CARES Research Study Protocol

CAIFOS (Calcium Intake Fracture Outcome Study) Age Related Extension Study (CARES)

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Background

The Bone and Calcium Research Group of the School of Medicine and Pharmacology, Sir Charles Gairdner Hospital Unit at the University of Western Australia has been conducting the Calcium Intake Fracture Outcome Study (CAIFOS) since 1998. This is a population based study of women aged >70 years with the aim of determining the efficacy of calcium supplementation in preventing fracture. The treatment phase of this two-arm, randomised, double-blind, 5 year study of calcium supplementation (1200mg/d) versus placebo in 1,500 women will conclude in 2003. Of 24, 800 women aged over 70 on the electoral roll women 6.2% were recruited. The mean age of the population at baseline was 75.1±2.7 years. These women had no significant current illness, were not taking bone active agents and were likely to survive the 5-year study. Although the subjects entering the study were weighted in favour of those in higher socio-economic categories they do not differ from the whole population in health resource utilisation. A remarkable 85% of the study subjects remain active in the study. Due to the low mortality rate (3.6%) that has occurred during the study, retention statistics suggest that more than 1200 subjects will be measured at the end of the treatment phase during 2003. Studies during 2002 indicate that 93% of these are willing to be followed up for a further 5 years. At their "end of treatment phase" appointment we have approval to re-consent willing study participants to a 5-year follow up trial.

Osteoporosis has been the main focus of the CAIFOS trial, as this condition is largely preventable yet during 2001, 1.9 million people, or one in ten Australians, have osteoporotic fracture. Every year 64,514 osteoporotic fractures occur, which is one fracture every 8.1 minutes [1]. The prevalence of osteoporosis is three times greater in women than men, with a lifetime risk of fracture estimated to be 42% commonly comprising of hip, vertebral and wrist (Colles') fractures [2]. In Western Australia the lifetime risk of hip fracture is 17% [2, 3] and the associated excess mortality is between 5% and 20% depending on age at fracture [4]. Osteoporosis is more expensive than diabetes or asthma (which are also National Health Priorities) with estimated costs of $1.9 billion per annum. Assuming constant age specific rates for fracture, the number of hospital admissions and associated costs for hip fracture alone are likely to increase by 63%, based on ABS projections, and cost more than $200 million annually [5].

Environmental and metabolic factors, namely hormonal status, genetics, medications use, co-morbidity and lifestyle factors affect the pathogenesis of osteoporosis. Lifestyle factors such as diet clearly influence the likelihood of developing osteoporosis later in life. It has be known for many years the importance of dietary calcium in the attainment of peak bone mass during growth, maintenance of bone during adulthood and prevention of bone loss during later life. Consequently the recommended dietary intake (RDI) for calcium is relatively steady throughout life and women aged >54 years should consume 1,000 mg of calcium daily [6]. There are no studies of elderly Australian women, however, that show that this intake is adequate to prevent fracture. The National Nutrition Survey (NNS) has reported that the dietary intakes for women >65 years remain inadequate at 686 mg/d and only 10.9% of this age group take calcium supplements [7]. We have shown in elderly women who were given calcium supplements to improve their intake, at follow-up 70% reported supplement use; however, only 30% consumed the recommended 1,000 mg calcium daily [8]. The baseline calcium intake of this CAIFOS population (854±321 mg/d) is slightly higher than a younger group previously reported by us (827±316 mg/d) [9], however, both groups of women have mean intakes below the RDI. We have shown that 1500 mg of calcium from the diet prevents bone loss in postmenopasual women [10], but we do not know whether this amount prevents fracture. Consequently the CAIFOS study that is now nearing completion has the power to determine whether a calcium supplement of 1,200mg per day is adequate to prevent fracture in elderly women.

Equally important is the ability of the CAIFOS study to examine other factors that influence osteoporosis and other diseases. The database currently consists of baseline and longitudinal measurements of phenotypic data related to bone health, cardiovascular health and neurocognitive clinical function. Specifically we have assessed bone strength and structure, hormone

concentrations, blood pressure, intimal medial thickness, neurocognitive clinical function, physical function and falls risk. We have extensive details of adverse events and medication use recorded throughout the last five years which will be ongoing in the follow up study. Some of these factors relate to all three-focus research areas and can be advantageous when determining links between metabolic systems.

