American Journal of Food and Nutrition, 2016, Vol. 4, No. 2, 55-62
Available online at http://pubs.sciepub.com/ajfn/4/2/4

DOI:10.12691/ajfn-4-2-4

Effect of the Level of Dietary Fat and Fat Type on Postprandial Calciuria and Involvement of Insulin

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Abstract Postprandial calciuric responses to high and low protein meals are usually involves making meals isocalorific by adding extra fat to the low protein meals. In this study the effect of high fat meals and the type of fat on calciuria is examined to evaluate any effect it can have on evaluating protein induced hypercalciuria. Changes in urinary calcium, creatinine and phosphate are compared with serum calcium, creatinine, urea, protein and insulin. Group result show that the average postprandial calciuria was affected by the high fat meals compared to low fat meals and the type of fat had little effect. 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 individuals with exaggerated insulinemia responses showed reduced hypercalciuria. Discussion is providing regarding the significance of the insulin in regard to hypercalciuria and possible mechanisms for it cause. A suggestion is made that diet induced calciuria could be used as a non-invasive measure of insulin insensitivity.

Keywords: *hypercalciuria. Lipids, insulin*

Cite This Article: B.W. Brazier, "Effect of the Level of Dietary Fat and Fat Type on Postprandial Calciuria and Involvement of Insulin." *American Journal of Food and Nutrition*, vol. 4, no. 2 (2016): 55-62. doi: 10.12691/ajfn-4-2-4.

1. Introduction

When studying the calciuric effect of high dietary protein (HP) the meals are usually made isocaloric by adding edible oils and some glucose to the low protein meals [1] BW. Brazier (2016) and [2] Alien *et. al.,* (1979); [3] Spencer *et. al,* (1978a); [4] Kerstetter and Allen, (1990) while keeping other ingredients constant. The study of the effect of high protein diets is important because of the reported hypercalciuric effect of HP meals and the danger that hypercalciuria could contribute to nephrolithiasis [5] Maalouf (201) and osteoporosis [7] Stefania S et.al. (2008). Nephrolithiasis, with a population incidence of up to 13%, results in significant morbidity and economic costs from medical treatment and time loss from work [6} Kevin K. F and. Bushinsky (2002). In addition to osteoporosis which can cause economic loss and misery.

The effect of high protein and high fat diets are particularly significant these days because of the continued popularity of high protein diets such as those promoted by Robert Atkins ,[8] Grant, C,(2002) and the 'The CSIRO Total Wellbeing Diet, [9] Noakes M, and Clifton, P, (2005), This study of the effect of high fat diets is not only relevant in regard to interpreting the studies of the effect of high protein diets but also it is relevant in its own right because the HP diets promoted by Atkins and others are also high fat diets and all aim to reduced carbohydrate intake.

Wood et al. (1984) [10] showed with rats that an increase in amino acid content of their diet produce a linear relationship in regard to renal calcium excretion but there was no change when an the low protein meals were made isocalorific with lipid or glucose. However there has been some evidence that lipids can change calcium uptake and excretion. It is one of the inevitable consequences of nutritional research that one component of a diet cannot be altered without affecting at least one other item.

Changing the fat content of a meal may modify the apparent effect of the protein induced changes by at least two different mechanisms. Hypercalciuria can be classified as either absorptive or renal [11] Santos *et. al.* (1987), that is, in normal individuals a dietary induced calciuric change can result from changing either intestinal absorption or renal handling of calcium. In the later handling of calcium can be changed by affecting the glomerular filtration rate (GFR) or the fractional reabsorption (FR). There appears to be little published work on the effect of dietary lipid on any of these parameters on calciuria.

Individuals with fat malabsorption have been found to excrete higher levels of faecal calcium and less in calciuria [12] Pike and Brown (1984); [13] Lange (1979). Pike and Brown reported however that diets ranging from 1 % to 38% fat and equal calcium contents have shown equal calcium balances in healthy individuals. But this balance could have been achieved by changing calciuria. Lange (1979) [13] suggest that high fat diets could influence calcium balance by forming insoluble fatty acid/calcium complexes in the gut lumen.

Potter *et al*. (1990) [14] has shown in man that calcium increases faecal loss of fatty acids by forming insoluble

calcium complexes and [15] Appleton (1991) has shown that increased dietary calcium reduces FFA in faeces by 37%. The fatty acid complexing property of calcium has been used to explain the role that calcium has in reducing colo-rectal cancer, i.e., by combining with fatty acid anions, calcium stops fatty acids soaps acting as mitogens [16] Wargovich *et. al* (1991); and [17] Lapre *et. al* (1993); These reports indicate that as calcium complexes with fatty acids and it follows that increased dietary fatty may reduce the bioavailability of calcium particularly in the high pH environment of the small intestine and could thereby reduce absorption of calcium.

