Modulation of Age-Related Changes in Serum 1,25-Dihydroxyvitamin D and Parathyroid Hormone by Dietary Restriction of Fischer 344 Rats[1]

H. JAMES ARMBRECHT,† RANDY STRONG,*† MONICA BOLTZ,* DAVID ROCCO,* W. GIBSON WOOD*† AND* ARLAN RICHARDSON‡

**Geriatric Research, Education and Clinical Center, Veterans Administration Medical Center, St. Louis, MO 63125, †Departments of Internal Medicine, Biochemistry, Pharmacology and Neurology, St. Louis University School of Medicine, St. Louis, MO 63104 and ‡Department of Chemistry, Illinois State University, Normal, IL 61761*

ABSTRACT The purpose of this study was to determine the effect of food restriction on age-related changes in serum 1,25-dihydroxyvitamin D and PTH, two important regulators of Ca metabolism. Starting at 6 wk, male F344 rats were fed a purified diet either ad libitum (non-restricted) or 60% of ad libitum (restricted). Rats from each group were killed at 5, 13, 22 and 28 mo of age. Dietary restriction increased the median lifespan from 24 to 31 mo. It delayed the rapid decrease in serum 1,25-dihydroxyvitamin D from 1.5–5.0 mo in the non-restricted group to 5–13 mo in the restricted group. It also completely suppressed the marked rise in serum PTH which occurred at 22 and 28 mo in the non-restricted group. Dietary restriction had these effects even though both groups of animals consumed the same amount of Ca per gram body weight. Diet had no effect on serum Ca and P, except at 28 mo. These effects of dietary restriction on serum 1,25-dihydroxyvitamin D and PTH may result in altered Ca metabolism in dietary restricted F344 rats. *J. Nutr.* 118: 1360–1365, 1988.

INDEXING KEY WORDS:

• *dietary restriction* • *aging* • *1,25-dihydroxyvitamin D* • *25-hydroxyvitamin D* • *parathyroid hormone*

Food restriction has been shown by a number of laboratories to increase the mean and maximal lifespan of laboratory animals (1–3). In addition, it delays a wide range of diseases and physiological changes that are associated with aging (4–7). It has been suggested that dietary restriction modulates the rate of aging itself (8). Therefore, dietary restriction may be a useful experimental tool for differentiating between time-dependent and aging-dependent changes in physiological function.

Serum Ca must be maintained at about 10 mg/100 ml throughout the lifespan for proper function of nerve, muscle and bone. Ca homeostasis and skeletal metabolism are maintained by a complex regulatory mechanism involving primarily the hormones 1,25-dihydroxyvitamin D and parathyroid hormone (PTH) with calcitonin (CT) playing a lesser role (9). In young animals, PTH enhances the resorption of Ca from bone, and it stimulates the production of 1,25-dihydroxyvitamin D from 25-hydroxyvitamin D by the kidney (10). 1,25-Dihydroxyvitamin D, the hormonal form of vitamin D, stimulates the intestinal absorption of Ca and modulates bone resorption.

The serum levels of 1,25-dihydroxyvitamin D and PTH change with age in both laboratory animals and humans. Serum 1,25-dihydroxyvitamin D decreases with age while serum PTH and CT increase with age (11–13). These changes affect Ca homeostasis and may contribute to the loss of bone which is seen with age. For these reasons, it is of interest to determine whether the age-related changes in these calcium-regulating hormones can be influenced by dietary restriction.

Only one study on the effect of dietary restriction on calcium-regulating hormones has been reported (14, 15). The basic protocol of this study was to restrict the diet of a group of male Fischer 344 rats by feeding them 60% of the diet consumed by an ad libitum group. Dietary restriction abolished the rise in serum PTH seen in later life (15) and the increase in serum CT seen throughout life (14). Dietary restriction also abolished the loss of bone seen in the oldest group of animals

[1]This work was supported by the Medical Research Service and the Geriatric Research, Education, and Clinical Center of the Veterans Administration and by NIH Grants Nos. AM32158 (H. J. A.) and AG01548 (A. R., R. S.)

0022-3166/88 $3.00 Received 20 November 1987. Accepted 20 July 1988.

