Dietary Perturbation of Calcium Metabolism in Normal Man: Compartmental Analysis

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ABSTRACT The effect of dietary calcium intake on calcium metabolism was studied in eight normal volunteers by multicompartamental analysis of radiocalcium and balance data. In paired studies of six normal subjects on normal and high or low calcium intakes, necessary and sufficient criteria were used to determine changes in calcium metabolic parameters produced by alterations in dietary calcium. These changes involved gastrointestinal calcium absorption rate, renal and endogenous fecal rate constants, and bone resorption rate. Bone accretion rate and compartment sizes need not change between the paired studies. The changes of parameters involving kidney, gut, and bone were in a direction to support calcium homeostasis and were compatible with the pattern of changes produced by parathyroid hormone. However, the source of the stimulus for hormone secretion was not apparent since plasma calcium concentrations showed no significant difference between paired studies. The implications of these findings relative to control of hormone secretion, calcium regulatory mechanisms, and metabolic bone disease are discussed.

INTRODUCTION

The clinical significance of dietary calcium intake is well recognized. High or low calcium dietary intakes have been included in the therapeutic regimen for a number of

metabolic disorders in man (1-4). To date, the evidence supporting these dietary approaches to therapy has been accumulated through animal studies and analysis of various disease states by balance and tracer techniques (5-10). However, no distinct pattern of metabolic response to dietary calcium perturbation has yet emerged. It is the purpose of this report to demonstrate the response in calcium metabolism to dietary calcium perturbation in normal human subjects and to relate these responses to calcium homeostasis.

METHODS

Materials. Eight normal volunteers were studied on the Metabolism Branch of the National Cancer Institute. Each had normal screening studies which included physical examination, complete blood count, serum calcium, phosphorus, creatinine, alkaline phosphatase, urinalysis, sterile urine culture, and chest and bone X-rays. All studies were done with the subjects kept indoors in air-conditioned wards and with controlled physical activity. Metabolic diets of constant composition were ingested by the subjects for the duration of the study. Paired metabolic balance and kinetic studies were done on six of the volunteers. In the paired studies, each subject was equilibrated for 4-6 wk on 800 mg of calcium and 1200 mg of phosphorus daily dietary intake before the initial study. Another 4-6 wk were allowed for reequilibration at a new level of dietary calcium intake before starting the second calcium kinetic and metabolic balance study. Three of the six subjects had their dietary calcium intakes reduced to 200 mg/day. The other three had their calcium intake increased to 2000 mg of calcium per day. The increase in calcium intake was accomplished with milk in the case of K. B. (subject No. 6) with an accompanying increase in phosphorus intake. In R. M. (subject No. 4) and S. C. (subject No. 5), calcium lactate tablets were given. Two other subjects (Nos. 7 and 8) were studied on high and low calcium intakes without a preceding study on 800 mg of calcium intake. In total, four subjects were studied on low calcium intake and four subjects on high calcium intake. The summary of the studies on the eight subjects is given in Table I.

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Summary of Balance and ^{47}Ca Kinetics Studies Performed on Eight Subjects

Subject	Sex	Age	Weight	Height	Study	Diet		Remarks
						Ca	P	
			<i>kg</i>	<i>cm</i>		<i>g/day</i>		
1. S. G.	F	21	79	173	I	0.81	1.23	Normal calcium dietary intake
					II	0.22	0.97	Low calcium dietary intake
2. A. M.	F	21	45	152	I	0.78	1.10	Normal calcium dietary intake
					II	0.19	0.72	Low calcium dietary intake
3. J. K.	M	27	56	172	I	0.95	1.26	Normal calcium dietary intake
					II	0.22	0.80	Low calcium dietary intake
4. R. M.	F	24	60	155	I	0.83	1.13	Normal calcium dietary intake
					II	2.06	1.16	High calcium intake; calcium lactate supplements
5. S. C.	M	26	85	181	I	0.93	1.71	Normal calcium dietary intake
					II	2.10	1.71	High calcium intake; calcium lactate supplements
6. K. B.	M	23	78	179	I	0.79	1.67	Normal calcium dietary intake
					II	2.14	2.62	High calcium intake; increased milk intake. This volunteer had isolated renal glycosuria.
7. V. M.	M	21	72	183	I	0.24	1.07	Low calcium dietary intake
8. N. B.	M	21	67	187	I	2.12	2.26	High calcium intake; increased milk intake