The second option is that dietary fat could have an effect on renal handling of calcium as well as intestinal absorption. This could be due to lipids reported action on the renin angiotensin system or it could alter the insulin response to dietary carbohydrates or amino acids. In the renin angiotensin system, dietary polyunsaturated fat have been shown to increase renal prostaglandins PGE_2 and PGE_3 by [18]Fische *et. al* (1988). These prostaglandins stimulate cAMP in renal vascular tissue [19] Schlondorff *et. al.*, (1980) and thereby reducing GFR.

Dietary fat could influence calcium excretion by a second completely independent method via its involvement with insulin release. Although it is known that triglycerides do not stimulate insulin release they are known to be a potent secretagogue of gastric inhibitory polypeptide (GIP) [20] Ebert and Creutzfeldt, (1978). GIP in turn is known to be a potentiator of insulin secretion increasing the effect of glucose so that meals with fat and glucose produce higher plasma insulin levels than dietary glucose alone [21] Scratchard, (1979). However the potentiating effect of GIP does not appear to assist arginine stimulation of insulin, [22] Lerner (1978). This could mean that dietary proteins stimulation of insulin may not be influenced by fat. Also, [23] O'Dea (1981) found no change to plasma insulin response when fat was included with carbohydrate or protein meals although fat did increase the postprandial GIP when taken with glucose.

The insulin that may be produced in greater quantities by a high fat diet could act directly on glomerular epithelial cells to influence FR as indicated in by a previous experiment in this laboratory [1] Brazier (2016) and [24] Brazier (2016b) or indirectly by influencing the Renin-Angiotensin System to reduce GFR. [25] Beretta-Piccoli (1980) has suggested an insulin-potassium-aldosterone axis that could have a physiological relevance. Insulin enhances potassium uptake into intracellular spaces causing lower plasma potassium levels. Lower potassium levels then gives rise to reduced aldosterone production thus reduced sodium reabsorption and producing a rise in renin output, angiotensin II and hence reduced GFR. In this experiment studies are conducted to try and detect the effect of dietary fat on calciuria and the possible complications this could have in studies of protein induced hypercalciuria.

In Part A of this experiment the calciuric response of high fat meals is compared with the calciuric response of low fat meals. In Part B low protein meals made with high fat composed of saturated fat are compared with high fat meals made with unsaturated fat to see the type of fat can change the calciuric effect.

In the first experiment (Part A) seven subjects were fed high fat meals (48%) HFM and low fat meals (6%) LFM. Urine samples are collected each half hour for three hours after the meal and analysed for calcium, ammonia and urea.

The second experiment (Part B) meals were similar to those described in experiments used to measure the effect of high and low proteins meals [1] Brazier (2016) . In this experiment the subjects consuming normal protein meals composed with a high liquid fat (USFM) or high solid fat (SFM) content. Samples of urine and blood were collected at hourly intervals from a half hours before and one half hour after the meal and each additional hour and analysed for calcium, phosphate, creatinine, blood samples were also analysed for insulin and a range of blood constituents.

2. Method (PART A)

2.1. Subjects

Seven active healthy male individuals ranging in age from 21 to 24 volunteered for this test. All had normal body mass indexes. No restriction was placed on their eating habits on the day prior to the experiment however they were requested to maintain their normal dietary behaviour. Consent forms were required and subjects were advised of the nature of the experiment and told that they could leave the experiment at any time if they wished.

2.2. The Meals

Two breakfast-type meals were designed using food composition tables [26] Thomas and Corden, (1977) and analysed with the diet analysis computer program; Foodzone (2013). [27] The two meals contain the same levels of protein (15.1 % of energy) and were approximately isocalorific 3.7 MJ and 3.6 MJ the high fat meal contained 48.8% fat and 36.1 % carbohydrate whereas the low fat meal contained 6% fat and 78.6% carbohydrate.

The meals were composed of normal food materials that free living people could consume for breakfast in real life rather than the semi synthetic meals usually used in many experiments. Diet analysis and meal composition are provided in Table A1 and Table A2. Subjects consumed each meal seven days apart and in random order.