(15). The serum 1,25-dihydroxyvitamin D levels of the animals in this study were not reported. However, the diet of the food-restricted rats was supplemented with vitamins and minerals. Thus, the food-restricted rats ingested more dietary Ca per body weight than the ad libitum group. The authors of this study suggested that the increased dietary Ca could account for the beneficial effects of the restricted diet (15).

The purposes of the dietary restriction studies reported here were twofold. First, we wished to study the effect of dietary restriction on serum 1,25-dihydroxyvitamin D, because 1,25-dihydroxyvitamin D is an important regulator of Ca homeostasis. Second, we wished to determine whether the effect of dietary restriction on Ca homeostasis was independent of the Ca content of the diet. Thus, we used the same dietary protocol that the previous study used (14, 15), but we did not supplement the diet of the restricted group with Ca.

MATERIALS AND METHODS

Rat maintenance and dietary restriction. Male Fischer 344 rats, purchased from Harlan Industries (Indianapolis, IN) at 4 wk of age, were used in these studies. They were housed individually in plastic cages (14" × 10" × 5"), and the cages were placed in laminar flow racks (Lab Products, Rochelle Park, NJ). The laminar-flow filtration system consisted of a prefilter and a high-efficiency, particulate-absorption (HEPA) filter capable of filtering 99.9% of particulate matter greater than 0.3 μm. The temperature was maintained at 22–24°C with a relative humidity of 50%. The animals were maintained on a 12-h light/dark cycle (lights on at 0630 h/lights off at 1830 h). The racks were kept in a single room which was isolated from all other animals. Entry to the room was restricted to the laboratory personnel who maintained the animals, and all personnel wore protective clothing while in the room. Cages and associated equipment were periodically sterilized, and sterilized corn cob bedding was used.

Rats were initially fed ad libitum on a purified diet which has been previously described (7). The diet (TD 80012) was obtained from Teklad Test Diets (Madison, WI) and consisted of the following: 21% casein, 15% sucrose, 43.6% dextran, 5% corn oil, 5% lard, 3% cellulose, 0.15% DL-methionine, 0.2% choline chloride, and mixtures of minerals (TD 80013) and vitamins (TD 80014). The diet contained 0.75% Ca, 0.45% P, and 2.2 IU of vitamin D-3 per g diet. Rats were also given distilled, acidified water ad libitum.

At 6 wk of age, the rats were divided into two groups. The first group was fed the diet ad libitum throughout life (non-restricted). The second group (restricted) was fed 60% of the diet consumed by the ad libitum group. The diet fed the second group was identical to the diet fed the first group. It was not specially fortified with vitamins, Na, P, and Ca to match the daily intake of these substances by the first group. In this way, the present study is significantly different from previous studies (3, 14, 15).

At the beginning of the study, 206 rats were placed in the non-restricted group, and 130 rats were placed in the restricted group. At 5, 13, 22 and 28 mo of age, eight rats from each diet group were killed for biochemical measurements. A group of 6-wk-old (1.5 mo) rats fed the ad libitum diet was also killed. This group was used to obtain initial biochemical measurements prior to the start of the dietary restriction study.

Collection of blood and urine. Unfasted rats were anesthetized with diethyl ether, and the abdominal cavity was exposed by a midline incision. Blood was withdrawn from the inferior vena cava using a syringe and allowed to clot on ice for 30 min. The clotted blood was then centrifuged, and the serum was removed and frozen for later analysis.

Urine was collected for a 24-h period before the animals were killed. Urine was collected by housing animals individually in stainless steel metabolic cages. Urine was collected into test tubes containing 1 ml of 1 *N* HCl.

Analytical measurements. Serum Ca was determined using a fluorometric technique (16). Serum and urinary P were measured by the method of Fiske and Subbarow (17). Serum and urinary creatinine were measured by a colorimetric method (18). Glomerular filtration rate was estimated from creatinine clearance, and percentage of tubular reabsorption of P was calculated as previously described (19).

Serum 1,25-Dihydroxyvitamin D was measured by competitive binding assay as previously described (20). 1,25-Dihydroxyvitamin D was partially purified by C-18 and silica Sep-Pak cartridges (Waters Associates, Milford, MA). Intestinal cytosol from rabbits was used as the source of binding protein. The standard curve was linear over the region 1.25–50 pg/tube. The lower limit of detection in serum was 4 pg/ml.

