CALCIUM RETENTIONS OF YOUNG ADULT WOMEN ON A CONTROLLED WEIGHT REDUCTION DIET

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by

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INTRODUCTION

The problem of weight reduction is an important one for the young woman, both from an esthetic and physiologic standpoint. The physiological strain of excess body tissue has been receiving increasing attention within the past few years and has resulted in a number of popular dietary regimes for weight reduction.

Obesity is frequently the result of poor nutritional habits with an over balance of high calorie fats and carbohydrates in the diet to the exclusion of essential minerals and vitamins. A weight reduction diet should not only cause reduction in excess fatty tissue, but should attempt to promote good dietary habits by providing the necessary food nutrients. This is particularly true of the young adult woman who should receive a diet which will be both adequate for completion of adolescent growth and in preparation for the child-bearing years.

The development and refinement of methods in nutrition research and the accumulation of scientific information in this field have resulted in an appreciation of the complexity of the interrelationships which exist between essential body nutrients. A change in the quantity of one or two dietary essentials for any prolonged period may alter the accepted pattern of food intake and result in an impairment of physiological processes; therefore, it is important to give consideration to interrelationships of dietary essentials.

The possible interrelationship of calcium and fat metabolism has been of interest since the early studies of Bosworth (1918) who reported that calcium was excreted as calcium soaps of the higher molecular fatty acids, and Holt (1920) who found that maximum calcium absorption was related to a definite fat to calcium ratio.

The purpose of the present study was to investigate the calcium retention of young college women on a weight reduction diet in which the calories were supplied chiefly by protein and fat. The possible interrelationship of the dietary constituents was also a matter of consideration.

REVIEW OF LITERATURE

The literature on calcium utilization in human nutrition indicates that calcium absorption is subject to considerable variation, not only between individuals, but also in respect to the same individual. The amount of calcium which is available for the body depends, as with other food nutrients, upon the ability of the body to absorb it from the food ingested. The form of calcium present in the diet may be insoluble in the digestive tract; therefore absorption is limited. Further it has been shown that various factors may influence both the solubility of calcium in the intestinal tract, and the absorption of calcium from the intestinal tract. The following review includes some of these factors with particular reference to the literature which deals with dietary factors that influence calcium absorption and retention.

Factors Affecting Calcium Retention

Dietary fat. Many early studies on the interrelationship of dietary fat and calcium were concerned with infant feeding problems. Bosworth et al. (1918) studied certain bottle-fed infants in respect to an apparent inability of the infants to digest and absorb fat. The modified cow's milk formula regulated the intake of organic constituents so that the composition approximated that of breast milk; however, the same adjustment was not made in respect to the inorganic constituents; thus the intake of calcium by these infants was much greater than those receiving breast milk. Observation of the stools of the breast-fed and bottle-fed infants revealed a greater excretion of calcium in the latter. The increase in fecal calcium was characterized by an increase in calcium soaps and was attributed to the imbalance of calcium to fat in the formula. The greater excretion of calcium with the modified formula was cited as a method of protecting the body from an excess of this element, but the point was made that in so doing nutritional disturbances might result. By decreasing the calcium content of the modified formula it was found that the calcium eliminated as soap was decreased and the infants were able to utilize the fat present in a normal manner. Investigation of the stools of infants receiving the modified formula before the calcium content was adjusted indicated the presence of high molecular fatty acids with a range in melting point from 50°-54° centigrade. To study the solubility of the calcium-fatty-acid complex pure butter was saponified with sodium ethylate. The solubility of this soap was found to be excellent in fresh ox bile, but upon the addition of calcium chloride, calcium soaps were precipitated. Further investigation showed the non-volatile fraction of the fatty acids obtained from butter fat to be soluble in ox bile but heavily precipitated by calcium chloride. The volatile fatty acid fraction, on the other hand, remained in solution on the addition of calcium chloride. The soaps formed by the non-volatile fatty acid fraction were similar to the type of soap found in the stools.

Holt <u>et al</u>. (1920b) found no relationship between a large intake of fat and excessive excretion of calcium; moreover, the calcium lost as soap was insignificant. In fourteen of seventy-nine instances the calcium excreted by infants as soap did not exceed 10 per cent. When the form of calcium which was excreted in the stools was analysed, it was found that the calcium found as soap was seldom more than three-tenths and never more than five-tenths of the total calcium excreted. These investigators found that greatest calcium absorption took place when the fat intake was greater than 3.0 grams per day with a fat: calcium oxide ratio of one gram of fat to 0.03-0.05 grams of calcium oxide.

Tefler (1921) observed infants suffering from congenital biliary atresia and concluded that an increase in the excretion of calcium soaps was evidently correlated with an increased concentration of fat in the diet and, moreover, that the form in which calcium and phosphorus was eliminated was dependent upon the concentration of the fatty acids in the intestine. Tefler suggested that calcium phosphate was dissolved in the lower molecular fatty acids present in the intestinal tract with subsequent precipitation of calcium as calcium soaps by the high molecular fatty acids. These secondary calcium salts could not be absorbed. Boyd et al. (1932) studied the relation of dietary fat to calcium utilization in white rats and found that the lower molecular fatty acids readily dissolved insoluble calcium carbonate and calcium phosphate, thus rendering them available for absorption. However, in the presence of high molecular fatty acids, increased formation of insoluble calcium soaps was found. When pure calcium soaps were fed in a synthetic food mixture which was otherwise free from calcium, the following order of utilization of calcium was found: lactate, 68.5 per cent, butyrate, 52.5 percent, oleate, 68.4 per cent, mixed soaps from lard, 46.7 per cent, palmitate, 39.4 per cent, and stearate, 30.0 per cent. When calcium chloride was the source of calcium on a fat free diet, the calcium lost in the feces amounted to 34 per cent. When lard* was added to the diet, the calcium lost in feces dropped to 22 per cent. It was calculated that 20.8 milligrams of calcium were retained on the fat free diet, compared with 29.3 milligrams when lard was included in the diet. The fat free

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* Lard was added to the amount of 10 per cent by weight; adjustment in the diet was made by a decrease in carbohydrate.

diet containing calcium chloride caused a neutral or alkaline reaction in the lower intestine and cecum. The addition of fat to the diet shifted the reaction of the upper half of the small intestine to greater acidity (pH 6.44 to 5.81). With this shift in acidity the amount of calcium retained by the experimental animals increased. When calcium lactate provided the source of calcium on a fat free diet, addition of fat caused little shift in intestinal reaction and increased the per cent absorptionof calcium only slightly. The indications from this work were that moderate amounts of fat might benefit calcium absorption through the maintenance of a favorable intestinal acidity. The studies of Jones (1940) on the addition of lard to rachitogenic diets of rats indicated that when lard was included in the diet in amounts of 5 to 25 per cent of the diet, on the basis of both weight and calories, definite antirachitic properties were observed which could not be accounted for in terms of the non-saponifiable portion. This work also suggested a beneficial effect of the addition of fat in the form of lard to calcium absorption.

French (1942) studied weanling male rats and found that the utilization of calcium decreased moderately and consistently as the fat content of the diet was increased. When the concentration of fat in the diet amounted to five per cent, a more acid condition existed in the intestinal tract and the calcium utilization paralleled the increased acidity. Average values for the pH of the jejunum contents were 5.81, 6.02, 6.18, and 6.65. These corresponded with the diets containing 5, 15, 28, 45 per cent fat respectively. The percentage of utilization for the four diets showed a progressive decrease as the fat content of the diet was increased, resulting in the following values: 80.0 per cent, 77.7 per cent, 74.1 per cent and 46.8 per cent. A diet with a relationship of one gram of fat to 0.06 grams of calcium oxide was found to be most efficient for calcium absorption; as the ratio of fat to calcium increased, the utilization of calcium was decreased. This work would seem to confirm that of Holt (1920) which reported the optimal effect of a certain calcium to fat ratio on calcium absorption.

Bosworth et al. (1918) observed that the extent of soap formation, and the ultimate fecal excretion of calcium, were dependent upon the fatty acid of the soap formed. Calcium oleate being more readily soluble in bile than either calcium palmitate or calcium stearate was absorbed from the intestine in large quantities, even with an excess of calcium ions. Chen, Morehouse and Deuel (1949) found that fecal calcium was increased when there was poor absorption of fats and fatty acids. They also found that calcium storage decreased under these circumstances wven when the calcium intake was high. The minimum loss of calcium occurred when the dietary fats had a low melting point; when the melting point exceeded 50° centigrade, there was marked loss of calcium which was proportional to the quantity of the fat fed.

In an additional study of the relation of fat to fecal excretion of calcium, Calverley and Kennedy (1948) also found that fecal calcium increased with an increase in the melting point of the dietary fat. The animals on a fat free diet excreted the least amount of fecal calcium. The free fatty acids calculated as soap in a study of the neutral fat and fatty acid in feces were found to be greatest when the diet included hydrogenated cottonseed oil. With the short chain saturated fatty acids, or long chain unsaturated acids, the increase in fecal fat was less marked.

Mattil and Higgins (1945) studied the relationship of glyceride structure to fat digestibility and found that stearic acid was poorly digested, whether it was fed as mixed glyceride or as simple triglyceride. The reaction of stearic acid with calcium ion resulted in fecal elimination of calcium. Hoagland and Snider (1943a) found no consistent relationship between the melting point of fats tested and the coefficient of digestibility; however, none of the fats mentioned in this study had a melting point above 50° centigrade. The tendency was in the direction of decreased digestibility with increasing melting point at both 5 per cent and 15 per cent intake of fat by weight. In a later study

(1943b) these same workers compared the digestibility of stearic, palmetic, myristic and lauric acids. The digestibility was found to be 21 per cent for stearic acid, 39.6 per cent for palmetic acid and 100 per cent for each of the other fatty acids.