Table M1. Computer Analysis of High Fat Meal

Nutrient	Quantity	Unit
Protein	32.5	g
Fat	46.6	g
Carbohydrate	77.4	g
Energy	3.6	Mj
Calcium	470	mg
Iron	5.5	mg
Sodium	1036.9	mg
□carotene equivalents	457.4	µg
Retinol	568.9	µg
Retinol equivalents	645.1	µg
Thiamine	710.6	µg
Riboflavin	1.5	mg
Niacin	5.5	mg
Niacin equivalents	10.9	mg
Ascorbic Acid	2.5	mg
Alcohol	0	g
Phosphorus	578	Mg

Energy Source

Protein	15.1%
Fat	48.8%
Carbohydrate	*36.1%*

Table M1 Food in High Fat Meal Composition		
Quantity (g)		Food
33		Cornflakes
250		Milk (fluid whole)
14		Margarine (table)
100		Crumpet
100		Egg (fried)
20		Cheddar Cheese (packet and sliced)

Table M1 Computer Analysis of Meals

Results of computer analysis are shown and the food used in the high fat meals used in Experiment Part A. The program from [27] Foodzone (2013) referred the food to the Table of Composition of Australian Foods (Thomas and Corden, 1971[26]) using the food codes given

Table M2. Computer Analysis of Low Fat Meal

Nutrient	Quantity	Unit
Protein	35.8	g
Fat	6.2	g
Carbohydrate	183.2	g
Energy	3.7	Mj
Calcium	423.1	mg
Iron	6.4	mg
Sodium	1326.3	mg
βcarotene equivalents	838.7	µg
Retinol	8	µg
Retinol equivalents	147.8	µg
Thiamine	1007.4	µg
Riboflavin	1.4	mg
Niacin	12.9	mg
Niacin equivalents	18.8	mg
Ascorbic Acid	147.9	mg
Alcohol	0	g
Phosphorus	504	mg

Energy Source

Protein	15.3%
Fat	6%
Carbohydrate	78.6%

Quantity (g)		Food
40		Tuna (canned in water)
33		Cornflakes
50		Bread (White)
250		Milk (fluid skimmed)
250		Orange Juice (fresh)
175		Banana (ripe commercial variety)
90		Pears (raw)
50		Rye Crispbread

Table M2 Computer Analysis of Low Fat Meal

Results of computer analysis are shown and the food used in the low fat meals in Experiment Part A. The analysis used a diet analysis program by [27] Foodzone (2013) which referred the food to the Table of Composition of Australian Foods by [26] Thomas and Corden (1971) using the Food Codes given.

2.3. Sample Collection

Subjects were required to empty their bladder on awakening to discard that urine, note the time and then drink approximately 250 cm^3 of water. On arrival at the Rusden laboratory subjects provided a fasting urine sample and drank 250 cm^3 of water before eating the meal. A urine sample was collected and another 250 cm^3 of water was drunk half an hour after the meal and each subsequent half hour for three hours.

Urine samples were measured for volume then acidified with one cm^3. of concentrated HCI and kept for analysis in sealed glass containers.

2.4. Analysis

Calcium concentration were analysed by atomic absorption spectrophotometry (AA) (AOAC) [28] Willis (1960), Ammonia and urea concentration were determined by the indophenol method of [29] Chaney and Marbach (1962).

2.5. Calculations

Rate of excretion of calcium was calculated by multiplying concentration of each substance by the volume of urine collected and dividing by the clearance time. Total excretion was obtained by adding the products of concentration and volume for each collection

3. Method (PART B)

3.1. Subjects

In this experiment ten subjects commenced the experiments ... There were four female and one male volunteers aged between 20 and 22. 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.

3.2. Meals

The meals were prepared by mixing ingredient into non-fat cottage cheese to provide compositions as per Table M3 and were similar to those used in Chapter Two except that one group contained hydrogenated vegetable fat instead of safflower oil. The composition of the meals was analysed for calcium, sodium and Mg by AA, phosphate by the phosphomolybdate method [30] Fiske and Subbarrow (1925) and protein by Kjeldahl method [31] Scales and Harris, (1920).

Table M3. Meal Composition (Experiment B)

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

Table M3 Meal Composition

This table shows the amount of each ingredient used to prepare meals for postprandial calciuria and insulinemia experiments in Pan B. The fat used was either hydrogenated vegetable oil (SFM) or safflower oil (USFM)

3.3. Sample Collection

Tests were conducted at Royal Melbourne Hospital Department of Medicine. Urine samples were collected by the same method as in part A. 2 ml aliquots of each sample of urine were placed in vials and forwarded to the RMH Biochemistry Department for analysis of calcium, creatinine, phosphate, by routine autoanalysis.

