American Journal of Food and Nutrition, 2016, Vol. 4, No. 1, 20-29
Available online at http://pubs.sciepub.com/ajfn/4/1/3

DOI:10.12691/ajfn-4-1-3

Effect of Insulin Hypercalciuric Effect of High Protein Diets

B.W. Brazier*

Bendigo Kangan Institute, Bendigo, Victoria, Australia
*Corresponding author: sail_bad@yahoo.com

Abstract Postprandial calciuric responses to high and low protein meals is examined and changes in urinary Ammonia. Urea, pH, creatinine, phosphate are compared with serum calcium, creatinine, urea, protein and insulin. Group result show that the average postprandial calciuria increase was greater after the high protein meals compared to low protein meals. But there was considerable variation between subjects. However the increase in calciuria in every case showed a plateau effect during the time of maximum insulinemia Examination of individual results show that the individuals with less insulinemia exhibited greatest hypercalciuria and those with greater insulinemia show less calciuria and even one individual with an exaggerated insulinemia response showed reduced hypercalciuria following the high protein meal. Discussion is provided regarding the significance of the protein induced hypercalciuria and possible mechanisms for it cause. The result indicate that plasma insulin had a significant effect on calciuria.

Keywords: *hypercalciuria, protein, insulin*

Cite This Article: B.W. Brazier, "Effect of Insulin Hypercalciuric Effect of High Protein Diets." *American Journal of Food and Nutrition*, vol. 4, no. 1 (2016): 20-29. doi: 10.12691/ajfn-4-1-3.

1. Introduction

High protein diets have been proposed as the cause of the higher incidences of osteoporosis in western societies compared to less affluent countries [1] (Abelow et. al., 1992) and it has been suggested that metabolic acidosis may be the cause of protein induced hypercalciuria. This has been demonstrated with adult young women by [2] Kaneko et. al. (1990) and with rats by [3] Fernandez-Repollet et. al. (1989). They suggested that acidosis resulted from oxidation of sulfur containing amino acids from protein. However when [4] Funaba et. al. (1989) studied the hypercalciuria of high protein diets on rats they found that calcium excretion could not be greatly reduced by adding extra dietary sodium bicarbonate and that calciuria did not correlate with net acid excretion. On the other hand Schneider and Menden (1988) [5]using long term experiments (61 weeks) with rats found that high protein diets showed a positive correlation between renal hydrogen ion and sulphate excretion. However Schneider and Menden (1988) [5] found that increasing net acid excretion with extra phosphorus intake produce less hypercalciuria counteracting the suggestion that acid from increased dietary protein could be the cause of hypercalciuria.

The hypercalciuric effect of high protein diets has been demonstrated several times, e.g.,[6] (Allen et. al., 1979) and [7] Hegsted and Linkswiler, 1981). Increased renal calcium excretion has been shown to correlate with increased urinary sulphate and acidity [8] (Zemel et. al., 1981) and the increase in total renal acid excretion resulting from high protein meals was correlated with increased excreted inorganic sulphate and ammonium by [9] Lutz and Linkswiler (1981).

When high protein diets were consumed the increased ammoniagenesis appears to be associated with a reduction in sodium excretion [10] (Schuette et. al., 1980) indicating that sodium is being replaced by ammonium under increased acid load. Sodium is reabsorbed while protons are secreted into the urine via an anti-portal protein found in the brush border membranes. This is a secondary active transport using energy from the primary active, ATP driven sodium pump. Thereby lowering the pH of the ursine

This was also shown by [11] (Rhoades and Pflanger 1989) indicating ATP is used to actively secrete H+ ions into urine and thereby lowering the tubular pH. [12] This lowering of pH stimulates ammoniagenesis in the proximal tubular cells releasing ammonia into the tubular lumen to buffer the acid in the urine as shown by [12] Nissim and States (1989)

The increased calcium loss appears to result from increased glomerular filtration rate (GFR) and a reduced fractional renal tubular reabsorption rate (FR) [10] Schuette et. al., (1980, [8] Zemel et. al., (1981; [7] Hegsted and Linkswiler, (1981). Zemel et. al. (1981) [8], Although others have shown that the magnitude of the calciuric response to different dietary proteins correlates with their sulfur content, the increased calciuria could not always be fully accounted for by increased sulfur intake indicating the involvement of other factors, possibly hormonal.

[13] Allen et. al. (1981), [14] Wood (1983) and [15a]Howe (1990) have reported a correlation between the

increased postprandial insulin release found with protein diets and the increased urinary calcium excretion. However no change was found in the levels of parathyroid hormone, active vitamin D or cyclic AMP [10] (Schuette, et. al. 1980).

The involvement of insulin and calciuria may be significant as indicated by several report of the difference in occurrence of osteoporosis in subjects with type I and type II diabetes mellitus. Eg, [16] Leidid-Buckner and Ziegler (2001). They report that people with type I diabetes exhibit low bone density (i.e. less calcium) and people with type II diabetes have normal or greater bone density (i.e. more calcium). [17] Osteoporosis Australia (2014) suggest that although people with type II diabetes are more likely to have bone fractures than normal people this is probably due to increased falls and inactivity even though they have normal bone density.

These observations with diabetes suggest that the effect of insulin on calciuria should be considered in this experiment

In order to look for a relationship between changes in calciuria and plasma insulin levels it was decided to use a study of acute effects following consumption of single meals because any related variations in the levels of these two components from time to time could be masked or average out over time if more lengthy experiments were used.

The choice of examining the postprandial calciuric responses had the added advantage in that it is easier to control free living subjects for the short period of time involved and it is possible to supervise the collection of urine and take blood samples with some degree of accuracy. Accurate collecting whole day samples of urine are difficult even when subjects are confined.

The purpose of this study was to look for variations in protein induced postprandial calciuria in relation to plasma insulin levels. This could be significant because it has been shown that greater plasma insulin levels are produced followed the high protein meals than the normal protein meals by Floyd *et. al.*(1966) [18].

2. Methods

2.1. Subjects

The subjects included seven healthy Caucasian nutrition students, five female and two male, age between 20 and 25 years and had BMI around 25 who normally consumed mixed western style diets. They were all fit, active and of normal weight for height, and showed normal GTT. A short medical history was taken from each to ensure that there was no indication of kidney stones, diabetes mellitus, hypertension or kidney disorders.

Subjects were required to sign consent forms showing that they had been well briefed before the experiment and that they knew they could withdraw from the activity at any time. The experimental procedures and protocol were approved by both the Melbourne University and Royal Melbourne Hospital Human Ethics Committees.

2.2. Food Intake

Meals were prepared on the day before each test by blending the ingredients shown in Table 1 with a metal spoon. The meals had a thick cream cheese texture and sour taste. On two separate occasions, one week apart, subjects ate a high protein meal (HPM) on one visit and the normal protein meal (NPM) on the other visit. Not more than three subjects were tested at one time. The order of consuming the high protein meal or the normal protein meal was randomised among the subjects.

The meals were made isocalorific by making additions to 100 g or 300 g samples of non-fat cottage cheese (0.5% fat) and standardised for sodium, calcium, lactose and phosphate as per Table 1. Atomic absorption spectrophotometry was used to measure Ca concentration (AOAC 1975, method. 2.109) and phosphate by the photometric molybdate reduction method (AOAC 1975 method. 24.015n.120) and lactose by the Lance-Eynon method (AOAC 1975 method. 31.061).

