avoid the artifact on EBT measurement. Our study cohort may be subject to selective survival bias of a healthy cohort. The lack of association may due to the greater extent of coronary calcification in our study participants. In some cross sectional studies reporting correlation, the extent of coronary calcification was far less than the distribution of coronary artery calcification of the current study (Barengolts et al., 1998). Moreover, the significant relationship was often attributable only to the presence of aortic or coronary calcification not the extent of coronary calcification. As we noted in the determinants of coronary calcification analysis, age and total calcium intake were significant correlates of coronary calcification. We were not able to observe significant or independent associations of traditional cardiovascular risk factors including hypertension, diabetes, low physical activity and current smoking. This may indicate that our study sample is quite healthier than other study groups, and the study results are affected by survivor bias. Also, the quantity of coronary calcification in older or oldest older population, especially in men, may be greatly influenced by aging rather than active atherosclerotic progression (Vogt et al., 1997; Aoyagi et al., 2001).

Some studies have suggested that several etiologic factors are common to both diseases, yet just act as confounders when these factors were not considered in the analysis. Estrogen deficiency has been recognized as major etiologic links, which account for the development of postmenopausal osteoporosis and possibly with aortic/coronary calcification (Banks et al., 1994). In addition, vitamin D excess and vitamin K deficiency have been proposed as etiologic links in several studies (Moon et al., 1992: Jie et al., 1996). In vitro and in vivo studies demonstrated that excess vitamin D induced both atherosclerosis and osteoporosis.

We hypothesized that gender specific hormonal factors, such as estrogen deficiency, unique to women may also play a role in the presence or progression of coronary calcification in men. Hormonal environments unique to women may influence the gender specific underlying pathophysiology of both diseases. Estrogen deficiency has been associated with decreased BMD (Riggs et al, 1998), with decreased HDL-cholesterol, or increased LDL cholesterol (Gaspard et al., 1995). In addition, cross

sectional studies and longitudinal studies in women have shown that increased incidence of CVD after menopause results from the loss of estrogen protection during menopause. Kiel et al (2001) reported that exogenous estrogen users had significantly less progression of aortic calcification compared with non-users. This result was supported by a recent autopsy study. The independent and inverse relationship of estrogen use (use of estrogen replacement therapy) to coronary calcium, plaque burden, and the proportion of calcium to plaque were demonstrated in postmenopausal and premenopausal women (Christian et al., 2002). Interestingly, these associations were not explained by different prevalence of known cardiovascular risk factors (age, diabetes, or hypertension) or osteoporosis.

2. QUS and coronary calcification

Our findings of no significant association between quantitative ultrasound (QUS) parameters and coronary calcification are consistent with the BMD findings. To our knowledge, there are no other studies available for comparison.

QUS measurement is a useful noninvasive technique to assess bone quality e.g. mechanical properties and microarchitecture, as well as bone density (Gluer, 1997; Bouxsein et al., 1997). Low calcaneal QUS parameters were associated with lower BMD at the calcaneous and hip, and these values have been reported independent predictors of hip and spinal fractures in older women (Bauer et al., 1997; Gregg et al., 1997;Hans et al., 1996). Current study showed a similar correlation between BUA and hip BMD measurements ($r=0.39$-0.42, $p<0.001$) to those results from other studies (Bauer et al., 1997; Frost et al., 2001).

Low QUS parameter is associated with cardiovascular mortality. The most recent study based on the Study of Osteoporotic Fractures (SOF) reported that each one standard deviation (17.0 dB/MHz) decrease of calcaneal BUA was significantly associated with a 16% increase in total mortality and 19% increase in cardiovascular mortality (Bauer et al., 2002). QUS measurements have been also negatively related to age in women (Gregg et al, 1997). However, we did not observe any significant

correlation between age and QUS parameters in men. The age effect on QUS parameters was reported to be small and relatively stable up to 60 years and begins to decline in men more slowly than in women (Sosa et al., 2002). Therefore, it is possible that we may not find that an association between age-related coronary calcification and age related changes of structural and architectural bone quality.