Subjects remained supine throughout the experiment except while self-collecting urine samples. A butterfly cannula was inserted into a cupital vein (and taped to the forearm) with a 10 ml plastic syringe attached. Samples of blood were withdrawn at hourly intervals, one before the meal and one half an hour after the meal and then one each hour for three hours. Samples were distributed between two heparinised viles and one fluoride vial the heparinised vials were immediately centrifuged to remove red blood cells then forwarded to RMH Biochemistry Laboratory for routine autoanalysis of plasma constituents and to the Endocrinology Laboratory for insulin analysis by routine radioimmunoassay. The fluoride vial of blood was used for blood glucose determination by the RMH Biochemistry Department.

4. Results (PART A)

Statistical analysis using student t- test of hypercalciuria following the high fat meal and low fat meal showed a significant difference between the two at only one time period. The thirty minute period showed significant difference at the 0.05 level, while all other time periods were not significant (see Table A1).

Table A1. Rate of Calcium Excretion (graph is Figure Al and Figure A4)

Time min	30	60	90	120	150	180
LFM mg/h	1.234	0.429	0.603	0.596	0.359	0.292
HFM mg/h	0.521	0.338	0.411	0.53	0.672	0.32
t test	2.35	0.604	1.03	0.3558	2.348	0.161
DF	10	12	12	10	6	4
Significance	0.05	no	no	no	no	no

Figure A1 shows the difference between the two meals at each time point. The average rate of calcium excretion in the low fat meal is more than twice that of the high fat meal, at the 30 minute at this point. However, calcium excretion levels out after this point remaining slightly higher in the low fat meal until the 120 minute the high fat meal produces slightly greater calcium excretion.

Figure A4 shows that the low fat meal produced greater total excretion of calcium (3.512 μg) than the high fat meal (2.8099 μg). This result is due largely to the difference between the two meals at thirty minutes. The total calcium excretion difference was not Significant between the two meals (0.05).

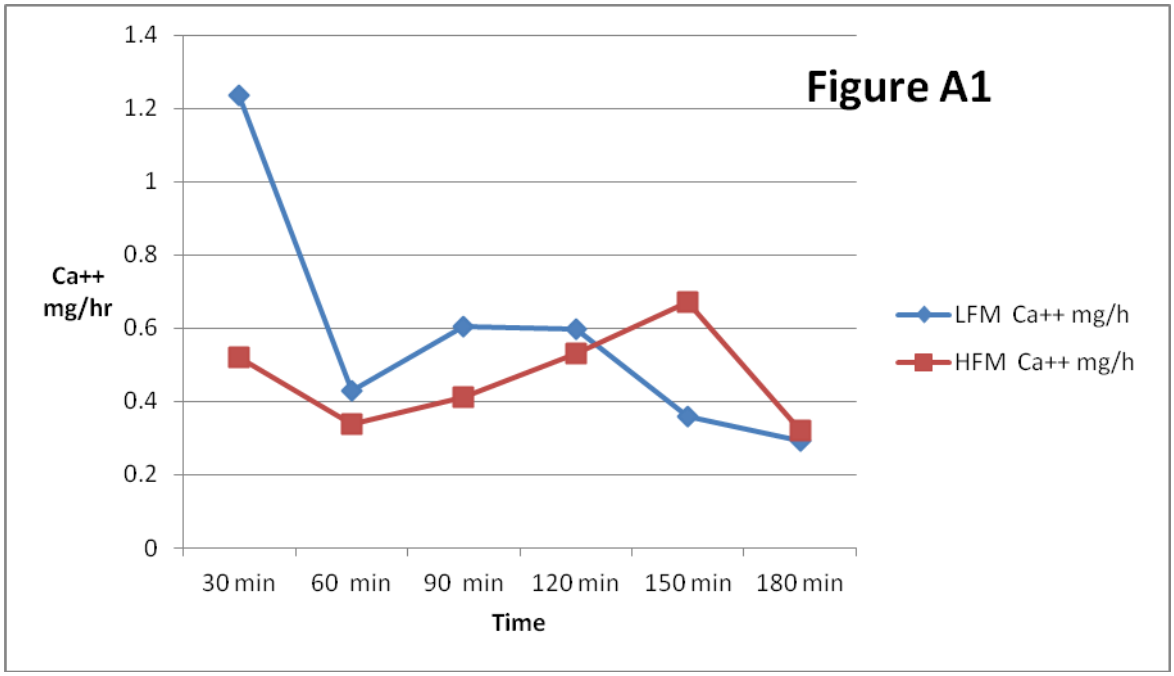


Figure A1. Rate of excretion of Calcium for high fat and low fat diets

Rate of excretion of calcium each time period t test are shown in Table A1 and indicate that only the 30 min time period shows significant difference.