FIGURE 1 A schematic representation of calcium metabolism. V s are transfer rates expressed in g/day of calcium. V_i = ingested, V_a = absorbed, V_f = endogenous fecal, V_F = total fecal, V_u = urinary excretion, V_o^+ = bone accretion, V_o^- = bone resorption. λ s represent the rate constant from a compartment expressed as day^{-1} . λ_t = gastrointestinal rate constant from compartment 1 into feces. λ_u = rate constant from compartment 1 into urine; λ_o^+ = rate constant from compartment 4 into nonexchanging calcium in bone. M_1, M_2, M_3, M_4, M_T = pool sizes in grams of calcium for compartments 1, 2, 3, 4, and total exchangeable calcium pool, respectively, α = fractional absorption of calcium and Δ = calcium balance in g/day.

TABLE II
Calcium Metabolic Parameters Derived from Multicompartmental Analysis
of ^{47}Ca Kinetics and Metabolic Balance Studies

		V_i	V_a	V_F	Δ	V_a	α	λ_u	λ_f	V_o^+	V_o^-	M_T
Low calcium intake												
1. S. G.	I	0.812	0.178	0.602	+0.032	0.333	0.410	0.153 ± 0.009	0.099 ± 0.007	0.417 ± 0.005	0.385	6.7 ± 0.4
	II	0.221	0.125	0.170	-0.074	0.152	0.688	0.080 ± 0.005	0.081 ± 0.006		0.491	
2. A. M.	I	0.781	0.125	0.691	-0.035	0.210	0.267	0.122 ± 0.007	0.112 ± 0.008	0.298 ± 0.005	0.333	6.3 ± 0.3
	II	0.192	0.101	0.169	-0.078	0.119	0.620	0.104 ± 0.006	0.097 ± 0.006		0.376	
3. J. K.	I	0.947	0.278	0.601	+0.068	0.477	0.504	0.251 ± 0.019	0.119 ± 0.010	0.547 ± 0.009	0.479	8.0 ± 0.4
	II	0.224	0.174	0.168	-0.118	0.165	0.737	0.176 ± 0.014	0.098 ± 0.009		0.665	
7. V. M.		0.236	0.210	0.190	-0.164	0.149	0.631	0.192 ± 0.013	0.087 ± 0.009	0.382 ± 0.021	0.543	16 ± 11
High calcium intake												
4. R. M.	I	0.833	0.143	0.627	+0.063	0.297	0.357	0.129 ± 0.006	0.087 ± 0.005	0.272 ± 0.005	0.209	5.2 ± 0.2
	II	2.055	0.184	1.712	+0.159	0.457	0.222	0.170 ± 0.007	0.109 ± 0.006		0.113	
5. S. C.	I	0.927	0.203	0.709	+0.013	0.377	0.407	0.158 ± 0.007	0.112 ± 0.009	0.363 ± 0.024	0.350	11 ± 2
	II	2.099	0.205	1.914	-0.020	0.367	0.175	0.158 ± 0.007	0.126 ± 0.009		0.383	
6. K. B.	I	0.785	0.358	0.601	-0.174	0.369	0.461	0.288 ± 0.002	0.133 ± 0.013	0.531 ± 0.011	0.705	8.7 ± 0.4
	II	2.136	0.356	1.670	+0.110	0.638	0.299	0.269 ± 0.020	0.151 ± 0.014		0.421	
8. N. B.		2.118	0.307	1.778	+0.033	0.495	0.234	0.230 ± 0.009	0.105 ± 0.007	0.530 ± 0.014	0.497	11 ± 1

The parameter values on high or low calcium intakes are shown below the values on base line (800 mg/day) dietary calcium intake. For explanation of the symbols, see Fig. 1. The parameter values from ^{47}Ca kinetics were obtained from the constrained paired studies (see text), in which V_o^+ and M_T were held constant.

10 μc of $^{47}\text{CaCl}_2$ dissolved in 2.0 ml of sterile isotonic saline was given in a rapid intravenous injection on the morning of the 1st day of the study. Serum calcium specific activity, as well as urine and fecal cumulative radioactivity, were monitored for 18 days. During this same period, balance data for calcium, phosphorus, and nitrogen were collected in three 6-day pools. The isotope counting techniques and the methods used for balance collections and chemical determinations have been previously described (11).