3. Biochemical markers of bone turnover and coronary calcification

We examined the correlation between biochemical bone turnover markers and coronary calcification in our study sample. We hypothesized that men with greater bone turnover markers would have greater extent of calcification. However, we did not observe any relationship of osteocalcin and NTx to coronary calcification.

In elderly men, a relative increase of bone resorption and stable bone formation result in a remodeling imbalance which cause bone loss with aging (Orwoll 1999). Orwoll (1998) and others (Schneider and Barrett-Connor, 1997; Krall et al 1997) showed that biochemical markers of bone turnover were correlated to BMD and bone loss. One study reported that men with both osteocalcin and NTX in the highest quartile had 11% lower BMD at the femur neck compared with the men with both markers in the lowest quartile (Schneider and Barrett-Conner, 1997). We observed similar patterns of bone turnover markers and BMD in our study sample. Similarly, correlation coefficients were significant between markers and the rate of change in BMD at all sites. The correlations remained significantly independent of age and weight changes during follow up. Studies have reported that elderly men with high levels of biochemical markers, especially increased bone resorption, have lower BMD and greater bone loss (Krall et al., 1997).

The lack of association between CAC and markers of bone turnover may due to the sensitivity or specificity of measurements of bone turnover markers. Mainly, biochemical bone markers were useful for the studies of bone disease, but they may also reflect metabolic disturbance independently from diseases (Szulc and Delmas,

2001). Osteocalcin in serum samples undergoes a rapid catabolism resulting in formation of smaller fragments. Currently available Immunoassays can measure not just intact osteocalcin but also smaller byproducts of its degradation. Therefore, these methods may yield different results in various populations (Garnero et al., 1992;Seibel 2000). Urinary NTx is also useful for measurement of bone resorption. Nevertheless, this measurement is subject to large analytical variability dependent on time of specimen collection and the assay variability of inter and intra-subjects (Orwoll et al., 1998).

Secondly, some biochemical markers are present in other tissues, and other diseases can influence their levels. For instance, osteocalcin is expressed in osteoblasts, but it is also expressed in osteoblast-like calcifying vascular cells (Wallin et al., 2001). Osteocalcin is present in calcium deposits in advanced human lesions (Bini et al., 1999). Therefore, it is possible that the measurement of osteocalcin may partially reflect both mechanisms in bone and cardiovascular disease. The association between osteocalcin and vascular calcification can be masked by the measurement of serum osteocalcin levels.

Moreover, one epidemiological study reported that serum osteocalcin levels were increased in women with aortic atherosclerosis (Jie et al., 1995). The authors reported atherosclerotic women with calcifications had higher free osteocalcin concentration with a low affinity for hydroxyapatite binding capacity. This result also suggests that osteocalcin found in calcium deposit from atherosclerotic lesions might have impaired functions for binding calcium hydroxyapatite and lost an inhibitory role against calcification (Dhore et al., 2001). Therefore, the measured osteocalcin as an early marker of bone turnover may reflect not only early stage of calcification but also advanced and stabilized calcified lesions. Also, the decline in bone mass with aging in men is not clear and different from the mechanism (accelerated bone resorption) that seen in postmenopausal women.

E. Estrogen, C-reactive protein in relationship with BMD, bone loss and CAC score

Estrogen has been recognized as an important factor in skeletal homeostasis in the male skeleton (Riggs et al., 1998: Khosla et al., 1998: Greendale et al., 1997: Szulc et al., 2001). Estrogenic effects on male skeleton are mediated by estrogen receptors (Riggs et al., 1998). Epidemiological data suggest that estrogen deficiency may be a common etiologic factor for CVD and osteoporosis (Barengolts et al., 1998: Banks et al., 1994). We investigated possible independent or mediating effects of estrogen deficiency with aging on both diseases. We found that bioavailable estrogen was related to cross-sectional measurement of total hip BMD but not to the rates of bone loss. Serum bioavailable estrogen was more strongly related to BMD than was bioavailable testosterone. These results are consistent to those of cross sectional studies of men (Greendale et al., 1997: Szulc et al., 2001). However, few studies were conducted to examine the association between age related change of BMD and estrogen levels in men (Khosla et al., 1998). Bioavailable estrogen levels were inversely related to age in age-stratified sample of men, aged 23-90 year. Furthermore, bioavailable estrogen concentrations were found to be an independent correlate of change in BMD of the femur neck in multivariate analysis (Khosla et al., 1998).