Table 1. Meal Composition

Meal	NPM[1]	HPM[2]
Ingredients	g	g
Cottage cheese'	100	300
Safflower oil	25.5	6.5
Sucrose	11	11
Corn-starch	10	10
Lactose	4	0
Glucose monohydrate	6.4	0
Sodium chloride	1.47	0
Phosphoric acid, cone.	0.76	0
Calcium phosphate	1.03	0.38
1	18 g protein/meal	
2	54 g protein/meal	
3	<0.5% fat cottage cheese	

This table shows the amount of ingredients used to prepare the meals consumed as part of the postprandial calciuria experiment.

2.3. Sample Collection

After an overnight fast subjects on awakening emptied their bladder, recorded the time and discarded the urine; the subjects then drank approximately 250 ml water. On arrival at the laboratory the subject drank 250 ml water, and again each half hour throughout the experiment.

On arrival at the Royal Melbourne Hospital School of Medicine metabolic laboratory the subjects were rested at least 20 min by lying on examination couches. Urine and blood samples were collected before the meal and then each hour after the meal. The subject remained prostrate except while eating or collecting urine samples. A butterfly cannula was inserted into a cubital vein and a plastic syringe taped to the forearm. Blood samples were withdrawn through the cannula at hourly intervals and distributed between two heparinised vials for insulin measurement and for blood chemistry and one fluoride tube for blood glucose determination.

2.4. Analysis

Urine samples were measured for volume then each sample was acidified with 1 cm^3 concentrated HCI and returned to the Rusden laboratory for determination of total nitrogen by the macro Kjeldahl method [19] (Scales and Harrison, 1920) urea and ammonia by the indophenol method of [20] Chaney and Marback (1962). Calcium was measured by atomic absorption [21] (Willis, 1960), creatinine by the method of Hare (1950) [22] and

phosphate by the photometric molybdate reduction method of [23] Fiske and Subbarow (1925). Serum calcium, creatinine, phosphate, urea and protein were measured by routine autoanalyser methods at RMH Biochemistry Department and serum insulin by routine radio- immunoassay in the Endocrinology Department of RMH.

Results for each time interval were averaged and the standard errors calculated using the following formula:

Calculation of Standard Error Formula:

$$SE_{\bar{x}} = \frac{S}{\sqrt{n}}$$

where

$SE_{\bar{x}}$ = Standard Error of the Mean

s = Standard Deviation of the Mean

n = Number of Observations of the Sample

The standard errors are displayed on the Table 2 and Figure 9 and Figure 1 to show the degree of validity of the results [20a] Maths worksheet (2015).

Table 2. Excretion of Urinary Calcium, Phosphate, Ammonia and Plasma Insulin

DO	Urinary Calcium		Urinary Ammonia		Urinary Phosphate		Plasma Insulin	
Time	NP	HP	NP	HP	NP	HP	NP	HP
hr.	µg/hr	µg/hr	µg/hr	µg/hr	µg/hr	µg/hr	µg/hr	µg/hr
0	13.7	14.3	4.3	1.1	4.74	3.45	19	19.5
1.42	10.1	16.6	2.3	1.8	4.55	3.66	43	105
2.34	21.7	20.3	3.2	2.6	5.41	4.68	26	53
3.29	25.6	26.3	2.9	3.8	5,34	10.57	29	51
3.59	14	29	2.6	4.6	3.55	9.15	35	25

RR	Urinary Calcium		Urinary Ammonia		Urinary Phosphate		Plasma Insulin	
Time	NP	HP	NP	HP	NP	HP	NP	HP
hr.	µg/hr	µg/hr	µg/hr	µg/hr	µg/hr	µg/hr	µg/hr	µg/hr
0	90.7	51	6.3	7.6	18.7	23.1	21	12
0.9	130	55	25	24.1	12	60	12	44
2	130	99.4	12.2	34.3	52	47.6	19	24
2.98	72	86.7	3.8	28.3	43	37.1	25	13.5
4.15	24	45	3.1	51	43	56.7	15	18.6

PB	Urinary Calcium		Urinary Ammonia		Urinary Phosphate		Plasma Insulin	
Time	NP	HP	NP	HP	NP	HP	NP	HP
hr.	µg/hr	µg/hr	µg/hr	µg/hr	µg/hr	µg/hr	µg/hr	µg/hr
0	5.36	36.4	0.84	0.59	5.9	23.5	21	11
1.5	4.43	25	0.36	1.41	1.91	21.3	38	37
2.5	6.97	21.73	2.62	1.55	13.49	18.5	29	59
3.8	10.21	33	1.89	1.76	59.98	21	11	25
4.9	6.96	40	1.14	1.65	7.86	27.2	8	12

DW	Urinary Calcium		Urinary Ammonia		Urinary Phosphate		Plasma Insulin	
Time	NP	HP	NP	HP	NP	HP	NP	HP
hr.	µg/hr	µg/hr	µg/hr	µg/hr	µg/hr	µg/hr	µg/hr	µg/hr
0	61	13	4.85	4.8	3.2	4.5	17.1	18
1.05	84	20	5.8	5.28	4.7	8.7	24	52.8
2.4	7.3	42.2	2	5.76	3.34	22.4	18	24
3.53	13.6	47.6	4.6	1.9	12.3	30.4	19.5	21
4.4	15.8	45	4	4.56	19.22	27.5	7.5	19.5
5.3	20.1	62.4	4.1	3.94	18.7	29.8	6.3	40

W.T'H	Urinary Calcium		Urinary Ammonia		Urinary Phosphate		Plasma Insulin	
Time	NP	HP	NP	HP	NP	HP	NP	HP
hr.	µg/hr	µg/hr	µg/hr	µg/hr	µg/hr	µg/hr	µg/hr	µg/hr
0	53	88	4.98	15.8	0.65	24	13.5	21
1.03	99.8	108	8.63	17.9	3.86	19.7	34.2	29
2.33	80.6	135	20.4	32.9	22	31	21	30
3.16	88.5	120	10.2	34.6	52	27.7	18.6	22.5
4.16	52	125	3.6	24.7	44.7	18	2.6	16.5

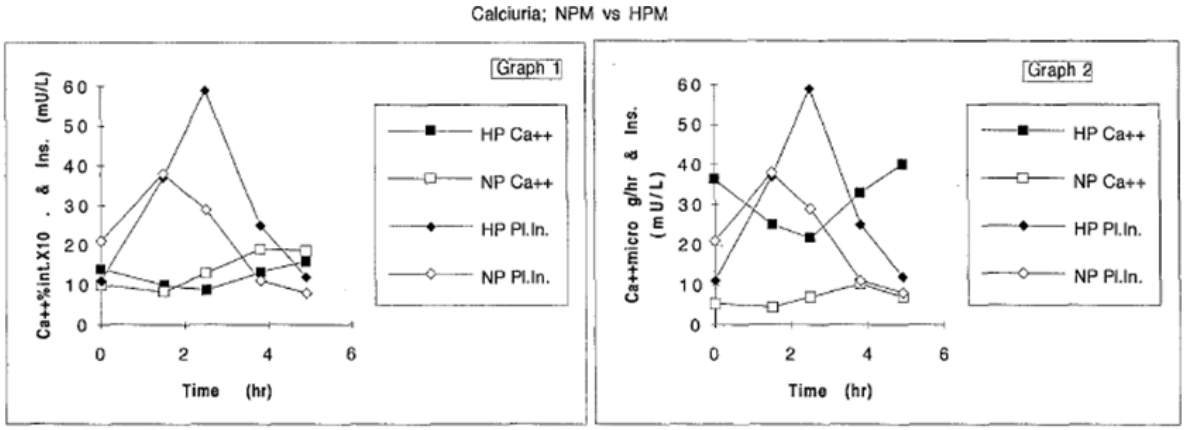
JT	Urinary Calcium		Urinary Ammonia		Urinary Phosphate		Plasma Insulin	
Time	NP	HP	NP	HP	NP	HP	NP	HP
hr.	µg/hr	µg/hr	µg/hr	µg/hr	µg/hr	µg/hr	µg/hr	µg/hr
0	29.6	14	3.8	9.6	22.1	0.55	19.5	15
1.08	69.1	18.5	2.45	23	45	4.75	34.2	11
2.4	55.6	18.3	5.6	36.5	47	1.63	30.9	32
3.4	105	30.5	1.6	17.9	52.6	7.33	48	18
4.28	95.8	36.9	4.75	14.6	45	10.55	24	13