For example, the lipid transport protein apolipoprotein ε4 (APOE-ε4) is an important risk factor for cardiovascular disease and has been associated with increased plasma cholesterol levels [11]. Other studies have shown that patients suffering from cardiovascular disease and those with high plasma cholesterol exhibit increased neuropathology characteristic of Alzheimer's Disease (AD) [12]. In addition in the CAIFOS population we have associated reduced bone density in subjects with the APOE-ε4 genotypes [13]. Recently, findings from the Study of Osteoporotic Fracture has shown that women with rapid hip bone loss were more likely to develop cognitive decline than those with who had slower bone loss [14]. The mechanism of the association is still unclear but suggestive of an APOE-ε4 phenotype.

In the area of neurocognitve health, APOE-ε4 is one of the major genetic risk factors for AD [15-17]. Recent studies have demonstrated that polymorphisms in the APOE promoter also promote risk, which is independent of the APOE-ε4 allele. In particular the AA polymorphism at position -491 of the APOE promoter is associated with an increased risk of AD and its presence is consistent with increased expression of the apoE protein [18, 19]. It is interesting to note that the levels of apoE may be regulated by estrogen. There is considerable interest in the role of estrogen on cognitive performance and the CAIFOS study has allowed for such studies to examine the association between endogenous estrogen levels with cognitive performance. Hormone levels may have important regulatory effects, directly or indirectly, that modify the complex genetic mechanism of regulation of apoE expression. It has been reported that estradiol up-regulates apoE gene expression by increasing levels of apoE mRNA in the polysomal translating pool [20] and region specific up-regulation of apoE by estrogen (although in non physiological concentration) has also been reported [21]. Additionally, the neuro-protective role for estrogen in global ischaemia is apoE-dependent [22]. These studies also suggest that each of these mechanisms are largely mediated by estrogen receptors (ERα and ERβ). As such the effect of polymorphisms within ERα would be of interest to investigate in correlation with cognitive performance – especially in light of recent publications linking such polymorphisms with an increased risk of developing AD in females [23].

In the area of skeletal and cardiovascular medicine, estrogen has long been recognized as a protective factor in women prior to menopause. Its action after menopause is pronounced in maintaining bone density [24]. Estrogen's protective action against cognitive decline involves regulation of cholesterol metabolism analogous to its action against cardiovascular disease. Thus it may be predicted that identification of factors that modulate estrogen's action against cognitive decline may be applicable to its action against cardiovascular disease.

Another metabolic factor that is considered a risk factor cerebrovascular disease and may play an important role in the pathogenesis of AD is total plasma homocysteine (tHcy) levels [25]. High values of plasma tHcy and low levels of vitamin B12 and folate are frequently present in AD patients [26, 27] and CVD patients [28]. Moreover, the homozygous mutation (C677T) of the methylene tetrahydrofolate reductase (MTHFR) gene, related to a thermolabile type of the encoded enzyme, causes hyperhomocysteinemia by reducing the 5-methyltetrahydrofolate availability. As such there is interest in determining the validity of the association of this mutation with both AD and pre-dementia cognitive impairment [29]. Homocysteine and folate levels, which have been measured, can be examined in relation to ongoing assessments of cardiovascular and cognitive endpoints, which include time to event.

In these cases there is an interaction between genetic and environmental factors that have significant commonalties between all research areas. Consequently we will be able to utilize the CAIFOS cohort to examine many different metabolic and environmental factors associated with morbidity in the three focus areas and mortality in elderly women who have not yet developed significant disease. We will attempt to understand why some women with relatively normal estrogen levels still

show skeletal and cognitive decline and have greater tendency to develop osteoporotic fracture, ischaemic heart and cerebrovascular disease. We will evaluate selected metabolic and environmental factors for their role in antagonising the protective action of estrogen.