Whereas there seems to be little evidence to support the theory that calcium is detrimental to fat absorption as advanced by Bosworth (1918), evidence has accumulated which is in agreement with the observations of these investigators on the poor utilization of fatty acids of high molecular content (Hoagland and Snider, 1943b; Mattil and Higgins, 1945), and the tendency of these fatty acids to bind dietary calcium in the form of insoluble soaps (Westerlund, 1934; French, 1942; Boyd, 1932; Calverly, 1948; Kane, 1949).

The relationship of calcium absorption to fat intake shows considerable variation with human subjects. The work of Holt (1920a, 1920b) with infants and young children demonstrated no close relationship of calcium absorption to fat intake; however, there was a definite ratio of calcium to fat which produced the best calcium absorption. Telfer (1921) found improved calcium utilization with moderate amounts of fat in the form of lard. Studies by Holt (1923) with children from two to six years of age supported the theory that fat has a beneficial effect on calcium absorption. Five of the seven children studied showed marked reduction in calcium absorption when the dietary fat was reduced from approximately 30 to 5 per cent of the daily calorie intake. Mallon *et al*. (1930) found no evidence of an influence of fat intake on the calcium retentions of two young women on intakes of 105.6 grams of fat per day, and 5.8 grams per day. A later study (Mallon *et al*., 1933) gave similar findings.

The work of Kane *et al*. (1949) using rats showed no difference in calcium absorption on diets containing one and twenty-one per cent of fat when young animals were the subject of investigation but a decrease in absorption was evident with increasing age. At 48 weeks female rats showed negative retentions of approximately the same magnitude on both diets; at 72 weeks the animals on the high fat intake showed a slightly greater negative balance than those animals on the lower fat intake. At the age of 96 weeks there was marked difference between the calcium balances of animals on the two diets; the animals which received a diet containing one per cent fat showed a balance of -1.1 milligrams per day, while those with intake of twenty-one per cent fat showed a balance of -3.0 milligrams per day.

These studies with experimental animals seem to parallel the findings of Holt (1920a, 1920b and 1923) for infants and young children, and Mallon (1930, 1933) for young women. As the maintenance of a positive calcium retention seems to become more difficult with increasing age in the human subject, the relationship of fat to calcium retention at different ages gains in importance.

Dietary protein. McCance et al. (1942) using adult male subjects found that an increase in the amount of protein in the diet markedly increased the absorption of calcium. On the basal diet, which contained 45-70 grams of protein, the average absorption of calcium was 32 milligrams per day, whereas on the protein supplemented diet the average calcium absorbed was 94 milligrams per day. Hall and Lehmann (1944) theorized that the addition of protein to the diet, and therefore an increase in amino acid content of the intestine, should increase the solubility of calcium salts. These workers studied the effect of protein supplement using an increase in urinary calcium as an indication of increased calcium absorption. Fourteen of 18 subjects showed a higher urinary calcium after the intake of peptone powders as against control powders which contained no form of protein or protein derivative.

Two studies of calcium retention by young women (Kunerth and Pittman, 1939; Pittman and Kunerth, 1939) also indicated that improved calcium retention paralleled an increase in dietary protein. Three young women acted as subjects in each study of forty-five days including fifteen 3-day experimental periods. During the study of low protein intake approximately 25 grams of protein per day were supplied. The second study provided a "medium" protein diet containing approximately 68 grams of protein per day. In each case meat accounted for 85 per cent of the protein allowance. Calcium intake was maintained at approximately the same intake in each study (mean per day, 0.458 grams and 0.459 grams respectively). In both studies all subjects were in negative calcium balance. However, the beneficial effects of the higher protein intake were evident from the improved retention in the subjects during this period. Individual balances for the three subjects on the low protein intake were -0.054, -0.099, -.090 grams per day. These same subjects during the period of higher protein intake showed retentions of -0.025, -0.040, -0.039 grams per day respectively.

The observation of Leverton and Marsh (1942) on the metabolism of young women would indicate that the calcium intake provided in these two diet studies was below that necessary for calcium equilibrium in this age group, and considerably lower than that given by Leverton and Marsh as the "minimal" requirement (0.83 grams per day). In eighteen studies of young women on self-chosen diets these investigators found that 61 per cent were in negative balance when the intake of calcium was in the range of 0.400 to 0.599 grams per day.

Adolph et al. (1932) studied the calcium utilization of three Chinese adults, 18 to 27 years of age, on diets which provided protein in the form of milk and soybean curd. They found that an increase in protein, whether supplied by milk or by soybean curd, facilitated the establishment of calcium equilibrium. With a low protein intake all subjects were in negative balance. When the amount of protein was increased with the calcium intake remaining approximately the same, calcium retention improved in all cases.

Additional factors influencing calcium absorption. The intestinal output of calcium has been shown to be increased by an excess of phosphorus in the diet (Fearon, 1947); absorption of the calcium on a low calcium:phosphorus ratio possibly is prevented by the formation of insoluble tricalcium phosphate (Knapp, 1943). Tisdall et al. (1938) observed that rats fed a preliminary low calcium diet with subsequent increase in calcium only, improved in retention of both calcium and phosphorus and concluded that the retention of phosphorus seemed to be dependent upon the retention of calcium. Sherman (1948) concluded that the ratio of calcium to phosphorus would be satisfactory if an ample allowance for each nutrient were provided in the dietary. In the case of children Stearns(1931) advocated a retention ratio ranging from 1.5 to 2 : 1. The recommended dietary allowance for phosphorus for adults, as stated by the Food and

Nutrition Board of the National Research Council (1948), is approximately 1.5 times the intake of calcium; this ratio could be maintained readily if foods adequate in calcium were included in the diet.

Other dietary factors which have been shown to decrease the absorption of calcium from the intestinal tract are phytic and oxalic acid (Fearon, 1947) and carbonate and sulphate ions (Kleiner, 1948). Stewart and Percival (1928) in reviewing the literature on calcium metabolism included the presence of excess potassium and magnesium ions as detrimental to calcium absorption whereas chloride ion appeared to have a beneficial effect upon calcium absorption. Aub *et al.* (1937) found that additions of sodium chloride were effective in increasing calcium absorption only in massive doses.

Anchau (1930) studied the effect of increased bulk on output of fecal calcium and phosphorus in dogs. With one exception the findings showed a greater calcium excretion with a high roughage diet. In the studies of Kunerth and Pittman (1939) previously mentioned it was found that the one subject who received a more bulky diet excreted a greater amount of fecal calcium than the other two subjects. However, this same subject in a later study (Pittman and Kunerth, 1939) also excreted a larger amount of fecal calcium on a diet containing approximately the same amount of bulk as the others on the study. Aub et al. (1937) found no relationship between calcium excretion and fecal volume.

Brieter et al. (1942) in a study of the utilization of calcium from carrots found that the addition of 700 grams of carrots per day resulted in an increased volume of fecal material and in most cases an increased intestinal activity. Five of the subjects studied showed poor utilization of calcium. The subject with good utilization of the calcium supplied by the carrot diet reported a decrease in fecal excretion. Despite the increase in bulk produced by a diet providing 93 per cent of the calcium intake through leaf lettuce, Mallon et al. (1933) reported superior utilization when this diet was compared with a diet supplying approximately the same quantity of calcium through whole pasteurized milk.

The effect of cellulose upon mineral absorption in adults was studied by Morgan (1934) with two periods of nine days each differing only in the addition of 20 grams of regenerated cellulose per day during the second period of a basically low roughage diet. The additional cellulose increased intestinal activity; however, six of eight subjects showed a positive calcium balance during the first period, while five of the eight subjects showed a negative balance during the second period of increased bulk. The composite calcium deficit during this period was 12.9 per cent; about

12.5 per cent of the calcium was eliminated in the feces. Morgan concluded from this study that a high roughage diet had a definite limiting effect upon calcium absorption.

Mention should also be made of the influence of Vitamin D upon calcium absorption. Although the mechanism by which the absorption is increased is not fully understood, it has been demonstrated that Vitamins D_2 and D_3 are most effective in promoting absorption. (Eddy, 1949). While a deficiency of Vitamin D is unlikely to occur in the adult, it may be a factor in border line cases of calcium deficiency (Youmans, J. B., 1950).

In addition to the influence of dietary pattern on calcium absorption McCance and Widdowson (1949) observed a seasonal variation. During July and August three of six subjects showed markedly greater absorption of calcium than in February and March, although the calcium intake was practically constant during the different periods. Administration of 2,000 I. U. of Vitamin D in March did not materially improve calcium absorption; this indicated that the seasonal change was not due to greater exposure to sunlight during the summer months. A similar study of magnesium and phosphorus failed to show any seasonal rhythm. Sherman, Gillette, and Pope (1918) studied the calcium metabolism of healthy women and found no variation due to the menstrual cycle; however, a comparison of the retention values for one of the subjects during successive months suggested a possible seasonal variation. During February, March and May this subject was in negative calcium balance; the average retention in February was -0.125 grams and in May the average retention was -0.05 grams. During June the subject showed a positive retention of 0.08 grams. Leverton and Marsh (1942) reported the calcium retentions of young women. In several cases subjects were studied over a period of months. Examination of these figures, and interpretation from information given regarding age at the time of each balance period, failed to suggest a seasonal rhythm. However, if seasonal variation is a true characteristic of calcium retention it must be taken into consideration in the evaluation of metabolic studies over a limited period of time.