In the current study, however, we were not able to demonstrate that the serum levels of estrogen were associated with the percentage rate of change in BMD of the total hip and other regions. Serum levels of testosterone were weakly associated with the bone loss of the total hip ($r=-0.16$, $p=0.07$) and intrertrochanter ($r=-0.16$, $p=0.05$), but these relationships were no longer statistically significant once we adjusted for age and body weight.

Serum levels of total and bioavailable estrogen were not related to coronary calcification in the present study. Men had approximately four times higher serum concentrations of estrogen compared to postmenopausal women at same age groups (Greendale et al., 1997). Instead, we found borderline significance in inverse

association between bioavailable testosterone levels and coronary calcification (p= 0.05) in univariate analysis, but the inverse relationship was no longer significant after adjusted for age. Although estrogen plays an important role in male skeleton, little is known about the roles of estrogen in vascular calcification in men.

Studies suggest that the extent of conversion of testosterone to estrogen or dihydrotestosterone (DHT) in tissue levels seem to play a more important role in determining the antiatherogenic or proatherogenic role of testosterone (Mukherjee et al., 2002). In vitro studies have demonstrated that local conversion of circulating testosterone to estradiol by the aromatase in endothelial cells (English et al., 2000). Other studies have suggested that the sensitivity to estrogen deficiency due to variability of the estrogen receptor gene expression may influence bone metabolism and cardiovascular system. Arterial walls as well as bone are target organs for estrogen. Several studies demonstrated that estrogen receptors were expressed on osteoblasts, osteoclasts, vascular endothelial and smooth muscle cells (Eriksen et al., 1988; Oursler et al., 1994; Mendelsohn et al., 1999).

Our lack of association of estrogen to both vascular calcification and bone loss in our study suggests that estrogen deficiency is not likely to be the full explanation. Estrogen deficiency may have indirect and opposite effects on arteries and bone by the differential cytokine responses even though estrogen trigger the same inflammatory markers, such as tumor necrosis factor α (TNF-α) (Tintut et al., 2000;Yanaga et al., 1992). For instance, estrogen deficiency has been linked to the upregulation of TNF-α, a cytokine mainly secreted by macrophages and found in bone and in atheroslcerotic lesions (Barath et al., 1990). Tintut et al (2000) and Bertolini et al. (1986) showed the opposite effects of TNF-α on the bone and vasculature by inducing different signaling pathways: 1) stimulation of the cAMP pathway in calcifying vascular cells promoting to mineralization and 2) stimulation of G-protein mediated pathway in osteoblasts by inhibiting bone formation.

The acute phase reactant C-reactive protein is a marker of inflammation, which has been related to cardiovascular disease. CRP has been shown to be an independent predictor of the atherosclerosis and unstable angina pectoris or recurrent risk of myocardial infarction. Moreover, increased plasma CRP level has been reported as an indicator of extent and severity of sub-clinical atherosclerosis even in apparently healthy population (Cushman et al., 1999: Ridker et al. 1999a). Estrogen deficiency during the menopause has been linked to stimulate the expression of cytokines including IL-1, IL-6, and tumor necrosis factor-α. Increased levels of these cytokines were associated with increased serum levels of CRP. Conversely, exogenous estrogen use also linked to the increased levels of CRP in studies of postmenopausal women (Shemesh et al., 1997: Herrington et al., 2001). In relationship with bone metabolism, some cross sectional studies suggested that CRP is significant predictor of bone loss in early rheumatoid arthritis patient (Gough et al., 1998). Therefore, we hypothesized that high serum CRP concentrations with low estrogen would be related to higher bone loss and greater vascular calcification. However, there was no relationship of serum CRP concentrations to BMD or bone loss in the present study. Furthermore, we could not find any relationship between CRP and coronary calcification in our study sample.