The mean age of the cohort is now 80±3 years and during the first 5 years we have observed some 75,101 reported adverse events i.e. approximately 1/month/person. With age, the incidence of adverse events is set to increase. In the CAIFOS population a 3-year incident fracture rate of 14% has been observed. Assuming linearity this is likely to increase to 23% over the coming 5 years. Hospitalization rates from coronary heart disease and stroke are 3.5 times and 8 times higher respectively in women aged 75 compared with women aged 55. The prevalence of cardiovascular disease or condition is 15.5% in the total population, the reason why this is a National Health Priority Area (NHPA) [30]. In the CAIFOS population thus far, 12.3% of subjects have reported incident ischaemic heart disease, peripheral vascular disease or stroke. The intima medial thickness measurements undertaken on the CAIFOS population were compared to one of the most quoted study's of IMT predictive value, the ARIC study [31]. This study which compared <1mm vs ≥1mm mean baseline IMT, found that women with IMT≥1mm ("extreme mean IMT") had a HR 5.07 for coronary heart disease incidence over 4 year follow-up when compared to those with IMT <1mm ("non-extreme mean IMT"). 4.9% of the CAIFOS women had an IMT ≥1mm placing them at increased risk of coronary heart disease. The CAIFOS study provides a unique opportunity to assess the interactions between evidence of early atherosclerosis, novel cardiovascular risk factors and cardiovascular outcomes in postmenopausal women. The women in the CAIFOS study have had their cardiovascular risk factors as well as their genomic profile and hormonal status well characterised, are now likely to develop cardiovascular events at an increasing rate.

Another NHPA is mental disorders where the prevalence is 5.8% of the total population [30]. Three percent of CAIFOS subjects have reported dementia, loss of memory or have been diagnosed with AD. Therefore this follow-up study will continue to provide valuable insight into the prevalence, incidence, prognosis, and predisposing risk factors for diseases in three focus areas: osteoporotic fracture, cardiovascular and cerebrovascular disease and neurocognitive clinical function in a cohort of elderly women.

Study Aim

The aim of this new five-year prospective epidemiological study is to determine health outcomes in a cohort of elderly postmenopausal women who in 2003 will have completed a five-year *population based* clinical trial of a *public health* intervention which commenced in 1998. The dietary intervention studied was the effect of an increased oral administration of calcium in the prevention of fracture. In this new study we will collect outcome data in three focus areas, musculoskeletal; cardiovascular and cognitive health, that will allow us to study the effects of parameters *already collected* including:

Environmental factors (eg diet, physical activity),

Anatomical measures (eg Bone mass and structure, carotid intimal medial thickness)

Physiological function (eg mobility, falls risk),

Metabolic function (eg ApoE levels, hormonal and iron status, homocysteine, beta-amyloid),

Genetic polymorphisms (eg estrogen receptor, TGF beta, ApoE 4, Cyp19),

that may predict increased risk of clinical events specifically osteoporotic fracture, cardiovascular events, and neurocognitive function.

Study Design

This is a prospective five-year follow-up trial of a cohort of elderly women who were participants in a five-year study of the effect of calcium supplementation on fracture prevention. As this is an extension to an existing study currently ongoing, new study participants will therefore not be recruited. All consenting participants of the CAIFOS trial will be included. Subjects who, in the opinion of the investigator, are not likely to complete the study for whatever reason will be excluded.

Withdrawals will be handled as missing values in the statistical analysis. Data for those subjects who withdraw will be used up to the time of withdrawal. Hospital morbidity data (HMDS) that is obtained routinely throughout the trial will be obtained on all subjects (withdrawn and active) unless the subject withdraws their consent for retrieval of HMDS data.

Subjects

As this is an extension to an existing study currently ongoing, new study participants will therefore not be recruited. All consenting participants of the CAIFOS trial will be eligible.

Withdrawals

Withdrawals will be handled as missing values in the statistical analysis. Data for those subjects who withdraw will be used up to the time of withdrawal. Hospital morbidity data (HMDS) will be obtained towards the end of the treatment phase on all subjects (withdrawn and active) unless the subject withdraws their consent for retrieval of HMDS data. Subjects who, in the opinion of the investigator, are not likely to complete the study for what ever reason will be withdrawn.

Drugs and Interventions

Nil.

Study Plan & Schedule of Assessments

End of treatment phase

During 2003 all active subjects will be reviewed within a four-week period of their baseline anniversary date as has been undertaken in the previous 4 years. Each subject will undergo a repeat of all baseline measurements. This visit will end the current treatment phase of the programme and the investigator will unblind the treatment code for each subject. The five-year change in bone density will be calculated and reported to each subject and reported to her GP. The final analysis will take place at the end of 2003 and all subjects will be invited to the presentation of the data in 2004. Letters relating the final outcomes of the study will be communicated to all subjects.

During the end of treatment phase visit, each subject will be asked whether they wish to continue with the follow up study. Those subjects that wish to continue will be required to sign a Patient Information and Consent Form entitled “Calcium Intake Fracture Outcome Study Five Year Follow up Study”.

