EFFECT OF CALCIUM-CARBONATE FORTIFIED BAKED PRODUCTS ON CALCIUM BALANCE AND RELATED METABOLISM IN ELDERLY FEMALES

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CHAPTER I

INTRODUCTION

Need for Investigation

Nutritional adequacy is one of the major problems for the rapidly growing population of elderly persons in the United States. The problem often arises because of limited income, lack of transportation to food markets, and poor health. Lack of social motivation can result in or be related to a poor diet (23, 55, 59).

Recent studies of disease free elderly people indicate that dietary calcium intake often was found to be below recommended minimal needs (9, 23, 28, 35, 53, 59, 63, 72). Low dietary calcium intake may precipitate the age-related degenerative disease of "osteoporosis" in the elderly, especially for post-menopausal women (31, 40). However, some investigators prefer to attribute the condition to lack of Vitamin D, and consequently, calcium malabsorption (31, 44, 51). Vitamin D deficiency is a rare condition in the United States, but it may occur in a population deprived of adequate exposure to sunshine and ultraviolent light. A comprehensive review of these physical and biochemical mechanisms has been discussed by a number of investigators (31, 34).

The dietary calcium intake of elderly people can be elevated by calcium supplementation. In fact, patients with osteoporosis are routinely given calcium tablets (3, 17, 32). This procedure assumes that no ancillary problems exist such as malabsorption of calcium.

Fortification of foods with calcium salts augments the absorption of calcium. Incorporation of calcium in food products was highly recommended by Epstein et al. (15), since absorption of calcium may be facilitated by wider distribution in the foods ingested.

Evidence has been obtained by Grotkowski and Sims (22) that calcium levels can be enhanced by fortifying "snack food" products which are popular with elderly persons. MacLeod, Judge, and Caird (41) also recommended the use of calcium fortified baked products including cookies, breads, and biscuits as foods which find favor with the elderly.

Objectives of the Study

The primary objective of this study was to determine if fortification of baked food products with a calcium salt is a worthwhile method of improving the calcium intake of healthy, elderly female subjects.

Secondary objectives encompassed the following:

1. To use calcium-carbonate to fortify baked products in order to provide an additional calcium intake equal

to one-half to two-thirds of the Recommended Dietary Allowances.

2. To determine calcium utilization from the baked products fortified with calcium carbonate as ascertained by the calcium balance method.

3. To observe the effect of Vitamin D supplementation on calcium utilization from the fortified baked products.

4. To ascertain if 1000 mg of calcium intake is an acceptable amount for maintaining positive calcium balance or calcium equilibrium in healthy, elderly women.

CHAPTER II

REVIEW OF LITERATURE

The elderly population group of the United States is large and expanding. There are now more than 42 million persons over 55 years of age, 32 million over 60, 8.5 million over 65, and 1.9 million over 85 years of age. A substantial majority of older persons are females. At the present time the ratio is 100 females for every 69 males over 65 years of age (66).

Increasingly nutritionists are called upon to consider nutritional requirements of the elderly. A number of surveys concerning dietary practices, food habits, and nutrient intake of this population group have been conducted (8, 10, 22, 23, 28, 33, 39, 53, 59, 63, 72). However, these studies do not justify making any broad generalizations for the entire country.

Dietary Calcium Intake of Elderly Americans

Dietary survey studies assessing the nutrient intake of older Americans have been summarized by O'Hanlon and Kohrs (53). These authors concluded that calcium is one of the nutrients most often deficient in the diets of persons past 59 years of age, with women more likely to exhibit a deficiency than men. The mean calcium intake for females falls short of the standard 533 mg (two-thirds of the Recommended Dietary Allowance) used in a number of studies as a guideline. These observations were conclusive irrespective of the methodology used to collect the dietary data.

The Ten-State Nutrition Survey (72) reported that the calcium intake of male subjects in low income states was 869, 539, and 461 mg for the ethnic groups of white, black, and Spanish-Americans, respectively. The comparable figures for female groups were 623, 488, and 586 mg, respectively.

The dietary intake and nutritional status of nursing home residents aged 63 to 93 years, in the state of Indiana, were reported by Justice, Howe, and Clark (33). Of the 44 subjects investigated, 19 consumed more than 800 mg of calcium per day; however, 20 subjects fluctuated between 400 and 800 mg. Five of the residents consumed less than 400 mg daily.

A study by Clark and Wakefield (10) investigated the food habits and food patterns of 99 elderly individuals living in nursing homes and 98 elderly who lived independently. The average nutrient intake for this group, all of whom were over 70 years of age, was adequate in every area except calcium. In this study 28 percent of the subjects failed to receive 50 percent of their calcium needs.

Brown et al. (9) studied a group of elderly individuals living in Rhode Island. Ten-day dietary intakes of 23 institutionalized elderly (9 men and 14 women), and 20 individuals (6 men and 14 women) living independently, between 70 and 96 years of age, were compared to the Recommended Dietary Allowances. Although 57 percent of the subjects had nutrient intakes below the Recommended Dietary Allowances, the average calcium intake of this group was higher than in the above mentioned studies. The mean calcium intake for women living at home was 570 mg;for those living in nursing homes it was 641 mg. The elderly males living at home consumed 665 mg, while those living in a nursing home consumed 614 mg of calcium.

Guthrie, Black, and Madden (23) examined the dietary practices of 109 elderly persons living in rural Pennsylvania, using a personal interview to obtain a 24-hour dietary recall. The investigators found that 65 percent of the 40 men and 65 percent of the 69 women received less than two-thirds of the RDA for calcium. The average intake of calcium for the males was 568 mg and for the female group was 493 mg.

Recently, Stiedemann, Jansen, and Harrill (70) investigated the nutritional status of 23 male and 24 female nursing home residents, aged 60 to 98 years. In general, the quantity of food provided the RDA for most nutrients for both sexes; however, calcium was the one nutrient least likely to be supplied in adequate amounts. Of the females, 43 percent received less than their necessary 530 mg per day.

In many instances, low dietary calcium in the aged results from the simple rejection or low consumption of milk and calcium rich foods. A 24-hour recall survey by Pelcovits (55) revealed that 20 percent of the 35,000 elderly surveyed did not consume milk products. The study in Indiana conducted by Justice, Howe, and Clark (33) also indicated that 50 percent of the elderly surveyed consumed not more than one cup of milk daily. In an across the board survey of six different nursing homes where milk and milk products provided generous quantities of calcium, in excess of 900 mg daily, Henrikson and Cate (28) found the ingestion of these foods was low. The mean calcium intake was less than 500 mg daily. More than 87 percent of these subjects failed to meet their calcium allowance, with the female subjects exhibiting generally lower calcium levels than males. The mean for females was 500 mg, with a range of 200 to 1100 mg. The mean for males was 600 mg, with a range of 200 to 700 mg.

Comparable results were observed among a group of elderly persons in England. In these subjects, milk provided 33 percent of the calcium intake for male subjects, and 44 percent of the calcium intake for female subjects. The remainder was provided by biscuits, cakes, bread, cheese, and cheese dishes (41).

In a survey study conducted with 104 senior citizens, aged 60 years of age and older, Rountree and Tinklin (63) indicated that 80 percent of these individuals understood the significance of calcium in their diet as well as good sources for calcium. However, less than two-thirds of them reported daily use of milk as well as some additional dairy products.

Low Dietary Calcium and Vitamin D Intakes

A low calcium intake results in negative calcium balance, and consequently a loss of body calcium. However, it is not always true that negative calcium balance is the only factor in bone calcium loss. In experimental animals, low dietary calcium does lead to osteoporosis, but not to osteomalacia or hypocalcemia. In man, a dietary deficiency of calcium has been postulated as a possible contributing factor to senile osteoporosis in alcoholics. Experimentally, it can be demonstrated that a low calcium intake is followed by an increase in parathormone secretion and an increase in bone resorption of calcium in spite of an increase in intestinal absorption. This increase in absorption, in itself, may be insufficient to prevent the incidence of negative calcium balance, paralleling low calcium intake (64).

In a survey of cortical bone density of institutionalized females, Justice, Howe, and Clark (33) found that the low bone densities could not be correlated, significantly, with the low calcium intake levels during the course of the study. However, a comparison of the bone density of these women with normal values for women of the same age suggested borderline osteoporosis.

A radiologic survey study conducted by Smith and Frame (67) included over 2000 ambulatory women, aged 45 to 90 years. Evidence of an age-associated, progressive loss of vertebral density was found. When matched for height, weight and age the amount of axial and appendicular bone was unrelated to the amount of ingested calcium.

Lutwak (39) has shown that, in reviewing some survey studies, osteoporosis and low dietary calcium intake tend to parallel each other. However, there seems to be no established relationship between compact bone density and calcium intake in a broad range of subjects, ranging in age from 20 to 75 years. The fact that compact bone remains relatively constant suggests that the minimum daily requirement for calcium may be below 400 mg per day (19).

As pointed out by Nordin (50) and Marshall, Nordin, and Speed (42), the calcium absorption rate in the intestines decreases with age, predominantly as a result of changes in the active transport mechanisms. Some investigators have considered the malabsorption a consequence of low Vitamin D levels (44, 50). In Scotland inadequate Vitamin D intake levels were observed in 300 subjects more than 60 years of age. In female subjects, the daily intake level was often below 100 I.U. per day. The radiological and biochemical evidence for the presence of osteomalacia was not clearly evident. There was a greater tendency for bone rarefaction to parallel low calcium intakes in men than in women (44). After observing ingested and net absorbed calcium in 212 cases of calcium balance, Nordin et al. (51) stated that calcium malabsorption in elderly subjects is not simply a matter of Vitamin D deficiency. The investigator pointed out that there are two possible explanations for this observation. These were exposure to the sun (ultraviolet light), and inadequate dietary calcium intake. This line of evidence was supported by the measurement of plasma 25-OH-D_3 levels in normal young people and in older people, and in cases of fractured neck of femur which show a highly significant reduction in plasma levels in old people. The second factor to be considered is a "resistance" to Vitamin D absorption, associated with impaired hydroxylation of 25-OH-D_3 to 1,25-$(OH)_2D_3$. The response of the elderly to supplementary Vitamin D therapy suggested that this may be a matter of concern. If this line of evidence can be supported, the presence or absence of adequate ultraviolet exposure, in itself, would not explain why people who live in areas with intense daily sunlight also suffer from osteoporosis and related deficiency conditions. Chronically ill persons, hospitalized for long periods, evidence Vitamin D deficiency conditions (32).

An experimental study of six elderly subjects who received supplementary calcium at the rate of 10 gm calcium-carbonate with their breakfast, resulted in a rise in serum calcium to a level of 0.69 mg/100 ml. However, this level was less than 50 percent of that of a matching group of young people (1.47 mg/100 ml). One week of pre-treatment, with 5000 units of calciferol daily, resulted in a rise to 1.39 mg/100 ml. Supplementation of the diets of the group of young people with Vitamin D did not increase their blood calcium levels. The study indicates that even with adequate ultraviolet exposure, an elderly person can exhibit sub-clinical Vitamin D deficiency (15).

Nayal et al. (49) studied dietary intake, sunlight exposure, and the 25-hydroxy-vit D level of the 62 patients admitted to a geriatric unit. After a nine-month observation period the investigators claimed that in old age dietary deficiency may be a more important cause of metabolic bone disease than limited sunlight exposure.

Requirements for Calcium

Calcium is one of the nutrients for which it is difficult to establish a minimum daily requirement, since it functions interdependently with many other nutrients and hormones. Food and Agriculture Organization and World Health Organization experts have stated that there is no clear-cut disease due to calcium deficiency which has been described in connection with the health of the adult human male or female (14). The Food and Nutrition Board of the National Research Council (47) has recommended a daily allowance of 800 milligrams for the older adult population.

Lutwak (40) collected 212 calcium balances on 84 normal subjects. Negative calcium balances were observed at intakes below 600 mg of calcium daily, but were positive at intakes above this level. The investigator suggested a dietary level of 800 mg daily to meet the minimum requirement of normal persons. The calcium requirement of post-menopausal women, however, is substantially higher. Some investigators believe that 800 milligrams daily is not enough for the elderly whose absorption capability may be impaired and who continue to exhibit obsteoporosis in the presence of seemingly adequate daily intake by present standards. Recommended intakes of 1000 mg of dietary calcium daily has been suggested by Albanese et al. (3) and by Lutwak (40). Jowsey (32) proposed as much as

1400 mg calcium daily. A study of 110 normal adult women between the ages of 35 to 50 years was conducted by Heaney, Recker, and Saville (25). The study revealed that the subjects were often in negative calcium balance ($\mp$ 0.065 gm/day) on their usual diet which provided 668 mg/daily. The investigators suggested that about 1200 mg calcium per day would be sufficient for those women. A further study by these investigators along this same line was conducted with 35 to 50 year old women, receiving 1240 mg calcium daily. These women were just on the borderline of meeting their calcium needs. Several of this group had slightly negative balances. The subjects exceeding 1240 mg of calcium daily maintained their calcium balance levels slightly on the positive side (26).

Calcium Supplementation and Fortification

Adequate dietary calcium intake of elderly people can be achieved by calcium supplementation. This is not a new idea; in fact, calcium tablets are frequently prescribed to patients with osteoporosis. A suggested supplementation of calcium is about 700 to 900 mg (3, 16). Khairi and Johnson (34) suggested 1500 mg of elemental calcium daily.

The optimal intake of calcium for absorption in man is uncertain. Data from a study of 180 adult human subjects indicated no detectable upper limit for absorption capacity.

Heaney, Saville, and Recker (24) found that at an intake load of 7.48 gm calcium, absorption averaged more than 1.0 gm per day. Absorption of calcium was observed as a simple linear function of intake up to 2 to 3 gm per day.

Berstad et al. (7) reported a study of ten healthy adult subjects, aged 23 to 60 years. The subjects ingested one of three different commercial calcium preparations (either NAF or Collett, each containing $CaCO_3$; or Sandoz, containing mainly calcium lacto-gluconate). Intestinal calcium absorption from the three preparations was not found to differ significantly. With each of the three supplements, the calculated minimal absorption of calcium was found to be about 100 mg on an intake of 500 mg of elemental calcium daily. A similar result was observed by Rao and Rao (58) in a comparison of calcium absorption from calcium-sulphate and calcium-lactate. No difference was observed in the absorption and retention of calcium from these two salts when four healthy, young men received about 200 mg of calcium daily, consumed with the meals.

Absorption of a single large oral dose of calcium-carbonate containing 4,000 mg of elemental calcium was investigated by Epstein et al. (15). Ingestion of this calcium salt, at the fasting stage, caused a rise in serum calcium level of the six young adult subjects. The mean rise in serum calcium of 1.47 mg/100 ml was observed at two hours.

The same load, given with breakfast, produced a higher mean peak value of 11.7 mg/100 ml, with hypercalcemia sustained for two hours.

A single dose of 500 mg of a $CaCO_3$ suspension administrated to 25 male patients with uncomplicated duodenal ulcer was investigated by Behar, Hitching, and Smyth (6). Serum gastrin and gastric acid output of the subjects was slight, but did not significantly change the serum calcium. A similar rise in serum calcium during an intravenous infusion of calcium gluconate failed to increase serum gastrin and gastric acid output. Both intragastric calcium actions were abolished by acidification of the calcium-carbonate solution. Unfortunately, the effect of calcium salts on the serum gastrin and gastric acid output of normal people was not tested and/or mentioned in the literature.

The benefit of calcium supplementation on bone density was studied by Albanese et al. (2). Two groups of healthy females, aged 53 to 88 years, participated in the study. One group of 17 subjects was given a dietary supplement of 750 mg of calcium as a calcium-carbonate salt, with 375 USP units of Vitamin D_2 per day. The bone density index was 90.6 mils, which was close to that of a matching placebo group density index of 90.3 mils. The diet provided calories and nutrients well within the range suggested for this age group by the Food and Agriculture Organization of the United

Nations. However, the self-selected calcium intake was 40 percent of the USRDA. During an average test period of eleven months, the supplemented group maintained pretest or slightly higher levels of bone density; whereas, the bone density of the phalanx 5-2 decreased significantly in the placebo group. During the same period, the serum cholestrol level decreased significantly in the experimental group. No changes were observed in serum calcium and phosphorus levels, Ca/P ratio, or urinary calcium in either the supplemented or placebo groups.

The effect of calcium supplementation on serum cholesterol level was investigated by Bhattacharyya et al. (8). Eleven males, aged 21 to 26 years, were fed in a switchback pattern diet containing either saturated or polyunsaturated fat, each with two different levels of calcium. Each diet was given for a two-week period. One gram of either calcium-carbonate or calcium-gluconate, incorporated into bread flour daily, reduced the cholesterol levels of the subjects on the saturated fat diet. No change in the serum cholesterol level was observed with the polyunsaturated fat diets containing either low or high levels of calcium. Also, serum phospholipids and triglyceride levels were not changed significantly.

An experimental study by Kilgore et al. (36) reported calcium utilization by rats fed hamburger fortified with calcium-carbonate. The patties were prepared from ground beef. The patties contained the following, either singly or in combinations: no calcium or 4 gm calcium; no Vitamin A or 15,000 I.U. Vitamin A; no ascorbic acid or 180 mg ascorbic acid. The added nutrients did not affect the flavor, texture, or the cooking losses of fat and water. The cooked patties were mixed with buns and mayonnaise, then fed to weanling rats. The serum calcium levels of the rats eating hamburgers supplemented with calcium, was doubled.

In India, a study was concerned with the enrichment of a rice-based diet with curry powder, fortified with calcium-carbonate at the level of 10 or 20 gm per 100 gm. The addition of calcium-carbonate did not change the acceptability of the product. There was no loss of nutrients during the use of the curry powder for the preparation of the national dishes (57).

CHAPTER III

PROCEDURES OF THE INVESTIGATION

The success of a nutritional investigation, especially a balance study, is entirely dependent upon the manner and to what degree the parameters of the study can be controlled. That is why collection of the data and analysis of it are given in detail.

Programming the Investigation

The experimental period began at 7:30 A.M. on May 31, 1978 and ended at 7:30 A.M. on June 27, 1978. This season of the year was especially chosen because the subjects could be expected to be physically active and yet not be faced with excessive perspiration that can occur during the summer. This time of the year was recommended by Morgan, Rivlin, and Davis (45). Holidays were avoided during this period.

Management of the Subjects

Selection

Potential subjects were chosen from a local residence home for the elderly, former participants in experimental programs at the Texas Woman's University and others suggested by the participants. Each subject was contacted by a personal letter.

The study required that the subjects met the following criteria: be female, over 65 years of age; be in good health and be free from any known major bone disorder, gastrointentinal disorder, or kidney disorder; had no major surgery or long-term period of bed rest for six months prior to the initiation of the study (16). Physically active people were recruited. Subjects were also selected for attitude (motivation), self-discipline, and the desire to cooperate.

All subjects were interviewed privately and a detailed explanation of the purpose and responsibilities was presented. Each subject signed a consent form approved by the Human Research Review Committee of the Texas Woman's University. Personal information about the dietary habits of subjects was obtained. Subjects selected were asked to complete a three-day dietary record for the purpose of evaluating their approximate intake of calcium and other nutrients. In a final evaluation, the subjects were examined by a physician for evidence of physical health status. A blood sample was screened, using SMAC (Sequence Multiple Analysis by Computer-Technicon).

Housing and Transportation

Eight females, aged 67 to 91 years, participated in the present study. Three of the subjects were living in the

Fairhaven Home. Fairhaven is a residence home for healthy, elderly individuals and is not a care facility. Five subjects were living alone at home. All of the subjects came to the Nutrition Department of Texas Woman's University for three meals daily, every day of the week. Texas Woman's University provided air-conditioned transportation for those living at Fairhaven and those living independently. One of the subjects drove her own car to the university daily. Two subjects lived within a short walking distance of the campus. Activities of the subjects were not restricted in any way. Daily weather temperatures were recorded from published records and are reported in Table 1.

All of these subjects were accustomed to eating three meals daily. As expected, their calcium intake (from dietary records) varied from individual to individual. The general cooperative level and motivation of the subjects was very high. With the exception of partaking of meals, the subjects were completely unrestricted and continued their normal, everyday activities. Logbooks maintained by all subjects (see Appendix A) provided helpful information concerning any possible emotional or physical stresses the subject encountered. This information was of broad general usefulness in motivating the subjects during the course of the investigation.

TABLE 1

DAILY ENVIRONMENTAL TEMPERATURE RANGE DURING THE PERIOD OF THE CALCIUM BALANCE STUDY

Date	Temperature Range (Fahrenheit Degrees)	
	Highest	Lowest
May 31	90	70
June 1	87	68
June 2	--	65
June 3	88	66
June 4	--	--
June 5	90	72
June 6	84	72
June 7	87	66
June 8	87	63
June 9	--	61
June 10	93	66
June 11	94	76
June 12	97	75
June 13	88	70
June 14	94	72
June 15	96	73
June 16	--	74
June 17	91	75
June 18	94	77
June 19	96	73
June 20	96	75
June 21	98	74
June 22	98	76
June 23	100	77
June 24	99	77
June 25	98	77
June 26	97	76
June 27	97	77
Average	93	72

Contractual Obligations of the Subjects

Although the subjects were all volunteers, additional motivation was provided in the form of a contract for one hundred fifty dollars upon completion of the study (see Appendix B and C). According to the terms of the contract, the subjects agreed to ingest the food provided for them and to collect daily urine and fecal samples. Subjects also gave permission to have blood samples taken periodically as well as making themselves available for X-rays of the hand.

Supervision

Although, in what might be referred to as "free living" conditions, the subjects remained under general supervision with respect to their daily eating habits. The daily feeding of all subjects was observed by the principal investigators. Special attention was given to the ingestion of vitamin supplements and the fecal markers. Subjects agreed not to eat outside of the laboratory, and were encouraged to report any food ingested other than the food served in the laboratory.

Basic Design of the Study

The balance study consisted of three, eight-day cycles. Eight subjects were randomly divided into two groups. Following a four-day period of adaptation, four of the subjects received the experimental diet, while the second group continued on a control diet which included identical food items except for the baked products which were not fortified with calcium carbonate. After the first cycle was completed, the diets of the two groups were reversed. The subjects were never aware of times when they received baked products fortified with calcium-carbonate. A Vitamin D supplement, in addition to the calcium fortified baked products, was ingested during the final eight-day experimental period by all subjects. Half of a cholecalciferol tablet, about 200 I.U., was given at breakfast to each subject.

