Excluded from the study were patients with malignant disease; with renal, hepatic, or gastrointestinal disorders; with endocrine disease associated with abnormal calcium metabolism that required therapy (diabetes mellitus type 2 or hypothyroidism were permitted for inclusion); who consumed >4 alcoholic drinks/d; or who had used estrogen, progesterone, glucocorticoids, anticonvulsants, vitamin D supplements, or other medications known to affect calcium or bone metabolism during the previous 12 mo. Other medications were continued during the study. Patients who had experienced fractures in the past were not excluded. The protocol was approved by the Research-Ethics Board of the Romanian Physicians' Council, and all patients gave written informed consent.

Study protocol

In this 1-y trial, all subjects received bread (buns) daily that had been fortified with 800 mg $CaCO_3$/d (320 mg elemental calcium) and 125 μg (5000 IU) vitamin D_3/d. Subjects were assessed initially by physical examination, blood was sampled, and urine was collected for 24 h. Bone mineral density (BMD) was measured at 2 sites to assess appendicular and axial changes to the skeleton. Vertebral fractures were identified by X-rays of the thoracic and lumbar spine. Subjects were seen every 3 mo to collect blood and urine. Urine was collected for 24 h to measure calcium and creatinine. Because of the poor reliability of 24-h urine collections in the elderly, data were obtained and presented as both the total urine volume of both compounds and as the ratio of calcium to creatinine, as was done previously (14), and was validated clinically as a sensitive measure of hypercalciuria (15–18).

Dietary records

At the beginning of the study, calcium intake in the group of institutionalized patients was assessed on the basis of the 10-d food records that were provided by the nursing home, which provided the same food to all patients; food records were not obtained from the patients individually. The evaluation of dietary intake was repeated at 4-mo intervals throughout the study.

Fortification of food

All subjects received daily one bread bun (100 g) fortified with 800 mg $CaCO_3$/d (320 mg elemental calcium) and 125 μg (5000 IU) vitamin D_3/d We added 0.5 mL of an oil solution containing vitamin D_3 (20,000 IU/mL, Vigantol; Merck KGaA, Darmstadt, Germany) into the dough used per bun. The dough was baked at 260–270°C for 15 min. Estimated loss during processing was 40–50%. The vitamin D_3 in samples of the bread was measured by solvent extraction (19) followed by HPLC and ultraviolet detection. The mean amount of vitamin D_3 was 5062.2 $\pm$ 459.7 IU/bun. The subjects were asked to consume all of the bread they were given.

Adherence and completion rate

Of the 45 patients enrolled, 40 completed the study (89%). Of those who withdrew, 1 did so for personal reasons, 1 left the nursing home, and the other 3 died (2 patients died of chronic heart failure and 1 of acute myocardial infarction). Seven additional patients were not available at the time the final dual-energy X-ray absorptiometry scans were performed (they had left the nursing home shortly after the final blood collection to be with relatives or were hospitalized elsewhere). Hence, initial and final pairs of BMD data were available for 33 subjects. Adherence to the study protocol was assessed by using a questionnaire at the end of the study and by measuring plasma 25(OH)D concentrations.

Analytic methods

Fasting blood and urine samples were collected at baseline and every third month during the 12-mo fortification period. Serum, plasma, and urine samples were kept frozen until analyzed. Serum calcium, phosphate, and creatinine were measured by using standard laboratory methods. Serum 25(OH)D was measured by immunoassay (Liaison Analyzer; DiaSorin, Stillwater, MN). Serum intact parathyroid hormone (PTH) was measured by an enzyme-amplified ''2-step'' sandwich-type immunoassay (DSL, Webster, TX) with an interassay CV that ranged from 6.0% to 6.3% (normal range for adults aged 40–70 y). Serum osteocalcin was measured with the use an enzymatically amplified ''one-step'' sandwich-type immunoassay (DSL) with an interassay CV between 3.7% and 10.1% (normal range in adults). Serum C-terminal telopeptides of type I collagen were assessed by using an enzyme-linked immunosorbent assay (CrossLaps CTX–Serum, Osteometer; BioTech, Herlev, Denmark) based on 2 highly specific monoclonal antibodies against the amino acid sequence of EKAHD-β-GGR, for which the aspartic acid residue (D) is β-isomerized. To obtain a specific signal in this assay, 2 chains of EKAHD-β-GGR have been cross-linked. The interassay CV was between 6.5% and 8.1%.