Contact 1, 2 and 3

The subjects will be reviewed at 6-months, 12-months and 18-months after their clinic visit in 2003 using a short telephone interview. The data collection will include:

Incident fracture

Each subject will be asked whether they have had a fracture in the previous 6 months. All incident fractures will

be verified by a radiological report.

Adverse event data

Adverse events have been monitored through the five years of the treatment phase by using a diary system that is completed by the subject, their GP, other health professional or specialist. These data have been entered into Microsoft Access that utilises ICPC2 Plus© a database of disease coding and CAPS©, a database of pharmaceutical's, developed and supplied by the Family Medicine Research Unit, Department of General Practice, University of Sydney [32]. This process of adverse event collection will be continued but administered by the clinical research assistant at the time of the telephone interview and recorded in the subject diary kept in the subjects source data.

The adverse event review will entail review of any significant adverse events and changes in medication use. This will include hospitalisation for any reason, change in state or severity of chronic or present disease, and an acute condition that required medication, new diagnosis of a condition not previously known in addition to any fall, fracture or/and death.

Contact 4

For contact 4, at the 2nd year of the study (CAIFOS Year 7: 2005) subjects will attend a clinic visit. Fasting blood and urine samples will be collected from all participants. The data collection will include:

1. Falls and Incident fracture

Each subject will be asked whether they have had any fall and/or fracture in the previous 6 months. All incident fractures will be verified by radiological reports.

2. Adverse event data

Review of any significant adverse events and changes in medication use. This will include hospitalisation for any reason, change in state or severity of chronic or present disease, and an acute condition that required medication, new diagnosis of a condition not previously known.

3. Anthropometry and assessment of physical function

Each subject will have repeat measurements of height, weight, hip and waist circumference, triceps skinfold and upper arm girth. Body mass index will be calculated.

For physical function assessments: **Romberg test** will be used to assess balance function, hand **grip strength** will be assessed by a hand dynamometer and mobility functioning will be measured by the **Timed Up and Go Test (TUAG)**.

4. Vertebral deformities and bone density

Measurements of bone parameters will be ascertained at the visit. These will include:

Quantitative ultrasound measurements (QUS) of left foot – Lunar Archilles

Dual energy X-ray absoprtiomtery (DXA) of hip and total body – Hologic QDR 4500A

Morphometry (MXA) - Hologic QDR 4500A

Peripheral quantitative computed tomography (pQCT) of distal radius (4% and 15%) and distal tibia (4% and 15%)- Stratec

5. Dietary intake

Dietary intake will be reassessed using the FFQ administered at baseline. The process of collection will be identical where by a dietitian supervises the completion of the questionnaire in small groups. Food models, cup and spoons and charts for frequency of seasonal fruits are used. The machine readable answer sheets are checked and sent to the Anti-Cancer Council of Victoria for processing. These data are received in a spreadsheet format and include individual food frequency data and mean nutrient intake for 31 nutrients. These data will be used to determine the change in calcium intake during the 2-years and 5-years since the end of the treatment phase.

6. Quality of life

After all tests have been undertaken each subject will be asked to complete a symptom related quality of life (SF36) questionnaire as employed at the baseline visit. The change in quality of life for those who sustained a fracture can be determined and compared to those who did not fracture. These data will also allow us to assess how quality of life changes with co-morbidity.

7. Other questionnaires

Apart from the above mentioned questionnaires subjects will be asked to complete the following questionnaires:

- **Beverage Frequency Questionnaire** for the assessment of beverage intakes.
- **WOMAC questionnaire** (Western Ontario & Mcmaster University Osteoarthritis Index) for the assessment of the pain and stiffness subjects experienced at knees and hips and the physical function of their knees and hips.
- **Abbreviated mini mental state test (AMS)** for the assessment of cognitive function.
- **Barthel questionnaire** for the assessment of activities of daily living.
- **Demographic questionnaire**

Contact 5, 6, 7, 8 and 9

The subjects will be reviewed 6-montly during 2006 and 2007 by a short telephone interview. The data collection will be as detailed for Contacts 1, 2 and 3.

Contact 10

Subjects will again be asked to attend a clinic visit in 2008 (CAIFOS Year: 10). For subjects who are unable to attend clinic, a home visit or phone review will be provided. The home visit will cover all parts of clinic except DXA and pQCT measurements. The phone review will cover adverse events and incident fracture collection as well as questionnaires. Fasting blood and urine samples will be collected as for contact 4 except subjects who only have a phone review.