Serum 25-hydroxyvitamin D was measured using a radioimmunoassay kit (INCSTAR, Stillwater, MN). The assay uses an antibody to 25-hydroxyvitamin D which was raised in goat. Lower limit of detection was 0.2 ng/ml.

Immunoreactive PTH was measured in serum samples using a radioimmunoassay kit for rat PTH (INCSTAR, Stillwater, MN). This assay uses a chicken antibody raised against the mid-molecule region of human PTH (amino acids 44–68). Rat PTH, which reacts with this antibody, was used as a standard. Serum PTH levels of TPTX rats were undetectable (less than 10 pg/ml) using this assay.

Statistics. When measurements were made on individual animals, data are reported as the mean ± standard error (SE) of 6–8 animals per group. Statistical analyses were performed using Student's two-tailed *t*-

test (21), and a confidence level of 95% or greater was considered significant.

RESULTS

The survival data for the rats in the present study (**Figure 1**) are similar to that reported previously for male F344 rats fed this diet. The ad libitum rats had a median lifespan of 24 mo, which is almost identical to the values reported previously (3). The restricted animals had a median lifespan of 31 mo, which is somewhat less than the 35 mo reported previously. The age at 10% survival was 26.5 mo for the non-restricted group and 34 mo for the restricted group. At 34 mo, the study was terminated by killing the remaining rats for biochemical studies.

Body weight of the dietary restricted group was less than the non-restricted group throughout the experiment (**Figure 2**). The body weight of the non-restricted animals increased rapidly until about 12 mo and then plateaued. The drop in body weight between 21 and 26 mo may be due to the high rate of mortality during that period of the lifespan (Figure 1). In the restricted animals, body weight increased rapidly until about 12 mo of age and then increased only slightly thereafter. These growth curves are similar to those previously published, including the decline in weight at 20 mo of age in the ad libitum group (3).

The food consumption of the restricted and non-restricted rats was monitored throughout the experiment (**Figure 3**). Initially, the restricted rats consumed less food per gram body weight than the non-restricted. However, by 4 mo of age, the food consumption per gram body weight for both groups was the same and remained the same throughout the study. The restricted animals matched the food consumption per gram body weight of the non-restricted animals by decreasing their rate of growth (Figure 2). Since the diet fed the restricted group was not fortified, both the restricted and non-restricted animals consumed the same amount of vitamins and minerals per gram body weight.

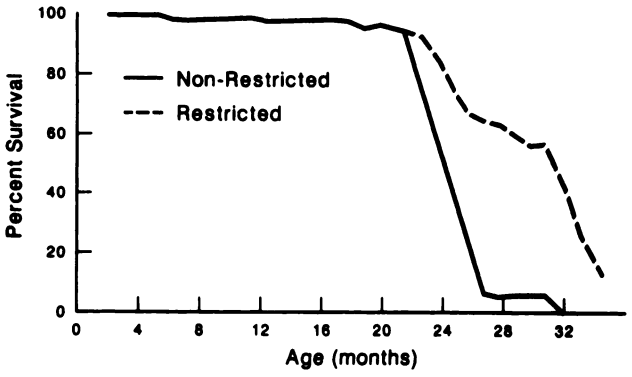

FIGURE 1 Effect of dietary restriction on survival of male F344 rats. Starting at 8 wk of age, 130 rats were placed on the restricted diet and 206 were fed ad libitum (non-restricted). The experiment was ended after 34 mo.

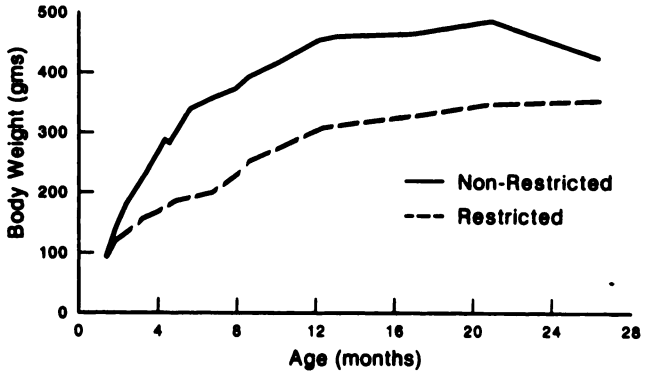

FIGURE 2 Effect of dietary restriction on average body weight. Rats were weighed every 2 wk from 2 to 8 mo of age and every 4 wk thereafter.