The rate and amount of ammonia (NH3) excreted showed differences following high fat meal and low fat meal that were not statistically significant during any period. Table A2 and Figure A2 illustrates that the low fat meal produced slightly more total NH3 excretion than the high fat meal.

The test for urinary urea indicated that there was a higher level of protein catabolism after the low fat meal. Table A3 and Figure A3, Only two time periods failed to show a significant difference at either the 0.05 or 0.01 levels. Total urea output for the low fat meal was much greater than the high fat meal.

Table A2. Rate of Ammonia Excretion (graph is Figure A2)

Time min	30	60	90	120	150	180
LFM mg/h	1.107	1.1187	1.19	1.697	1.566	0.582
HFM mg/h	1.391	1.375	0.8424	1.16	1.29	0.763
t test	0.543	0.329				
DF	10	10	8	8	4	4
Significance	no	no	no	no	no	no

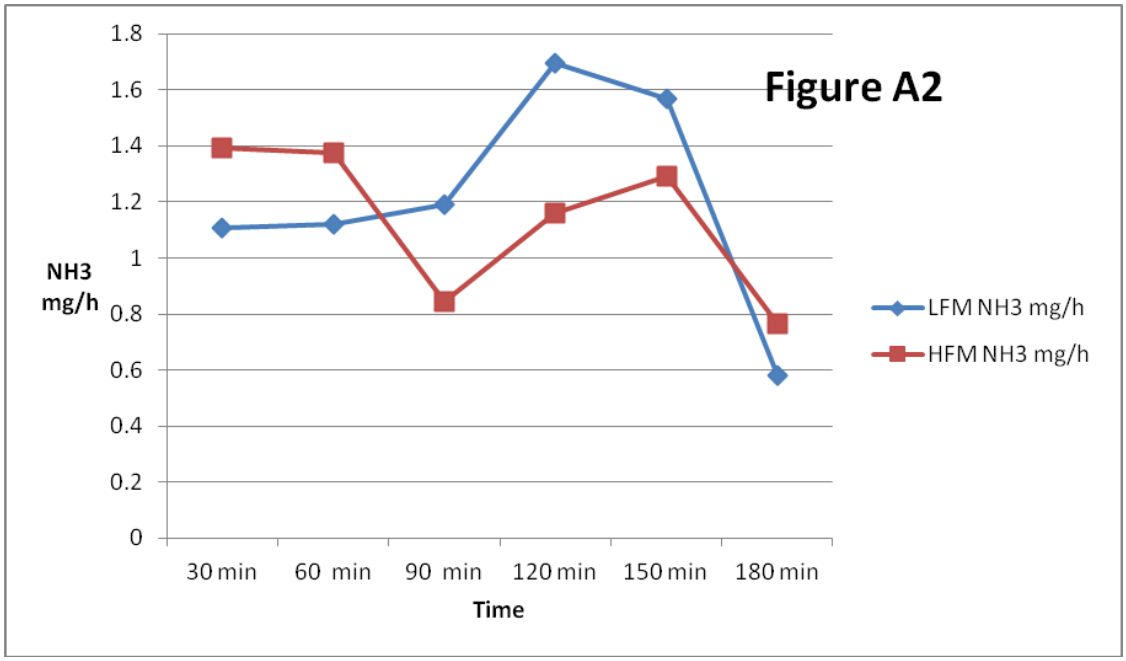


Figure A2. Rate of excretion of Ammonia for high fat and low fat diets

Rate of excretion of Ammonia each time period t test are shown in Table A2 and indicate that no time period shows a significant difference.

Table A3. Rate of Urea Excretion (graph Figure A3)

Time min	30	60	90	120	150	180
LFM mg/h	4.878	1.822	4.156	3.288	2.466	0.5112
HFM mg/h	0.60	0.662	0.06	0.894	0.85	0.29
t test	2.685	4.724 "	0.846	3.652	3.384	0.4506
DF	10	8	8	8	4	4
Significance	0.05	00.01	no	0.05	0.05	no