Urinary excretion rates of calcium. ammonia and phosphate are shown for each collection time together with the corresponding Plasma insulin levels. Results of each subject are shown separately.

3. Results

The excretion rate for Ca, total Nitrogen, ammonia, urea and creatinine was calculated for each hourly clearance period by multiplying the volume of urine collected by the concentration of each substance then dividing by the clearance time; the time between sample collections

Although the fasting calcium excretion rate varied considerably the rate of calcium excretion increased after both the high protein (HP) meal and normal protein (NP) meal for all but one subject who showed a sustained decrease after a high protein meal. All subjects showed increased ammonia, urea and phosphate excretion following all meals refer to Table 2. Because of the different starting levels of calcium excretion the difference in the effect of protein content of the meals on postprandial calciuria was most clearly illustrated by showing the calcium excretion for each clearance period as a percentage of the initial excretion rate.

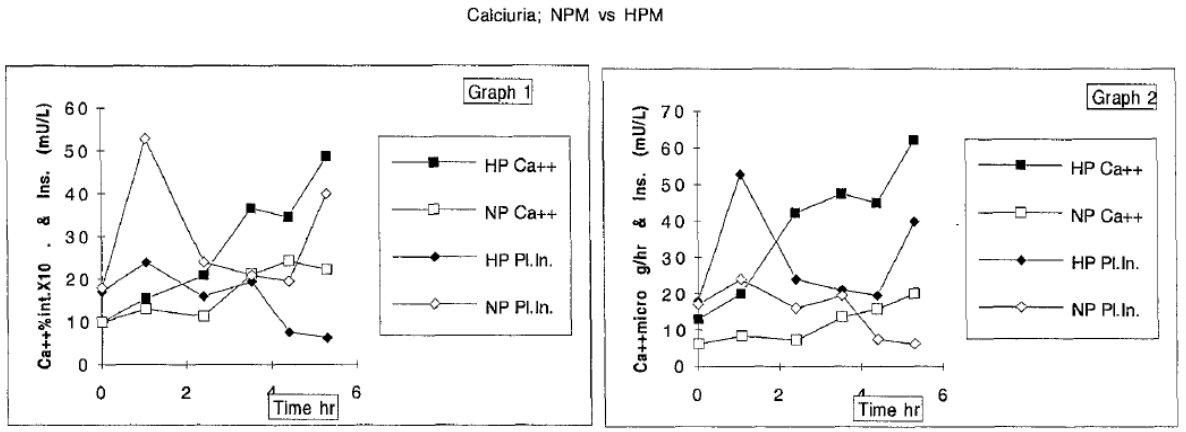


P.B. Time hr	Urine Ca++ % of int.X10 HP Ca+	NP Ca+	Plasma Insulin mU/L HP Pl.In	NP Pl.In
0	14	10	11	21
1.5	10	8.26	37	38
2.5	8.7	13	59	29
3.8	13.2	19	25	11
4.9	16	18.6	12	8

P.B. Time hr.	Urine Ca++ micro g/hr HP Ca+	NP Ca+	Plasma Insulin mU/L HP Pl.In	NP Pl.In
0	36.4	5.36	11	21
1.5	25	4.43	37	38
2.5	21.73	6.97	59	29
3.8	33	10.21	25	11
4.9	40	6.96	12	8

Figure 1. Postprandial Calciuria and Insulinemia

On the first graph for subject P.B. the postprandial excretion rates of urinary calcium are shown as percentages of the initial rates of excretion for each collection time after the high protein meats (HP Ca++) and after the normal protein meats (NP Ca++) and the corresponding plasma insulin values in mU/L following the HP and LP meats as (HP PI In.) and (NP PI In.) respectively are displayed as well. On the second group calcium excretion rates are shown as ug/hr for each collection time.

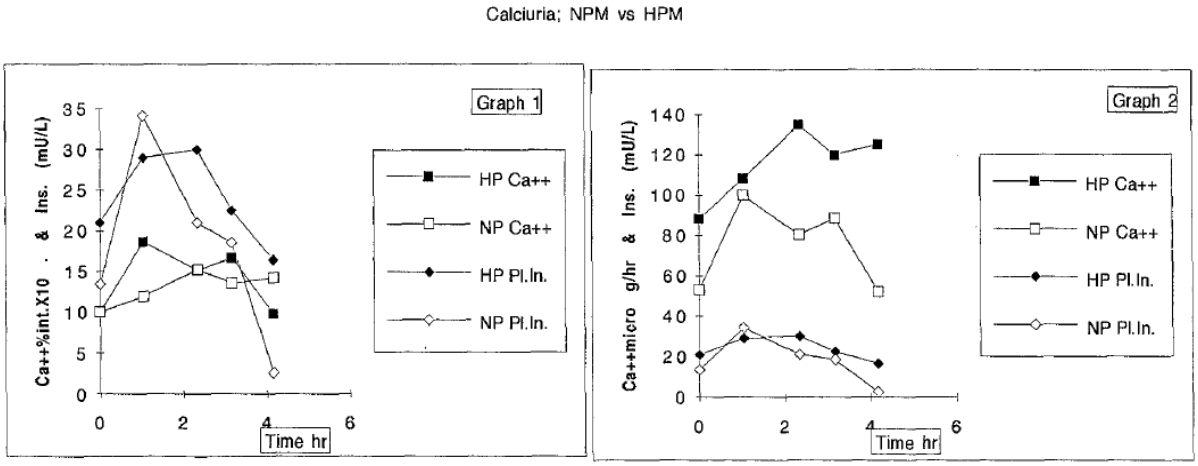


D.W. Time hr	Urine Ca++ % of int./10 HP Ca+	NP Ca+	Plasma Insulin mU/L HP Pl.In	NP Pl.In
0	10	10	17.1	18
1.05	15.5	13	24	52.8
2.4	20.9	11.3	16	24
3.53	36.6	21.2	19.5	21
4.4	34.6	24.2	7.5	19.5
5.3	48.8	22.3	6.3	40

D.W. Time hr.	Urine Ca++ micro g/hr HP Ca+	NP Ca+	Plasma Insulin mU/L HP Pl.In	NP Pl.In
0	13	6.1	18	17.1
1.05	20	8.4	52.8	24
2.4	42.2	7.3	24	16
3.53	47.6	13.6	21	19.5
4.4	45	15.8	19.5	7.5
5.3	62.4	20.1	40	6.3

Figure 2. Postprandial Calciuria and Insulinemia

On the first graph for subject D.W. the postprandial excretion rates of urinary calcium are shown as percentages of the initial rate of excretion for each collection time after the high protein meats (HP Ca++) and after the normal protein meats (NP Ca++) and the corresponding plasma insulin values in mU/L following the HP and LP meats as (HP PI In.) and (NP PI In.) respectively are displayed as well. On the second group calcium excretion rates are shown as ug/hr for each collection time.