The lack of correlation between CRP and coronary calcification is similar to several other reports (Redberg et al., 2000; Hunt et al., 2001). A possible explanation of the lack of link may reflect the fact that C-reactive protein is related to an early stage of atherosclerosis while coronary calcification is an accumulative process of atherosclerosis states over time. In other words, coronary calcification may be linked to a cumulative inflammatory response (Tintut and Delmer, 2001).

In summary, we were not able to find any significant relationship of serum levels of estradiol and CRP with the rate of change in BMD after adjusting for age. Furthermore, we could not find correlation between estrogen, CRP and coronary calcification.

F. Effect of osteoprotegerin gene variants on BMD, bone loss, and QUS parameters

Genes may be involved in possible etiologic pathways in both osteoporosis and vascular calcification. Osteoprotegerin (OPG) is a recently cloned soluble decoy receptor of the tumor necrosis factor receptor family (TNF) interacting with receptor activator of nuclear factor (NF-kB) ligand (RANKL), and its cellular receptor activator of NF-kb (RANK) in regulation of osteoclast differentiation and activity (Simonet et al., 1997; Yasuda et al., 1998). By binding to RANKL (or OPGL), osteoprotegerin can block the interaction of RANK and RANKL to inhibit mature osteoclast differentiation (Simonet et al. 1997; Yasuda et al., 1998; Tan et al., 1997).

Osteoprotegerin deficient mice showed severe vascular calcification as well as osteoporosis (Bucay et al., 1998:Mizuno et al., 1998). The observations raise the possibility, a common genetic contribution in variability of vascular calcification and osteoporosis. Studies of OPG transgenic mice also support the hypothesis of the involvement of OPG in both cardiovascular disease and osteoporosis (Min et al., 2000; Simonet et al., 1997).

Two single nucleotide polymorphisms (T-950C in the promoter region, G-1181C gene in the exon1) in the OPG gene were examined in the present study. We hypothesized that men with C/C genotypes at the promoter legion and exon1 of each osteoprotegerin polymorphisms would be associated with higher BMD, lower bone loss, and lower calcification. The significant linkage disequilibrium between these two polymorphisms in this study has been reported in a Danish study (Langdahl et al., 2002) and Japanese study (Ohmori et al., 2002). Linkage disequilibrium (D'=0.70) in our study was greater than that of the European study (Langdahl et al., 2002). The distribution of allelic variability in each polymorphism is more similar to Caucasian European studies (Wynne et al., 2002; Langdahl et al., 2002; Brandstrom et al., 2000) compared to the results from Japanese study (Ohmori et al., 2002). Both genotype frequencies were in Hardy Weinberg equilibrium. We investigated whether an assocation differed by the number of C allele in each loci i.e. allele-dose effect. The C/C genotype of T-950C

polymorphism has been associated with greater BMD in Swedish men (Brandstrom et al., 1999). In the present study, none of loci in the OPG gene was significantly related to the BMD. Out results are consistent with more recent reports, which also found a lack of association with BMD in Irish (Wynne et al., 2002) and Danish populations (Langdahl et al., 2002).