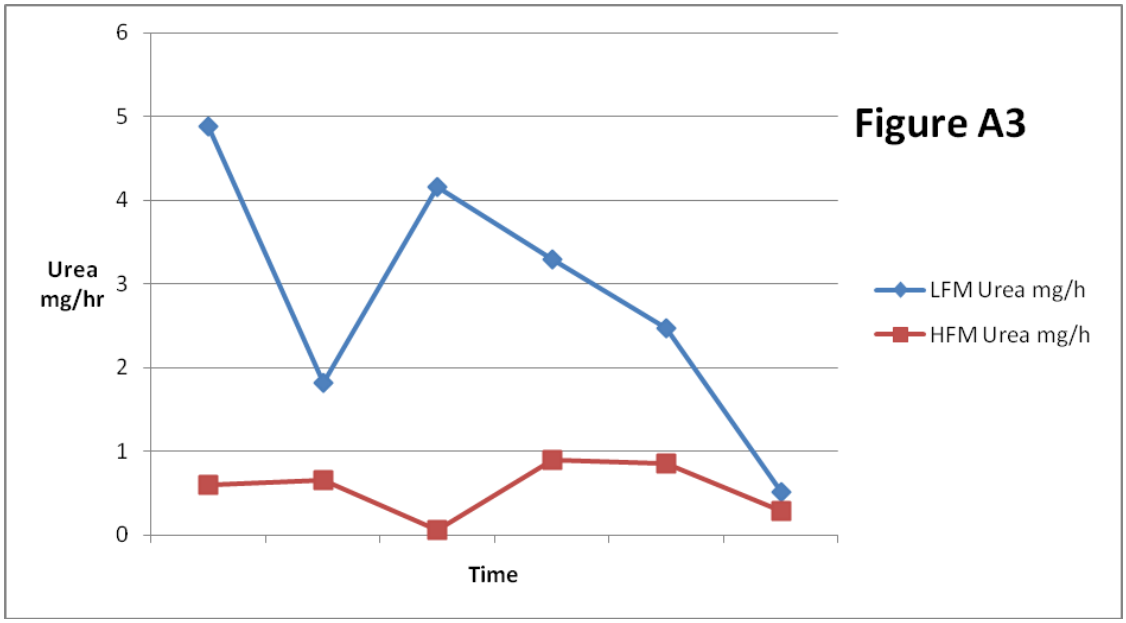


Figure A3. Rate of excretion of Urea for high fat and low fat diets

Rate of excretion of urea each time period t test are shown in Table A3 and indicate that only the 60 and 180 min time periods shows an insignificant significant difference.

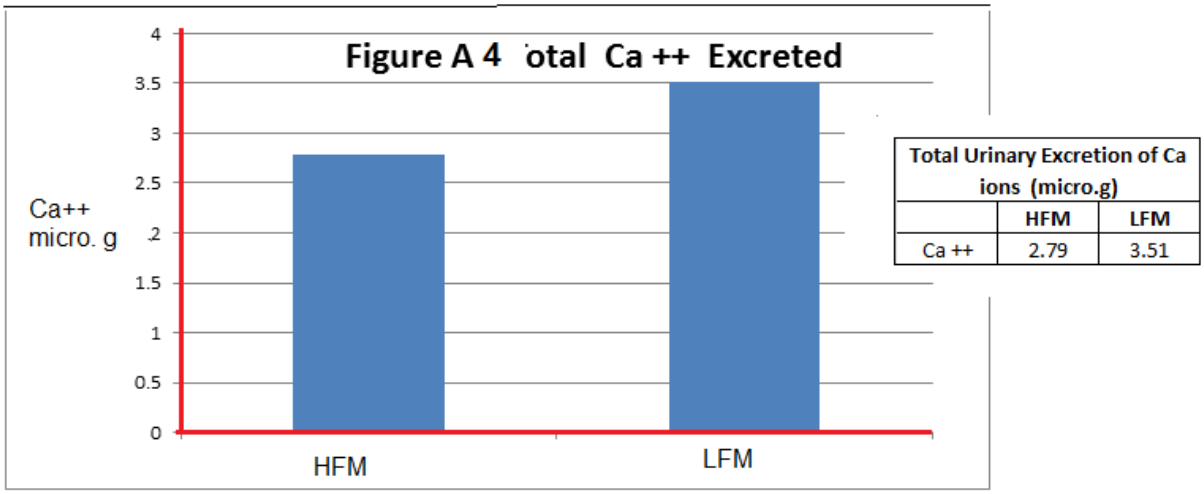


Figure A4. Total of excretion of urinary Calcium for high fat and low fat diets\

The average total excreted calcium is shown following the high fat meal (HFM) and after the low fat meal (LFM). The values were obtained from Figure A 1 by multiplying the rate excretion by the corresponding clearance times(30, 30, 30, 30 and 60 min.) and summing these products. The low fat meal is shown to be associated with greater calcium excretion.

The pattern of ammonia and calcium excretion shown in Figures A5 is very similar for both meals. The first period produced a high output of calcium, ammonium and urea which was followed by a decrease in all three over the next period. The third period resulted once again in an increase in the three species. Decreases then occurred after the 90 minute mark. This pattern of increase in excretion rate followed by decrease then a subsequent increase again was a similar pattern to that exhibited by subjects in a previous experiment [1] Brazier BW (2016).