Serum Ca and P were not significantly affected by dietary restriction except at 28 mo of age (**Figure 4**). Serum Ca did not change significantly with age regardless of diet. Serum P decreased markedly between 1.5 and 5.0 mo and then did not change until 28 mo of age. At this age, serum P was significantly lower in the restricted animals compared to non-restricted animals.

Dietary restriction had a significant effect on serum 1,25-dihydroxyvitamin D in all age groups studied (**Figure 5**). In the non-restricted animals, serum 1,25-dihydroxyvitamin D decreased with age as has been previously observed (11). The greatest decrease was between 1.5 and 5.0 mo of age. In the restricted animals, this decrease occurred between 5 and 13 mo of age. Serum 1,25-dihydroxyvitamin D levels in the restricted group were significantly above the non-restricted group at 22 and 28 months of age.

The effect of dietary restriction on serum 25-hydroxyvitamin D, the precursor to 1,25-dihydroxyvitamin D, was also determined in pooled serum samples from each age group (**Table 1**). In non-restricted rats,

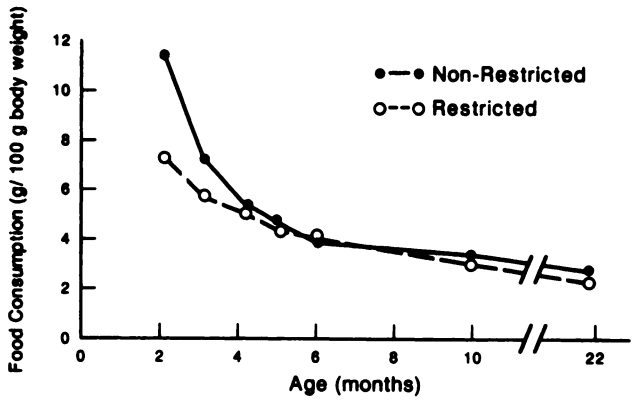

FIGURE 3 Effect of dietary restriction on food consumption. Average food consumption was measured at the indicated ages and divided by the average body weight.

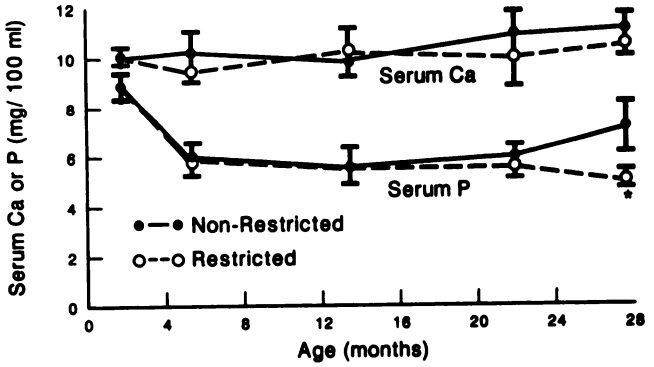

FIGURE 4 Effect of dietary restriction on serum Ca and P. Data points represent the mean ± SE of eight animals. The asterisk indicates a significant difference between the restricted and non-restricted groups ($P < 0.05$, *t*-test).

serum 25-hydroxyvitamin D was relatively constant from 1.5 to 13 mo, but it was markedly decreased at 22 and 28 mo. In restricted rats, there was a more gradual decrease in serum 25-hydroxyvitamin D with age.

Finally, dietary restriction had a marked effect on serum PTH levels later in the lifespan (**Figure 6**). In the non-restricted animals, serum PTH increased markedly with age, as has been reported previously (11). This increase was significantly blunted by dietary restriction at 22 and 28 mo of age. Because one of the physiological effects of PTH is to increase urinary P, tubular reabsorption of P (TRP) was measured at 1.5, 13 and 28 mo of age (**Table 2**). There was no difference in the TRP between diet groups at 13 mo. However, TRP was significantly decreased in the non-restricted group at 28 mo. This decrease in TRP correlates with the increase in serum PTH in the 28-mo-old non-restricted group (Figure 6).