Fairbanks and Mitchell (1936) investigated the relationship between calcium retention and the store of calcium in the body. In a preliminary period three paired groups of growing rats were fed diets providing 0.18 and 1.25 per cent 336 mg, 2336 mg), 0.32 and 1.25 per cent (610 mg, 2559 mg), 0.49 and 1.25 per cent (746 mg, 1916 mg) calcium respectively. At the end of the preliminary period, three or four pairs from each group were sacrificed for analysis of calcium to determine the body content. In all groups animals receiving the higher intake of calcium were found to have a greater store of body calcium. Retention studies during the preliminary period were inversely proportional to the calcium intake. When the diet provided 1.25 per cent calcium, retention was approximately 25 per cent while on the lower intakes (0.18, 0.32, 0.49 per cent) the percentage retentions were 106, 76, and 55 respectively. After the completion of the experimental period all animals were placed on a diet contributing 1.25 per cent calcium. Subsequent studies of the retention demonstrated that the degree of saturation of calcium stores in the preliminary period produced inequalities in calcium retention when a uniform high calcium diet was fed. Greater retention was found when the initial saturation of calcium was low. This would indicate the desirability of a period of adjustment to equalize calcium stores of experimental subjects, or information concerning previous calcium intake before interpreting data of calcium retention studies.

The Calcium Requirement of Young Adult Women

The periods of greatest calcium need are those associated with the years of growth and during pregnancy and lactation. After adolescent growth is completed, there is in the adult a relatively low but continuous deposition and withdrawal of calcium going on in body tissues including bone. Over a reasonable period of time the withdrawal should be balanced by deposition so that no net loss of calcium occurs (Leitch, 1937).

Mitchell (1949) discussed the nutrient requirements for adult growth in respect to the nitrogen and phosphorus balances of 23 male university students. Approximately one gram of nitrogen and 0.174 grams of phosphorus were retained daily from an adequate diet, presumably for adult growth including the skin, hair and nails and in the case of phosphorus for delayed calcification of bones and teeth. The possibility of continuing saturation of bone after growth has apparently taken place was shown in the investigations of Fairbanks and Mitchell (1936). McKay et al. (1942) also found in observations of young college students that retention of calcium was continued after apparent growth was completed when the calcium intake was adequate. Therefore it may be concluded that nutritional requirements of certain essentials continue in the adult over and above those required for endogenous losses.

Sherman (1920) estimated a daily requirement of 0.68 grams of calcium per day for the theoretical 70 kilogram man. Leitch (1937) analysed the calcium retentions of the literature by means of regression and suggested minimum requirements for young adults as follows: Eighteen to twenty years, 0.9 grams per day; twenty to twenty-four, 0.7 grams per day.

Leverton and Marsh (1942) concluded from 100 studies of young women that 0.83 grams per day represented the minimum allowance. An intake of 1.08 grams per day was suggested as being "optimal" intake for the group studied. McKay et al. (1942) in a study of the calcium, phosphorus, and nitrogen metabolism of 124 college women representing four states found that mean daily intakes of 0.8 grams calcium were required for equilibrium. Statistical analysis showed that place, age, and weight differences were not significantly related to calcium retention for this group. Steggerda and Mitchell (1946a) reported that the average calcium requirement for 43 adult subjects studied at the University of Illinois was 0.65 grams of calcium daily, with a coefficient of variation of 22.5 per cent. In a previous publication Steggerda and Mitchell (1946) suggested that 10 mg. of calcium per kilogram of body weight per day formed a good approximation of the calcium requirement of American college students and staff members on the accepted food pattern.

The recommended dietary allowance of the Food and Nutrition Board of the National Research Council (1948) for daily calcium intake of young women was 1.0 grams per day.

Calcium Metabolism During Weight Reduction

In the past decade studies of the metabolism of young women during weight reduction have been reported from two laboratories (Brown, 1946; Leverton, 1949, 1951). The investigation of calcium metabolism has had an important part in each study.

Brown (1946) observed the calcium retention of eight obese young women on a reduction diet which provided approximately 1200 calories per day with a distribution of 68 grams protein, 25-27 grams fat, and 125 grams of carbohydrate. Calcium retentions decreased in the period of weight reduction and the decrease continued in the post-reduction period. The mean coefficient of calcium absorption decreased from 30 per cent in the pre-reduction period to 22 and 20 per cent in the weight loss and post-reduction periods respectively. During the period of self-selected diet, at a mean daily intake of 1.253 grams, the average retention of calcium was 0.141 grams per day. The calcium intake during the period of low calorie intake was 1.096 grams per day and the calcium retention was 0.079 grams. Post-reduction intake of calcium for the subjects was 1.217 grams per day and the calcium retention was -0.029 grams.

Leverton and Rhodes (1949) studied the metabolism of fourteen young women during weight reduction. Seven of the group were maintained on a diet containing 60 grams of protein and 43 grams of fat. The other seven subjects received a diet containing 105 grams of protein and 34 grams of fat. The diets provided 1150 to 1250 calories per day. The calcium retention of the subjects on the low protein diet was 0.107 grams per day; this compared favorably with the value of 0.134 grams per day which was reported by Leverton (1942) for young women on self-selected diets with a daily intake of 1.20 to 1.680 grams per day. The group receiving the high protein diet, with a calcium intake of 1.410 grams per day showed a mean retention -0.074 grams.

A further study (Leverton, 1951) of twelve young college women utilized a low calorie diet of approximately 1200 calories per day with a customary distribution of food nutrients. Mean daily calcium intake was 1.10 grams with a retention of -0.03 grams per day.

Consideration of these studies would suggest that a weight reduction diet affects calcium retention during the period of controlled diet and may also influence the utilization of calcium during the post-reduction period. The diets cited provided a greater intake of calcium than the average intake reported for young women on their own self-chosen diets by either Leverton (1942) or McKay (1942).

Table 1 summarizes the mean daily intake and retention of calcium for young women on self-chosen and weight reduction diets reported in the literature.

TABLE 1

MEAN DAILY CALCIUM INTAKE AND RETENTION OF YOUNG COLLEGE WOMEN REPORTED IN THE LITERATURE

No. of Subjects	Intake	Retention	Experimental Conditions	Investigator
	gms. per 24 hr.	gms. per 24 hr.		
98	0.857	0.015	Self-chosen diet	Leverton(1942)
124	0.941	0.030	Self-chosen diet	McKay(1942)
3	1.253	0.141	Self-chosen diet	Brown(1946)
8	1.096	0.079	Reduction, 1200 calorie	Brown(1946)
5	1.217	-0.029	Post-reduction, controlled	Brown(1946)
7	1.221	0.107	Reduction, 60 gms. protein 43 gms. fat	Leverton(1949)
7	1.410	-0.074	Reduction, 105 gms. protein 34 gms. fat	Leverton(1949)
12	1.100	-0.030	Reduction, 1200 calorie	Leverton(1951)

EXPERIMENTAL PROCEDURE

Subjects

Six overweight college women served as subjects for this study. The young women were subjects included in a metabolism study on weight reduction carried on by the Department of Foods and Nutrition of Michigan State College. The age range was from 18 to 23 years. The range of weight in excess of that considered desirable for each subject was from 10.15 pounds to 60.08 pounds.[1]

Experimental Plan

The pattern of the dietary used differed from that accepted customarily for this age group in the relationship of protein, fat, and carbohydrate. The total caloric intake was 1500 calories per day, except where noted.

Preliminary to the period of weight loss, the subjects were maintained on a self-selected diet typical of the North Central region for a two week observation period. During the second week a balance study was conducted to establish calcium retention of the subjects on their usual food intakes; thus each subject served as her own control. Follow-

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[1] Optimum weight determined from Baldwin-Wood, height, weight tables for age, interpreted with reference to anthropometric measurements.

ing the preliminary period subjects were given the weight reduction diet. After two weeks on the diet a balance period was maintained for a period of one week. Six balance periods were completed for six subjects during the study which was continued from January, 1951, to June, 1951. The balance periods were scheduled approximately every third week except during the vacation periods.

The meals were prepared and served in the Home Economics Department of the College under the supervision of trained personnel. The confinements of a study of this length were appreciated and every effort was made to maintain a pleasant and companionable atmosphere. Subjects were kept on the controlled diet until satisfactory weight was reached, or until the termination of the school year. The subjects who reached their desired weight before termination of the study were given additional foods to study the dietary intake which would maintain the desired wieght.

Throughout the study all subjects (except S. S.) received 312 I. U. Vitamin D per day. The amount of salt to be given for each subject was determined at the beginning of the study according to the taste preference of each individual. All foods were prepared without salt and weighed amounts were supplied at the table. There was no restriction on liquid intake but a record of all water, tea and coffee was kept in the individual record books of each subject. In view of the studies indicating the sometimes appreciable amount of calcium in water, a sample was analysed for calcium and the intake of calcium from fluids was calculated for each subject for each balance period. Fifty per cent allowance was given for tea and coffee (Widdowson, 1943).

Collection and Treatment of Samples

During the second week of the self selection period records of the weight of all servings were kept. A weighed aliquot equal to one-fifth of the foods eaten was preserved by freezing. Similar aliquots of the weight loss diet were kept for all periods. Because of the volume of food collected daily aliquots were collected for two periods during the week. Liquids and solids were stored separately for ease in handling.