There is no study available for comparison of our results on the association of OPG polymorphisms and bone loss in men. In the analysis of bone loss with OPG genotypes, we found a trend (p= 0.09 at the femur neck) but it did not reach the statistically significant association across T-950C genotypes. Men with C/C genotype tended to lose more BMD at all sites. C/C genotype lost approximately 0.25SD (1.63 $mg/cm^2/yr$) of total hip BMD compared with T/T genotype. Therefore, we tried the two other approaches to see whether the C alleles act as a dominant allele or as a recessive allele. In dominant model, we expected that the effect of the allele should be similar in both CC and other heterozygotes (T/C or G/C). In recessive allele model, we expected that the effect of C allele is only present in the C/C genotype. In a recessive effect model, we found that the presence of C allele in the T-950C polymorphism was significantly associated with the rate of change in BMD. For instance, there was a significant association with bone loss at the intertrochanter in men with C/C genotypes compared with men with T/T genotypes or T/C genotypes. Similarly, we found a trend of bone loss at the femur neck (p=0.06) across G-1181C genotypes such that men with C/C genotype tended to lose more bone compared with other two genotypes (G/G or G/C genotype). However, we could not find any statistical significance using all three approaches, which C alleles considered as a recessive, a dominant allele or as an allele dose effect. Two previous studies reported similar results that BMD of the men with C/C genotype of the G-1181C polymorphism was not significantly different from those of the men with G/C or G/G genotypes (Landahl et al., 2002:Wynne et al., 2002: Ohmori et al., 2002).

Interestingly, G to C substitution in the exon1 was associated with QUS parameters measured at the calcaneus. As we hypothesized, men with C/C genotypes

showed approximately 10% higher mean values of BUA compared with men with G/G or G/C genotypes. Calcaneal QUS parameters are also influenced by genetic components in the literature. A twin study reported that the estimated habitability of BUA and SOS were 0.53 and 0.61, respectively (Arden et al., 1996). Another twin study demonstrated that approximately 80% of variability of QUS might be attributable to genetics factors like BMD (Howard et al., 1998). Furthermore, the authors reported the separate genetic factors might play an important role in the genetic variance of QUS so that the QUS related specific genetic factors remained independently from joint genetic components to BMD (Howard et al. 1998). Currently there is no association study of G-1181C polymorphism and QUS parameters for comparison.

G. Effect of OPG gene variants on coronary artery calcification

Although OPG is an important cytokine involved in bone metabolism, OPG may play an important protective role in the development of cardiovascular disease. Animal studies strongly suggest that osteoprotegerin plays an inhibitory role in vascular calcification (Bucay et al., 1998; Min et al., 2000). OPG has been found in bone, heart arteries, veins, and other various tissue types (Bucay et al., 1998; Dhore et al., 2001; Simonet et al., 1997; Yun et al., 1998). OPG was detected in arterial smooth-muscle cells (Hofbauer et al., 2001) and endothelial cells (Malyankar et al., 2000).

Serum levels of OPG are increased with age (Yano et al., 1999;Szulc et al., 2001) and regulated by various factors including estrogen (Hofbauer et al., 1998), parathyroid hormone (Lee et al., 1999), and cytokines such as TNF-α (Hofbauer et al., 1998). There have been relatively few clinical studies of osteoprotegerin and cardiovascular disease. One standard deviation (0.11 ng/mL) increase in serum levels of OPG was related to increased cardiovascular mortality among a large cohort of older women (Browner et al., 2001). The association between increased serum levels of OPG and cardiovascular mortality was opposite to the evidences from animal studies (Bucay et al., 1998). This opposite evidence may be due to the fact that OPG in human reflect a

result of the development or presence of atherosclerosis rather than a cause of disease (Browner et al., 2001). In other words, serum OPG concentrations act as an inhibitory factor and try to regulate the disease process. Some of In-vitro studies also support the regulatory (inhibitory) role of OPG. OPG was detected in non-diseased vessel wall as well as in the advanced lesions of cardiovascular diseases (Dhore et al., 2001; Collin_Osdoby et al., 2001). Furthermore, OPG expression was upregulated in diseased lesions such as fibrous cap and fibrocalcific plaque. From these observations, we hypothesized that the polymorphisms in OPG gene may be related to the extent of vascular calcification.

Even though there was no statistically significant difference in mean values CAC score across OPG T-950C genotypes, our results showed that men with C/C genotype had higher CAC score compared with men with T/T or T/C genotypes. This trend is similar to the report of a recent study, which showed a relationship between the C/C genotype and increased intima media thickness. Brandstrom et al (2002) analyzed the effects of OPG polymorphisms on carotid intima-media thickness (IMT) and maximal post-ischemic forearm blood flow (FBF). They found that individuals with C allele homozygous had higher IMT and lower FBF compared to individuals homozygous for T-allele or heterozygous for TC. Serum lipids, smoking or physical activity did not differ across the OPG T-950C genotypes.