Data analysis. The kinetic behavior of the calcium isotope as seen in serum, urine, and stool can be described by a four compartment model. Although many four compartment models can satisfactorily fit the data, the series model discussed by Neer, Berman, Fisher, and Rosenberg (11) was used as the basis of comparison. Steady-state compartment sizes, rate constants, and flow rates were calculated from the tracer and balance data by the Simulation, Analysis, and Modeling (SAAM) computer program of Berman and Weiss (12, 13).

A physiological interpretation of the four compartment series model is shown diagrammatically in Fig. 1. Flow rates and rate constants are designated by V 's and λ 's, respectively. V_i is the rate of dietary calcium entering the gastrointestinal tract. V_a is the rate of calcium absorption into the labile calcium pool. The fractional gastrointestinal absorption, α , is the ratio of the rate of absorption to the rate of ingestion ($\alpha = V_a/V_i$). V_o^- is the rate of calcium resorption from bone. Calcium may leave the labile pool through accretion into nonexchangeable bone at a rate, V_o^+ , and by losses to urine and feces at excretion rates V_u and V_f , respectively. The theory and assumptions for this multicompartmental analysis have been discussed in detail in a previous report (11).

Six of the subjects underwent paired studies on normal and either high or low calcium intakes, and therefore each served as his own control for analysis of the dietary perturbation. There is no reason to expect alterations in every parameter of the compartmental system after a dietary calcium change. The rationale in the analysis, therefore, was to determine a necessary and sufficient set of parameter changes as an hypothesis to explain the observations (14). To accomplish this computationally, both sets of data from paired studies were used jointly in the computer least squares fitting procedure, with the constraint that one or more of the kinetic parameters have identical values for both studies.

RESULTS

Model testing. Kinetic data from paired studies were first analyzed individually. The least squares fitting procedure demonstrated significant parameter changes only in λ_u and λ_f between each of the paired studies. Since other combinations of parameter changes may also fit the data, formal model testing was used to rigorously test these initial results.

No significant difference in serum calcium concentration was seen between the normal and high or low calcium intake periods (25 paired postabsorption blood samples from each subject; normal and low intake, 10.46 ± 0.05 mg/100 ml and 10.37 ± 0.04 mg/100 ml, respectively, $P < 0.2$; normal and high intake, 10.27 ± 0.04 mg/100 ml and 10.32 ± 0.04 mg/100 ml, respectively,

TABLE III
Intercompartmental Rate Constants, Transfer Rates, and Steady-State Pool Sizes for the
Studies on Normal Subjects

