Dietary Calcium and Fracture Study Protocol 1998

Calcium Intake and Fracture Outcome Study (CAIFOS)

Assoc Prof R L Prince and Amanda Devine
University Department of Medicine
Sir Charles Gairdner Hospital
Nedlands, Western Australia

Background

Osteoporosis is a major disease of the locomotor system. 50% of women will sustain an osteoporotic fracture. Of the 17% of women who will suffer a hip fracture 3% will die as a result. More women sustain hip fractures than develop breast, ovarian and cervical cancer together. Most of these fractures occur after the age of 70.

For many years evidence has been accumulating that a negative calcium balance is a major cause of bone loss in age related osteoporosis and that this can be corrected in part by increased dietary calcium supplementation. A recent consensus conference supported this view and recommended that future research should include "prospective longitudinal studies to investigate long-term effects of calcium intake on.. fracture incidence in postmenopausal women". This application is aimed at examining the effectiveness of calcium supplementation in preventing fractures in the elderly female Australian population as a whole.

Aims

We intend to institute a randomized placebo controlled double blind study in which we recruit 1500 female subjects over the age of 70 and randomize them to receive a 1200-mg calcium supplement per day or matched placebo for up to 5 years. The subjects will be derived from electoral role sources and thus allow extrapolation to the whole community. The end point, clinical fracture rate, will be validated by viewing of X-rays or reports. A health economic analysis will also be included as an end point.

Our group has extensive experience and expertise in running randomized controlled clinical trials and has the physical resources to undertake this study.

Significance

This project based as it is on 40 years of scientific data should be able to definitively indicate whether an major increase in dietary calcium intake can be recommended as a cost effective intervention reduce fracture in the whole elderly female Australian population. Thus this project has the potential to significantly improve the health and reduce costs in this segment of the population that have high morbidity and mortality rates from osteoporosis.

Background

Basic physiology of calcium homeostasis

The relationship between the major calcitropic hormones, PTH and calcitriol (1,25 dihydroxy vitamin D) and calcium balance indicate that under conditions of reduced calcium levels in the extracellular fluid compartment there is an increase in PTH which has three main effects: stimulation of calcium reabsorption in the kidney, increase in the reabsorption of bone to liberate calcium and in conjunction with a low ionised calcium stimulation of calcitriol formation in the kidney. The raised calcitriol levels stimulate active transport of calcium from the bowel lumen into the extracellular space. Thus PTH alters calcium fluxes at the bone, kidney and indirectly the gut to restore extracellular calcium homeostasis. PTH levels then fall under the influence of the raised calcium, sensed by the recently discovered calcium receptor in the parathyroid gland ((1), and by a short loop feed back action of calcitriol to inhibit PTH production (2).

The presence of these homeostatic responses has been demonstrated in pre and postmenopausal women subject to low calcium intake (3, 4). The critical role of PTH in relation to bone loss is its effect to increase bone resorption to correct the extracellular calcium deficit. This effect has been known for many years. This effect of PTH should not be confused with the effect of intermittent elevations in PTH to stimulate bone formation (5).

The regulation of calcium homeostasis by calcitropic hormones is relevant to our understanding of the negative calcium balance that occurs in elderly individuals. Two facts are generally agreed. Firstly with increasing age there is a rise in PTH levels (6, 7). An elevation in PTH is what would be expected in response to a negative calcium balance. Secondly bone turnover in elderly women is high relative to premenopausal individuals (7). It is important to realize that calcium supplementation in addition to reducing bone loss also reduces measures of bone resorption and secondarily bone formation (8, 9). It is highly likely that this is due to the suppression of circulating PTH levels noted following calcium supplementation (8, 9).

A second abnormality of calcitropic hormones that occurs with aging is a relative reduction in calcitriol levels (10-12) and free calcitriol levels (7). There has been some suggestion that there is a rise in total calcitriol with aging (13) but this may be due to a rise in vitamin D binding protein (7) and any way only continues until the mid sixties after which even the total calcitriol levels fall (14). The fall in calcitriol is associated with the reduction of gut calcium absorption with aging (10, 15). From what has been said above a fall in calcitriol would be most unexpected in the face of a negative calcium balance. Therefore it seems very likely that the fall in calcitriol is a pathophysiological abnormality that is implicated in the rise in PTH and excess loss of bone calcium with aging. Because of the reduction in gut calcium absorption with age the obvious response is to increase calcium absorption.