Data collection will be the same as for contact 4 and will include study procedures undertaken in 1998 except that a new questionnaire the **Patient Health Questionnaire (PHQ 9)** for the assessment of depression and mood will be added to contact 10. If the score of the PHQ-9 questionnaire indicates that the patient suffers from depression they will be followed up by the investigator for appropriate action.

After contact 10 seven and ten-year incident changes in BMD will be available on most participating subjects during the study. Moreover, using the 7^{th} and 10^{th}-year change in BMD, the

calcium intake required to prevent bone loss and fracture will be determined.(for further details, see information on Contact 4).

Summary of Assessment Schedule

Visit	1 Post CAIFOS	2 Post CAIFOS	3 Post CAIFOS	4 Post CAIFOS	5 Post CAIFOS
Year	2004	2005	2006	2007	2008
Type	Phone	Clinic	Phone	Phone	Clinic
Adverse events	**X**	**X**	**X**	**X**	**X**
Anthropometry		**X**			**X**
Bone structure		**X**			**X**
Dietary assessment		**X**			**X**
Mini Mental State		**X**			**X**
SF 36/Barthel		**X**			**X**
Updated Demographic Questionnaire		**X**			**X**
Beverage Questionnaire		**X**			**X**
WOMAC Questionnaire		**X**			**X**
PHQ 9					**X**

Statistical Analysis

All primary analyses of osteoporotic fracture, cardiovascular events (ie stroke, TIA, myocardial infarction, unstable angina and sudden cardiac death using ICD definitions), neurocognitive impairment and death from any other cause will use time-to-event methods. For a given outcome, the time of event is determined from study commencement (during 1998) to the first diagnosis as determined by reporting in the adverse event diary retained by the centre. Participants without a diagnosis will be censored for that event at the time of last follow up. Participants who withdraw from the study will be censored at the time of withdrawal. Primary outcome comparisons will be presented as hazard ratios (HRs) and 95% confidence intervals from Cox proportional hazards analyses after adjustment for age, years since menopause, weight, metabolic and environmental factors.

These data will also be analysed using logistic regression at time of last follow up. The effect of post study calcium supplementation on incident fracture (yes, no) and new vertebral deformities (yes, no) will be analyzed by logistic regression analysis (LRA) adjusting for age, weight and other metabolic and environmental factors. These include diet and alcohol intake, physical activity, biochemical parameters and sociological factors from questionnaires. Being a prospective follow up study we can calculate the predictive risk of osteoporotic fracture, cardiovascular events, and

neurocognitive impairment based on their baseline characteristics, randomisation status (placebo or treatment) and prior disease and medication use.

The subjects will be grouped by incident fracture status (0, 1, or >1) and vertebral deformity status (0, 1 or >1). In the case of three groupings cumulative logits will be modeled by performing ordered LRA using the proportional odds model (McCullagh, 1980). This analysis will assess the effect of randomisation status, prior disease and medication use.

To determine the metabolic and environmental predictors of change in bone density, quality of life, and change in neurocognitive endpoints, stepwise multiple regression will be used to determine which factors play a significant role.

For all repeated measures categorical variables, for example vertebral deformities or questionnaire data on falls, the effects of baseline categorical factors will be assessed using Generalised Estimating Equations (GEE). This analysis will assess the effects of these factors and their interaction with time after adjusting for age, socio-economic status and co-morbidity. The procedure “proc genmod” implemented in SAS version 8 will be used to analyse these data (Categorical Data Analysis using the SAS System. Stokes M et al., 1995. SAS Institute, Cary, USA).

Data Management and Reporting

All data from telephone interviews and clinic visits will be entered in to the same relational database as utilised for Caifos study ie. Real Beach which runs in parallel to Site Safe system, that front ends Microsoft Access. This utilises ICPC2 Plus© a database of disease coding and CAPS©, a database of pharmaceutical’s, developed and supplied by the Family Medicine Research Unit, Department of General Practice, University of Sydney (Britt et al., Aust Family Physician 1997;26:S79-82). These adverse event data will be forwarded to Clinical Drugs Trial Committee after independent statistical analysis is used to determine the safety issues of the trial. A report will be forwarded to the Clinical Drug Trials Committee each year.

