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Effect of Serum Insulin on Calciuria Following Protein Meals in Humans

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Abstract Postprandial calciuric responses to normal protein meals is examined and urinary, calcium, creatinine, and phosphate are compared with plasma insulin levels. Group result show that the average postprandial calciuria varied greatly from individual to individual. The calciuria in every case showed a reduction 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. Graphs produced show a strong inverse relationship between insulin and calciuria. Discussion is provide regarding the significance of and possible mechanisms of plasma insulin's effect on calciuria.

Keywords: *calciuria, prortein, insulin, osteoporosis, diabetes*

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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).

The increased calcium loss appears to result from increased glomerular filtration rate (GFR) and a reduced fractional renal tubular reabsorption rate (FR)[4] (Schuette et. al., 1980, [5]Zemel et. al., 1981; [6] Hegsted and Linkswiler, 1981). Zemel et. al. (1981) [5], 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.

[7] Allen et. al. (1981), [8] Wood (1983) and [9] 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 [4] (Schuette, et. al. 1980).

The involvement of insulin and calciuria may be significant as indicated by several report of the difference in occurance of osteoporosis in subjects with type I and type II diabetes mellitus. Eg, [10] 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). [11]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.

Experiments at this laboratory looking at the hypercalciuric effect of high protein diets revealed considerable variation between individuals in regard to the calciuric response. These variations showed an relationship with the postprandial plasma insulin responses. This experiment aims to take a more detailed look at the correlation of plams insulin and postprandial calciuria with all individuals consuming similar meals

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.

Normal protein meals were chosen because it has been shown that greater plasma insulin levels are produced followed protein meals than the non-protein meals by Floyd *et. al.*(1966) [12].

2. Method

2.1. Subjects

In this experiment ten subjects commenced the experiments. There were nine female and one male

volunteer aged between 20 and 22, Caucasian nutrition students. All appeared healthy and presented no histories of diabetes mellitis or any other diseases. Subjects signed informed consent forms and were fully briefed in regard to the nature of the experiment. They were all apparently fit and normally consumed omnivorous diets. They were all of average build with average weight for height. The experiment was approved by both the RMH and Melbourne University Medical School ethics committees.

2.2. Meals

The meals were prepared by mixing ingredient into non fat cottage cheese to provide compositions as per Table B1 The composition of the meals was analysed for calcium, sodium and Mg by AA, phosphate by the phosphomolybdate method [13] Fiske and Subbarrow (1925) and protein by Kjeldahl method [14] Scales and Harris, (1920).

Table 1. Meal Composition

Ingredient	g/meal
Cottage cheese[3]	23.5
Fat	11
Sucrose	10
Corn starch	4
Lactose	6.4
Glucose monohydrate	1.4
Sodium chloride	0.76
Phosphoric acid, conc.	1.03
Calcium phosphate	

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 Calcium was measured by atomic absorption [15] (Willis, 1960), creatinine by the method of Hare (1950) [16] and phosphate by the photometric molybdate reduction method of [17] 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.

Two meal were tested for each individual and Ca /creatinine values were calculated for each clearance period, A table was prepared to show the max Ca/Cr ratio and the corresponding plasma insulin value. as well as the maximum plasma value and its corresponding Ca/Cr reading.

A graph was prepared showing Plasma insulin vs Ca/Cr and a second graph showing the inverse of the Plasma insulin vs Ca/Cr Correlation coefficients were calculated for each graph.

Calculation of Standard Correlation coefficient:

Results were recorded on a Microsoft Excel spreadsheet and the Excel facilities used to draw the graphs and calculate the Pearson' Correlation Coefficients

3. Results

The excretion rate for Ca, 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

Rate of excretion was measured as the ratio of urinary calcium or urinary phosphate to creatinine this is more accurate as the measurement of urine volume each void is not very accurate due to incomplete emptying of bladder or loss due to subject error. Using this method the fasting values were closer together for each subject and so absolute values can be used rather than percentage changes.