The BMD of the lumbar spine, femoral neck, and trochanter were measured by dual-energy X-ray absorptiometry (Delfi A; Hologic, Waltham, MA). The bone densitometry measurements were calibrated against the European Spine Phantom, and the same experienced technicians performed all of the bone measurements.

Statistical analysis

We performed an intention-to-treat analysis. Statistical calculations were performed by using SPSS version 13.5 (SPSS Inc, Chicago, IL). All data are expressed as means $\pm$ SDs. Initial comparisons between the male and female subjects were based on the independent-samples t test, without assuming that SD values are the same for both groups. Comparisons for variables measured at multiple time points were conducted by using the general linear model, with repeated-measures analysis of variance with post hoc testing, looking for interactions between month and sex. Effects of the intervention were examined by using a paired t test, comparing the initial and final values for each subject. Statistical significance was based on a P value < 0.05.

RESULTS

Dietary intakes of calcium and vitamin D (not including what was in the fortified bread) based on the group's 10-d food records of the institution, taken on three 10-d occasions during the study were 717 mg/d and 84 IU/d, respectively. Adherence to vitamin D intake from fortified bread was monitored by questionnaire and

受試者與研究方法

受試者

我們研究了 45 名(男性 17 位，女性 28 位)，年齡在 58–89 歲(平均數±SD: 71 ± 6.9 歲)之間的患者，均爲羅馬尼亞雅西市(Iasi)(緯度：47°N))護理之家住民。本試驗在 2003 年 11 月和 2004 年 12 月之間進行。

研究中已排除患有惡性疾病患者，包括腎臟、肝臟、腸胃失調、需治療的內分泌疾病伴隨鈣新陳代謝不正常(但不排除第二類型糖尿病或甲狀腺機能不足者)、每天喝酒 > 4 杯、及曾經攝取雌激素、黃體素、糖皮質激素(glucocorticoids)、鎮痙劑、維他命 D 補充劑，或在過去 12 個月，曾接受過其他療程，已知會影響鈣或骨骼新陳代謝的藥劑。其他療程在本研究進行中則仍持續進行。並未排除曾經罹患骨折的患者。本試驗協議經羅馬尼亞醫師會研究倫理委員會核可，所有患者均簽有同意書。

研究模型

在本次爲期 1 年試驗中，所有受試者每天均食用強效麵包(圓狀)，其中已添加 800mg 的 $CaCO_3$/d(320mg 元素鈣)，及 125 μg (5000 IU)維他命 D_3/d。受試者在一開始均先體檢、採血及收集 24 小時的尿液樣本，由 2 個點量測骨質密度(BMD)，以評估骨骼肢骨及軸向變動。脊椎骨骨折透過胸柱和腰椎骨的 X 光確定。每 3 個月對受試者採血及收集尿液。收集 24 小時的尿液量，以量測鈣和肌氨酸酐值。由於要老年人收集 24 小時尿液的可靠度甚差，所取得及所呈現的數據，均係總尿量中，鈣與肌氨酸酐兩種化合物的比例，如先前已進行過的研究(14)，已獲臨床確認可做爲高尿鈣症之靈敏量測(15-18)。

飲食記錄

在研究一開始，機構組患者的鈣攝取情形，係根據護理之家所提供的—所有患者都一樣的 10 天飲食紀錄評估，食物紀錄並非患者個人所提供。飲食攝取量評估於 4 個月間隔下重複進行。