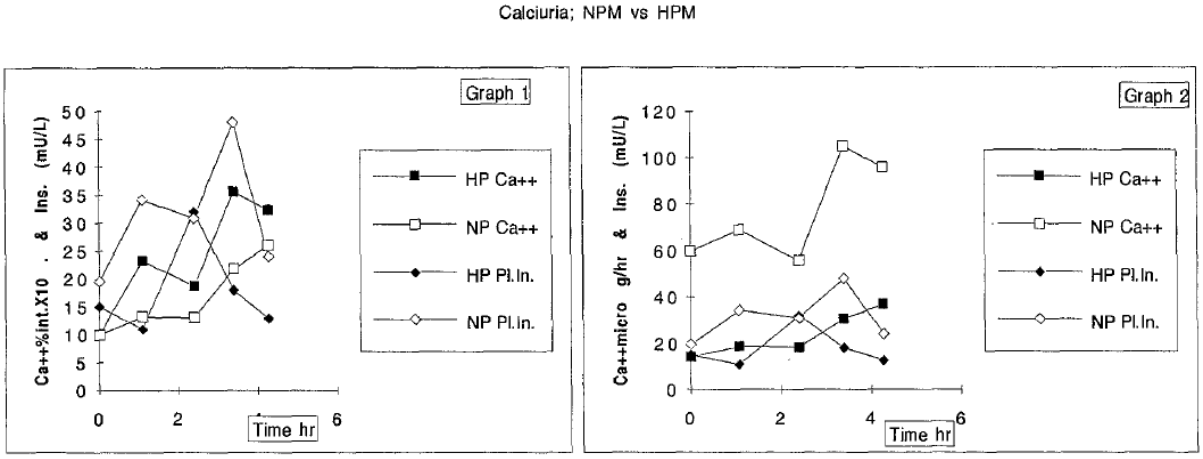


W.T'H. Time hr	Urine Ca++ % of int./10 HP Ca+	NP Ca+	Plasma Insulin mU/L HP Pl.In	NP Pl.In
0	10	10	21	13.5
1.03	18.7	12	29	34.2
2.33	15.2	15.3	30	21
3.16	16.7	13.6	22.5	18.6
4.16	9.8	14.2	16.5	2.6

W.T'H Time hr.	Urine Ca++ micro g/hr HP Ca+	NP Ca+	Plasma Insulin mU/L HP Pl.In	NP Pl.In
0	88	53	21	13.5
1.03	108	99.8	29	34.2
2.33	135	80.6	30	21
3.16	120	88.5	22.5	18.6
4.16	125	52	16.5	2.6

Figure 3. Postprandial Calciuria and Insulinemia

On the first graph tor subject W. T'H. the postprandial excretion rates of urinary calcium are shown as percentages of the initial rate of excretion for each collection time after the high protein mea/s (HP Ca++) and after the normal protein mea/s (NP Ca++) and the corresponding plasma insulin values in mU/L following the HP and LP mea/s as (HP PI In.) and (NP PI In.) respectively are displayed as well. On the second graph calcium excretion rates are shown as ug/hr for each collection time.



J.T. Time hr	Urine Ca++ % of int./10 HP Ca+	NP Ca+	Plasma Insulin mU/L HP Pl.In	NP Pl.In
0	10	10	15	19.5
1.08	23.3	13.2	11	34.2
2.4	18.8	13.1	32	30.9
3.4	35.6	21.8	18	48
4.28	32.4	26.1	13	24

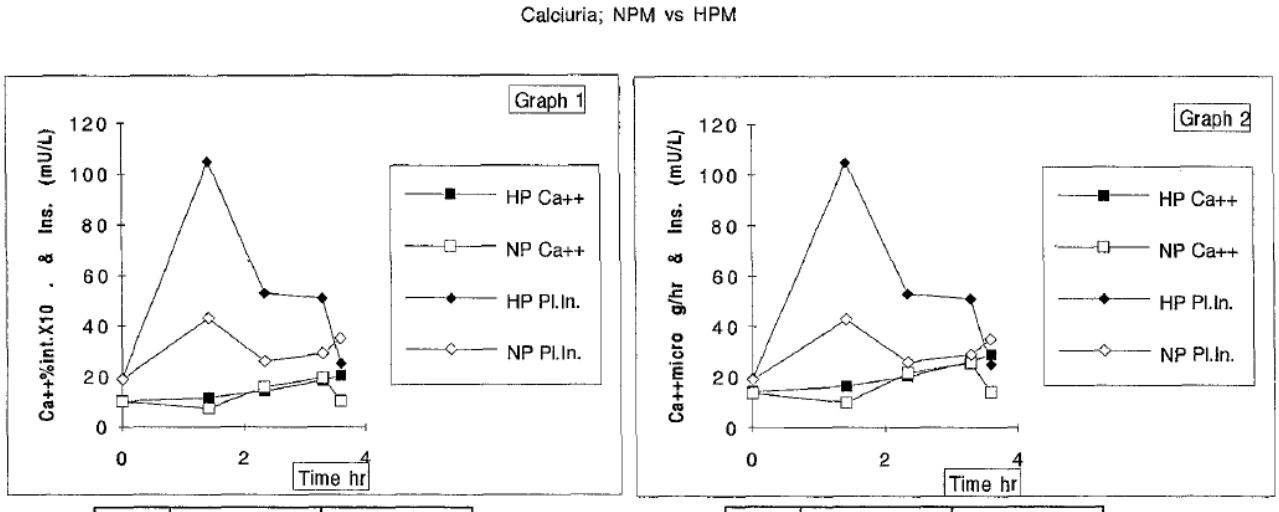
J.T. Time hr.	Urine Ca++ micro g/hr HP Ca+	NP Ca+	Plasma Insulin mU/L HP Pl.In	NP Pl.In
0	14	59.6	15	19.5
1.08	18.5	69.1	11	34.2
2.4	18.3	55.6	32	30.9
3.4	30.5	105	18	48
4.28	36.9	95.8	13	24

Figure 4. Postprandial Calciuria and Insulinemia

On the first graph tor subject J. T. the postprandial excretion rates of urinary calcium are shown as percentages of the initial rate of excretion for each collection time after the high protein mea/s (HP Ca++) and after the normal protein mea/s (NP Ca++) and the corresponding plasma insulin values in mU/L following the HP and LP mea/s as (HP PI In.) and (NP PI In.) respectively are displayed as well. On the second graph calcium excretion rates are shown as ug/hr for each collection time.

Figure 1 to Figure 7; see [25] Brazier BW (2016), show both the percentage change and absolute amounts of urinary calcium excretion rate and plasma insulin secretion. Each subject is identified by their initials PB, OW. etc. The average difference between the excretion rate after the high protein meal and the excretion rate after the normal protein meal each hour is shown in Figure 8.