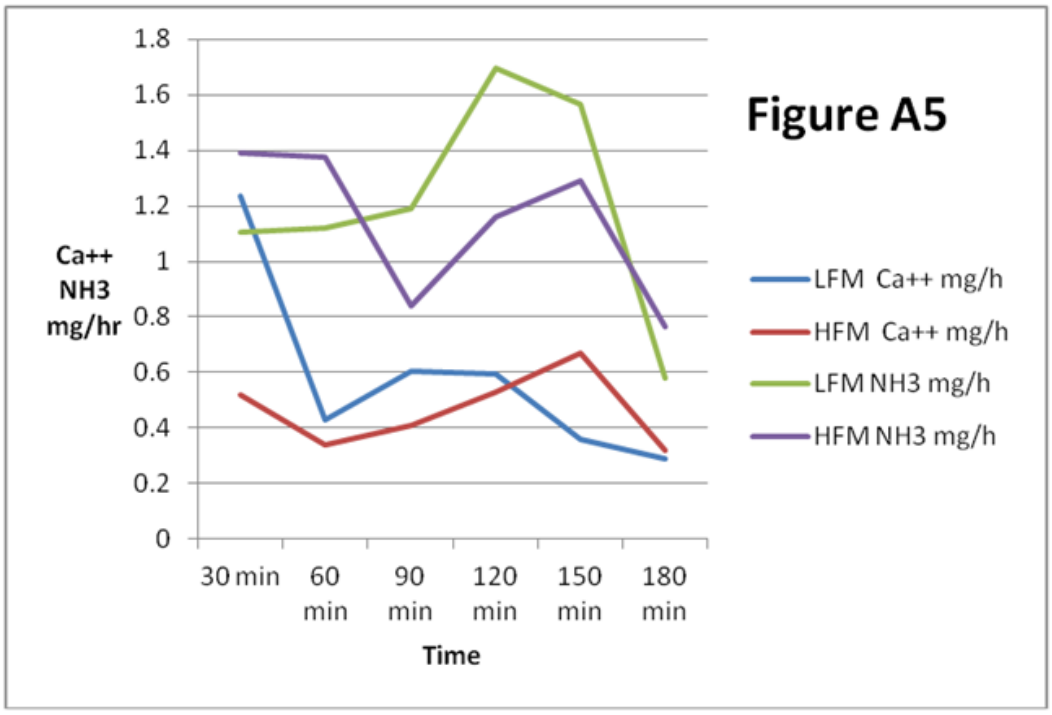


Figure A5. Rate of excretion of Ca++ and NH4 for high fat and low fat diets

Rate of excretion of urinary calcium and ammonia each time period are shown as per Table A3 and A4 and indicate that only the 60 and 180 min time periods.

5. Results: PART B

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

There was no recognisable pattern relating oil or solid fat diets (USFM vs SFM) with calcium excretion. However some individuals showed substantial difference between the two diets while others did not. For calcium excretion four showed SFM > USFM, three showed USFM > SFM and three showed USFM = SFM (refer Figures B.3 to B.7 at [32] Brazier BW. (2016c)

There was however a clear pattern in regard to insulinemia and renal calcium excretion rate. This was irrespective of the type of meal consumed. The insulin responses showed no correlation with meal type. When insulin showed a peak, plasma concentration calcium / creatinine ratio was low and when urinary calcium /creatinine showed a peak the plasma insulin levels were low (Table B1).

Table B1. Calcium Excretion vs Insulinemia

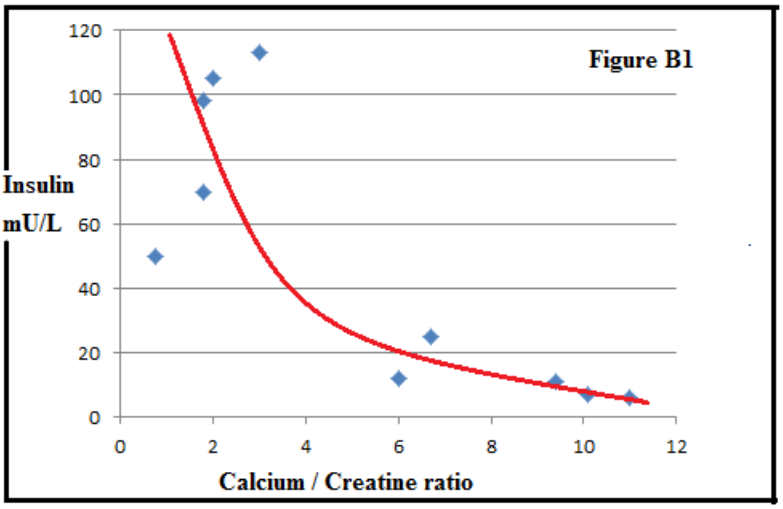
peak [In]	113	50	105	98	70
Ca/Cr x 10	3.0	0.75	2.0	1.8	1.8
peak Ca/Cu x 10	6.0	6.7	11.0	10.1	9.4
[In]	12	25	6	7	11