	1. S.G.	2. A.M.	3. J.K.	7. V.M.	Mean low Ca diet	4. R.M.	5. S.C.	6. K.B.	8. N.B.	Mean high Ca diet	Combined mean with standard deviation
M ₁	1.24 ±0.08	0.99 ±0.07	1.10 ±0.08	1.1 ±0.2	1.1	1.05 ±0.04	1.44 ±0.09	1.29 ±0.09	1.4 ±0.1	1.3	1.2 ±0.2
M ₂	0.96 ±0.09	0.65 ±0.05	1.14 ±0.11	1.5 ±0.4	1.1	0.75 ±0.08	2.1 ±0.4	1.4 ±0.2	0.8 ±0.1	1.3	1.2 ±0.9
M ₃	2.1 ±0.2	1.9 ±0.1	2.4 ±0.2	3.4 ±1.2	2.4	1.4 ±0.2	2.7 ±0.3	2.8 ±0.5	2.6 ±0.2	2.4	2.4 ±0.6
M ₄	2.3 ±0.3	2.8 ±0.2	3.4 ±0.2	9.6 ±8.8	4.5	2.0 ±0.2	4.6 ±1.6	3.1 ±0.4	5.9 ±0.7	3.9	4.2 ±2.5
M _T	6.7 ±0.4	6.3 ±0.3	8.0 ±0.4	16 ±11	9.2	5.2 ±0.2	11 ±2	8.7 ±0.4	11 ±1	8.9	9 ±4
λ ₂₁	24 ±6	21 ±6	28 ±7	22 ±4	24	26 ±5	15 ±2	24 ±7	25 ±4	23	24 ±4
λ ₂₁	30 ±8	32 ±8	27 ±6	16 ±3	26	36 ±8	10 ±3	22 ±6	42 ±5	28	27 ±10
λ ₃₂	5.1 ±1.0	6.4 ±0.8	5.3 ±1.1	2.3 ±0.5	4.8	5.5 ±1.4	1.5 ±0.7	4.2 ±1.1	8.7 ±1.5	5.0	4.9 ±2.3
λ ₃₃	2.1 ±0.4	2.0 ±0.2	2.3 ±0.5	0.9 ±0.2	1.8	2.7 ±0.7	1.0 ±0.3	1.8 ±0.6	2.6 ±0.4	2.03	1.9 ±0.7
λ ₄₃	0.42 ±0.11	0.42 ±0.05	0.55 ±0.12	0.26 ±0.06	0.41	0.62 ±0.16	0.21 ±0.02	0.53 ±0.22	0.62 ±0.06	0.50	0.46 ±0.16
λ ₃₄	0.21 ±0.08	0.18 ±0.05	0.22 ±0.05	0.06 ±0.03	0.17	0.30 ±0.07	0.042 ±0.004	0.31 ±0.11	0.18 ±0.03	0.21	0.19 ±0.10
λ ₀₊	0.18 ±0.02	0.11 ±0.01	0.16 ±0.01	0.04 ±0.04	0.12	0.14 ±0.01	0.08 ±0.03	0.17 ±0.02	0.09 ±0.01	0.12	0.12 ±0.05
ρ ₂₁	30 ±6	21 ±5	31 ±6	24 ±6	26	27 ±5	21 ±3	32 ±6	35 ±6	29	28 ±5
ρ ₁₂	29 ±6	21 ±5	30 ±6	24 ±6	26	27 ±5	21 ±3	31 ±6	34 ±5	28	27 ±5
ρ ₃₂	5.0 ±0.6	4.2 ±0.3	6.0 ±0.8	3.4 ±0.8	4.7	4.1 ±0.7	3.1 ±1.0	5.8 ±1.0	7.0 ±2.0	5.0	4.6 ±1.4
ρ ₂₃	4.5 ±0.6	3.9 ±0.3	5.5 ±0.8	3.1 ±0.8	4.3	3.8 ±0.7	2.8 ±1.0	5.3 ±1.0	6.8 ±2.0	4.7	4.2 ±1.4
ρ ₄₃	0.9 ±0.1	0.80 ±0.06	1.3 ±0.2	0.9 ±0.2	1.0	0.9 ±0.1	0.55 ±0.06	1.5 ±0.4	1.6 ±0.3	1.1	1.1 ±0.4
ρ ₃₄	0.5 ±0.1	0.50 ±0.06	0.8 ±0.2	0.5 ±0.2	0.6	0.6 ±0.1	0.19 ±0.06	1.0 ±0.4	0.11 ±0.4	0.48	0.54 ±0.3

M₁, M₂, M₃, M₄, M_T = pool sizes in grams of calcium for compartments 1, 2, 3, 4, and total exchangeable calcium pool, respectively. The λ_{ij}s designate the rate constants from compartment j into compartment i, and ρ_{ij}s are the corresponding transfer rate. These rate constants and pool sizes were obtained from the paired analysis and were constrained at identical values between the studies on base line and high or low calcium dietary intakes.

FIGURE 2 Typical metabolic balance data on normal and low calcium diets. Data are expressed according to the convention of Reifenstein. The normal study is shown on the left and the low calcium study on the right. There was an intervening equilibration period of 6 wk between the studies. Negative calcium balance was produced without significant changes in phosphorus or nitrogen balances.

FIGURE 3 (a) Serum and urine specific activity curves from the same subject on normal and low calcium intake plotted semilogarithmically vs. time in days. (b) Cumulative excretion of ^{45}Ca in urine and feces with normal and low calcium intake in the same subject.