At the completion of each week the frozen food composites were thawed. The thawed material was transferred quantitatively to a Waring food blender using warm water to wash out the container. This was blended for five minutes or until the mixture was homogeneous. The slurry was then transferred quantitatively to a two-liter volumetric flask and made to volume with distilled water. After mixing by inverting and rotating fifty times two samples were measured into 250 milliliter volumetric flasks. These samples were transferred quantitatively to weighed evaporating dishes containing a glass stirring rod. Samples were placed in an oven at 40 degrees centigrade until dry (three to four days). Samples were stirred at intervals during drying to permit more even evaporation of moisture. After they were dried to constant weight, the weight of the sample was determined. The dried sample was then scraped from the sides of the dish and the entire contents ground in a mortar. A portion of the dried sample was transferred to a bottle and stored in a desiccator until analysed. Each slurry was analysed separately and the results added to give the total for the period. Calcium determinations were made on balance period samples only.

During the balance periods daily fecal collections were made in waxed containers; carmine was used as a marker. At the end of each balance period the fecal collections were transferred to a blender and blended for five minutes. The sample was then transferred quantitatively to a two-liter volumetric flask and made to volume. After mixing fifty times three samples were measured into 100 ml. volumetric flasks and transferred quantitatively to weighed evaporating dishes supplied with stirring rods. Samples were dried partially over a steam bath. Drying was completed under infra red lamps. When drying was complete, (constant weight reached), the weight of the sample was determined. The dried sample was scraped, ground, and stored in bottles in a desiccator until analyzed.

Twenty-four hour urine collections were made during balance periods. After determination of the volume of the sample in a glass stoppered mixing cylinder the urine was diluted with distilled water to a volume easily divisible by five. The sample was mixed by inverting the cylinder twenty times and one-fifth of the daily sample transferred to a three-liter brown bottle and stored in the refrigerator. The procedure was repeated daily until a composite for the balance period was obtained.

Chemical Methods

Weighed portions of the dried samples of food and feces were prepared for calcium determinations by dry ashing in a muffle furnace at a temperature of approximately 490° centigrade (A.O.A.C., 1940). The temperature was maintained for approximately 48 hours. The furnace was cooled and the crucibles removed to a desiccator. The crucibles were reweighed and the weight of the ash determined. Completeness of the ashing process was checked by reheating for 12 hours and again determining the weight of the ash. After the first two periods of the controlled diet, reheating was discontinued. Completeness of ashing was accepted on the basis of the percentage ash obtained. The ash was taken up with hydrochloric acid (1 + 4), transferred quantitatively to a 100 milliliter volumetric flask, and made to volume.

Urine samples were prepared for determination of calcium by a process of wet ashing. An aliquot of fifty milliliters of the urine composite for each balance period was transferred by pipette to a 150 milliliter erlenmeyer flask. Five milliliter portions of concentrated sulphuric acid, concentrated nitric acid and hydrogen peroxide[1] were added slowly in the order mentioned. Samples were kept on a hot plate between medium and fast heat until clear and colorless. During ashing additions of nitric acid were made when the contents became charred due to carbonization of organic material, or when the contents tended to become dry. Several additions were found necessary for satisfactory ashing. Complete ashing required one to two weeks. When ashing was completed, the ash was transferred quantitatively to a 100 milliliter volumetric flask and made to volume. In some cases a precipitate formed during ashing so that hydrochloric acid (1 + 4) was used to transfer the ash.

Solutions of both dry and wet ash were analyzed for calcium using a modification of McCrudden's method (1911). Aliquots of five milliliters each of the solutions of ashed

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1 Supersol.

feces and urine, and aliquots of ten milliliters of the solutions of food were taken for analysis.

Brom cresol purple was used in adjusting the pH (Kramer, 1926). Care was necessary in adjusting the pH to avoid interference with magnesium, phosphorus and iron. Concentrated ammonia was added until the sample turned purple. Sufficient concentrated hydrochloric acid was then added to return the color to yellow. This pH has been found satisfactory for calcium determination in this laboratory. After satisfactory adjustment of pH, 10 ml. each of 2.5 per cent oxalic acid and 20 per cent sodium acetate (12 per cent anhydrous) were added slowly in order given to precipitate the calcium as calcium oxalate. The following day the sample was filtered through Jena glass filters, the precipitate washed with dilute ammonia to remove traces of oxalic acid, dissolved in approximately normal sulphuric acid and titrated between 70° and 90° centigrade with approximately 0.01 normal potassium permanganate. Standard sodium oxalate was used to determine the normality of the potassium permanganate.at frequent intervals.

Experimental procedures were checked by recovery of known quantities of calcium and are recorded in Table 7 (Appendix).

RESULTS AND DISCUSSION

The body weights of the subjects at the beginning of the experiment and during weight reduction are presented in Table 2. These data indicate that the subjects lost weight satisfactorily on the experimental diet. Two of the six young women (AW, SS) reached desired weight within the period of study. The remaining subjects (MJ, EH, FG, VA) showed steady weight losses, although they did not complete the recommended reduction before the end of the experimental period.

The Weight Reduction Diet

The distribution of calories in the weight reduction diet differed from that ordinarily accepted. The general pattern of the high fat, high protein diet presented by Pennington (1949) was modified to include a wider variety of foods. The diet reported by Pennington was low in calcium since milk was not included. Omission of milk from the diet makes it difficult to provide the necessary dietary requirement of calcium. The weight reduction diet used in this experiment included one pint of milk per day.

The recommended distribution of protein, fat and carbo-

TABLE 2

AGE, HEIGHT AND WEIGHT SCHEDULE FOR INDIVIDUAL SUBJECTS

Subject	Age	Height	Weight				
			Initial	Desired	Excess	Final	Loss
	years	cm.	kg.	kg.	kg.	kg.	kg.
EH	18	161	86.4	59.1	27.3	75.0	11.4
AW	18	167	78.5	65.9	12.6	65.4	13.1
MJ	19	168	82.8	68.2	14.6	74.2	8.6
FG	18	167	83.0	65.9	8.1	72.5	10.5
SS	22	157	59.2	54.5	4.7	54.5	4.7
VA	18	165	83.8	65.9	17.9	72.4	11.4

hydrate in the normal diet as compared with the distribution provided in the diet study is presented in Table 3.

The Food and Nutrition Board of the National Research Council (1948) recommended 75 grams of protein for women 16-20 years of age. This would provide 300 calories from protein in the diet. It was further recommended that the fat in the diet should provide at least 20-25 per cent of the total calories. On the basis of 1500 calories this would amount to approximately 350 calories from dietary fat. The remaining 850 calories would be derived from carbohydrate. The weight reduction diet used in this study contained approximately 90 grams of protein and 80 grams of fat. Thus the diet provided about 360 calories from protein and 720 calories from fat. The remaining 420 calories would be supplied by about 80 grams of carbohydrate.

Typical menus for one week during weight reduction are given in Table 8 (Appendix). The calculated calcium and phosphorus intake per day (Bureau of Human Nutrition and Home Economics, 1945) were 0.66 and 1.1 grams per day respectively, giving a calcium : phosphorus ratio of 1 : 1.6. The calculated value for calcium (0.66) was 13 per cent lower than 0.76 grams per day found by analysis of food composites for the corresponding weight loss period; however, variation between calculated and analyzed values has been

TABLE 3

COMPARISON OF RECOMMENDED DISTRIBUTION OF FAT, PROTEIN AND CARBOHYDRATE WITH DISTRIBUTION PROVIDED IN WEIGHT REDUCTION DIET

Recommended Allowance	Fat	Protein	Carbo-hydrate	Total
Percent of calories	25.0	21.4	53.5	99.99
Total calories	350.0	300.0	850.0	1500.0
Present study				
Percent of calories	51.4	25.7	22.8	99.99
Total calories	720.0	360.0	420.0	1500.0

noted to be particularly characteristic of calcium (Bassett, 1931; Hummel, 1942; Widdowson, 1943).

Calcium Intakes and Retentions of Individual Subjects

Intake and retention during self-selection. The calcium metabolism of individual subjects for the period of self-selection, weight reduction and maintenance (subject AW) is given in Table 4. Individual values for all subjects in all periods are given in Table 9 (Appendix). Individual retention values for calcium retentions during all periods are shown graphically in Figure I..

The mean daily intakes during the period of self-selected diets ranged from 0.93 grams for MJ to 1.16 grams for SS. The retention values for these subjects were 0.06 and o.o5 grams per day, respectively. The greatest retention value was that for VA (0.27 grams per day) on an intake of 1.11 grams per day. The remaining three subjects (EH, FG, AW), on intakes of 1.06, 1.07, and 0.98 grams per day, showed corresponding retentions of 0.10, 0.14, and 0.04 grams per day.

When these values are compared with values reported by McKay (1942) for young college women on self-selected diets, it is apparent that the young women subjects of the weight reduction study were retaining calcium in greater quantities than those on comparable intake ranges in the McKay study. Thirteen subjects reported by McKay on a mean daily intake

TABLE 4

MEAN DAILY CALCIUM METABOLISM OF INDIVIDUAL SUBJECTS DURING SELF-SELECTION AND DURING WEIGHT REDUCTION PERIODS

Subject	Period	Excretion		Intake	Retention
		Urinary	Fecal		
		gms/d	gms/d	gms/d	gms/d
EH	self-selection	0.24	0.72	1.06	0.10
	weight reduction	0.22	0.65	0.84	-0.03
		(0.18-0.26)*	(0.48-0.79)	(0.79-0.91)	(-0.14- +0.15)
AW	self-selection	0.23	0.71	0.98	0.04
	weight reduction	0.30	0.67	0.90	-0.08
		(0.28-0.32)	(0.53-0.72)	(0.79-0.93)	(-0.06- +0.08)
	maintenance	0.38	0.93	1.17	-0.14
MJ	self-selection	0.21	0.66	0.93	0.06
	weight reduction	0.27	0.62	0.82	-0.06
		(0.23-0.31)	(0.35-0.93)	(0.77-0.91)	(-0.32- +0.19)
FG	self-selection	0.28	0.65	1.07	0.14
	weight reduction	0.28	0.56	0.83	-0.02
		(0.22-0.36)	(0.46-0.72)	(0.77-0.91)	(-0.20- +0.09)
SS	self-selection	0.14	0.97	1.16	0.05
	weight reduction	0.15	0.67	0.81	-0.004
		(0.09-0.18)	(0.55-0.73)	(0.71-0.92)	(-0.18- +0.16)
VA	self-selection	0.09	0.75	1.11	0.27
	weight reduction	0.12	0.81	0.83	-0.09
		(0.09-0.14)	(0.56-1.07)	(0.78-0.89)	(-0.32- +0.06)

* Range of weight reduction periods.