DISCUSSION

This study demonstrates that the age-related decline in serum 1,25-dihydroxyvitamin D is delayed by dietary restriction. It also confirms the action of dietary restriction on serum PTH. However, in this study dietary restriction had these effects even though both groups of animals were consuming the same amount of Ca per g body weight (Figure 3). These effects were not due to a greater percentage of Ca in the restricted diet, a possible complication in the interpretation of other studies (14, 15). It is of interest that dietary restriction increased the median survival and 10% survival in this study (Figure 1), even though the diets were not fortified with vitamins and minerals.

The changes in serum 1,25-dihydroxyvitamin D with age and dietary restriction are not explicable by changes in serum 25-hydroxyvitamin D. For example, at 5 mo of age there is a twofold difference in serum 1,25-hydroxyvitamin D levels between diet groups (Figure 5). However, there is no difference in serum 25-hydroxyvitamin D levels at this age (Table 1). Likewise, the greatest decrease in serum 1,25-dihydroxyvitamin D is in the 1.5–13 mo age range in both age groups, but

TABLE 1

Effect of dietary restriction on serum 25-hydroxyvitamin D

Age	Serum 25-hydroxyvitamin D	
	Non-restricted	Restricted
mo	*ng/ml*	
1.5	14.0	–
5	18.9	17.2
13	20.9	15.0
22	6.1	13.2
28	6.2	9.0
33	–	9.4

Table entries are values obtained from pooled serum. Serum was pooled from eight rats in each age and diet group. The SE, based on measurements of individual rats from the same group, was 7–9% of the mean.

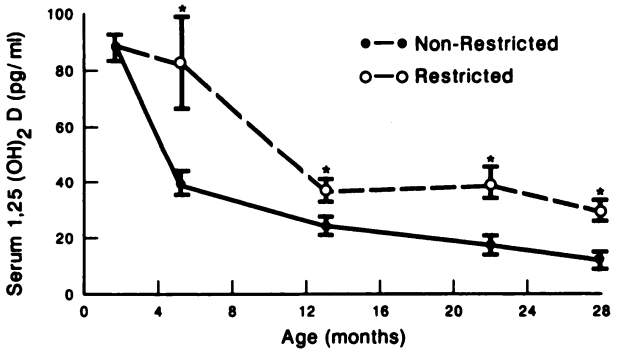

FIGURE 5 Effect of dietary restriction on serum 1,25-dihydroxyvitamin D. Data points represent the mean ± SE of eight animals. The asterisk indicates a significant difference between the restricted and non-restricted groups ($P < 0.05$, *t*-test).

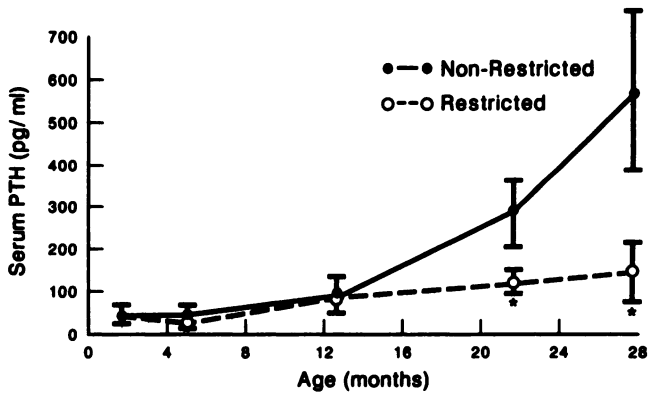

FIGURE 6 Effect of dietary restriction on serum PTH. Data points represent the mean ± SE of eight animals. The asterisk indicates a significant difference between the restricted and non-restricted groups ($P < 0.05$, *t*-test).

TABLE 2

Effect of dietary restriction on tubular reabsorption of phosphorus

	Tubular reabsorption of phosphorus		
	1.5 Months	13 Months	28 Months
		%	
Non-restricted	73 ± 2	75 ± 5	63 ± 4
Restricted	–	79 ± 4	83 ± 2[1]

[1]Significantly different from non-restricted ($P < 0.05$, t-test). Table entries are the mean ± SE of eight animals.

serum 25-hydroxyvitamin D is relatively constant (14–21 ng/ml) in these animals. However, in the 22–33 mo age range, serum 25-hydroxyvitamin D levels are consistantly higher in the restricted animals. This may contribute to the increased 1,25-dihydroxyvitamin D levels seen in these animals.