$P < 0.4$). However, there were distinct differences in renal excretion rate V_a (Table II). Since there was no significant change in serum calcium concentration, the difference in renal calcium excretion rates was likely due to a change in renal calcium clearance. To test whether such a change would necessitate a change in λ_u , the paired studies were fitted jointly with common λ_u values, while changes in all other parameters and pool sizes were free to adjust. A good fit of the tracer data could not be obtained for this hypothesis. A similar test was performed for the gastrointestinal rate constant, λ_t , with comparable results. Thus, the testing led to the conclusion that both λ_t and λ_u must change with an alteration in the level of calcium dietary intake.

Since λ_u and λ_t had to change, the next question asked was whether all the differences in tracer kinetics produced by changes in dietary calcium intake could be explained by changes only in λ_u and λ_t . This was tested by fitting both sets of data jointly with the constraint that all corresponding compartment sizes and λ_s , except λ_u and λ_t , have the same value between the paired studies. A good fit of both sets of data was obtained. These results rigorously corroborated the initial unconstrained analysis and demonstrated that the changes in λ_u and λ_t were not only necessary but also sufficient to account for the changes in ^{45}Ca kinetics on the low and high calcium diets. In addition, the alterations in total fecal calcium and calcium balance with dietary calcium per-

LOW CALCIUM INTAKE

HIGH CALCIUM INTAKE

FIGURE 4 Typical results of simultaneous multicompartmental analysis of paired studies in two volunteers on low (subject S.G.) and high (subject R.M.) dietary calcium intakes. Parameter values were obtained from the paired analysis in which pool sizes, V_o^+ , and intercompartmental rate constants were constrained at identical values. Using necessary and sufficient criteria, changes were seen in V_a , V_o^- , λ_r and λ_u (see text). The values for these four parameters on base line (800 mg/day) calcium intake are shown above the value on high or low calcium intake.

turbation required changes in bone resorption rate, V_o^- , and gastrointestinal calcium absorption rate, V_a . Thus, changes in calcium metabolism secondary to dietary calcium perturbation may be characterized by changes in model parameters representing kidney (λ_u), gut (λ_r, α), and bone (V_o^-). The values calculated by the computer for normal and high or low calcium intakes subject to these constraints are summarized in Tables II and III.

Low calcium intake. With a 200 mg/day calcium intake, the calcium balance became negative in all patients (Fig. 2), but no significant changes were seen in phosphorus or nitrogen balances. Excretion of radioactivity into urine and stool was decreased (Fig. 3 b). Although the early points in the serum specific activity curves for normal and low calcium intakes were coincident (Fig. 3 a), there was a definite decrease in the

slope of the curve on low calcium intake beginning at about day 3. Since bone accretion rates could be held constant in the face of negative calcium balance, bone resorption rates had to be increased ($V_o^- = V_o^+ - \Delta$). In all three subjects studied on low calcium intake, similar changes at kidney, gut, and bone were seen. The renal rate constant, λ_u , decreased by a mean of 31%, the gastrointestinal rate constant, λ_r , fell by 19% and the bone resorption rate, V_o^- , increased by 20%. Although the rate of dietary calcium absorption, V_a , fell from a mean of 0.36 g/day on normal intake to a mean of 0.14 g/day on low calcium intake, the fractional absorption, α , increased by a mean of 81%. A typical response pattern is shown in Fig. 4.

High calcium intake. The direction of the change was, in general, opposite that for the low calcium stud-

FIGURE 5 Typical balance data on normal and high calcium intakes. Positive calcium balance was produced but no changes were seen in phosphorus or nitrogen balances. There was an intervening equilibration period of 6 wk between studies.

dies. In subject R. M., V_a rose from 0.30 to 0.46 g of calcium per day on calcium lactate supplementation. The calcium metabolic balance was positive (Fig. 5), and the excretion of radiocalcium in urine and feces increased (Fig. 6b). There was an increase in the slope of serum specific activity curve but, as in the low calcium intake studies, early points in the specific activity curves were coincident in the paired studies (Fig. 6a). Necessary and sufficient changes to explain the response to high calcium intake involved the same parameters as those for low calcium intake, i.e., λ_u , λ_r , V_a , and V_o^- (Fig. 4).

In subject S. C., there was no increase in V_a despite supplementation with calcium lactate to a daily intake level of 2000 mg of calcium (Table II). There were also no other changes in model parameters except for λ_r . The gastrointestinal rate constant, λ_r , and fractional absorption, α , both fell while total fecal calcium excretion

rate, V_F , was greatly increased. Thus, it appears that in this subject, the gastrointestinal tract alone was adequate to maintain homeostasis in the face of high dietary calcium intake.

