The effect of alcohol withdrawal on bone turnover in women with alcohol dependence

By

Pallavi Wyawahare

A thesis
Submitted to Victoria University of Wellington
in partial fulfillment of the requirements for the degree of
Master of Clinical Research

Victoria University of Wellington

2013

ABSTRACT

Objective:

Several studies have highlighted the detrimental effects of alcohol dependence upon bone health, but the majority of data relate to male alcoholics. In general, these effects are considered to be either a direct toxic effect on bone or related to confounding lifestyle factors linked to alcohol dependence. Given the rising prevalence of alcohol dependence in young women, data relating to this group are timely. We performed a study to assess bone health in this population and specifically to study change in bone turnover following admission to an alcohol detoxification unit in New Zealand.

Materials and methods:

We studied 20 women admitted to the Kenepuru alcohol withdrawal unit. After obtaining written consent, women completed lifestyle questionnaire detailing demographic information, and questions relating to cigarette and alcohol consumption, physical activity, dietary calcium intake. Fall and fracture history was also elicited. Heel ultrasound was performed with a GE Achilles instrument, to obtain measures of Speed of Sound (SOS), Broadband Ultrasound Attenuation (BUA), Stiffness Index (SI) and T score bone mineral density (BMD).). We supplemented our study with a similar data collection protocol in 16 healthy staff members without a diagnosis of alcohol dependence. Fasting blood samples were obtained in a subset of 6 premenopausal alcoholic women and a marker of bone formation [serum procollagen type 1 N propeptide (P1NP)] and bone degradation [serum C-terminal cross-linking telopeptide of type 1 collagen (CTX)] measured. Matched samples were obtained fasting on the day of admission (day 1) and the day of discharge (day 5). Ethical approval was given by the Central Health and Disability Ethics Committee.

Among women with alcohol dependence, the mean age was 45.2 (SD 8.97) years. Risk factors for osteoporosis were common, and much higher in this group than in the control group of healthy volunteers of similar age. Women had been drinking heavily for a mean of 16.3 years, and 75 % were current smokers. The mean

calcium intake in the group was very variable (range 121.41 mg to 2838.9mg daily). Five women (25%) reported moderate physical activity over the preceding 7 days; 15 women (75%) reported falls in the preceding year.

Table 1 shows the mean marker values on day 1 and day 5, available in a subset of 6 premenopausal, Caucasian women. While significant differences were seen in P1NP over the admission period, change in CTX failed to attain statistical significance in this small sample.

	Day 1 mean (SD)	Day 5 mean (SD)	P value difference
P1NP (µg/l)	23.08 (9.52)	27.88 (8.04)	0.04
CTX (µg/l)	0.14 (0.072)	0.14 (0.09)	0.85

Discussion:

In conclusion, lifestyle factors associated with poor bone health are prevalent in this population. Even within the limited sample size, significant differences were seen in change in BTM over a 5 day period following abstinence from alcohol in an alcohol dependent group; further work is now indicated in a larger sample size to assess speed and size of response.

Acknowledgement:

I would like to express my heartfelt gratitude and zillions of thanks to many key people who have helped me put this elaborate thesis together. First and foremost, I want to thank my Prof. Elaine Dennison for giving me such a great exposure. She is an institution in herself and hence an inspiration. Moreover, I want to thank our biostatistician Prof. Dalice Sim for her contribution in this effort. Her expertise helped me generate the most significant data which is an integral part of this thesis. Needless to mention, Dr. Geoff Robinson and Ms. Moira Gilmour for allowing me to carry out the thesis related procedures at the alcohol dependence unit at Kenepuru hospital. Their coordination has been truly outstanding.

Last but not the least, I specially, want to thank my family for encouraging me to take up this research. Their support truly meant a lot and has made me deliver what I thought was an enormous task.

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List of abbreviations

7-DHC 7-Dehydrocholesterol

25-OHD 25Hydroxychoecalciferol

ALP ..... Alkaline Phosphatase

ANOVA..... Analysis Of Variance

BASP/BALP ..... Bone Specific Alkaline Phosphatase

BMD ..... Bone Mineral Density

BUA..... Broadband Ultrasound Attenuation

Ca..... Calcium

CCDHB ..... Capiti and Coast District Health Board

CRF..... Clinical Risk Factors

CTX..... Carboxy Terminal Region

DEXA ..... Dual Energy X Ray Absorptiometry

DPD..... Deoxypyridinoline

ER ..... Estrogen Receptor

ERR..... Estrogen Receptor Related Receptor

GLOW ..... Global Longitudinal Study of Osteoporosis in Women

GPs ..... General Practitioners

GR..... Gradient of Risk

HPM ..... Health Promotion Model

HR-QOL ..... Health Related-Quality of Life

HR-MRI ..... High Resolution Magnetic Resonance Imaging

HR-pQCT ..... High Resolution peripheral Quantitative Computed Tomography

HR-µCT ..... High Resolution micro Computed Tomography

HUS..... Heel Ultrasound

IL ..... InterLeukins

MrOS..... Osteoporotic Fracture in Men study

NOF..... National Osteoporosis Foundation

NOS ..... National Osteoporosis Society

NSDUH ..... National Survey on Drug Use and Health

NTX..... Amino Terminal Region

NZ ..... New Zealand

OPP..... Osteoporosis Prevention Program

OPG ..... Osteoprogesterin

P..... Phosphorous

P1CP..... Procollagen Type I C Propeptide

P1NP..... Procollagen Type I N Propeptide

Pi..... Serum Phosphorous

$PreD_3$..... Previtamin D_3

PTH..... ParaThyroid Hormone

PYD..... Pyridinoline

QA..... Quality Assurance

QUS ..... Quantitative Ultrasound

RANK-L ..... Receptor Activator of Nulcear Factor-kB Ligand

RIA ..... Radio ImmunoAssay

s-CTX ..... serum-C Terminal Cross linked Telopeptide

S-OC ..... Serum Intact Osteocalcin

S-Total OC ..... Serum Total Osteocalcin

S-cOC ..... Serum g-carboxylated Osteocalcin

SOS..... Speed of Sound

SI..... Stiffness Index

SD ..... Standard Deviation

TRACP ..... Tartarate Resistant Acid Phosphatase 5b

U.S. ..... United States of America

U.K. ..... United Kingdom

UV ..... Ultraviolet

U-OC ..... Urine Osteocalcin

WHO ..... World Health Organization

Chapter One: Introduction

1.0 Introduction:

This thesis investigates the association between alcohol dependence and bone health in a group of New Zealand women admitted to an alcohol detoxification unit. While the intention of this study was to recruit 50 – 100 women, slow accrual rates necessitated the recruitment of a healthy control group without a history of alcohol dependence from staff at the Unit to provide a comparison group. We investigated relationships between alcohol intake and heel ultrasound measures; interrelationships between alcohol intake and other lifestyle factors detrimental to bone health; and change in bone turnover markers over a 5 day period following admission to the alcohol detoxification unit.

1.1 Osteoporosis – an overview:

Osteoporosis is defined by the World Health Organization (WHO) as a disease characterized by reduced bone mass and micro architectural deterioration of bone tissue [1]. This low bone mass makes the bone brittle and increases the risk of bone fracture [2].

Osteoporosis can be developed as a primary disorder or may be a result of other medical diseases such as gastrointestinal diseases, Rheumatoid Arthritis or endocrine diseases, surgical procedures or medications such as corticosteroids which lead to bone loss [3].

The main cause of postmenopausal osteoporosis is a drop in the circulating oestrogen level in women after the menopause, while in men a corresponding decrease in oestrogen and testosterone occurs. Women over the age of 50 and men over 70 years are hence more susceptible to osteoporosis [4]. Other identified risk factors for osteoporosis include:

1. Family history of Osteoporosis
2. Rheumatoid Arthritis,
3. Chronic Kidney disease
4. Hyperparathyroidism
5. Vitamin D deficiency
6. Inadequate dietary calcium intake
7. Consumption of high amounts of alcohol
8. Smoking
9. Amenorrhea, possible in association with eating disorders
10. Low body mass index for other reasons
11. Drugs used in breast cancer and prostate cancer therapy

At the early stages, before fragility fracture occurs, many patients are asymptomatic and hence undiagnosed. However, with greater reduction in bone density and consequent fracture, the patient may remark on tenderness/ bone pain due to micro fracture or low back pain and neck pain due to vertebral fracture which may also result in kyphosis. [4]

1.2 The Bone Remodelling process:

There are multiple reasons why the bone may become fragile. These include :a failure to produce sufficient bone mass and strength (peak bone mass), excessive bone resorption which reduces bone mass and results in micro-architectural deterioration of the skeleton, often coupled to an inadequate formation response to increased resorption during bone remodelling[5]

Bone remodelling is the process which helps in formation of new bone in the human body by preforming the resorption of old bone [5]. Osteoclasts and osteoblasts are the main cells which in addition with other accessory cell types perform bone remodelling [5].

Below is a schematic representation of the phases which take part in bone remodelling:

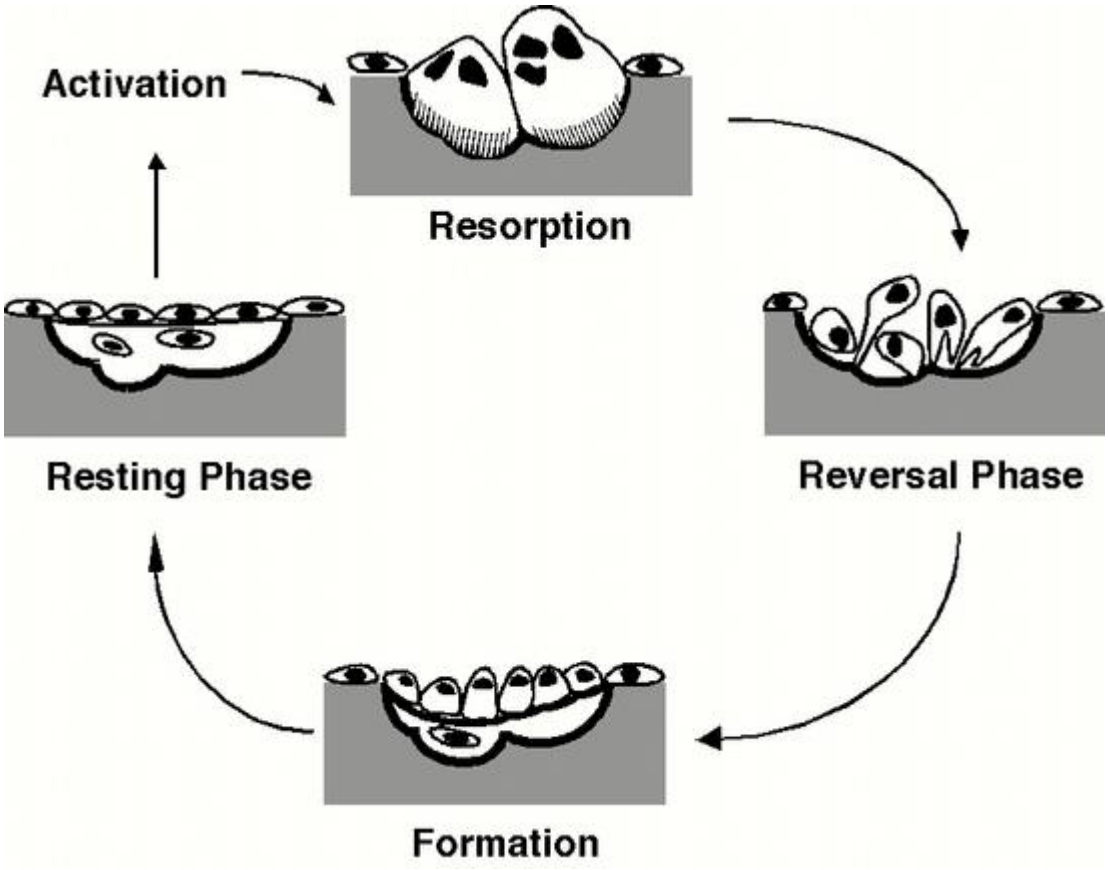


Figure 1: **Bone remodelling schematic representation**

The first step in bone remodelling is activation of osteoclasts [5]. During activation, the mononuclear osteoclasts precursors meet on the bone surface and fuse to form osteoclasts [5]. Receptor activator of nuclear factor-kB ligand (RANKL), IL-1, IL-6 (InterLeukins), systemic hormones like PTH, 1,25-dihydroxyvitamin D3 and calcitonin are the local cytokines which help in formation, activation and activity of osteoclasts[5]. RANK, which is the cognate receptor of RANKL is the main regulator of the osteoclast [5].

The reversal phase is the next step after the resorption phase. Apoptosis causes the death of osteoclasts and mononucleated resorbing cells [5]. Osteoblasts are the cells which then replace the osteoclast and proceeds the cycle in formation phase [5].

Osteoblasts traverse the bone surface via cortex where they refill the cortex by depositing it within osteon; a lamellar bone [5].

Throughout the formation phase in the matrix, the osteoblasts are buried and formed into osteocytes [5]. Osteocytes maintain contact with one another as well as with the cells on the surface of the bone; this helps in sending signals to the cells on the surface to regulate the bone remodelling process whenever necessary [5].

The resorption and reversal phase of the bone remodelling process are short while the formation process is long. In instances where there is any increase in the rate of the remodelling of bone, the bone mass will be reduced [5]. The important factor in osteoporosis is thus the inadequate formation response during bone remodelling [5].

1.3 The role of oestrogen in bone remodelling:

As mentioned previously one of the major factors in the pathogenesis of osteoporosis in postmenopausal women is oestrogen. Research studies show that oestrogen plays a significant role in bone loss in older women [5]. Although oestrogen is important for bone turnover in both the sexes, it has been seen that with a decrease in oestrogen, the rate of bone remodelling increases and amount of bone lost per remodelling cycle is also increased with oestrogen deficiency in the body [5].

Oestrogen acts on the body via its 2 receptors ERα and ERβ. Studies show that there is also an orphan nuclear receptor: oestrogen receptor-related receptor α (ERRα) which is also present in bone cells along with receptors ERα and ERβ [5]. This receptor may interact with receptors ERα and ERβ or may directly modify the bone cell function [5].

The schematic representation of oestrogen action shown overleaf in Figure 2 [40] helps to explain the process:

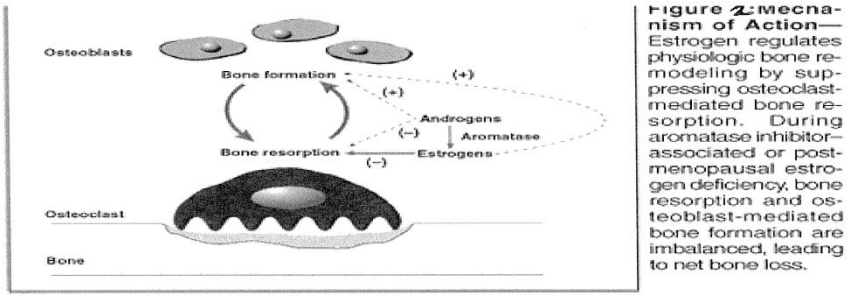


Figure 2: **Mechanism of action of oestrogen**

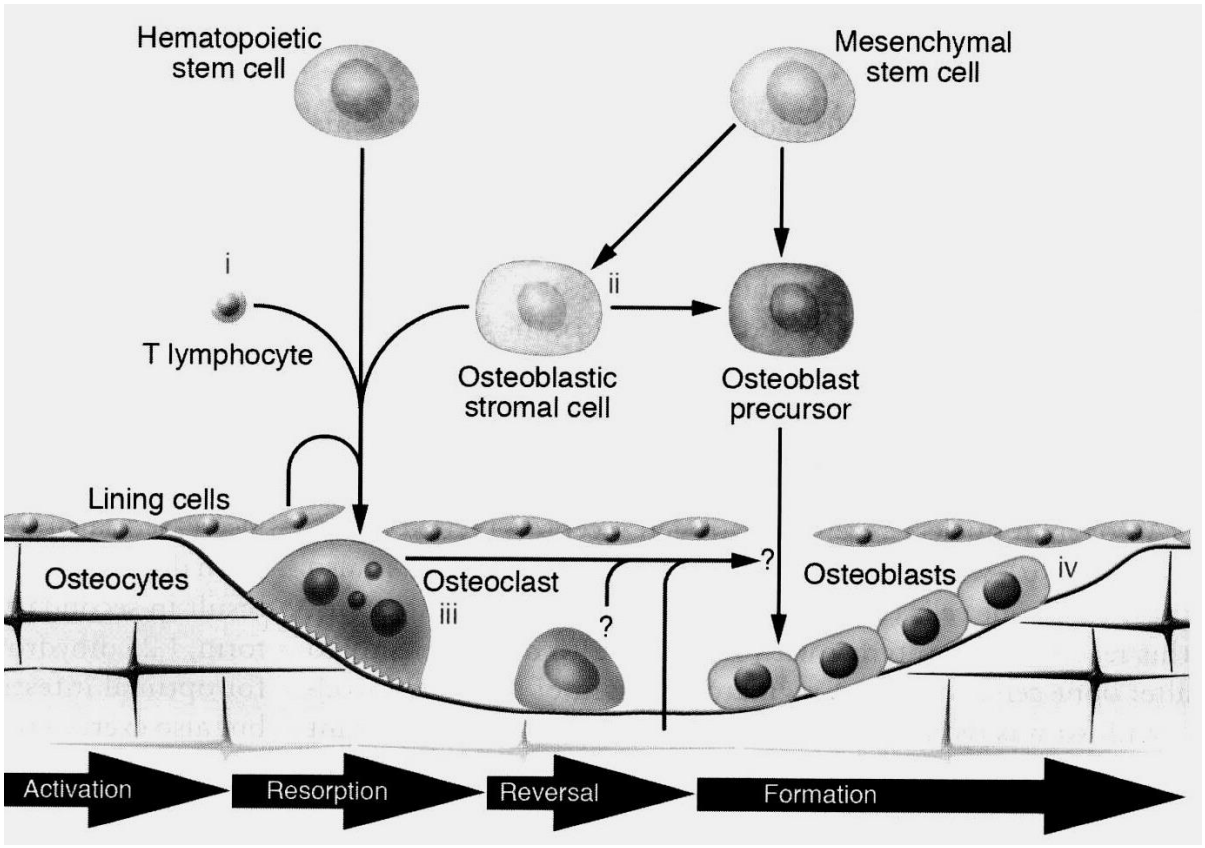


Figure 3: **process of bone remodelling on trabecular bone**

Figure 3 shown above shows the process of bone remodelling on trabecular bone. The osteoclasts are activated when hematopoietic precursors interact with cells in osteoblastic lineage. After the formation of osteoclasts, the resorption phase starts

which is then followed by the reversal phase. In the reversal phase, the mononuclear cells cover the bone surface. The next phase involves the formation phase. In this last phase of the bone remodelling process, the matrix is developed with the help of osteoblastic waves. These are then formed into osteocytes which then embed in the bone or die due to apoptosis.

Specifically, the action of estrogen shows an effect on:

i. T cell cytokine production.
ii. Effect on osteoblasts to alter their production of RANKL or OPG (osteoprogesterin).
iii. Direct inhibition of osteoclasts.
iv. Effect on formation of bone by osteoblasts.

In osteoporosis, the rate of bone resorption exceeds the rate of bone formation. With a decrease in oestrogen level, cytokines level increases which lead to increase in bone resorption [6].

1.4 Causative factors for osteoporosis:

As mentioned previously there are many factors which may lead to osteoporosis and the risk increases with the number of possible factors present.

1. **Genetic factors**:

Family history of hip fracture or any other osteoporotic fracture increases the risk of fracture in the individual.

2. **Hormones:**

The role of oestrogen has been discussed in detail above.

3. **Age:**

It has been observed that as age increases, bone mineral density (BMD) decreases increasing the chances of osteoporotic fragility fracture [6].This is demonstrated graphically in figure 4 [6].