Gut calcium absorption consists of two mechanisms an active, high affinity, low capacity, calcitriol regulated system (16), that is especially important under circumstances of a low calcium intake and a passive, paracellular, high capacity system that is driven by the concentration gradient of calcium between the lumen of the gut and the extracellular fluid. Increasing luminal calcium by dietary supplement will increase absorption by both pathways.

Vitamin D status

In subjects with low sunlight exposure, not compensated for by dietary vitamin D, there is a fall in vitamin D production in the skin with resulting fall in 25 hydroxy vitamin D the substrate for the formation of calcitriol in the kidney (17). Because of difficulties with the assay of calcitriol it has not always been possible to show a fall in calcitriol in the presence of vitamin D deficiency. Vitamin D deficiency is however associated with a raised PTH and is thereby associated with bone loss (18, 19). Thus vitamin D deficiency and a negative calcium balance are intimately related at a mechanistic level so that arguments over the relative importance of vitamin D and calcium intake miss the main point which is that adequate supplies of both are essential for maintenance of calcium balance and prevention of bone loss. Thus if an individual only has a deficient calcium intake replacement of that nutrient will slow bone loss whereas if that individual is short of both vitamin D and calcium both will be required for an optimal effect on calcium balance.

Because of a possible confounding effect of vitamin D status on the response to dietary calcium intake this study will be confined to ambulant subjects in whom vitamin D deficiency is uncommon (20, 21)

Evidence for the importance of calcium balance in maintenance of bone mass and the prevention of fracture

Although it is not always fully appreciated, studies of the effects of calcium intake on bone density or fracture rate address the question of the importance of calcium balance because increasing calcium intake is in fact a way of improving calcium balance. Previous difficulties in demonstrating the importance of dietary calcium intake in epidemiological studies (22, 23) are due to difficulties in measuring habitual calcium intake over long periods of time using dietary questionnaires. Furthermore these studies are not able to separate the effects of dietary calcium from other dietary constituents which are co-correlated, often do not allow for body size effects and do not allow for the effects of sodium which is notoriously difficult to measure in dietary questionnaires (24). Thus it is not surprising that dietary calcium intake does not always emerge as a significant factor in these types of study.

However, there are now numerous randomized controlled clinical trials of calcium supplementation in postmenopausal women, which have demonstrated a significant reduction in bone loss for up to four years compared to placebo. These are inherently more precise in assessing the effect of dietary calcium because they are prospective and use tablets to define the calcium intake (8, 9, 25-27). These data have now been extended to four years. Our own data on this topic show no overall loss of bone at the hip or lower leg and support the data of Reid et al in showing a prolonged beneficial effect of calcium supplementation (9). Thus recent consensus conferences have underlined the probable desirability of increasing dietary calcium intake in postmenopausal women to 1500 mg per day (28, 29)

Recent trials have shown a reduction in fracture rate with supplemental calcium. The largest of these trials studied the effect of calcium 1.2g and vitamin D 800u per day in French women with a mean age of 84. Actual non-vertebral fracture rate was around 10% per year with a reduction in hip fracture of 43% and a reduction in non-vertebral fractures of 32%. Because the population we will be studying will be not be vitamin D deficient it is reasonable to expect that a calcium supplement alone will be equally efficacious. A recent small-randomized trial of calcium alone carried out in New Zealand support the effectiveness of calcium supplementation. In that study with an

underlying fracture rate of 5% per year for clinically detected fractures calcium supplementation reduced fracture rates by 75% (9). Finally a small study of vertebral fractures in elderly Swiss women showed an underlying fracture rate of 10% per year with a reduction in fractures in women receiving a 800 mg calcium supplement of 32% (30). Thus current data is consistent with the concept that increasing diet calcium by 1000 mg may reduce fractures by about 35%.

Effects on other disease processes

In considering calcium supplementation of large populations it is important to consider effects on other disease processes. To date no significant deleterious effects of calcium supplementation has been found. Indeed there is some evidence that it may result in a reduction in kidney stones (31), bowel cancer (32) and blood pressure (32, 33). A few subjects may have hypercalcaemia, which may be exacerbated if it is due to sarcoidosis. Most cases of primary hyperparathyroidism will not be affected as usually the problem is a set point one, which is not altered by increased gut calcium absorption. It is likely that most cases of malignancy in which there is likely to be hypercalcaemia will be excluded on the criteria that the subjects must be likely to survive five years.