Dietary Plan

A three-day menu cycle was used. Certain vegetables, such as spinach and legumes, were excluded because of the relationship to calcium absorption (21, 47). The master menus provided a sufficient amount of kilocalories, protein, fat, carbohydrate, vitamins, and minerals for women of this age group (see Appendix D). Nutrient content of the three-day master menu cycle is shown in Table 2. During the adaptation period, there was a small change made in the amount of food served for two subjects. This change was provided to satisfy their hunger and current eating habits.

TABLE 2

CALCULATED* NUTRIENT CONTENT OF THE MASTER MENU

Nutrient	Day 1		Day 2		Day 3	
	Unit	% RDA	Unit	% RDA	Unit	% RDA
Kilocalories	1508	84	1576	88	1549	86
Protein (gm)	65	140	63	136	57	124
Fat (gm)	53	--	61	--	54	--
Carbohydrate (gm)	163	--	198	--	218	--
Phosphorus (mg)	912	114	832	104	797	100
Vitamin A (I.U.)	3199	80	6015	151	3403	85
Iron (mg)	11	105	9	93	12	121
Thiamin (mg)	0.96	96	0.92	92	1.05	105
Riboflavin (mg)	1.47	134	1.18	107	1.34	122
Niacin (mg)	17.48	144	14.21	118	12.42	104
Vitamin C (mg)	93	206	95	211	104	231
Calcium** (mg)	495	62	670	84	577	73
Vitamin D (I.U.)	81	--	56	--	56	--
Calcium (mg) (diet plus fortification)	908	114	1078	134	1022	128
Vitamin D (I.U.) (diet plus supplement)	281	--	256	--	256	--

*Ohio State Dietary Data Base (54).
**Atomic Absorption Spectrophotometer (Perkin-Elmer Model 303).

The nutrient intake of each individual was kept as constant as possible on a day-to-day basis.

The control diet provided about 426 to 508 mg of calcium daily, calculated according to the Ohio State Dietary Data (54). The experimental diet contained approximately 500 mg additional elemental calcium from the fortified baked products. One serving of each of the baked products, as shown in Table 3, did not exceed 175 mg of calcium.

The subjects were free to use sugar, sugar substitute,[1] non-dairy creamer,[2] black pepper, salt, vinegar, and lemon juice with the meals if they so desired. Subjects were free to take any prescribed medicine. A few of the subjects received drugs for high blood pressure as prescribed by their personal physician.

Food Preparation

The foods fortified with calcium-carbonate included yeast rolls and chocolate-chip, sugar, and oatmeal cookies. These products were prepared in the research kitchen of the Nutrition Department of the Texas Woman's University. The recipes are given in Appendix D. The additional amounts of calcium-carbonate were based on one serving of the products. One serving provided approximately 150 mg of elemental calcium. Table 3 gives the calcium

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[1]Sweet 'n Low: Cumberland Packing Corp., Brooklyn, N.Y. 11205.

[2]Non-dairy Creamer: Luckerne, Oakland, CA 94660.

TABLE 3

CALCIUM CONTENT OF ONE SERVING OF BAKED PRODUCTS SERVED TO EIGHT HEALTHY ELDERLY WOMEN, AGED FROM 67 TO 91 YEARS, DURING EACH EXPERIMENTAL PERIOD

Food	Serving Size (Uncooked dough)	Analysis of Calcium Content of Baked Products	
		AAS*	Calculated**
	gm	mg	mg
Chocolate Chip Cookie	21	7	7
Chocolate Chip Cookie with calcium-carbonate	21	148	155
Sugar Cookie	20	15	16
Sugar Cookie with calcium-carbonate	20	188	163
Oatmeal Cookie	20	8	8
Oatmeal Cookie with calcium-carbonate	20	140	157
Yeast Rolls	40	24	21
Yeast Rolls with calcium-carbonate	40	160	172

*Atomic Absorption Spectrophotometer (Perkin-Elmer Model 303).

**Composition of Foods, Agricultural Handbook No. 8, Agricultural Research Service, USDA (71).

contents of the baked products. The uncooked dough was weighed in individual serving sizes before being baked. The products were kept in a freezer until they were to be served.

Groceries for the meals were bought by contract from a local supplier. In order to keep the same brand names and batches, frozen dinners, canned vegetables, frozen fruits juice, and cereals were purchased at one time. Fresh vegetables (tomatoes and lettuce), and fruits (apples and bananas), milk, and bread were bought as needed.

Foods, except for those foods packaged individually such as frozen dinners, mayonnaise, sugar, etc., were accurately weighed on a Hanson dietetic Scale shortly before serving. Cookies were brought to room temperature shortly before serving, but the yeast rolls were warmed in a micro-wave oven.

Calcium content of the foods for which the food composition values (71) indicated over 50 mg in a 100 gm portion was analyzed by the Atomic Absorption Spectro-photometer. Table 4 gives the calcium content of the foods served during the study. The food samples for analysis were taken during the first days of the study. Samples for baked products were taken randomly from the batches of baked products. The samples were stored in a plastic bag and kept in the freezer.

TABLE 4

CALCIUM CONTENT OF 100 GRAMS OF EDIBLE FOODS SERVED TO EIGHT HEALTHY ELDERLY WOMEN, AGED FROM 67 TO 91 YEARS, DURING EACH EXPERIMENTAL PERIOD

Foods	Analysis of Calcium Content of Foods	
	AAS*	Calculated**
	mg	mg
Asparagus--canned	15	19
Bacon	31	14
Beans--green, canned	39	45
Beef--ground	22	12
Beef--roast beef with gravy, frozen	16	16
Bread--white, enriched	93	96
Chicken--whole, dark and white	11	10
Cornflakes	22	17
Cream of wheat, prepared with water	20	9
Cake--pound	76	79
Crackers--graham	90	88
Eggs--scrambled	45	45
Ham--cold cut	45	10
Ice-cream--vanilla	114	78-146
Macaroni and Cheese--frozen	115	181-83
Milk--nonfat, fluid	138	143
Peas--canned	26	25
Potato--new, boiled, canned	12	10
Potato--mashed, prepared with water, milk, and fat	16	10
Salami	44	14
Spaghetti with meat balls	17	95-45
Turkey with gravy--frozen	26	10
Turnip greens--frozen	124	118
Vanilla Wafers	97	41

*Atomic Absorption Spectrophotometer (Perkins-Elmer, Model 303).

**Composition of Foods, Agricultural Handbook No. 8, Agricultural Research Service, USDA (71).

Analytical Procedures

Collection of Urinary and Fecal Samples

All urine and fecal samples were collected during the entire period of the investigation. Fecal and urine samples were collected separately in special polyproylene containers (Specipan) which were placed beneath the commode lid. This facilitated the ease of sample collection.

Subjects brought urine samples contained in plastic bottles to the laboratory. All urine samples for a given subject, for the same 24-hour period, were pooled in a larger bottle and kept in the refrigerator. A few drops of acetic acid were added to each sample. After the total 24-hour sample was collected total volume was recorded and an aliquote was placed in a small plastic bottle and frozen for chemical analysis.

Autoclaved Carmen Red Dye in Number 0 gelatin capsules was used as fecal marker to separate each experimental period. Fecal samples were deposited upon a sheet of aluminum foil within the Specipan. Each speciman was then placed in a plastic bag and labelled with the name of the subject and the date of collection. Four days of fecal samples for each individual were pooled and blended with the addition of equal or double amount by weight of distilled water. An aliquot was taken in a jar and frozen for further chemical analysis.

Chemical Analysis

Calcium content of metabolic body excretion and food samples was analyzed by a procedure using the Atomic Absorption Spectrophotometer Pelkin-Elmer Model 303 (56) and/or by methods adapted from Robinson (62) and Heanley, Recker and Saville (26). The samples were burned in Atomic Absorption Spectrophotometer after the final dilution with 0.5 percent lanthanum-oxide.

Urine Analysis

Urinary calcium content was determined in duplicate for each subject every day. Two milliliters of the urine sample were diluted up to 100 ml with 0.5 percent lanthanum oxide as the direction given by the Atomic Absorption Spectrophotometer (56).

Creatine excretion of each subject was determined in order to check validity of the total volume of 24-hour urine. A modificated Folin Method (4) was applied.

Fecal Analysis

Fecal calcium excretion of each subject for four-day periods was determined. Duplicate samples, approximately 5 gm, were placed in an acid washed porcelain crucible and burned overnight in a high temperature oven (approximately 500° C). The dry ash was dissolved in approximately 15 to 20 ml of 6 N HCl, then filtered through a Whaltman No. 42 paper. The filtrate was collected in a 100 ml volumetric flask, brought to the final volume with 0.5 percent La_2O_3. Further dilution with 0.5 percent La_2O_3 was made up to 1:100 dilution in duplicate from each sample (56).

Blood Analysis

A fasting blood sample of each subject was drawn at the end of each experimental period. Approximately 5 ml of blood were drawn each time with the exception of 10 ml at the final test period. Serum was prepared immediately after the blood was drawn.

Calcium analysis. For the calcium determination, serum protein was coagulated by 10 percent of TCA and then 0.1 ml of serum was diluted to 10 ml with 0.1 percent La_2O_3 for analysis with an Atomic Absorption Spectrophotometer.

Phosphorous analysis. Serum phosphorous of each subject was assessed in duplicate or triplicate by a modified molybdate, Fiske and Subbarous method (4).

Alkaline phosphate analysis. Total alkaline-phosphatase enzyme activity was determined in triplicate by using the kits of the Sigma Chemical Company (65). Fresh serum was used each time, with one exception, on June 19, 1978. This test was repeated.

Alkaline phosphatase isoenzyme separation. The kit of Helena Laboratory was used for alkaline phosphatase isoenzyme separation. Five micromilliliter of fresh serum was dropped over a cellulose acetate plate and substrates were prepared for visual and densitometric examination. Isoenzyme bands were scanned in a densitometer of Quick-Scan with a green filter (nm 510) (27).

Food Analysis

Approximately 5 gm of the food samples in duplicate were burned overnight in a high temperature oven at 400-500° C. Dry ash from the samples was dissolved with approximately 15 to 20 ml of 6 N HCl and filtered through Whaltman No. 42 paper filtrate paper into a 100 ml or 50 ml of volumetric flash, brought to the final volume with 0.5 percent La_2O_3. Further dilution with 0.5 percent La_2O_3 varied from one food item to another, based on the possible calcium contents.

Milk and Ice Cream Analysis

Fresh milk and frozen ice cream samples were prepared in triplicate for determination of calcium content. Approximately 5 gm samples were coagulated by 50 ml of 24 percent TCA (w/v) in 100 ml volumetric flash, and brought to volume with distilled water, then filtrated through Whaltman No. 42 filtrate paper. The 5 ml aliquot of the filtrates was diluted with 0.5 percent La_2O_3, in duplicate (56).

Bone Density

Roentgenograms of the left hand of each subject were made at the end of each experimental period in order to assess bone density. Texas Woman's University bone density assessment method was applied (5, 73). Roentgenograms were made with a six-kilovolt peak GE on Imperial Unit and Kodak Industric TREX film AA2. An aluminum alloy wedge was placed adjacent to the hand. By using the Joyce Leob Microdensitometer, 3 CS, three single densitometric scans were made across the roentgenogram of the non-immersed middle phalanx of the little finger. These scans were: one approximately 1 mm distal, another 1 mm proximal end, and the third at the mid-shaft of the phalanx.

Radiologic cortical thickness of metacarpal III-2 and III-4 of the left hand were also determined. Postero-anterior radiographs of the left hand of the subjects were projected at a magnification of approximately 10 times onto drawing paper and the projected cortical limits of the metacarpal III-2 and III-4 were marked in ink. With a millimeter ruler, thickness of the "medial" and "lateral" cortices of the shaft at the cortex were measured, then divided by the lateral diameter of the shaft at that level. This fraction was multiplied by 100 (5).

Calculations of Balance

The following formula was used to calculate the calcium balance of subjects per day.

$$\text{Calcium Balance} = \text{Intake Calcium (actual)} - \text{Output Calcium (Urinary + Fecal)}.$$

The calcium intake of each subject was based on the chemical analysis (by the Atomic Absorption Spectrophometer) of the foods served during the study. Similarly, the calcium excretion, via fecal and urine of each subject, was analyzed and calculated by the same chemical method.

Statistical Analysis

The nutrient content of the menus and the three-day dietary records of the subjects were computed by Ohio State Nutrient Analysis (54).

In order to detect significant differences between the dietary treatments (low calcium; calcium-carbonate fortified baked products; and calcium-carbonate fortified baked products, with Vitamin D supplementation), a single factor analysis of variance test, with repeated measures, was used. This procedure was applied to the evaluation data for blood, calcium, phosphorous, calcium to phosphorous ratio, and total alkaline-phosphatase enzyme activity, as well as to the calcium balances and bone densities. All the data were analyzed by Tukey test at $P = 0.05$ level. A regression line for the amount of calcium intake versus calcium balance was obtained for each subject individually.

Simple correlations were calculated for each subject between the three-day dietary records and calcium balance at the adaptation period, and between the three-day dietary record and bone densities.

CHAPTER IV

RESULT AND DISCUSSION

Calcium Balance

Fortification of baked products with calcium-carbonate improved calcium retention by the subjects. Table 5 summarizes the calcium balance of the subjects. Six out of the eight subjects were in negative balance during the adaptation period. Based on the three-day dietary record for each subject collected before the study, the average intake of calcium was in the range of 213 to 1181 mg daily.

The correlation between previous dietary intake and calcium balance during the adaptation period was moderate ($r = 0.602$). Subjects who had previously consumed more calcium-rich foods exhibited greater negative balance.

Figure 1 illustrates calcium balance for both experimental groups for each eight-day experimental period. Significantly more calcium was retained by the subjects of Group I, who were continued on the same control diet following the adaptation period. The calcium balance of these subjects improved significantly, when they were fed the

TABLE 5

SUMMARY OF THE CALCIUM BALANCE OF EIGHT HEALTHY ELDERLY WOMEN, AGED FROM 67 TO 91 YEARS, DURING EACH EXPERIMENTAL PERIOD

Subject No.	Adaptation 497-670 mg Ca/day	Experimental Periods: Low Calcium 497-670 mg Ca/day		High Calcium 408-1078 mg Ca/day		High Calcium with Vitamin D 908-1078 mg Ca, 200 I.U. Vit D Day	
		First 4 Day	Second 4 Day	First 4 Day	Second 4 Day	First 4 Day	Second 4 Day
Group I							
3368	- 12	+ 50	+185	- 16	+281	+ 68	+257
3370	-108	+184	+ 68	+381	+295	-161	+148
3372	+ 22	- 23	- 20	+294	+ 41	+308	+153
3377	-365	-339	- 62	+199	+531	+ 2	+151
Mean	-116	- 32	+ 43	+215	+149	+ 54	+177
Group II							
3369	-328	+157	+191	+539	+ 44	+275	+393
3371	+ 45	+174	+114	+261	+246	+169	+246
3374	- 83	-101	+149	+ 35	+487	+120	+418
3375	-401	-261	- 83	+144	+ 17	+267	- 17
Mean	-192	- 8	+ 92	+245	+199	+208	+260
Group Mean	-154	- 20	+ 63	+230	+174	+131	+219
Group Mean for 8 Days		+22		+202		+175	

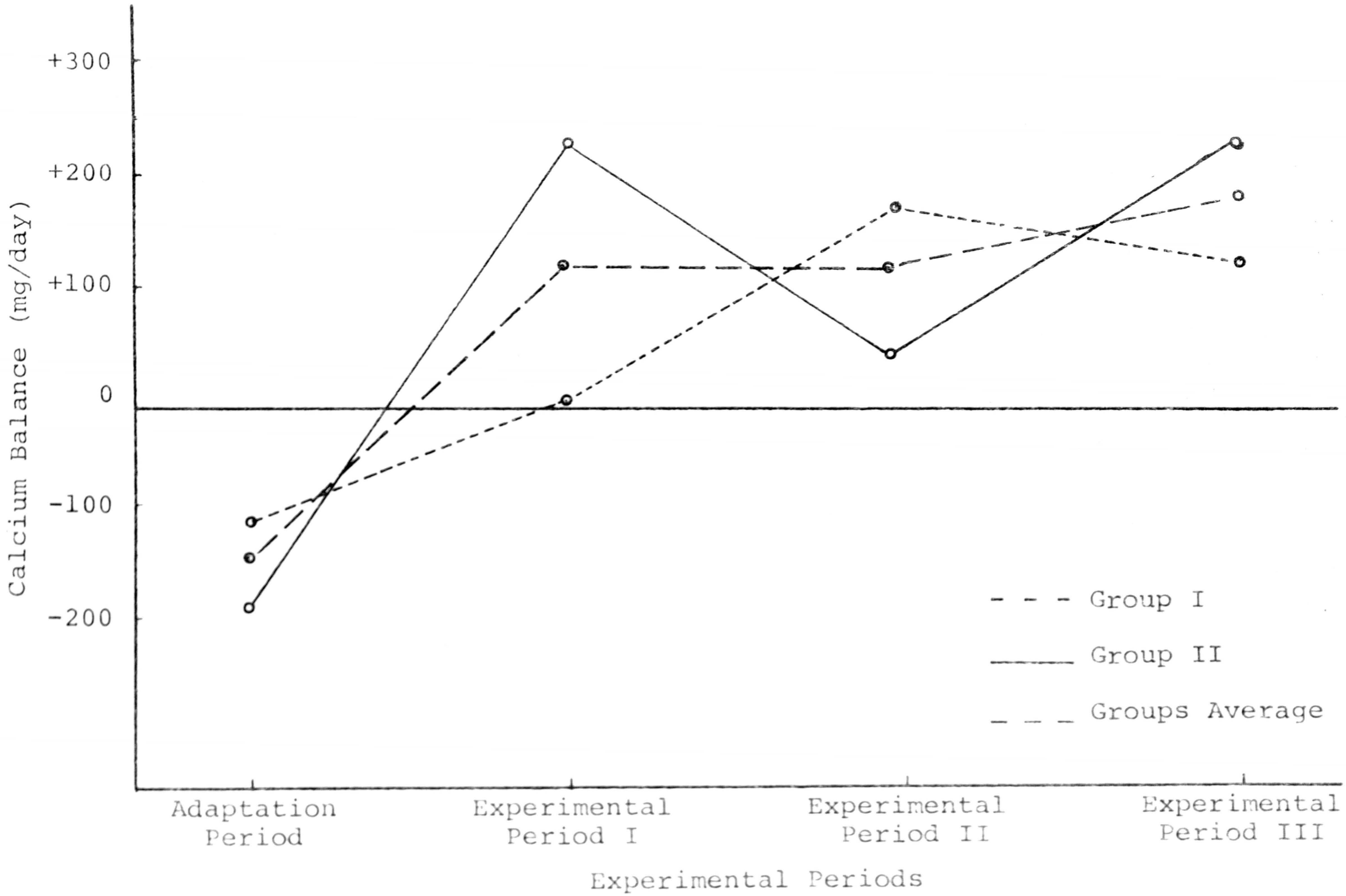

Fig. 1. Mean Calcium Balance of Two Groups of Elderly Healthy Women, Aged From 67 to 91 Years, During a 4-Day Adaptation Period and Three 8-Day Experimental Periods. Experimental Period I - Group I - Low Calcium Diet, Group II - High Calcium Diet. Experimental Period II - Group I - High Calcium Diet, Group II - Low Calcium Diet. Experimental Period III - Both Groups on High Calcium Diet with Vitamin D Supplementation.

high calcium diet. Subject 3368 was in negative balance during the first four days of the high calcium diet. This negative balance could possibly be attributed to emotional stress, since she had retained significantly more calcium during the following four day period on the same diet. On the other hand, Group II, the subjects who received the high calcium diet during first experimental period, had a sharp increase in calcium balance. The calcium balance declined significantly when the subjects were switched to the low calcium diet. Figure 2 illustrates the calcium balance of both groups during each of the four experimental periods.

Calcium retention of the subjects at the different experimental periods varied from one individual to another. For this reason, Figures 3 through 10 are given to show patterns for each subject.

The addition of 200 I.U. of cholecalciferol (Vitamin D_3) supplementation to the high calcium diet had no significant effect on the calcium retention when comparing to the high calcium diet alone. There was a large variation in calcium retention among the subjects during the Vitamin D supplementation period. In fact, two Subjects, 3370 and 3377, showed a decline in calcium retention during this period. Subject 3377 had mild gastrointestinal discomfort and slight diarrhea during this time.

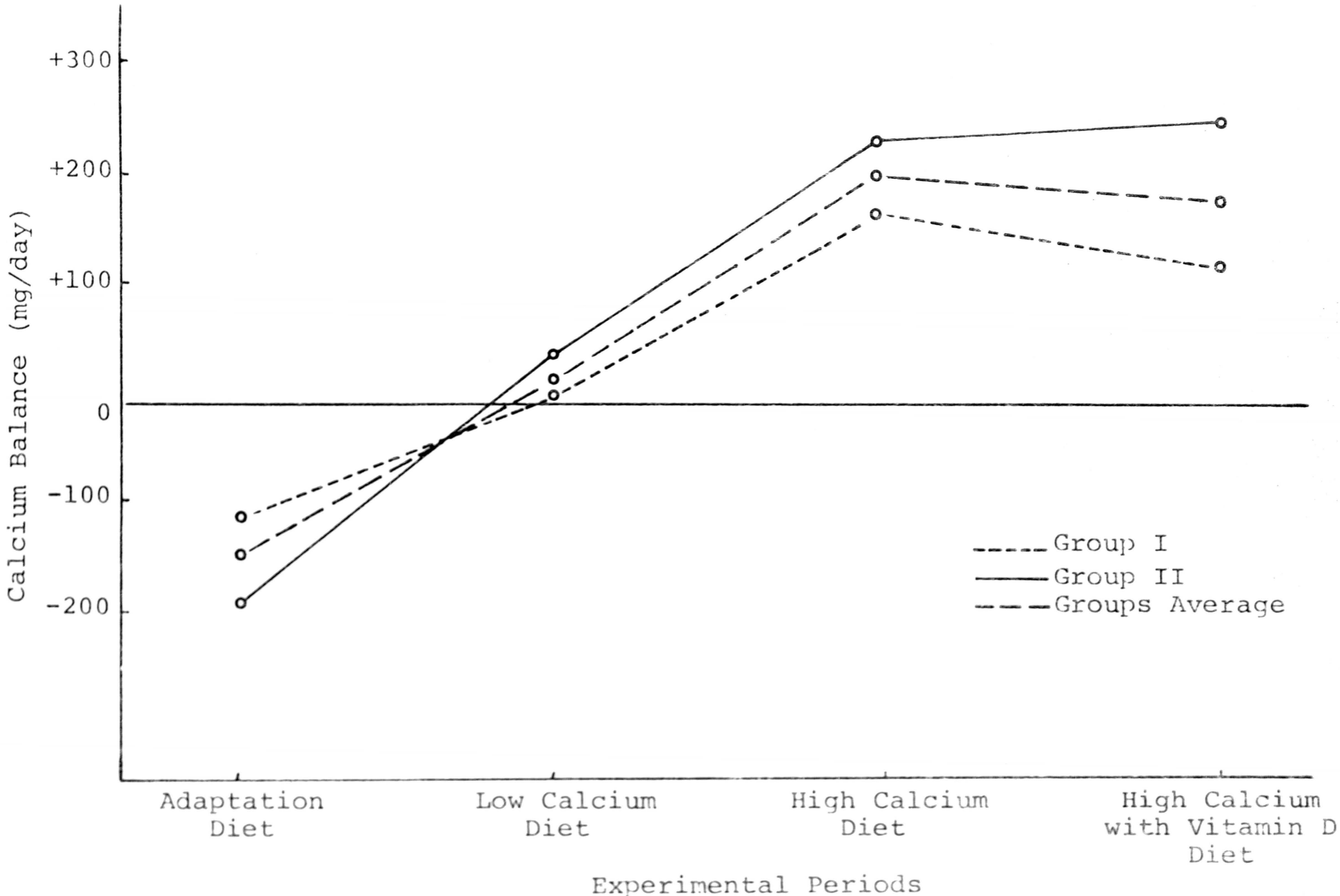

Fig. 2. Mean Calcium Balance for Elderly Healthy Women, Aged From 67 to 91 Years, During a 4-Day Adaptation Period and Three 8-Day Experimental Periods.