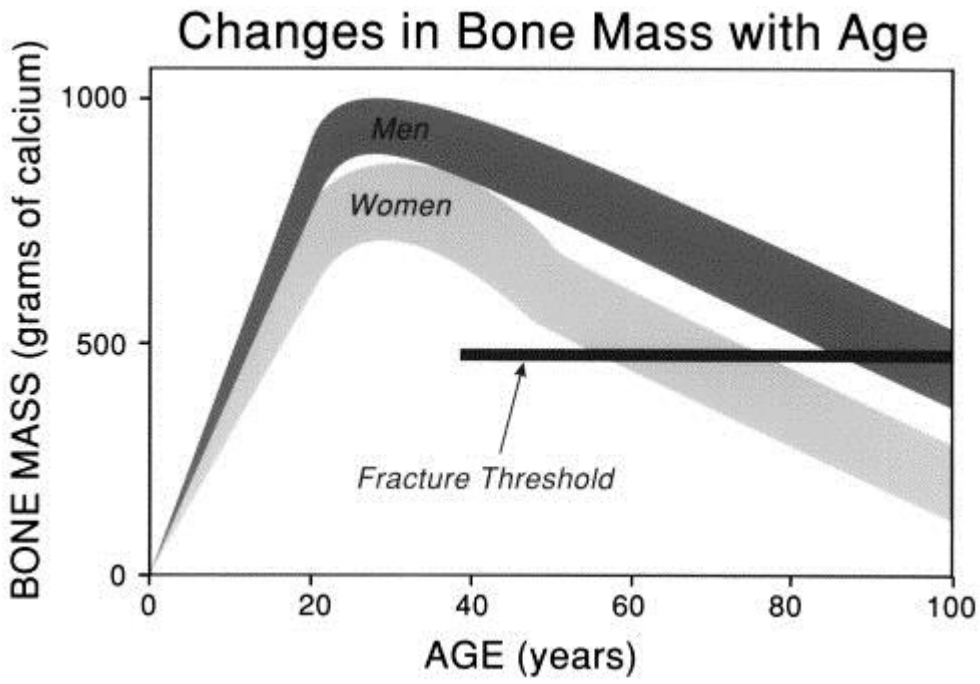


Figure 4: **Changes of bone mass with Age**

In both males and females, bone mass reaches its peak in early adult life with a gradual decrease thereafter [7]. In women, after the menopause concludes, a more gradual decrease in bone mass has been observed. The fracture threshold here shows that with a decrease in bone mass, the risk of fracture increases [7].

4. **Previous history of fractures:**

Several studies have shown that individuals with a prior fragility fracture have a greater risk of suffering a further fracture [6, 7].

5. **Smoking:**

A meta-analysis of the relationships between smoking and BMD has shown that BMD in smokers is decreased by up to 2% [6, 7]. Furthermore, the risk of being osteoporotic is greater in current smokers as compared to previous smokers. [7]

6. **Excessive alcohol consumption:**

Excessive alcohol consumption is also one of factors that may increase the chance of osteoporosis and will be discussed in greater detail later.

7. **Vitamin D and calcium:**

Vitamin D plays an important role in the absorption of calcium and bone health. Figure 4 shows how vitamin D is manufactured and taken into the body, and how its metabolism interacts with calcium absorption to ensure bone health. The role of this hormone and the consequences of deficiency are described in more detail later in this chapter.

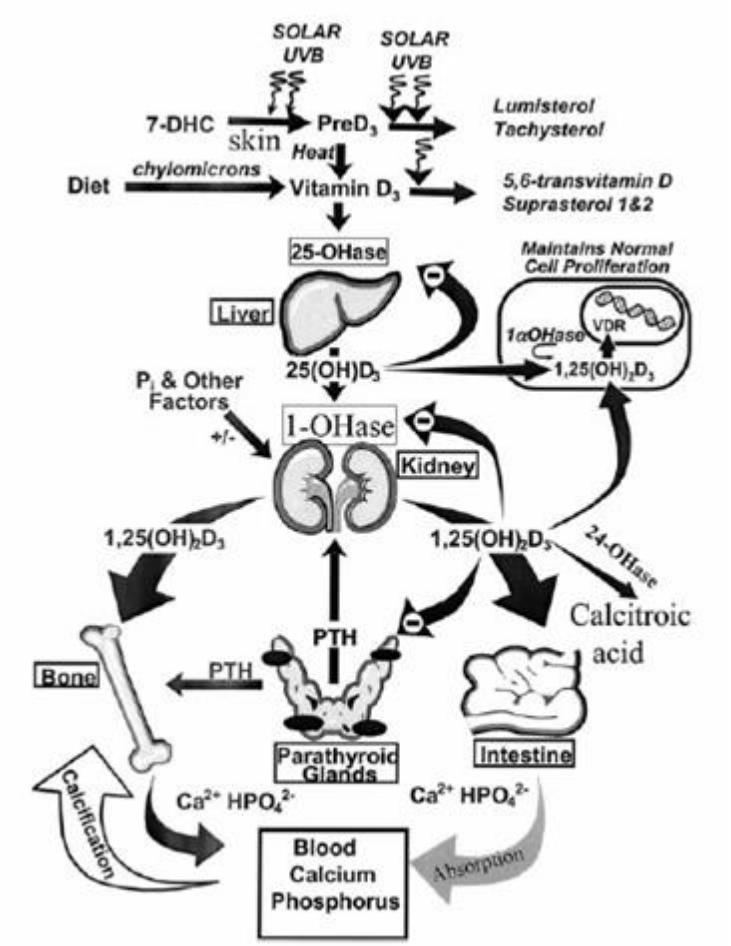


Figure 5: **Schematic representation of cutaneous production of vitamin D**

When the skin is exposed to sunlight, 7-dehydrocholesterol (7-DHC) which is present in the skin absorbs the UV rays and is converted to previtamin D_3 ($PreD_{3)}$. This previtamin D_3 goes through a thermally induced transformation and converts into vitamin D_3. Vitamin D which is produced from diet or skin enters into the circulation and gets metabolised by vitamin D 25-hydroxylase (25-OHase) to 25 $(OH)D_3$ in the liver. In the kidney, 25 $(OH)D_3$ gets converted to 1, 25$(OH)_2D_3$ with the help of the enzyme 25 (OH) D_3 1α- hydroxylase (1-OHase). Factors such as P_i (serum phosphorous) and PTH help in regulating the production of 1, 25$(OH)_2$D, which helps in calcium metabolism through interacting with bones and the intestines. 1, 25$(OH)_2$D, gets converted to 25 (OH)D 24-hydroxylase (24-OHase). 25(OH)D is metabolised in other tissues for cell growth regulation.

8. **Exercise:**

Numerous studies have shown that physical activity protects against osteoporosis. Individuals with a sedentary lifestyle are at higher risk for osteoporosis as compared to those who exercise daily [6, 7]. Performing weight bearing exercise has been shown to increase bone density.

9. **Secondary Osteoporosis:**

In addition to the above risk factors, there are some secondary causes which may cause osteoporosis.

1. Clinical disorders such as anorexia, chronic liver disease, coeliac disease, hyperparathyroidism, irritable bowel syndrome, male hypogonadism, renal disease, rheumatoid arthritis may also lead to secondary osteoporosis [7].

2. Corticosteroids:
   Use of long term corticosteroids increases the risk of non-vertebral fracture within 3 months of treatment initiation. Studies prove that even with a low dose of glucocorticosteroids, the chances of secondary osteoporosis increase [7].

1.5 The classification of osteoporosis:

Using the WHO definition, osteoporosis is based on the T score derived from the measurement of Bone Mineral Density. T score is measured as the number of standard deviations below the average for a young adult at peak bone density when compared to a reference population [7].

WHO has defined the below mentioned categories on the basis of bone mineral density in women:

a. T-score better than -1 : Normal
b. T-score between -1 and -2.5 : Osteopenia
c. T-score less than -2.5 : Osteoporosis
d. T-score less than -2.5 with fragility fracture : established osteoporosis

In addition to the T-Score, a Z-score is derived; A Z-score can be defined as the number of standard deviations from which a person's BMD differs from the mean BMD *for the same age group* of people [6,7]. Hence while the T-score is used as a score which can help in determining whether the patient is classed as suffering from osteoporosis, in young populations, it is less predictive for determining osteoporotic fracture risk [7]. For each one standard deviation decrease in BMD, the risk of fracture approximately doubles [7].

FRAX:

Studies have shown that T-Score alone cannot predict the risk of fracture, Hence the WHO has developed a model called the FRAX tool. This tool was developed to calculate the 10-year risk of osteoporotic fractures of an individual and is calculated using information on clinical risk factors and BMD [7]. The clinical risk factors that are included in the FRAX algorithm are: Age, Sex, Weight (kg), height (cm), prior fracture status, family history of hip fracture, Rheumatoid arthritis, 3 or more units of alcohol consumption daily, and other causes of secondary osteoporosis.

The FRAX tool calculator screen is shown below.

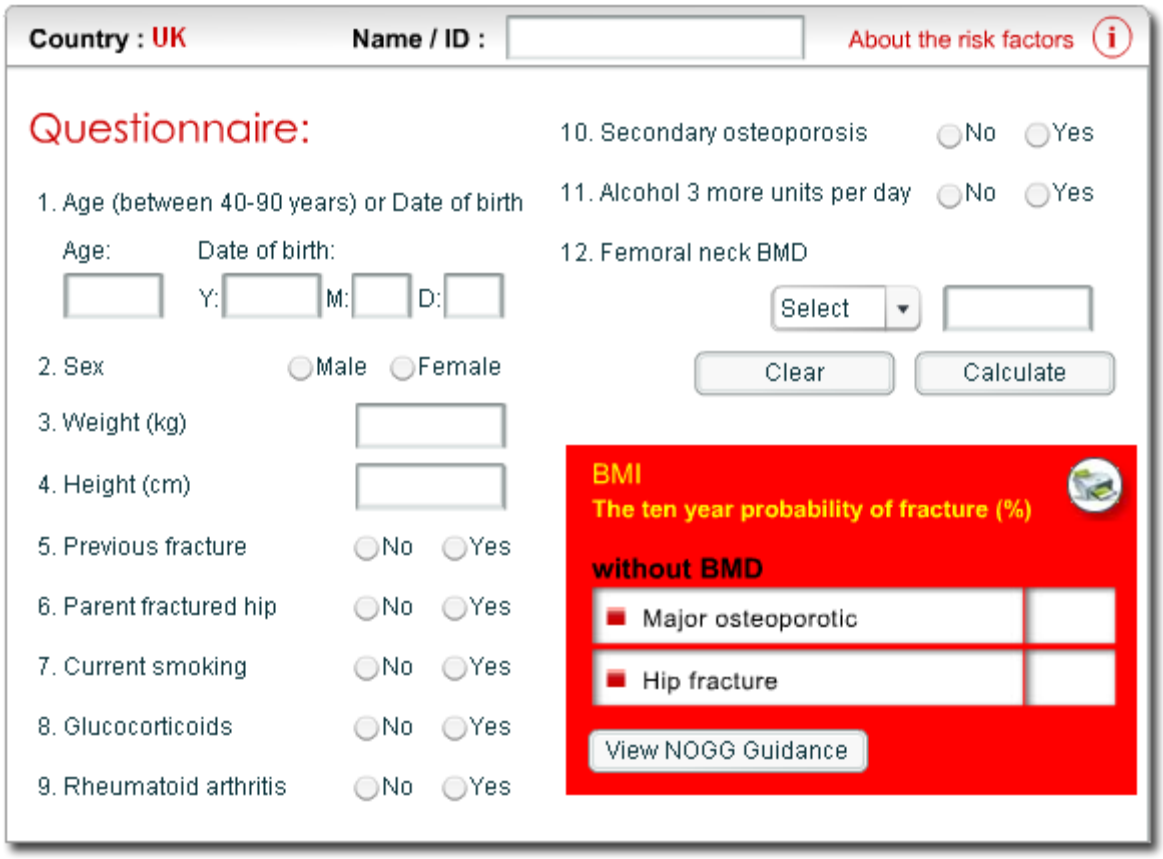


Figure 6: **FRAX tool**

The National Osteoporosis Foundation (NOF in U.S.) and National Osteoporosis Society (NOS in U.K) have added the FRAX tool to BMD scores in their guidelines for identifying the individuals with high fracture risk [7].

Despite the above mentioned advantages of the FRAX algorithm, there are certain limitation that has been observed. Firstly the FRAX algorithm does not take into account the ‘dose effect’ of risk factors like glucocorticoids. Furthermore, no data is collected about prior fractures.

1.6 Bone quality:

The strength of the bone is determined by its BMD, geometry and the quality of the bone [8]. The quality of the bone depends on many components including bone turnover, microarchitecture and composition of the bone matrix [8]. All these components are mutually dependent on each other and thus if any of the component has some abnormality, the effect is observed on all the others.

Of all the components of bone quality, bone turnover plays a pivotal role in determining these. Section 1.2 summarises the bone remodelling process.

The most common method of assessing bone turnover is by measurement of biochemical markers of resorption and formation [8]. These markers show inconsistency both within and between individuals and are dependent on diet. Therefore, samples should be collected with the patient in a fasting stage [8]. Another less often used method to assess the bone turnover is by performing histomorphometric assessment of bone, with the help of tetracycline labelling prior to biopsy [8].

Bone microarchitecture also contributes to bone quality. Any changes in microarchitecture have a direct effect on the strength of the bone thereby damaging bone quality [8]. These architectural features can be assessed using high resolution magnetic resonance imaging (HR-MRI), high resolution peripheral quantitative computed tomography (HR-pQCT) and micro-CT (µCT).

Finally bone quality is also affected by bone mineralisation. Mineralisation of the bone occurs in 2 phases: deposition of bone mineral in the bone remodelling process is called primary mineralisation. Secondary mineralisation is defined as the process of mineralisation after the completion of the bone remodelling process [8]. The degree of bone mineralisation is measured by bone mineral density measurements, but is not the main factor in determining the BMD [8].

1.7 Bone Turnover:

As has been discussed, in section 1.2 bone turnover consists of 2 activities namely formation of bone and resorption of bone. The rate of formation of bone is calculated by measuring the enzymatic activity of bone forming cells [8]. Based on their origin, bone turnover markers are divided into formation and resorption markers. In disease like osteoporosis where both formation and resorption process are coupled together and may change in the same direction, these markers will reproduce the overall rate of bone turnover [8]. Bone turnover markers are the biochemical products present in blood or urine. They have the capability to reflect metabolic activities of bone and are said to be markers for bone formation and bone resorption [9]. During the activation of osteoblasts, direct and indirect products are released which are said to be bone formation markers [9]. During bone formation process, precursor procollagen of type I collagen is secreted [9]. At both the ends of procollagen molecule there are extension peptides namely, procollagen type I N propeptide (PINP) and procollagen type I C propeptide (PICP) [9]. These extension peptides are released into circulation during the process of bone formation are called as bone formation markers [9]. During bone formation, osteoblasts produce osteocalcin [9]. The kidneys play a pivotal role in extraction of Osteocalcin and it can found in urine [9].

Another bone formation marker is ALP (alkaline phosphatase). ALP can be measured in serum as it is secreted in blood by osteoblasts [9]. As ALP, is mostly in hepatic origin, assays help in identifying bone derived isoform of ALP called as BALP [9].

The most common bone resorption markers used are tartarate resistant acid phosphatase 5b (TRACP). During bone resorption, pyridinium crosslinks, pridinoline (PYD) and deoxypyridinoline (DPD) are released and excreted in urine in peptide forms. The peptide form of PYD and DPD are C and N terminal crosslinking telopeptides (CTX and NTX) of type I collagen molecule [9].

1.7.1 Bone formation markers:

1. Procollagen I extension peptides:

Osteoblasts produce the type I collagen as procollagen with extension peptides in carboxy (C) and amino (N) ends [10].

2. Bone specific alkaline phosphatase:

Bone specific alkaline phosphatases (BSAP) are produced by osteoblasts at the time of matrix production.

3. Osteocalcin:

Osteocalcin is a non-collagen protein located in bone. Osteocalcin plays an important role in mineralisation of bone.

1.7.2 Bone resorption markers:

1. Telopeptides of type I collagen:

Degradation of non-helical region of type I collagen in amino-terminal region (NTX) and carboxy-terminal region(CTX) produces these peptides [10]. NTX and CTX can be measured in serum or urine.

2. Pyridinium cross-links:

After the catabolization of collagen, the cross links are released in the circulation [10]. These cross link are available in serum or urine in 2 types namely pyridinoline and deoxypyridinoline [10].

3. Acid phosphatase:

Acid phosphatase is an iso-enzyme which is produced by osteoclasts [10].

1.8 Use of bone turnover markers

With an increase or decrease in these bone turnover markers, it may be possible to predict risk of osteoporotic fracture [10]. Research shows that age and sex are important factors in influencing the level of bone turnover markers. It has been observed that in children and postmenopausal women, bone turnover markers are generally higher than is usually observed in an adult group [10]. Many cohort studies have been conducted with some suggesting that in general the higher levels of resorption markers the higher the risk of non-vertebral and vertebral fractures [10].

However, the main use of bone turnover markers is now in monitoring the response to drug therapy [10]. There are many factors that govern the changes in bone turnover markers including: route of drug administration, marker type and cellular mechanism of the drug's action [10]. For example bisphosphonates administered orally decrease bone resorption markers in 3 weeks. Bone resorption markers decrease as the dose of bisphosphonates increases [10].

During the menopause, estrogen levels decrease, which leads to increased bone turnover and disturbance in the balance between bone formation and bone resorption. In postmenopausal women, bone turnover is normally high with high rate of bone loss, thereby leading to bone fragility and increased risk of fracture [11].

A study was conducted to assess the capability of biomarkers bone specific alkaline phosphatase (BALP) and creatinine corrected urinary C-telopeptide (CTX) to predict osteoporotic fracture risk in post- menopausal women [11]. 721 women participated in the trial and during 1992-1994 serum samples were collected from 512 women [11]. With the help of single energy X-Ray densitometry, bone density was measured [11]. At baseline, urine samples were also collected along with serum samples [11]. With the help of immunoradiometric assay, serum BALP was measured, while urinary C-telopeptide was measured with an enzyme linked immunosorbent assay [11]. To reduce urinary volume effects the results for this biomarkers were measured as a ratio of C-telopeptide to urinary creatinine (urinary CTX) [11]. In 33

women new vertebral fractures were identified, while in 25 women new non spine fractures were diagnosed [11]. Also, 10.7% of the women had suffered from at least 1 fracture since first visit [11]. Baseline BALP and CTX levels were increased in women with osteoporotic fractures as compared to women with no fracture recorded [11]. The odds ratio for calcaneus BMD (for spine fractures was 1.49), BALP (for spine fractures was 1.49) and CTX (for spine fracture was 1.33) showed that they play a significant role in predicting the fractures in these women [11].
The study results suggest that in postmenopausal women, bone turnover markers play a significant role in predicting fractures [11].

A further study assessed the ability of 7 bone turnover markers to predict risk of fractures in 1040 elderly women [12]. The study follow up period was 9 years [12]. All women included in the trial were aged 75 years and above [12]. Serum and urine samples were collected at baseline and follow up was at 1 year [12]. A real BMD was obtained for 931 women at total body, 926 at total hip, and 974 at lumbar spine [12]. The 7 bone turnover markers that were assessed included: a) Bone resorption markers: tartarate resistant acid phosphatase 5b (TRAP) and serum C-terminal cross linked telopeptide (S-CTX-I) and b) Bone formation markers: serum bone specific alkaline phosphatase (BALP), and 3 assays for different molecular forms of osteocalcin [12]. The 3 forms of osteocalcin assessed were: Serum intact osteocalcin (S-OC[1–49]), serum total osteocalcin (S-TotalOC), and serum g-carboxylated osteocalcin (S-cOC) [12]. With addition to serum, osteocalcin assays were also performed for urine osteocalcin (U-OC) [12]. The fracture history of the patients who participated was obtained and for the follow up in predicting fractures, date of first fracture was considered as an endpoint [12]. The results of this study showed that during the 9 year follow up, of 1040 women, 363 women sustained at least 1 fracture [12]. In total 116 women had a hip fracture and 103 were reported to have vertebral fractures [12]. It was also observed that women who reported a fracture at baseline had low BMI and low weight when compared with women who did not report any fracture at baseline [12]. They were also reported to have a lower BMD [12]. The results also suggested that baseline bone resorption markers helped in identifying the fracture risk as the hazard ratio for S-TRACP5b was 1.16 and for S-

CTX-1 was 1.13 [12]. These markers also predicted an increased risk of vertebral fractures with HR 1.22 for S-TRACP5b and 1.32 for S-CTX-I [12]. However, bone formation markers S-OC, S-Total OC, S-c0C and S-BALP were not able to predict fracture risk [12]. The study also found that none of the 7 bone turnover markers were able to predict the risk of fracture for hip [12].