This shows that on average the rate of calcium excretion is greater after the HP meal than the NP meal i.e. in Figure 8 the average difference in excreted calcium is always more than zero. However at the 1.5 hr. time period the average calcium excretion rate difference is less than at other times. This drop in the excretion rate of calcium corresponds with a high in the average plasma insulin level that is also shown on Figure 8.

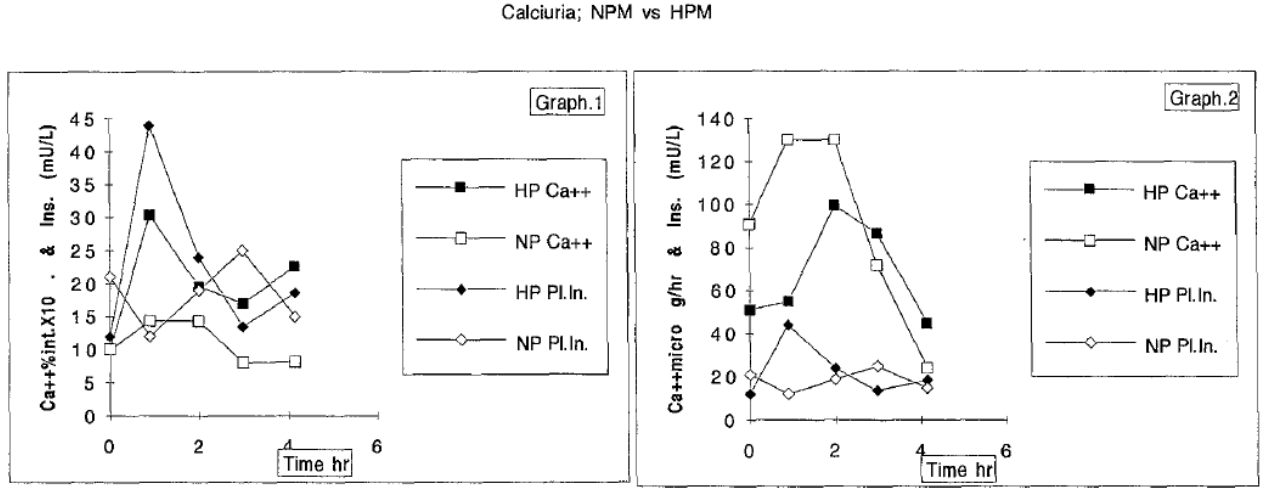


DO Time hr	Urine Ca++ % of int./10		Plasma Insulin mU/L	
	HP Ca+	NP Ca+	HP Pl.In	NP Pl.In.
0	10	10	19.5	19
1.42	11.6	7.3	105	43
2.34	14.2	15.8	53	26
3.29	18.4	19.4	51	29
3.59	20.3	10.2	25	35

D.O. Time hr.	Urine Ca++ micro g/hr		Plasma Insulin mU/L	
	HP Ca+	NP Ca+	HP Pl.In	NP Pl.In.
0	14.3	13.7	19.5	19
1.42	16.6	10.1	105	43
2.34	20.3	21.7	53	26
3.29	26.3	25.6	51	29
3.59	29	14	25	35

Figure 5. Postprandial Calciuria and Insulinemia

On the first graph for subject D. O. the postprandial excretion rates of urinary calcium are shown as percentages of the initial rate of excretion for each collection time after the high protein mea/s (HP Ca++) and after the normal protein mea/s (NP Ca++) and the corresponding plasma insulin values in mU/L following the HP and LP mea/s as (HP PI In.) and (NP PI In.) respectively are displayed as well. On the second graph calcium excretion rates are shown as ug/hr for each collection time.



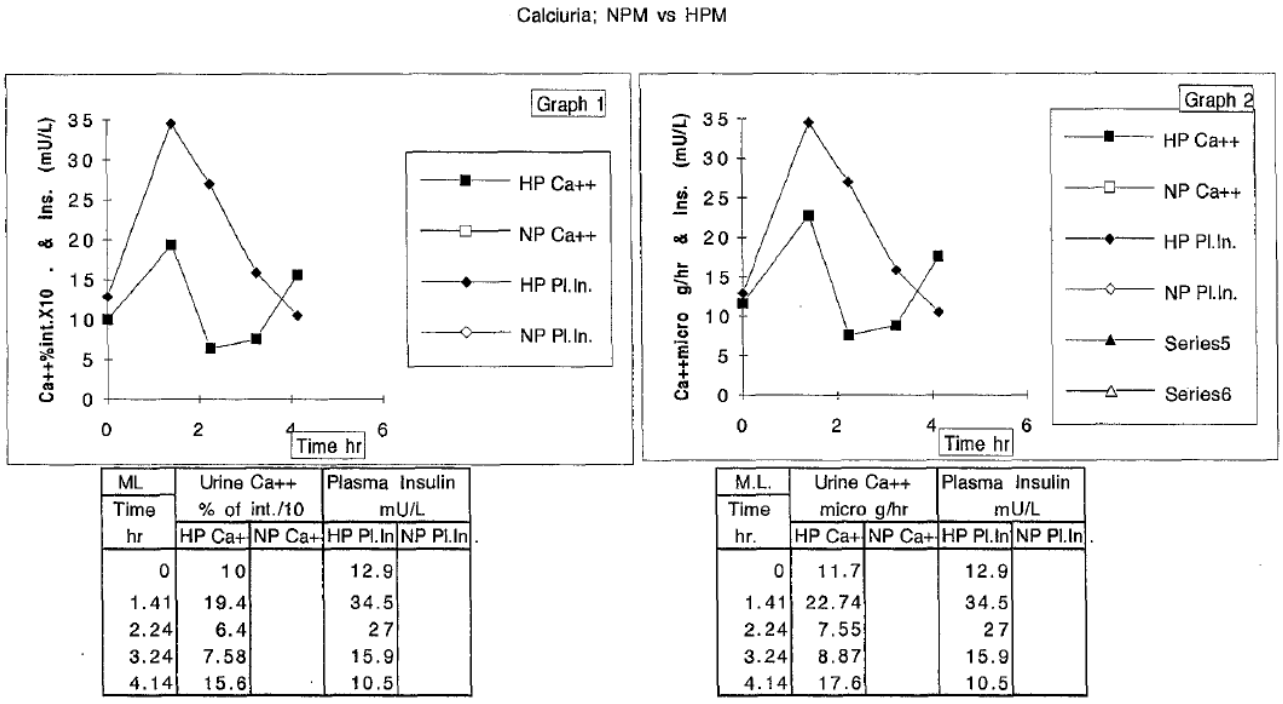
R.R. Time (hr)	Urine Ca++ % of int./10		Plasma Insulin mU/L	
	HP Ca+	NP Ca+	HP Pl.In	NP Pl.In.
0	10	10	12	21
0.9	30.4	14.4	44	12
2	19.5	14.4	24	19
2.98	17	8	13.5	25
4.15	22.6	8.2	18.6	15

R.R. Time (hr.)	Urine Ca++ micro g/hr		Plasma Insulin mU/L	
	HP Ca+	NP Ca+	HP Pl.In	NP Pl.In.
0	51	90.7	12	21
0.9	55	130	44	12
2	99.4	130	24	19
2.98	86.7	72	13.5	25
4.15	45	24	18.6	15

Figure 6. Postprandial Calciuria and Insulinemia

On the first graph for subject R. R. the postprandial excretion rates of urinary calcium are shown as percentages of the initial rate of excretion for each collection time after the high protein mea/s (HP Ca++) and after the normal protein mea/s (NP Ca++) and the corresponding plasma insulin values in mU/L following the HP and LP mea/s as (HP PI In.) and (NP PI In.) respectively are displayed as well. On the second graph calcium excretion rates are shown as ug/hr for each collection time.