Fracture epidemiology in an Australian population

There have only been three studies of total symptomatic fractures in Australian populations (34-36). The Adelaide study had a rate of about 3% in 70 year olds, in Dubbo the rate was about 2% whereas in the healthy 60% of the Busselton population, it was 2.7% in 70 to 79 year olds. In the Dubbo population the rate rose to 3.5% in 75 to 79 year olds and 5% in 80 year old plus patients. These rates are conservative in relation to samples from other countries. In general this can be explained on age differences or the fact that other studies have selected subjects on the basis of previous fractures or low bone mass.

Cost effectiveness study

In parallel with the introduction of a public health campaign it would be important to evaluate its effectiveness fracture propensity, disability and quality of life. These last two are especially important in osteoporosis prevention as the major impact of the disease is to increase morbidity rather than mortality.

The cost effectiveness of a calcium intervention in the current age group is potentially one of net gain, if a 35% reduction in fracture can be ascertained. At present rates of hip fracture, which double for each five year increase in age, an expected six hip fractures per year over the five year period would occur for 1000 women aged 75 years. This amounts to a total saving of $84,000 with a 35% reduction in hip fracture for hospital and medical costs. From previously calculated nursing home costs, (37) there is an additional saving of $373,000 in nursing home costs. This represents a net saving in hip fractures alone, given that the costs of calcium for 1000 women amounts to $365,000 (1992 dollars). Further savings due to reductions in vertebral and other osteoporosis-related fractures have not been included.

Given that a health promotion campaign could substitute part of the calcium increase with supplementation in the diet, this would conceivably further halve the cost equation, making this intervention very cost effective.

Study design

Protocol

A randomized placebo controlled double blind approach will be adopted. The study will run for up to five years. We plan to randomize subjects by block randomization into two regimens.

a) Calcium 600 mg twice daily.
b) Matched placebo twice daily.

End points

The major end point will be the relative proportion of fractures that the two groups will sustain. An important subsidiary end point will be the cost effectiveness of the intervention on fracture end points.

Safety data will be analyzed from adverse event reports collected during the study. This data may provide support for the beneficial effect of calcium supplementation on other disease processes or indicate problems with calcium supplementation. A committee separate from the study will view adverse events by treatment category on an ongoing basis to detect significant increases in adverse events in the treatment category.

Subsets of subjects may have secondary end points such as markers of bone turnover, calcitropic hormones, bone densitometry by dexa or ultrasound, or spinal morphometry measured should other applications for grant support be successful.

Subjects.

We will recruit 1500 healthy women over the age of 70. The principle exclusion will be subjects who have preexisting metabolic bone disease or are receiving bone active agents and those who are unlikely to survive a five-year study. The inclusive nature of the recruitment to this study is planned so that its results, if successful, will be applicable to the whole population. We will use the electoral role to define this population, which in this age group accesses over 90% of the population (35), and then approach specified individuals by letter. We have already used this method of recruitment for 2 previous demanding clinical trials and have achieved a 30% response rate. Because of the minimal demands of this study and the essentially safe agent that we are studying we would hope to achieve at least the same or better entry rate into the study. We currently have 33000 names and addresses of females over the age of 70 in the Perth metropolitan area on this database, which we have not yet accessed. Thus this study will be population based and the study results can therefore be extrapolated to other populations.

Data collection

Subjects replying to our letter will be contacted by phone to explain the study and to apply the exclusion criteria. 10-12 subjects per day will then be invited into our clinical research center. Following an introductory seminar they will be asked to sign the consent form previously mailed to them. They will then have a blood and urine collection. Data collection from the patient will be from preprinted questionnaires that can be filled out by the patient and then checked by research personnel prior to data entry by the research secretary into a relational data entry program. This database will be used to generate follow up letters to patients and GP's which will be sent during the study. Telephone contact will be undertaken at four months with face to face review at twelve monthly intervals. Medication for 12 months will be dispensed at entry.. Thus most contact and data collection can be undertaken by phone, mail and fax.