Hence while some studies suggest that bone turnover markers can help in predicting risk of fracture, there are some limitations which were observed in the use of bone turnover markers. It is recognised that several factors influence bone turnover markers, including menopausal status, sex, age, gender, food intake. [13]. For example, menopausal status and female sex increases bone turnover markers level, while food intake affect serum –CTX-1 level (bone resorption markers) but does not have any effect on bone formation markers [13].

1.9 Public health importance of osteoporosis:

Osteoporosis is a disease that causes premature mortality and morbidity [14]. Studies show that 1.5 million annual fractures including hip fractures are attributable to osteoporosis [14].

Research shows that in United States, more than 10 Million people above 50 years of age suffer from osteoporosis and fragility fractures, while in United Kingdom, one in every 8 women over 50 years of age have osteoporosis fractures [14]. 1.67 million hip fractures had been reported globally in 1990, leading to 740000 deaths due to fractures per year [14]. Hip fractures are said to be the main reason for healthcare burden because of the need for immediate hospitalisation and long term care [14].

By contrast vertebral fractures are often asymptomatic. In the US, the estimated lifetime risk of clinically-diagnosed vertebral fracture is 16% in white women and 5% in white men. In Great Britain, the rates are slightly lower at 11% in white women and 2% in white men [14].

Hence public health initiatives aim to educate and spread awareness in women and their clinicians about osteoporosis, in an attempt to reduce fracture incidence and associated morbidity and mortality. [14]. Lifestyle measures that will protect against osteoporosis and are often promoted in public health initiatives include maintaining an adequate calcium and vitamin D intake, maintain weight bearing physical activity, avoiding cigarette smoking and keeping alcohol intake to within recommended levels (14 units per week in women)[14].

1.10 Perception of Osteoporosis risk:

Several studies have been conducted on osteoporosis education. After the completion of such programs, improvement in terms of knowledge about osteoporosis, and what steps to be taken to prevent osteoporosis have been assessed in those women [15]. In one study, an osteoporosis prevention program (OPP) focused on non-compliance concerning recommendations which were provided to prevent osteoporosis [15]. One of the observation noted while this program was taken was a belief that osteoporosis is not serious [15]. This program consisted of 3 sections: education classes, bone mineral density testing and individual consultation [15].

Participants were told about the importance of physical activity and exercise in osteoporosis. Benefits of weight bearing exercise were shared with the participants [15]. Bone mineral density testing was performed for all participants using dual energy X-ray absorptiometry (DEXA) [15] and results fed back. The participants were educated regarding diet intake, which exercises should be done and how to take care if you are suffering from osteoporosis [15].

The Global Longitudinal Study of Osteoporosis in Women (GLOW) also focused in part on women's perception of fracture risk [16]. This study was performed in 10 countries and 60393 women over 55 years of age participated [16].

This study highlighted several risk factors for fracture including age, low weight, family history of hip fracture, personal history of fracture, 2 or more falls in last one year, use of corticosteroids and consumption of alcohol daily [16]. This study, like the others, highlights the knowledge and perception gap among women at risk of osteoporosis.

Several other studies have been conducted on promoting the health and awareness about osteoporosis. The inclusion criteria for the trial were postmenopausal women with over 50 years of age [17]. Calcium intake was measured based on dietary intake [17]. A note was taken of the exercise levels among participants. [17]. Another study was based on Pender's Health Promotion Model (HPM) [18]. HPM had three components: cognitive factors, modifying factors and cue of action [18]. The cognitive factors consist of perception of benefits and barriers to behaviour, self-efficacy, health control and importance [18]. Demographic, biologic, interpersonal and behavioural factors come under modifying variables [18]. Transient stimuli to behaviours are the cue of action [18]. The study concluded that awareness of osteoporosis should be heightened among young women.

1.11 Impact of osteoporosis on quality of life

Health related quality of life (HR-QoL)is affected in women suffering from osteoporosis. An instrument has been developed called the osteoporosis-targeted quality of life survey [19]. This survey focuses on three sections: physical difficulty, adaptation to daily life and fears about the future [19]. This method helped in identifying the difference between those with normal bone mineral density, osteopenia and osteoporosis [19].

A cross sectional survey was performed to check the impact of HR-QoL on premenopausal and postmenopausal women suffering from osteoporosis [19]. The survey consisted of questions based on 3 sections along with health related questions such as prior fractures etc [19]. The results showed that the chance of

suffering from osteoporosis, and consequent adverse effects such as, postural changes, were observed mainly in older women. Women over the age of 65 years had a mean domain score of 17.9 to 34.1 as compared with a mean domain score of 7.4 to 9 in women below 65 years of age [19,20]. The adaptation and fear score were worse in women whose T-scores were in the range of osteoporosis [19, 20]. The study results suggest that fractures and fear of fracture have a great impact on quality of life. Fear was another factor which had an impact on QOL [19, 20]. The QoL score was compared with BMD and was observed that those with low BMD had worst QoL score [20].

1.12 Prevalence of vitamin D insufficiency in New Zealand:

Lack of vitamin D is associated with osteomalacia and osteoporosis and considerable research is being carried out on this topic globally. A high rate of vitamin D insufficiency is often reported in women with osteoporosis, although this is often also the case in healthy women as well [21].

A diagram showing vitamin D metabolism is shown in section (figure 5) earlier in this chapter in the section 1.4 ‘Causative factors for osteoporosis’. Vitamin D is available in 2 forms namely ergocalciferol (vitamin D2) and cholecalciferol (vitamin D3) [22]. Vitamin D is transported to the liver via the circulation where it is converted to 25-hydroxycholecalciferol (25OHD) by the enzyme 25-hydroxylase [22]. This is further converted to 1,25-dihyroxycholecalciferol in the kidney [22]. 125-dihydroxycholecalciferol is pivotal in promotion of bone mineralisation and calcium absorption [22].

The most abundant source of vitamin D is usually UV rays in sunlight. Research shows that vitamin D play an important part in enhancing the activity of osteoclasts and stimulates bone formation and maturation [22]. It has also been suggested that vitamin D and parathyroid hormone (PTH) help in the metabolism of Ca and P [22].

Many studies have shown that vitamin D deficiency causes bone loss in women and low BMD when compared with women with no vitamin D deficiency [22].

The groups of people in which the chance of low vitamin D is highest are, according to a recent study [22]:

a. Patients with hip fracture
b. Mothers of infants having rickets.
c. Older people admitted to hospital
d. People with no exposure to sun for a long period of time [22].

Research on vitamin D levels has been conducted in Australia and New Zealand populations [22]. Researchers characterized vitamin D deficiency into 3 categories:
Mild vitamin D deficiency, when range of the serum 25-OHD is 25-50nmol/L, leading to increased bone turnover and high level of PTH secretion [22].
Moderate vitamin D deficiency, when the range of serum 25-OHD is 12.5-25 nmol/L [22]. Bone turnover is increased in this group, who may also have low BMD and an increased risk of hip fracture in older people [22]. Finally
Severe vitamin D deficiency, in which category levels of serum 25-OHD are less than 25 nmol/L and patients suffer from osteomalacia [22]. The study results concluded that while there were rare chances of severe vitamin D deficiency in Australia and New Zealand, most cases were mild deficiency [22].

In another study conducted in the NZ population on individuals of age 15 years and older to determine the serum level of 25-hydroxy vitamin D [23], researchers found that the mean serum 25-hydroxyvitamin D concentrations were 47 (45–50) nmol/l in women and 52 (49–55) nmol/l in men. 3% of the New Zealanders were identified with deficiency in serum 25-hydroxy vitamin D levels [23]. It has been suggested that people in NZ may be at risk of low serum levels of vitamin D for the following reasons: high latitude in the country with over 35– 47° S; high use of sunscreen; lifestyle which leads to less outdoor activity [23].

Research also shows that ethnicity plays a role in vitamin D levels and as the New Zealand population has 3 main different populations viz. Maori, Pacific and Europeans (among others) the level of vitamin D in all the 3 categories vary [23]. As the Maori and Pacific Origin populations have a comparatively darker skin compared to their European origin counterparts, they tend to have low levels of serum vitamin D. Furthermore, food intake also plays a significant role in vitamin D levels in the body. In New Zealand, it had been observed that fatty fish and organ meats are not consumed on a regular basis, which may be another factor contributing to low vitamin D levels [23].

The National Nutrition survey was conducted in 1996-1997 in NZ on adults aged 15 years and older [23]. 3946 participants were included in this study whose results showed that the lowest serum 25-hydroxy vitamin D was observed in pacific women with a mean concentration of 34 nmol/L (29-40) [23], while European men had the highest mean serum 25-hydroxyvitamin D concentration (53 nmol/L) [23]. Half of the participants were classified as deficient in vitamin D as serum 25-hydroxyvitamin D concentrations were less than 50 nmol/L and more than 80% of the population had serum concentration less than 80 nmol/L [23]. Women had lower levels of vitamin D serum concentration than men by 5 nmol/L [23]. It was also noted that the mean serum concentration of 25 (OH) vitamin D in Maori women was 11 nmol/L and in Pacific women was 16 nmol/L lower when compared to European women [24], while the mean serum 25 (OH) vitamin level in Maori men was 10 nmol/L lower and in Pacific men was 12 nmol/L as compared with European men [24].

1.13 Techniques to assess bone mass:

Heel Ultrasound:

The most widely used methods to measure BMD are dual energy X-ray absorptiometry (DEXA), heel ultrasound (HUS) and quantitative computerised tomography (QCT) [25].

Quantitative heel ultrasound is a technique which was first launched in 1984 [25]. QUS uses sound waves [25]; the physical and mechanical properties of bone modify the speed, shape and intensity of the wave [26]. While QUS cannot be used as a diagnosis for osteoporosis, heel ultrasound has been shown to be able to predict hip and all osteoporotic fractures in elderly women [26]. A meta-analysis of QUS of the heel and its ability to predict fractures was performed [26]. The eligibility criteria for a trial to be a part of this meta-analysis study were: prospective studies with baseline measurements in units done with a QUS method; fractures to be the main outcome and relative risk estimate should be provided for the fractures. The studies which were included had used QUS from 4 different manufacturing companies and had different parameters recorded. For example, studies using the Walker Sonix UBA device has reported broadband ultrasound attenuation (BUA); studies which used the McCue CUBA device have recorded BUA and speed of sound (SOS); studies that used the GE-Lunar Achilles device have recorded BUA, SOS and Stiffness Index (SI) and studies which had ultrasound device of Hologic Sahara had recorded BUA, SOS and the quantitative ultrasound index (QUI) [26]. Results showed variance among studies between the BUA and SOS measure and associated risk of fractures [26].

According to this meta-analysis, the 2 studies which used GE Achilles and Sahara devices in the same age group of the elderly population showed significant relationships between measured parameters and risk of fractures [26]. From this meta-analysis it can be concluded that quantitative heel ultrasound can predict fractures [26].

In one such sample study conducted on women aged between 45 to 75 years in Bournemouth, U.K, women aged between 45 to 75 and registered with 2 general practices in area of Bournemouth, UK , were asked to participate [27]. Measurement of the heel of the dominant foot was taken with a Achilles ultrasound densitometer [27]. The results of the ultrasound were obtained as BUA, SOS and SI parameters [27]. 4018 women in total were scanned for the study [27]. An inverse relationship

was observed between age and QUS results [27]. The RR of fracture with a one unit change in BUA was 1.4 (1.26-1.56).

In another study conducted in more than 7000 women of >= 70 years of age [28] the (Swiss Evaluation of Methods of Measurement of Osteoporotic Fracture Risk) study, 3 different ultrasound devices (2 of heel and 1 of phalanges) were assessed in detecting the risk of hip fracture in women aged 70 years or more [28]. The trial was conducted between 1997-1999 and 7609 women participated. QUS devices that were used in this study were the Achilles+, Sahara and DBM Sonic 1200 (for phalanges) instruments [28]. Eligible women were aged more than 70 years and able to walk and perform daily activities, while exclusion criteria were a history of hip fracture or bilateral hip replacement [28]. Every 6 months follow up was performed and participants were asked questions about any changes, illness, or medications or fractures with the exact locations [28]. It was observed during follow up that 425 women had started treatment for osteoporosis [28].

All the 3 QUS devices measured different parameters:

- Achilles+: This device is water based. It measures BUA in dB/ MHz, SOS in m/s and SI with a formula as SI= (0.67 x BUA) + (0.28 x SOS) – 420.
- Sahara: This is a dry system which uses an oil based gel [20]. This device also measures BUA and SOS along with QUI. QUI can be calculated as QUI = 0.41 x (BUA + SOS) – 571.
- DBM Sonic 1200: This calculates the propagation of the US pulse through the first phalanges of last 4 fingers of the hand [20]. The parameter that the device measure is Amplitude Dependent speed of sound (AD-SOS) in m/s [28].

In this study, the participants were followed for almost 3 years and of 7609 recruits, data were available for 7062 women. [28]. Eighty women suffered a low-trauma hip fracture during follow-up. For Achilles+ Machine, the mean T score calculated was -1.7± 0.9 SD. For the Sahara, the mean T score was –1.4 ± 1.1 SD [28] while for the

DBM Sonic 1200, the mean T-Score was −3.1 ± 1.3 SD for AD-SOS [28]. Hence the mean score of the other 2 heel ultrasound devices were higher than the mean score for the DBM Sonic 1200 machine [28]. This difference may be because of variation in the reference population of the devices used. It was also noticed that the majority of participants who sustained a hip fracture had low heel QUS when compared with subjects having no risk of fractures [28]. When these results were compared with another study (French EPIDOS Study), results were comparable. In the EPIDOS study, 5662 women were included and participated for 2 years [28]. In this study, the HR of fracture per decrease of 1 SD, was 2.0 for Achilles+ BUA and 1.7 for SOS [28].

Another study was conducted in 12958 elderly women [29]. The aim of EPISEM prospective cohort study was to assess the 10 year probability of osteoporotic hip fracture as assessed by combining heel bone ultrasound scores and clinical risk factors [29]. The EPISEM study was performed by combining databases of the EPIDOS and SEMOF studies [29]. 12958 women aged between 70 to 100 years participated in the trial. The Stiffness Index (SI) parameter was used for analysis [29]. Along with this, 22 clinical risk factors combined from both the EPIDOS and SEMOF studies were used in this study, in which during the follow up period 307 hip fractures were reported [29]. In a multivariate study model, 6 out 22 clinical risk factors statistically significantly predicted fracture risk. These were: BMI, history of fracture after 50 years of age, chair test results, history of fall for past 12 months, current cigarette smoking and diabetes mellitus]. The study showed that for predicting hip fracture for all ages, a model with SI alone, had a higher gradient of risk (GR) as compared with model using clinical risk factors alone (CRF) [29]. For example, in women aged 70 yrs; the gradient of risk for SI was 2.16 as compared to 1.87 of CRF alone but when SI and CRFs were combined together an increase in GR was observed. For hip fractures, the average GR observed was 1.77 for SI and 1.52 for CRFs respectively. [29]. But when both of them were combined, the GR observed was 2.10 [29]. Using these combined results, the study calculated the 10 yr probability of hip fractures.

For example the 10 year probability of fracture for an 80 yr old women varied from 16.9% at a SI score of +2 to 52..6% at SI score of -3 [29]. When this was combined with 2 CRFs for these women, the score increased from 16.9 to 26.6% and from 52.6% to 70.5% respectively [29].

From all the above the role of QUS might be summarised as:

a. QUS is an inexpensive (as compared with DEXA) method to predict risk of hip fracture. [29].
b. QUS is easy to perform, quick and safe as it does not use X-rays [29].
c. QUS can be used as an initial screening method for osteoporosis, but it cannot be yet considered as a test to diagnose osteoporosis [29].

1.14 Alcohol and its effect on bone:

1.14.1 Overview:

It is well- known that alcohol consumption worldwide has increased significantly in recent years. It is thought that consuming low amounts of alcohol can increase bone density and bone mass while consumption of high amounts of alcohol has deleterious effect on bone [30]. There are many more studies conducted on men than on women with regard to the relationship between consumption of alcohol and its effect on bone [31]. This research project focuses on women who are alcoholics and admitted to an alcohol dependence unit to determine the effect of heavy alcohol consumption on bone as assessed by QUS; to look at the prevalence of other lifestyle factors associated with low bone density in this group and to relate the findings obtained to a control group of local women without a history of alcohol dependence.

One of the few studies of female alcoholics to be conducted to determine bone mineral density and risk of fractures was conducted on 834 Caucasian women [31]. The required age for participation in the trial was 18 to 70 years [31].

These 834 women were divided in 3 groups: 228 women who were undergoing treatment for alcohol dependence, 156 women with a history of alcohol dependence and currently recovering, and 447 women in a group of non-alcoholic women [31]. BMD was measured for all participants and detailed questions were asked to determine the history of fractures, demographic information and history of smoking, reproduction and medical history [31]. Results of the study showed that women from the group who were currently undergoing treatment reported 10.8 years of drinking with consumption of about 124.6 oz of alcohol each week before admission [31]. Women in the group of recovery subjects had a mean duration of drinking problem of 13.4 years and were consuming 110.1 oz of alcohol each week when they were alcoholics [31]. Data collected from the women admitted in the recovery group showed that on an average these women had abstained from alcohol for about 8.3 years [31]. It was seen that of women in the non-alcoholic group, 110 women reported no regular consumption of alcohol [31].

The study results showed that among women who belonged to the treatment group, BMD was lower at femoral neck (7.7%) and lumbar spine (6.3%) when compared with non- alcohol drinking women [31]. 1, 25-dihydroxyvitamin D levels were measured in women who participated and results were obtained in mean and adjusted for age [31]. It was noted that women undergoing treatment had low levels of serum vitamin D as compared with non-alcoholic women, though there was no significant difference between serum vitamin D levels in the non-alcoholic group and recovery group women [31]. This study also predicted the relation between alcohol consumption and fractures. Results of the study showed that 60.5% women in the treatment group and 63.5% women in the recovery group had reported a minimum of 1 fracture in their lifetime [31]. By contrast, women who belonged to the non-alcoholic group had 36.5% women who reported fractures [31]. This study complements the above findings by providing pilot data relating to another modality of bone quality as assessed by heel ultrasound.

1.14.2 Alcohol abuse in New Zealand and elsewhere

It has been observed that young adults are more prone to drinking alcohol and often happen to binge drink. Binge drinking can be defined as consumption of more than 5 drinks at a single occasion [32]. Many surveys had been conducted on the prevalence of alcohol abuse in young adults across the world and a related survey was conducted in the US [33]. The national survey on drug use and health (NSDUH) is the longest survey that provides proof on binge drinking through the collected samples for years from 1979 to 2006 [33]. The survey was conducted on young group people aged 12-34 years who were divided in 5 different groups as: 12 to 14, 15 to 17, 18 to 20, 21 to 23 and 24-34 as a reference population [33]. The survey includes young adults from both sexes [33]. It was observed that men belonging to age group 21-23 had the highest rate of binge drinking. The odd ratios (OR) of alcohol abuse for this age group was 2.1 [33]. Results were different for female group in these categories. For females belonging to the age group 21-23 and 18-20 the odd ratios were approximately 1.0 for constant number of years [33]. The odds ratio was observed for all the categorised age group vs 24-34 group of reference population [33]. For the females who belonged in the age group 15-17, the odd ratio was observed in the range of 0.2 to 04 , while those who belonged to age group of 12-14, an odd ratio of 0.01 to 0.14 was noted [33]. The survey results state that relative risk of binge drinking was lower in young adults below 20 years of age. This has been attributed to the fact that any regulations and rules have been slated for alcohol consumption below 21 years of age and seems to be working as per the results of the survey [33]. Another issue which was highlighted in this survey was an increasing trend of alcohol and binge drinking in young females [33].

