PIMES Data Overview

Description	Variable	Baseline	1 year	2 years
Structural measures	**DXA of hip and whole body**	222	198	183
	VFA	222	198	183
	pQCT of forearm and tibia	222	197	168
	QCT of hip and spine	193	-	172
Physical function	**Muscle strength (hand grip and lower limb)**	222	194	181
	Timed Up & Go	222	195	179
	Clinical Romberg test	222	195	181
	Clinical Chair Rise	-	195	181
	Force Platform (Romberg, tandem, chair rise)	-	192	161
Physical measures	**Blood Pressure**	221	197	182
	Anthropometry	222	198	183
Questionnaires	**Demographic questionnaire**	222	198	183
	Physical activity questionnaire (IPAQ)	222	198	183
	SF-36	222	198	183
	Mini mental state	221	-	-
Dietary	**Food frequency questionnaire**	218	188	-
	3-day food record	222	195	182
Biochemistry	**Cholesterol (TC, triglyc, HDL, LDL)**	188	187	-
	Insulin	188	186	182
	IGF-1	188	188	182
	Glycerol	188	188	-
	Non esterified fatty acids (NEFA)	188	188	-
	Glucose	222	196	182
	Creatinine	222	196	182
	eGFR	222	196	182
	Urinary nitrogen	222	195	-
	Urinary Na, K and Ca	221	195	181
Other	**TRAK1 genotyping**	222	-	-
	Compliance data	Complete		

Values indicate the number of people measured at that time point for that assessment

Subjects and Methods

Participants

Study subjects were recruited from April to September 2007 using a population-based approach in which a random selection of women ($n = 6065$) aged 70 to 80 years on the electoral roll in the metropolitan area of Perth, Western Australia, received a letter inviting them to join the study. Over 98% of women of this age are on the Western Australian electoral roll. Of the 829 women who responded to the letter, 256 attended clinic screening, and 219 women who met the inclusion criteria joined the study. The exclusion criteria were participation in another clinical trial during the last 12 weeks, previous osteoporotic fracture, currently or within last year taking medication for osteoporosis apart from calcium or vitamin D, taking steroid tablets in the past 3 months or have taken more than 7 g in total in lifetime, metabolic bone disease apart from osteoporosis, total-hip bone density more than 2 SD below the mean for age, lactose intolerance or dislike of milk products, high protein intake as assessed by a food frequency questionnaire (equivalent to protein intake of more than 1.5 g/kg of body weight per day), cognitive impairment (Mini Mental State score < 24), body mass index (BMI) greater than 35 kg/m^2, malabsorption disorders, celiac disease, clinical hepatic or renal insufficiency, clinical diagnosis of diabetes, and participants who in the opinion of the investigator would not be likely to complete the study for any reason. All procedures followed were in accordance with institutional guidelines and were conducted at the Sir Charles Gairdner Hospital in Perth. The study was approved by the Sir Charles Gairdner Hospital Human Research Ethics Committee, and all participants provided written informed consent. The study was conducted in compliance with the ethical principles of the Declaration of Helsinki and the International Conference on Harmonization Good Clinical Practices Guidelines and registered with the Australian Clinical Trials Registry (Registration Number ACTRN012607000163404).

Study design

A 2-year randomized, double-blind, placebo-controlled, prospective parallel study was undertaken. Eligible participants were randomized to one of two treatment groups (protein and placebo groups). The study used a computer-generated randomization sequence with a block size of 10 to assign participants to either a protein drink or a placebo drink in a ratio of 1:1. The randomization code was generated by one of the investigators who did not have direct contact with participants, and the code was kept at the School of Public Health, Curtin University. In addition to assigning participants to intervention, Curtin University staff labelled the drinks and organized the delivery of the study drinks to participants' homes. The study participants and researchers at the Sir Charles Gairdner Hospital responsible for recruitment and assessment of outcomes measures remained blinded as to group assignment.

Drink supplements

The supplemental drinks were developed by an experienced food technologist. The test drinks were provided in three flavours—coffee, chocolate, and strawberry—and the nutrient content of the test drinks is shown in Table 1. Test drinks were delivered to participants' homes every 3 months, and participants were instructed to take a daily test drink before breakfast for the duration of the study.

Table 1. Nutritional Composition of the Test Drink (per 250 mL)

	Protein drink	**Placebo drink**
Energy (kJ)	809.5	819.5
Protein (g)	30.1	2.1
Fat (g)	2.3	2.0
Cholesterol (mg)	8.6	8.6
Carbohydrate (g)	13.2	42.3
Calcium (mg)	602.7	600.1
Sodium (mg)	47.9	33.0

Protein supplement

Participants in the protein group received a 250-mL skim milk–based high-protein supplement drink reconstituted with cold water from a powder that provided 30 g of protein, 600 mg of calcium, and 3.2 kJ/mL of energy. The high-protein product used skim milk plus whey protein isolate (Alacen 894, Fonterra Brands, Ltd., Palmerston North, New Zealand) to increase protein content.