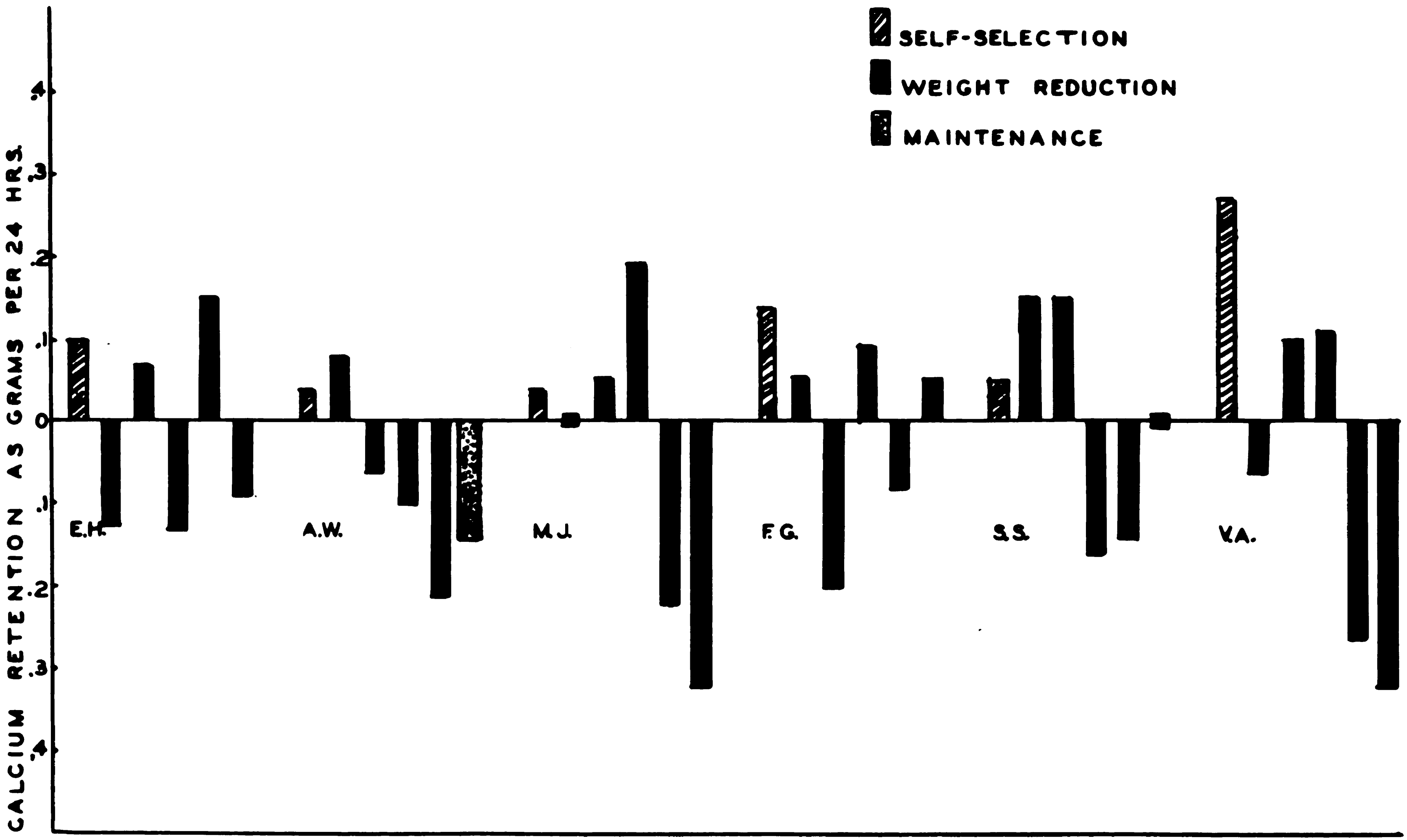

FIG. I THE CALCIUM RETENTIONS OF SUBJECTS ON SELF-SELECTED DIETS AND DURING WEIGHT REDUCTION of 0.939 grams per day showed a mean retention of -0.019 grams per day. The two subjects who had comparable calcium intakes were AW and MJ, both of whom showed a positive retention of 0.04 and 0.06 grams respectively. In McKay's study the mean retention for eighteen subjects who had intakes which ranged from 1.000 to 1.099 grams and averaged 1.045 grams was 0.044 grams of calcium per day. Two subjects of the present study were within this range of intake. These subjects, EH and FG, had retentions of 0.10 and 0.14 grams per day respectively. The remaining two subjects, SS and VA, were within the intake range of 1.100 and 1.199 grams per day observed by McKay for twelve subjects. The mean intake of the group studied by McKay was 1.145 grams per day and the mean retention was 0.111 grams per day. The retention of one subject (SS) with an intake of 1.16 grams per day was 0.05 grams, which was below this value, while the value for VA, whose intake was 1.11 grams per day, had a retention of 0.27 grams per day, well above the mean value for the McKay subjects on comparable intake.

Since individual retention of calcium on a controlled diet is influenced by previous intake of calcium (Fairbanks, 1936), information about the usual intake of milk by the subjects, previous to the diet study, was obtained. All subjects reported inclusion of at least a pint of milk per day on the usual self-chosen diet. The calcium retention values for the period of self-selected diet, and the information concerning usual intake of milk, would indicate that the subjects were receiving an adequate amount of calcium for their individual needs previous to the diet study.

A decrease in retention for all subjects could be expected during the weight reduction period, since the average intake of calcium from the controlled diet was 0.82 grams per day compared with the average for the self-selected period of 1.04 grams per day.

From Figure I it is apparent that values for three subjects (EH, FG, SS), during weight reduction, varied about calcium equilibrium. In the other three cases (AW, MJ, VA) calcium retention became increasingly negative as the duration of the diet increased. The nervous tension of the final examination period might account for part of the increased loss of calcium for MJ, who was not a strong student and worried considerably about her work; however, VA, who also showed greater loss of calcium than the other members of the group during the last part of the study, appeared to be well adjusted, was an excellent student with a calm and cheerful disposition.

Urinary calcium. Average values for urinary calcium varied from 0.12 to 0.30 grams per day during weight reduction; these values were within the normal range given by Knapp (1947) for comparable subjects. Two subjects that showed high retentions during the period of self-selected diet (EH, FG) showed stable urinary excrĕtion of calcium during the preliminary period and during weight reduction.

The values for SS show remarkable constancy in urinary excretion during pre-reduction and reduction periods. This appeared to suggest that the shift from positive to negative retention of calcium when the subject was given the weight reduction diet was due to decreased intake during this period. Moreover, evidence that the body may have adjusted to this lower intake is suggested by the fact that the subject was in calcium equilibrium during the last period.

Three subjects (MJ, VA, AW) showed greater variation in urinary excretion of calcium than the rest of the subjects. Increase in "ketone" bodies due to improper oxidation of fat in the body might influence urinary calcium elimination by the resulting increase in body acidity. Analyses of urine for acetone were made during the study and the findings were available for observation. Qualitative tests for acetone were positive for subject EH during period of self-selected diet (period 1) and two weight loss periods (4,5); for FG during period one and one weight loss period (4); for SS and VA during weight loss periods 3 and 4 respectively; and MJ during period one. However, the periods in which findings for acetone were positive did not coincide with periods of increased urinary excretion.

Fecal calcium. During the weight reduction period the fecal excretion of calcium ranged from 67 per cent to 97 per cent of the intake. Excluding this one high value (VA) the percentage excretion ranged from 67 to 82 per cent; this was within the range for the preliminary period in the self-selected diet. Two subjects that showed high retentions during the preliminary period (EH, FG) showed stable urinary excretion of calcium during weight reduction but an increase in fecal calcium which corresponded to nine and seven per cent of the intake respectively.