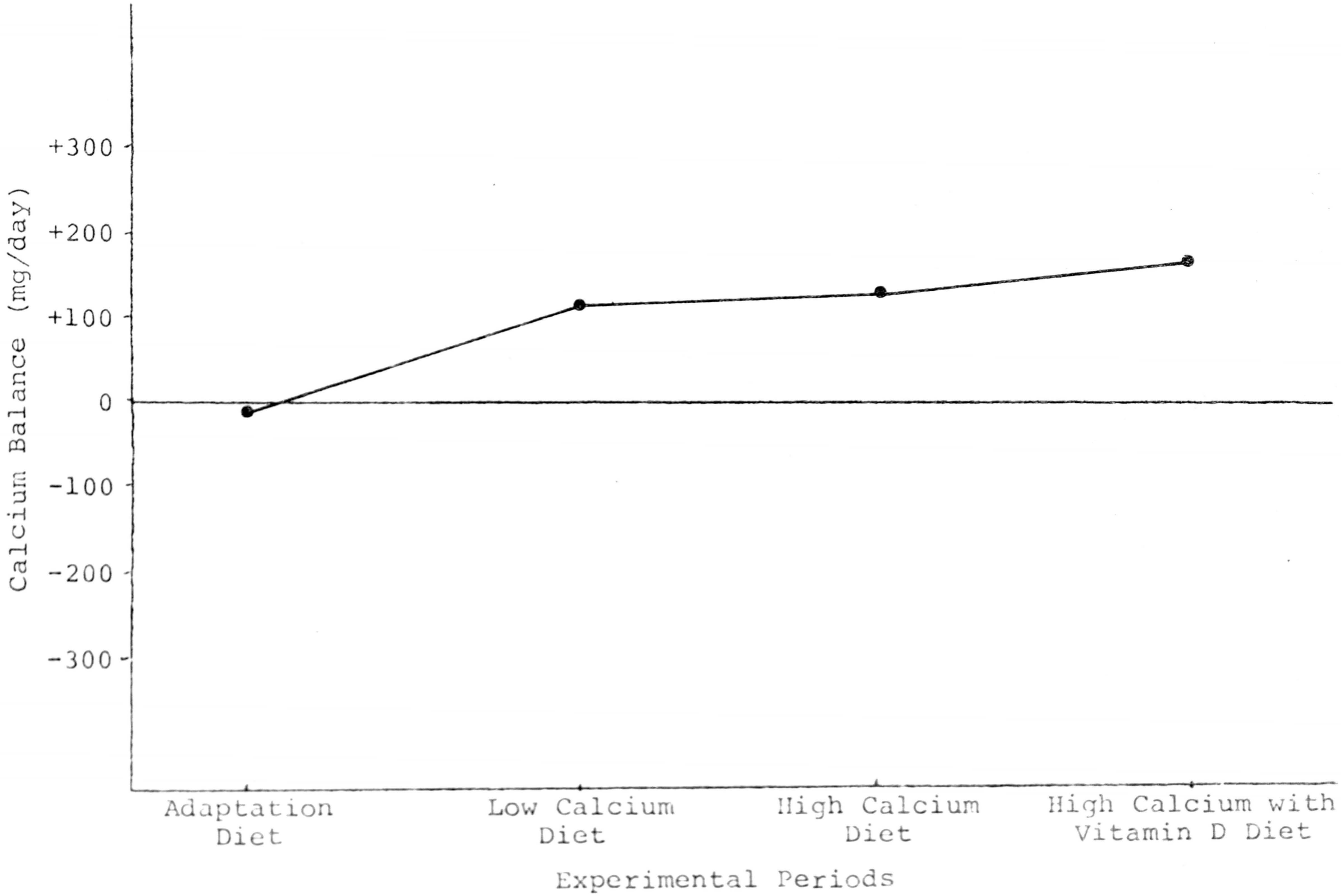

Fig. 3. Mean Calcium Balance for Subject 3368 During a 4-Day Adaptation Period and Three 8-Day Experimental Periods.

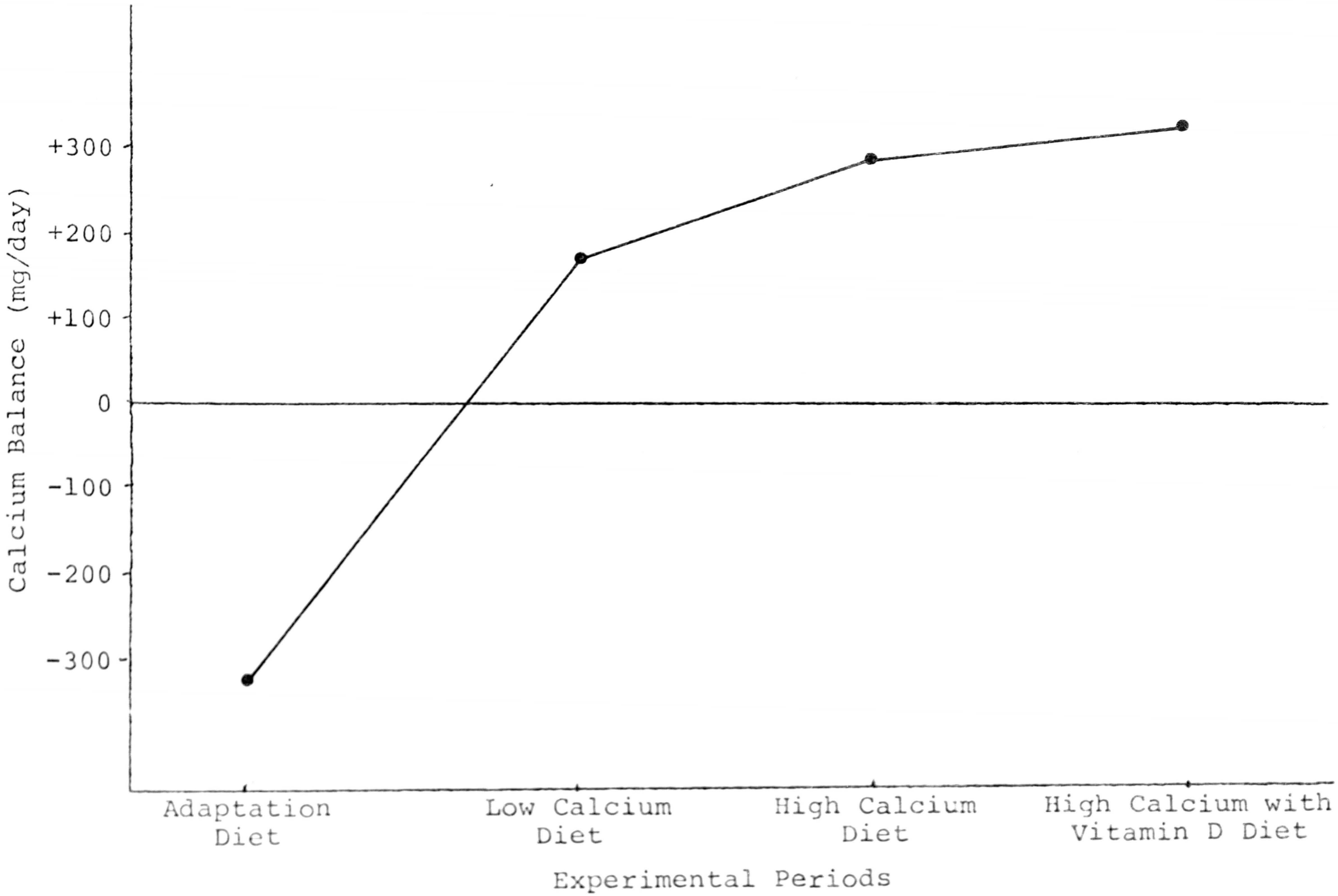

Fig. 4. Mean Calcium Balance for Subject 3369 During a 4-Day Adaptation Period and Three 8-Day Experimental Periods.

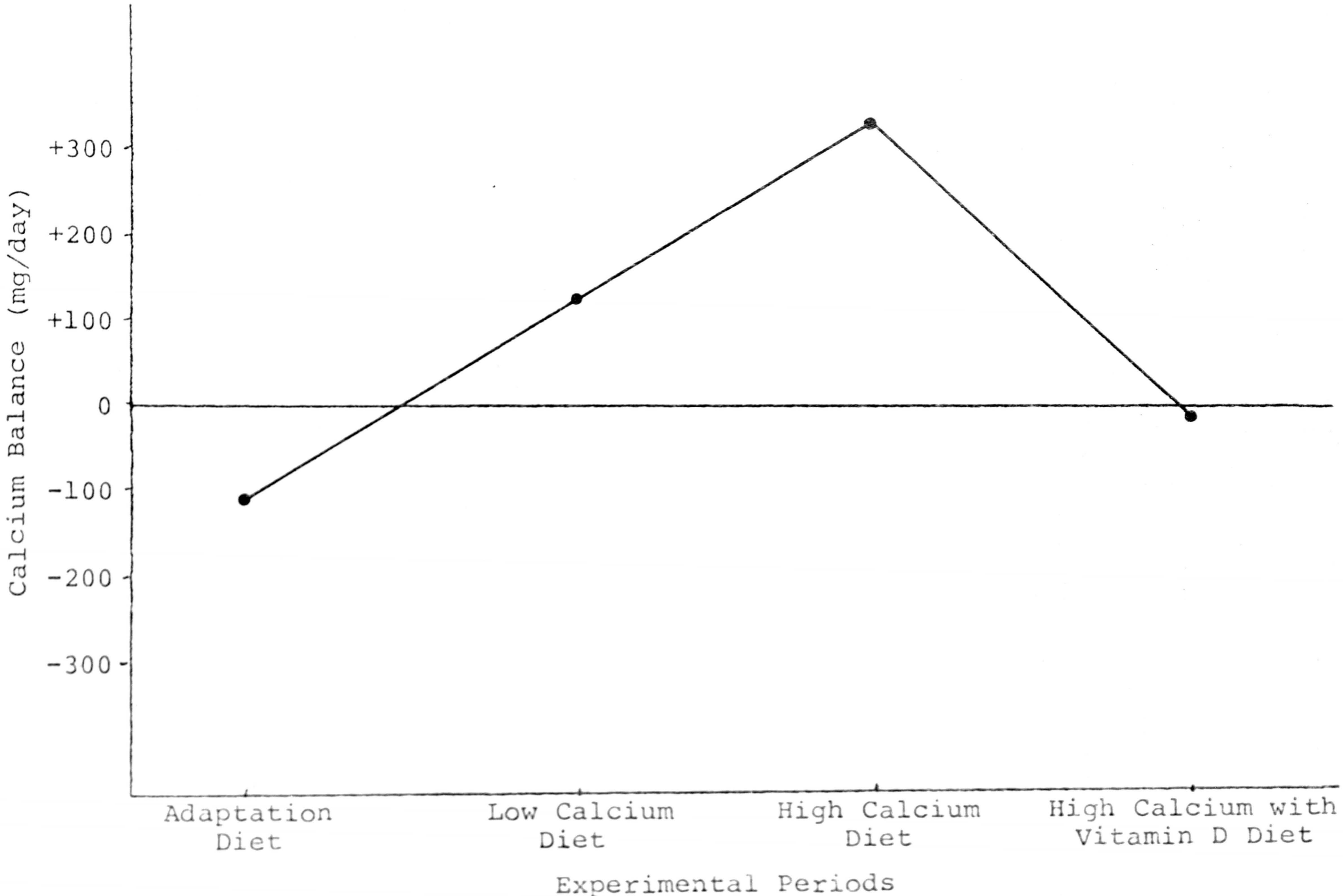

Fig. 5. Mean Calcium Balance for Subject 3370 During a 4-Day Adaptation Period and Three 8-Day Experimental Periods.

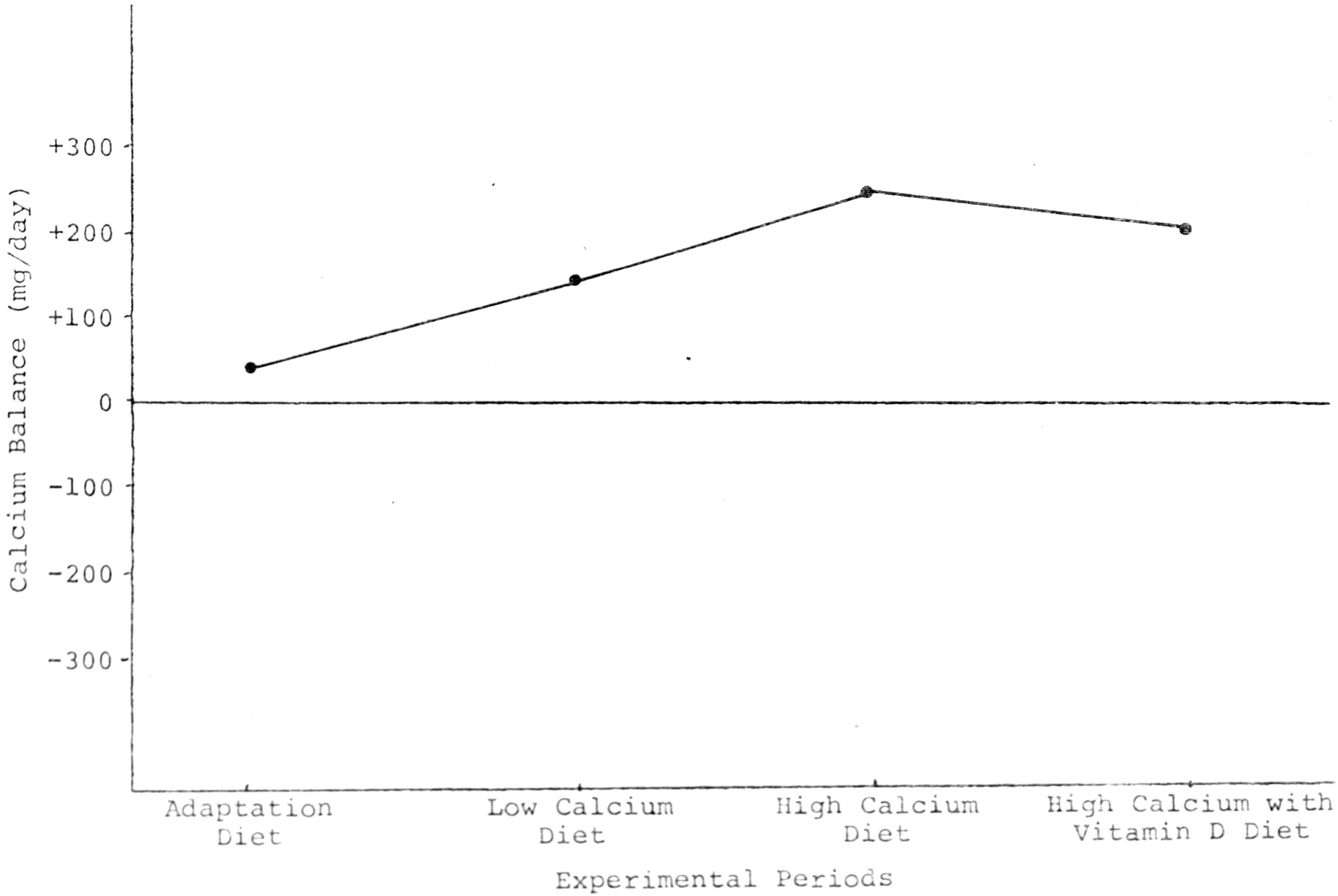

Fig. 6. Mean Calcium Balance for Subject 3371 During a 4-Day Adaptation Period and Three 8-Day Experimental Periods.

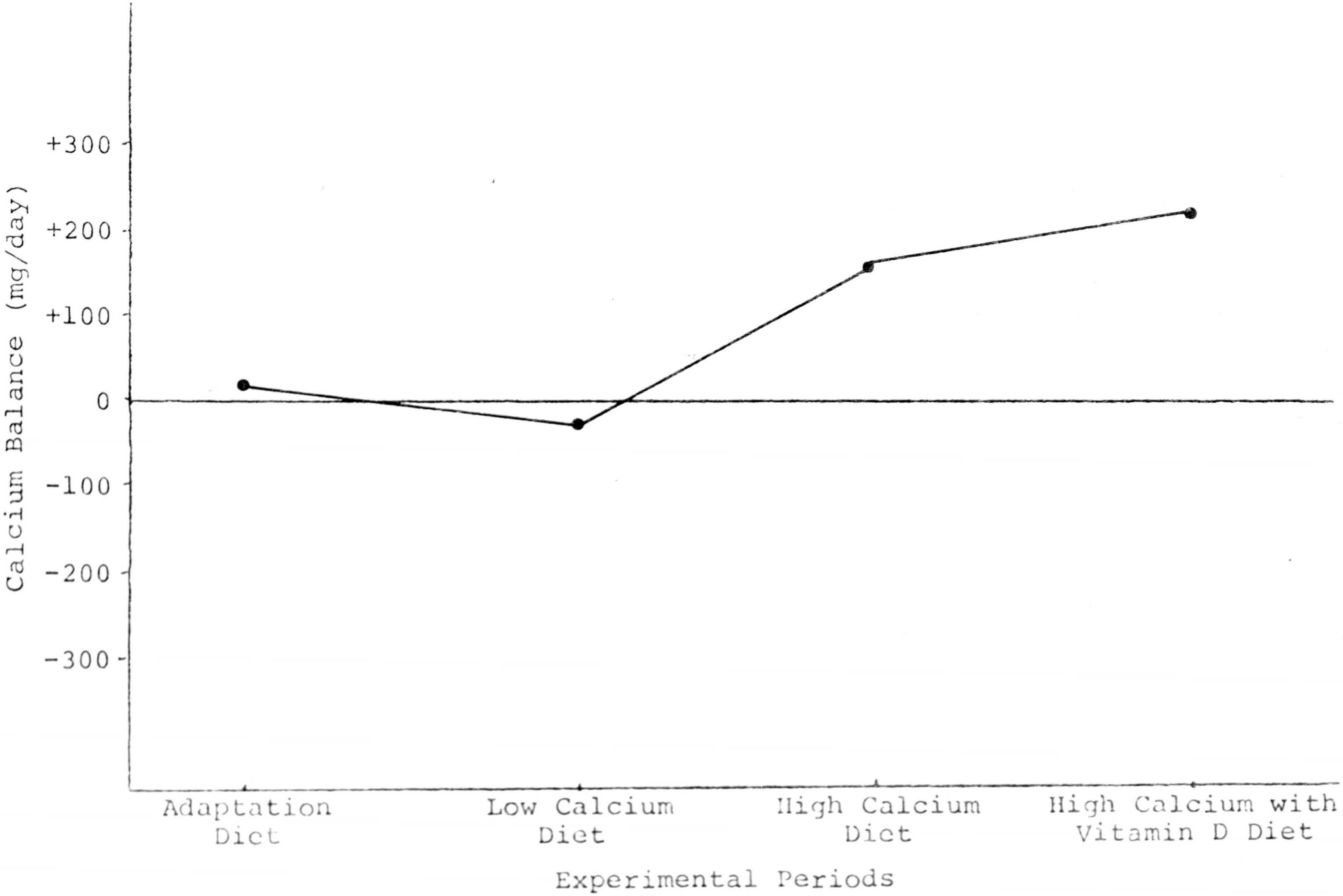

Fig. 7. Mean Calcium Balance for Subject 3372 During a 4-Day Adaptation Period and Three 8-Day Experimental Periods.