The mechanism by which dietary restriction delays the decrease in serum 1,25-dihydroxyvitamin D (Figure 5) is not clear. It may be that dietary restriction enhances production of 1,25-dihydroxyvitamin D by the kidney or that it decreases the rate of 1,25-dihydroxyvitamin D degradation. Three factors that have been implicated in the regulation of renal 1,25-dihydroxyvitamin D production are serum PTH, serum Ca and serum P (10). The greatest effect of dietary restriction is at 5 mo of age (Figure 5). At this age the serum 1,25-dihydroxyvitamin D levels of the restricted group are more than twice as high as those of the non-restricted. However, there is no significant difference in the serum levels of PTH, Ca or P between restricted and non-restricted animals at this age (Figures 4 and 6). This suggests that some other factor may be stimulating renal 1,25-dihydroxyvitamin D production at 5 mo.

Dietary restriction also suppresses the rise in serum PTH which is seen in later life (Figure 6). This effect of dietary restriction is similar to the results of a previous study (15). In both studies there was a marked increase in serum PTH after 12–13 mo of age in the ad libitum group and no increase in the restricted group.

The mechanism by which dietary restriction prevents the late rise in serum PTH is unclear. Serum Ca is thought to be the major regulator of PTH secretion by the parathyroid glands (22). Serum Ca did not change with age or diet in these studies (Figure 4). However, dietary restriction may retard the age-related decrease in the sensitivity of the parathyroid glands to Ca (23). Another possibility is that the high serum PTH levels in the older animals are due to the presence of renal lesions, which could result in decreased clearance of PTH. Dietary restriction may decrease PTH levels by decreasing renal lesions (3) and increasing PTH clearance. The serum PTH in the ad libitum animals appears to be biologically active, since the TRP of these animals is decreased (Table 2). Interestingly, the high serum PTH levels in these animals did not result in increased serum 1,25-dihydroxyvitamin D levels. This may reflect the fact that the adult and old kidney are refractory to PTH in terms of increased 1,25-dihydroxyvitamin D production (20).

The effects of dietary restriction on Ca homeostasis are interesting since the changes observed—increased serum 1,25-dihydroxyvitamin D and decreased serum PTH—would be expected to preserve bone mineral. Indeed, it has been reported that dietary restriction abolishes the bone loss that is seen late in the lifespan of the F344 rat (15). It is possible that dietary restriction decreases bone loss by first enhancing renal production of 1,25-dihydroxyvitamin D. The resulting increase in serum 1,25-dihydroxyvitamin D then enhances the intestinal absorption of Ca. The increased supply of Ca via the intestine then suppresses PTH secretion and the mobilization of Ca from bone. To test this hypothesis further, studies of the effect of dietary restriction on renal 1,25-dihydroxyvitamin D production, intestinal Ca absorption, and PTH secretion are needed.