Table B1 Calcium Excretion vs. Insulinemia

This table shows the peak levels of calciuria and the corresponding levels of plasma insulin (mU/dm^3] as well as the peak levels of plasma insulin and their corresponding levels of calciuria. These values are used to plot the graph in Figure B1.

When these values were plotted against each other a hyperbolic relationship was observed as shown by: Figure B1.

No other significant changes were noticed in blood plasma constituents or urine composition.



URINARY Ca/Cr	PLASMA INSULIN
3	113
0.7	50
2	105
1.8	98
1.8	70
6	12
6.7	25
11	6
10.1	7
9.4	11

Figure B1. Plasma Insulin vs Corresponding Calciuria

Plasma insulin levels in mU/L are plotted against corresponding urinary calcium/creatinine ratios. Only the maximum plasma insulin values and their corresponding urinary calcium/ creatinine ratios and maximum calcium/creatinine ratios and their corresponding plasma insulin levels are used. The values were obtained from the postprandial calciuria and insulinemia responses of the human subjects following consumption of high fat meals with saturated and unsaturated fats

6. Discussion

The results of Part A of this study tend to indicate that a high fat content in the diet can affect the level of urinary calcium.

In the case of the low fat meal, the first thirty minutes period produced more than twice the rate of calcium excretion than the high fat meal. This could be due to a larger amount of calcium available for absorption in the low fat meal as compared to the high fat meal, in the early stages of the experiment. Fat and other nutrients begin to be broken down immediately they enter the stomach and small intestine. The rate of breakdown is dependent on both amount of substrate and enzymes present. If the amount of substrate exceeds its enzyme Vmax, the rate cannot increase. Furthermore, the capacity for a particular nutrient to be absorbed exhibits similar Michaelian kinetics. If a high fat meal stretches lipolysis to its limit it may be likely that this may exceed the rate of absorption.

Free fatty acids not immediately absorbed can accumulate in the gut lumen and react with calcium ions to form insoluble calcium complexes. These compounds are not able to diffuse through epithelial cell membrane of the intestinal microvilli. Therefore, they are voided with faeces as has been reported by [14] Potter (1990) and [15] Appleton (1991) when they examined the FFA content of faeces after HF diets and LF diets.

The large initial difference between the calciuric response of the low and high fat meals could be associated with nutrients found in the meals other than fat. The use of 'Skinny Milk' in the low fat meal, with its greater lactose content, compared to whole milk in the high fat meal, and may have contributed to the result. Lactose has been shown to facilitate calcium absorption [13] Lange, (1979). As more lactose was present in the low fat meal compared to high fat meal (HFM=11 g), calcium absorption may have been boosted. This combined with a low fat level could have contributed to the significant difference in the results during the first thirty minute period.

The protein content was constant between the two meals and no significant difference was found in the excretion of ammonia. Ammonia is excreted in urine to neutralise the acid excreted as a result of the acid ash content of the amino acids catabolism.

The results show that after the first half hour no significant differences between calciuric response of the low and high fat meals. Figure Al clearly illustrates the large fall in calcium excretion after this period in the low

fat meals. These results may be attributable to the rate of fatty acid digestion approached the rate of fatty acid absorption increasing so that there is less excess free fatty acid in the latter periods to complex Ca ++.

The difference in urea production between high fat meal and low fat meals without corresponding to change in renal ammonia production could have been because the keto-acids from fatty acid catabolism equalled the acid from amino acid production. The difference in apparent protein catabolism could have been due to different levels of insulinemia (not measured in this experiment).

In Part B of this experiment no difference could be observed between the solid fat compared to liquid fat in regard to the postprandial calciuric effect. However a Significant inverse relationship is observed between postprandial insulinemia and calciuria. This is the opposite of the results obtained by [5] Wood and Allen (1983) and [27] Howe (1990) who produced a direct relationship between plasma insulin and calciuria when arginine or glucose was infused into. A two further studies has been done in this laboratory [19] Brazier (2016b) to examine the effect of plasma insulin on calciuria and [34] Brazier (2016d) to examine the effect of insulin on calciuria calcium exchange rate across isolated kidney tubules membranes using Ca^{45} isotopes.

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