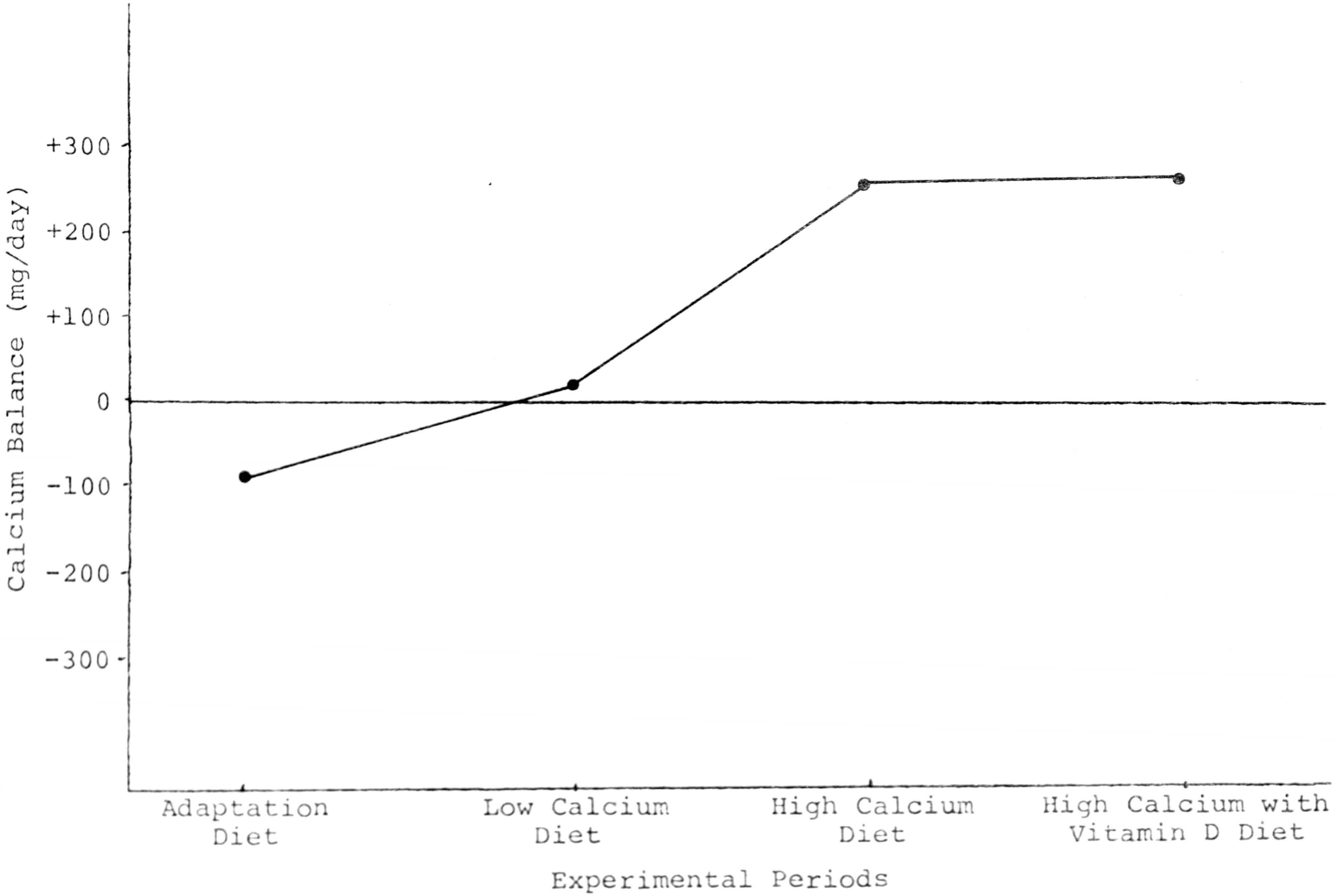

Fig. 8. Mean Calcium Balance for Subject 3374 During a 4-Day Adaptation Period and Three 8-Day Experimental Periods.

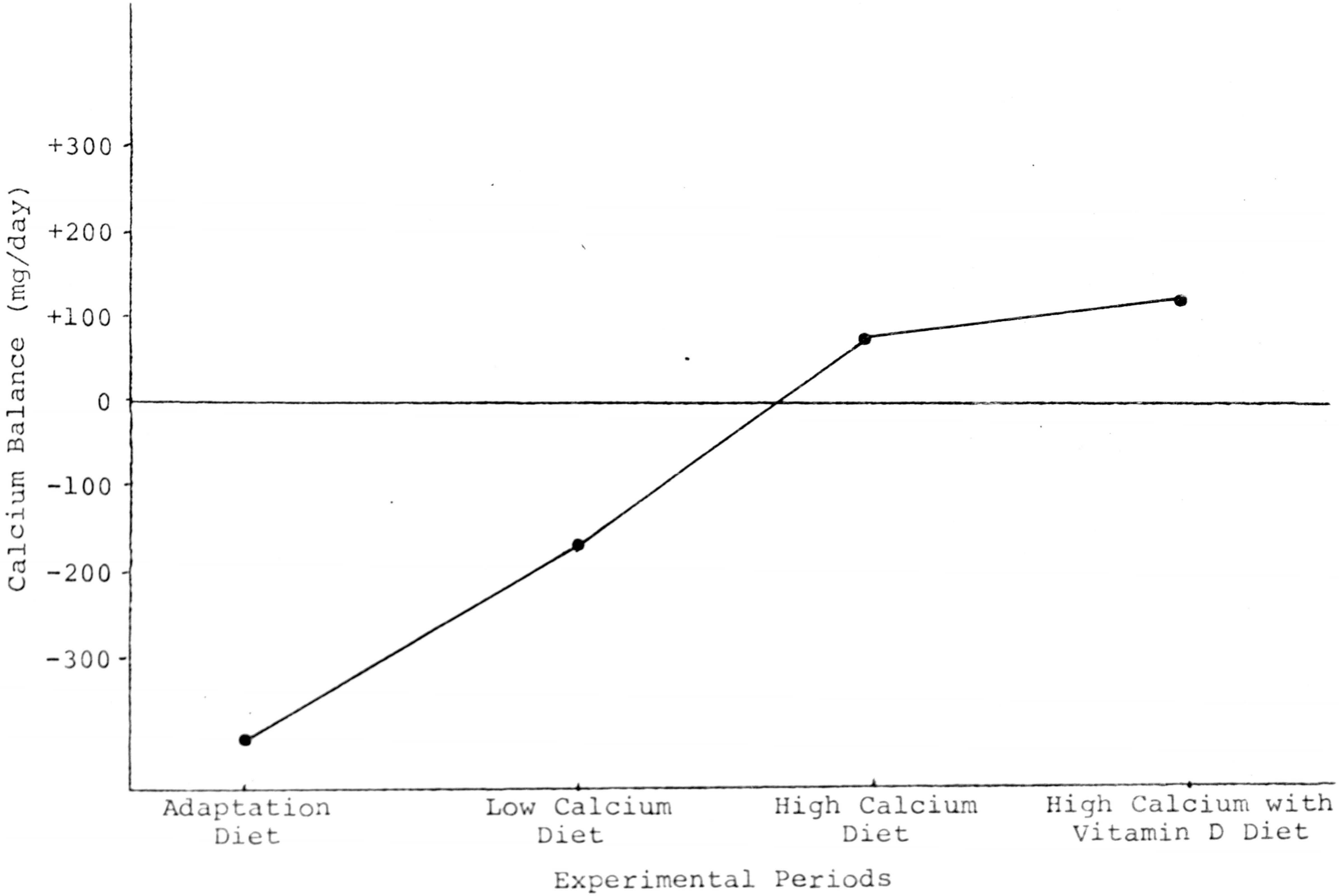

Fig. 9. Mean Calcium Balance for Subject 3375 During a 4-Day Adaptation Period and Three 8-Day Experimental Periods.

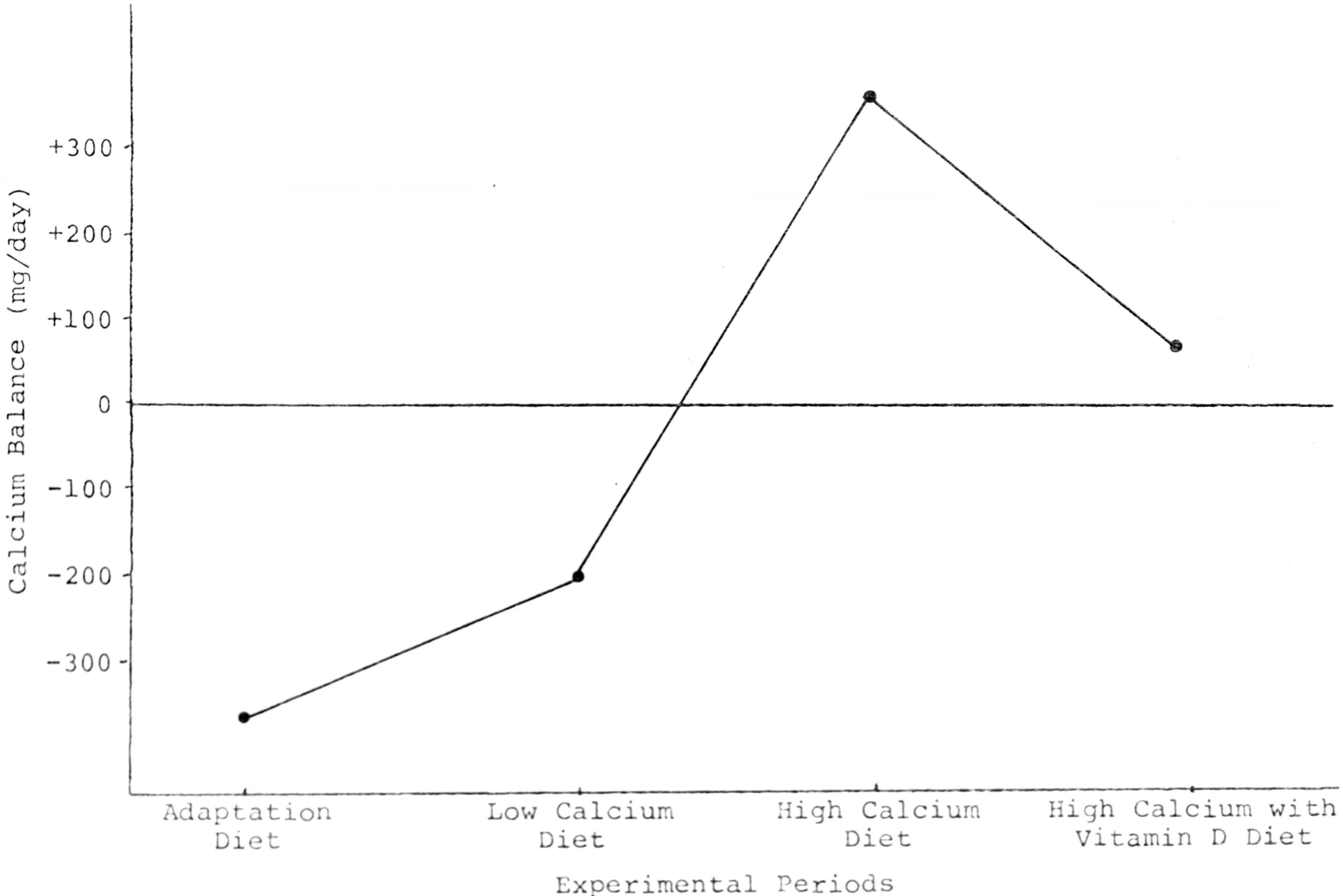

Fig. 10. Mean Calcium Balance for Subject 3377 During a 4-Day Adaptation Period and Three 8-Day Experimental Periods.

This condition could reduce calcium and/or Vitamin D absorption. However, no clear explanation was evident in the case of Subject 3370.

In a study by Lutwak (39), a group of four patients with osteoporosis did not show a response to calcium supplementation alone. The subjects retained more calcium when the Vitamin D supplement was given orally, in doses of 5,000 to 10,000 I.U. daily. Calcium retention of one subject, who had shown adequate retention of calcium with increasing intake of calcium, did not improve when the Vitamin D supplement was given.

In the present study, the results of Vitamin D addition to the diet was unexpected. One might suppose that the subjects had a sufficient amount of dietary Vitamin D intake and/or exposure to sunshine. On the other hand, the dosages of Vitamin D supplementation to enhance calcium absorption in the Lutwak study (39) was considerably higher than in the present study. The suggested amount of Vitamin D supplementation given in the literature ranges from a minimum of 1,000 I.U. to 400,000 I.U. of ergocalciferol per day (39, 52).

Nordin et al. (51) observed that the subjects over 70 years old showed no response to the addition of 1,000 I.U. of Vitamin D daily. A significant response to 10,000 I.U. daily and a further improvement in the response to dosages of 20,000 I.U. daily was shown. The investigators suggested that the malabsorption of calcium in elderly subjects is not simply a matter of Vitamin D deficiency, but may include Vitamin D resistance.

The response of some of these subjects to Vitamin D supplementation may be the result of using Vitamin D_3 in the study. Small amounts of Vitamin D_3 may be more effective than large amounts of Vitamin D_2 which has been used in many studies.

Urinary Calcium Excretion

The urinary calcium excretion of the subjects was much lower than the values observed in the literature. The average urinary and fecal calcium excretion of the subjects are given in Table 6.

The collective data for urinary calcium excretion reported in the literature for healthy elderly females ranged from 50 to 250 mg daily (46). In the present study, urinary calcium excretion was greater when the subjects were on the high calcium diet (82 mg/day) than when they were on the low calcium diet (57 mg/day). The difference, however, was not statistically significant. Heaney, Saville, and Recker (24) observed that dietary calcium intake at normal levels has a slight effect but excretion is significantly elevated when high absorption is forced by very high intakes.

TABLE 6

AVERAGE URINARY AND FECAL CALCIUM EXCRETION OF EIGHT HEALTHY ELDERLY WOMEN, AGED FROM 67 TO 91 YEARS, DURING EACH EXPERIMENTAL PERIOD

Subject No.	Urinary Calcium Excretion mg/day				Fecal Calcium Excretion mg/day			
	Adaptation 497-670 mg Ca/day	Low Calcium 497-670 mg Ca/day	High Calcium 908-1078 mg Ca/day	High Calcium with Vitamin D 908-1078 mg Ca/day 200 I.U./Day	Adaptation 497-670 mg Ca/day	Low Calcium 497-670 mg Ca/day	High Calcium 408-1078 mg Ca/day	High Calcium with Vitamin D 908-1078 mg Ca, 200 I.U. Vit D Day
3368	49	68	70	79	501	387	807	727
3369	43	31	76	61	840	365	633	598
3370	168	165	205	195	525	324	475	824
3371	63	45	92	82	450	377	675	710
3372	40	44	52	43	518	564	783	729
3374	18	23	21	28	628	534	764	706
3375	63	55	88	71	895	698	945	807
3377	29	29	54	21	897	753	585	860
Mean	59	57	82	72	657	479	708	745

A number of dietary factors affect urinary calcium excretion. The effect of protein and phosphorous intake are well known. A large dietary intake of protein increases urinary calcium excretion (74). The ratio of dietary calcium to phosphorous is another factor that affects calcium excretion. An increase in calcium intake, without a proportional increase in phosphorous intake, is reflected in greater urinary calcium excretion (31, 60). Therefore, in the present study, the diet of the subjects was kept constant during each experimental period for all nutrients except for calcium.

A number of studies have observed that a reduction of calcium intake leads to a reduction in urinary calcium excretion (32, 49). Furthermore, the change in urinary calcium correlates with a change in calcium absorption rather than with a change in calcium intake (46).

Low calcium excretion of the subjects in the present study may be related to their calcium intake. Even though they received about 1,000 mg of calcium per day, this amount might be less than bodily needs. On the other hand, the creatine excretion in urine for these subjects was also lower than suggested normal values. Both urinary calcium and creatine excretion of the subject did not differ significantly from day to day.

Fecal Calcium Excretion

The level of calcium intake affected calcium excretion in the feces. The low calcium diet resulted in significantly less fecal excretion of calcium than the high calcium diet or the high calcium diet with the Vitamin D supplementation. During the adaptation period, the fecal calcium excretion was greater than the dietary calcium intake. The differences could be attributed to endogenous calcium or that calcium of the digestive juice which is not reabsorbed.

Apparent absorption of calcium was calculated using the following formula (58):

$$\text{Percent apparent Calcium absorption} = \frac{\text{Intake} - \text{Fecal Excretion}}{\text{Intake}} \times 100$$

Apparent calcium absorption during the low calcium diet was 12.9 percent. The absorption was increased significantly, up to 29.2 percent, on the high calcium diet, and slightly reduced to 25.6 percent when Vitamin D supplement was given.

High calcium excretion during the high calcium intake has been explained by Spencer et al. (68) as due to absorbed calcium, not to increased excretion of endogenous fecal calcium. An experimental study reported by Heaney, Saville, and Recker (24) with over 180 adult humans, demonstrated that high calcium intake provides more available calcium for absorption in the gastrointestinal tract.

Intake ranging from 0.163 to 7.48 gm, calcium per day increased calcium absorption. Furthermore, it was demonstrated that active transport (i.e., saturable at high intakes) was greater for any given perfusate concentration, in subjects who had previously adapted to a low calcium intake.

Nordin et al. (51), on the basis of 57 balance studies, reported that net absorption (ingested calcium minus fecal calcium) increased as the level of dietary intake of calcium increased. Maximum absorption capacity of normal subjects for calcium was thought to be 10 to 15 mg intake per kg body weight.

The addition of 200 I.U. of cholecalciferol to the high calcium diet in the present study, increased calcium excretion slightly, but the increase was not statistically significant. In other words, the apparent calcium absorption was 25.6 percent, which is less than the 29.2 percent absorption on the high calcium diet alone. That an administration of Vitamin D increases calcium abosrption has been well documented (13, 30). The conflicting results for this study could be explained as follows: (1) the endogenous calcium excretion may have been altered, thereby increasing calcium excretion, and (2) the more reasonable responses of two subjects (3370 and 3377) may have decreased the average for the group. These two subjects showed a decline in calcium balance. When these subjects are eliminated from the data, the average of the group becomes 767 mg instead of 708 mg for the high calcium diet. The 745 mg for the high calcium diet and with Vitamin D would change to 712 mg.

Calcium Requirements

The minimum calcium requirement to maintain calcium equilibrium for these subjects was estimated to be 640 mg per day. The information obtained from individual regression lines for calcium intake was plotted against calcium balance (Figures 11 through 18). There is a wide variation among the subjects of 142 mg to 883 mg per day to maintain calcium balance. Actually, some subjects were in negative balance when they received 550 mg calcium per day. This result is not different from that reported in a study by Steggerda and Mitchelle (69). These investigators observed the effect of high and low dietary fat intake on calcium metabolism. Calcium balance studies were obtained from 65 observations made on 13 men eating controlled diets for a period of eight to 20 days. The regression line was plotted for calcium intake (mg) against calcium retention (mg/kg/day). Calcium balance ranged from -2.70 to +1.99 mg/kg/day. Positive balance occurred with intakes as low as 2 to 3 mg/kg, and negative balance were observed with

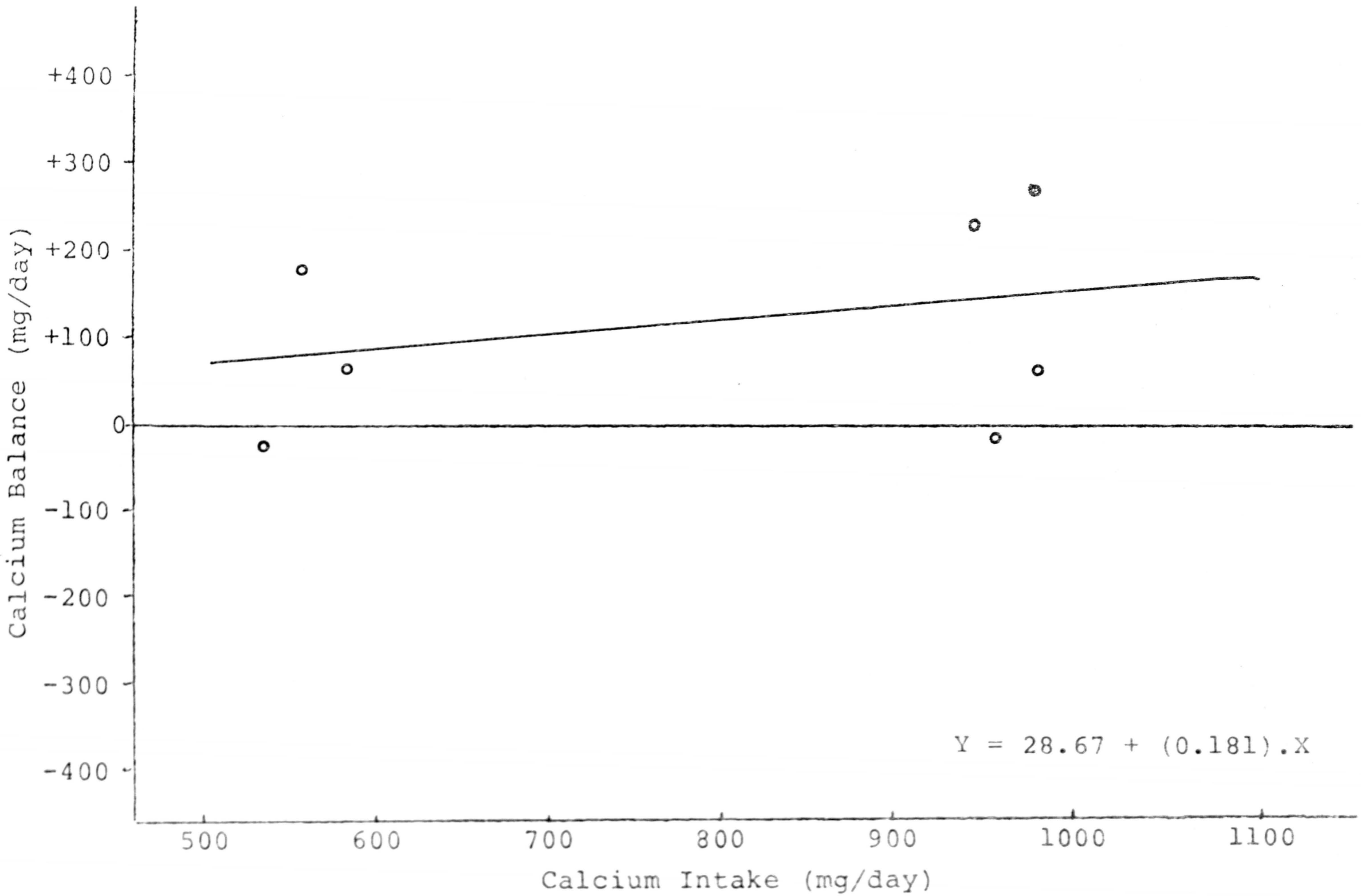

Fig. 11. Regression Line for Subject 3368 Showing the Relationship Between Calcium Intake and Calcium Balance During a 4-Day Adaptation Period and Three 8-Day Experimental Periods (Each Value Represents One 4-Day Period).