There are fewer data available in the form of surveys on alcohol consumption and the data that is available is mostly for large economies like the US, UK and to some extent for Australia. But, for the population in New Zealand, a minimal quantity of data on alcohol consumption patterns is available for review [34]. One such study was conducted to report the trends of alcohol drinking in the New Zealand population taking into account data from surveys conducted in 1995, 2000 and 2004 [34].

Households were selected from households who participated in 1995, 2000 and 2004 surveys and a random based selection method was used. Survey results showed no significant difference in the number of male drinkers in year 1995 and 2000 [34]. Results suggested that female drinkers had increased in number among women aged between 14-17 years [34]. Results also show that heaviest female drinkers of the group were in the age group 20-24 and 30-39 years.

A history of NZ shows that NZ had a culture of alcohol drinking. According to the sociology report of 2010, binge drinking has increased in women to the same extent as men. The reasons for these changes might include factors such as an increase in the number of hospitality outlets where late night drink and parties are organised, changing and emerging role of women and liberalization of the liquor law [35]. Another survey conducted in 2009 showed that of young adults of the age group 16-17 years, 9% females and 15% males are involved in chronic alcohol consumption on a weekly basis [35].

A web based survey was conducted on university going students in 5 NZ universities [36]. Samples were collected randomly from students of age groups 17-25 years who were full time university students [36]. 430 Maori and 430 Non Maori students were invited from each university [36]. Questions were asked to participants about the number of time they engaged in binge drinking for last 1 year. Binge drinking was defined as more than 4 drinks for women and more than 6 drinks for men at one time [36]. Questions were also asked regarding any alcohol related issue/problem they faced [36]. Out of the students who participated, the majority of students were of European origin (71%), while 15% were Maori and the remainder were of other ethnicity [36]. The result of the survey showed that 88% of both men and women were involved in binge drinking for past 1 year [36]. Hence a review of the effect of alcohol on bone health is timely.

1.14.3 Effect of Alcohol on bone:

Alcohol has both positive and negative effect on bone depending on consumption. Alcohol consumption can be divided in 3 sections: light, moderate and heavy [37]. There are several definitions and a fixed quantity is not defined to characterize the 3 [37]. However, one study was conducted on alcohol and its effects on bone and defined the 3 consumptions in the following ways [37]:

a. Light alcohol consumption: 1-10 g of ethanol/day
b. Moderate alcohol consumption: 11-30g of ethanol/day
c. Heavy alcohol consumption: more than 30 g of alcohol/day.

Effect of light and moderate alcohol consumption*:*

At least one study has showed that consuming small amounts of alcohol (wine) is associated with positive effects on BMD of the lumbar spine [37]. The effect of alcohol are dependent on age, sex, hormonal factors and beverage consumed [38]. A further study showed that post- menopausal women drinking 11-29 g (moderate) alcohol per day had increased BMD at the trochanteric site when compared with non-drinkers [38]. By contrast a further study showed that when a pre-menopausal women consumed 5-24 g of alcohol/day, the risk of fracture is increased [39]. With low alcohol consumption, BMD is typically increased. This is mirrored by BTM changes [39].

1.14.4 Effect of alcohol on bone turnover:

In long term consumption of alcohol, bone microarchitecture and remodelling processes are altered [39,40]. Fewer data are available on changes in bone microarchitecture after alcohol consumption, but research shows that cortical and trabecular thickness are decreased along with decreased bone formation rate in subjects with alcohol induced bone disease [39,40].

The available literature on bone and alcohol effects shows that the bone formation rate is decreased and bone resorption rate increased in subjects chronically consuming alcohol (120-150 g/day) [39,40]. A decrease in the rate of bone formation can occur because of inhibition of proliferation and activity of osteoblasts [39-40]. Some studies also show an increase in bone resorption markers, but less data is available [39, 40]. Consumption of alcohol in a chronic or high amount can also lead to an increased risk of fractures. People consuming about more than 100 g of ethanol per day have high reported rates of skeletal trauma and vertebral fractures [39, 40].

Though the effect of alcohol on bone is not completely clear, they can be divided into direct and indirect mechanism of action, listed below.

Indirect mechanism of action:

Body composition:

a. **Leptin**: Leptin is a protein that is secreted by white adipose tissue. Studies shows that leptin regulates bone mass and has the ability to decrease bone mass through the CNS. Study results suggest that alcohol consumption decreases leptin and modifies the balance between adipogenesis and osteogenesis [39, 40].
b. **Cell differentiation**: Studies show that alcohol consumption causes a decrease in cell recruitment required for the osteoblast lineage when compared with samples from non-alcoholic subjects [39, 40].
c. **Vitamin D and PTH**: Few studies are conducted on effect of alcohol on Vitamin D and PTH levels but some data suggest that PTH levels and vitamin D levels in the body decreases [40]. PTH plays a pivotal role in vitamin D activation and vitamin D has a positive effect on osteoblast differentiation and function [40]. Thus, chronic alcohol consumption decreases serum level of vitamin D [40]

d. **Calcitonin**: Some studies report that with moderate consumption of alcohol, calcitonin levels increases [40]. Calcitonin is a thyroid hormone that inhibits bone resorption [40].
e. **Sex steroids**: A few studies show a reduction in serum estradiol levels in females [40]. Low serum estradiol acts as a major factor in ethanol-induced bone loss [40]. Study results also suggest that consumption of low /light alcohol can increase serum estradiol levels [40].

Direct mechanism of action :

a. Osteoblasts and osteoclasts: Studies report that alcohol causes a decrease in activity and differentiation of osteoblasts [40]. Minimal data is available on effect of alcohol on osteoclasts.

Osteocyte: Osteocytes play a pivotal role in bone remodelling process [40]. Osteocytes also helps in preventing activation of osteoclast when dying osteocytes send signals to osteoclasts [40]. Studies conducted on animals shows that osteocytes are modified because of alcohol and BMD was noted to decrease as osteocyte apoptosis increased [40].

b. **Wnt pathway/DKKI**: Wnts are glycoproteins. The most common pathway for activation of Wnt is Wnt/β pathway [40]. The pathway is activated by binding of Wnt to a co-receptor [40]. B catenin has an ability to affect the gene transcription by staying in the cytoplasm [40]. B catenin has the capability of regulating genes involved in differentiation, proliferation and apoptosis of bone [40]. Alcohol tends to suppress the Wnt activation pathway thereby causing negative effects on bone [40].

DKKI acts as an antagonist to the Wnt pathway and its levels are increased due to alcohol consumption and can cause apoptosis of bone cell [40].

1.15 Summary:

Thus, whether it be in a direct or indirect way, alcohol induces changes in bone health with far fewer data available in young alcoholic females than other groups. The aim of this thesis is therefore:

1. To compare bone health among healthy women (controls) with women admitted to an alcohol dependence unit at Kenepuru hospital using a heel ultrasound machine, with administration of a questionnaire about risk factors for low bone density.

2. To examine bone turnover in 10 premenopausal women at the start and end of a 5 day admission to an alcohol detoxification unit to assess changes on bone turnover.

Chapter Two: Methods

2.0 Introduction:

This research was conducted at the alcohol dependence unit based at Kenepuru hospital, Porirua. Kenepuru Hospital was chosen because there is an independent drug and alcohol dependence unit with a high rate of admissions and dedicated junior medical staff.

Figure 7: **KENEPURU hospital**

The alcohol withdrawal services provided at CCDHB is a residential based program lead by Medical Director Dr. Geoff Robinson. Patients may self-refer or be referred by a clinician. The unit has 4 beds dedicated to the alcohol detoxification programme. Both male and females can be enrolled into the unit for the detoxification program. They are typically admitted for a 5 day period and many of them have further rehabilitation thereafter.

2.1 Specific Research and Objectives:

In this research study, we considered only female participants enrolled in the detoxification program; we did not include males admitted to the unit as far more data and research articles are available relating alcohol consumption and its effect on bone in men. There were about 130 admissions to the alcohol detoxification unit over the last year and managers have predicted a total admission number of 150 for this year inclusive of both male and female participants. In 2011, the numbers of female participants at the unit were 63 (48%), while the number of males was 67 (52%).

Age distribution is presented graphically in Figure 8 which shows the age distribution of admissions to the Unit during 2011, and demonstrates a wide age range of 20 – 80 years.

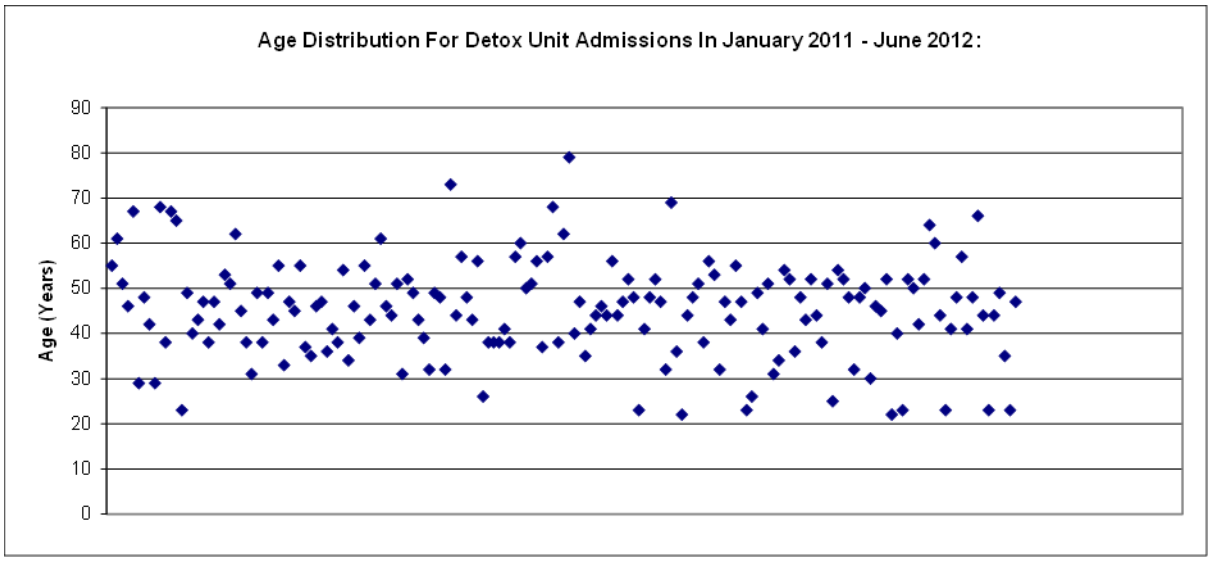


Figure 8: **Graphical representation of Age distribution**

The main aims of conducting this study were:

1. To report bone turnover in a group of women with alcohol dependence at admission to an alcohol dependence unit and after 5 days of abstinence from alcohol
2. To examine bone health as assessed by heel ultrasound at different ages among alcoholic females (premenopausal, menopausal, postmenopausal) and to compare the results with a group of healthy control women who also completed a questionnaire detailing lifestyle factors for bone health.

2.2 Ethical considerations and Recruitment procedures:

Ethics approval was sought and gained from Central Health and Disability Ethics Committee.

Women were typically admitted to the alcohol dependence unit for a 5 day stay, with admissions anticipated at least one week in advance. Hence the researcher travelled to the unit each week to explain the study and after obtaining informed consent, to administer the questionnaire and perform the heel ultrasound scan. Staff at the unit were briefed about the study before it commenced and were consulted before any subject was approached to confirm that they were medically fit to be approached. Blood samples were taken on suitable subjects who had given informed consent by medical staff when routine blood tests were collected on admission and discharge.

Participants were briefed about the study and were asked their willingness to participate. If they agreed, they were given a patient information sheet which contained detailed information about the study and were asked to sign the informed consent form. Once their consent was obtained, participants were asked to fill in a questionnaire.

2.2.1 Questionnaire:

The research was conducted using a questionnaire based method. Subjects were asked to complete a detailed questionnaire starting with baseline data regarding their height and weight, their date of birth, GP's name.

Next, they were asked to complete set of 19 questions which were divided into 3 sections:

a. **Questions related to alcohol and smoking, personal and family history of fractures and diseases:**

1. Whether they smoked or not, if they smoked the number of cigarettes, or amount of roll-up tobacco. Details of pipes or cigars smoked were asked. This datum was converted to pack years.
2. Age at which they first started the consumption of alcohol.
3. Amount and quantity of alcohol (options given as shandy, low alcohol beer, lager, cider, low alcohol wine, wine, sherry, port, martini, cinzano, spirits or liqueurs). This data was converted to units of alcohol consumed each week.
4. Whether they experienced any falls in the last year. If yes, age, broken bone history, details of x-ray and name of the hospital and date of the broken bone was recorded.
5. Family history of hip fracture was recorded.
6. The participant was asked to name any long lasting illness which they were suffering from such as overactive/ underactive thyroid gland, diabetes I, asthma, bronchitis, Rheumatoid arthritis and Coeliac disease.
7. Menopausal history of the participants was also recorded and the date was noted if the patient had ceased menstruating.

b. Questions related to physical exercise and medications:

8. Questions related to physical exercise were also asked. The exercise related questions were divided into 3 parts: brisk pace walking (number of days and time spend in doing the same), moderate physical exercise (number of days and time spend doing the same) and vigorous physical exercise (number of days and time spend doing this).
9. Subjects were asked to describe their regular physical activity. They were given 5 options and were asked to select one which suited them the most.
10. List of any type of medications which the subject had been taking was noted.

c. Questions related to dietary calcium intake:

The last section of the questionnaire was based on the dietary calcium intake of the subject. The information gathered was used to estimate a daily dietary calcium intake and for this reason food frequency questions were asked only about calcium rich food and drinks.

11. In this section, they were asked to give the intake of total amount of milk consumed throughout the day.
12. Subjects were asked to select 1 of the options out of none, once a month, once every 1-2 weeks, 1-22 days/ week, 3-5 days/week and every day for describing their intake of tea, coffee, any other milk drinks like Milo, Bournvita, Horlicks, hot chocolate etc. and milk alone. Along with this, subjects were asked to select the type of milk they consumed viz. none, liquid milk (whole, semi-skimmed, skimmed, UHT, sterilised and powdered made-up), tinned milk and powdered milk.
13. In the diet section, questions were also asked describing the amount of consumption of bread, desserts made with milk, cheese, cakes, scones, biscuits and green vegetables.

Subjects were asked to select of the options from none, once a month, once every 1-2 weeks, 1-22 days/ week, 3-5 days/week and every day. This information was converted to dietary calcium intake per week.

2.3 Heel Ultrasound:

Once the questionnaire was completed, a heel ultrasound was performed on the subjects using a GE Achilles+ heel ultrasound machine. The GE Achilles+ heel ultrasound machine uses BUA, SOS and Stiffness Index (SI) as parameters to display the result, as shown below. It also gives a T-Score and a Z-Score which helps in identifying the risk of fracture for that particular subject as per WHO standards.

Quality Assurance (QA) was performed of the machine before each use using a phantom. Once this was completed, each subject was seated comfortably as shown in Figure 9, and asked to insert their bare heel into the machine after liberal application of alcohol gel. Once positioned, the measurement section on the machine was selected and participant's age and side of heel to be measured (left or right) was entered. The typical scan time was a few minutes.

Figure 9: **Heel ultrasound scan**

Below are the steps provided by the manufacturer of the ultrasound machine which were to be followed while performing the heel ultrasound scan:

1. Position the device and person:

3.1.2. Position the device and person

1) The device should be positioned in an appropriate place where meets the Environmental Requirements in Section 1.4.3.

2) Refer to Figure 3-1 to lift the Calf Support.

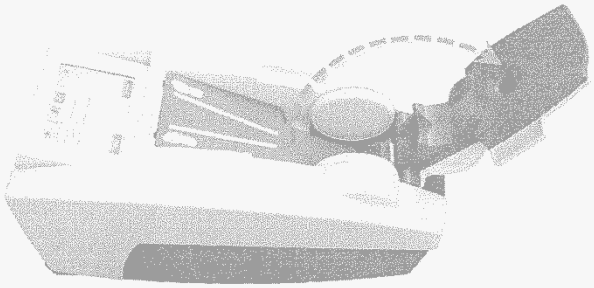


Figure 3-1 Position the Calf Support

3) Seat the person in a stable chair without wheels, directly in front of the device. Make sure the person faces the device in a comfortable, upright position. Refer to Figure 3-2. If necessary, adjust the position of the unit so that the middle of the calf rests gently on the Calf Support. Be sure the Foot, Calf, Thigh and body are well lined. Refer to Figure 3-3.

Notes: Resting the calf too heavily on the Calf Support may cause the heel to move too far forward causing erroneous readings. **Correct positioning is important for valid measurement results.**

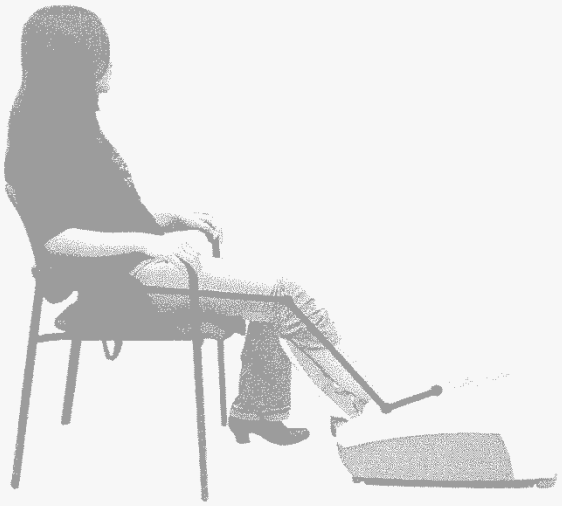


Figure 3-2 Position the Person (side view)

2. Entering information and application of alcohol:

3.1.3. Entering Information

The device requires entering Age, Gender and Foot Measured.

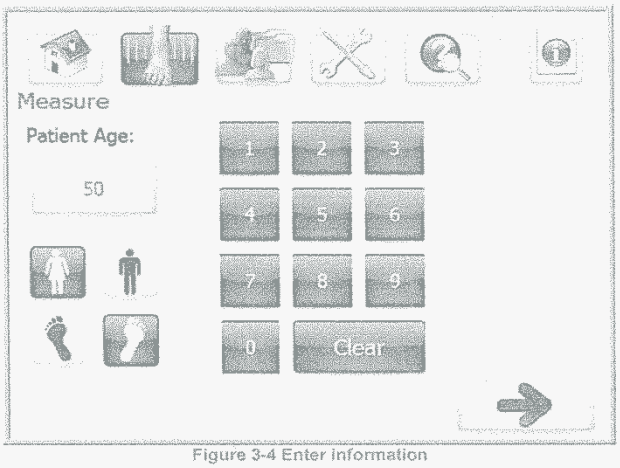


Figure 3-4 Enter information

- **Age**

  Use the number pad to enter the person's age. You can enter any age between 5 and 120.

  Z-Score is dependent upon the age and gender, and will not be displayed if the age of the person is outside of the age range covered by the current Reference Population or if the current Reference Population does not include males.

- **Gender**

  Use  or  to select Gender. The blue background means selected.

  T-Score and Z-Score values are dependent upon the age and gender, and may vary based on the Reference Population.

- **Foot**

  Use  or  to select FOOT. The blue background means selected.

  Click  to continue when above information are completed.

3.1.4. Apply alcohol

1) Use the Spray Bottle to apply a layer of alcohol to both sides of the heel (Figure 3-5), two times at least for each.

   This is to condition the skin and ensure proper ultrasonic coupling between membranes and heel. The person can rest heel lightly on the side of the device while the next steps are completed. Use 70% Isopropyl Alcohol as coupling agent.