Table 2. Calcium Excretion vs Insulinemia

Subjects	PS				M B				R de B			
Meal No	1	2	1	2	1	2	1	2	1	2	1	2
[In] mU/L	10	5	15	22	58	12	10	10	100	35	15	18.
Ca/Cr x 10	28	70.1	32	37	43	70	95	40	17	52	75	50

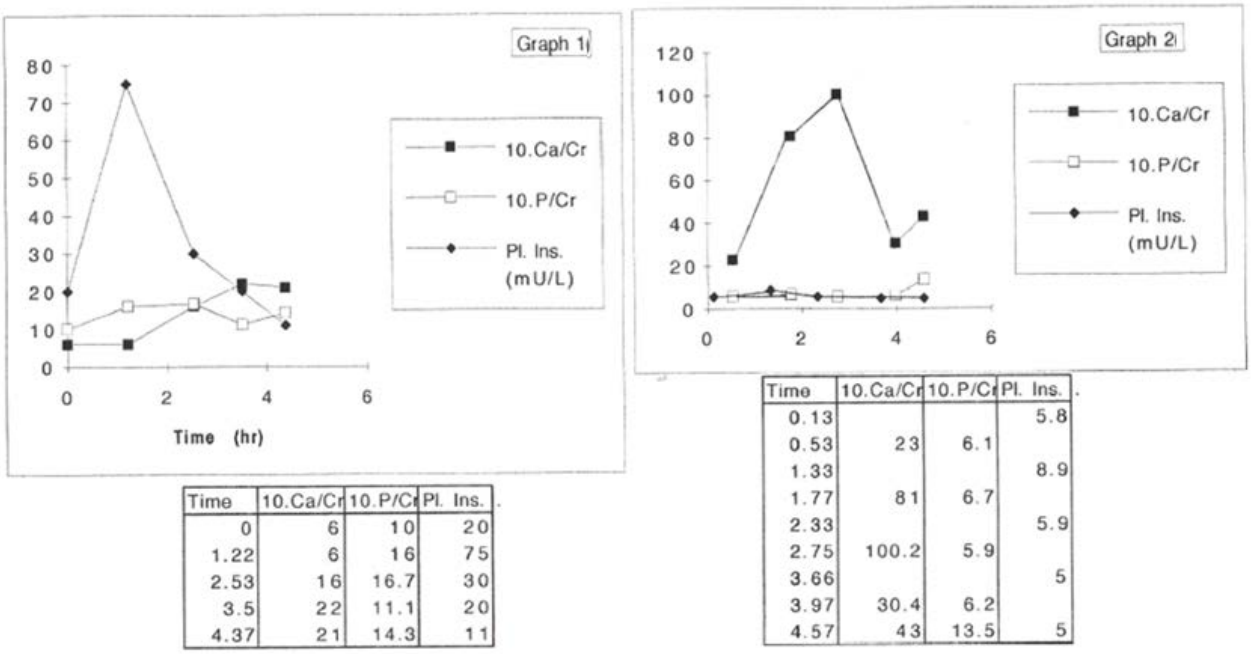
Subject	MC				SC				DO			
Meal No	1	2	1	2	1	2	1	2	1	2	1	2
[In] mU/L	30	43	22	27	107	17	65	8	75	5	22	4
Ca/Cr x 10	10	23	37	38	20	22	22	27	8	80	20	100

Subjects	AG				PW				AS				RR			
Meal No	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2
[In] mU/L	100	40	20	5	100	20	30	3	35	8	50	15	50	45	120	10
Ca/Cr x 10	30	65	40	120	5	65	45	100	12	60	5	40	45	50	5	18

Plasma insulin (In) is showed in mU/L for each a peak insulin levels with the corresponding the urinary calcium /creatinine ratio. Also each peak urinary calcium /creatinine is shown with its corresponding the plasma insulin level. These results are shown of Figure 2 and Figure 3.

Some individuals showed substantial difference between their two meal and between each other subjects. There was however a clear pattern in regard to insulinemia and renal calcium excretion rate. When insulin showed a peak plasma the urinary calcium /creatinine ratio was low and when urinary calcium /creatinine showed a peak the plasma insulin levels were low as shown in Table 2 and Figure 2 and Figure 3.

The values in Table 2 are taken from Figures 3 to 12; see [17] Brazier BW (2016), that show the change of urinary calcium excretion rate and plasma insulin secretion. Each subject is identified by their initials PS, PW. etc.



Time	10.Ca/Cr	10.P/Cr	Pl. Ins.
0	6	10	20
1.22	6	16	75
2.53	16	16.7	30
3.5	22	11.1	20
4.37	21	14.3	11

Time	10.Ca/Cr	10.P/Cr	Pl. Ins.
0.13			5.8
0.53	23	6.1	
1.33			8.9
1.77	81	6.7	
2.33			5.9
2.75	100.2	5.9	
3.66			5
3.97	30.4	6.2	
4.57	43	13.5	5

Figure 1. is displayed here as an example of the correlation of plasma insulin and calciuria

Figure 1 Shows Plasma Insulin (Pl Ins) values in mU/L at each collection time and the corresponding Ca/Creatine ratios and corresponding Phosphate/Creatine for subject PW.