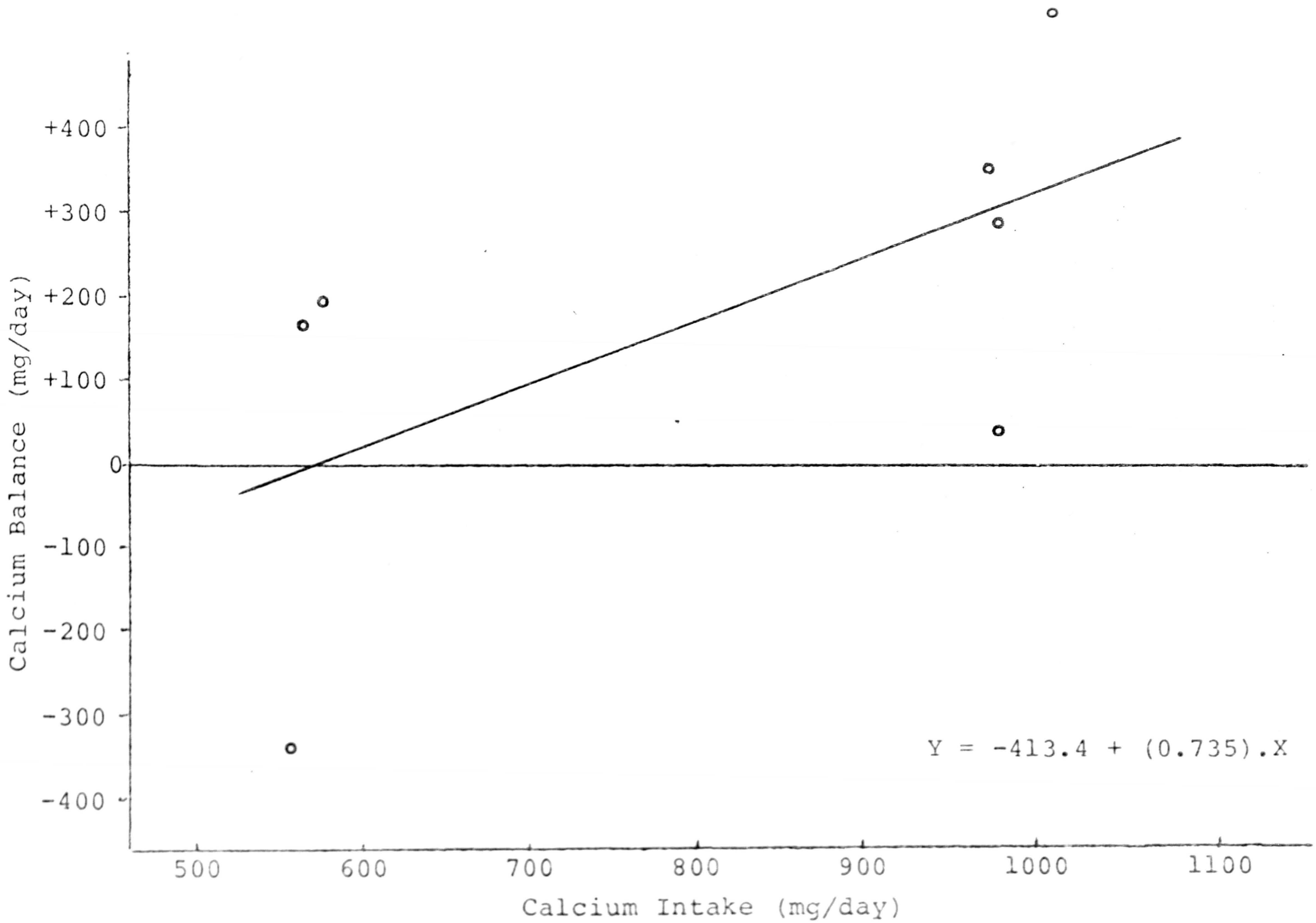

Fig. 12. Regression Line for Subject 3369 Showing the Relationship Between Calcium Intake and Calcium Balance During a 4-Day Adaptation Period and Three 8-Day Experimental Periods (Each Value Represents One 4-Day Period).

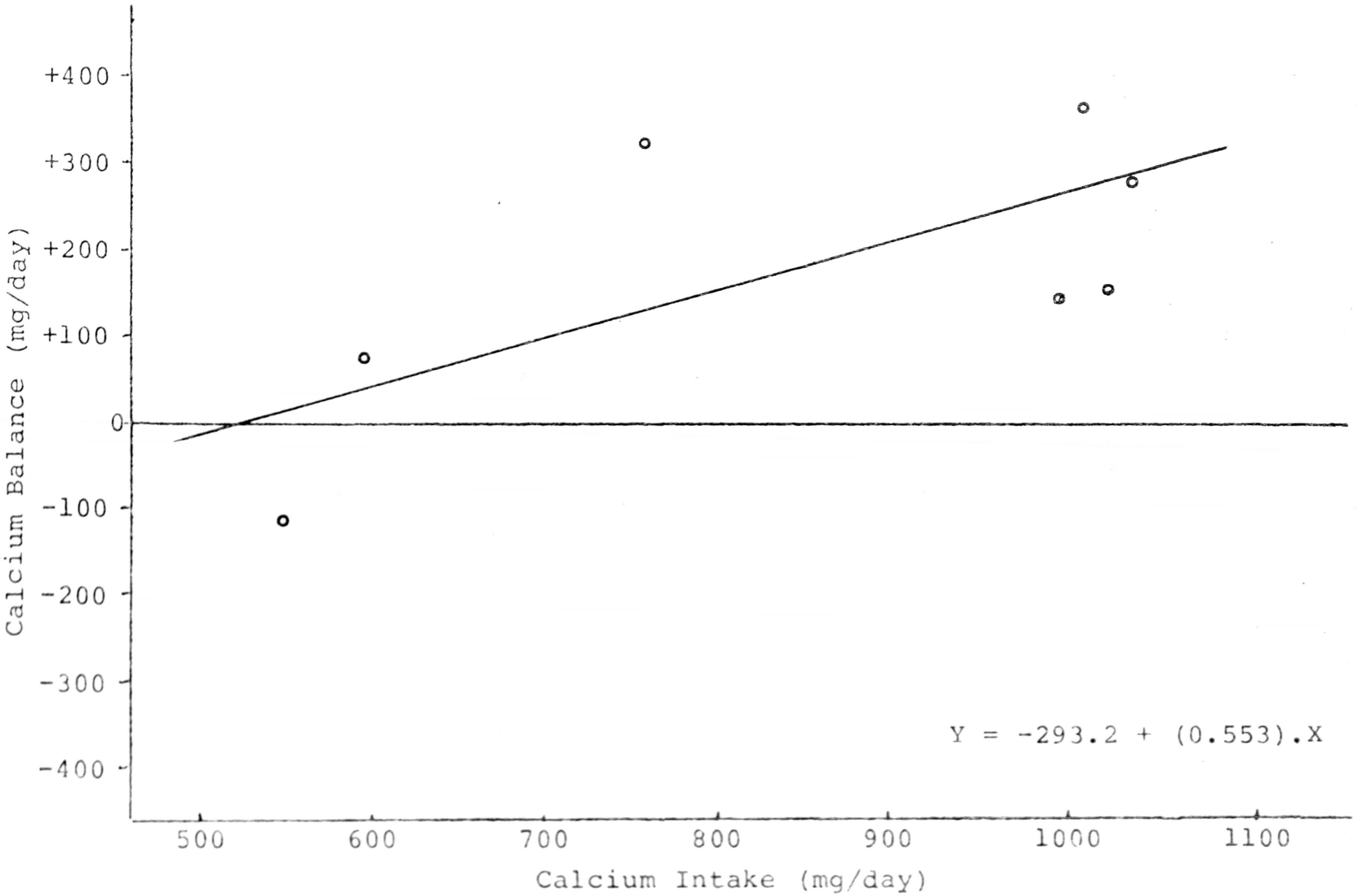

Fig. 13. Regression Line for Subject 3370 Showing the Relationship Between Calcium Intake and Calcium Balance During a 4-Day Adaptation Period and Three 8-Day Experimental Periods (Each Value Represents One 4-Day Period).

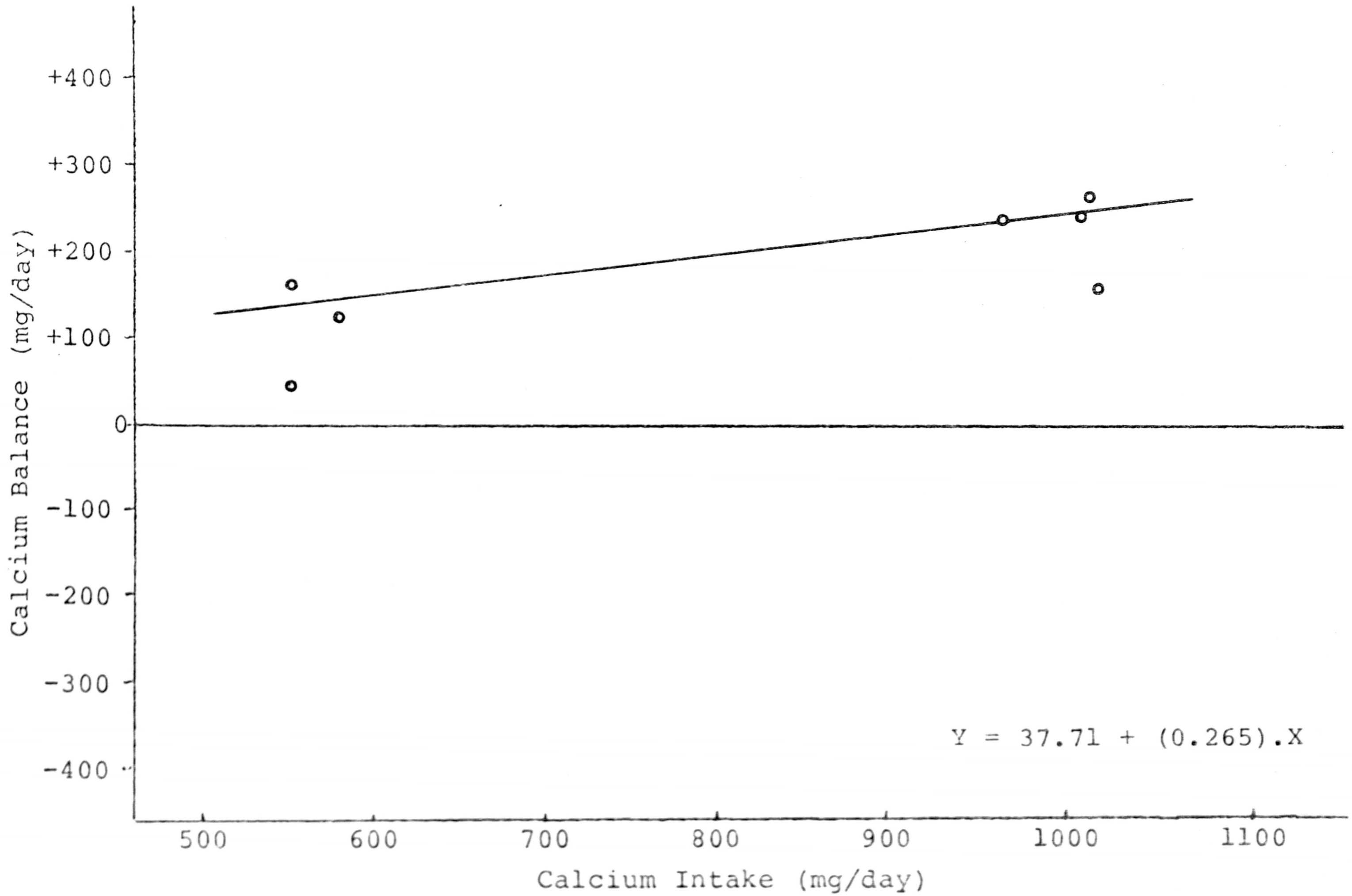

Fig. 14. Regression Line for Subject 3371 Showing the Relationship Between Calcium Intake and Calcium Balance During a 4-Day Adaptation Period and Three 8-Day Experimental Periods (Each Value Represents One 4-Day Period).

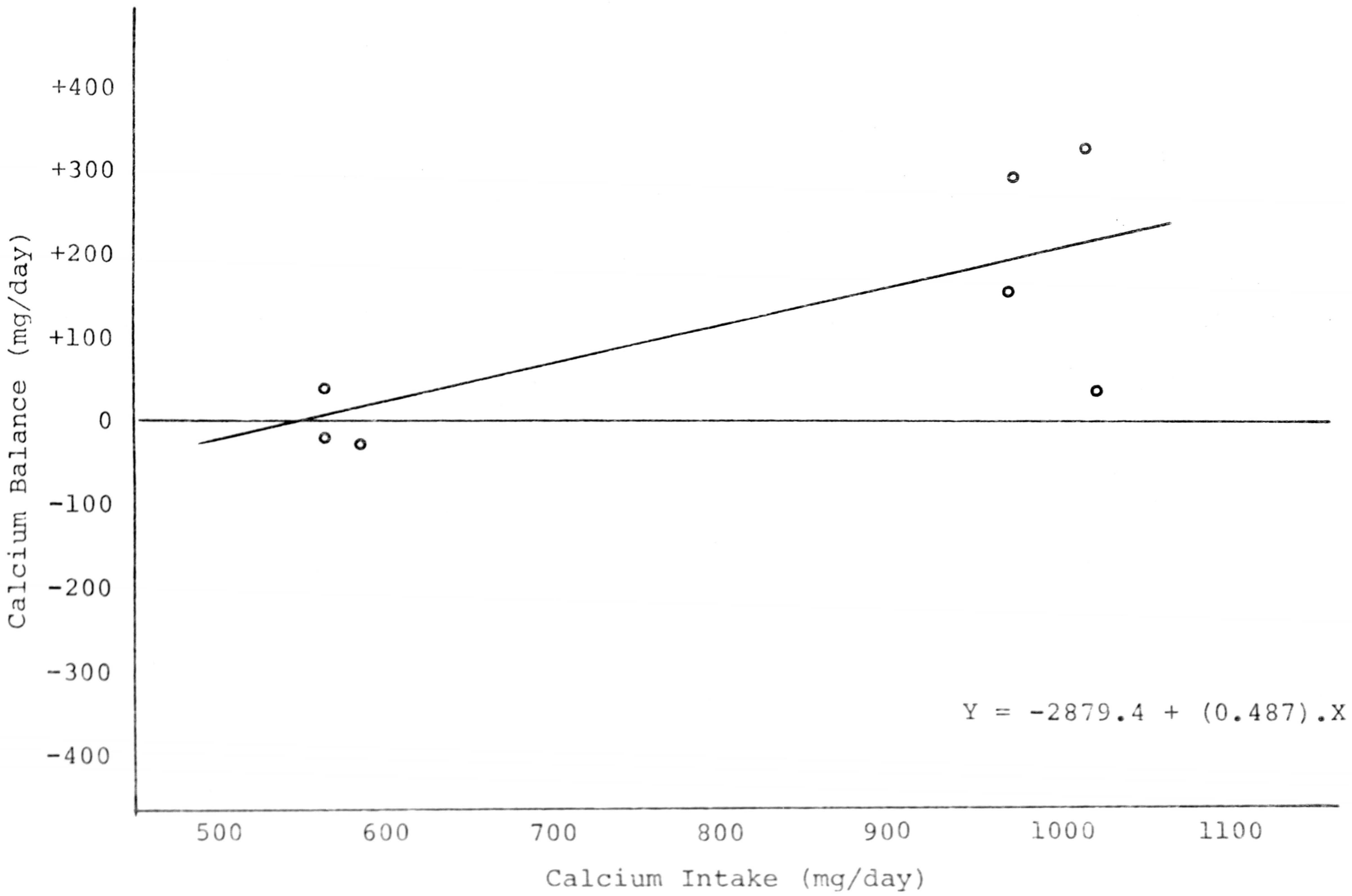

Fig. 15. Regression Line for Subject 3372 Showing the Relationship Between Calcium Intake and Calcium Balance During a 4-Day Adaptation Period and Three 8-Day Experimental Periods (Each Value Represents One 4-Day Period).

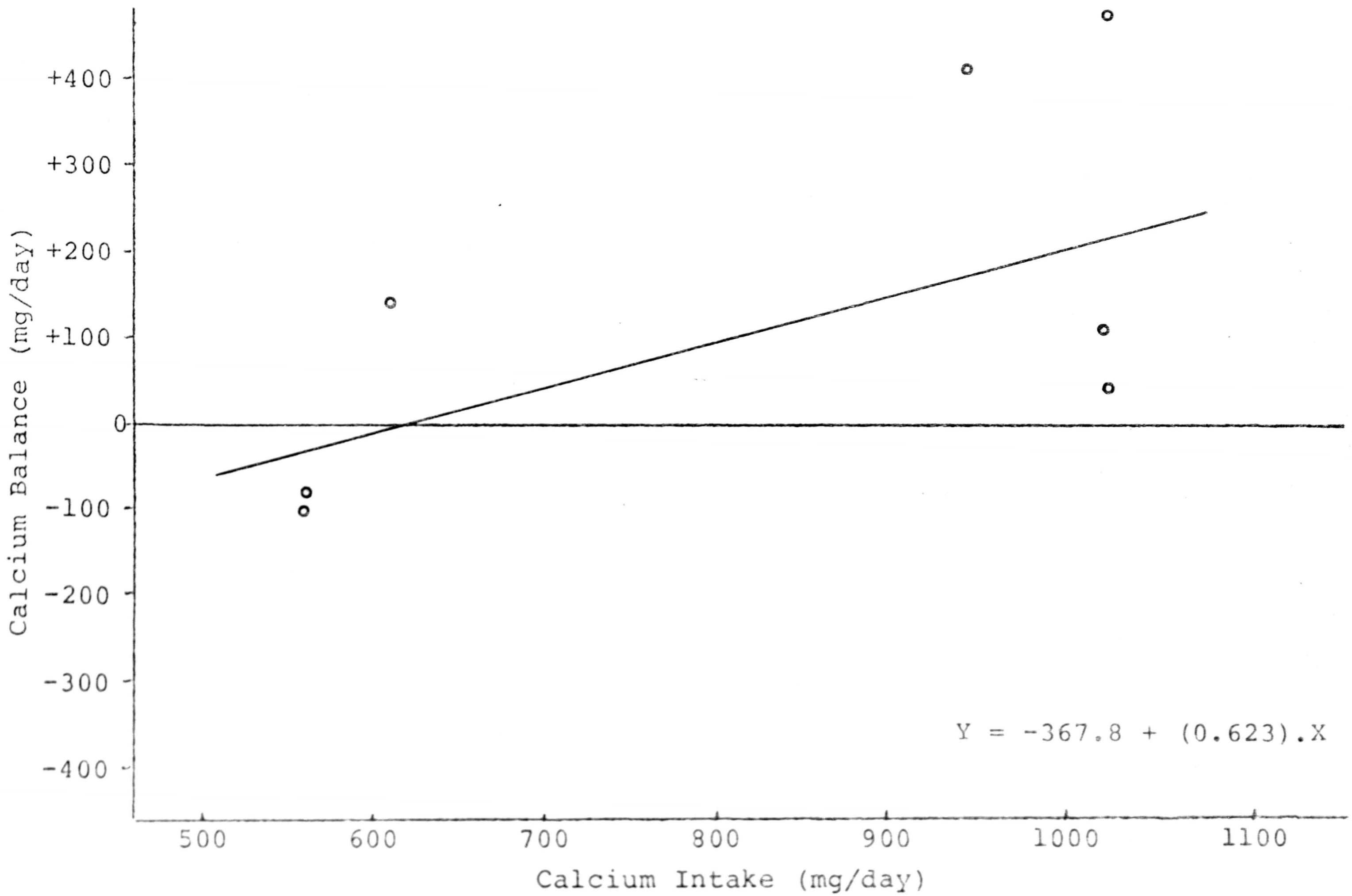

Fig. 16. Regression Line for Subject 3374 Showing the Relationship Between Calcium Intake and Calcium Balance During a 4-Day Adaptation Period and Three 8-Day Experimental Periods (Each Value Represents One 4-Day Period).

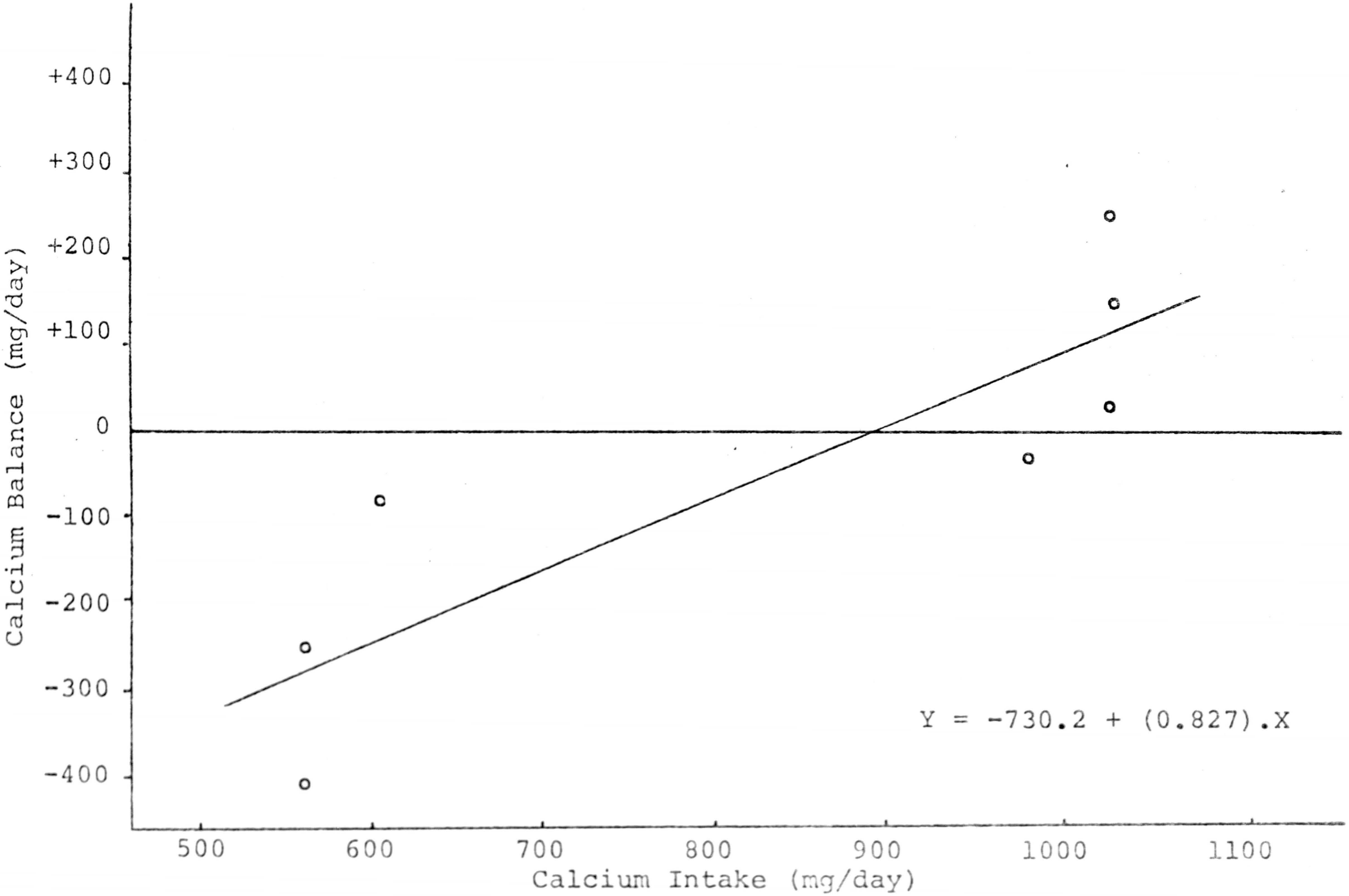

Fig. 17. Regression Line for Subject 3375 Showing the Relationship Between Calcium Intake and Calcium Balance During a 4-Day Adaptation Period and Three 8-Day Experimental Periods (Each Value Represents One 4-Day Period).