LITERATURE CITED

1. McCay, C. M, Crowell, M. F. & Maynard, L. A. (1935) The effect of retarded growth upon the length of life span and upon the ultimate body size. *J. Nutr.* 10: 63–79.
2. Barrows, C. H., Jr. & Roeder, L. M. (1965) The effect of reduced dietary intake on enzymatic activities and life span of rats. *J. Gerontol.* 20: 69–71.
3. Yu, B. P., Masoro, E. J., Murata, I., Bertrand, H. A. & Lynd, F. T. (1982) Life span study of SPF Fischer 344 male rats fed ad libitum or restricted diets: longevity, growth, lean body mass and disease. *J. Gerontol.* 37: 130–141.
4. Weindruch, R. & Walford, R. L. (1982) Dietary restriction in mice beginning at 1 year of age: effect on life-span and spontaneous cancer incidence. *Science* 215: 1415–1418.
5. Levin, P., Janda, J. K., Joseph, J. A., Ingram, D. K. & Roth, G. S. (1981) Dietary restriction retards the age-associated loss of rat striatal dopaminergic receptors. *Science* 214: 561–562.
6. Masoro, E. J., Compton, C., Yu, B. P. & Bertrand, H. (1983) Temporal and compositional dietary restrictions modulate age-related changes in serum lipids. *J. Nutr.* 113: 880–892.
7. Birchenall-Sparks, M. C., Roberts, M. S., Staecker, J., Hardwick, J. P. & Richardson, A. (1985) Effect of dietary restriction on liver protein synthesis in rats. *J. Nutr.* 115: 944–950.
8. Sacher, G. A. (1977) Life table modification and life prolongation. In: *Handbook of the Biology of Aging* (Finch, C. E. & Hayflick, L., eds.), pp. 582–638, Van Nostrand Reinhold, New York.
9. Bringhurst, F. R. & Potts, J. T. (1979) Calcium and phosphate distribution, turnover, and metabolic actions. In: *Endocrinology, Volume 2* (DeGroot, L. J., Cahill, Jr., J. F., Martini, L., Nelson, D. H., Odell, W. D., Potts, Jr., J. T., Steinberger, E. & Weinegrad, A. I., eds.), pp. 551–585, Grune & Stratton, New York.
10. Henry, H. L. & Norman, A. W. (1984) Vitamin D: Metabolism and biological actions. *Annu. Rev. Nutr.* 4: 493–520.
11. Armbrecht, H. J., Forte, L. R. & Halloran, B. P. (1984) Effect of age and dietary calcium on renal 25(OH)D metabolism, serum 1,25-dihydroxyvitamin D, and PTH. *Am. J. Physiol.* 246: E266–E270.

12. GALLAGHER, J. C., RIGGS, B. L., EISMAN, J., HAMSTRA, A., ARNAUD, S. B. & DELUCA, H. F. (1979) Intestinal calcium absorption and serum vitamin D metabolites in normal subjects and osteoporotoic patients. *J. Clin. Invest.* 64: 729–736.
13. WISKE, P. S., EPSTEIN, S., BELL, N. H., QUEENER, S. F., EDMONDSON, J. & JOHNSTON, C. C. (1979) Increases in immunoreactive parathryoid hormone with age. *N. Engl. J. Med.* 300: 1419–1421.
14. KALU, D. N., COCKERHAM, R., YU, B. P. & ROOS, B. A. (1983) Lifelong dietary modulation of calcitonin levels in rats. *Endocrinology* 113: 2010–2016.
15. KALU, D. N., HARDIN, R. H., COCKERHAM, R. & YU, B. P. (1984) Aging and dietary modulation of rat skeleton and parathyroid hormone. *Endocrinology* 115: 1239–1247.
16. KEPNER, B. L. & HERCULES, D. M. (1963) Fluorometric determination of calcium in blood serum. *Anal. Chem.* 35: 1238–1240.
17. FISKE, C. H. & SUBBAROW, Y. (1925) The colorimetric determination of phosphorus. *J. Biol. Chem.* 66: 375–400.
18. HEINEGARD, D. & TIDERSTROM, G. (1973) Determination of serum creatinine by a direct colorimetric method. *Clin. Chim. Acta* 43: 305–310.
19. ARMBRECHT, H. J., ZENSER, T. V., GROSS, C. J. & DAVIS, B. B. (1980) Adaptation to dietary calcium and phosphorus restriction changes with age in the rat. *Am. J. Phsyiol.* 239: E322–E327.
20. ARMBRECHT, H. J., WONGSURAWAT, N. & PASCHAL, R. E. (1987) Effect of age on renal responsiveness to parathyroid hormone and calcitonin in rats. *J. Endrocrinol.* 114: 173–178.
21. DIXON, W. J. & MASSEY, F. J. (1969) *Introduction to Statistical Analysis*. McGraw-Hill, New York.
22. BROWN, E. M. (1982) PTH secretion in vivo and in vitro. Regulation by calcium and other secretagogues. *Mineral Electrolyte Metab.* 8: 130–150.
23. WONGSURAWAT, N. & ARMBRECHT, H. J. (1987) Comparison of calcium effect on in vitro calcitonin and parathyroid hormone release by young and aged thyroparathyroid glands. *Exp. Gerontol.* 22: 263–269.