In subject K. B., V_a was increased on a 2000 mg/day calcium intake. Accompanying this increase in absorbed calcium was an increase of λ_r and a decrease in V_o^- . There was no change, however, in V_u or λ_u . The additional calcium for this subject was given by increasing milk intake, and therefore there was a commensurate increase in phosphorus intake. This may, in part, explain the lack of change in renal calcium excretion (see Discussion).

Pattern of homeostatic response. The constancy of the size of compartment 1, M_1 , reflected the unchanged mean values for postabsorptive serum calcium concentrations between paired studies. Thus, calcium homeostasis was preserved in the face of dietary calcium

FIGURE 6 (a) A comparison of serum and urine specific activity curves on normal and high calcium intake in the same subject. (b) Cumulative excretion of ^{45}Ca in urine and feces of normal and high calcium intake in the same subject.

manipulation. This preservation was possible because changes in calcium absorption rate, V_a , were balanced by compensatory changes in λ_u , λ_t , and V_o^- . The fractional changes in λ_u , λ_t , and V_o^- between paired studies were plotted against fractional changes in V_a (Fig. 7). Fitting these data by the method of least squares, it was found that $\delta V_o^- / V_o^- = -0.33 \delta V_a / V_a$; $\delta \lambda_u / \lambda_u = 0.53 \delta V_a / V_a$; $\delta \lambda_t / \lambda_t = 0.27 \delta V_a / V_a$.

DISCUSSION

Model testing. The set of parameter changes determined by necessary and sufficient criteria is not mathematically unique but provides the simplest hypothesis to explain the observations in tracer data. In particular,

no changes in intercompartmental λ_s and λ_o^+ were necessary. This finding implies that the paired serum ^{45}Ca disappearance curves were superimposable if corrected for urinary and fecal excretion (λ_u , λ_t). It may be that two or more parameters were changed in combination to produce this result fortuitously, but this is unlikely. It is also possible that there may have been subtle changes beyond the resolution in our data.

It is likely that the hypothesis for parameter changes in tracer data (λ_s) is correct for the above considerations. However, the likelihood of the sufficient hypothesis for steady-state parameters, i.e., compartment sizes, V_o^+ , V_o^- , cannot be forcefully argued. These parameters are sensitive to variations in the model for the site of steady-state input. Although there is little evidence supporting the entry of gastrointestinal calcium, V_a , or resorbed calcium from bone, V_o^- , into a pool other than plasma, one must leave open these possibilities. Future studies may require extensions of the hypothesis proposed in this report.

Calcium regulation. Plasma calcium concentration in man is very closely regulated by parathyroid hormone (PTH). Thyrocalcitonin (TCT) may also be involved in this regulation. PTH secretion is stimulated by hypocalcemia, and the hormone acts on kidney, gut, and bone to elevate plasma calcium levels (15). TCT decreases plasma calcium concentration by decreasing bone resorption and is secreted in response to hypercalcemia (16). Acting alone, or perhaps in concert with TCT, PTH maintains plasma ionized calcium in normal individuals with little measureable fluctuation.

The independent variable in our studies is the level of ingested calcium. Kinetics and balance data provide quantitative estimates of the homeostatic responses at kidney, gut, and bone after the subject has adjusted to the change in calcium intake. This regulatory adjustment may be complex and involve a number of factors.

Low calcium intake. With a decrease in dietary calcium intake, V_i , there was a distinct pattern of metabolic response. Absorbed calcium, V_a , fell from 0.34 ± 0.013 g/day on 0.8 g/day intake to 0.15 ± 0.02 g/day on 0.2 g/day intake. Accompanying this decrease in V_a , there was an increase in bone resorption rate (V_o^-) and a decrease in renal and gastrointestinal rate constants (λ_u , λ_t). The fractional changes at these three sites ($\delta V_o^- / V_o^-$, $\delta \lambda_u / \lambda_u$, $\delta \lambda_t / \lambda_t$) were of a similar magnitude ($\approx 25\%$), and suggest a common mechanism. Since PTH has effects on kidney, gut, and bone which parallel the observed changes, it is attractive to hypothesize that increased PTH activity is the common factor mediating the target organ responses. Unfortunately, it has not yet been possible to test this directly. However, there is evidence from animal studies which supports this hypothesis (17-19).