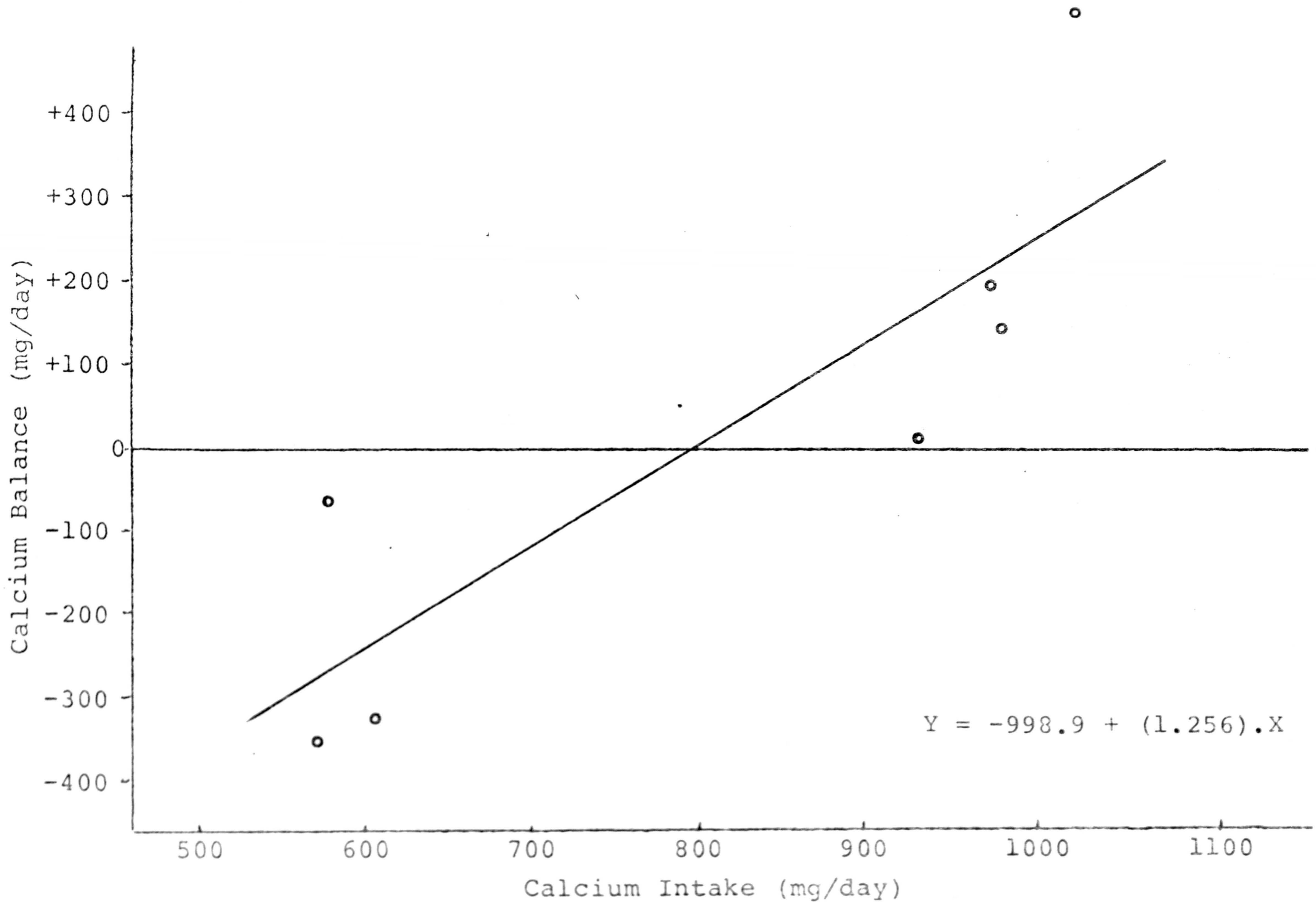

Fig. 18. Regression Line for Subject 3377 Showing the Relationship Between Calcium Intake and Calcium Balance During a 4-Day Adaptation Period and Three 8-Day Experimental Periods (Each Value Represents One 4-Day Period).

intakes as high as 10 mg/kg. The regression line indicated that for these male subjects, aged 19 to 44 years, the mean calcium intake necessary for equilibrium was 7.40 mg/kg body weight. The average body weight of the subjects was 71.5 kg; thus the average calcium intake would be 529 mg per day.

Irwin and Kienholz (30) reported data obtained in the study of controlled dietary calcium intake and excretion for 10 inmates in a Peruvian prison. The calcium requirement for equilibrium for these men was calculated to range from 0 to 596 mg/day, with an average of 216 mg calcium per day. These investigators reviewed another study, conducted with 36 men. The regression equation indicated that a calcium intake of 600 to 700 mg per day was necessary for equilibrium.

An early study was conducted by Ackermann and Toro (1), with eight elderly women, aged 48 to 80 years, to determine minimum calcium intake requirements. The equation line was plotted for the intake of calcium in mg/kg body weight. The equilibrium value was found to be 16.7 mg/kg/day (i.e., approximately 927 mg/calcium/day). Those subjects were free from any infectious disease, but one had a marked osteoporosis, and three subjects were low in renal clearance. They had received 150 to 250 I.U. of Vitamin D per day, the amount given being based on body weight. A high calcium intake for the subjects was obtained by additional calcium-gluconate, equivalent to 255 mg of calcium per day. Duration of each experimental period was 40 to 60 days. All subjects were placed on the high calcium diet during the experimental period. Therefore, the data do not confirm the level of calcium intake essential for equilibrium since the duration of the experimental period was too long and the calcium content of the diet did not vary.

Consideration of the previous dietary calcium intake of an individual is important to establish the minimum requirement. Among the subjects of the present study, those previously accustomed to consuming more dietary calcium, required more calcium to maintain balance. The degree of correlation between the three-day dietary records and calcium balance, however, was not high ($r = 0.478$). The three-day dietary record of a few of the subjects was not representative of their usual diets.

If the sweat losses of calcium are included in the balance calculations, the amount of calcium intake to maintain equilibrium would be higher. The calcium balance studies in the literature, generally, did not include dermal calcium losses. Dermal calcium losses under high sweating conditions can be a serious problem. Calcium can be lost through the skin, up to 30 percent of the daily excretion (11). However, dermal calcium losses could be negligible under nonsweating conditions. A recent study by Gitelman and Lutwak (19) determined a 15.8 $\mp$ 10.8 mg Ca/day dermal calcium loss in four elderly women with osteoporosis and one with osteitis deformaties, under non-sweating conditions. This result was close to the 20 mg Ca/day suggested by Irwin and Kienholz (30) and by Lenther et al. (38). Gitelman and Lutwak (19) also found no correlation between measured body dermal loss and body size, dietary intake, urinary excretion, mineral balance, or mineral content of forced sweat.

The subjects in the present study did not face a sweating problem. The estimated dermal losses of 20 mg calcium in a day would be a reasonable amount of dermal loss for these subjects. This amount is included in the calculation of the recommended amount of daily calcium intake.

Daily recommended calcium intake for the subjects was estimated to be not more than 1,000 mg. The amount of dietary calcium intake to provide calcium balance during the adaptation period ranged from 513 mg to 958 mg of calcium per day with an average of 716 mg of calcium daily. With the possibility of a 5 percent error (38) and the estimated 20 mg daily dermal losses, the minimum daily calcium requirement of the subjects was approximately 771 mg of calcium with a range of 559 mg to 1026 mg.

The subjects who required less calcium intake to maintain calcium balance had low bone density of phalanx V-2 which will be discussed later. Only two of the subjects who required over 900 mg of calcium per day had desirable values for bone density. Therefore, 1,000 mg calcium per day is the recommended amount to maintain calcium nutriture and prevent osteoporosis for these subjects. The results of this study, although confined to a small group of subjects, was close to the previously suggested daily calcium intake (3, 40).

Bone Density

Supplementation with calcium-carbonate from the baked products did not affect the bone density of the subjects during the 28 days of experimental study. The average values of phalanx V-2, metacarpal II-4 and metacarpal III-4 for the experimental periods--adaptation, low calcium, high calcium, and high calcium with Vitamin D--are given in Table 7.

An eight-day high calcium diet and an eight-day high calcium diet with Vitamin D fortification are actually too short a period of time to observe mineral deposition in the bones. A number of studies have indicated that an increase in bone density of osteoporotic patients by calcium supplementation took several months (about 24 to 36 months) (3, 39).

TABLE 7

MEAN CALCULATED CALCIUM* INTAKE FOR THREE-DAY DIETARY RECORDS AND MEAN BONE DENSITY OF PHALANX V-2, METACARPAL II-4, AND METACARPAL III-4 OF THE EIGHT HEALTHY ELDERLY WOMEN, AGED FROM 67 TO 91 YEARS

Subject No.	Mean Calcium Intake (mg/day)	Bone Density (Aluminum Equivalent)		
		Phalanx V-2	Metacarpal II-4	Metacarpal III-4
3368	288	0.149	40.77	40.07
3369	---	0.146	45.75	42.32
3370	213	0.164	40.08	41.39
3371	323	0.149	38.42	35.66
3372	568	0.189	47.63	44.50
3374	987	0.165	43.03	41.89
3375	492	0.189	56.87	53.48
3377	1181	0.247	49.14	52.93
Mean		0.175	45.21	44.03
Correlation of Dietary Calcium to Bone Density		r = 0.77	r = 0.42	r = 0.57

*Ohio State Dietary Data Base (54).

A noticeable increase in bone density over a term of six months was observed by Lee and Johnson (37) among elderly female subjects with osteoporosis. An additional 701 mg of calcium and 401 I.U. of Vitamin D, along with their normal diet, increased bone density of the 3 -2 phalanx from .83$\pm$ 17 to 1.04 $\pm$ 16 alimunium equivalent (AE) for 11 out of the 20 subjects studied.

The Texas Woman's University roentrographic method, at the middle phalanx of the little finger, is an accepted method for diagnosis of osteoporosis. In the literature, the bone density value of the osteoporotic subjects was not clearly distinguished from that of the non-osteoporotic subjects, because there is a wide variation among individuals. The data provided for normal subjects for the middle phalanx (non-immersed) ranges from .163 to .342 AE, with an average of .234 AE. The range for slightly osteoporotic individuals is from .143 AE to .250 AE, with an average .196 AE (20). Based on this information, some of the subjects in this study were at least slightly osteoporotic. In order to evaluate osteoporosis among the subjects, previous dietary calcium intake, serum calcium phosphorous, and alkaline-phosphatase enzyme activity were determinated.

Blood Analysis

Calcium Analysis

The fasting serum calcium levels of the subjects were not changed by the dietary calcium intake and Vitamin D supplementation. The data are given in Table 8. The serum calcium level of all the subjects were within the normal range of values, 8.6 to 11.0 mg/100 ml (56). These results are in agreement with a number of studies related to high and low dietary calcium intake and oral calcium supplementation (2, 37). Increased blood calcium level has been observed shortly after intravenous administration (15).

Phosphorus Analysis

Fasting serum phosphorus levels were affected by Vitamin D supplementation, but were not affected by the dietary calcium intake. The phosphorus levels of the subjects are summarized in Table 9. The serum phosphorus levels of the all subjects were within the normal range, except for Subject 3369. Serum phosphorus concentration for this subject was low during the high calcium diet, but almost doubled when Vitamin D was given. When data for this subject were eliminated, the statistically significant difference found by the Tukey test was not observed between the high calcium with Vitamin D diet, and the high calcium diet, without Vitamin D or the low calcium diet, but not

TABLE 8

FASTING SERUM CALCIUM CONCENTRATIONS* OF EIGHT HEALTHY ELDERLY WOMEN, AGED FROM 67 TO 91 YEARS, DURING EACH EXPERIMENTAL PERIOD

Subject No.	Experimental Periods			
	Adaptation 497-670 mg Ca/day	Low Calcium 497-670 mg Ca/day	High Calcium 908-1078 mg Ca/day	High Calcium with Vitamin D 908-1078 mg Ca, 200 I.U. Vit D Day
	mg/100 ml	mg/100 ml	mg/100 ml	mg/100 ml
3368	9.30	9.67	10.12	9.53
3369	8.61	9.64	8.54	8.42
3370	9.89	10.08	9.73	9.26
3371	9.83	8.47	9.42	9.11
3372	9.27	9.83	9.10	9.89
3374	9.39	9.37	8.37	9.26
3375	9.50	10.00	8.67	9.41
3377	9.61	9.17	8.61	9.13
Mean	9.42	9.53	9.07	9.25

*Note: Normal range is 8.6 to 11.0 mg/100 ml (56).

TABLE 9

FASTING SERUM PHOSPHORUS CONCENTRATIONS* OF EIGHT HEALTHY ELDERLY WOMEN, AGED FROM 67 TO 91 YEARS, DURING EACH EXPERIMENTAL PERIOD

Subject No.	Experimental Periods			
	Adaptation 497-670 mg Ca/day	Low Calcium 497-670 mg Ca/day	High Calcium 908-1078 mg Ca/day	High Calcium with Vitamin D 908-1078 mg Ca, 200 I.U. Vit D Day
	mg/100 ml	mg/100 ml	mg/100 ml	mg/100 ml
3368	3.81	3.60	4.15	4.19
3369	3.54	3.82	2.90	5.65
3370	4.54	4.34	4.50	4.66
3371	4.07	4.05	3.24	4.37
3372	4.11	3.53	3.63	3.85
3374	3.32	3.26	3.24	3.98
3375	3.49	3.73	3.49	3.88
3377	3.49	3.49	3.94	3.75
Mean	3.80	3.73	3.63	4.29

*Note: Normal range is 2.7 to 4.5 mg/100 ml (4).

when compared to the adaptation diet. However, the serum phosphorus differed significantly among the four periods of the study when analyzed by the Duncan test.

A study conducted by Albanese et al. (2) with over 200 elderly females demonstrated there were no changes in blood calcium and phosphorus level during supplementation with 750 mg of calcium from calcium-carbonate and 375 USP units of Vitamin D_2 per day. Similar results were observed by Lee and Johnson (37).

Calcium to Phosphorus Ratio

The calcium to phosphorus ratio (Ca/P), because of the change in phosphate level, was significantly different between the high calcium diet with Vitamin D supplementation and the low calcium diet. The Ca/P ratio of all the subjects with each dietary treatment is given in Table 10. Figure 19 illustrates serum calcium and phosphorus levels of the subjects for each experimental diet.

Vitamin D supplementation in this study did not affect the serum calcium level, but the phosphorus level rose, and consequently reduced the Ca/P ratio. The effect of Vitamin D supplementation on phosphorus metabolism is not well documented, but such an increase was not expected. The increase in serum phosphorus level with Vitamin D supplementation may be related to bone uptake of calcium or phosphorus. A further study in metabolism of phosphorus and its relation to Vitamin D, would be helpful.

TABLE 10

FASTING SERUM CALCIUM/PHOSPHORUS RATIOS OF EIGHT HEALTHY ELDERLY WOMEN, AGED FROM 67 TO 91 YEARS, DURING EACH EXPERIMENTAL PERIOD

Subject No.	Experimental Periods			
	Adaptation 497-670 mg Ca/day	Low Calcium 497-670 mg Ca/day	High Calcium 908-1078 mg Ca/day	High Calcium with Vitamin D 908-1078 mg Ca, 200 I.U. Vit D Day
3368	2.4	2.7	2.4	2.3
3369	2.4	2.5	2.9	1.5
3370	2.2	2.3	2.2	1.9
3371	2.4	2.1	2.9	2.1
3372	2.3	2.8	2.5	2.6
3374	2.8	2.9	2.6	2.3
3375	2.7	2.7	2.5	2.4
3377	2.8	2.6	2.2	2.4
Mean	2.5	2.6	2.5	2.2

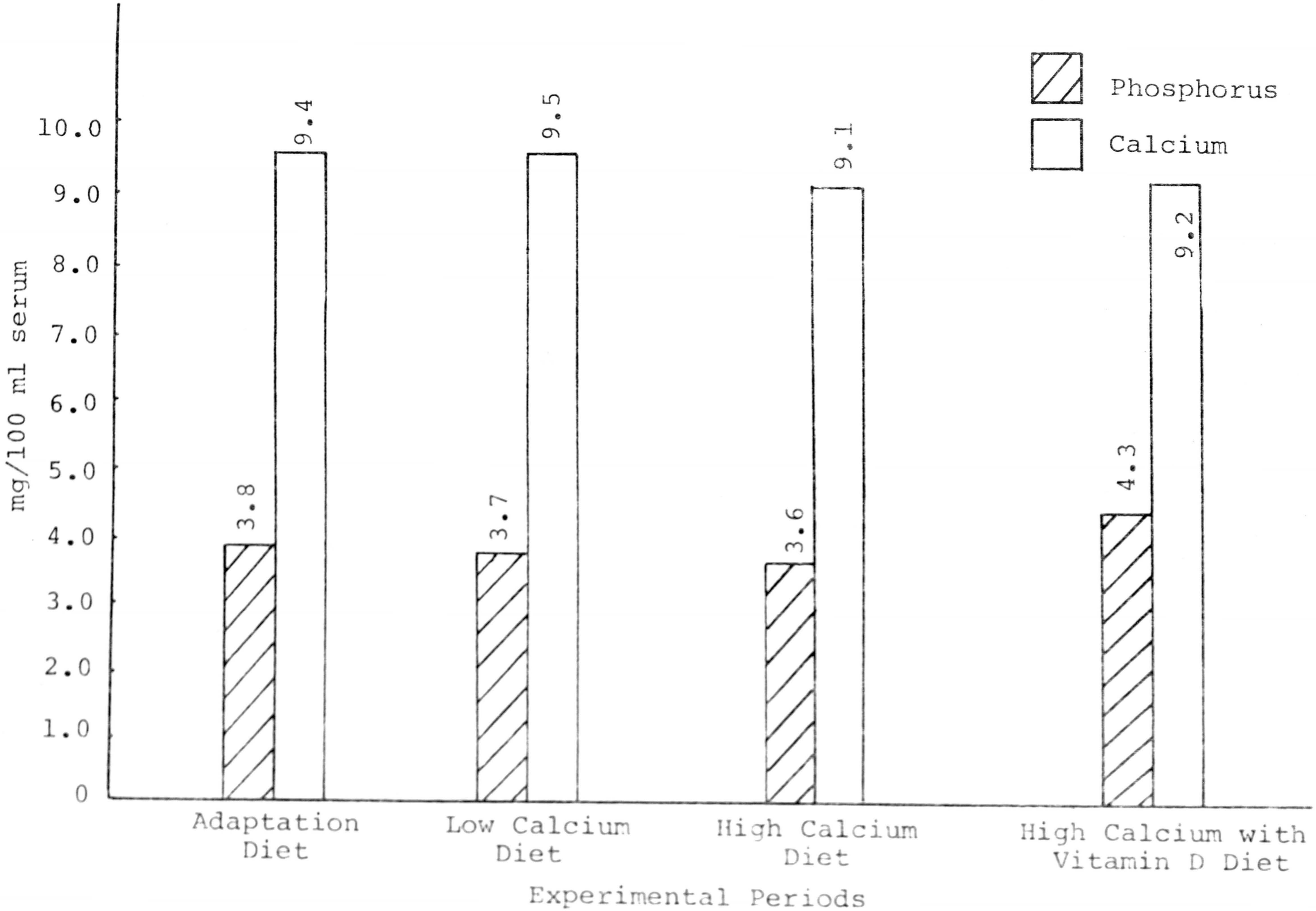

Fig. 19. Serum Calcium and Phosphorus Concentrations of Eight Healthy Elderly Women, Aged from 67 to 91 Years, During Each Experimental Period.

Alkaline-Phosphatase Enzyme Activity

Total alkaline-phosphatase enzyme activity of the subjects was not affected by the experimental diets according to the Tukey test. However, a statistically significant difference was noted when data for the high calcium diet with Vitamin D diet supplementation and the low calcium diet were analyzed by the Duncan test. Table 11 gives the alkaline-phosphatase enzyme activity of the subjects at each experimental period. A few subjects showed a response to the level of calcium intake. The alkaline-phophatase enzyme activity for Subject 3372 was in the osteoporotic range according to the method applied for determination (normal range 0.8 to 2.3 sigma unit/100 ml). There was a noticeable reduction in alkaline phosphatase enzyme activity from the adaptation diet (3.42 sigma units) to the high calcium diet (3.26 s.u.), and the high calcium with Vitamin D diet (3.06 s.u.). Subject 3377 showed a noticeable increase in alkaline-phosphatase enzyme activity when she was on the low calcium diet. The enzyme activity rose slightly with the high calcium diet with Vitamin D supplementation.

Serum alkaline-phosphatase activity increases in a number of clinical conditions as a result of tissue breakdown, necrosis, or catabolism. The highest levels are found in bone disorders associated with increased osteoblastic activity, Paget's disease, rickets, osteomalacia,

TABLE 11

FASTING SERUM TOTAL ALKALINE PHOSPHATASE ENZYME ACTIVITY* OF EIGHT HEALTHY ELDERLY WOMEN, AGED FROM 67 TO 91 YEARS, DURING EACH EXPERIMENTAL PERIOD

Subject No.	Experimental Periods			
	Adaptation 497-670 mg Ca/day	Low Calcium 497-600 mg Ca/day	High Calcium 908-1078 mg Ca/day	High Calcium with Vitamin D 908-1078 mg Ca, 200 I.U. Vit D Day
	sigma unit/100 ml	sigma unit 100/ml	sigma unit/100 ml	sigma unit/100 ml
3368	2.18	2.03	1.72	1.78
3369	1.73	1.55	1.79	1.55
3370	1.83	1.76	1.84	1.61
3371	1.70	1.78	1.59	1.57
3372	3.42	3.30	3.26	3.06
3374	1.47	1.75	2.06	1.49
3375	2.24	2.34	2.62	2.24
3377	2.41	3.14	2.14	2.53
Mean	2.12	2.21	2.13	1.98

Note: Normal range is 0.8 to 2.3 sigma units/100 ml.
Senile Osteoporosis 1.5 to 3.0 sigma units/100 ml.
Generalized Osteoporosis 2.7 to 5.5 sigma units/100 ml (65).

hyperparathyroidism, osteogenic sarcoma, and tumors metastatic to bone (27, 35).