Placebo supplement

Participants in the placebo group received a 250-mL skim milk–based supplement drink reconstituted with cold water from a powder that provided identical calories and calcium (600 mg of calcium and 3.2 kJ/mL of energy) but only 2.1 g of protein The extra energy content in the placebo drink was supplied by carbohydrate. Alginate natural flavoring and natural emulsifying agents were used to provide a similar texture and flavor to the drinks. Adherence to the study drinks was established by counting empty tins returned at the clinic visits at 1 and 2 years.

Bone measurements

Total hip BMD was the primary outcome measure of this study and was measured by dual-energy X-ray absorptiometry (DXA) using a Hologic Discovery A fan-beam densitometer (Hologic Corp., Waltham, MA, USA) at baseline and years 1 and 2. The coefficient of variation (CV) at this site was less than 2% in our laboratory.

Quantitative computed tomographic (QCT) scans of the hip were undertaken at baseline and 24 months using a Phillips Brilliance 64-slice spiral QCT scanner (Philips Medical Systems, Andover, MA, USA). Scans were acquired at 120 kVp and 100 mA with a 1-mm slice thickness and a pitch of 1. The patients were supine on the CT scanner table, lying on top of a QCT PRO (Mindways Software, Inc., Austin, TX, USA) calibration phantom and bolus bag so that the calibration phantom extended from the lumbar vertebrae to below the lesser trochanter (TR). The scans were analyzed using the QCT PRO analysis software (Mindways Software, Inc., Austin, TX, USA), and volumetric BMD (vBMD) and geometric engineering values were obtained from the analysis. These included femoral neck cross-sectional bone area (CSA) related to strength in compression, the polar cross-sectional moment of inertia (CSMI) related to strength in torsion, and the buckling ratio (BR) related to strength in buckling.

Biochemistry

Serum and 24-hour urine samples were collected at baseline and at 1 and 2 years for assessment of the secondary outcomes of this study—serum IGF-1 and 24-hour urinary serum. Venous blood samples were collected following a 12-hour fast from the antecubital vein of the forearm. Blood was collected into BD Vacutainer SST tubes (BD, Belliver Industrial Estate, Plymouth, UK). Analyses of IGF-1 were performed on sera stored at −80°C, and serum IGF-1 concentration was determined using a solid-phase, enzyme-labeled chemiluminesent immunometric assay (IMMULITE 2000 IGF-1, Siemens Medical Solutions, Los Angeles, CA, USA).

The participants collected a 24-hour urine sample on the third day of the food recording period in a 5-L plastic collection bottle that contained 20 mL of 1 M HCl. They discarded the first urine specimen of the morning and collected all specimens for 24 hours. The urine sample was weighed, and a 5-mL sample was stored at −20°C until analyzed. Urinary calcium concentration was determined using an Architect c16000 Analyser with calcium reagent (Abbott Diagnostics, Abbott Laboratories, Abbott Park, IL, USA).

Dietary intakes

Dietary intake was assessed by a 3-day weighed food record (2 weekdays, 1 weekend day). Participants were asked to record everything they ate and drank for 3 consecutive days using either the electronic food scales provided or household measures. They watched a training video on how to complete their food record prior to undertaking the food record. When the food record was returned 1 week later, the participant was interviewed to clarify types and amounts of food or beverages recorded. The food record was analyzed using the AUSNUT99 database (Foodworks Professional Edition, Version 3.02, Xyris Software Pty Ltd, Highgate Hill, QLD, Australia) by nutritionists trained in dietary assessment. The net endogenous acid production (NEAP) was estimated with the following formula using energy-adjusted protein and potassium intakes [1, 2]:

$$\text{Estimated NEAP (mEq/d)} = [54.5 \times \text{protein (g/d)}/\text{potassium (mEq/d)}] - 10.2$$

Other assessments

Anthropometric measurements were performed with subjects in light clothes and without shoes. Physical activity level was assessed by the International Physical Activity Questionnaire (IPAQ) short form (www.ipaq.ki.se). Demographic information for participants, including health history, education, past occupation, and smoking history, was collected using a demographic questionnaire. The Mini Mental State Test was administered at baseline with the aim of excluding participants who demonstrated significant cognitive impairment.

Adverse events

Using a previously validated method, [3] participants were asked to fill out an adverse-event diary in which each contact with a physician was recorded. At 6-month intervals, the diary was return to the study center at clinic visit or by mail. The adverse events were coded using the International Classification of Primary Care (ICPC2 Plus) system database of disease coding, a validated method of event recording developed for use in general practice. [4]

Sample size calculation

Power calculations were performed prior to commencement of the study. A sample size of 85 in each group was required to detect a difference of 3% on change in hip BMD, the primary outcome variable of the study, assuming an SD of 6% based on our previous study, at 90% power and 5% level of significance. A 3% increase was considered reasonable based on our previous epidemiologic studies. [5] This number was increased to 110 per group (total of 220) to allow for a 30% predicted dropout rate that we reported in previous studies of a similar age group.

References

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5. Devine A, Dick IM, Islam AF, Dhaliwal SS, Prince RL. Protein consumption is an important predictor of lower limb bone mass in elderly women. Am J Clin Nutr. 2005;81:1423–1428.