Compliance with Good Clinical Practice & Ethical Considerations

This study will be conducted in compliance with the conditions stipulated by the Clinical Drug Trials Committee and Research Institute Ethics Committee, informed consent regulations and NH&MRC Guidelines. All amendments to the trial will be placed before the Research Institute Ethics Committee and the Clinical Drug Trials Committee for approval. Any information that may affect either committee's decision to continue approval for the trial will be forwarded to the committees without delay. An annual report will be issued, in accordance with the guidelines provided by the Research Institute Ethics Committee

Informed Consent

The investigator, or a person designated by the investigator will explain the benefits and risks of participation in the study to each subject and obtain written informed consent prior to the subject entering the study by signing a consent form approved by CDTC and RIEC. Each subject’s original consent form, signed and dated by the subject will be retained by the investigator. All subjects will receive a copy of the document at the end of the visit

Record retention

Records will be retained for 15 years in a locked facility, in accordance with NH&MRC guidelines. Access to the records will be limited to treating clinician, chief investigator and his/her nominated co-investigators, and authorised representatives of drug regulatory bodies. Patient confidentiality

will be maintained in accordance with local requirements.

Withdrawal criteria

Participants will be withdrawn from the study under the following conditions:

Patient request

Circumstances which, in the opinion of the investigator will prevent the patient from completing the trial procedures

Liability & Indemnity

The risks of participating in this study are low. In the unlikely event of liability for an adverse event related to a per protocol event the University of Western Australia will carry liability.

Documentation of study findings

The results of this study will be published in peer reviewed journals and presented at scientific meetings. This will be after all investigators have agreed to the manuscript. Authorship will be determined by mutual agreement in relation to normal standards relating to having played a substantial role in the study.

Study dates

Starting date: August 2003

Finish date: December 2008

References

[1] Access Economics Pty Ltd. The burden of brittle bones: Costing osteoporosis in Australia. September ed. Canberra: Access Economics Pty Ltd, 2001.

[2] Doherty DA, Sanders KM, Kotowicz MA, Prince RL. Lifetime and five-year age-specific risks of first and subsequent osteoporotic fractures in postmenopausal women. Osteoporos Int 2001;12:16-23.

[3] Hockey RL, Hobbs MST, Goldswain PRT. Femoral neck fractures in Western Australia 1971-1988. September ed. University WA, Department of Eds.) (1990;Medicine:Perth.37pages.

[4] Sanders KM, Seeman E, Ugoni AM, Pasco JA, Martin TJ, Skoric B, et al. Age- and gender-specific rate of fractures in Australia: a population-based study. Osteoporos Int 1999;10:240-7 (http://link.springer.de/link/service/journals/00198/bibs/9010003/90100240. htm).

[5] Geelhoed EA, Criddle A, Prince RL. The epidemiology of osteoporotic fracture and its causative factors. Clin Biochem Revs 1994;15:173-8.

[6] National Health and Medical Research Council. Recommended dietary intakes for use in Australia. Canberra: Australian Government Publishing Service, 1991.

[7] Australian Bureau of Statistics, Department of Health and Family Services. National nutrition survey selected highlights Australia 1995. Canberra: AGPS, 1997.

[8] Devine A, Prince RL, Bell R. Nutritional impact of calcium supplementation by skim milk powder or calcium tablet on total nutrient intake in post menopausal women. Am J Clin Nutr 1996;64:731-7.

[9] Devine A, Prince RL, Bell RR. Nutrient intake of postmenopausal women in relation to skeletal and cardiovascular disease. Aust J Nutr Diet 1996;53(4):144-50.

[10] Prince R, Devine A, Dick I, Criddle RA, Kerr D, Kent N, et al. The effects of calcium supplementation (milk powder or tablets) and exercise on bone density in postmenopausal women. J Bone Miner Res 1995;10:1068-75.

[11] Jarvik GP, Wijsman EM, Kukull WA, Schellenberg GD, Yu C, Larson EB. Interactions of apolipoprotein E genotype, total cholesterol level, age, and sex in prediction of Alzheimer's disease: a case-control study. Neurology 1995;45:1092-6.

[12] Sparks DL. Coronary artery disease, hypertension, ApoE, and cholesterol: a link to Alzheimer's disease? Ann N Y Acad Sci 1997;826:128-46.

[13] Dick IM, Devine A, Marangou A, Dhaliwal SS, Laws S, Martins RN, et al. Apolipoprotein E4 is associated with reduced calcaneal quantitative ultrasound measurements and bone mineral density in elderly women. Bone 2002;31:497-502.