In spite of a response pattern to conserve calcium, a

FIGURE 7 Linear regression of the fractional change in V_o , λ_u , and λ_t on fractional change in V_a . For each subject, fractional change in these parameters was calculated as the ratio of the difference produced by high or low dietary calcium intake to the value on normal calcium intake in that subject. M is the slope of the straight line passing through the origin and was calculated by the method of least squares. Subject No. 6 is K.B. who had increased phosphorus intake accompanying the high calcium intake and also had isolated renal glycosuria.

negative calcium balance was produced in all four subjects on a 200 mg/day calcium intake. This is a well-known phenomenon. One may argue that negative balance is only temporary and that additional time is required for complete metabolic adjustment. Even so, metabolic balance can only be achieved after there has been a definite decrease in total body calcium; i.e., plasma calcium homeostasis occurs at the expense of calcium in bone.

Phosphorus intake. The inorganic phosphorus content of the diet was decreased ($\approx 30\%$) during the low calcium intake studies (Table I). Phosphorus balance, however, remained unchanged (Fig. 2). Dietary phosphorus is known to affect renal, intestinal, and osseous calcium metabolism (20), but these effects were related to hypophosphatemia and marked negative phosphorus balances, neither of which were present in our studies. Furthermore, a decrease in phosphorus intake would

alter urinary calcium in a direction opposite that observed in our studies. It is therefore unlikely that the response seen in the low calcium studies was significantly altered by the small change in phosphorus intake.

High calcium intake. A high dietary calcium intake produced changes in kidney and bone metabolism of calcium when there was an increased rate of calcium absorption from the gastrointestinal tract. In subject R.M., V_a increased from 0.30 to 0.46 g/day with calcium lactate supplementation, but in subject S. C., V_a remained unchanged despite the same high level of calcium intake as in R. M. This finding suggests that there may be individual variation in the ability to utilize an increased dietary calcium load. The previous dietary history has been suggested as a factor in determining the level for V_a with increased dietary calcium intake (9, 10). It has also been suggested that positive calcium balance from an increased calcium intake may be maintained only for a limited duration (5). It may be that the subjects with an increased V_a will undergo further equilibration after a period of positive calcium balance.

It is clear that an increase in V_a produced a physiologic response pattern involving changes in calcium metabolism at kidney, gut, and bone. Resorption of calcium from bone was decreased, whereas renal and gastrointestinal calcium clearances were increased. That this pattern included the same organ sites as those seen with calcium deprivation again suggests a change in hormone secretion may be the mediating factor.

In subject K. B., there were gastrointestinal and bone responses consistent with those observed in the other subjects, but no renal response was seen. λ_u remained unchanged despite the increase in V_a . However, since this subject was given milk to increase his calcium intake, there was a concomitant increase in phosphorus

FIGURE 8 Rate of calcium absorption, V_a , plotted against rate of calcium intake, V_i . Mean values for three intake rates are shown. The curve was obtained by least squares fit using the expression: $V_a = k_1 V_i / (k_2 + V_i)$.

intake. It has been shown that high phosphorus intake will decrease the urinary excretion of calcium in normal subjects (21, 22). The lack of any increase in urine calcium excretion in K. B. may have been due to this effect. Also, K. B. had isolated renal glycosuria (11). Although no other renal tubular defects were found, the failure of renal adaptation to calcium loading might represent a subtle form of nephropathy.

Gastrointestinal absorption of calcium. The rate of calcium absorption, V_a , is dependent on the rate of calcium ingestion, V_i . PTH control of this absorption process has been proposed by several investigators (23, 24). The efficiency at which the ingested calcium is absorbed presumably is altered by PTH concentration which, in turn, is sensitive to plasma calcium concentration. A plot of V_a vs. V_i in our studies suggests that V_a approaches saturation with increasing V_i (Fig. 8), and that their relationship may be expressed reasonably well by a Michaelis-Menten type of equation,

$$V_a = \frac{k_1 V_i}{k_2 + V_i}$$

with values for k_1 and k_2 of 0.66 and 0.77, respectively. This suggests the possibility of an intrinsic (nonhormonal) control of calcium absorption. Such a local control mechanism has been suggested by the studies of Kimberg, Schachter, and Schenker in rats (25). Hormonal control of the gastrointestinal absorption process, however, is not inconsistent with our data, and it is possible that both local and hormonal control mechanisms are present.