For the overall results the standard error for calcium excretion is very large because there is considerable variation between individuals. Four of the six subjects showed substantial increases in calciuria whereas three subjects show little increase in calciuria. In fact one showed a decrease in calciuria following the high protein meal. In Figure 1 the results for representatives of the two groups are shown. DO shows that a high plasma insulin resulted in less calciuria and the individual DW with low plasma insulin showed a high level of calciuria.



ML Time hr	Urine Ca++ % of int./10 HP Ca+	NP Ca+	Plasma Insulin mU/L HP Pl.In	NP Pl.In
0	10		12.9	
1.41	19.4		34.5	
2.24	6.4		27	
3.24	7.58		15.9	
4.14	15.6		10.5	

M.L. Time hr.	Urine Ca++ micro g/hr HP Ca+	NP Ca+	Plasma Insulin mU/L HP Pl.In	NP Pl.In
0	11.7		12.9	
1.41	22.74		34.5	
2.24	7.55		27	
3.24	8.87		15.9	
4.14	17.6		10.5	

Figure 7. Postprandial Calciuria and Insulinemia

On the first graph for subject M. L. the postprandial excretion rates of urinary calcium are shown as percentages of the initial rate of excretion for each collection time after the high protein mea/s (HP Ca++) and after the normal protein mea/s (NP Ca++) and the corresponding plasma insulin values in mU/L following the HP and LP mea/s as (HP PI In.) and (NP PI In.) respectively are displayed as well. On the second graph calcium excretion rates are shown as ug/hr for each collection time.

Figure 9 shows that in four out of six cases the total calcium excretion was greater after the HP meals than the NP meals. One case (DO) who showed a very small level of calciuria after both HP and NP meals exhibited exaggerated plasma insulin levels. Subjects WT'H and RR. who both showed exaggerated total calcium excretion had suppressed insulin secretions.

The average total calcium excretion for the six subjects and average total plasma insulin results are shown in Figure 1. Total excreted calcium by each subject is obtained by multiplying the excretion rate for each clearance period by the clearance time then adding together the amounts excreted for all clearance times. A value for total insulin secretion is obtained by adding the plasma insulin level for all the collections from one subject during the experiment. The total calcium excreted and insulin secreted is then averaged over the six subjects. The high protein meal is seen to have definitely greater calciuric response. However the average insulin output is only slightly greater for the HP meals compared to the NP meals.

It can be observed that in subjects who showed large plasma insulin rise the calcium excretion rate was suppressed at the time of maximum plasma insulin concentrations. The calcium excretion often increased after the insulinemia declined. Two subjects DO and PB showed no hypercalciuria following high protein meals and one of these (PB) showed reduced calciuria.

The one subject (PB) (Figure 1) who showed a decline in calcium excretion following the high protein meal also showed an exaggerated insulinemia. Both of these subjects and one other (DO) who showed less calciuria following high protein meal than normal protein meal had much greater plasma insulin levels following the high protein meal than the normal protein meal and both had maximum plasma insulin concentrations twice as high as the other subjects (> 100 vs <50).

The composition of blood plasma showed no significant changes for sodium, potassium, chloride, creatinine urate, calcium, phosphate, albumin, total phosphate or alkaline phosphatase. Plasma glucose, urea and insulin increased following all meals. The plasma urea concentration increase was greater following the high protein meal than the normal protein meal.

4. Discussion

The greater calciuria following high protein meals identified by [13] Allen (1981), [14] Woods (1983) and [15] Howe (1990) were confirmed in five out of six cases. However there seems to be a strong indication that insulin may have an effect that can greatly modify the hypercalciuria of dietary protein. [26] Gollaher et. al. (1984); [14] Wood and Allen (1983) and [15] Howe (1990) reported that the higher plasma insulin responses following high protein meals correlated with the high dietary protein induced hypercalciuria. The results in this experiment do not appear to support the claims of these workers. In fact when looking at the individual results of each subject it appears that when a subject produces a high plasma insulin response the calciuria is markedly reduced.

This is indicated in Figure 9 for subjects DB, DW and DO. The Subjects with low plasma insulin levels (WT'H, JT and RR) had the most exaggerated levels of calciuria.

These results suggest that insulin may have a suppressing effect on calciuria which is the opposite effect suggested by [14] Wood and Allen (1983) and [15] Howe (1990). This difference is revealed by looking at the average results at each clearance time (Figure 8) and the

individual results as per Figure 9 instead of relying on overall averages like those shown in Figure 1. This later type of data was used by other workers and tends to mask the acute relationships by averaging results over time and between subjects who respond differently to each other.

The results reported in this experiment show that postprandial hypercalciuria is likely to be the major form of calcium loss from the body. This is because most people consume three meals a day at least four hours apart so that they are in a postprandial condition for 12 out of 24 hours a day. The average calciuria for all postprandial clearance times and all subjects involved in this Experiment is 137% greater than their fasting urinary calcium level.

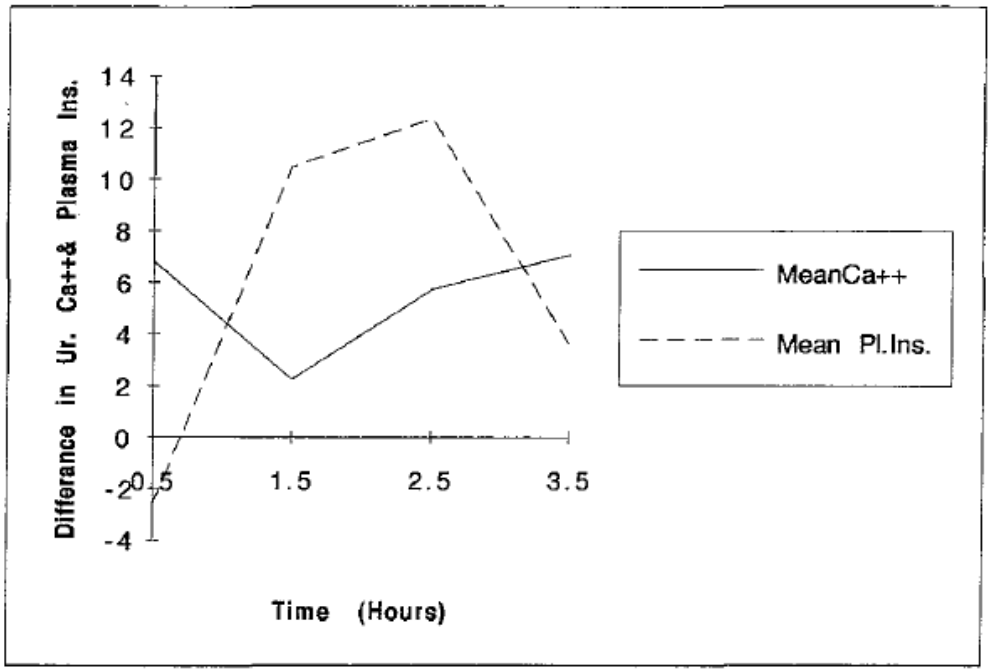


Figure 8

Time	0.5	1.5	2.5	3.5
MeanCa++	6.82833	2.25833	5.75	7.11667
Standard E	2.22137	2.17319	3.43742	2.42053
Mean Pl.In	-2.6	10.5167	12.4	3.58333
Standard E	2.60768	11.6319	5.22303	7.41891

Figure 8. Total Calcium Excretion after NP and HP meals by Each Subject In Relation To Plasma Insulin Secretion

The average differences in postprandial hypercalciuria (ug/hr) after HP meals compared to after normal protein (NP) meals is shown for each collection time after consuming the receptive meals. The mean plasma insulin levels are also shown for each corresponding collection time.