The initial data collected will document risk factors for osteoporosis, dietary intake, including current calcium intake, by a food frequency method produced by the Anti Cancer Council of Victoria, and a list of current diseases and medications. Clinical assessment including, heel ultrasound measurements, seated and postural blood pressure, anthropometric tests and tests of functionality including, Timed Up and Go, balance and grip strength will be undertaken at baseline. The subject will complete a symptom related quality of life questionnaire, an abbreviated Mini Mental test, and a Barthel test. The subjects will be provided with a diary for documentation of encounters with their general practitioner during the five years of the study as a measure of adverse events. These will be returned every four months, photocopied for our records and returned to each subject. These baseline tests will be repeated at twelve months.

Follow up data will collect information on compliance and adverse medical events especially including fractures. Where fractures occur, the site, degree of trauma, and X-ray or report will be collected. Tablet counting will be undertaken at the yearly visits. Compliance will be enhanced with newsletters and meetings where possible.

Bone mineral density measurements
A randomly selected sample of subjects will undertake bone density measurements at the hip, spine, and whole body site using the Hologic Acclaim 4500 detector fan beam densitometer. The subject will also collect a 24-hour urine sample on the day before the test.

Dietary measurements
Every third subject randomised into the trial will be invited to join a sub sample of subjects who will be asked to complete a 24 hour diet recall administered by a dietitian. These subjects will complete a food related issues questionnaire (FRIQ) that has been designed by our unit, to assess factors that influence food preparation, purchase and consumption at this time. The subject will also collect a 24-hour urine sample on the day before the test.

Prospective cost data collection:

Collection of fracture costs will include hospital and medical costs in addition to after care costs of fracture and will be collected prospectively. Hip fracture costs are clearly defined because of clear-cut diagnosis and the necessary hospitalization. Prospective cost collection can provide validation of published ANDRG costs. Vertebral fracture costs are less clearly defined because cases are rarely hospitalized, and unlike hip fractures, do not resolve. As a consequence, the costs of vertebral fractures have largely been ignored in studies of osteoporosis. Since fracture costs will be collected

close to the time of fracture, the costs of disability in addition to direct medical and hospital costs can be assessed. Costs collected for other osteoporosis related fractures will have additional relevance to overall costs for osteoporosis. A questionnaire dealing with fracture costs will be completed after each fracture episode. Costs will be revalued according to the AIHW health expenditure deflators where required, to reflect an annual time period.

Power calculations and data management

The assumptions underlying the power calculations for this application are conservative. We assume a fracture rate of 3.5% per year in the placebo group and a reduction in fractures of 35% in the calcium group (see Background for validation of these assumptions). At a power of 0.8, an alpha of 0.05, 516 patients per group over five years will be required (Fischer's exact test). Because of the expected drop out rate (33%) we would plan to recruit 774 patients per group.

For analysis data will be entered into SPSS for Windows. The primary method of analysis will utilize survival analysis and chi squared testing.

A Randomised Controlled Study of the Effects of Calcium and Calcium plus ergocalciferol on Bone Density, Calcitropic Hormones, Safety, and Quality of Life in Elderly Postmenopausal Women (The CalD Study)

Assoc Prof R L Prince and Amanda Devine
University Department of Medicine
Sir Charles Gairdner Hospital
Nedlands, Western Australia

Aims

Osteoporotic fracture is major community problem. We are currently recruiting subjects for a two arm, randomised, double blind, five year study of calcium supplementation (1200mg/ day) versus placebo in 1500 women over the age of 70 to determine whether calcium is safe and effective in preventing fracture. This study is called the Calcium Intake Fracture Outcome Study (CAIFOS) and will recruit approximately 5% of the 30000 women over 70 in Perth. In the first 3 months of full activity we have recruited over 400 subjects suggesting that we will meet our target recruitment in one year as planned. This application is addressed to an additional surrogate end point sub-study in 120 extra women, the CALD study. Current data in a few Northern Hemisphere studies suggests that calcium or calcium plus vitamin D can stop fractures and bone loss. A large vitamin D supplementation study failed to show benefit from vitamin D alone. To date no direct comparison of calcium to calcium and vitamin D has been undertaken in a population with a vitamin D status similar to elderly Australians.
We plan to undertake a 3 arm, 5 year, randomised, placebo controlled, double dummy surrogate end point study in women selected using identical criteria to our current study. The three treatment regimens will be calcium 1.2g with ergocalciferol 1000U daily, calcium 1.2g alone with placebo vitamin D and placebo calcium and vitamin D (the double dummy).