3. Measuring the Heel:

❷ Position the foot to ensure the white rib is between the first two toes.

❸ Push the heel fully back

❹ Rest the calf lightly against the Calf Support

Click → to continue. Dry skin or membrane will cause poor coupling, which may result in an erroneous reading.

3.1.6. Measuring

1) The membranes will fill, and measurement will start immediately. Two waveforms display on the screen (Figure 3-8).

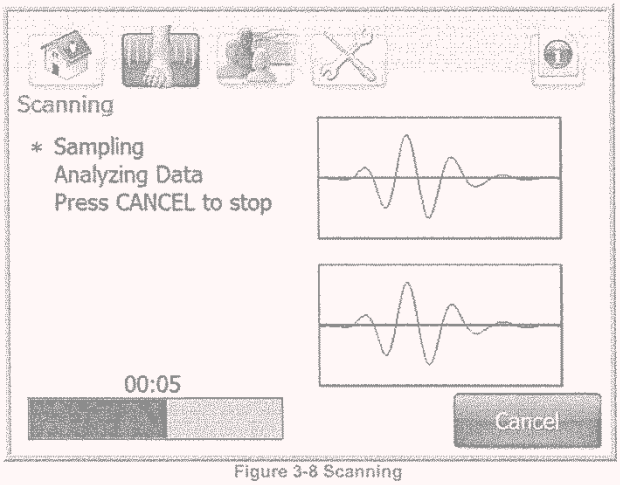


Figure 3-8 Scanning

➢ Make sure the person does not move their foot during the measurement as this can lengthen the measurement time or cause a measurement failure.

Note: Do not power off the device during person measurement, to avoid foot be clamped by inflated membrane.

CAUTION: The operator should keep the person in full view at all times and never leave the person unattended while measuring.

2) The membranes deflate after the measurement is completed

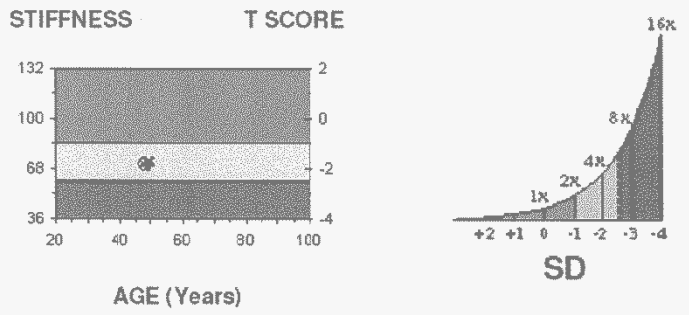
3) Remove the foot from the footplate

4) Wipe any remaining alcohol from the foot and membranes. Conducting cleaning as addressed in section 3.1.1

4. Results:

Facility Name
Street Address
City, State, Zip
Phone Number

Name:		Patient ID:	
Age / Date of Birth:	50 yrs.	Physician:	
Sex:	Male	Test Date:	
Heel:	Left		



Stiffness Index: 72	T-Score: -1.8	BUA: 54.4
% Young Adult: 72	Z-Score:	SOS: 1500.0
% Age Matched:		

Clinical Risk Factors:

Previous fracture, Smokes, Drinks

Comments: Medium risk of osteoporotic fracture.

Follow Up: None 6 Months 12 Months 18 Months 24 Months

GE Healthcare Lunar Achilles EXPII SN: 99999 Software Version 1.0

2.4 Pilot study:

Before we started the main study, we performed a pilot trial measuring heel density on a group of healthy subjects using the heel ultrasound machine. This was conducted on 10 participants at the clinical trials unit, level 8 at Wellington Hospital, and was undertaken to familiarise the researcher with the machine, and to assess whether any difference existed between left and right heel measurements to inform

whether both sides should be measured in the main study. The data obtained was as follows:

Table1: T-Score for pilot study

Left heel T Score	Right heel T Score	Comparison between left and right
0.7	0.3	Left predominant
-1.1	-1.7	Left predominant
-0.1	-0.6	Left predominant
-1.6	-1.0	Right predominant
-0.7	1.8	Right predominant
-1.5	0.7	Right predominant
0.7	-1.1	Left predominant
-1.5	-1.5	NA
0.9	0.8	Left predominant
-0.8	-1.5	Left predominant

According to these readings, left heel ultrasound T-score results were greater than the right heel ultrasound T-score results in 6 out of 10 participants, while for 3 subjects, right heel ultrasound results were greater than the left heel and for 1 subject left and right heel ultrasound data were similar. This pilot study suggested that given the variability it would be prudent to perform measurements on both sides and to take an average of the two readings.

2.5 Measurement of controls:

Heel ultrasounds results were compared firstly with the manufacturer reference database. However, since no local New Zealand referent data was available, we also performed heel ultrasound on 20 further healthy control women with no history of alcohol dependence who were closely matched in age to the cases admitted to Kenepuru.

Volunteers were recruited from the general nursing staff at Kenepuru hospital, who gave informed written consent after receiving the information about the study. The same lifestyle questionnaire was administered to compare prevalence of other possible lifestyle factors for osteoporosis that we hypothesized might cluster in women with alcohol dependence, including reduced levels of physical activity, smoking and a low dietary calcium intake.

2.6 Blood sample collection:

Routine measures of renal function (urea and creatinine) were performed in all women admitted to the unit; serum magnesium levels were also available in all admissions. Blood samples were collected on Day1 in a subset; serum procollagen type 1 N propeptide (s-P1NP) and serum C-terminal cross-linking telopeptide of type 1 collagen (s-CTX) were also taken. Samples were collected again on Day 5 for s-P1NP and s-CTX. Blood samples of bone turnover were taken only for 10 women who were premenopausal and of European origin. This restriction was performed to minimise variability due to age; menopausal status; ethnicity and to increase the likelihood of detecting change in bone turnover marker status from day 1 to day 5.

2.6.1 Sample segregation techniques:

Different techniques/assays were performed to measure Vitamin D, PTH, s-CTX and P1NP in selected blood samples. The detailed methods are outlined below.

a. <u>Technique for s-CTX:</u>

s-CTX was isolated from blood using the Roche Elecsys Beta-Crosslaps methd. The assay is specifically used for an octapeptide in –terminus of type I collagen which reflects the bone resorption mediated by the osteoclasts. Samples were collected fasting early in the morning because of significant diurnal variation which is blunted after fasting. The reference interval is less than 0.75 µg/L in fasting in early morning,

while the uncertainty of measurement is 0.01µg/L for less than 0.1 µg/L and 10% for more than 0.13 µg/L

b. Technique for P1NP:

P1NP was isolated from blood with the Roche Elecsys 2010 method. P1NP is a bone formation marker which is a specific indicator of type I collagen disposition. P1NP is released as a trimeric structure, but it degrades into a monomer. P1NP is used to assess the response to osteoporosis treatment because it increases in state of high bone turnover. The reference interval for P1NP is not specific as it is age related.

c. Technique for Vitamin D (25-Hydroxycholecalciferol):

Vitamin D was separated from blood using a radioimmuno assay (RIA) after the extraction with acetonitrile. Below is the suggested reference range:

a. Optimal target range for bone health : 50-150nmol/L
b. Moderate to severe deficiency : < 225 nmol/L
c. Mild deficiency : 25-50 nmol/L

d. Technique for Parathyroid hormone (PTH):

PTH was separated from blood using an Elecsys assay. The elecsys assay employs a sandwich test principle in which a biotinylated monoclonal antibody reacts with N-terminal fragment and a monoclonal antibody labelled with Ruthenium complex reacts with C-terminal fragment. The measuring range specified is 1.20-5000 pg/mL.

Chapter Three: Results

3.0 Characteristics of population:

a) Women admitted to alcohol dependence unit

Table 2: Characteristics of women admitted to the alcohol unit

	N	Minimum	Maximum	Mean	Std. Deviation
Age at admission	**20**	**27**	**67**	**45.2**	**9.0**
Age of starting alcohol	**20**	**12**	**41**	**16.3**	**6.2**
Dietary Ca (mg/week)	**20**	**849.9**	**19872.3**	**4624.1**	**4202.7**

Table 2 shows the summary characteristics of women admitted to the alcohol dependence unit. Among the 20 women who had been admitted to the unit for alcohol detoxification, the mean age was 45.20 (SD = 8.97) years. Women reported that they had been drinking alcohol for a mean of 16.25 (SD = 6.61) years prior to admission to the unit. The mean alcohol consumption per day was 19.80 units with a standard deviation of 9.90. Dietary calcium intake was very variable; the range was (849.90-19872.30) mg weekly. The mean dietary calcium intake per week was 4624.08 mg.

Nine women (45 **%**) reported that their periods had stopped by the time of interview. Fifteen women (75 %) were current smokers; 85 % were ex-smokers and 15 % were lifelong non-smokers. Among smokers the mean pack years were 18.60**.** Fifteen (75 %) women reported at least one fall in the previous year; 7 women reported a previous fracture.

2 (10%) women out of 20 reported any vigorous physical activity in the preceding week. 5(25%) women out of 20 reported any moderate physical activity in the previous week. Seven women out of 20 (35 %) reported a co-morbidity; in 4 cases this was a diagnosis of depression, but 2 women reported seizures, 4 a history of bronchitis and one a history of cardiovascular disease. Others reported degenerative lumbar spine disease and non-specific abdominal pain.

b) Healthy controls

Table 3: Characteristics of healthy women as controls

	N	Minimum	Maximum	Mean	Std. Deviation
Age at admission	**16**	**21**	**67**	**42.3**	**130**
Age of starting alcohol	**14**	**12**	**21**	**16**	**2.42**
Dietary Ca (mg/week)	**16**	**705.9**	**8164.0**	**3503.8**	**2187.4**

Table 3 shows the summary characteristics of healthy women who had been included as controls in the study. Sixteen healthy women participated in the study. The mean age was 42.31 (SD = 12.95) years. Women reported that they had been drinking alcohol for a mean of 16 (SD = 2.42) years. The mean alcohol consumption per day was 1.77 units with a standard deviation of 2.74**.** Dietary calcium intake was

very variable in the range of (705.90 – 8164.00) mg weekly. The mean dietary calcium intake per week was 3503.83 mg.

Six women (37.5%) reported that their periods had stopped by the time of interview. One woman (6.3 **%**) was a current smoker; 7 women (43.8 **%**) were ex-smokers and 9 women (56.3 %) were lifelong non-smokers. Among smokers the mean pack years were 6. Four (25%) women reported at least one fall in the previous year; 7 women reported a previous fracture.

Nine (56.5%) women out of 16 reported any vigorous physical activity in the preceding week. Eleven (69%) women out of 16 reported any moderate physical activity in the previous week. Four women out of 16 (25 %) reported a co-morbidity; 2 a history of bronchitis and one cardiovascular disease. 1 woman reported degenerative lumbar spine disease and non-specific abdominal pain.

The significant differences in lifestyle factors likely to influence bone mass are age at admission, age of starting alcohol and dietary Ca intake as highlighted in Table 1 and 2.

3.1 Heel ultrasound (HUS) parameters:

The heel ultrasound results among healthy controls and alcohol dependent women are shown below in Table 4 and displayed graphically in figure 10

Table 4: HUS results of alcohol dependent women vs healthy controls

	Alcohol dependent women		**Healthy controls**	
	Mean	SD	Mean	SD
BUA	107.38	14.63	121.16	27.28
SOS	1550.58	38.07	1570.6	44.51
SI	85.65	18.42	95.53	18.66
T score	-1.09	1.42	-0.31	1.40
Z score	-0.44	1.29	0.28	1.41

.

Of the 20 women admitted to the alcohol detoxification unit, those with a |T| score classed as normal was 9 (45%); with a T score between -1 and -2.5 was 6 (30%) and lower than -2.5 was 4. (20%)

The mean average SOS for the 20 women admitted to the unit for both heels was 1550.58 m/sec and the mean average BUA was 107.39 dB/MHz, while the mean average SOS for the 16 women who participated in the study as controls for both the heels was 1570.62m/sec and mean average BUA was 121.17dB/MHz.

However, the crude difference in mean value for each of these variables between the alcohol dependent women and the controls was not statistically significant as shown in the below table:

Table 5: Difference in mean values of HUS outcomes between both the groups

HUS outcomes	Df	F	P value
Avg BUA	1	0.77	0.38
Avg SOS	1	0.03	0.86
Avg T Score	1	0.40	0.53
Avg Z Score	1	0.34	0.56
Avg SI	1	0.36	0.54

Thereafter, the values for all 36 women were combined into one data set to increase statistical power when evaluating relationships between alcohol consumption and bone quality. The mean values of each variable in the combined data set are shown below; results are plotted graphically to demonstrate normality.

Table 6: Mean value for both groups for HUS outcomes

	Alcohol dependent group		Healthy women	
Heel Ultrasound Parameters	Mean	SD	Mean	SD
Avg BUA	107.39	14.63	121.17	27.28
Avg SOS	1550.58	38.07	1570.62	44.51
Avg T score	-1.10	1.422	-0.32	1.40
Avg Z score	-0.45	1.29	0.28	1.41
Avg SI	85.66	18.42	95.53	18.66

The age bands with the HUS outcomes were assessed together for both the groups to increase the number and power of the data. The mean value by age bands for the grouped women was:

Table 7: Mean value of HUS outcomes as per age bands for both the groups together

HUS outcomes	Mean	SD	P value
Avg BUA			.176
0-36	114.38	7.96	
37-44	122.65	12.65	
45-50	100.48	13.42	
51+	117.65	39.66	
Avg SOS			0.023
0-36	1552.25	27.28	
37-44	1595.08	39.16	
45-50	1542.96	34.71	
51+	1547.29	47.69	
Avg T Score			0.002
0-36	-0.70	0.65	
37-44	0.63	1.24	
45-50	-1.62	1.36	
51+	-1.34	1.37	
Avg Z Score			0.007
0-36	-0.62	0.70	
37-44	1.07	1.31	
45-50	-0.91	1.41	
51+	0.03	1.22	
Avg SI			0.002
0-36	90.77	8.47	
37-44	108.22	16.50	
45-50	78.88	17.72	
51+	81.87	18.06	

Figure 10: Histogram to show Distribution of BUA among women admitted to the alcohol dependence unit

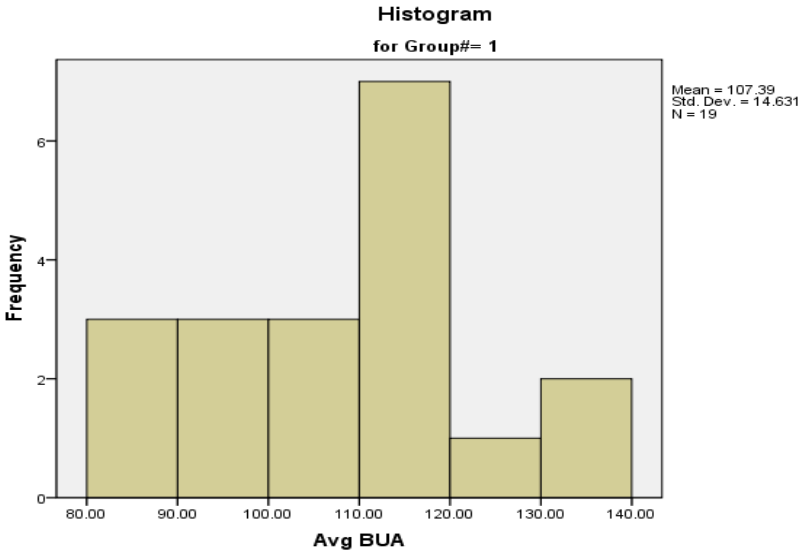


Figure 11: Histogram to show Distribution of BUA among women enrolled in the control group

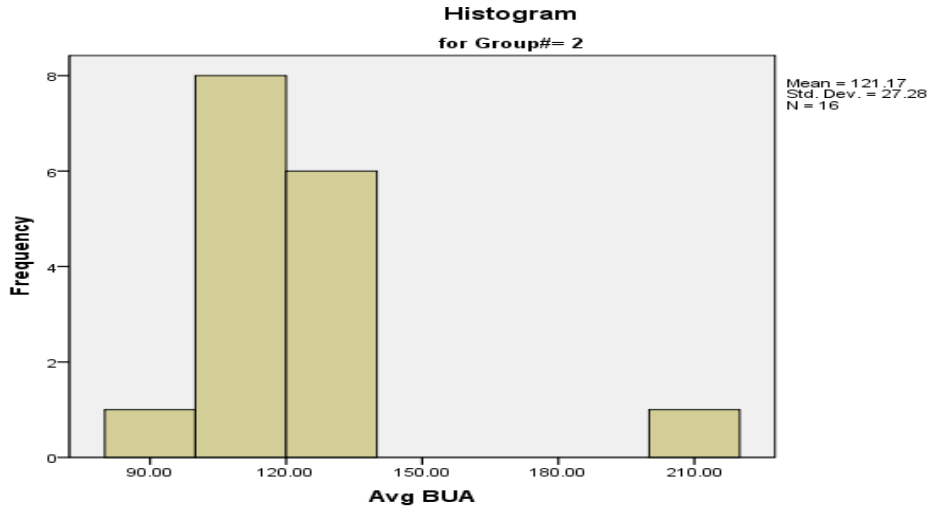


Figure 12: Histogram to show Distribution of SOS among women admitted to the alcohol dependence unit

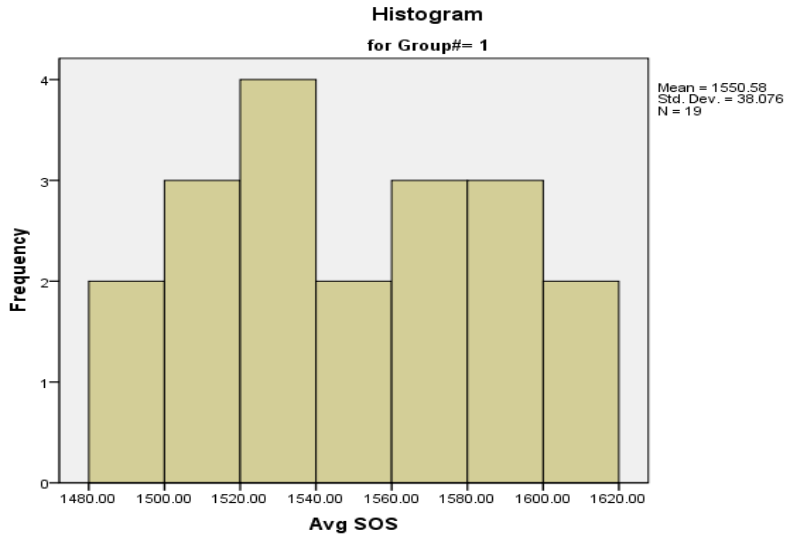


Figure 13: Histogram to show Distribution of SOS among women enrolled in the control group

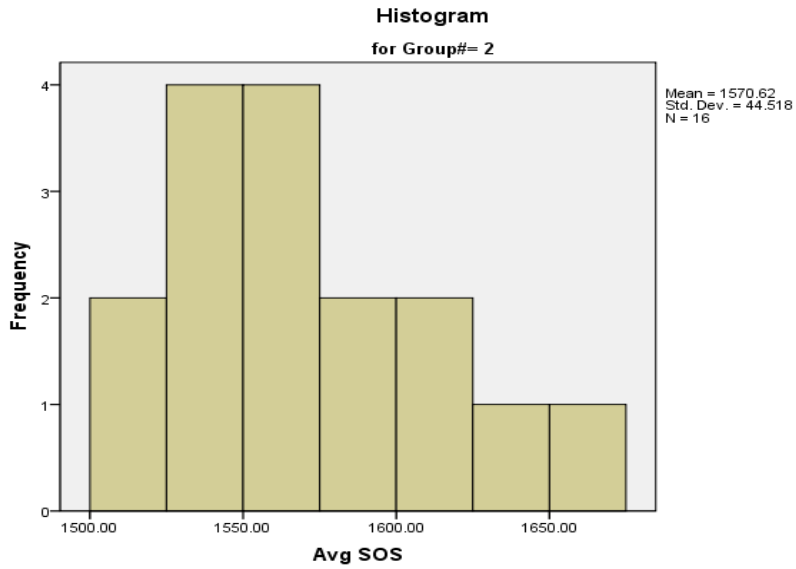


Figure 14: Histogram to show Distribution of T Score among women admitted to the alcohol dependence unit