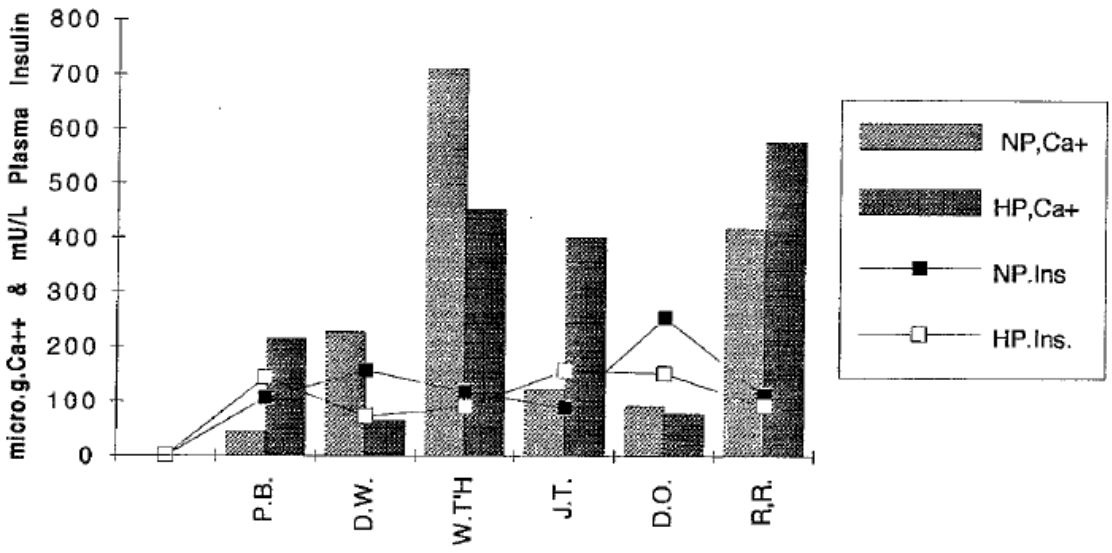


Figure 9. Total Calcium Excretion after NP and HP meals by Each Subject In Relation To Plasma Insulin Secretion

Total Ca^{++}ion excretion (microgram) after NP and HP meals by each subject is shown together with their corresponding total postprandial plasma insulin values. (Mu/l) The unusually high insulin production levels of subject D.O. is seen to correspond with a much reduced level of calciuria and the opposite situation is seen in regard to subjects W.T'T. and R.R.

If this were repeated three times a day we would lose 37% more calcium each day then during our overnight fasting period. For two subjects the average percentage rise in calciuria after the meals was 260 and 240% above their fasting level.

The fasting level of calciuria is largely determined by the plasma levels of parathyroid hormone and active Vitamin D. These levels of hormones are slow to respond to stimuli and have little effect on postprandial responses. [10] Schuette et. al. (1980); [6] Allen et. al. (1979) found no change in circulatory levels of immunoreactive parathyroid hormone, dihydroxycholicalciferol or cyclic AMP over a study period of several days involving high and low protein diets and calciuria. [7] Hegsted and Linkswiler (1981) found no change in circulating cAMP throughout similar six day studies.

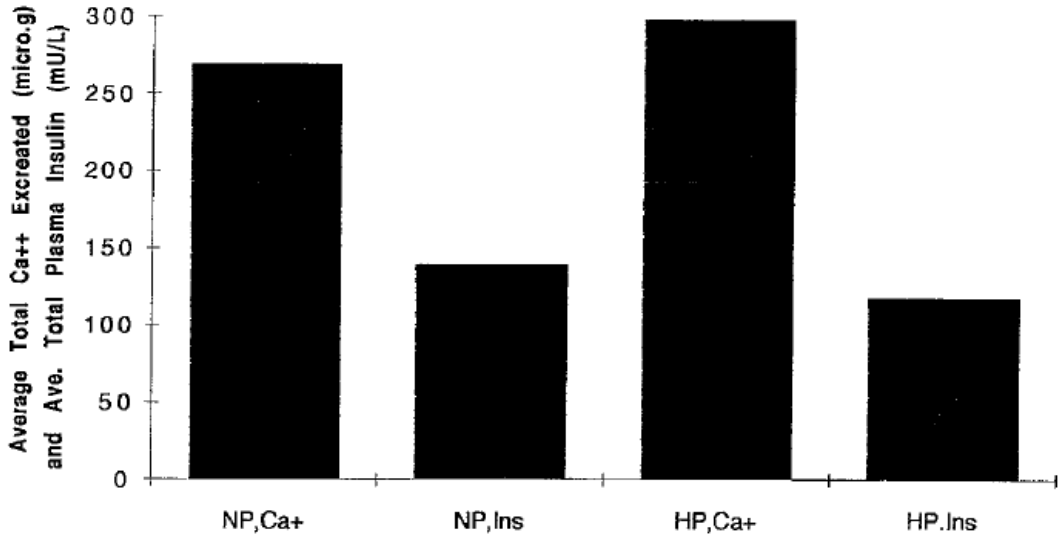
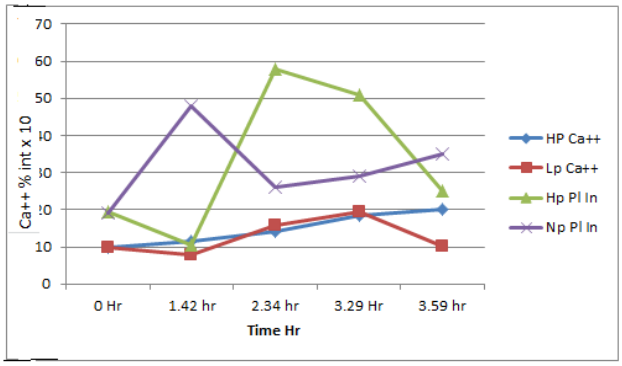
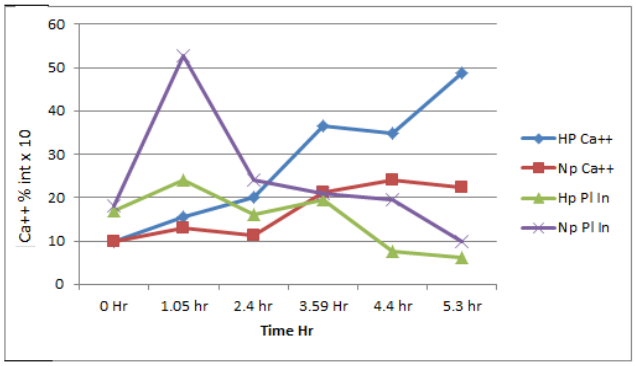


Figure 10. Average Total Calcium Excretion and Plasma Insulin Secretion After NP and HP Meals

This graph shows the average postprandial total calciuria (microgram) after HP meals compared to after normal protein (NP) meals. This is the total of calcium collected over all collection time after consuming the respective meals. The average total postprandial plasma insulin (mU/L) secretions are also shown after each corresponding meal type.