Hypothesis 1) Calcium and vitamin D and calcium alone will suppress the secondary hyperparathyroidism of ageing equally when compared to placebo. There is much information indicating that the bone loss of ageing is due to secondary hyperparathyroidism, this study will be able to examine the mode of action of the treatment regimens and compare them.

Hypothesis 2) Calcium and vitamin D and calcium alone will suppress markers of bone turnover to a similar extent. This end point will allow direct comparison of the treatments on bone function.

Hypothesis 3) Calcium and vitamin D and calcium alone will have similar effects on fractional gut calcium absorption. This end point will directly examine the effect of vitamin D status on calcium absorption.

Hypothesis 4) Calcium and vitamin D and calcium alone will prevent bone loss in this elderly population compared to placebo. Calcium alone will be as effective as calcium and vitamin D.

Study Design

Subjects:
As a sub-study of CAIFOS we will recruit 120 women using the CAIFOS inclusion and exclusion criteria. These are that the subject must be over the age of 70, likely to survive a five-year study, and who are not receiving bone active agents. There are no other specific exclusions so that the results will be generalisable to the whole ambulant population. Subjects will be recruited from the electoral role that we have available for CAIFOS, by means of a letter inviting participation in the study. This will allow us to evaluate possible selection bias in the recruitment to the study and therefore make our conclusions more generalisable to the population under consideration. Subjects will be randomized by block randomization into three regimens.
1. Calcium (600mg) twice daily with vitamin D (1000 IU) daily for five years
2. Calcium (600mg) twice daily with placebo vitamin D for five years.
3. Placebo calcium twice daily with placebo vitamin D for five years.

Protocol:
At the first visit the following will be performed:
1. A fasting blood and urine samples including, parathyroid hormone, calcitriol, vitamin D binding protein, osteocalcin, 25 hydroxyvitamin D, serum ionised calcium, total calcium, albumin, phosphorus, creatinine, alkaline phosphatase, and urine deoxypyridinoline/creatinine ratio.
2. A gut calcium absorption test using calcium 45. We will use a method which utilizes a 10mg calcium carrier and a 5 uCi tracer dose. This method is currently in use in our institution. Specific precautions include the use of deionised water before the study and careful timing of the administration and blood sampling.
3. A clinical assessment including, heel ultrasound measurements, anthropometric tests and tests of functionality which include, Timed Up and Go, balance and grip strength.
4. A symptom related quality of life questionnaire, an abbreviated Mini Mental test, Barthel test and a food frequency questionnaire will be completed by the subject.
5. Bone density measurements at the hip, spine, and whole body site. These will be carried out using the Hologic Acclaim 4500 detector fan beam densitometer.
6. The subjects will be provided with a diary for documentation of encounters with their general practitioner during the five years of the study. These will be returned every four months, photocopied for our records and returned to each subject.

At four months the following will be performed
1. A fasting blood and urine samples including, parathyroid hormone, calcitriol, vitamin D binding protein, osteocalcin, 25 hydroxyvitamin D, serum ionised calcium, total calcium, albumin, phosphorus, creatinine, alkaline phosphatase, and urine deoxypyridinoline/creatinine ratio.
2. A gut calcium absorption test using calcium 45. We will modify the method by using a 600 mg calcium carrier with a 10 uCi radioactive calcium tracer. Specific precautions include the use of deionised water before the study and careful timing of the administration and blood sampling.
Every twelve months the baseline tests will be repeated, with exception of the gut calcium absorption test.

Background

Osteoporosis is a major disease of the locomotor system. 40% of women will sustain an osteoporotic fracture at some time in their life (35, 38). More women sustain hip fractures than develop breast, ovarian and cervical cancer together. Most of these fractures are sustained after the age of 70. In view of the very high incidence of osteoporosis, it is appropriate to examine a population based prevention approach. Calcium treatment may potentially fulfil the requirements for such a community based intervention. There is uncertainty as to whether vitamin D would provide extra benefit without extra side effects in an Australian population.