There was a slight reduction in alkaline-phosphatase enzyme activity with a dietary increase in calcium intake, both with and without Vitamin D supplementation, over that found for a low calcium diet. These nutrients may have prevented bone breakdown or altered calcium absorption from the intestine.

Alkaline-Phosphate Isoenzyme Separation

The densitometer scans of fasting serum alkaline-phosphatase isoenzyme separation did not indicate any bone diseases. The darkness of the isoenzyme band on cellulose acetate media is related to blood ABO groups, age, and serum bilirubin concentration (61). Such information for these subjects was not obtained, but each subject had the same pattern for the isoenzyme band at each dietary treatment. The band of isoenzyme separation for Subject 3372, who had highest total alkaline-phosphatase enzyme activity, had a wider and darker band than the other subjects. Subject 3377 had the next darkest band and Subject 3370 had the second widest band. Representative graphs of alkaline-phosphatase isoenzymes separation on cellulose-acetate media for each subject are given in Appendix F. Because of technical difficulties (poor blotting) some of the graphs were not uniform. The overlap at the left side of the graphs does not clearly indicate an increase in bone enzyme activity. By visual comparison, the location of the isoenzyme bands, of the blood samples were at the same locations as the subjects in the Fritsche and Park study (17). Determination of urinary hydroxyproline for the suspected individuals, Subjects 3371, 3374 and 3375, would have been necessary for diagnosis in bone phosphatase activity (52).

General Health of the Subjects

The subjects were in good health during the entire study. No side effects of calcium carbonate fortification of baked products was observed. Three individuals, Subjects 3368, 3374 and 3375, who had previous constipation problems, continued with the problem during the study. Subject 3369 had a cold during the time of low calcium dietary intake, and had used Tylenol. Her cold did not affect her calcium balance. Subject 3377 developed gastro-intestinal discomfort the last few days of the study. She vomitted shortly after lunch of the twenty-first day of the study.

The study was designed to keep body weight constant; however, some of the subjects hoped to lose and some of them wanted to gain weight. There was only a small variation in body weight at each four-day weighing period for each subject. Table 12 gives the body weight of each

TABLE 12

BODY WEIGHT IN POUNDS OF EIGHT HEALTHY ELDERLY WOMEN AGED FROM 67 TO 91 YEARS MEASURED AT FOUR DAY INTERVALS DURING EACH EXPERIMENTAL PERIOD

Study Periods	Subject No.							
	3368	3369	3370	3371	3372	3374	3375	3377
Initial Weight 5/31/78	95	145	119	130	125	156	138	106
Adaptation Period 6/4/78	96	145	118	130	124	155	135	106
Experimental Period I 6/8/78	96	145	118	130	123	155	135	107
6/12/78	96	145	118	131	125	153	135	105
Experimental Period II 6/16/78	95	145	117	129	123	152	135	106
6/20/78	94	145	117	129	123	154	135	104
Experimental Period III 6/20/78	96	145	118	129	123	153	134	104
6/28/78	95	144	117	128	122	151	134	104
Mean	95	145	118	129	123	153	135	105

subject at four-day intervals. The variations in body weight could be attributed to difference in clothing at the time of weighing, gastro-intestinal elimination, and body fluid alterations. The losses in body weight did not exceed more than 5 percent of total body weight for each subject.

Osteoporosis Among the Subjects

Although the subjects of this study were carefully selected as healthy elderly females, at least two of the eight women were suspected of having osteoporosis. The clinical parameters and the factors that cause osteoporosis were examinated for diagnosis of the disease for these subjects.

The level of dietary calcium intake is one of the major causes, but not a single factor in causing osteoporosis. A number of investigators have shown that osteoporotic patients did not have lower calcium intake than non-osteoporotic individuals (18, 39, 49). A study involving 398 individuals, some osteoporotic and some non-osteoporotic, aged from 15 to 90 years, demonstrated that the effect of the lifetime calcium intake on bone density is not pronounced until the later years of life (29). Furthermore, Matkovic et al. (43) stated that dietary factors influence bone formation rather than bone loss. Justice, Howe, and Clark (33) observed that among aged women, the dietary pattern before and during the middle years of life may be more important than that during old age in the development of osteoporosis. Nevertheless, a negative calcium balance eventually leads to greater bone resorption than bone formation (29). In this study, a three-day dietary record was not adequate to determine the lifetime calcium consumption. Subjects 3368, 3370 and 3375 were in negative balance when they were on the adaptation diet (550 mg calcium per day). According to the three-day dietary records these women had been consuming less than 500 mg of calcium per day.

Increases in serum total alkaline-phosphatase enzyme activity is not a recognized parameter to diagnosis osteoporosis. The highest serum alkaline-phosphatase enzyme activity was found for Subject 3372. However, alkaline-phosphatase isoenzymes separation for this subject did not indicate any bone disease, and her bone density measurements were comparatively more than those of other subjects. Blood alkaline-phosphatase activity of Subject 3375 fluctuated with the type of dietary treatment. The higher value for her was noted when she was on the low calcium diet (2.62 sigma units, which is slightly more than the normal range of 0.8 to 2.3 sigma units/100 ml serum). The alkaline-phosphatase activity of Subjects 3368 and 3370 did not alter significantly with the type of dietary treatments. However, for both subjects, the phalanx V-2 values were lower than the group averages, .146 and .164 aluminum equivalents, respectively.

CHAPTER V

SUMMARY AND CONCLUSION

This study determined that baked food products fortified with calcium-carbonate was a worthwhile method of imporivng calcium intake of elderly female subjects. The study also considered improvement of calcium retention with Vitamin D supplements. An addition of 200 I.U. cholecalciferol did not provide further calcium retention. An intake of 1000 mg of calcium daily was recommended for these female subjects to maintain calcium equilibrium and prevent bone losses.

Eight elderly women were examined with respect to calcium-carbonate fortified baked products. After a four-day adaptation diet, the subjects were divided into two groups. One group continued on a diet which provided approximately 500 mg calcium per day. The second group was placed on the high calcium diet, approximately 1,000 mg daily, which was provided by calcium-carbonate fortification of yeast bread, chocolate chip cookies, sugar cookies and oatmeal cookies. Using a crossover design the diets of the subjects were reversed after an eight-day experimental period. During the third, eight-day experimental period, all of the subjects were given the high calcium diet also supplemented with Vitamin D_3 (200 I.U. of cholecalciferol).

Urine and fecal samples were collected throughout the investigation and analyzed for calcium content using an Atomic Absorption Spectrophotometer. Representative samples of the foods served to subjects were analyzed by the same method. Calcium balance for each subject was calculated through chemical analyses, by using the formula:

Calcium Balance = Intake Calcium - Output Calcium (Fecal + Urinary).

Mean calcium balances demonstrated that baked products fortified calcium-carbonate improved calcium retention. The mean calcium balances were -154 mg, +22 mg, + 202 mg, and +175 mg daily for the adaptation period, the low calcium diet, the high calcium diet, and the high calcium with Vitamin D supplemented diet, respectively. Vitamin D supplementation did not affect the calcium retention any more than the high calcium diet itself.

Urinary calcium excretion was not significantly altered by varying the calcium intake. However, fecal calcium excretion was increased when extra calcium was provided in the diet. Calcium absorption values ranged from 12 percent for the low calcium diet to 29 percent for the high calcium diet.

From regression data for calcium intake, plotted against the calcium balance, 532 mg daily was the mean minimum calcium requirement. The range was from 142 to 883 mg calcium per day.

Daily recommended calcium intake for the subjects was estimated to be not more than 1,000 mg. The amount of dietary calcium intake to provide calcium balance during the adaptation period ranged from 540 mg to 895 mg of calcium per day with an average of 716 mg of calcium daily. With the possibility of a 5 percent error and the estimated 20 mg daily dermal losses, the minimum daily calcium requirement of the subjects was approximately 771 mg of calcium with a range of 589 mg to 960 mg.

An examination of the bone density (phalanx V-2, metacarpal II-2 and metacarpal III-4 were x-rayed) revealed that six of the eight subjects exhibited low bone density levels.

Blood calcium concentration of the subjects remained within the normal range, and were not significantly different with the various dietary regimens. Statistically significant increases in blood phosphate concentrations were observed during the high calcium diet supplemented with Vitamin D. The differences are small and not readily explained. This effect of Vitamin D supplementation on serum phosphorus level is recommended as a subject for future investigation.

No statistically significant difference was noted on total serum alkaline-phosphatase activity during the investigation. However, a few subjects had higher than suggested normal level for alkaline-phosphatase. These subjects exhibited a response to the amount of calcium intake. The alkaline-phosphatase activity of these subjects rose when they were on the low calcium diet and decreased on the high calcium diet with Vitamin D. On the other hand, serum alkaline-phosphatase isoenzyme separations did not indicate the presence of any bone disease. The baked products, fortified with calcium-carbonate, were found palatable. No side effect of the fortified baked products with calcium-carbonate up to two-thirds of the Recommended Daily Allowance was observed.

Calcium fortification of baked products with calcium-carbonate was found to be a suitable method to improve calcium intake and retention with elderly subjects. Future studies should focus on to development of new recipes to incorporate calcium salts. Another research investigation of worth could be, to determine the maximum, safe amount of calcium salts used in fortification and/or enrichment without harmful side effects. A long term research study could be designed to observe the effect of baked products fortified with calcium-carbonate on serum cholestrol concentration, and bone density of human subjects.

LIST OF REFERENCES

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1. Ackermann, P. G., and G. Toro, 1954, Calcium balance in elderly women, J. Gerontol., 9(4):446-449.

2. Albanese, A. A., A. H. Edelson, M. L. Woodhull, E. J. Lorenze, E. H. Wein, and L. A. Orto, 1973, Effect of a calcium supplement on serum cholesterol calcium, phosphorus and bone density of normal, healthy elderly females. Nutr. Report Internat., 8(2):119-130.

3. Albanese, A. A., A. H. Edelson, E. J. Lorenze, M. L. Woodhull, and E. H. Wein, 1975, Problems of bone health in elderly, N.Y. State J. Med., 75(2):326-336.

4. Annino, J., and R. W. Giese, Clinical Chemistry Principles and Procedures, Boston, Little Brown and Company, Fourth Ed., 1976.

5. Barnett, E., and B. C. Nordin, 1960, The radiological diagnosis of osteoporosis; A new approach, Clin. Radiol., 11(1):166-174.

6. Behar, J., M. Hitching, and R. D. Smyth, 1977, Calcium stimulation of gastrin and gastric acid secretion, effect of small doses of calcium carbonate, Gut, 18(4):442-448.

7. Berstad, A., H. Jorgersen, H. Frey, and E. M. Lund, 1976, Intestinal Absorption of calcium from three commercial calcium preparation in man. Scand. J. Gastroenterol., 11(7):747-51.

8. Bhattacharyya, A. K., D. Phil, C. Thera, J. T. Anderson, F. Granda, and A. Keys, 1969, Dietary calcium and Fat: Effect on serum lipids and fecal excretion of cholesterol and its degradation products in man, Am. J. Clin. Nutr., 22(9):1161-1174.

9. Brown, P. T., J. G. Bergan, E. P. Parsons, and I. Krol, 1977, Dietary status of elderly people, J. Am. Diet. Assoc., 71(6):41-45.

10. Clark, M., and L. M. Wakefield, 1975, Food choices of institutionalized vs. independent-living elderly, J. Am. Diet. Assoc., 66(6):600-604.

11. Consolazio, C. F., L. O. Matoush, R. A. Nelson, L. R. Hackler, and E. Preston, 1962, Relationship between calci-m in sweat, calcium balance and calcium requirement, J. Nutr., 78(1):78-88.

12. Davis, R. H., D. B. Morgan, and R. S. Rivlin, 1970, The exeretion of calcium in the urine and its relation to calcium intake, sex and age, Clin. Sci., 39(7): 1-12.

13. DeLuca, H., 1975, Calcium metabolism, Acta Orthop. Scand., 46(3):284-314.

14. Egg-Larsen, N., and L. Myhre, 1976, Early signs of calcium and phosphorus deficiency, Bibl. Nutr. Dieta., 23:129-136.

15. Epstein, S., W. V. Mieghem, J. Sagel and W. P. U. Jackson, 1973, Effect of single large doses of oral calcium on serum calcium levels in the young and the elderly, Metabolism, 22(9):1163-1173.

16. Exton-Smith, A. N., 1976, Osteoporosis, Proc. R. Soc. Med., 69(12):931-934.

17. Fritsche, O. A., and H. R. Adam Park, 1972, Cellulose acetate electrophoresis of alkaline phosphatase isoenzymes in human serum and tissue, Clin. Chem., 18(5):417-421.

18. Garn, S. M., E. M. Pao, and C. G. Rohman, 1965, Calcium intake and compact bone loss in adult subjects, Fed. Proc., 24:(3):567. (Abstract)

19. Gitelman, H. J., and L. Lutwak, 1963, Dermal loses of minerals in elderly women under non-sweating conditions, Clin. Res., 11:42. (Abstract).

20. Goldsmith, N. F., J. O. Johnston, H. Urg, G. Vose and C. Colbert, 1971, Bone-mineral estimation in normal and osteoporotic women: A comparability trial of four methods and seven bone sites, J. Bone Joint Surg., 53(1):83-100.

21. Goodhart, R. S., and M. E. Shills, Modern Nutrition in Health and Disease, Philadelphia, Lea & Febiger, Fifth Ed., 1973.

22. Grotkowski, M. L., and L. S. Sims, 1978, Nutritional knowledge attitudes, and dietary practices of the elderly, J. Am. Diet. Assoc., 72(5):499-506.

23. Guthrie, H., K. Black, and J. D. Madden, 1972, Nutritional practices of elderly citizens in rural Pennsylvania, Gerontologist, 12(4):330-336.

24. Heaney, R. P., P. D. Saville, and R. R. Recker, 1974, Calcium absorption as a function of calcium intake, J. Lab. Clin. Med., 85(6):881-890.

25. Heaney, R. P., R. R. Recker, and P. D. Saville, 1974, Calcium balance and calcium requirement in middle aged women, Clin. Res., 22:649. (Abstract).

26. Heaney, R. P., R. R. Recker, and P. D. Saville, 1977, Calcium balance and calcium requirements in middle-aged women, Am. J. Clin. Nutr., 30(9):1603-1611.

27. Helena Laboratories, Alkaline Phosphatase isoenzyme procedure, Procedure No. 5, 1978.

28. Henrikson, B., and H. D. Cate, 1971, Nutrient content of food served vs. food eaten in nursing home, J. Am. Diet. Assoc., 59(8):126-129.

29. Hurxthal, L. M., and G. P. Vose, 1969, The relationship of dietary calcium intake to radiographic bone density in normal and osteoporotic persons, Calcif. Tissue Res., 4(5):245-256.

30. Irwin, M. I., and E. W. Keinholz, 1973, Calcium requirements of man, J. Nutr., 103(7):1011-1095.

31. Jowsey, J., 1977, Osteoporosis: dealing with a crippling bone disease of the elderly, Geriatrics, 32(7):41-50.

32. Jowsey, J., 1978, Why is mineral nutrition important in osteoporosis, Geriatrics, 33(8):39-48.

33. Justice, K. L., N. M. Howe, H. E. Clark, 1974, Dietary intakes and nutritional status of elderly patient, J. Am. Diet. Assoc., 65(12):639-645.

34. Khairi, M. R., and C. C. Johnson, 1978, What we know and don't know about bone loss in the elderly, Geriatric, 34(11):67-76.

35. Kaplan, M., 1972, Alkaline phosphatase, N. Engl. J. Med., 286(4):200-202.

36. Kilgore, L. T., K. Watson, N. Wren, R. W. Rogers, and F. Windham, 1978, Fortification of hamburger with calcium, vitamin A and ascorbic acid, J. Am. Diet. Assoc., 71(2):135-139.

37. Lee, J. C., and G. H. Johnson, 1979, Effect of supplementary calcium and calcium rich foods on bone density of elderly females with osteoporosis, Fed. Proc., 38(3):772. (Abstract).

38. Lenther, C., T. Laufferburges, J. Guncaga, M. A. Dambacher, and H. G. Haas, 1975, The metabolic balance technique: A critical Reappraisal, Metabolism, 24(4):461-471.

39. Lutwak, L., 1964, Osteoporosis - A mineral deficiency disease, J. Am. Diet. Assoc., 44(3):173-175.

40. Lutwak, L., 1974, Continuing need for dietary calcium throughout life, Geriatrics, 29(5):171-178.

41. MacLeod, C. C., T. G. Judge, and F. I. Caird, 1975, Nutrition of the elderly at home III. Intakes of minerals, Age Ageing, 4(1):49-57.

42. Marshall, D. H., B. E. C. Nordin, and R. Speed, 1976, Calcium, phosphorus and magnesium requirements, Proc. Nutr. Soc., 35(2):163-173.

43. Matkovic, M., K. Kostial, I. Simonovic, A. Broadree, and A. Buzina, 1977, Influence of calcium intake: age and sex on bone, Calfic. Tissue Res., 21 Suppl:393-396.

44. McLennan, W. J., F. I. Caird, and C. C. MacLeod, 1972, Diet and bone rarefaction in old age, Age Ageing, 1(1):131-140.

45. Morgan, D. B., R. S. Rivlin, and R. H. Davis, 1972, Seasonal changes in the urinary excretion of calcium, Am. J. Clin. Nutr., 25(7):652-654.

46. Morgan, B., and W. G. Robertson, 1974, The urinary excretion calcium: An analysis of the distribution of values in relation to sex, age and calcium deprivation, Clin. Orthop., 101:254-267.

47. National Research Council, Food and Nutrition Board, Recommended Dietary Allowances, National Academy of Sciences, 8th Ed., Washington, 1974.

48. National Dairy Council, Dairy Council Digest, Nutrition of the elderly, 48(1):1-5, 1977.

49. Nayal, A. S., W. J. MacLennan, J. C. Hamilton, P. Rose, and M. Kong, 1978, 25 - hydroxy- vitamin D, diet and sunlight exposure in patients admitted to a geriatric unit, Gerontology, 24(10):474-480.

50. Nordin, B. E. C., 1974, Calcium, phosphorus and vitamin D, Practitioner, 212(4):474-480.

51. Nordin, B. E. C., R. Wilkinson, D. H. Marshall, J. C. Gallagher, A. Willians, and M. Peacock, 1976, Calcium abosrption in the elderly, Calcif. Tissue Res., 20 Suppl.:448-451.

52. Nordin, B. E. C., 1978, Diagnostic procedures in disorders of calcium metabolism, Clin. Endocrinol., 8(1):55-67.

53. O'Hanlon, P. L., and M. B. Kohrs, 1978, Dietary studies of older Americans, Am. J. Clin. Nutr., 31(7): 1257-1269.

54. Ohio State Data Base - The Ohio State University Hospitals Department of Dietetics Nutrient Calculation Data Base. 1975.

55. Pelcovits, J., 1971, Nutrition to meet the human needs of older Americans, J. Am. Diet. Assoc., 60(4): 297-300.

56. Perkin-Elmer, Atomic Absorption Spectrophotometer, Instruction Model 303, 303-0073, Norwalk, Connecticut, USA, June 1966.

57. Ramasastri, B. V., 1974, Improving the nutritive value of diets through fortification of accessory foods, Indian J. Nutr., Diet., 11(4):213-215.

58. Rao, C. N., and B. S. Rao, 1974, Absorption of calcium from calcium lactate and calcium sulphate by human subjects, Indian J. Med. Res., 62(3):426-429.

59. Rawson, I. G., E. I. Weinberg, J. A. Herold, and J. Holtz, 1978, Nutrition of rural elderly in southwestern Pennsylvania, Gerontologist, 18(1):24-29.

60. Renner, R., 1977, Nutritional aspects of bone loss, J. Can. Diet. Assoc., 38(2):111-115.

61. Rhone, D. P., F. M. White, and H. Gidaspow, 1974, Isoenzymes of liver alkaline phosphatase in serum of patients with hepatobiliary disorders, Clin. Chem., 19(10):1142-1147.

62. Robinson, J. W., Atomic Absorption Spectrocopy, Marcel Detcher, Inc., New York, 1966.

63. Rountree, J. L., and G. L. Tinklin, 1975, Food beliefs and practices of selected senior citizens, Gerontologist, 15(6):537-540.

64. Russell, R. G. G., 1978, Regulation of calcium metabolism, Ann. Clin. Biochem., 13(6):518-539.

65. Sigma Chemical Company, Alkaline phosphatase, Techn. Bul. No. 104, 1963.

66. Siegel, J. D., M. D. Herrebruck, D. S. Akers, and J. S. Parrell, Demographic aspects of aging and the older population in the United States, U.S. Department of Commerce Bureau of the Census, Ser., No. P-23: No. 59. 2nd print. 1978.

67. Smith, R. W., and B. Frame, 1965, Concurrent axial and appendicular osteoporosis: Its relation to calcium consumption, N. Engl. J. Med., 273(2):73-78.

68. Spencer, H., J. Menczel, I. Lewin, and J. Samachson, 1964, Absorption of calcium in osteoporosis, Am. J. Med., 57(8):223-233.

69. Steggerda, F. R., and H. H. Mitchelle, 1951, The calcium balance of adult human subjects on high and low fat diets, J. Nutr., 45(1):201-211.

70. Stiedmann, M., C. Jansen, and I. Harrill, 1978, Nutritional status of elderly men and women, J. Am. Diet. Assoc., 73(2):132-139.

71. U.S. Department Agriculture Handbook No. 8. Composition of foods - raw, processed, prepared, Agriculture Research Service, Washington, D.C., 1963.

72. Ten-State Nutrition Survey, 1968-1970: V Dietary and Highlights, DHEW. Pubs., No. (HSM) 72 - 8134, 1972.

73. Vose, G. P., and A. Engel, 1973, Relationship of radiographic cortical thickness vs. age in thirteen bones of the hand, J. Gerontol., 28(1):46-49.

74. Walker, R. M., and H. M. Linkswiler, 1972, Calcium retention in adult human male as affected by protein intake, J. Nutr., 102(11):1297-1302.

APPENDIX A

RECORD OF DAILY ACTIVITIES

CASE NO.______________

DATE__________________

RECORD OF DAILY ACTIVITIES

Time Arose:

Time Retired:

What types of physical activities did you participate in today and for how long? (Over and above typical activities.)