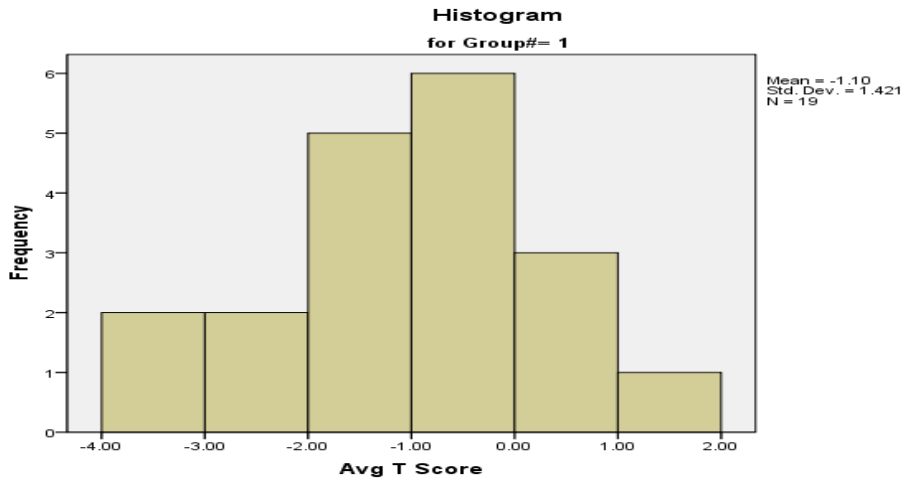


Figure 15: Histogram to show Distribution of T Score among women enrolled in the control group

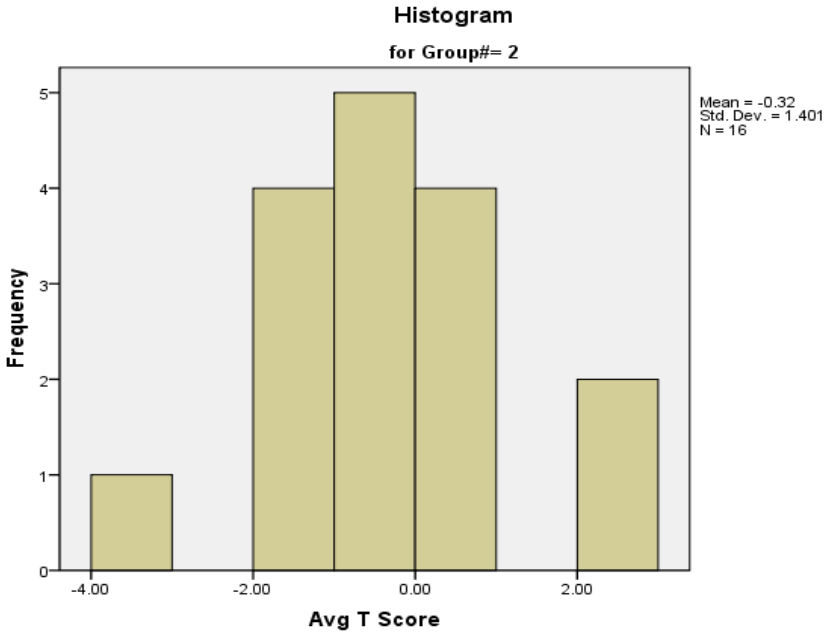


Figure 16: Histogram to show Distribution of Z Score among women admitted to the alcohol dependence unit

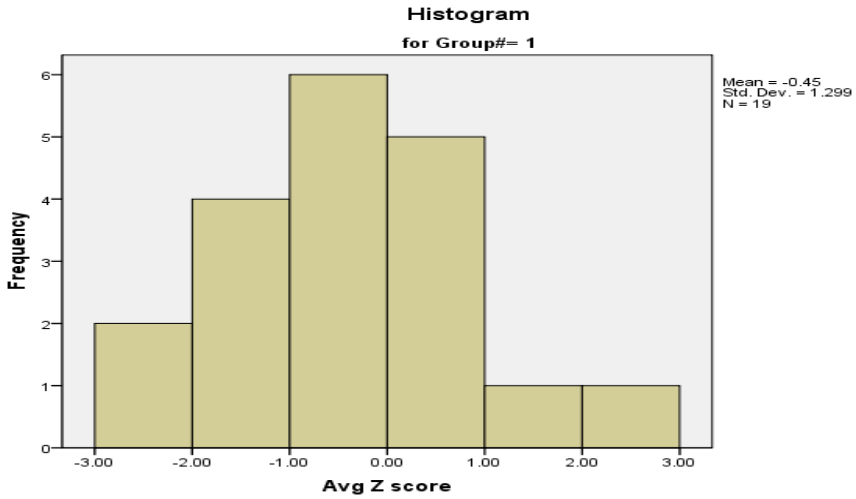


Figure 17: Histogram to show Distribution of Z Score among women enrolled in the control group

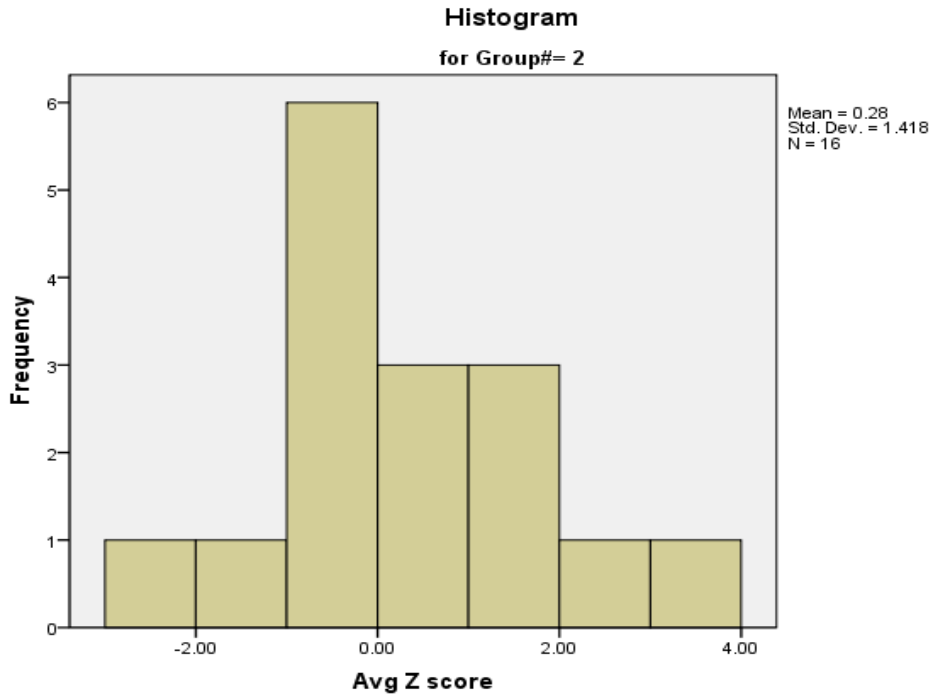


Figure 18: Histogram to show Distribution of SI among women admitted to the alcohol dependence unit

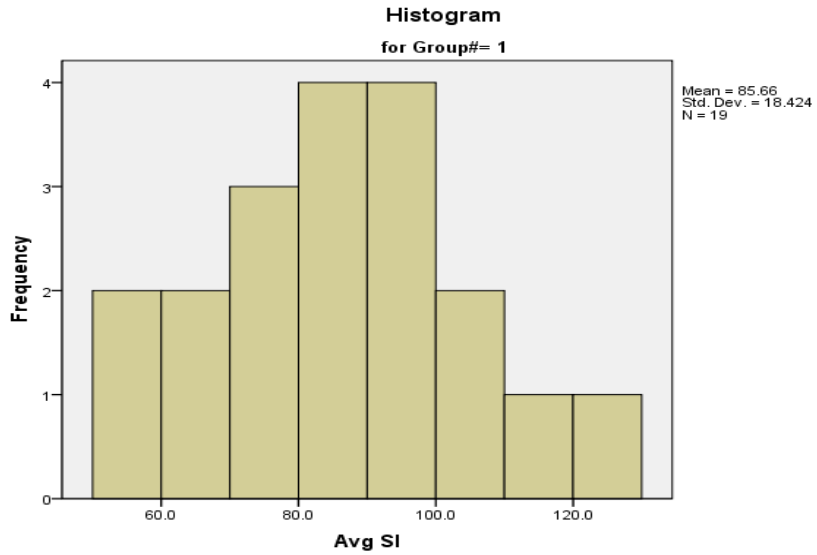
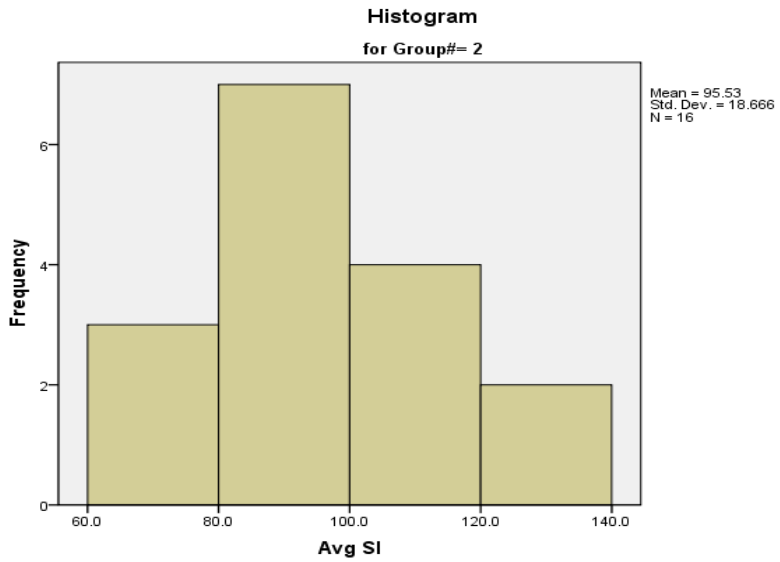


Figure 19: Histogram to show Distribution of SI among women enrolled in the control group



3.2 Menstruation status and association with heel ultrasound outcomes:

We assessed whether menstrual status was a determinant of heel ultrasound variables in this dataset. We analysed this parameter of whether ‘still having periods = y/n’ with all the 5 heel ultrasound outcomes using Analysis of variance (ANOVA) for all 36 women together as a cohort for different age bands. After adjustment for age, by analysing in predefined age bands, we found higher values among women who still reported menstruation, as shown below in Table 8.

Table 8: Mean HUS values by menstrual status

Age bands	Mean	SD	Df	F	P value
Avg BUA			3	7.74	0.002
0-36	114.33	8.51			
37-44	125.15	8.71			
45-50	98.08	12.38			
51+	118.1				
Avg SOS 0-36			3	3.68	0.03
37-44	1554.90	27.90			
45-50	1593.73	44.21			
51+	1530.28	37.52			
	1611.90				
Avg T score			3	6.86	0.003
0-36	-0.65	0.68			
37-44	0.73	1.22			
45-50	-2.02	1.37			
51+	0.75				
Avg Z score			3	5.84	0.006
0-36	-0.57	0.73			
37-44	1.16	1.30			
45-50	-1.29	1.44			
51+	1.65				
Avg SI			3	6.73	0.003
0-36	91.50	8.76			
37-44	109.50	16.32			
45-50	73.80	17.67			
51+	110.00				

We used an independent T-test to calculate the effect of smoking status on HUS outcomes and found significant differences with lower values among women who had ever smoked for Avg SOS (p value = 0.02) and for Avg SI (p value = 0.04) (Table 9)

Table 9: Means between subjects and controls according to smoking status

	T	Df	p value
Avg BUA	0.114	33	0.91
Avg SOS	2.390	33	0.02
Avg T score	2.004	33	0.05
Avg Z score	1.764	33	0.08
Avg SI	2.056	33	0.04

For the variables 'family history of hip fracture' and 'still smoked regularly', we showed no significant effect on the mean for all the 5 outcome variables Avg BUA, Avg SOS, Avg T Score, Avg Z Score and Avg SI.

3.3 Bone Turnover markers:

Bone turnover markers were measured among a subgroup of premenopausal women admitted to the alcohol dependence unit. The aim was to obtain bone turnover samples for 10 women from the subjects group. However, at the time of this data set construction, we had available only the results for 6 women.

Table 10 shows the mean (SD) values for day 1 P1NP, day 5 P1NP, day 1 s-CTX, day 5 s-CTX.:

Table 10: Mean (SD) bone turnover markers:

Paired Samples Statistics

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	Day 1 P1NP (Ref range: 15-60 µg/L)	23.08	6	9.52	3.88
	Day 5 P1NP	27.88	6	8.03	3.28
Pair 2	Day 1 s-CTX(Ref Range : <0.75 µg/L)	.14	6	.07	.029
	Day 5 s-CTX	.13	6	.08	.03

The mean value for day 1 P1NP was 23.08µg/l with SD = 9.52; while the mean value for day 5 P1NP was 27.88µg/l with SD of 8.03.

The mean value for day 1 s-CTX was 0.14µg/l (SD = 0.07); while the mean value for day 5 s-CTX was 0.13µg/l (SD = 0.08). These differences were significant, even in this small pilot study, for PINP but not CTX.

Table 11: Mean difference between Day 1 and Day 5 Bone markers

	Mean	SD	t	Df	p value
Day 1 P1NP – Day 5 P1NP	-4.8000	4.3731	-2.689	5	0.043
Day 1 s-CTX – Day 5 s-CTX	0.00833	0.10068	0.203	5	0.847

3.4 Blood Samples:

Blood samples were obtained from 20 women who participated in the subjects group who were admitted at the alcohol detoxification unit. Creatinine, Magnesium, PTH and Vitamin D levels were checked from the blood samples obtained.

Relationships between these biochemical values and the heel ultrasound outcomes BUA, SOS, T score, Z score and SI were investigated, but none were significant. This may reflect the small sample size and limited power for this aspect of the study, particularly as results were often missing from the clinical system.

Table 12: Relationship between biochemical values and HUS outcomes

	Avg BUA	**Avg SOS**	**Avg T score**	**Avg Z score**	**Avg SI**
Creatinine	0.11 P value = 0.65	-0.12 P value = 0.61	-0.004 P value = 0.98	-0.08 P value = 0.74	-0.009 P value = 0.97
Magnesium	-0.08 P value = 0.75	0.14 P value = 0.59	0.03 P value = 0.90	0.09 P value = 0.71	0.03 P value = 0.88
PTH	-0.03 P value = 0.95	-0.06 P value = .0.91	-0.05 P value = 0.93	-0.20 P value = 0.73	-0.04 P value = 0.93
Vit. D	-0.77 P value = 0.22	-0.29 P value = 0.70	-0.51 P value = 0.48	-0.43 P value = 0.56	-0.52 P value = 0.48

Please note that bone turnover markers and blood samples had not been obtained for the women included in the control group

3.5Summary:

In conclusion, the main findings of the study were as follows:

a. The mean alcohol content/day in women admitted to the alcohol detoxification unit was 19.80/day compared to women who participated in the control group (1.77/day)

b. In women admitted to the alcohol dependence unit, the mean average outcome for BUA, SOS, T score, Z score and SI was low as compared to women participated in control group; the mean Z Score (age related outcome) was observed to be lower in women admitted to the alcohol unit with a value of -0.44; whereas, the mean Z score in control group women was 0.28.

c. 7 out of 20 women (35%) admitted to the alcohol dependence unit had reported a history of fracture ; while 7 out of 16 healthy control women (43.8%) had reported a history of fracture, suggesting that healthy women at risk of osteoporosis may have volunteered to participate in this study as they were interested in their risk.

d. 15 out of 20 women (75%) with alcohol dependence had reported falls in the preceding year before admission to the unit, whereas only 4 women out of 16 (25%) who participated as controls in the study had reported falls, highlighting the high falls risk in alcoholics.

e. Dietary calcium intake in both the groups was similar but below the recommended weekly intake of 5600 to 7000 mg weekly; the dietary intake among the alcoholic women was 4624.80 mg/week while the calcium intake in the healthy control group was 3503.83 mg/week.

f. Women admitted to the alcohol unit were less physically active than women who participated in the control group. Five out of 20 alcohol dependent women

(25%) performed regular moderate physical activity and 2 out of 20 women (10%) performed regular vigorous physical activity. By contrast, 11 out of 16 healthy control women (68.75%) performed regular moderate physical activity while 9 out 16 (56.25%) performed regular vigorous physical activity.

g. Women who had ever smoked regularly have significantly lower Avg SOS and Avg SI results than lifelong non-smokers.

h. After adjustment for age, by analysing in predefined age bands, HUS results were significantly higher among women who still reported menstruation when compared with women whose periods had stopped

i. Even in this small pilot study, there was a significant rise in the bone formation marker P1NP between the day of admission to an alcohol dependence unit and after 5 days of abstinence

Chapter Four: Discussion

4.0 Findings:

We have performed a study administering a lifestyle questionnaire and performing heel ultrasound in two groups of women; women with a history of alcohol dependence and staff members from the same unit. The questionnaire we administered detailed age, personal and family history of fracture, cigarette smoking (pack years), alcohol history (duration and level of drinking; type of alcohol consumed), physical activity, dietary calcium intake (food frequency questionnaire), past medical history (to elicit information on possible other secondary causes of osteoporosis e.g. insulin dependent diabetes, hyperthyroidism), drug history and reproductive history.

As anticipated, we found a number of lifestyle differences other than levels of alcohol consumption between the two groups studied. That the mean age at admission for alcoholic women was 45.20 years, while that in healthy women was 42.50, so matching by age was reasonable. The mean age of starting drinking alcohol was 16.25 in alcoholic women, which was similar to healthy women. Dietary Ca intake and physical activity levels were higher in healthy women when compared with women admitted to the alcohol unit. As expected, there was a significant difference in the mean alcohol content/day in women admitted to the alcohol unit (mean intake was 19.80 units/day) to women participated in control group (mean intake was 1.77 units/day).

Fifteen women out of 20 admitted to the alcohol unit had reported falls in the preceding year with 7 women (35%) having fracture history. These results may be compared with results found in one study conducted on alcoholic men with an ethanol consumption of more than 150g/day. 81 men participated in the study and in those men, the prevalence of fracture was 49.3% mainly rib fracture [41]. When our study results were compared with similar studies conducted on men and women we

found a similar percent of people suffering from fracture risk. In our group of alcoholic women, 6 women out 20 had osteopenia (30%), whereas only 25% controls were classified as osteopenic. Though other studies conducted on alcoholics reported a significant difference in fracture history between alcoholics and controls [41]; this is in contrast to our own study results; even in our control population, 7 out of 16 women (43.8%) had reported a previous history of fracture. This may reflect an awareness of osteoporosis risk in our control group, who volunteered for this study for this reason. Among controls, only 4 women out of 16 (25%) had reported a fall in the preceding year of admission.

We evaluated the dietary Ca intake /week for both groups and found that dietary Ca intake in both the groups was below the recommended levels of 6300 to 8000 mg/ week. Dietary Ca intake in the subject group was 4624.80 mg/week while Ca intake in the control group was 3503.83 mg/week i.e lower than among the alcoholic group.

The drug history we took showed that 1 out of 20 alcoholic women (5%) were on steroids at admission to the alcohol detoxification unit, while 2 out of 20 women (10%)were on vitamin D supplements. In women enrolled in the control group, no woman was on any of these supplements. Blood samples were obtained from women admitted to the alcohol unit to test vitamin D and PTH levels. According to national guidelines, a serum concentration of 25(OH)D is defined as insufficient if less than 50 nmol/L, if less than 25 nmol/L it is classified as deficient and severe deficient is defined as less than 12.5 nmol/L. Vitamin D levels were obtained only in 4 subjects out of 20 (20%), a severe limitation of our study, and were in the range of 11 to 70 nmol/L. The mean level of vitamin D was 46.50 nmol/L. Our study results were similar to other study results which showed that vitamin D levels were significantly deficient in alcoholics [42]. In a study conducted by Ulrich Bang et al., out of 146 participants enrolled (66 men and 80 women), the mean level of vitamin D was 47 nmol/L [42]. In this group, vitamin D levels were comparatively higher in participants who were on vitamin D supplements [42]. Unfortunately vitamin D levels

were not measured in participants who were on oral vitamin D supplements in our study.