We and others (26, 39) have shown that extra calcium in the diet is very effective in slowing bone loss in perimenopausal women (8). The treatment effect on bone density is more marked in women more than ten years past the menopause (24, 40). There are now three small studies showing that calcium supplementation alone can prevent fracture (9, 30, 41). Two larger studies of calcium and vitamin D supplementation have also resulted in fracture reduction (42, 43). Finally a large vitamin D supplementation study did not show any fracture prevention efficacy (44). Thus it is uncertain as to whether vitamin D supplementation is required if high doses of calcium are used. Clearly it is likely that the vitamin D status of the population under study will be important in determining whether vitamin D supplementation is necessary. Thus in the Australian context it is important to know whether vitamin D supplementation will add anything to a calcium supplementation regimen.

We have already commenced a large five-year calcium supplementation study aimed at a fracture end point. If a one year surrogate end point study of effects of calcium versus calcium and vitamin D suggests a greater efficacy of calcium and vitamin D in the subjects under study, it will be possible to alter the study design of our main fracture outcome study, to include vitamin D and calcium as the main intervention.

Outcomes

The outcome variables have been carefully selected to advance our understanding of the mechanism of action of calcium and vitamin D supplementation on bone and calcium. There is now much evidence to link the rise in PTH observed with ageing with calcium deficiency and bone loss (7). Our previous studies of calcium supplementation have shown evidence of suppression of PTH and bone turnover (40). The important question is whether in the context of the vitamin D status of ambulant elderly Australians, vitamin D supplementation will improve these specific outcomes by lowering PTH, alkaline phosphatase and deoxypyridinoline. These end points can be addressed as shown by our power calculations.

Over the five years of the study the bone density measurements at the hip, spine and whole body will help to define likely clinical benefits. Previous data have reinforced the concept that in trials of calcium supplementation the effects on bone density have translated into clinical benefits of fracture prevention (45). As indicated below the study is powered to measure clinically significant treatment effects of calcium versus calcium and vitamin D.

Gut calcium absorption

Dosing studies in young subjects which used bowel washout techniques, clearly show dependence of gut calcium absorption on 1,25 dihydroxyvitamin D concentrations and calcium intakes across the physiological range (16). Sheikh et al were able to calculate that in their subjects the vitamin D independent fraction of calcium absorption was about 30% across the range of calcium intakes. 1,25 dihydroxyvitamin D regulated calcium absorption across the physiological range and was able to stimulate fractional calcium absorption from close to zero at low 1,25 dihydroxyvitamin D concentrations to over 60 % at very high concentrations. Thus in a normal individual with a 1,25 dihydroxyvitamin D concentration of 120 pmol/l, consuming a meal containing 300 mg of calcium, approximately 75 mg of calcium would be absorbed by vitamin D dependent processes while approximately 25 mg would be absorbed by passive processes. However by extrapolation if a high calcium intake were consumed, for example a 600 mg calcium tablet, it could be calculated that 180 mg of calcium would be absorbed by passive means with no increment in active absorption. These fractional absorption data have been supported by other studies carried out at high calcium doses (46, 47). Thus assuming that there is no deterioration in the passive absorption of calcium with age and if high calcium meals are ingested, the vitamin D status of the individual would have little impact on calcium absorption. If however low calcium meals are consumed, as is common in elderly subjects, then the vitamin D status becomes critical. Under these circumstances and if there is deficiency of 1,25 dihydroxyvitamin D there may well be net calcium loss from the bowel (16).

The gut calcium absorption tests have been designed to answer a variety of questions. The first relates to determinants of active gut calcium absorption in women over the age of 70. It is known that active gut calcium absorption falls with age (10, 48). In this part of the study, we intend to examine a variety of potential determinants of active gut calcium absorption including vitamin D status, total and free calcitriol, renal function, dietary intake and functional factors. The second test using a 600mg calcium load is designed to determine whether under normal consumption conditions at a high calcium load, vitamin D supplementation increases calcium absorption. In the control subjects, we will be able to compare the fractional absorption of calcium administered at low and high calcium carrier loads.