In An attempt to determine whether there might be a relationship between calcium excretion and increased excretion of organic material, the values for fecal calories (unpublished data from this laboratory) of individual subjects were compared with the values for fecal calcium. If it is assumed that an increase in fecal calories might indicate a higher content of calcium binding substances (fatty acids) in the feces, an increase in fecal calcium might be expected to parallel an increase in fecal calories. The comparison of fecal calories and fecal calcium for the individual subjects during the successive experimental periods is shown in Figure II. The lines drawn for fecal calcium for all subjects seem to follow the general direction of lines

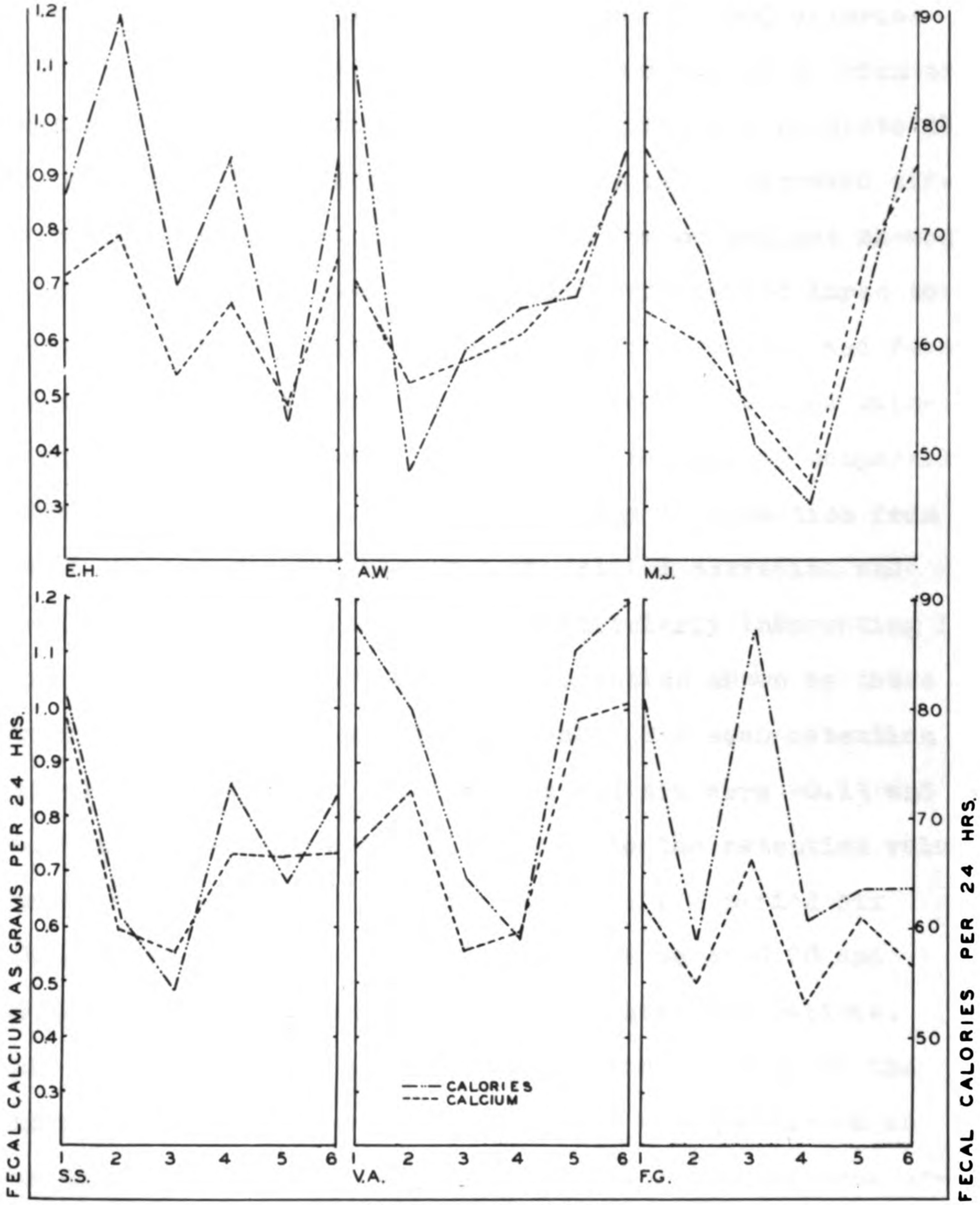

FIG. II FECAL CALCIUM AS RELATED TO FECAL CALORIES FOR ALL SUBJECTS drawn for fecal calories; the increase in fecal calories was reflected in most cases with an increase in calcium excretion. The relationship was most marked for subjects AW, MJ, and VA. The situation for AW must be interpreted differently from that of MJ or VA. The former subject showed a tendency to a reverse relationship from period three to period five with a sharp increase in both calcium and fecal calories in period six. As this period represented maintenance for the subject, it cannot be considered comparable to the other periods. The sharp change in direction from rapid decline to increase in both calcium excretion and fecal calories for MJ and VA is particularly interesting in view of the marked difference in retention shown by these two subjects during the same periods. The mean retention values of calcium for periods five and six were -0.13 and -0.15 grams per day respectively, while the retention value for MJ during period five was -0.22 and for period six -0.32 grams per day. Retentions for VA were -0.28 and -0.32 grams per day respectively for these two periods. This sharp decrease in calcium retention, in view of the sharp increase infecal calories, might be interpreted as due to an interference with calcium absorption by some organic, calcium binding material subsequently eliminated in the feces.

<u>Post-reduction retention of calcium</u>. The two subjects reaching desired weight, SS and AW, indicate different reactions to the post-reduction regime. During the third reduction period SS was given a diet providing 80 per cent of the regular weight reduction diet. The final period represents a return to the full weight loss diet; hence this period of maintenance provided the same food distribution as the weight loss periods. The daily retention for this period was at equilibrium, which would seem to reflect response to the variation in intake, or perhaps a final adjustment to the dietary distribution of food. The response of AW is interesting because this subject was given the regular weight maintenance diet, which increased the calcium intake for this subject to 1.17 grams per day. Despite this increase, which provided a greater intake than during the preliminary period, the retention remained negative with a value of -0.14. This retention was an improvement over the previous period and might indicate that the period of adjustment to the new intake required time.

Group Response to Weight Reduction

Individual variation of small experimental groups tends to limit the value of interpretations of group averages. However, as mass metabolism studies, comparable to the weight reduction study reported, are impractical, it is of value to observe the group trend in relation to the diet regime. Group averages are also of value in comparing studies from different laboratories.

The mean daily intake, excretion, and retention of calcium for all subjects are given in Table 5 and are shown graphically in Figure III.

The mean retention for the period of self-selection on a mean intake of 1.05 grams of calcium per day was 0.11 grams per day. During the first two periods of weight reduction when the mean intake of calcium was 0.86 grams per day, the average retention was 0.02 grams per day. This is similar to the value for calcium retention of 0.034 which was reported by McKay (1948) for subjects on an intake of 0.85 grams per day. The retentions for the remaining weight reduction periods were -0.01, -0.13 and -0.15 grams per day respectively.

The initial decrease in average calcium retention could be accounted for by the decrease in intake following the change from self-selected dietary to the controlled weight reduction diet. If this were the only factor influencing calcium retention, the retention might be expected to stabilize at the new value. In the present study a slight downward trend continued in period four, with a sharp drop in period five. Period six showed continuing decrease in calcium retention, although less marked than in the previous period. The continued decline in retention

TABLE 5

MEAN DAILY INTAKE, EXCRETION, AND RETENTION OF CALCIUM FOR ALL SUBJECTS DURING SELF-SELECTION AND WEIGHT REDUCTION PERIODS

Diet Period	Intake	Excretion		Retention
		Urinary	Fecal	
	gms. per 24 hr.	gms. per 24 hr.	gms. per 24 hr.	gms. per 24 hr.
1[a]	1.05 ± 0.08[b]	0.20 ± 0.07	0.74 ± 0.12	0.11 ± 0.09
2	0.92 ± 0.01	0.26 ± 0.09	0.64 ± 0.16	0.02 ± 0.10
3	0.79 ± 0.01	0.21 ± 0.08	0.57 ± 0.08	0.02 ± 0.13
4	0.77 ± 0.03	0.21 ± 0.07	0.57 ± 0.14	0.01 ± 0.15
5	0.79 ± 0.03	0.21 ± 0.08	0.72 ± 0.17	0.13 ± 0.15
6	0.90 ± 0.01	0.22 ± 0.07	0.83 ± 0.19	0.15 ± 0.14

[a] Self-selection period: remaining periods weight reduction.

[b] Standard deviation.