____

____

____

Were you under any unusual emotional stress today? (Please be be specific.)

____

____

____

____

Physically how did you feel today? (Please describe symtoms and be specific.)

____

____

____

APPENDIX B

CONSENT FORM I

TEXAS WOMAN'S UNIVERSITY
P.O. Box 23975
DENTON, TEXAS 76204

DEPARTMENT OF NUTRITION AND FOOD SCIENCES,
COLLEGE OF NUTRITION, TEXTILES,
AND HUMAN DEVELOPMENT

PHONE (817) 382-5611
(817) 387-5305

Consent Form I

I, ______________________, do hereby agree to participate in the study entitled, " Calcium Balance Study for $CaCO_3$ Enriched Baked - Products for Older Adults". I agree to eat meals which will be served to me during about one month of a study period. I agree to take Vitamin D supplement during the last eight days of the study. I agree to collect my urine and feces daily, and I give my permission for taking blood samples during the study period. I agree to cooperate with the physician and the nutritionist, and to follow their instructions explicitly.

I thoroughly understand that there is no known health risk in the study.

I understand that my name will never be divulged to the public.

I understand I am free to withdraw from the study anytime.

X-ray's will be taken with an extremely low level of radiation exposure.

Signed ______________________

Date ______________________

Witness ______________________

Date ______________________

APPENDIX C

CONSENT FORM II

TEXAS WOMAN'S UNIVERSITY
P.O. BOX 23975
DENTON, TEXAS 76204

EPARTMENT OF NUTRITION AND FOOD SCIENCES,
LLEGE OF NUTRITION, TEXTILES,
AND HUMAN DEVELOPMENT

PHONE (817) 382-5611
(817) 387-5305

Consent Form II

I, ____________________ , do hereby agree to participate in the study entitled " Calcium Balance Study for $CaCO_3$ Enriched Baked Products for Older Adults ". I understand I am free to withdraw from the study anytime. I understand I will receive $ 150 (One hundred and fifty dollars) if I complete the entire study, but not otherwise.

Signed ______________________

Date ______________________

Witness ______________________

Date ______________________

APPENDIX D

THE MASTER MENUS

THE MASTER MENUS

Food Item	Amount (gram)	Measure
Day One		
Breakfast		
Grapefruit juice	123	1/2 c.
Scrambled eggs	56	1 serving
Bacon	7	1 slice
Margarine	5	1 tsp.
Jelly	14	1 Tbsp.
Roll	40	1 roll
Coffee/tea		
Lunch		
Spaghetti with meat balls	206	1 serving
Margarine	5	1 tsp.
Bread, white, enriched	23	1 slice
Apple	180	1 medium
Chocolate chip cookie	17	1 cookie
Ice tea/coffee		
Dinner		
Baked chicken	85	1 serving
Peas, green	57	1/3 c.
Potato, new, boiled	75	3/4 c.
Margarine	5	1 tsp.
Roll	40	1 roll
Ice tea/coffee		
Night Snack		
Vanilla wafers	15	4 wafers

THE MASTER MENUS--Continued

Food Item	Amount (gram)	Measure
Day Two		
Breakfast		
Orange juice	123	1/2 c.
Corn flakes	19	1/2 c.
Milk (2% fat)	123	1/2 c.
Roll	40	1 roll
Margarine	5	1 tsp.
Jelly	14	1 tbsp.
Coffee/tea		
Lunch		
Hot turkey sandwich		
Turkey with gravy	115	1 serving
Bread	43	2 slices
Salad dressing	10	1 tbsp.
Lettuce	56	3/4 c.
Tomato	50	1/2 c.
Fruit jello	120	1/2 c.
Oatmeal cookie	20	1 cookie
Iced tea/Coffee		
Dinner		
Ground beef pattie	50	1 small pattie
Macaroni and cheese	227	1 serving
Turnip greens	50	1/3 c.
Sliced tomato	50	1/2 c.
Lettuce leaf	20	1 leaf
Margarine	5	1 tsp.
Roll	40	1 roll
Pound cake	29	1 slice
Iced tea/coffee		
Night Snack		
Graham crackers	14	2 squares

THE MASTER MENUS--Continued

Food Item	Amount (gram)	Measure
Day Three		
Breakfast		
Orange juice	125	1/2 c.
Cream of wheat	123	1 individual pkg.
Milk (2% fat)	123	1/2 c.
Jelly	14	1 tbsp.
Margarine	5	1 tsp.
Roll	40	1 roll
Coffee/tea		
Lunch		
Ham and salami sandwich		
Ham	28	1 slice
Salami	28	1 slice
Bread	46	2 slices
Salad dressing	10	1 tbsp.
Lettuce leaf	20	1 leaf
Green beans	68	1/2 c.
Canned peach halves	96	3/4 c.
Sugar cookie	20	1 cookie
Iced tea/coffee		
Dinner		
Roast beef with gravy	140	1 individual pkg.
Mashed potatoes	105	1/2 c.
Asparagus	121	1/2 c.
Margarine	5	1 tsp.
Roll	40	1 roll
Ice cream	67	1/2 c.
Night Snack		
Banana	175	1 medium

APPENDIX E

RECIPES

OATMEAL COOKIE

Sugar	100 gm
Brown Sugar	200 gm
Crisco	150 gm
Egg	50 gm
Flour (all purpose)	115 gm
Oatmeal	240 gm
Salt	1 tsp.
Vanilla	1 tsp.
Baking Soda	1/2 tsp.
Water	60 ml
Calcium Carbonate	15.50 gm (for Ca fortified oatmeal cookie)

1. Cream shortening and add sugars, eggs, and water and vanilla.
2. Sift together flour, salt, soda. Add to creamed mix.
3. Stir in oatmeal.
4. Drop by 20 gm of dough on greased cookie sheet.
5. Bake in preheated oven at 350 F. for 12 minutes.
6. Remove from the sheet in 2 to 4 minutes. Pack and freeze.

CHOCOLATE CHIP COOKIES

Sugar	100	gm
Crisco	100	gm
Brown Sugar	110	gm
Flour (all purpose)	115	gm
Eggs	50	gm
Vanilla	1	tsp.
Salt	3/4	tsp.
Chocolate Chips	63.5	gm (0.57 gm each chip)
Calcium Carbonate	10.5	gm (for Ca fortified chocolate chip cookies)

1. Cream Crisco, add sugars then vanilla and egg.

2. Combine dry ingredients and add into the mix. Stir well.

3. Weigh 17 gm dough and place on a greased cookie sheet.

4. Stick 4 even pieces of chocolate chips into each cookie dough.

5. Bake in preheated oven at 375 F. for 9 minutes.

6. Pack and freeze.

YEAST BREAD

Yeast	16 gm	
Sugar	24 gm	
Salt	5 gm	
Flour	400 gm	
Crisco	40 gm	
Milk	300 ml	
Flour	40 gm	(for kneading)
Calcium Carbonate	7.65 gm	(for Ca fortified yeast bread)

1. Heat the milk and let it cool to 40° C.

2. Allow yeast to hydrate in milk for five minutes.

3. Add sugar, salt, fat and $CaCO_3$ (if needed) and approximately half of the flour. Beat with a wooden spoon for 2 minutes. (Do not be concerned if fat does not disperse well at this stage.)

4. Add half of the remaining flour and beat for one minute.

5. Flour bread board lightly with additional flour. Knead the dough for 100 strokes. (Grease hands for this step.)

6. Shape dough into a ball and place it into a greased mixing bowl.

7. Place a thermometer in dough, touching the bottom of the bowl, and proof for 20 minutes at 30 C.

8. Punch down dough and knead an additional 50 strokes.

9. Weight out 40 gm dough and shape it into a roll and place into a greased muffin pan.

10. Keep the rolls at 30 C. until it doubles in size, about 15 minutes.

11. Bake in a preheated oven at 425 F. for 5 minutes. Then turn the temperature down to 400 F. for 10 minutes.

12. Brush top of the rolls lightly with margarine and cool on the rack for a few minutes.

13. Pack and freeze right away.

SUGAR COOKIES

Sugar	150 gm
Crisco	135 gm
Eggs	50 gm
Milk	15 gm
Flour (all purpose)	230 gm
Baking powder	4.5 gm
Vanilla	1 tsp.
Salt	1/4 tsp.
Calcium carbonate	10.80 gm (for Ca fortified sugar cookies)

1. Thoroughly cream shortening, add sugar then vanilla.

2. Add egg, beat until fluffy, stir well.

3. Sift dry ingredients, mix well.

4. Chill dough 1 to 2 hours.

5. Weigh out 20 gm and shape round. Chill the cookie dough again 10 to 15 minutes.

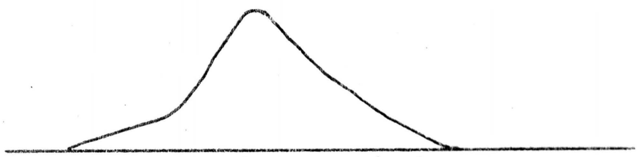
6. Bake in preheated oven at 375 F. for 10 minutes.

7. Pack and freeze.

APPENDIX F

ALKALINE PHOSPHATASE ISOENZYME SEPARATION FIGURES

Part 1: Subject 3368



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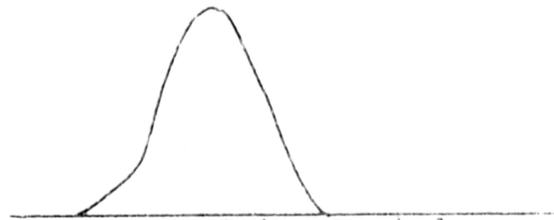

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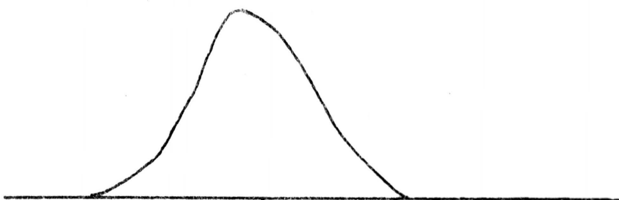

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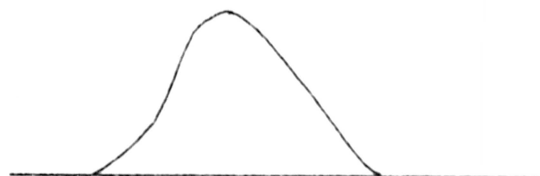
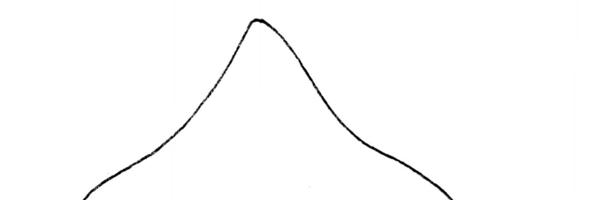


High Calcium with
Vitamin D Supplemented

Part 2: Subject 3369



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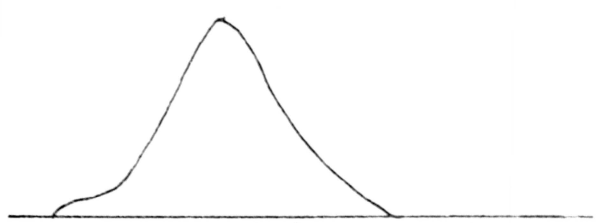

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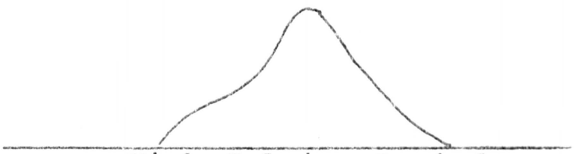

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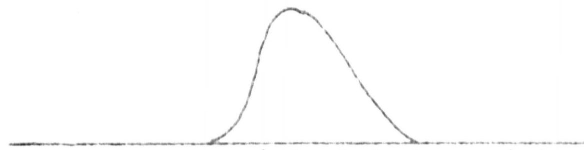
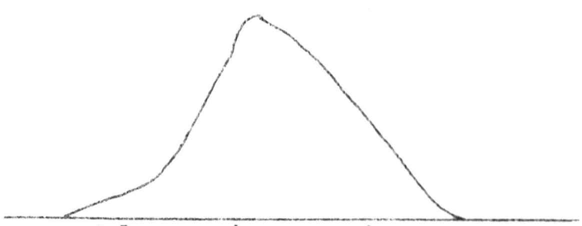


High Calcium with
Vitamin D Supplemented

Part 3: Subject 3370



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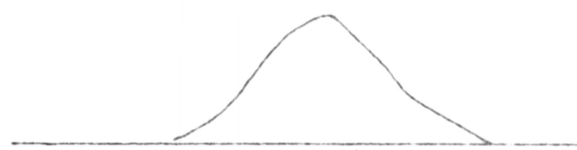

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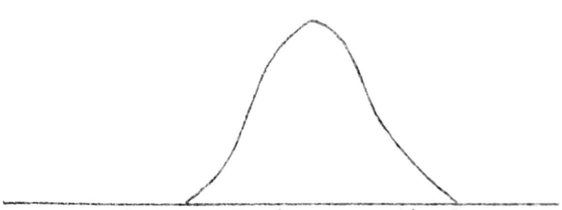

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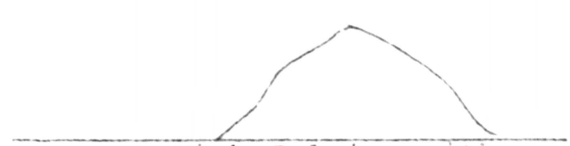
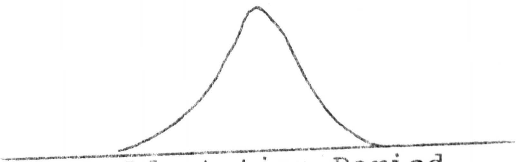


High Calcium with
Vitamin D Supplemented

Part 4: Subject 3371



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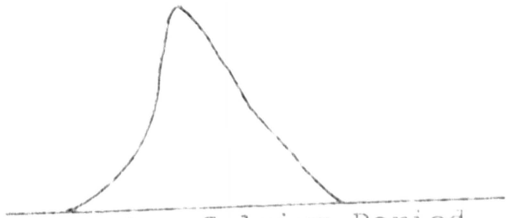

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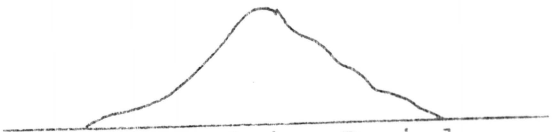

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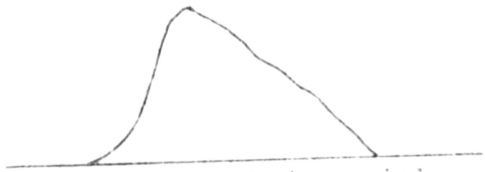
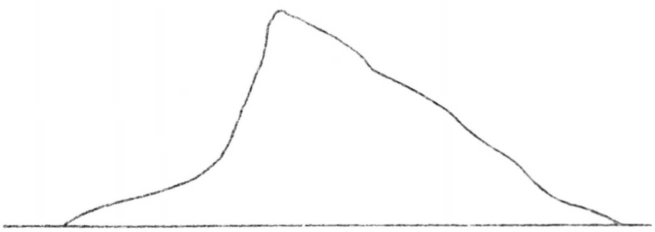


High Calcium with
Vitamin D Supplemented

Part 5: Subject 3372



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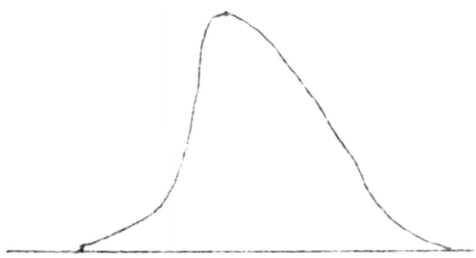

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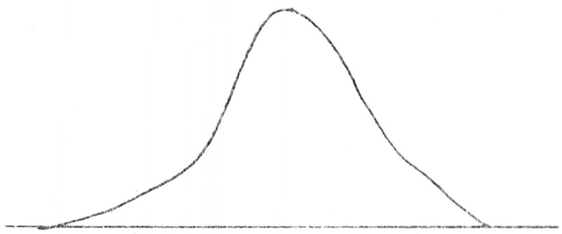

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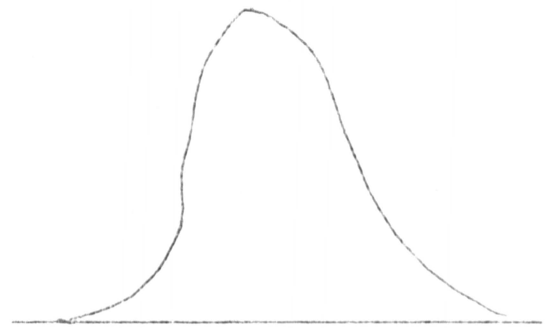
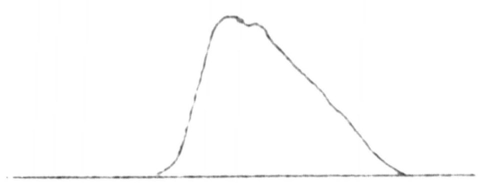


High Calcium with
Vitamin D Supplemented

Part 6: Subject 3374

Adaptation Period



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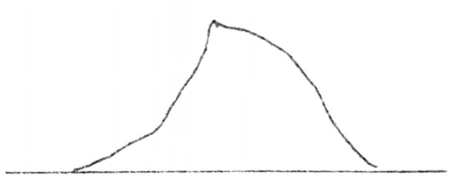
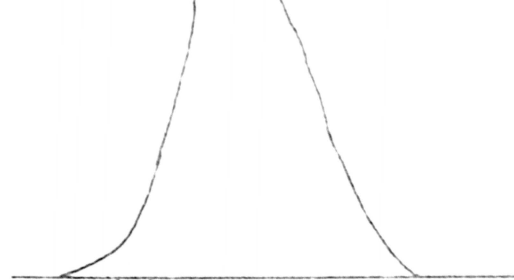
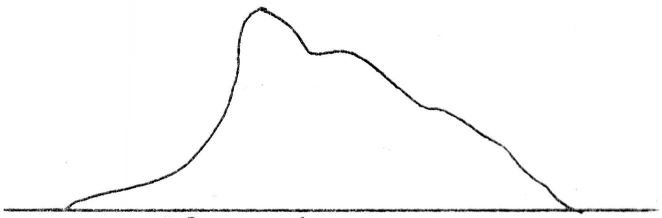


High Calcium Period

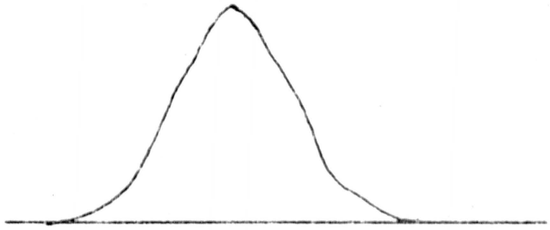


High Calcium with
Vitamin D Supplemented

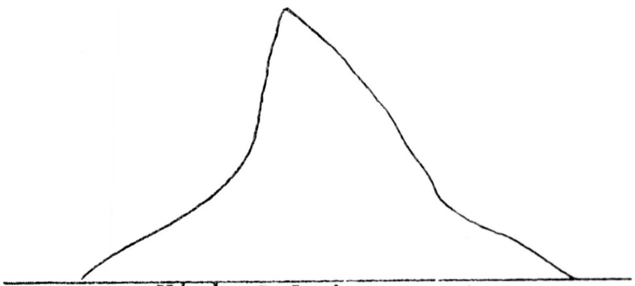
Part 7: Subject 3375



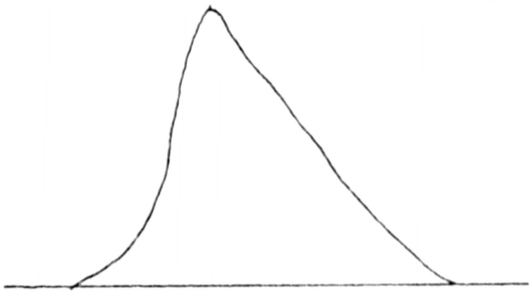
Adaptation Period



Low Calcium Period

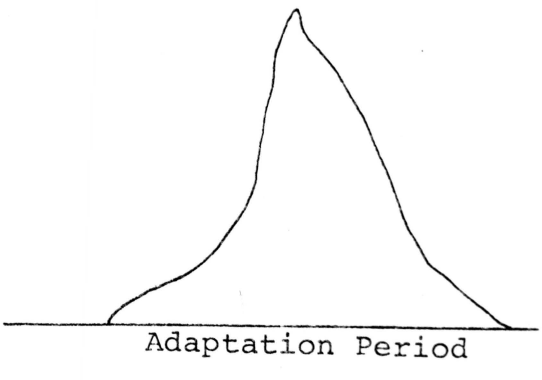


High Calcium Period

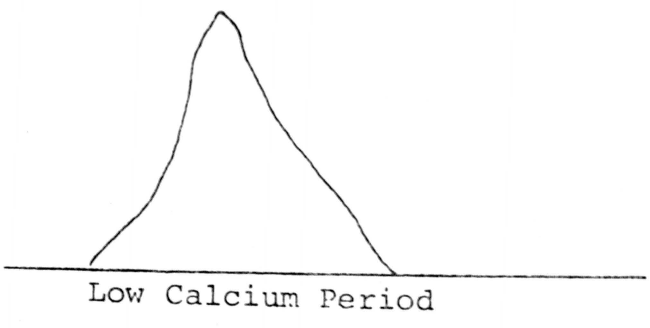


High Calcium with
Vitamin D Supplemented

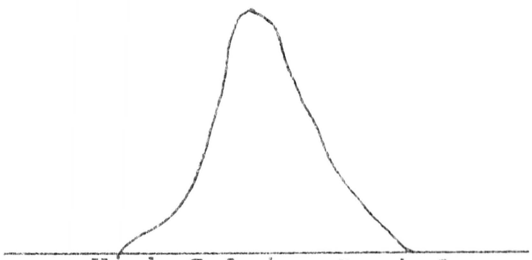
Part 8: Subject 3377



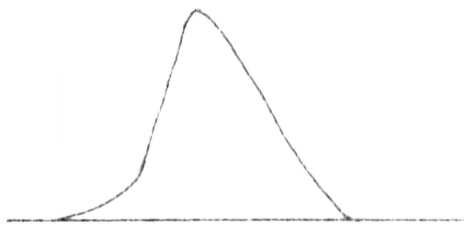
Adaptation Period



Low Calcium Period



High Calcium Period



High Calcium with
Vitamin D Supplemented