[14] Lui LY. Bone Loss Predicts Subsequent Cognitive Decline in Older Women: The Study of Osteoporotic Fractures. J Am Geriatr Soc 2003;51:38-43.

[15] Strittmatter WJ, Weisgraber KH, Huang DY, Dong LM, Salvesen GS, Pericak-Vance M, et al. Binding of human apolipoprotein E to synthetic amyloid beta peptide: isoform-specific effects and implications for late-onset Alzheimer disease. Proc Natl Acad Sci USA 1993;90:8098-102.

[16] Corder EH, Saunders AM, Strittmatter WJ, Schmechel DE, Gaskell PC, Small GW, et al. Gene dose of apolipoprotein E type 4 allele and the risk of Alzheimer's disease in late onset families. Science 1993;261:921-3.

[17] Martins RN, Clarnette R, Fisher C, Broe GA, Brooks WS, Montgomery P, et al. ApoE genotypes in Australia: roles in early and late onset Alzheimer's disease and Down's syndrome. Neuroreport 1995;6:1513-6.

[18] Laws SM, Taddei K, Martins G, Paton A, Fisher C, Clarnette R, et al. The -491AA polymorphism in the APOE gene is associated with increased plasma apoE levels in Alzheimer's disease. Neuroreport 1999;10:879-82.

[19] Laws SM, Hone E, Taddei K, Harper C, Dean B, McClean C, et al. Variation at the APOE -491 promoter locus is associated with altered brain levels of apolipoprotein E. Mol Psychiatry 2002;7:886-90.

[20] Srivastava RA, Srivastava N, Averna M, Lin RC, Korach KS, Lubahn DB, et al. Estrogen up-regulates apolipoprotein E (ApoE) gene expression by increasing ApoE mRNA in the translating pool via the estrogen receptor alpha-mediated pathway. J Biol Chem 1997;272:33360-6.

[21] Levin-Allerhand J, McEwen BS, Lominska CE, Lubahn DB, Korach KS, Smith JD. Brain region-specific up-regulation of mouse apolipoprotein E by pharmacological estrogen treatments. J Neurochem 2001;79:796-803.

[22] Horsburgh K, Macrae IM, Carswell H. Estrogen is neuroprotective via an apolipoprotein E-dependent mechanism in a mouse model of global ischemia. J Cereb Blood Flow Metab 2002;22:1189-95.

[23] Brandi ML, Becherini L, Gennari L, Racchi M, Bianchetti A, Nacmias B, et al. Association of the estrogen receptor alpha gene polymorphisms with sporadic Alzheimer's disease. BiochemBiophysResCommun 1999;265:335-8.

[24] Prince RL, Smith M, Dick IM, Price RI, Garcia Webb P, Henderson NK, et al. Prevention of postmenopausal osteoporosis: A comparative study of exercise, calcium supplementation, and hormone-replacement therapy. N Engl J Med 1991;325:1189-95.

[25] Miller JW. Homocysteine and Alzheimer's disease. Nutr Rev 1999;57:126-9.

[26] Levitt AJ, Karlinsky H. Folate, vitamin B12 and cognitive impairment in patients with Alzheimer's disease. Acta Psychiatr Scand 1992;86:301-5.

[27] Clarke R, Smith AD, Jobst KA, Refsum H, Sutton L, Ueland PM. Folate, vitamin B12, and serum total homocysteine levels in confirmed Alzheimer disease. Arch Neurol 1998;55:1449-55.

[28] Ward M. Homocysteine, folate, and cardiovascular disease. Int J Vitam Nutr Res 2001;71:173-8.

[29] Postiglione A, Milan G, Ruocco A, Gallotta G, Guiotto G, Di Minno G. Plasma folate, vitamin B(12), and total homocysteine and homozygosity for the C677T mutation of the 5,10-methylene tetrahydrofolate reductase gene in patients with Alzheimer's dementia. A case-control study. Gerontology 2001;47:324-9.

[30] Australian Institute of Health and Welfare. Australia's health 2002. Canberra: AIHW, 2002.

[31] Chambless LE, Folsom AR, Clegg LX, Sharrett AR, Shahar E, Nieto FJ, et al. Carotid wall thickness is predictive of incident clinical stroke: the Atherosclerosis Risk in Communities (ARIC) study. Am J Epidemiol 2000;151:478-87.

[32] Britt H. A new coding tool for computerised clinical systems in primary care - ICPC Plus. AustFamPhysician 1997;26 (Supp 2):S79-82.