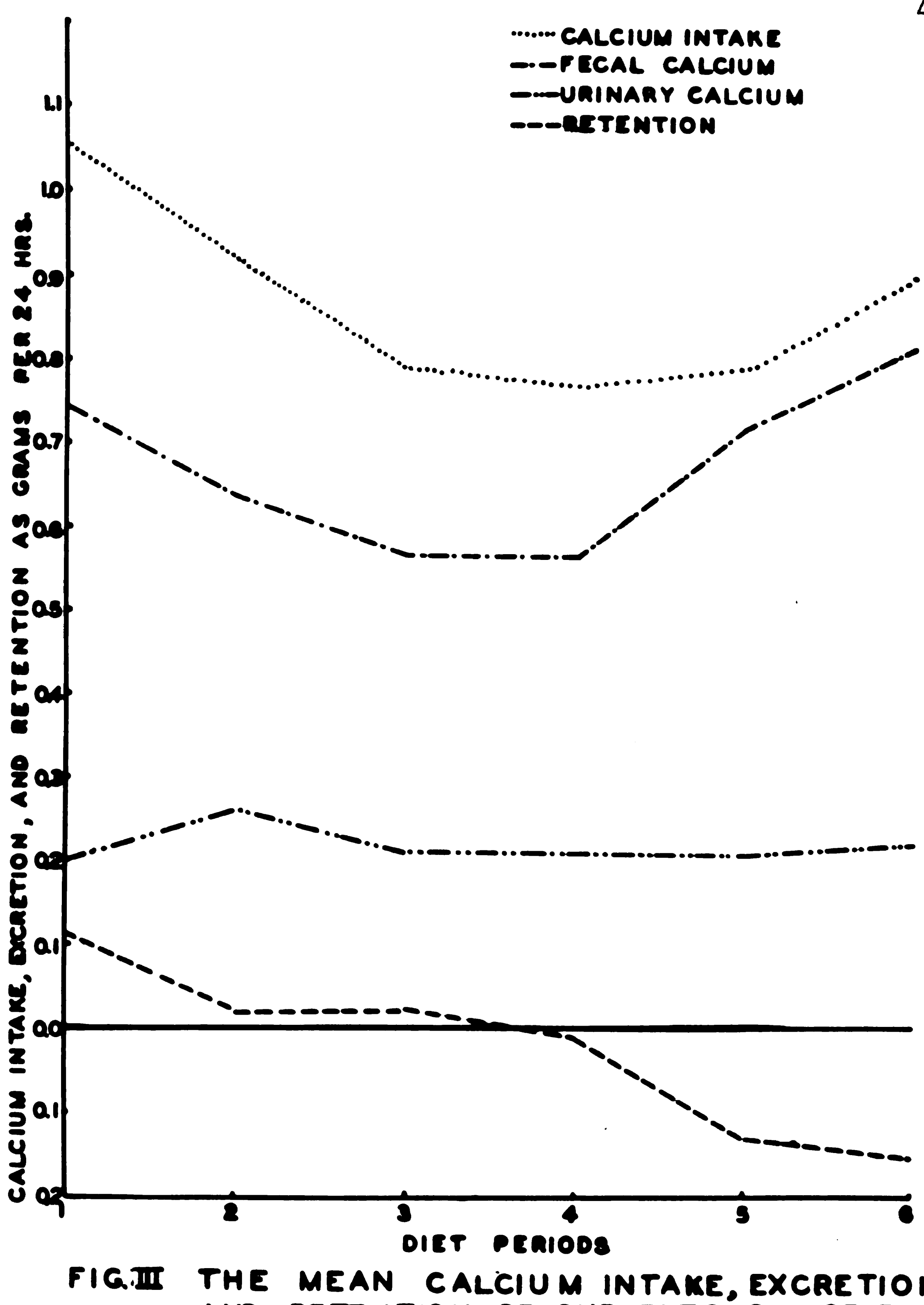

FIG. III THE MEAN CALCIUM INTAKE, EXCRETION, AND RETENTION OF SUBJECTS ON SELF-SELECTED DIET (PERIOD 1) AND DURING WEIGHT REDUCTION (PERIODS 2 - 6) might indicate some alteration in endogenous calcium metabolism. The values for urinary excretion would reflect endogenous changes, but with the exception of period two, in which the mean urinary excretion was 0.26 grams per day, the mean values were constant and in keeping with the mean value for the period of self-selection. Increased percentage excretion of calcium in the feces might represent impairment of absorption. The values for fecal excretion are more consistent with the decreased retention values than are the urinary excretion values.

The individual values for calcium retentions of subjects on the diet used in this study, and the calcium retention values from other published weight reduction studies, Brown (1946), Leverton (1949, 1951), are shown in Figure IV plotted about the regression line drawn from the predicting equation for calcium retention against calcium intake reported by McKay (1942),[1] from observations of 124 normal young women of college age. The mean calcium retention values during weight reduction for these same studies are given in Table 6.

The regression line for the data from the present weight reduction study was determined.[2] Comparison of the

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[1] $y = 0.030 + 0.2392\ (x - 0.941)$

[2] $y = 0.019 + (-0.0773x)$

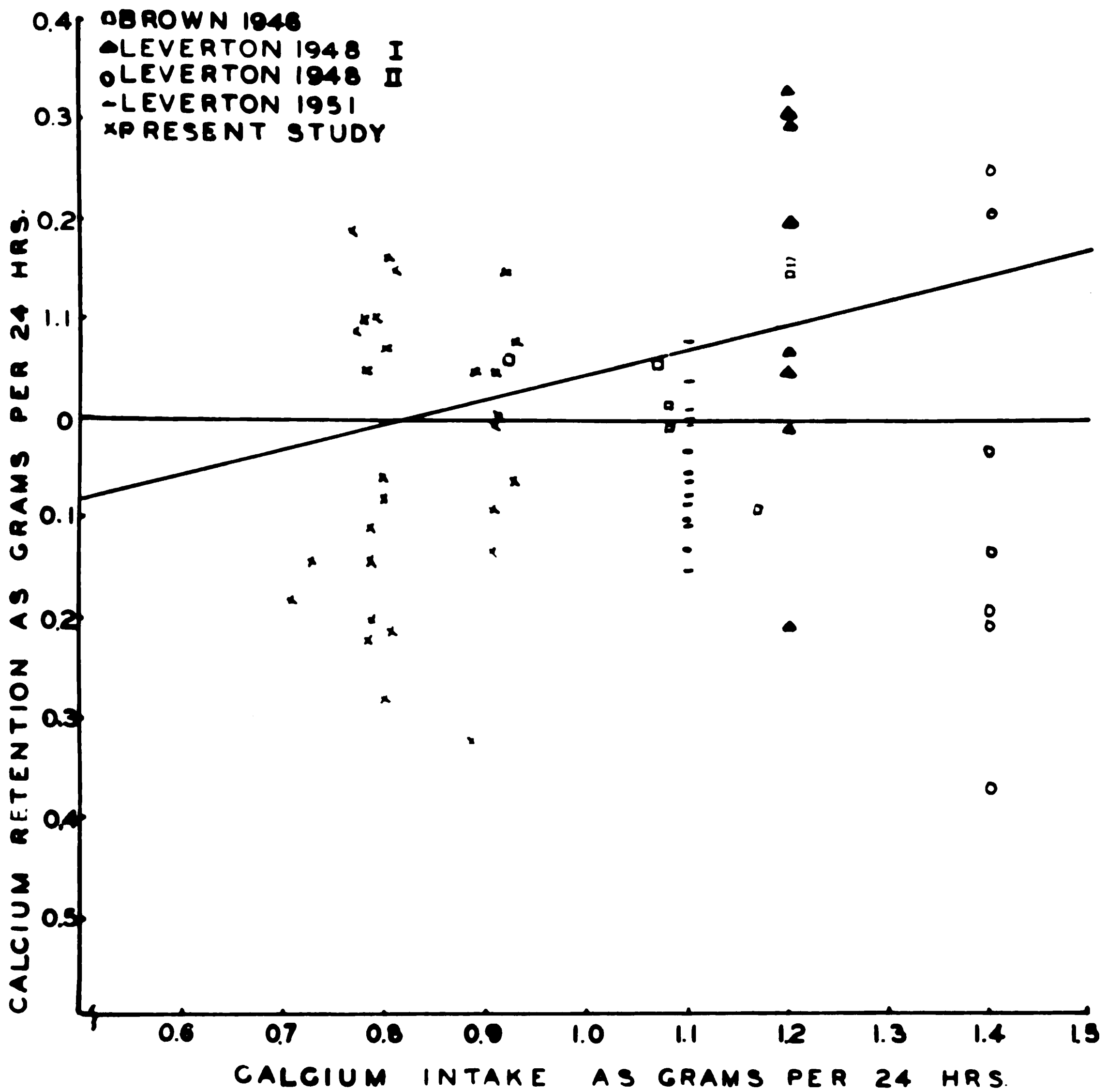

FIG. IV REGRESSION OF CALCIUM RETENTION ON CALCIUM INTAKE FOR YOUNC COLLEGE WOMEN ON WEIGHT REDUCTION DIETS PLOTTED ABOUT REGRESSION LINE FOR 124 COLLEGE WOMEN ON SELF-SELECTED DIETS (MCKAY, 1942)

TABLE 6

COMPARISON OF MEAN CALCIUM RETENTIONS FROM THE PRESENT STUDY AND WEIGHT REDUCTION STUDIES REPORTED IN THE LITERATURE

Investigator	No. of Subjects	Approximate Length of Study	Mean Intake	Average Retention
		days	gms. per day	gms. per day
Brown(1946)	8	98	1.096	0.079
Leverton(1947)				
I. Low Protein	7	56	1.221	0.107
II. High Protein	7	56	1.410	-0.074
Leverton(1951)	12	54	1.11	-0.03
Present Study	6	112	0.83	-0.05

slope of the line of the present study (b = -0.0773) with the slope of the line from the McKay study (b = 0.2392) was made using the "t" test[1] (Snedecor, 1946); there was not a significant difference. This indicated that the relationship between calcium intake and retentions for the subjects of the study was similar to that found for the subjects of average height and weight studied by McKay (1942).

Indication that calcium retentions of subjects of this study decreased steadily during weight reduction was presented in both the individual data (Figure I) and by the group trend (Figure III). A decrease in ability to retain calcium during weight reduction has been observed in each of the other studies mentioned (Brown, 1946; Leverton, 1949, 1951), and suggests some change in calcium metabolism caused by weight reduction. The studies discussed represent considerable variation in diet regime. Brown (1946) and Leverton (1951) provided low calorie diets with a generally accepted food distribution; Leverton (1949) used a moderate protein intake for one group, and a high protein intake for a second group during the same study; the present study provided a high protein, high fat diet. Thus the decrease in calcium retention seemed to occur irrespective of the diet employed, which might support the hypothesis

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[1] $t = \frac{b - b_1}{\sigma b}$

that the decrease in retention may be due to metabolic disturbance during weight reduction.