Another lifestyle factor that plays an important role in bone health is physical exercise. We assessed physical activity levels for both the groups and found that women admitted to the alcohol unit were less physically active than women who participated in the control group. 5 out of 20 alcoholic women (25%) performed regular moderate physical activity and 2 out of 20 women (10%) performed regular vigorous physical activity. By contrast, 11 out of 16 control women (68.75%) performed regular moderate physical activity while 9 out 16 performed regular vigorous physical activity. 5 women out of 20 (25%) alcoholic women reported being regularly physically active for past 6 months and 13 out of 16 (81.25%) women in the control group were regularly physically active in the past 6 months. Sampson [46] showed that among women who were chronic alcohol drinkers and who undertook some regular physical activity, BMD was higher as compared to non-physically active alcoholic women [46]. Also, when he compared the results with healthy controls who undertook regular physical activity, BMD was slightly higher than in the alcoholic women who undertook no physical activity [46].

Preclinical studies have been performed on alcohol fed animals to evaluate the effect of exercise on them [47]. The study results showed that exercise did not lessen the side effects caused by the alcohol on the bone [47]. Though there is less literature available on whether exercise can improve bone density in alcoholics, some studies showed that any mechanical stress either by doing any physical exercise or by carrying heavy weights can increase bone density [48].

Earlier studies and literature available shows that most of the studies on effect of alcohol on bone mineral density have been performed in men. A study conducted by Mulleman et al compared the BMD in alcoholic patients and healthy men and they

observed that BUA, SOS and SI were higher in controls when compared with alcoholic patients [49].

In summary the main aim of our study was ‘To compare bone health among healthy women (controls) with women admitted to an alcohol dependence unit at Kenepuru hospital using a heel ultrasound machine’. We performed a heel ultrasound scan for all the 36 participants (20 alcoholic women and 16 controls) and results were obtained on 5 parameters viz. BUA, SOS, T score, Z score and SI for both the heels. We added the results for both the heels and obtained average results for all these 5 outcomes. It was seen that in general the mean average outcome for BUA, SOS, Z score and SI was low as compared to women who participated in control group. Further work in a larger study group is now indicated.

4.1 Animal studies of alcohol and bone:

As discussed in earlier chapters, osteoporosis is a disease which is defined as micro architectural deterioration of bone which thereby lowers the BMD [50]. This deterioration of bone can lead to fragility and fractures of bone [50]. There have been several studies published on the effect of alcohol on bone; in general the literature shows that moderate alcohol consumption is associated with an increase in bone strength whereas, chronic consumption of alcohol can decrease BMD and increase the chance of fractures. It is yet not known how alcohol causes bone loss. Many pre-clinical studies have been performed to understand the mechanism and effect of alcohol on bone. This section discusses the findings of this area of research, and its possible relevance to our own study. One such study was conducted on long boned rats to check the effect of different concentrations of alcohol on them [50]. 18 rats were evaluated in acute and chronic studies and were divided into 3 groups for both the studies [50]. In control animals (group A), normal saline was administered for 5 days intraperitoneally while 20 % and 30% alcohol was administered to treated animals (group B) and (group C) [50]. The acute study was terminated after 6 days

of exposure while the chronic study was halted after 6 weeks of exposure to alcohol [50]. After the study was terminated, the femur and tibia were dissected from the body of the rats [50]. The results in the acute study showed that bone weight and strength increased at 20% alcohol exposure; whereas bone weight and strength decreased at 30% of alcohol exposure [50]. In the chronic study which was terminated after 6 weeks of treatment, study results showed that bone weight and strength decreased in both treated animal groups [50]. These results of the study suggested that bone strength and weight decreased as alcohol concentration is increased [50]. This may be relevant in understanding the aetiology of bone loss in chronic alcoholics.

To understand more about the biological aspect of the effect of ethanol, bone research has been undertaken and shows that there are many cytokines that play an important factor in bone resorption process; IL-6 is one of them [51]. Studies have shown that ethanol can lead to a rise in IL-6 gene expression in bone marrow [52]. A study was performed on mice based on a hypothesis that ethanol can cause bone loss as it has an ability to induce IL-6 mediated osteocalstogenesis [52]. To measure bone loss, DEXA was performed on the mice [52]. The mice were divided into IL6+/+ and IL6-/- groups [52]. The whole body BMD was reduced by 3.01 % in the ethanol-fed IL6+/+ mice, when compared with the control-fed IL6+/+ mice [52]. However, there was no significant difference between whole body BMD of IL6-/- mice for both alcohol fed and control fed mice [52]. The femoral BMD of both the groups of mice was also measured and it was observed that in alcohol fed IL6+/+mice BMD decreased by 5.37% and no difference was observed in IL6-/- mice in both the groups [52]. These results suggest that excessive abusive alcohol consumption can lead to increased bone loss and implicates IL-6 can cause bone loss when estrogen levels are low [52].

Animal studies have also been performed to check whether chronic alcohol consumption can decrease the mineral constituents of bone.

As we know, bone is mainly composed of Ca and P [53]. At the time of menopause, estrogen levels in the women's body decrease thereby causing an imbalance in the Ca and P concentrations in the bone [53]. Studies have shown that alcohol induced estrogen deficiency can lead to an imbalance in bone mineralisation thereby decreasing BMD [53]. In a study which was conducted on 54 rats,[53] rats were divided in 2 groups. Group 1 consisted of rats who had been ovariectamised with low estrogen levels, while group 2 consisted rats with ovaries not removed [53]. These were again divided and were fed 20% alcohol and control solution [53]. The results showed that the Ca and P concentrations in the bones extracted were decreased in the alcohol fed rats. It was also observed that there was a significant difference in Ca and P levels in estrogen deficient alcohol fed rats and control fed rats [53]. This study results also highlighted that when food and nutrients were fed to the rats, a significant increase in Ca and P was seen thereby improving the quality of the bone [53]. Other studies have shown lower BMD at the tibia and the femur in alcohol fed animals as compared to control animals [54].

In addition to the studies above, research has been undertaken to check whether chronic alcohol use damages fracture repair properties and whether abstinence from alcohol improves bone quality and strength [55]. Research was conducted on 36 rats divided in 4 different groups: Group A and B were on a Lieber - Decarli liquid diet with either 36% ethanol or control diet [55]. AIN-93M ad libitum diet was given to rats in group C, and group D rats were fed with AIN-93M liquid ethanol diet [55]. The study results showed that rats in group C had a higher bending rigidity than group A (48%) and group B (47%) [55]. Group D rats had bone quality results similar to group C rats [55]. In group A and group B rats, the mineral constituent of bone was 16 % and 13 % respectively; while in group C and group D the mineral content was normal [55]. Thus, these study results suggested that the bone repair function was damaged in Group A rats (alcohol fed) as compared to their counterparts in group B who were kept on control liquid diet [55]. The results also suggested that after bone injury, if there is abstinence from alcohol the bone repair mechanism improved thereby improving bone quality [55].

Studies have showed that bone loss which occurs because of alcohol happens mainly because of an imbalance in the bone remodelling cycle [56]. Research shows that some inherited polymorphisms at particular gene loci can be a risk factor for post - menopausal osteoporosis [56]. A study had been conducted on rats to recognise changes in expression profiles for bone formation and bone resorption-related genes [56]. Adult male rats included in the study were divided into 2 groups with either 1 week of treatment or 4 week of treatment. The rats were fed either binge alcohol or as saline-treated controls [56]. To measure the blood ethanol levels, NAD^+ reduction assay was performed and to measure the biomechanical properties of lumbar vertebrae compressive strength tests were performed [56]. There was no significant decrease in BMD of vertebral cortical bone among rats treated with moderate binge alcohol as compared to controls [56]. In rats fed with chronic binge alcohol, a significant reduction in vertebral cancellous bone BMD was observed [56]. The compressive strength was also decreased in these rats when compared with controls [56]. This study also highlighted that bone formation markers gene expressions (eg. Alkaline phosphatase and osteocalcin) decreased after exposure to moderate binge alcohol consumption with a p value of 0.015 and 0.003 respectively [56]. For bone resorption markers (eg. RANKL); after moderate binge alcohol exposure the levels increased and normalised after chronic binge alcohol exposure [56]. This study demonstrated gene expression changes for both bone resorption and bone formation markers after exposure to binge alcohol treatment [56]. It suggests that bone loss due to alcohol intake may be a result of changes in expression of genes that plays an important role in the bone remodelling process [56].

4.2 Alcohol bone studies in men:

Heavy alcohol can be a reason for secondary osteoporosis in men and thus research had been conducted to see how deleterious the effects of chronic alcohol are on men. In one study conducted on male chronic alcohol drinkers [57], 138 male subjects participated in the research. Patients included in the study had 3 years or

more history of alcohol dependence. Alcohol content was calculated and heel ultrasound was performed [57]. 20 patients were included in a lifestyle modification program for two and half months where they went into alcohol detoxification. Lifestyle modification of these 20 patients included increased levels of physical activity, dietary improvements, and general education programs. Chronic consumption of alcohol was associated with a decrease in BMD in older alcohol dependent people [57]. Lifestyle modification was seen to be beneficial and achievable.

A study conducted by Kim et al [58] included 18 alcoholic men who consumed >= 40 g of alcohol/day and 18 controls who consumed less than 20 g of ethanol/day and all of whom were ambulatory patients [58]. The study results showed that daily Ca and protein intake was the same in alcoholics and controls. Osteocalcin and vitamin D levels were higher in the control group than heavy drinkers [58]. The study results support the earlier discussed findings in pre-clinical studies.

In an older population, the majority of fractures are because of falling. As discussed above, high consumption of alcohol leads to decrease in bone density and thus there can be a relation between alcohol intake and falls and fractures [59]. Furthermore, alcoholic intoxication is strongly associated with falling. At least one study suggest that excessive chronic alcohol intake is associated with a higher risk of hip fracture [60]. In the MrOS (Osteoporotic Fracture in Men study), 5995 older men above 65 years of age with no hip replacement history were recruited. A questionnaire based method was used and questions detailing the alcohol type and amount, problem drinking, falls and fractures were asked. BMD was calculated using DEXA. The study results showed that light to moderate alcohol consumers were not at higher risk of falls and fractures while a history of chronic excessive consumption of alcohol led to an increased risk of falls and fractures [61]. Though there are only a few studies that compared excessive alcohol intake and fracture risk, the above suggest that there is a link, and this is mediated through a combination of falls and low BMD.

4.3 Alcohol bone studies in women:

Research shows that women are at higher risk of suffering from osteoporosis than men. Lifestyle factors associated with alcohol dependence may be important; smoking and alcohol can decrease BMD [62], whereas lifestyle factors such as physical activity and high dietary calcium intake can improve bone health [63]. Educational programmes to modify lifestyle have been undertaken. A study was conducted on 669 healthy pre-menopausal women aged 15-35 years to evaluate the effect of educating about osteoporosis [64]. Women were asked to complete a questionnaire before the education program about BMD testing and osteoporosis and after the program [64]. Follow- up was at a year. The study results showed an increase in Ca intake, physical activity, vitamin D supplements and decrease in smoking and alcohol content after 1 year of follow–up [64]. The study results were similar to another study which was conducted on 263 pre-menopausal women who underwent an education programme about osteoporosis [65]. Both the studies showed that in women with normal BMD, there was no subsequent significant difference in Ca intake and physical activity before and after the education program, but in women with a low BMD, there was a significant increase in Ca intake and physical activity [64, 65].

Several methods have been introduced to predict the risk of osteoporosis by measuring the BMD including DEXA and quantitative heel ultrasound. While several studies have evaluated one or the other technique, fewer studies have been performed to identify which is the best way to predict this fracture risk. One such study had been performed using a questionnaire based method and involving use of both the techniques (DEXA and QUS) to predict risk of fractures in 668 healthy women [66]. BMD was measured at the hip and lumbar spine by DEXA, while heel SI, BUA and SOS was measured using a heel ultrasound machine. The study showed that 50 women out of 668 had low bone mass at the hip and 100 women out of 668 had low bone mass at the lumbar spine. 122 women had a low bone mass as assessed by SI and QUS. In total, there were 61 women who had decreased BMD

as measured by both DEXA and QUS. The factors associated with low bone mass in this study were: low body weight, late age at menarche (>15 years) and low levels of physical activity [66].

Studies have also shown that side effects of alcohol are much greater physiologically in women than in men [68]. The negative consequence of heavy drinking includes alcohol related falls leading to injuries, heart disease and cognitive disease. In our study more than 50% of the women had experienced falls because of chronic alcohol consumption. Comorbidities are also more frequent; in the National Comorbidity study, comorbidities were higher in alcoholic women with 72.4% as compared to that in alcoholic men with 56.8% [68]. These comorbidities included depression and anxiety, as in our own study.

4.4 Alcohol dependency in New Zealand:

New Zealand, like other developed countries of the world faces the problem of chronic excessive alcohol consumption in society. It has been also observed that alcohol consumption is more common in the older population as compared to the younger generation in both men and women [69]. Studies conducted among older adults in NZ in different parts of the country showed that 83% people in the age group of above 65 years consumed alcohol and in 9.9 % this was at a hazardous level. The study also predicted a prevalence rate of 24.8% of lifetime alcohol dependence for 35 out of 141 participants in a community in Christchurch. The study also involved an evaluation of GPs (General Practitioner) regarding alcohol use and misuse. This study showed that either there is no full awareness amongst GPs about the hazardous use of alcohol in the community or the consumption had not reached the anticipated levels [69]. Several other literatures have also suggested that alcohol use [70] and misuse is not recorded or missed by GPs amongst elderly people [71].

Studies have also been conducted on university students in NZ to assess the level of binge and alcohol drinking in this population [72]. Many university students are heavy drinkers. This may be in part because the legal age enabling purchase of alcohol is 18 years, and so students have easy access. There was no significant difference amongst drinking levels in men and women. It was also noted that participants who were hazardous drinkers had experience of unsafe and unwanted sex as compared to moderate drinkers [72]. A survey conducted in NZ included 217 participants who were all workers in alcohol and drugs treatment service companies. The survey results suggest that men are the main clients for these services as compared to women and the average age of referral was 31 years. Most subjects were Caucasians, with a high prevalence of drug abuse [73].

Research has also been conducted in NZ to evaluate why there has been a sudden increase in consumption of alcohol by adolescents and university students and how the media might have played an important role [74]. Broadcasting of beer advertisements has increased in the past few years and consumption of beer was seen to be higher than wine and spirits. Age factor played an important role in this survey. It was noted that males who remembered the alcohol advertisement at the age of 15 had consumed 3 times more alcohol at 18 years of age than those who could not remember the advertisements at 15 years of age [74].

4.5 Limitations:

There were certain significant limitations for our study as follows:

1. The important covariates used for the analysis in the study were age at admission, BMI, alcohol content/day, smoking status, pack years of cigarettes, comorbidities, fracture history of bone, age at menarche, physical activity status, dietary calcium, any vitamin D supplements or steroids intake. We could not investigate the effect of all these covariates together on HUS outcomes because often data for all the covariates was not available. This

prohibited us from evaluating the effect of all of them together. We performed multiple regression to check effect of age at admission, alcohol content/day, fracture history and age at menarche on 34 out of 36 participant altogether, and found no significant effect of these covariates together on heel ultrasound outcomes (Avg BUA, Avg SOS, Avg T score, Avg Z score and Avg SI).

2. Due to limitations of funds, we performed bone markers on 10 premenopausal women only who were admitted at the alcohol unit. We could not collect blood samples for the women who participated as controls in the study. Furthermore, slow recruitment to the study meant that we were unable to reach even this target; future work in larger numbers and incorporating a control group is now planned.

3. After the time required to obtain ethical approval, there was only a 5 month time period for data collection. Hence we were able to obtain data only for 20 women who were admitted to the unit and fitted the inclusion criteria in the period of Jul 2012-Nov 2012.

4.6 Future work:

We plan to recruit more female participants to this study so that more significant results can be obtained. Future work include our aim to obtain extra funding which will allow recruitment of more participants for the bone markers sub-study, and to incorporate a control group.

A1: Ethical approval

Health and Disability Ethics Committees

Northern Y Regional Ethics Committee
c/- Ministry of Health
Level 3, Bridgewater Building
130 Grantham St
Hamilton 3204
Phone: (07) 9293612
Email: northerny_ethicscommittee@moh.govt.nz

21 June 2012

Elaine Dennison
Victoria University
PO Box 600
Wellington 6140

Dear Elaine -

Re:	Ethics ref:	**NTY/12/04/024** (please quote in all correspondence)
	Study title:	The effect of alcohol withdrawal on bone turnover in women with alcohol dependence
	Investigators:	Elaine Dennison, Dr Geoff Robinson, Ms Pallavi Wyawahare

This study was given ethical approval by the Northern Y Regional Ethics Committee on 21 June 2012.

Approved Documents

- Protocol version 1 dated 26/3/2012
- Information sheet and Consent form version 1 dated 26/3/2012
- Questionnaire version 1 dated 26/3/2012
-

This approval is valid until 1 May 2015, provided that Annual Progress Reports are submitted (see below).

Amendments and Protocol Deviations

All significant amendments to this proposal must receive prior approval from the Committee. Significant amendments include (but are not limited to) changes to:

- the researcher responsible for the conduct of the study at a study site
- the addition of an extra study site
- the design or duration of the study
- the method of recruitment
- information sheets and informed consent procedures.

Significant deviations from the approved protocol must be reported to the Committee as soon as possible.

Annual Progress Reports and Final Reports

The first Annual Progress Report for this study is due to the Committee by 21 June 2013. The Annual Report Form that should be used is available at www.ethicscommittees.health.govt.nz.

Please note that if you do not provide a progress report by this date, ethical approval may be withdrawn.

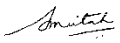
A Final Report is also required at the conclusion of the study. The Final Report Form is also available at www.ethicscommittees.health.govt.nz.

Statement of compliance
The committee is constituted in accordance with its Terms of Reference. It complies with the *Operational Standard for Ethics Committees* and the principles of international good clinical practice.

The committee is approved by the Health Research Council's Ethics Committee for the purposes of section 25(1)(c) of the Health Research Council Act 1990.

We wish you all the best with your study.

Yours sincerely



Amrita Kuruvilla
Northern Y Ethics Committee Administrator

Email: amrita_kuruvilla@moh.govt.nz

A2: Ethical Approval for protocol amendment

Health and Disability Ethics Committees

Health and Disability Ethics Committees
1 The Terrace
PO Box 5013
Wellington
6011

0800 4 ETHICS
hdecs@moh.govt.nz

19 October 2012

Professor Elaine Dennison
Clinical Trials Unit
Private Bag 7902
Wellington 6021

Dear Professor Dennison

Re:	**Ethics ref:**	**NTY/12/04/024**
	Study title:	The effect of alcohol withdrawal on bone turnover in women with alcohol dependence

I am pleased to advise that this amendment has been *approved* by the Central Health and Disability Ethics Committee. This decision was made through the HDEC Expedited Review pathway.

Non-standard conditions:

1. Please ensure correct ethics committee quoted.

Please don't hesitate to contact the HDEC secretariat for further information. We wish you all the best for your study.