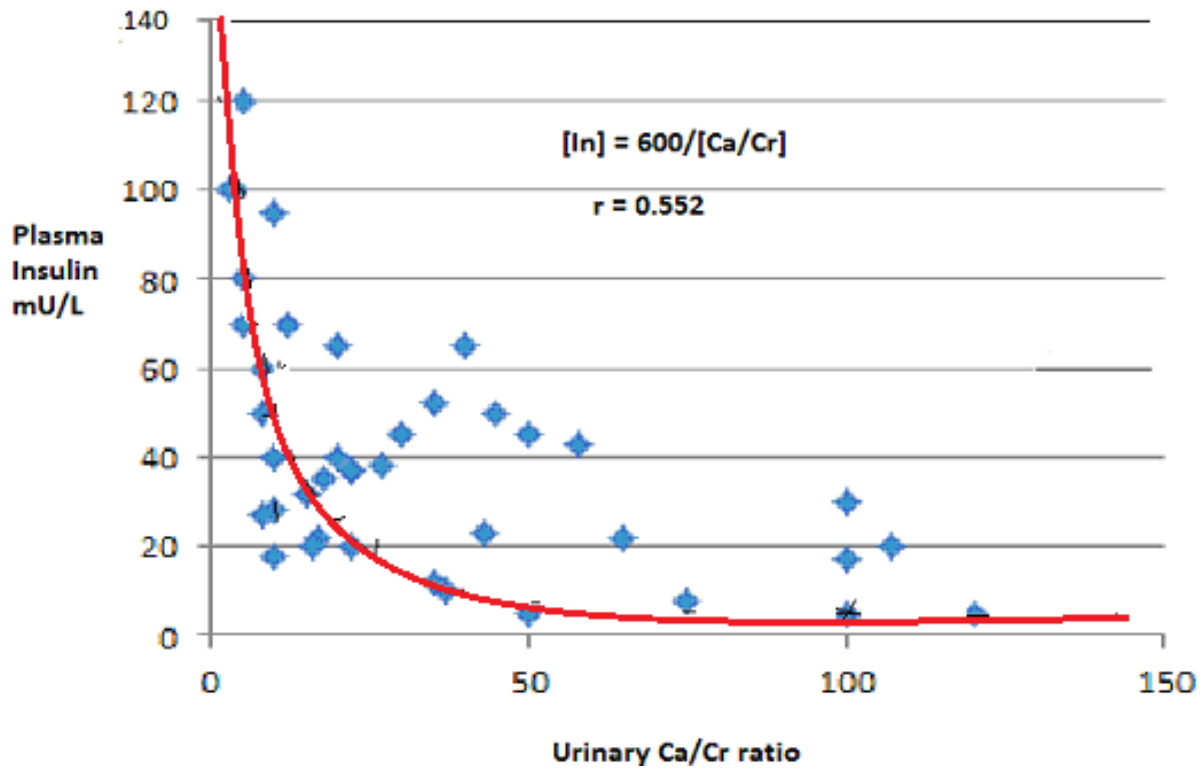


Figure 2. Plasma Insulin vs Urinary Ca/Cr ratios

Figure 2 shows the Plasma Insulin concentrations in mU/L and the corresponding Urinary Ca/Cr ratios. The Pearson's correlation coefficient for the graph in Figure 1 is r = -0.552 which indicates there is a definite inverse relationship. The Line of best fit is drawn using points calculated from points on the line of best fit from Figure 3.