強效食物

每天提供給所有受試者的強效食物，爲添加(100 g)與 800mg 的 CaCO3/d (320mg 元素鈣)及 125 μg (5000 IU)維他命 D_3/d 的圓麵包，我們在每個圓麵包用麵糰中，添加 0.5 mL 的含維他命 D_3 (20,000 IU/mL，Vigantol; Merck KGaA、德國 Darmstadt)油溶液。麵糰在 260-270℃烘烤 15 分鐘。估計在處理過程中的損失爲 40-50%。麵包中的維他命 D_3 樣本，係以溶劑中萃取，採用 HPLC 和紫外線探測器(19)量測。維他命 D_3 的平均數爲 5062.2 ± 459.7 IU/bun。要求所有受試者均必須把麵包吃完。

順適性(adherence)及完成率

登記有案的 45 位患者中，有 40 位完成試驗(89%)。未完成者中，有 1 位係個人因素、1 位離開護理之家，其他 3 位死亡(其中 2 位死於慢性心力衰竭及 1 名急性心肌梗塞)。另外 7 位患者是在最後的雙能量 X 光骨質密度儀掃瞄的時候不在場(在最後採血後，立即和親戚離開護理之家，或轉院)。因此，最初和最後的 BMD 數據配對，只有 33 位受試者可資利用。根據研究模型設計，在試驗結束時進行問卷調查，及量測血漿 25(OH)D 濃度評估。

分析方法

在基線值時及 12 個月的加強期間，每隔 3 個月採集一次空腹血液和尿液樣本。 血清、血漿和尿液樣本，

一直冷凍保存到分析階段。血清鈣、磷酸鹽和肌氨酸酐，係使用標準實驗室方法量測。使用免疫分析法(Liaison Analyzer; DiaSorin, Stillwater, MN)量測血清 25(OH)D。使用「2-step」酵素增效三明治型免疫分析法(DSL, Webster, TX)，量測血清完整副甲狀腺激素(PTH)，其組間變異係數 CV 範圍爲 6.0%至 6.3% (40-70 歲成人的正常範圍)。使用「one-step」酵素增效三明治型免疫分析法(DSL)，量測血清骨鈣素(osteocalcin)，其組間變異係數 CV 範圍在 3.7%和 10.1% 之間(成人正常範圍)。使用酵素連結免疫吸附法 (CrossLaps CTX–Serum, Osteometer; BioTech, Herlev, 丹麥)，基於 2 種高度特異的抗氨基酸序列 EKAHD-β-GGR 單克隆抗體，天門冬氨酸殘基(D)被 β-異構化，評估血清 I 型膠原蛋白的碳末端胜鏈。爲由本分析試樣取得具體訊號，2 條 EKAHD-β-GGR 鏈須相互交聯(cross-linked)。組間變異係數 CV 在 6.5%和 8.1%之間。

使用雙能量 X 光骨質密度儀(Delfi A; Hologic,Waltham, MA)，量測腰椎、髖骨頸和轉節(trochanter)的 BMD。骨質測密度量測器依 European Spine Phantom 標準校準，而且由同一批技術純熟的技術員，進行所有的骨質量測作業。

統計分析

我們執行治療意向分析法。使用 SPSS 13.5 版(SPSS 公司、芝加哥、IL) 統計演算。 所有數據以平均數± SDs 表示。男性和女性受試者之間的初始比較，採用獨立樣本 t 檢定，不假設兩群組的 SD 值相同。不同時間點所測得變數之比較，透過一般線性模型，以重複測量變異數分析檢定因果關係，尋找月數和性別之間的相互作用。相互作用採用配對 t 檢定調查，比對每位受試者的初始值和最終值。統計顯著水準爲 $P<0.05$。

研究結果

根據 10 天群組飲食的機構記錄，在研究期間的三個 10 天階段，鈣和維他 D (不包括添加在麵包中的劑量)的攝取量分別爲 717 mg/d 和 84 IU/d。經由強效麵包攝取維他命 D 的情形，係透過問卷調查表監控，結果如下：

91%的患者表示每天均食用強效麵包。每位受試者一般食用的麵包比例如下：

76%的患者吃完整個麵包、9%只吃 75%、12%只吃 50%、3% (1 人)通常僅食用 25%的麵包。無人抱怨麵包口味。