Methods

Examination

This will consist of (a) anthropometry involving height, weight, skin thickness of the upper arm, and a measure of kyphosis using a flexible curve (b) a validated functional assessment the Timed Up and Go Test, involving standing up from a chair walking 3 meters and sitting down, (c) a timed standing balance test, (d) grip strength measured using a dynamometer, and (e) seated and postural blood pressure using a Dynamap machine.

Questionnaires

These will include:

(a) a specific questionnaire related to bone and functional end points;
(b) a symptom related quality of life questionnaire, the SF-36, which has already been used extensively in our unit;
(c) an assessment of activities of daily living using a Barthel questionnaire,
(d) an abbreviated Mini Mental State test,
(e) a validated food frequency record using food models and a computer read answer sheet developed by the Victorian Anti Cancer Council.

Bone densitometry

This will be carried out on the Hologic Acclaim 4500A 248 detector fan beam densitometer. This was the first of these new densitometers to be installed in Australia and we were amongst the first centres world wide to have access to this new technology. The bone density will be measured at the spine, hip and whole body. The CV on the Acclaim at the various sites ranges from 1.8% to 1.0%. The Acclaim also has the capability of measuring the heights of vertebral bodies with the patient lying in the supine position in approximately five minutes and will automatically calculate the hip axis length which may prove to be useful in predicting fracture risk. This study will allow us to evaluate the technical feasibility of these new morphometric capabilities of this machine on a clinical population.

Biochemistry

Calcitropic hormones to be measured in the Dept. of Endocrinology and Diabetes, Sir Charles Gairdner Hospital, include PTH by a chemiluminometric method which does not have the non linearity problems of other assays at high PTH concentrations, calcitriol by our previously validated extraction method and 25 hydroxyvitamin D by protein binding after extraction. Ionised calcium will be measured using a Corning calcium electrode with pH correction. Total calcium, albumin, phosphorus and creatinine on blood and urine will be measured using routine techniques at Pathcentre. Parameters of bone turnover including alkaline phosphatase and urine deoxypyridinoline will be measured by state of the art assays available at Pathcentre under the direction of Dr. G Neil Kent who has an international reputation in this area.

Gut calcium absorption

The technique of gut calcium absorption using calcium 45 has been set up in our institution using the method of Nordin et al (49). This technique uses a 10mg calcium carrier and a 5uCi calcium tracer. It is a measure of active calcium transport which appears to correlate with other physiological variables. In a modification in order to achieve steady state levels we will delay the blood test to 2 hours. At 4 months we will measure calcium absorption using a 600 mg calcium load and a 10uCi tracer concentration. The blood test will be taken at 2 hours. This will be a measure of fractional calcium absorption at the calcium supplement dose that is being used in the study. It will be administered with at standard breakfast.

Adverse event reporting

Adverse event reporting will be by the use of a diary to be filled out by the patient each time they see a medical practitioner. These diaries will be reviewed at 4 monthly intervals.

Power calculations and data management

To allow for withdrawals over the five years of the study the power calculations have been done on the basis that 30 subjects remain in the study. The main biochemical outcome variables will be PTH and urine Dpd/creat. At a power of 0.8, an alpha of 0.05, 30 subjects completing the study and a within group measurement error of 60 %, a difference in the Dpd/creat between groups of 40 % could be detected. For PTH at a power of 0.8, alpha of 0.05, 30 subjects and a within group measurement error of 16% we could detect a change of 11%. This equates with the magnitude of the effect of calcium alone on these variables that we have detected in a previous study (40). A previous study of vitamin D plus calcium showed a fall of over 30 % in PTH. (50). This indicates that we have the power to detect a clinically meaningful treatment effect of the addition of vitamin

D to the calcium treatment. Thus we would be able to detect a clinically significant treatment difference between calcium and calcium and vitamin D if it were to occur.

For the bone density outcome, at a power of 0.9, an alpha of 0.05, 25 subjects completing the study and a within group measurement error of 2 % a difference in the rate of bone loss between groups of 1.8 % over the study could be detected. Thus the clinically significant value of 4% difference could be detected.

Data will be entered into Access, a relational data entry programme. For analysis it will be entered into SPSS for Windows. All these software packages are available, as are the computers for the analyses. The primary method of analysis will utilise repeated measures ANOVA. A regression line will be fitted to the data where appropriate to provide a summary statistic for evaluation in covariance models.

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