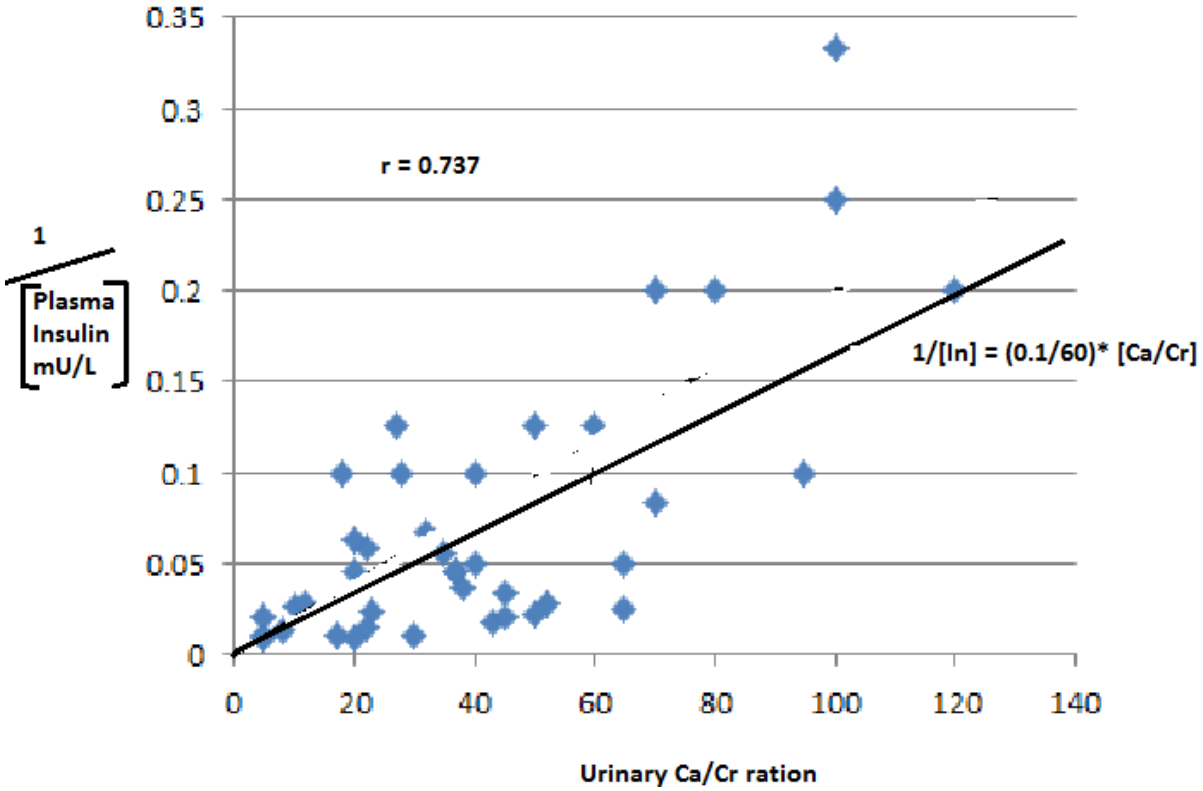


Figure 3. shows the reciprocal of the plasma insulin concentration vs the corresponding urinary Ca/Creatine ratios

The graph has a Pearson's Correlation Coefficient of r = 0.737. The line of best fit is drawn in by hand and use to calculate the points used on Figure 1 to draw its hyperbolic line of best fit. Both Figure 1 and Figure 2 indicate a strong inverse relationship of Plasma insulin and calciuria.

It can be observed that in subjects who showed large plasma insulin values the corresponding calcium excretion rate was suppressed at the time of maximum plasma insulin concentrations. The calcium excretion often increased after the insulinemia declined.

4. Discussion

There is shown in these results a strong indication that insulin may have an effect that can greatly modify the hypercalciuria. [18] Gollaher et. al. (1984); [8] Wood and Allen (1983) and [9] 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.

These results show that insulin has a suppressing effect on calciuria which is the opposite effect suggested by [8] Wood and Allen (1983} and [9] Howe (1990). This is shown clearly in Figure 1 and Figure 2. Reduced calcium excretion can be caused by reduced glomerular filtration rate or by increased fractional reabsorption. The effect of insulin on fractional reabsorption is a subject of a further study at this laboratory which looks at the effect of insulin on membrane transport of Ca in isolated renal tubules and an additional study looks at possible biochemical causes this insulin effect.

The observation that the level of calciuria following a meal has an inverse relationship to plasma insulin means that it could be used as a non-invasive method to measure insulin insensitivity.

There was a considerable difference in insulin responses between individuals in this and the experiment at this laboratory looking at the hypercalciuric response of high protein meals. [19] Brazier (2016b) and also the study of the effect of dietary fat on calciuria, [All subjects in these tests showed normal GTTs and no sigh of diabetes or pre-diabetes. This indicates that these young individuals were all able to regulate plasma glucose at normal levels but some had to produce more insulin to achieve this norm.

It is possible that some of these individuals who produce large insulin responses may have developed an early stage of insulin resistance and were what could be called "pre-pre-diabetics". If Type II diabetes is caused by a build-up of amyloid deposits on insulin sensitive tissues it may be that the build-up starts in early life. May be the precursor amyloid oligomers are acquired by epigenetic means

A measure of the calciuric response to protein meals could be used as an early stage screening tool for people who have a family history of type II diabetes.

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