One might expect λ_r to be affected in a manner paralleling the changes in the efficiency of calcium absorption, since the gastrointestinal rate constant is a composite of calcium secretion from the plasma (λ_s) and reabsorption from the gastrointestinal tract ($\lambda_r \cdot \alpha$) such that $\lambda_r = \lambda_s (1 - \alpha)$. The actual changes in λ_r observed, however, are smaller than predicted from the above relationship, a fact which suggests that the reabsorption of calcium secreted from the plasma is less than absorption of ingested calcium. This hypothesis was first proposed by Heaney and Skillman (26). They suggested that secreted calcium may be divided into absorbable and non-absorbable fractions such that only the absorbable portion is affected by changes in α . Recent studies by Birge¹ demonstrating variations in calcium absorption along the human gastrointestinal tract elaborates on Heaney and Skillman's hypothesis. As in the case of V_a , changes in λ_r reflecting changes in α may be explained with or without invoking hormone regulation.

Kidney. The changes in λ_u occurred in the face of constant serum calcium concentration. It would be difficult to exclude the possibility that small changes in ultrafiltrable calcium or glomerular filtration rate were responsible for the changes in λ_u . More likely is a

change in renal tubular reabsorption of calcium. A number of studies show that renal tubular reabsorption of calcium is increased by parathyroid hormone (27, 28). It is reasonable and consistent, therefore, to hypothesize hormone regulation at the kidney in response to changes in calcium dietary intake.

A small but significant difference in serum calcium concentration with low calcium diets was reported by MacFadyen, Nordin, Smith, Wayne, and Rae (29). These authors then correlated changes in urinary calcium excretion with changes in renal calcium filtered load. In our study, no significant difference in serum calcium concentrations was found between the normal and high or low calcium intake periods. This discrepancy between our data and that of MacFadyen et al. may be due to the different durations employed for equilibration on the low calcium diet. In MacFadyen's study, blood calcium determinations were obtained on 2 successive days with an acute perturbation in dietary calcium intake on the 2nd day. It may be that they observed differences in serum calcium concentration during a transient phase before completion of homeostatic responses in the gastrointestinal tract and bone.

Bone. In view of constant plasma calcium concentrations, changes in bone resorption rate (V_o) are most likely due to hormonal control. This has been suggested by Jowsey and Gershon-Cohen (18) in studies of low calcium dietary effects in cats.

Stimulus for hormone secretion. The changes at kidney, gut, and bone produced by perturbations in dietary calcium intake are consistent with the regulatory effects of PTH. The stimulus for secretion of this hormone, however, is not apparent from our studies. It has been clearly demonstrated in cows that plasma PTH concentration is proportional to the degree of acute change in plasma calcium levels (30). In our study, no significant difference in mean serum calcium concentrations could be detected at high and low calcium intakes. This would suggest either that hormone secretion changes its sensitivity to plasma calcium with chronic stimulation, or that another triggering mechanism for PTH secretion exists. The former hypothesis has been supported in part by studies in cows with milk fever (31). The latter is suggested by studies in other hormone controlled metabolic systems where intestinal absorption may act as a triggering mechanism (32, 33), but there has been no investigation of this possibility in calcium regulation in animals or in man. It must be pointed out, however, that we found small, hour to hour, fluctuations in plasma calcium concentration, and it is conceivable that these fluctuations may be factors in the regulation of PTH or TCT secretion.

Pattern of response. The correlation of bone resorption rate and renal and gastrointestinal rate constants to gastrointestinal calcium absorption rate may be of diagnostic and therapeutic significance. A number of rela-

tively simple techniques are available for determining calcium absorption¹ (34, 35). Using these techniques, one may predict normal patterns of response in V_0 , λ_0 , and λ_1 following a change in V_A . Future kinetic studies may demonstrate abnormal patterns of response. These abnormal patterns may help to elucidate primary pathogenetic mechanisms in calcium disorders and metabolic bone disease.

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