Although the subjects in the study reported by Brown (1946) were in equilibrium on a self-selected diet, it is of interest to note that the tendency toward negative calcium retention of the weight reduction period continued into the period of post-reduction. The study of Brown showed a lower retention during this period than during weight reduction. On an average calcium intake of 1.096 grams per day, during weight reduction the average retention of eight subjects was 0.079 grams. During the post-reduction period with an intake of 1.211 grams of calcium per day the retention decreased to -0.029 grams per day. Subject AW of the study reported here was given a post-reduction diet of accepted food distribution. Although the retention was improved during this period, -0.14 grams per day compared with -0.21 grams for the preceding period on weight reduction, the subject did not attain calcium equilibrium during the three weeks that she was on the weight maintenance diet. Since Brown also found negative balance during the post-reduction period, it would appear that further study in calcium retention during weight loss and during adjustment to weight maintenance is needed.

SUMMARY AND CONCLUSIONS

The effect of weight reduction on the calcium retention of six young women has been studied. The subjects were part of a group of overweight women of college age taking part in a weight reduction study carried on by the Nutrition Department of Michigan State College. Observations of the calcium retentions of these six subjects were made at regular intervals from January to June, 1951. A period of self-selected diet preceded the weight reduction periods. The diet used during weight reduction provided approximately 80 grams of fat and 90 grams of protein per day; the intake of carbohydrate was adjusted to keep the total calorie intake at approximately 1500 calories per day.

During the course of weight reduction a decrease in calcium retention was apparent for the group as a whole. Individual subjects showed wide variation. All subjects were in positive calcium balance during the period of self-selection on an average intake of 1.05 grams of calcium per day with a range in retention of 0.04 to 0.27 grams per day. During weight reduction, with an average calcium intake of 0.83 grams per day, the range in retention was 0.19 to -0.32 grams per day. Calcium retentions averaged -0.05

grams per day, a value which was not significantly different from that of McKay (1942) for college women.

Decreased calcium intake during weight reduction periods did not seem to give an entirely satisfactory explanation for the decrease in calcium retention. Urinary calcium values during weight reduction remained close to the pre-reduction average for the group, suggesting little evidence of decreased retention which might be due to unsatisfactory calcium to phosphorus ratio in the body, or to formation of ketones due to incomplete fat oxidation. Fecal calcium increased during successive weight reduction periods, indicating decreased absorption of calcium from the intestinal tract. The possibility of a relationship between this increase in fecal calcium and fat digestion is discussed.

Data have been discussed for individual subjects and averages for the subjects considered for indications of group trends. Findings of the study have been discussed in relation to the studies on weight reduction from other laboratories (Brown, 1946; Leverton, 1949, 1951).

APPENDIX

TABLE 7

RECOVERY OF CALCIUM FROM SOLUTIONS PREPARED FROM WET AND DRY ASHING PROCEDURES

Form of Calcium Added	Gms. Calcium in Sample	Gms. Calcium Added	Calculated Gms. Calcium (sample added)	Actual Gms. Calcium	% Recovery
Wet ash--Urine					
Calcium carbonate	0.00869	0.00048	0.00917	0.00917	99.8
Calcium lactate	0.000614	0.00065	0.00065	0.000614	94.5
Dry Ash--Food					
Calcium lactate	0.00294	0.05743	0.05743	0.05784	100.7
Calcium lactate	0.00271	0.01298	0.01569	0.01524	97.1

TABLE 8

A. FOODS INCLUDED IN MENUS DURING SEVEN DAY PERIOD OF WEIGHT REDUCTION DIET

Meal	Food	Weight
First Day		
		gms.
Breakfast	Grapefruit and orange juice	100
	Egg[1]	100
	Bread	20
	Butter (total for day)	10
	Milk[2]	150
Lunch	Pork chop	125
	Broccoli	100
	Milk	150
Dinner	Hamburg	125
	Creamed potato	100
	Milk	150
	Fresh grapefruit sections	100
Second Day		
Breakfast	Orange juice	100
	Egg	100
	Bread	20
	Butter (total for day)	20
	Milk	150
Lunch	Lamb patties	100
	Lettuce	15
	Pears (drained)	100
	Milk	150

[1] Approximate weight of two eggs. Eggs scrambled in 5 gm. portion of butter from day's allowance.

[2] Whole milk.

TABLE 8 (continued)

Meal	Food	Weight
Dinner	Roast veal	125
	Peas	75
	Applesauce	100
	Milk	150
	Third Day	
Breakfast	Orange juice	100
	Egg	100
	Bread	20
	Butter (total for day)	20
	Milk	150
Lunch	Meat loaf	125
	Cottage cheese	20
	Fresh tomato	30
	Lettuce	10
	Milk	150
Dinner	Roast beef	125
	Boiled potato	80
	Sweet cherries	80
	Milk	150
	Fourth Day	
Breakfast	Grapefruit juice	100
	Egg	100
	Bread	20
	Butter (total for day)	20
	Milk	150
Lunch	Swiss steak	125
	Cauliflower	100
	Milk	150
Dinner	Roast pork	125
	Beets	100
	Apricots	100
	Milk	150

TABLE 8 (continued)

Fifth Day		
Meal	Food	Weight
		gms.
Breakfast	Tomato juice	100
	Egg	100
	Bread	20
	Butter (total for day)	15
	Milk	150
Lunch	Baked whitefish	125
	Butter sauce	15
	Baked sweet potato	100
	Milk	150
Dinner	Salmon salad	125
	Celery	25
	Lettuce	10
	Mayonnaise	25
	Green beans	100
	Milk	150
Sixth Day		
Breakfast	Grapefruit and orange juice	100
	Egg	100
	Bread	20
	Butter (total for day)	15
	Milk	150
Lunch	Hamburg	125
	Tossed salad	100
	French dressing	5
	Milk	150
Dinner	Pork roast	125
	Peas	75
	Lettuce	50
	Canned peaches	100
	Milk	150

TABLE 8 (continued)

Seventh Day		
Meal	Food	Weight
		gms.
Breakfast	Orange (1)	
	Sweet roll	25
	Butter (total for day)	20
	Milk	150
Dinner	Roast chicken	150
	Fresh tomato	100
	Lettuce	50
	French dressing	5
	Milk	150
Supper	Cold meat loaf	125
	Bread	30
	Apple	150

B. FOODS ADDED TO WEIGHT REDUCTION DIET DURING WEIGHT MAINTENANCE PERIOD

First Day	
Food	Weight
	gms.
Plums	100
Raw carrot	15
Green beans	100
Cookies	20
Butter	30
Second Day	
Asparagus	100
Butter	20
Cake	35

TABLE 8 (continued)

Food	Weight
Third Day	
	gms.
Pineapple	100
Cottage cheese	50
Butter	20
Lettuce	50
Cookies	20
Fourth Day	
Fruit cocktail	100
Potato	100
Butter	20
Fifth Day	
Orange sections	75
Canned tomato	90
Royal Ann cherries	100
Cupcake with frosting	30
Butter	20
Sixth Day	
Fresh grapefruit	100
Cheddar cheese	30
Potato	100
Butter	20
Seventh Day	
Baked potato	80
Ice cream	100
Cookies	20
Butter	15

TABLE 9

INDIVIDUAL DATA ON AVERAGE DAILY CALCIUM METABOLISM OF ALL SUBJECTS DURING SELF-SELECTION AND DURING WEIGHT REDUCTION PERIODS

Subject	Period	Excretion		Intake	Retention
		Urinary	Fecal		
		gm/d	gm/d	gm/d	gm/d
EH	1	0.24	0.72	1.06	0.10
	2	0.25	0.79	0.91	-0.13
	3	0.19	0.54	0.80	0.07
	4	0.26	0.67	0.79	-0.14
	5	0.18	0.48	0.81	0.15
	6	0.23	0.77	0.91	-0.09
	Mean[a]	0.22	0.65	0.84	-0.03
AW	1	0.23	0.71	0.98	0.04
	2	0.32	0.53	0.93	0.08
	3	0.29	0.57	0.80	-0.06
	4	0.28	0.62	0.79	-0.11
	5	0.30	0.72	0.81	-0.21
	6[b]	0.38	0.93	1.17	-0.14
	Mean	0.30	0.61	0.84	-0.08
MJ	1	0.21	0.66	0.93	0.06
	2	0.31	0.60	0.91	0.00
	3	0.26	0.47	0.78	0.05
	4	0.23	0.35	0.77	0.19
	5	0.25	0.76	0.79	-0.22
	6	0.28	0.93	0.89	-0.32
	Mean	0.27	0.62	0.82	-0.06

[a] Mean of weight reduction periods(2,3,4,5,6).

[b] Maintenance diet values, not included in mean.

TABLE 9 (continued)

Subject	Period	Excretion		Intake	Retention
		Urinary	Fecal		
		gm/d	gm/d	gm/d	gm/d
FG	1	0.28	0.65	1.07	0.14
	2	0.36	0.50	0.91	0.05
	3	0.27	0.72	0.79	−0.20
	4	0.22	0.46	0.77	0.09
	5	0.26	0.62	0.80	−0.08
	6	0.31	0.53	0.89	0.05
	Mean	0.28	0.56	0.83	−0.02
SS	1	0.14	0.97	1.16	0.05
	2	0.18	0.59	0.92	0.15
	3	0.09	0.55	0.80	0.15
	4	0.16	0.73	0.71	−0.18
	5	0.14	0.73	0.73	−0.14
	6	0.16	0.74	0.90	0.00
	Mean	0.15	0.67	0.81	−0.00(4)
VA	1	0.09	0.75	1.11	0.27
	2	0.14	0.85	0.93	−0.06
	3	0.13	0.56	0.79	0.10
	4	0.09	0.59	0.78	0.10
	5	0.10	0.98	0.80	−0.28
	6	0.14	1.07	0.89	−0.32
	Mean	0.12	0.81	0.83	−0.09

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