DO	Urine Ca++		Plasma Insulin	
Time	% of int/10		mU/L	
Hr	HP Ca++	LPCa++	HP Pl In	NP P lIn
0	10	10	19.5	19
1.42	11.6	7.8	10.5	48
2.34	14.2	15.8	58	26
3.29	18.4	19.4	51	29
3.59	20.2	10.2	25	35



DW	Urine Ca++		Plasma Insulin	
Time	% of int/10		mU/L	
Hr	HP Ca++	LPCa++	HP Pl In	NP P lIn
0	10	10	17.1	18
1.05	15.5	13	24	52.8
2.4	20	11.3	16	24
3.59	36.6	21.2	19.5	21
4.4	34.8	24.2	7.5	19.5
5.3	48.8	22.3	6.3	10

Figure 11. On the two graphs DO and DW the postpradial excretion rates of urinary Calcium are shown as percentages of the initial rate of excretion for each time period after the high protein meal (HP Ca++) and after the normal protein meal (Lp Ca++) and the corresponding plasma insulin values in mU/L following both HP (HP PI In) and NP (Np PI In) meals

As most studies on the calciuric effect of dietary protein have observed no change in plasma PTH and DHCC and that insulin is the only hormone that shows significant change and also appears to have some effect on the calciuric response. Further examination of the effect of insulin is included in further experiments by at this laboratory. This proposed effect of insulin could explain the different bone density observed in patients with type I and type II diabetes

The effect of insulin could be directly on the renal tubular epithelial cells modifying calcium ion reabsorption or it could act on peripheral tissues to reduce catabolism of amino acids and reduce sulphate production and metabolism acidosis. However the plasma urea concentration showed steady increases following high protein meals even for the subject who exhibited a sustained decrease in calciuria and higher than normal

insulinemia. The effect of insulin of tubular calcium exchange is examined in a further experiment.

The meals were made isocalorific by adding extra fat to the normal protein meal in the form of safflower oil and some extra glucose. Lactose was added to the normal protein meal to make both meals equal in lactose content because lactose is reported to help calcium absorption from the intestine.

The extra fat in the normal protein meal may lower the calcium absorption from the intestine and change the calcium excretion results this problem will be examined in another future separate experiment.

References

[1] Abelow, B.J., Holford, T.R and lnsogna, KL. (1992) 'Cross-cultural association between dietary animal protein and hip fracture: a hypothesis', Calcif.Tissue Int., 50(1): 14.

[2] Kaneko, K, Masaki, U., Aikyo, M., Yabuki, K, Haga, A, Matoba, c., Sasaki, H. and Koike, G. (1990) 'Urinary calcium and calcium balance in young women affected by high protein diet of soy protein isolate and adding sulfur containing amino acids and/or potassium', J.Nut.Sci.Vitaminol., Tokyo, 36(2): 105-16.

[3] Fernandez-Repollet, E., Van Loon, P. and Martinez-Maldonado, M. (1989) 'Renal and systemic effects of short-term high protein feeding in normal rats', Am.J.Med.Sci., 297(6): 348-454.

[4] Funaba, M., Yano, H. and Kawashima, R (1989) 'Effects of high protein diet and sodium bicarbonate supplementation on calcium metabolism in rats', J.Nur.Sci.Vitaminol., Tokyo, 35(5): 495-503.

[5] Schneider, W. and Menden, E. (1988) 'The effect of long term increased protein administration on mineral metabolism and kidney function in the rat. Il. Kidney function and bone mineralization', Z-Ernahrungswiss., 27(3): 186-200.

[6] Allen, L.H., Oddoye, E.A and Margen, S. (1979), 'Protein-induced hypercalciuria: a longer term study', American Journal of Clinical Nutrition, 32: 742-49.

[7] Hegsted, M. and Linkswiler, H.M. (1981) 'Long-term effects of level of protein intake on calcium metabolism in young adult women', J.Nutr., 111(2): 244-5l.

[8] Zemel, M.B., Schuette, S.A, Hegsted, M. and :~inkswiler, H.M. (1981) 'Role of the sulfur- containing amino acids in protein-induced hypercalciuria in men', J.Nut., 111(3): 545- 52.

[9] Lutz, J. and Linkswiler, H.M. (1981) 'Calcium metabolism in postmenopausal and osteoporotic women consuming two levels of dietary protein', Am.J.Clin.Nut., 34(10); 2178-86.

[10] Schuette, S.A, Zemel, M.B. and Linkswiler, H.M. (1980) 'Studies on the mechanism of protein-induced hypercalciuria in older men and women', J.Nut., 110(2): 305-15.

[11] Rhoades, Rand Pflanzer, R (1989) Human Physiology, Saunders, Philadelphia

[12] Nissim, I. and States, B. (1989) 'Ammoniagenesis by cultural human renal cortical epithelial cells: study with 15N [see comments], Am.J.Physiol., 256(1 Pt 2): F187-96.

[13] Allen, L.H., Block, G.D., Wood, Rl. and Bryce, G.F. (1981), 'The role of insulin and parathyroid hormone in the protein induced calciuria of man', Nut.Res., 1981, 1: 3-11.

[14] Wood, RJ. and Allen, L.H. (1983) 'Evidence for insulin involvement in arginine and glucose- induced hypercalciuria in the rat', J.Nutr., 113(8): 1561-67.

[15] Howe, J.C. (1990) 'Postprandial response of calcium metabolism in postmenopausal women to meals varying in protein level/source', Metabolism, 39(12): 1246-52.

[16] Leidid-Buckne,G and Ziegler, R (2001) 'Diabetes mellitus a risk for osteoporosis?'. Experimental and Clinical Endocrinology & Diabetes 109 (suppl 2): 493-514.

[17] Osteoporosis Australia (2014),'Diabetes and osteoporosis Consumer Factsheet', 1st Edition 08/14

[18] Floyd, JC Jr. SS Fajans, JW.Conn, RF Knopf and J. Rull, (1966) 'Stimulation of Insulin Secretion by Amino Acids', J of Clinical Investigation 45 (9): 1487-1502

[19] Scales, F.M. and Harrison, AP. (1920) 'Boric acid modification of Kjeldahl Method for crops and soils analysis', J.Ind.Eng.Chem., 12: 350-52.

[20] Chaney, AL. and Marbach, AP. (1962) 'Modified reagents for determination of urea and ammonia', Clin.Chem., 8(2): 130-32.

[21] Willis, J.B. (1960) 'Determination of calcium in blood serum by atomic absorption spectroscopy', Nature, 186: 249-50.

[22] Hare, RS. (1950) 'Endogenous creatinine in serum and urine',Proc.Soc.Exp.BioI.Med., 74: 148.

[23] Fiske, CH; Subbarow, Y. (1925) 'The colorimetric determination of phosphorus', J.Biol.Chem., 66: 373-400.

[24] Maths worksheet (2015) 'How to Calculate Standard Error'. http://ncalculators.com/math-worksheets/calculate-standard-error.htm.

[25] Brazier, BW., (2016a) 'Calciuric effect of dietary protein data' http://barry-bwb2.tripod.com/proten_calcium_data.htm.

[26] Gollaher, CR., Wood, RJ., Holl, M. and Allen, L.H. (1984) A comparison of amino acid- induced hypercalciuria in sham-operated and parathyroidectomized rats, In.Nut., 114(3) 622-6.