It is not known how this polymorphism exerts its effect. It is also uncertain that the activity of T-950 C promoter polymorphism is involved in altered transcription leading to increased or decreased serum levels of OPG. To date, there is no study available for explaining this novel single nucleotide polymorphism in the promoter regions of OPG and its association with cardiovascular disease and/or vascular calcification. Results from our study and Brandstrom et al. (2002) suggest an association between the OPG polymorphism and cardiovascular disease but this observation needs to be confirmed in other cohorts.

Surprisingly, the studies on osteoprotegerin G-1181C single nucleotide polymorphism yielded a significant association with coronary calcification. The results of our study show for the first time that the OPG G-1181C gene allelic variation is associated with coronary artery calcification.

Animal studies strongly suggest that osteoprotegerin play an inhibitory role in vascular calcification (Bucay et al., 1998; Min et al., 2000). We found a significant positive correlation between the presence of C allele of OPG G-1181C gene and higher prevalence of coronary artery calcification and family history of cardiovascular disease.

There are, however, several disadvantages in association studies of single gene (e.g. osteoprotegerin) for complex phenotypes such as BMD or bone strength. Firstly, the functionality of a T to C change in the promoter region has not been studied. The T 950-C polymorphism is located in the promoter, 129 bp upstream from the TATA box and 233bp upstream from the translation initiation site. One possibility of the functional significance of this polymorphism may be associated with the alteration of promoter activity. TGF-β was related to accelerate the expression of OPG gene in vitro study (Thirunavukkarasu et al., 2001).

In addition, it is not clear whether there is an association between genetic variations of OPG and cardiovascular risk factors or outcomes. Secondly, the relationship between the OPG G-1181C polymorphism and CAC may reflect an altered response resulting from the differential secretion of OPG containing the lysine to asparagines mutation. G to C polymorphism at the first exon of OPG gene causes a change in 3rd amino acid of 10 amino acid residues of the signal peptide from lysine to asparagines. Biochemical characteristics of these changes of two amino with respect to OPG protein physiological changes have not been studied yet. The biochemical property of lysine is a basic amino acid while asparagines is a polar, uncharged amino acid. Allelic variation encoding third amino acid of signal peptide may be involved in the sequence discrepancy (Morinaga et al., 1998). In addition, Ohmori et al (2002) suggested that a basic amino acid in the signal peptide could cause the difference in the secretion of protein like an angiotensinogen gene (Nakajima et al., 1999).

H. Public health implications of the present study

The present study failed to find a link between coronary calcification and BMD in older men. Therefore, this study suggests that these two diseases may not be etiologically linked. Thus, public health efforts to prevent CAD and osteoporosis in men need to focus on each disease separately. In addition, our study findings suggest that larger intakes of calcium or vitamin D in older populations may affect the development of vascular calcification. Vitamin D and calcium supplementation are widely recommended for the prevention of osteoporosis in older persons. Several studies have reported that adequate calcium intake was weakly related to a reduced risk of hypertension and cardiovascular disease in older persons (Allender et al., 1996). However, our study and others (Hines et al., 1985, Price et al., 2000; Aoyagi et al., 2001) demonstrated that a higher intake of total calcium and vitamin D may be related to a higher risk of vascular calcification. The observation of a link between calcium and vitamin D intakes on vascular calcification needs to be further evaluated. Another important public health issue is our observation of the relationship between genetic variations in OPG and coronary calcification. G-1181C polymorphism was significantly associated with coronary calcification in older Caucasian-American men. The prevalence of G-1181C C allele was high (49%) among our study population, consistent with other reports (Langdahl et al., 2002; Ohmori et al. 2002). Thus, the finding of the relationship between G-1181C C allele and coronary calcification, especially in light of its high prevalence, may be of importance in identifying individuals who have this polymorphism to investigate the genetic effect on vascular calcification and coronary artery disease.