Yours sincerely,

Mrs Helen Walker
Chairperson
Central Health and Disability Ethics Committee

Encl: appendix A: documents submitted
appendix B: statement of compliance and list of members

Appendix A
Documents submitted

Document	Version	Date
Protocol: Modified protocol	2	08 October 2012
Survey/questionnaire: Modified information sheet and consent form for controls	2	08 October 2012
Post Approval Form		

Appendix B
Statement of compliance and list of members

Statement of compliance

The Central Health and Disability Ethics Committee:

- is constituted in accordance with its Terms of Reference
- operates in accordance with the *Standard Operating Procedures for Health and Disability Ethics Committees*, and with the principles of international good clinical practice (GCP)
- is approved by the Health Research Council of New Zealand's Ethics Committee for the purposes of section 25(1)(c) of the Health Research Council Act 1990
- is registered (number 00008712) with the US Department of Health and Human Services' Office for Human Research Protection (OHRP).

List of members

Name	*Category*	*Appointed*	*Term Expires*
Mrs Helen Walker	Lay (consumer/community perspectives)	01/07/2012	01/07/2015
Dr Angela Ballantyne	Lay (ethical/moral reasoning)	01/07/2012	01/07/2015
Mr Paul Barnett	Lay (the law)	01/07/2012	01/07/2014
Mrs Gael Donoghue	Non-lay (health/disability service provision)	01/07/2012	01/07/2014
Mrs Sandy Gill	Lay (consumer/community perspectives)	01/07/2012	01/07/2014
Dr Patries Herst	Non-lay (intervention studies)	01/07/2012	01/07/2015
Dr Dean Quinn	Non-lay (intervention studies)	01/07/2012	01/07/2015
Dr Lynne Russell	Non-lay (observational studies)	01/07/2012	01/07/2014

http://www.ethics.health.govt.nz

A3: Patient information sheet

Patient Information Sheet

Study Protocol Title: **Alcohol and bone health: a case control study**

Principal Investigator:

Professor Elaine Dennison, Professor of Clinical Research, Victoria University, Wellington 6140 tel: 04 463 5233

You are invited to participate in the clinical research study named above. In order to determine whether or not to be part of this research study you should understand enough about its potential benefits and risks to make an informed judgement. The purpose of this information sheet is to give you that information. You can keep this to take away and read and consider before making up your mind. Your participation is entirely voluntary (your choice). You do not have to take part in this study, and if you choose not to take part you will receive the usual treatment/care. If you do agree to take part you are free to withdraw from the study at any time, without having to give a reason and this will in no way affect your future health care. Participation in this study will be stopped if the study doctor feels it is not in your best interest to continue.

What is the standard treatment?

This study will assess your bone health, by performing a heel ultrasound test.

What are the aims of the study?

1. To compare the bone health among healthy women (controls) with women admitted to alcohol dependence unit at Kenepuru Hospital using a heel ultrasound machine.

Description of Study

This is a non-randomised study, which means that everyone will get the same treatment. This study will analyse the results from up to 150 patients from one study site in New Zealand. It will take approximately 1 year to accumulate this number of patients.

If you agree, after obtaining written consent, you will be invited to complete a questionnaire detailing : age, personal and family history of fracture, cigarette smoking (pack years), alcohol history (duration and level of drinking; type of alcohol consumed), physical activity, dietary calcium intake (food frequency questionnaire), past medical history (to elicit information on possible other secondary causes of osteoporosis e.g. insulin dependent diabetes, hyperthyroidism), drug history and reproductive history. You do not have to answer all the questions, and you may stop the interview at any time. Height and weight shall be recorded. Finally, we would like to measure the strength and thickness of your bones using a heel ultrasound machine.

Are there any risks involved and will I experience any discomfort?

This clinical research may involve unforeseen risks for you. The heel ultrasound test does not involve any exposure to radiation.

Are there any benefits?

Through participating in this study, we will learn more about your risk of breaking a bone in the future. We can use this information to help you and the doctors looking after you make sure that your bones are kept as healthy as possible. The information obtained will also help us advise future patients.

Are there alternatives to entering this study?

Participation in the study is completely voluntary and your usual care will not be affected if you decide not to take part.

Will I be made aware of new information regarding the treatment of my condition?

We will feedback your results and some general guidance about your bone health

Confidentiality

Your name or any other personally identifying information will not be used in reports or publications resulting from the study. Authorised study representatives of the research group at Victoria University who coordinate the study, may have limited access to medical records. Study records will be kept in a locked room.

Statement of approval

'This study has received ethical approval from the Northern Y Regional Ethics Committee'.

Further Information or Any Problems

If you require further information or if you have any problems concerning this project (for example, any side effects or injury), you can contact one of the researchers or the study coordinator. Contact numbers are:

Professor Elaine Dennison

Telephone: 04 4635233

Rights

If you have any queries or concerns regarding your rights as a participant in this study you may wish to contact a Health and Disability Advocate, telephone

- Northland to Franklin

  0800 555 050
- Mid and lower North Island
  0800 42 36 38 (4 ADNET)
- South Island except Christchurch
  0800 377 766
- Christchurch
  03 377 7501

If you have any concerns or complaints about the conduct of a research study can contact the Chairperson of the Wellington Ethics Committee on (04) 385 5999 ext 5185.

If you would like advice or support from the Maori Health Unit please contact: Jackie Davis, Wellington Hospital, on (04) 385 5999 ext 4074.

Compensation

Include for form A studies

In the unlikely event of a physical injury as a result of your participation in this study, you may be covered by ACC under the Injury Prevention, Rehabilitation and Compensation Act. ACC cover is not automatic and your case will need to be assessed by ACC according to the provisions of the 2002 Injury Prevention Rehabilitation and Compensation Act. If your claim is accepted by ACC, you still might not get any compensation. This depends on a number of factors such as whether you are an earner or non-earner. ACC usually provides only partial reimbursement of costs and expenses and there may be no lump sum compensation payable. There is no cover for mental injury unless it is a result of physical injury. If you have ACC cover, generally this will affect your right to sue the investigators.

A4: Patient Consent form

Capital & Coast
District Health Board
ŪPOKO KI TE URU HAUORA

Patient Consent Form

Study Protocol Title:

Alcohol and bone

English	I wish to have an interpreter.	Yes	No
Maori	E hiahia ana ahau ki tetahi kaiwhakamaori/kaiwhaka pakeha korero.	Ae	Kao
Samoan	Ou te mana'o ia i ai se fa'amatala upu.	Ioe	Leai
Tongan	Oku ou fiema'u ha fakatonulea.	Io	Ikai
Cook Island	Ka inangaro au i tetai tangata uri reo.	Ae	Kare
Niuean	Fia manako au ke fakaaoga e taha tagata fakahokohoko kupu.	E	Nakai
	Other languages to be added following consultation with relevant communities.		

I have read and I understand the information sheet dated 18/05/12 for volunteers taking part in this study. I have had the opportunity to discuss this study and I am satisfied with the answers I have been given.

I understand that taking part in this study is voluntary (my choice) and that I may withdraw from the study at any time and this will in no way affect my continuing health care.

I understand that my participation in this study is confidential and that no material which could identify me will be used in any reports on this study.

I understand that the treatment, or investigation, will be stopped if it should appear harmful to me.

I understand the compensation provisions for this study.

I have had time to consider whether to take part.

I know whom to contact if I have any side effects from the study.

I know whom to contact if I have any questions about the medication or the study.

I consent to my GP being informed of my participation in this study.
YES/NO

I …….hereby consent to take part in this study.

……

……

Patient signature

Date

……

……

Witness signature

Date

To be completed by Doctor (Investigator)

I have discussed with

……..……(patient's name)

The aims of, procedures and risks involved in this study.

……

……

Doctor's signature

Date

A5: Bone Alcohol Questionnaire for data collection

Alcohol and bone questionnaire version 2 17/7/2012

QUESTIONNAIRE

Name: _____

Address: _____

_____

_____

Telephone: _____

GP _____

Date of birth _____-

Height _____ **Weight** _____

Interviewer _____ ☐

Date of Interview:

d	d	m	m	y	y

Q1a Have you ever smoked regularly?
(i.e. at least once a day for a year or more)

0. No 1. Yes ☐

If yes, continue
If no, Go to Q3

b Do you still smoke regularly?

0. No 1. Yes ☐

If yes, continue
If no, *Go to Q3*

c How old were you when you last smoked regularly? ☐☐

Q2 How much do you smoke now?

Cigarettes/day ☐☐☐

Roll-ups tobacco/week (g) ☐☐

Cigars/week ☐☐

Pipe tobacco/week (g) ☐☐

Q3 How old were you when you started to drink alcohol? ☐☐

Q4a How often do you currently drink shandy/low alcohol beer/lager/cider? (don't include alcohol free lager etc.)

0. Never
1. Once every 2-3 months
2. Once a month
3. Once a fortnight ☐
4. 1-2 times per week
5. 3-6 times per week
6. Once a day

7. More than once a day

When you drink these, how many glasses would you normally have? ☐☐•☐

Q4b How often do you currently drink low alcohol wine?

0. Never
1. Once every 2-3 months
2. Once a month
3. Once a fortnight ☐
4. 1-2 times per week
5. 3-6 times per week
6. Once a day
7. More than once a day

When you drink these, how many glasses would you normally have? ☐☐•☐

Q4c How often do you currently drink Wine/Sherry/Port /Martini /Cinzano?

0. Never
1. Once every 2-3 months
2. Once a month
3. Once a fortnight ☐
4. 1-2 times per week
5. 3-6 times per week
6. Once a day
7. More than once a day

When you drink these, how many glasses would you normally have? ☐☐•☐

Q4d How often do you currently drink spirits/liqueurs?

0. Never
1. Once every 2-3 months
2. Once a month
3. Once a fortnight ☐
4. 1-2 times per week
5. 3-6 times per week
6. Once a day
7. More than once a day

When you drink these, how many glasses would you normally have? ☐☐ • ☐

Q5a Have you had any falls in the last year?

0. No 1. Yes ☐

Q5 b ***If yes,*** how many? ☐☐

If no, Go to Q6.

Q5c. At what age did you first fall?

Q6 Have you broken any bones ?
If yes, please continue 0. No 1. Yes ☐

If No, please Go to Q7.

Q6b Did you receive medical attention for the broken bone?

0. No 1. Yes ☐

If yes, where did you receive this attention?

_____

Q6c Was an x-ray taken of the broken bone? 0. No 1. Yes ☐

Q6d What was the approximate date you broke your bone?

_____

Q7 Has a first degree relative (mother, father, brother, sister) broken their hip?

If yes, how old were they when they broke it?

_____

Q8a Have you had any longstanding illness, diseases or medical conditions for which you have sought treatment?

0. No 1. Yes ☐

If yes, please list below

_____

_____

Q9 Have you had any of these conditions diagnosed by a doctor?

		Yes	No
a.	Coeliac disease	☐	☐
b.	Overactive thyroid gland?	☐	☐
c.	Underactive thyroid gland?	☐	☐
d.	Type 1 Diabetes	☐	☐

e. Asthma, bronchitis, emphysema, COPD requiring steroid ☐ ☐

f. Rheumatoid arthritis ☐ ☐

Q10 a How old were you when your period started? ☐

Q10b Are you still having periods? ☐

Q10c If no, when did they stop? ☐

Q10d Have you had any children?

If yes, how many? ☐

Q11 During last 7 days, on how many days did you walk at a brisk pace – a brisk pace is a pace at which you are breathing harder than normal? This includes walking at work or school, while getting from place to place, at home and at any activities that you did solely for recreation, sport, exercise or leisure.

Think only about brisk walking done for at least 10 minutes at a time. (kindly circle any 1 of the below options)

a. .....days per week (Go to Q12)
b. None (Go to Q13)

Q12 How much time did you typically spend walking at a brisk pace on each of those days?
..... hours..... minutes

Q13 During last 7 days, on how many days did you do moderate physical activities? 'moderate' activities make you breathe harder than normal, but only a little – like carrying light loads, bicycling at a regular pace. Do not include walking of any kind.

Think only about those physical activities done for at least 10 minutes at a time. (kindly circle any 1 of the below options)

a. ..... Days per week (Go to Q14)
b. None (Go to Q15)

Q14 How much time do you typically spend on each of those days doing moderate physical activities?

..... Hours .....minutes

Q15 During last 7 days, on how many days did you do vigorous physical activities? 'vigorous' activities makes you breathe a lot harder than normal ('huff and puff') – like heavy lifting, digging, aerobics, fast bicycling.

Think only about those physical activities done for at least 10 minutes at a time. (kindly circle any 1 of the below options)

a. ..... Days per week (Go to Q16)
b. None (Go to Q17)

Q16 How much time did you typically spend on each of those days doing vigorous physical activities?

.....hours.....minutes

Q17 Thinking about all activities over last 7 days (including brisk walking), on how many days did you engage in :

a. At least 30 minutes of moderate activity (including brisk walking) that made you breathe a little harder than normal OR
b. At least 15 minutes of vigorous activity that made you breathe a lot harder than normal ('huff and puff')? (kindly circle any 1 of the below options)

i. .....days per week
ii. None

Q18 Describe your regular physical activity over the past 6 months. Regular physical activity means at least 15 minutes of vigorous activity (makes you 'huff and puff') or 30 minutes of moderate activity (makes you breathe slightly harder than normal) each day for 5 or more days each week. Includes brisk walking.

(kindly circle any 1 of the below options)

a. I am not regularly physically active and do not intend to be so in the next 6 months.
b. I am not regularly physically active but am thinking about starting in the next 6 months.
c. I do some physical activity but not enough to meet the description of regular physical activity.
d. I am regularly physically active but only began in last 6 months.
e. I am regularly physically active and have been so for longer than 6 months.

Q19 What regular medicines/tablets/eye drops/inhalers etc. do you use?
PLEASE USE BLOCK CAPITALS
Please include regular pain killers such as paracetamol

1 _____
2 _____

3
4
5
6
7
8
9
10
11
12

Alcohol and bone questionnaire version 2 17/7/2012

Dietary Calcium Questionnaire

Please think about your usual eating habits over the last year.

C1. How much milk do you usually use in an average day? (Probe: Do you have milk delivered? Think about milk used in tea and coffee, or breakfast cereals or puddings, and in cooking). Give your answer to the nearest quarter pint .....

C2. Next, I would like to ask you about a number of different foods. Please tell me whether or not you eat the food, how often you have it if you do eat it, and how much you have on the days when you eat the food. (Ring the correct answers and fill in amounts).

	Not Eaten	1x per 3-4 wks	1x per 1-2 wks	1 -2d per wk	3-5d per wk	6-7d per wk	Amount per day	Milk?
Tea	1	2	3	4	5	6	_____ cups _____ mugs	1. None 2. Liquid Milk * 3. Tinned Milk 4. Powdered Milk____ g+
Coffee	1	2	3	4	5	6	_____ cups _____ mugs	1. None 2. Liquid* , 1/4 or less 3. Liquid , 1/2 4. Liquid , 3/4 5. Liquid , All 6. Tinned Milk 7. Powdered Milk____ g+ 8. Coffeemate

* Liquid milk includes: whole, semi-skimmed, skimmed, UHT, sterilised and powdered made-up.

	Not Eaten	1x per 3-4 wks	1x per 1-2 wks	1-2d per wk	3-5d per wk	6-7d per wk	Amount per day	Milk?
Other milky drinks e.g Horlicks Bournvita Ovaltine Hot Chocolate Cocoa Complan Build-up Milo etc.	1	2	3	4	5	6	_____ cups _____ mugs	1. None 2. Liquid * , 1/4 or less 3. Liquid , 1/2 4. Liquid , 3/4 5. Liquid , All 6. Tinned Milk 7. Powdered Milk ____g+
Milk alone	1	2	3	4	5	6	_____ small glasses _____ large glasses _____ cups _____ mugs	
Breakfast cereal (probe for porridge made with milk)	1	2	3	4	5	6	_____ portions	1. None 2. Small 3. Medium 4. Large

* Liquid milk includes: whole, semi-skimmed, skimmed, UHT, sterilised and powdered made-up.
+ Level tsp = 2g Rounded tsp = 3g Heaped tsp = 4g = 1/4 cup liquid

DIETARY CALCIUM QUESTIONNAIRE

	Not Eaten	1x per 3-4 wks	1x per 1-2 wks	1-2d per wk	3-5d per wk	6-7d per wk	Amount per day	
Bread	1	2	3	4	5	6	_____ slices	1. Small 2. Large
Cheese	1	2	3	4	5	6	_____ portions	1. Small 2. Medium 3. Large
Cakes	1	2	3	4	5	6	_____ no. of portions	
Scones	1	2	3	4	5	6	_____ no. of portions	
Biscuits	1	2	3	4	5	6	_____ no. of portions	
Desserts made with milk (+ custard or ice-cream on other desserts	1	2	3	4	5	6	_____ no. of portions	
Green vegetables	1	2	3	4	5	6	_____ no. of portions	

A6: Standard calculation used for dietary calcium content/week

Appendix One - Calculating Calcium Intake per Week

Basic Formula: Calcium Intake (mg/Week) = F * N * Ca

where F = Frequency
N = Number of portions (or slices, cups etc)
Ca = Calcium content of portion (mg)

If frequency code is	1	2	3	4	5	6
then F =	0	0.3	0.75	1.5	4.0	6.5

Calcium Contents (mg):

		Milk Code	Ca/Cup	Ca/Mug
1.	Tea	1	0	0
		2	42	51
		3	42	51
		4	12 mg/g	12 mg/g
2.	Coffee	1	0	0
		2	49	58
		3	108	147
		4	162	220
		5	216	294
		6	49	58
		7	12 mg/g	12 mg/g
		8	1	1
3.	Other Milky Drinks	1	0	0
		2	49	58
		3	108	147
		4	162	220
		5	216	294
		6	49	58
		7	12 mg/g	12 mg/g

		Measure	Ca
4.	Milk Alone	Small Glass	192
		Large Glass	348
		Cup	216
		Mug	294

		Milk Code	
5.	Breakfast Cereal	1	0
		2	72
		3	144
		4	216

	Portion Size	
	Small	24
	Large	33
	Portion Size	
	Small	160
	Medium	320
	Large	480
Cakes	Portion Size	
	Small	28
	Medium	55
	Large	83
9. Scones	Portion Size	
	Small	28
	Medium	55
	Large	83
10. Biscuits	Portion Size	
	Small	16
	Medium	32
	Large	53
11. Desserts	Portion Size	
	Small	60
	Medium	90
	Large	120
12. Green Vegetables	Portion Size	
	Small	0
	Medium	0
	Large	0

Appendix Two - Assumptions to be made for missing values

In general these are based on the mode values of non missing.

1. Tea - If number cups/mugs is missing assume 1 cup.
   If milk in tea missing assume liquid milk.

2. Coffee - If number cups/mugs missing assume 1 cup.

3. Other Milky Drinks - No missing values.

4. Milk Alone - No missing values.

5. Breakfast Cereal - No missing values.

6. Bread - If frequency is missing assume 6-7 days per week (6).
   If slices per day missing assume 4 slices.
   If slice size missing assume large slice.

7. Cheese - If portions per day missing assume 1.
   If portion size missing assume medium.

8. Cakes - If portion size missing assume medium.

9. Scones - No missing values.

10. Biscuits - If frequency missing assume 1-2 days per week.
    If portions missing assume 1.
    If portion size missing assume medium.
    If all 3 are missing assume not eaten (Calcium=0).

11. Desserts - If frequency missing assume 1-2 days per week.
    If portion size missing assume medium.

12. Green Vegetables - If portion size missing assume medium.

Appendix Three - Changes made to data file - Tea drinking

Tea drinking was very messy in some cases. The following is a summary of changes made.

Serial No. 004	- Changed from	4 cups Liquid Milk (x2) Powdered Milk 2g (x2)
	To	3 cups Liquid Milk
Serial No. 019	- Changed from	6 mugs Liquid Milk (x3) Powdered Milk 3g (x3)
	To	5 mugs Liquid Milk
Serial No. 021	- Changed from	10 cups Liquid Milk (x3) Powdered Milk 2g (x7)
	To	7 cups Liquid Milk
Serial No. 095	- Changed from	3 cups Liquid Milk (x2) Powdered Milk 2g (x1)
	To	3 cups Powdered Milk 3g

A7: Link for calculating pack years of cigarettes

http://smokingpackyears.com/

A8: Link for calculating alcohol contents/day

http://www.drinkaware.co.uk/tips-and-tools/drink-diary/

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