I. Strengths and limitations

Most studies on the link between osteoporosis and cardiovascular disease have focused on female populations. The advantages of the current study is that it focuses on a male population with wide age range, and it offer the opportunity to explore the

relationship of various bone related measurements, not just BMD, to coronary calcification and to examine the proposed etiologic mechanism for hypothesized link between osteoporosis and cardiovascular disease running nine years of follow-up. This study is unique in that no other studies have previous examined the bone quality measured using quantitative ultrasound (QUS) evaluations of bone density or bone turnover markers and coronary calcification. Moreover, the quantitative method of coronary calcification using EBT has been regarded as one of the most sensitive techniques to assess coronary artery calcification and to predict future coronary hard events. Finally, the present study may be the first study investigating the association of two genetic polymorphisms of the osteoprotegerin gene and coronary calcification in older men.

Despite several strengths, we need to address several limitations of this study.

First, we recruited study participants who were able to attend a clinic examination and didn't have a history of coronary bypass surgery reflecting no significant cardiovascular disease. Men who did not return for the EBT examination were older, and had poorer health status with more comorbid conditions than those who participated in the study. For instance, participants of current study were less likely to experience a myocardial infarction at baseline and follow-up examination than non-participants of each study examination. The study sample is also limited to those men who survived and participated at three examinations during the 9 year follow up. These men were younger and were less likely to lose bone than those who did not participate at baseline or follow up. This will limit the generalizability of study findings.

Second, we studied only elderly Caucasian-American men, and our findings may not be generalized to younger men or men of other ethnicities.

Third, it is important to emphasize that coronary calcification was measured cross-sectionally and we could not determine the causal relationship between the progression of atherosclerosis and bone loss.

Fourth, sex steroid hormone levels were measured once at the follow up examination, and the relationship of estrogen and the rate of changes in BMD were analyzed retrospectively. Thus, we are unable to determine the cause and effect of bone loss and

levels of sex steroid hormone. It is possible that the changes of sex steroid hormones in men may truly reflect the bone loss in men even though we could not establish the association in this study.

Finally, we could not determine the causal association between OPG genotypes and vascular calcification. It is uncertain whether osteoprotegerin genetic polymorphisms are associated with altered OPG expression and secretion levels interfering with the vasculature and vascular calcification mechanisms.

J. Directions for Future Study

Most studies on the relationship of coronary artery disease and osteoporosis have been conducted in pre- or post-menopausal women. For the most part, the correlation between BMD and vascular calcification is more significant in studies of women. Gender differences in the prevalence of coronary calcification and bone loss is prominent and comparison studies of men and women across specific age groups may provide insight into the role of possible association between osteoporosis and cardiovascular disease. In addition, prospective studies of coronary calcification in older men are needed to determine whether the progression of coronary calcification is associated with bone loss in older men. An unexpected finding was the observation that total calcium intake was related to vascular calcification needs to be further evaluated in other epidemiological and clinical studies.

Also, we need to further explore the influences of genetics polymorphisms of osteoprotegerin on serum levels of osteoprotegerin or osteoprotegerin promoter activity, which may relate to the presence of coronary calcification or bone mineral density. Investigations on the associations of coronary calcification and BMD and common pathways leading to deleterious health outcomes may provide an efficient prevention strategy of aging related diseases in an aging society.

K. Conclusions

In summary, the current study failed to provide convincing evidence of a link between osteoporosis and cardiovascular disease. Our results suggest that there is no significant relationship of BMD or bone loss to coronary artery calcification in older men. Serum levels of estrogen were related to cross -sectional BMD, but not to bone loss. Estrogen was not related to coronary calcification either. C-reactive protein, an inflammation marker, was not correlated with BMD or coronary artery calcification.

Our data suggest that osteoprotegerin (OPG) genetic polymorphisms may be associated with coronary calcification and/or BMD. However, future studies in this area are needed to corroborate and confirm these findings.